eISSN: 2564-6524

ANKARA ÜNİVERSİTESİ
ECZACILIK FAKÜLTESİ DERGİSİ

JOURNAL OF FACULTY OF PHARMACY OF ANKARA UNIVERSITY

Cilt / Vol : 44
Sayı / No : 1
Yıl / Year : 2020

eISSN: 2564-6524

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(*Ankara Ecz. Fak. Derg.*) **eISSN: 2564-6524**

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Baş Editör:

Prof. Dr. İlkay YILDIZ
Ankara Üniversitesi, Eczacılık Fakültesi, Farmasötik Kimya Anabilim Dalı,
06560 Yenimahalle-ANKARA,
Tel: 0 312 203 30 69
Faks: 0 312 213 10 81
e-posta: *iyildiz@pharmacy.ankara.edu.tr*

Editörler:

Prof. Dr. Canan HASÇİÇEK
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e-posta: ustundag@pharmacy.ankara.edu.tr
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e-posta: bkaskatepe@ankara.edu.tr
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e-posta: akoc@ankara.edu.tr
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Ankara Üniversitesi, Eczacılık Fakültesi, Farmakognozi Anabilim Dalı
e-posta: ozbilgin@pharmacy.ankara.edu.tr
Dr. Kayhan BOLELLİ
Ankara Üniversitesi, Eczacılık Fakültesi, Farmasötik Kimya Anabilim Dalı
e-posta: bolelli@ankara.edu.tr
Dr. M. Mesud HÜRKUL
Ankara Üniversitesi, Eczacılık Fakültesi, Farmasötik Botanik Anabilim Dalı
e-posta: mhurkul@ankara.edu.tr
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JOURNAL OF FACULTY OF PHARMACY OF ANKARA UNIVERSITY

(*J. Fac. Pharm. Ankara*) eISSN: 2564-6524

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Editor-in-Chief:

Prof. Dr. Ilkay YILDIZ
Ankara University Faculty of Pharmacy, Department of Pharmaceutical Chemistry
06560 Yenimahalle-Ankara/TURKEY
Tel: 0 312 203 30 69
Fax: 0 312 213 10 81
e-mail: efd.editor@ankara.edu.tr

Editors:

Prof. Dr. Canan HASÇİÇEK
Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Technology
e-mail: cogan@pharmacy.ankara.edu.tr
Assoc. Dr. Prof. İlker ATEŞ
Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology
e-mail: iates@pharmacy.ankara.edu.tr
Assoc. Dr. Prof. Özgür ÜSTÜNDAĞ
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e-mail: ustundag@pharmacy.ankara.edu.tr
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Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology
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Assis. Dr. Prof. Aslı KOÇ
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e-mail: ozbilgin@pharmacy.ankara.edu.tr
Dr. Kayhan BOLELLİ
Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry
e-mail: bolelli@ankara.edu.tr
Dr. M. Mesud HÜRKUL
Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany
e-mail: mhurkul@ankara.edu.tr
Dr. Gizem GÜLPINAR
Ankara University, Faculty of Pharmacy, Department of Pharmacy Business Administration
e-posta: gaykac@ankara.edu.tr

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J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 44(1): 1-17, 2020
Doi: 10.33483/jfpau.567757

ORIGINAL ARTICLE / ÖZGÜN MAKALE

HISTOLOGICAL STUDY OF A CORRECTIVE INFLUENCE OF A COMPOUND POTASSIUM 2-((4-AMINO-5-(MORPHOLINOMETHYL)-4*H*-1,2,4-TRIAZOL-3-YL)THIO)ACETATE (PKR-173) ON THE STATE OF CHICKEN'S LIVER UNDER INFECTION BY *PSEUDOMONAS AERUGINOSA*

PSEUDOMONAS AERUGINOSA İLE ENFEKTE EDİLMİŞ CİVCİVLERİN KARACİĞERİNE POTASYUM 2-((4-AMİNO-5-(MORFOLİNOMETİL)-4H-1,2,4-TRİAZOL-3-İL)TİYO)ASETAT (PKR-173) BİLEŞİĞİNİN DÜZELTİCİ ETKİSİNİN HİSTOLOJİK OLARAK ARAŞTIRILMASI

Yevgenia VASHCHYK[1], Roman SHCHERBYNA[1,*], Volodymyr PARCHENKO[2], Inna BUSHUEVA[2], Bogdan GUTYJ[3], Hanna FOTINA[1], Tatiana FOTINA[1], Yuriy STRONSKYI[3]

[1]Sumy National Agrarian University, Faculty of Veterinary Medicine, Department of Veterinary Sanitary Examination, Microbiology, and Zoohygiene Safety and Quality of Animal Products, Sumy, Ukraine

[2]Zaporizhzhya State Medical University, Faculty of Pharmacy, Department of Natural Sciences for Foreign Students and Toxicological Chemistry, Zaporizhzhya, Ukraine

[3] Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Lviv, Ukraine

ABSTRACT

Objective: *The aim of the research is the histological study of the corrective effect of the compound potassium 2((4-amino-5-(morpholinomethyl)-4H-1,2,4-triazol-3yl)thio)acetate (PKR-173) with different antibiotics on the state of chicken's liver under infection by Pseudomonas aeruginosa.*

Material and Method: *The histological study was carried out on the levers of intact chickens (intact control); chickens that were infected by the strain of Pseudomonas aeruginosa (controlled pathology); chickens that had (before being infected by Pseudomonas aeruginosa) preventive input of the compound PKR-173 with*

* **Corresponding Author / Sorumlu Yazar:** Roman Shcherbyna
e-mail / e-posta: rscherbyna@gmail.com

Submitted/Gönderilme: 20.05.2019 **Accepted/Kabul:** 01.09.2019

the classic antibiotic Enrofloxacin or with Saroflox (undergoing research); or were input Thiotriazolin with the classic antibiotic Enrofloxacin.

Result and Discussion: *As a result, it is stated, that preventive input of the compound PKR-173 with reseachable antibiotic Saroflox, as well as with classic antibiotic Enrofloxacin and Thiotriazolin with antibiotic Enrofloxacin, to chickens infected with pseudomonas aeruginosa, prevents from chickens' livers developing stereotypical morphological changes characteristic of nonspecific reactive hepatitis. Analysis of quantitative characteristics of pathological process that developed in chicken's liver infected with pseudomonas aeruginosa, and the corrective influence of researched methods showed that the compound PKR-173 with antibiotic Saroflox believably lowers displays of all signs of pathology compared to the controlled pathology.*

Keywords: *1,2,4-triazole, hepatitis, hepatoprotective effect, infection of pseudomonas, saroflox*

ÖZ

Amaç: *Araştırmanın amacı, potasyum 2-((4-amino-5-(morfolinometil)-4H-1,2,4-triazol-3-il)tiyo)asetat (PKR-173) bileşiğinin farklı antibiyotikler ile birlikte Pseudomonas aeruginosa ile enfekte edilmiş civcivlerin karaciğerine düzeltici etkisinin histolojik olarak incelenmesidir.*

Gereç ve Yöntem: *Histolojik araştırma, sağlam civcivler (kontrol), Pseudomonas aeruginosa ile enfekte edilmiş civcivler (kontrol edilen patoloji), (Pseudomonas aeruginosa ile enfekte edilmeden önce) PKR-173 bileşiği ile Enrofloxacin veya Saroflox gibi klasik antibiyotik yada tiotiazolin ile Enrofloxacin antibiyotiği verilen civcivlerin karaciğeri üzerinde yapılmıştır.*

Sonuç ve Tartışma: *Araştırmaların sonucunda; araştırılan antibiyotik olan Saroflox ile PKR-173 bileşiğinin ve önleyici bir şekilde Pseudomonas aeruginosa ile enfekte edilmiş civcivlere uygulanan Enrofloxacin ve Thiotriazolin- Enrofloxacin gibi klasik antibiyotiklein, nonspesifik reaktif hepatite özgü olan karaciğerdeki standart morfolojik değişiklikleri önlediği tespit edilmiştir. Pseudomonas aeruginosa ile enfekte edilmiş civcivlerin karaciğerinde gelişen patolojik sürecin kantitatif özelliklerinin analizi ve düzeltici etkisi araştırılan metot, Saroflox antibiyotiği ile PKR-173 bileşiğinin, kontroldeki patolojiye kıyasla patolojinin bütün belirtilerini azalttığını göstermiştir.*

Anahtar Kelimeler: *1,2,4-triazol, hepatit, hepatoprotektif etki, pseudomonas enfeksiyonu, saroflox*

INTRODUCTION

Hepatoprotective remedies are still in high demand [1]. They raise liver's resistance to the effects of toxic agents and normalize its metabolism in conditions of intensive detoxication function. The mechanisms of damage to the liver may be different, but among the many, used for liver treatment, a doctor has to choose the optimal one both from the perspective of etiology and pathogenesis, as well as clinical presentation of a disease. Unfortunately, the variety of the existent domestic hepatoprotective remedies isn't big.

Safety, tolerance to the drug, possibility of continuous intake, economical reasoning are the deciding factors in favour of choosing certain hepatoprotective remedies. The widening of hepatoprotectors' variety, caused by implementing new discoveries in biochemistry, molecular biology and nanotechnology, is on agenda for modern scientists [2, 3].

Many 1,3,4-oxadiazole, 1,2,4-triazole and 1,3,4-thiadiazole derivatives are of great interest and are associated with a broad spectrum of pharmacological activities [4-9]. Ribavirin, fluconazole and cefazolin are antiviral, antifungal and antibacterial drugs that contain units of 1,2,4-triazole and 1,3,4-thiadiazole. Furthermore, the 1,3,4-oxadiazole ring system has been found in the skeleton of fungicidal and bactericidal, analgesic, antipyretic, antiphlogestic, anticompulsive, paralytic hypnotic and sedative

agents [10-12] in addition to antiviral, antitumour [13] and tyrosinase inhibiting agents [14]. Moreover, various substituted 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles and their dihydro analogues have been shown to possess antimicrobial, antibacterial [15], anti-inflammatory [16], antifungal, CNS-depressant and antiviral [17] activities.

The data on studying hepatoprotective properties of the above-mentioned compounds under hepatitis, caused by the toxic influence of chemical substances, is present in scientific literature [18, 19]. The aim of our research was to study hepatoprotective properties of the compound PKR-173 under conditions of bacterial toxins on chickens' organisms. It is known that upon experimental infection by *Pseudomonas aeruginosa* chickens develop conditional characteristic of toxic liver damage [20, 21]. Thus histological study of the corrective effect of the compound “potassium 2((4-amino-5-(morpholinomethyl)-4H-1,2,4-triazol-3yl) thio) acetate” (PKR-173) with different antibiotics on the state of chicken's liver under infection by *Pseudomonas aeruginosa* we deem appropriate [22].

The aim of the research is the histological study of the corrective effect of the compound “potassium 2((4-amino-5-(morpholinomethyl)-4H-1,2,4-triazol-3yl) thio) acetate” (PKR-173) with different antibiotics on the state of chicken's liver under infection by *Pseudomonas aeruginosa*.

MATERIAL AND METHOD

As an object of research potassium 2-((4-amino-5-(morpholinomethyl)-4H-1,2,4-triazole-yl-3)thio)acetate (PKR-173) was used [23].

Experimental studies were held under conditions of the department of natural sciences for foreign students and toxicological chemistry of Zaporizhzhya State Medical University and departments of veterinary sanitary expertise, microbiology, veterinary public health and safety and quality of products of animal breeding of Sumy National Agrarian University. Histological research was conducted at central research laboratory of the National University of Pharmacy.

The study was conducted on chickens aged 7-18 days. The histological study was carried out on the levers of intact chickens (intact control); chickens that were infected by the strain clinical isolate of *P. aeruginosa* (controlled pathology) [24]; chickens that had (before being infected by *P. aeruginosa*) preventive input of the compound PKR-173 with the classic antibiotic Enrofloxacin or with Saroflox (undergoing research); or were input Thiotriazolin with the classic antibiotic Enrofloxacin. The strain *P. aeruginosa* ATCC 27853 were used as a control reference strain.

P. aeruginosa culture was intraperitoneally input in chickens on the 4th day of the experiment with a solution of 300.000 CFU/ml (by McFarland [25]), with the dosage of 0.2 ml (LD50 by the Kerber method in the modification of Ashmarin) [26]. The compound PKR-173 (in powder form) and Thiotriazolin (in powder form) was input per os during the 3 days before the infection and 5 days after,

with a dosage of 150 mg/kg and 50 mg/kg respectively [27, 28]. Antibiotics were input during the 5 days after the infection.

The selection of liver samples of each chickens' group of the experiment was carried out on the 6th day after the infection. The material was fixed in a 10% solution of formaldehyde, and was dehydrated in alcohols of increasing strength, and embedded in paraffin wax. The paraffin blocks' slices of 4-5 μ min width were produced on sled microtome “MC 2” and painted for observation microscopy with hematoxylin (Mayer's hemalum solution) and eosin [29]. Microscopic study of the microscope slide were conducted on Granum microscope, and the photography of the microscopic images was done with digital camera Granum DCM 310. The photographs were edited on Pentium computer with 2,4GHz frequency with an application Toup View.

For the conveniency of comparison and greater objectification of the results on hematoxylin and eosin painted microscope slides, the quntitative (graded) assessment of degrees of liver damage of chickens of different study groups was done. The distinctiveness of hepatocytes' dystrophy, presence and distribution of areas of breached beam pattern, cell necrosis, cell infiltration of blood vessels were assessed. It was based on semiquantitative visual assessment of output of histochemical reactions, by Sokolovsky method [30]. Each type of damage was assessed separately.

The dispersion analysis (Kruskal-Wallis criteria) was conducted for statistical results, and Mann-Whitney criteria with probability ≤0,05 – for comparing data samples [31-35]. For statistical processing the Statistuca 6.0 was used.

RESULT AND DISCUSSION

The results of bright-field microscopy analysis of intact chickens show that histostructure of their liver corresponds to the healthy organ and is inherent to the age of [30, 31]. The lobules structure is not shown. The inter-lobules connective tissue is underdeveloped. The borders of lobules were indentified by the portal triad (passing zone for branches of hepatic artery, portal vein and common bile duct). The passing zones themselves are quite narrow. The parenchymal lobules consist of hepatocyte plates, which consist of a few polygonal hepatocytes each, with rounded and well-bordered centre-placed nuclei (one for a cell), in which nucleoli and chromatin macromolecules are well visible. The hepatocyte cytoplasm is well coloured, doesn't show inserts visible on bright-field microscopic level. Radial direction of the liver plates is the most prominent close to central veins, at a distance from them – the plates are more round. Between the liver plates are sinusoidal hemocapillaries that show a very limited amount of lymphoid cells and nuclei erythrocytes. Central and inter-lobules veins and triad vessels are not expanded, as a rule, and not filled with blood, Kupffer cells have no distinctive features (fig. 1, fig. 2). In some chickens close to some inter-lobules veins the lymphocytic clusters of various sizes were discovered.

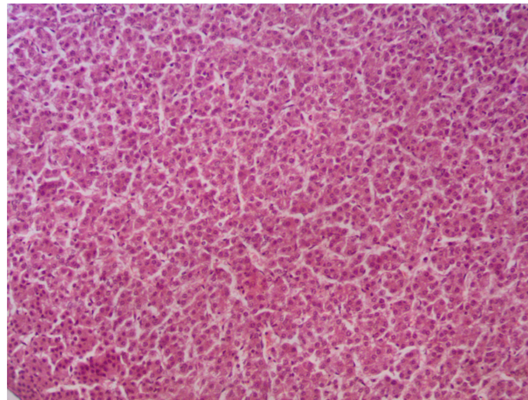


Figure 1. Area of an intact chicken's liver. The liver plates look clear, hepatocytes have evenly coloured cytoplasm, normal nuclei, sinusoidal hemocapillaries are moderately expanded. Hematoxylin-eosin. x200

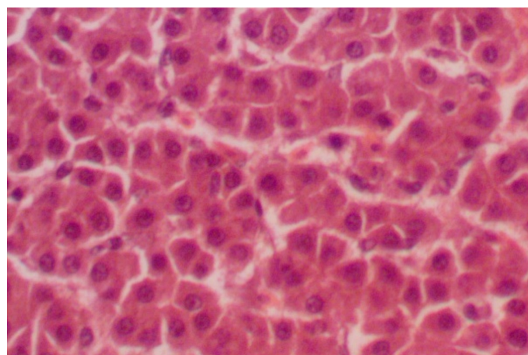


Figure 2. A fragment of fig. 1. Hematoxylin-eosin. x400

After infecting the chickens with *P. aeruginosa* their liver showed nonspecific morphological changes that occur under different diseases of liver and other organs and systems, and can be also caused by overall toxic influence endotoxins of a given microorganism. Chickens showed disorganisation of a outline of liver plates, vacuole (fatty and mainly hydropic) distrophy of hepatocytes, small focuses of necrosis, inflammatory cell infiltration. As a rule, prevalence of deviation zones of plates outline, distinctiveness of fatty and hydropic hepatocyte dystrophy, presence and numerosity focuses of parenchyma necrosis varied among chickens of the group. Moreover, different combinations of these pathological signs were observed. That way the structure disorganisation was combined more often with a dystrophy, and necrosis – with cell infiltration. Vecuoles had pepperish nature. Hydropic dystrophy is observed at certain places as well (fig. 3, fig. 4).

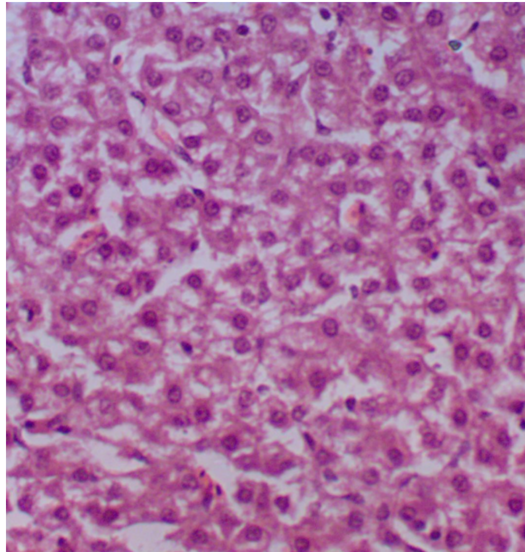


Figure 3. Area of a chicken's liver infected with *P. aeruginosa*. Hepatocyte dystrophy, deviations in plates outline. Hematoxylin-eosin. x400

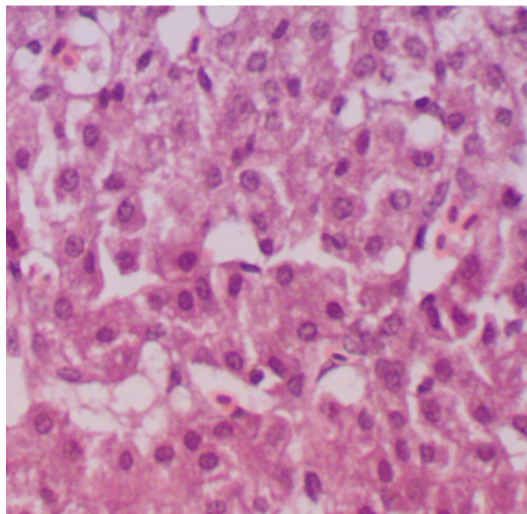


Figure 4. Area of a chicken's liver infected with *P. aeruginosa*. Hydropic hepatocyte dystrophy. Hematoxylin-eosin. x400

In zones of liver plates' disorganisation the hepatocytes were swollen, varied in size, had blurred boundaries, various-sized nuclei with poorly visible colouring or were in a state of karyolisys; visible focals of cell lysis (fig. 5).

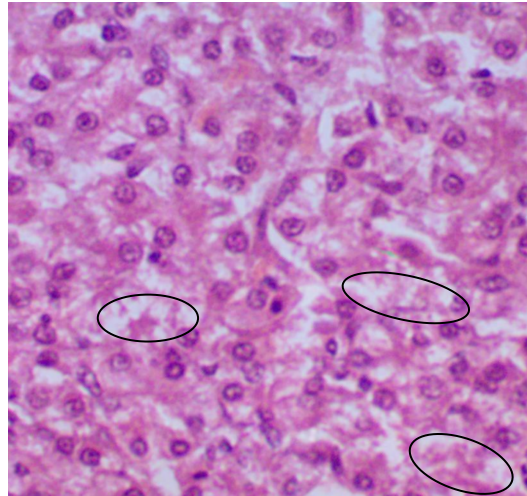


Figure 5. Area of a chicken's liver infected with *P. aeruginosa*. Lysis of part of hepatocytes (ellipses), different states of cell nuclei. Hematoxylin-eosin. x400

Sometimes apoptotic bodies (Councilman bodies) were visible as round eosinophile formations with pyknotic nuclei or without, the appearance of hepatocytes in a state of cell division (fig. 6). Moreover, a vacuolesation of bile ducts' endothelium in some triads, minor biliary hyperplasia was observed (fig. 7).

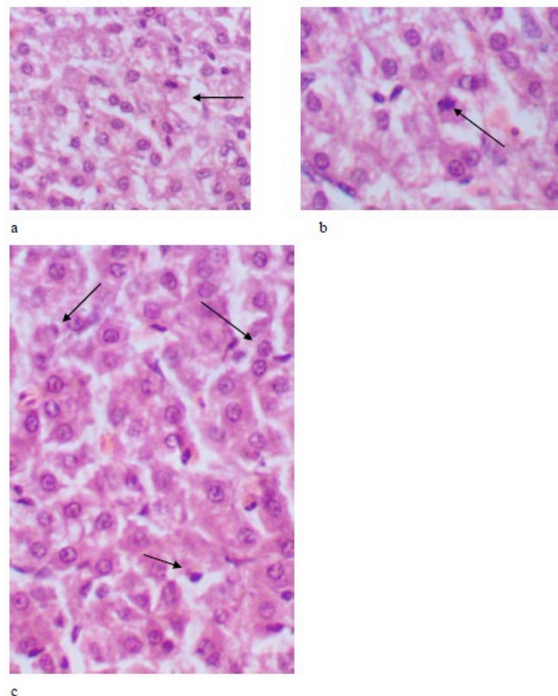


Figure 6. Area of a chicken's liver infected with *P. aeruginosa*. a-b – mitosis in hepatocytes; c – Councilman bodies (arrows). Hematoxylin-eosin. x400.

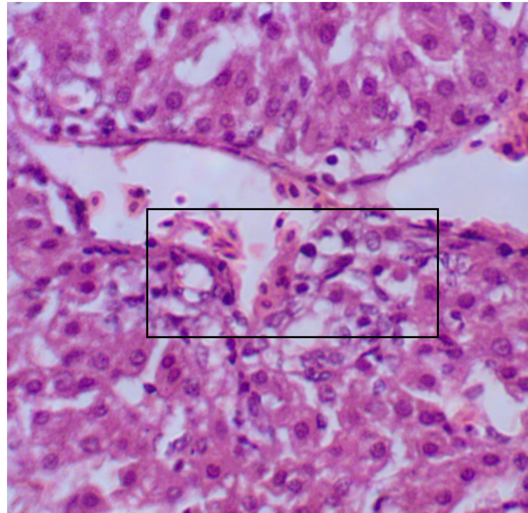


Figure 7. Area of a chicken's liver infected with *P. aeruginosa*. Vacuolesation of endothelium, minor biliary hyperplasia in a triad. Hematoxylin-eosin. x400.

Hepatocyte necrosis (colliquative, monocellular) was of minor focal necrosis character. Focuses of necrosis were localized in different areas of lobules, often had quite clear borders, and usually abundantly infiltrated by limphoid cells' plates. Sometimes the infiltrated cells "spread out" between liver plates. In some necrosis focuses, among infiltrated cells, "immured" groups of hepatocytes were visible (fig. 8).

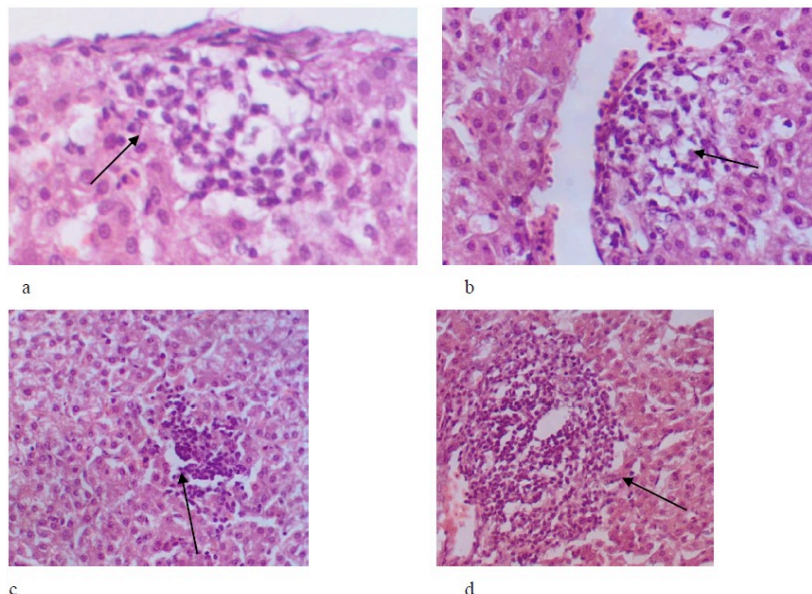
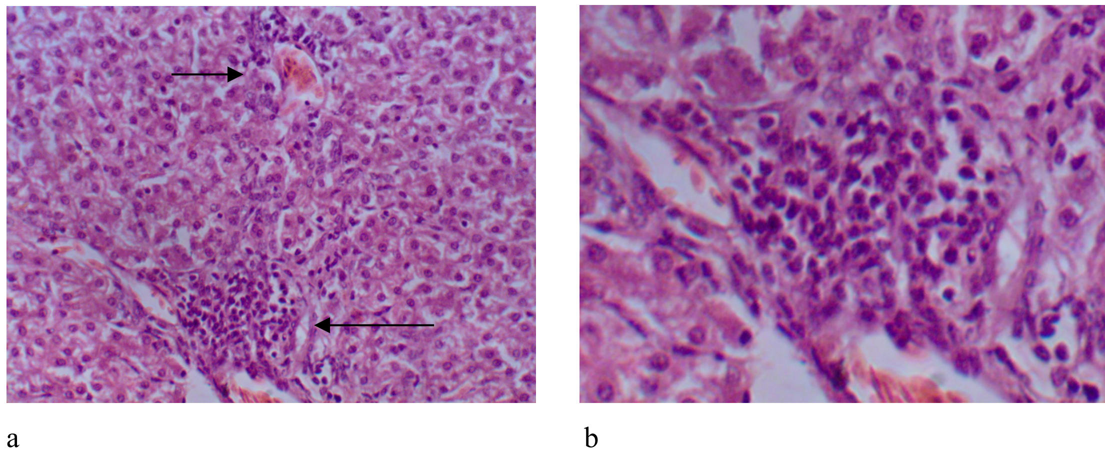


Figure 8. Area of a chicken's liver infected with *P. aeruginosa*. Minor focals of hepatocytes necrosis of different locations (a – subcapsular, b – near the inter-lobules vein, c – in parenchyma of a lobule, d – periportal), penetration of infiltrated cells between liver plates (c), "immured" hepatocytes among infiltrated cells (d). Hematoxylin-eosin. a,b,d - x250, c- x400

In some triads perivenularly, close to some central veins, moderate/minor inflammatory polymorphic cell infiltration is observed. Among the infiltrated cells lymphocytes, some neutrophile white blood cells, macrophages were visible (fig. 9).



a b

Figure 9. Area of a chicken's liver infected with *P. aeruginosa*. Moderate/minor inflamed cell infiltration close to central vein and triad vein (a, x200), lymphocytes, neutrophile white blood cells and macrophages in inflamed infiltration (b, x400). Hematoxylin-eosin.

Suggested microscope picture, by morphological features, corresponds to moderately shown nonspecific reactive hepatitis (fig. 8).

After getting the compound PKR-173 with researchable antibiotic Saroflox, 80% of chickens, infected with *P. aeruginosa*, almost fully restored typical outline of liver plates and normal morphology of hepatocytes. In some hepatocytes nuclei were visually hypertrophied, the contents of two-nuclei cells were visually increased (non-direct evidence of regenation). Fatty and hydropic dystrophy is absent in 40% of birds, or distinctly lowered – in 60% (fig. 10). In 20% of chickens minor inflammatory infiltration of veins in some triads was observed, as well as single minor focuses of necrosis (fig. 11, fig. 12). Microcircular blood vessels were moderately blood-filled in all chickens.

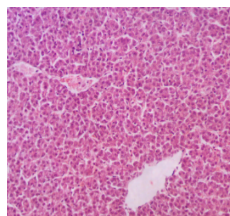


Figure 10. Area of a chicken infected with *P. aeruginosa* and input the compound PKR-173 with the antibiotic Saroflox. Full recovery of normal histostructure, absence of hepatocyte dystrophy, normal state of microcircular blood vessel. Hematoxylin-eosin. x250

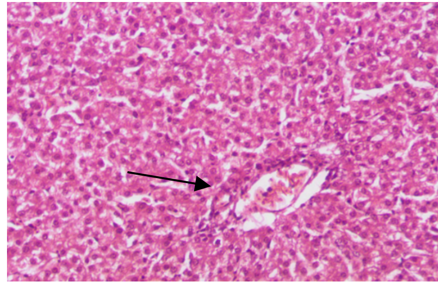


Figure 11. Area of a chicken infected with *P. aeruginosa* and input the compound PKR-173 with the antibiotic Saroflox. Minor inflammatory cell infiltration in triad zone. The distinctiveness of the dystrophy is lowered. Hematoxylin-eosin. x250

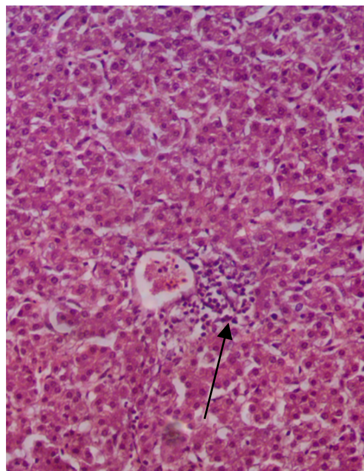


Figure 12. Area of a chicken infected with *P. aeruginosa* and input the compound PKR-173 with the antibiotic Saroflox. Small focus of necrosis infiltrated by round cell elements next to the central vein of lobules. Hematoxylin-eosin. x250

After preventive input of compound PKR-173 with a classic antibiotic Enrofloxacin in 80% of chickens the plate structure of liver parenchyma is restored, hepatocyte dystrophy is absent (fig. 13).

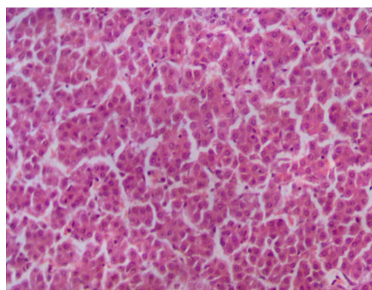


Figure 13. Area of a chicken infected with *P. aeruginosa* and input the compound PKR-173 with the antibiotic Enrofloxacin. Absence of breaches in liver plates outline, as well as hepatocyte dystrophy. Hematoxylin-eosin. x250

Single very small focuses of necrosis are present in 20% of chickens (fig. 14). At the same time, almost all chickens kept different distinctive inflammatory round cells infiltration close to central veins and vessels in many triads. Infiltrators consisted of lymphocytes, some neurophile white blood cells, macrophages (fig. 15a). In one case the focal expansion and full-bloodness of sinusoidal hemocapillaries, minor hemorrhages with destruction of parenchyma (fig. 15b).

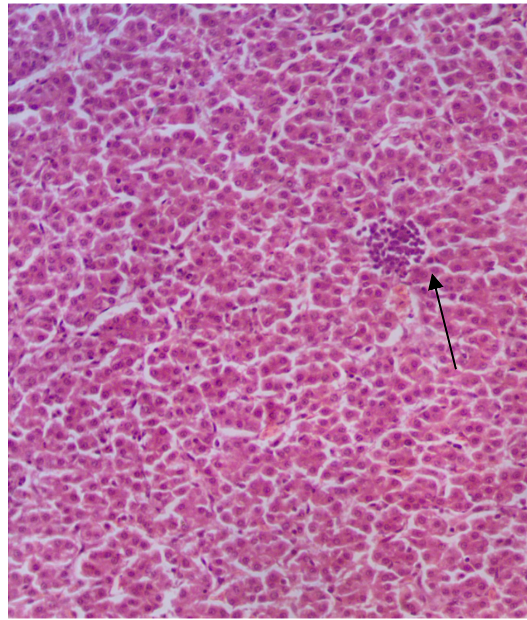
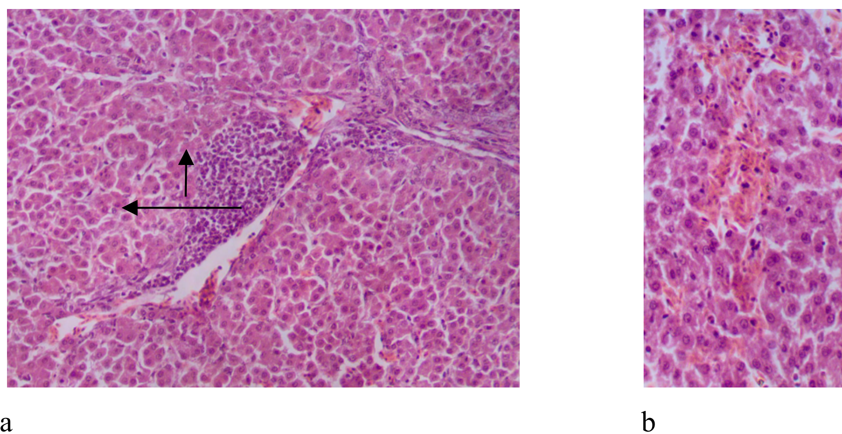


Figure 14. Area of a chicken infected with *P. aeruginosa* and input the compound PKR-173 with the antibiotic Enrofloxacin. Single small focus of necrosis in lobules parenchyma. Hematoxylin-eosin. x250



a b

Figure 15. Area of a chicken infected with *P. aeruginosa* and input the compound PKR-173 with the antibiotic Enrofloxacin. a - inflammatory cell infiltration close to a veins in a triad (x200); b - hemorrhage in lobules parenchyma (x250). Hematoxylin-eosin

After preventive input of Thiotriazolin with the classic antibiotic Enrofloxacin, a positive influence on the morphological state of liver was observed in 60% of chickens – recovery of typical outline of liver plates, absense of signs of fatty and hydropic dystrophy (fig. 16). Nevertheless, the rest of chickens had these indicators close to the level of controlled pathology (fig. 17).

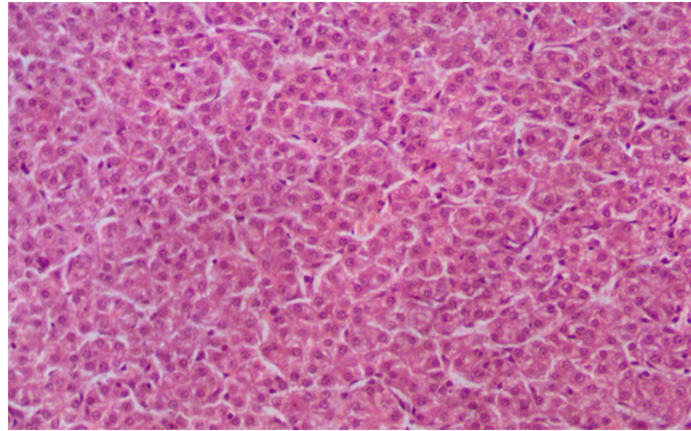


Figure 16. Area of a chicken infected with *P. aeruginosa* and input Thiotriazolin with the antibiotic Enrofloxacin. Normal outline with liver plates. Hematoxylin-eosin. x200

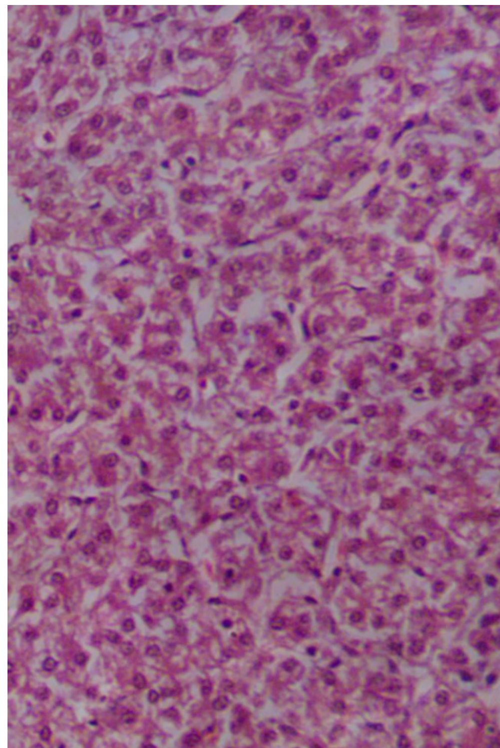
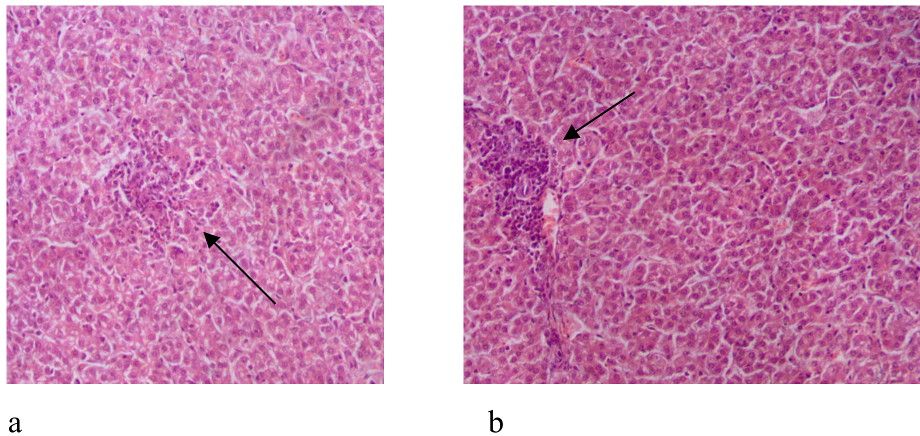


Figure 17. Area of a chicken infected with *P. aeruginosa* and input t Thiotriazolin with the antibiotic Enrofloxacin. Blurred outlineof liver plates, vacuole dystrophy of hepatocytes. Hematoxylin-eosin. x250

Hepatocytes necrosis and inflammatory perivascular round cell infiltration was absent in only 40% of chickens, and 60% of birds, even with lowered distinctiveness of these changes corresponding to the controlled pathology, were still quite visible (fig. 18).



a b

Figure 18. Area of a chicken infected with *P. aeruginosa* and input Thiotriazolin with the antibiotic Enrofloxacin. a – necrosis focus with blurred outlines, penetration of macrophages into a lobule; b – inflammatory round cell perivascular infiltration. Hematoxylin-eosin. x200

So, preventive input of the compound PKR-173 with reseachable antibiotic Saroflox, as well as with classic antibiotic Enrofloxacin and Thiotriazolin with antibiotic Enrofloxacin, to chickens infected with *P. aeruginosa*, prevents from chickens' livers developng stereotypical morphological changes characteristic of nonspecific reactive hepatitis which, obviously, is a result of overall toxic influence of endotoxins of a microorganism, facilitating the normalisation of organ histostructure, decrease of dystrophy and necrosis displays, inflammatory infiltration in vein sector of microcircular vessel. Visually the distinctiveness of hepatoprotective effect in all 3 groups is very close, even though it varies by some indicators. Thus, according to the set task, semiquantitative (graded) assessment of the corrective influence of the compound PKR-173 and Thiotriazolin with antibiotics on morphological state of chickens' liver parenchyma was conducted. The results of the graded assessment are shown in table 1.

Analysis of quantitative characteristics of pathological process that developed in chicken's liver infected with *P. aeruginosa*, and the corrective influence of reseached methods showed that the compound PKR-173 with antibiotic Saroflox believably lowers displays of all signs of pathology compared to the controlled pathology.

Based on the distinctiveness of hepatoprotective effect the compound PKR-173 with antibiotic Saroflox believably analogical to the effect of this same compound with a classic antibiotic Enrofloxacin.

Table 1. Semiquantitative assessment of corrective influence of the compound PKR-173 with different antibiotics on the 16-18 days old chickens' livers under conditions of infection by microorganisms *P. aeruginosa* (grades), M (min; max)

Experiment group	indications, grades (average)			
	distinctiveness of hepatocytes dystrophy	Necrosis changes in hepatocytes	breaches in plates' outline	inflammatory infiltration, connected to the vessels
Intact control	0(0;0)	0(0;0)	0(0;0)	0.5(0;2)
P. aeruginosa (US)	2.6(2;3)*	1.6(1;2)*	1.6(1;2)*	2.2(2;3)*
PKR-173+ antibiotic Saroflox	0.8(0;2)**	0.4(0;1)**	0.4(0;2)**	0.12(0;1)**
PKR-173+ antibiotic Enrofloxacin	0.12(0;1)**	0.12(0;1)**	0.12(0;1)**	2(2;2)*/#
Thiotriazolin + Enrofloxacin	0.8(0;2)**	0.6(0;1)**	0.6(0;2)	0.6(0;1)**/ψ
p	0.0044	0.0069	0.0156	0.0013

Notes: ρ – level of statistical significance while comparing samples by means of Kruskal-Wallis criteria;
* - level of statistical significance while comparing research groups to US group by means of Mann-Whitney criteria;
** - level of statistical significance while comparing research groups to PS group by means of Mann-Whitney criteria;
# - level of statistical significance while comparing research groups to PKR+Saroflox group by means of Mann-Whitney criteria;
ψ - level of statistical significance while comparing research groups to PKR+Enrofloxacin group by means of Mann-Whitney criteria.

Based on absolute data the researchable compound with antibiotic Saroflox as for its hepatoprotective effect, specifically influence on anti-inflammatory processes in a liver, despite statistical reliance, Thiotriazolin with antibiotic Enrofloxacin preveils in comparison.

Prospects for further research are in more depth study of potassium 2-((4-amino-5-(morpholinomethyl)-4H-1,2,4-triazol-3-yl)thio)acetate on the ability of display hepatoprotective effect on various models of hepatitis.

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J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 44(1): 18-32, 2020
Doi: 10.33483/jfpau.559509

ORIGINAL ARTICLE / ÖZGÜN MAKALE

NOVEL TACRINE AND HESPERETIN ANALOGUES: DESIGN, MOLECULAR DOCKING AND IN SILICO ADME STUDIES TO IDENTIFY POTENTIAL ACETYL CHOLINE ESTERASE INHIBITORS FOR ALZHEIMER'S DISEASE

YENİ TAKRİN VE HESPERETİN ANALOGLARI: ALZHEİMER HASTALIĞINDA POTANSİYEL ASETİLKOLİN ESTERAZ İNHİBİTÖRLERİNİN AYDINLATILMASI İÇİN TASARIM, MOLEKÜLER DOKİNG VE İN SİLİKO ADME ÇALIŞMALARI

Kondapalli NARESH*[1], Kanna SRUTHI[2]

[1]Department of Pharmaceutical Chemistry, G. Pulla Reddy College of Pharmacy, Osmania University, Hyderabad, India

[2]Department of Pharmaceutical Chemistry R.B.V.R.R Women's College of Pharmacy, Osmania University, Hyderabad, India

ABSTRACT

Objective: *Present investigationis aimed to design and identify new potential molecules to treat Alzheimer's disease from the Tacrine and Hesperetin structures via molecular modification. Acetyl cholinesterase (AChE) enzyme was selected as target, since inhibitors of AChE were successful in the management of dementia and alleviation of other symptoms.*

Material and Method: *In this study, two series of new Tacrine (T1-T9) and Hesperetin (H1-H9)derivatives on the basis of the structural characteristics of acetyl cholinesterase (AChE) inhibitors were designed and screened to identify potential analogues as Anti-Alzheimerdrug on the AChE (PDB ID:1DX4) using GLIDE employing extra-precision docking. The docking results (Glide score, XPscore, docking score and binding interactions) were compared with standard drug, Tacrine.*

Result and Discussion: *From the docking results it was found that T9 showed highest docking score among the designed compounds. The ADME properties also predicted using Qikprop application, from the above studies' potential analogues with highest AChE inhibition and excellent pharmacokinetic properties were identified.*

Keywords: *Acetylcholine esterase inhibitors (AChE), alzheimer's disease (AD), glide, tacrine*

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* **Corresponding Author /SorumluYazar:** Naresh Kondapalli
e-mail / e-posta: naresh4nani@gmail.com

Submitted/Gönderilme: 30.04.2019 **Accepted/Kabul:** 23.09.2019

ÖZ

Amaç: *Mevcut araştırma, Alzheimer hastalığını tedavi etmek için Tacrin ve Hesperetin yapılarından moleküler modifikasyonla yeni potansiyel moleküller tasarlamayı ve belirlemeyi amaçlamaktadır. Asetilkolinesteraz (AChE) enzimi hedef olarak seçilmiştir, çünkü AChE inhibitörleri demansın tedavisinde ve diğer semptomların hafifletilmesinde başarılı olmuştur.*

Gereç ve Yöntem: *Bu çalışmada, asetilkolinesteraz (AChE) inhibitörlerinin yapısal özelliklerinitemel alan iki seri yeni Takrin (T1-T9) ve Hesperetin(H1-H9) türevleri tasarlanmış ve AChE (PDB ID: 1DX4) üzerinde Anti-Alzheimer ilaç olarak potansiyel türevler belirlemek için ekstra hassasdoking uygulaması ile GLIDE kullanarak taranmıştır. Doking sonuçları (Glide skoru, XP skoru, doking skoru ve bağlanma etkileşimleri) standart ilaç, Takrin ile karşılaştırıldı.*

Sonuç ve Tartışma: *Doking sonuçlarından, T9'un tasarlanan bileşikler arasında en yüksek doking skoru gösterdiği bulundu. ADME özellikleri ayrıca Qikprop uygulaması kullanılarak da öngörülmüş, yukarıdaki çalışmalardan en yüksek AChE inhibisyonuna sahip potansiyel analoglar ve mükemmel farmakokinetik özellikleri tanımlanmıştır.*

Anahtar Kelimeler: *Alzheimer hastalığı (AD), asetilkolinesteraz inhibitörleri (AChE), glide, takrin*

INTRODUCTION

Alzheimer's disease (AD) is considered as severe neurodegenerative disease that progress with time and has complex pathophysiological events that are very difficult to diagnose in the early stage, as disease progresses it affects both the behavioral and physiological changes whichmake the treatment very challenging. Since it is most commonly seen in elder patients require a special care during the treatment period whichmakes a socio-economic burden [1]. As the older population in the United States estimated to be increased by 22% by the year 2050, there will be also increase in AD cases in the future [2]. There are very few drugs available for the treatment and management of symptoms of the disease specially to treat the most common symptom dementia. Most of the existing drugs act by increase the levels of acetylcholine in the brain such as acetylcholine esterase inhibitors. Though many other novel targets have been explored to target ADtill date, the acetylcholine esterase inhibitors occupied the first place as the drugs of choice and gained importance. Many other drugs that target variouspathways of AD are currently under clinical trials and some of them are stilling drugdiscovery pipeline [3].

AChE inhibitor Tacrine is the first drug discovered and approved for the treatment of early stage Alzheimer's symptoms such as memory and cognitive performance. But after 20 years of its discovery it has been withdrawn from the US market due to its considerable hepatotoxicity by elevating the levels of alanine transaminase. In spite of its toxicity Tacrine still remains as an interesting molecule for the researchers in many drug discovery programs for the development of drugs for AD. However, in the treatment of AD for the management of symptoms and prevent the disease progression, drugs with anti-inflammatory and antioxidant properties could helpful. They retard the neuro degeneration at a considerable level and alleviate the symptoms. Hence drugs acting on multiple targets of AD receive considerable interest due to their enhanced therapeutic effectiveness [4].

In the last few decades, many natural compounds have been reported for the treatment of AD. Among them flavonoids such as Luteolin, quercetin, fisetin, rutin and hesperidin were found to possess multi targeting abilities and play a crucial role in the neuroprotection. Hesperidin flavanone glucoside found in fruits of citrus family, structurally considered as β-7 rutinoside of Hesperetin in which glycon part rutinoseislinked to aglycon part Hesperetin. Recently, Parhiz*et al.*, extensivelyreviewed the molecular mechanisms responsible for hesperidin antioxidant and anti-inflammatory activity, Marziyeh*et al.*, also reviewed the neuroprotective effect of hesperidin [5,6].

In view of the above observation and increase in demand of new drugs for the treatment of AD, the present investigation emphasizes on the design and identification of potent acetylcholine esterase inhibitors from the promising drugs Tacrine and a natural flavonoid Hesperetin. Tacrine was selected as parent structure for the design of Tacrine analogues, since the molecular framework proven to be a potential anti AD drug, Hesperetin was also selected for its anti-inflammatory, antioxidant and neuro protective properties, hence these analogues could exhibit multi targeted actions in the AD.

Tacrine and Hesperetin analogues design strategies

The proposed molecules were designed (Figure 1) based on the original structure of Tacrineand Hesperetin moleculesconsidering the reported SAR of these analogues. Tacrine analogues were designed (T1-T9) by the molecular modification of Tacrine which contain planner tri cyclic ring system, in some of the designed analogues T1-T7 the try cyclic ring system was retained and modifications were made on the C5 position of the ring B one of the amine hydrogen is substituted with five member substituted heterocyclic ring systems such as imidazole, and pyrazole. The idea behind the substitutions is to design simple analogues by converting the primary amine on the B ring into a secondary amine in which the one hydrogen donor is less than the parent compound, in some derivatives hydrogen bond donor group incorporated in to the side chain heterocyclic ring system to compensate the hydrogen bond donors on ring B, in some of the analogues such as T8 and T9 the C ring was converted into an alkyl group and in some other analogues isosteric replacement has been carried out where trivalent –CH= of ring was replaced with trivalent Nitrogen-N=.

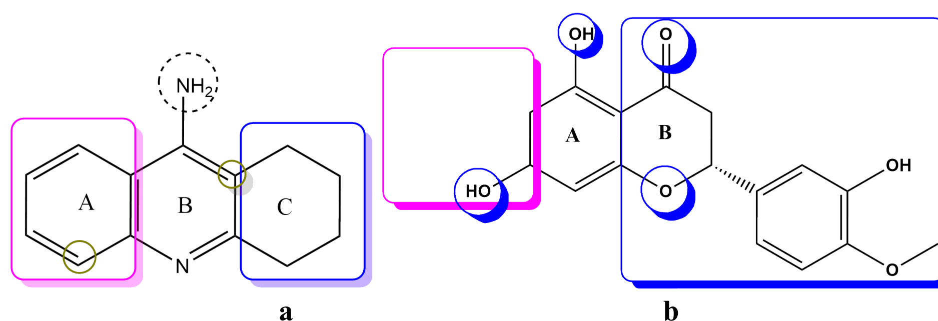

Figure 1. Design strategies for Tacrine(a) and Hesperetin(b) analogues for AChE Inhibition

In the design of Hesperetin analogues (H1-H9, Figure 1b) the parent drug Hesperetin is highly polar due to its polar functional group on the flavanone ring system. The structural moiety of Hesperetin and functional groups arrangement on it make the molecule to to cross the blood brain barrier and proven to exhibit CNS activity.

In view of the above observations, several molecular modification techniques have been employed in the design of analogues, in some of the analogues design one of the phenolic hydroxyl group in the ring A modified into hetero aryl ether derivatives (H9), in few analogues aromatic ring is fused with heterocyclic ring systems such as pyrrolidine and Piperidine ring (H1-H4), whereas in some derivative the ring oxygen atom is isosterically replaced with bivalent –NH-(H4,H5,H7). All the designed analogues of Tacrine and Hesperetin (Figure 2) are diverse in their structure and also carried the basic structural framework.

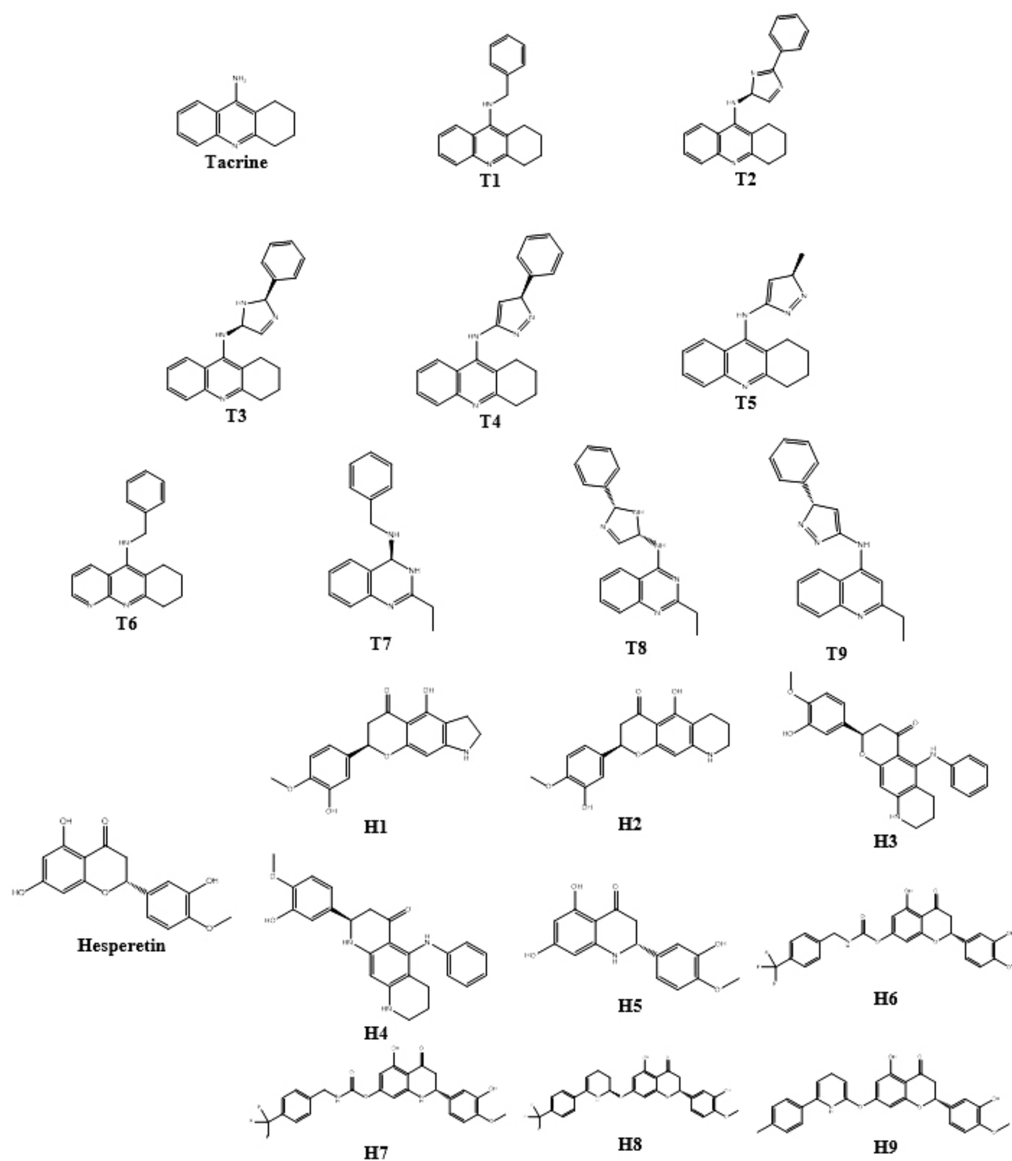

Figure 2. Structures of the designed Tacrine and Hesperetin analogues

The docking studies of these analogues using Schrodinger GLIDE [7] could reveal their binding potential within the active site of cholinesterase (Figure 3) and would helpful in the identification of potential scaffolds with enhanced inhibitory property. The druggability of designed molecules was further evaluated by computationally predicting ADME properties by Qikprop [8].

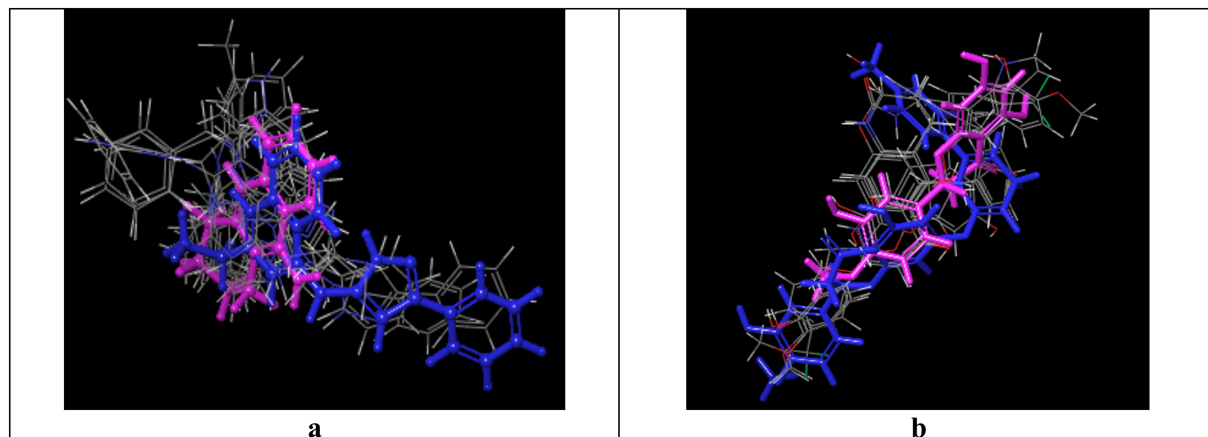

Figure 3. Clusters of binding conformation of the docked ligands a) clusters of tacrine analogues, Tacrine (Magenta) and Ligand T9 (blue) b) clusters of Hesperetin analogues, Hesperetin (Magenta) and Ligand H9 (blue)

MATERIAL AND METHOD

Molecular docking studies were carried out for the designed molecules employing standard docking protocol [9] using Schrodinger Glide software package on windows. To increase the docking speed water molecule from the protein AChE were removed and polar hydrogens were added, and the final structure was then energy minimized by employing OPLS forcefields. A receptor grid was identified from the position of co crystallized inhibitor molecule in the enzyme with grid points X:36.5, Y:65, Z:10.63 (Figure 3) and same was selected to facilitate the docking in this predefined grid. All the designed ligands, Tacrine, Hesperetin were prepared as a single energy minimized Ligprep file and they were used in docking. Extra precision docking was adopted for final docking and the results are presented (Table 1 and 2). The ADME properties (Table 4) also predicted from Ligprep files using Qikprop application in Schrodinger (Maestro).

RESULT AND DISCUSSION

In the present investigation efforts have been made to design and identify potential Anti Alzheimer’s drugs which targets Acetylcholine esterase enzyme. The newly designed Tacrine and

Hesperetin analogues were docked into the target protein AChE structure, PDB ID 1DX4. The target structure comprises a total of 585 amino acid residues and an inhibitor molecule.

Figure 4. Selected Grid box within the protein AChE (1DX4) for docking

All the designed ligands, Tacrine, Hesperetin were prepared as a single energy minimizedLigprep file and they were used in docking by selecting extra precision docking (XP) protocol. The docking predicts the most appropriate ligand receptor complexes of the docked ligands and ranked according to the binding energies, the XP docking gave docking score and glide score for all the docked ligands from which the preliminary evaluation was made. The entire docking protocol was validated by comparing the docked conformation of the representative ligand [10] with its Co-crystal structure conformation in the protein, since the RMSD value was less than 1 Aº, the procedure employed was considered as valid.

Once the docking job has been completed, the anticholine esterase potency of the new analogues was evaluated by considering the three parameters, the scores (Table 1), poses and binding interactions and compared with that of standard inhibitors (Tacrine and Hesperetin). For instance, a molecule is considered to be potent when it bounds with a least binding energy and mimics the binding pose and interactions of standard drug Tacrine in the active site of AChE. Therefore, from the docking scores ligand T9 was considered as most potent among the tested ligands with a score -16.11 which is much greater than the standard Tacrine (-11.16). The interactions of T9 with AChE also mimics the Tacrine binding (Figure 5), Aromatic ring of the T9 made a π-π interaction with the Trp 83, Tyr370 aromatic ring, the secondary amine hydrogen of ring B interacted with Hie480. In addition to these, the 3-phenyl pyrazolyl group on the amine of ring B was flexible enough to accommodate into the active site and its interaction with Trp85 and Trp472 of the AChE, could be the reason for its high binding affinity. Further, modification of C ring of the Tacrine into an ethyl group oriented toward Gly150 also influences

the binding. It is believed that these observations could make us to consider the T9 molecule as most potent AChE inhibitors useful for the development of new drugs for Alzheimer's disease.

Ligand T5 also considered to be potent since it bound to the target molecule active site with a docking score -15.009, Moreover, it mimics the Tacrine interactions, the Ring A and B of the tricyclic ring system is interacted via π-π stacking with Trp83 and the other interaction also observed with Hie480 and Tyr370(H bond)(figure 7), The pyrazole ring on the amine also mimics the orientation of T9 and also forms a Hydrogen bond with the Asn84.Ligand T4 (XP score -14.43) also bound to the AChE reproducing the same pose, orientation and binding interactions of Tacrine, it showed a π-π interaction with Trp83, Tyr370, the pyrazole made another π-π interaction with Trp472, Pyrazole NH forms a H Bond with Hie480 , but the amine nitrogen on the B ring did not participate in H bond as it is flipped away from Hie480 and the presence of ring C could prevent the rotation in the active site. Nevertheless, both ligands T5 and T4 were also considered as potent ligands for the AChE inhibition.

Table 1. Molecular docking results of Tacrine analogues on Acetylcholine esterase active site

Entry	Compound	Docking Score	Glide Score	XP Score
1	**T1**	**-13.631**	**-13.631**	**-13.631**
2	**T2**	**-12.33**	**-14.366**	**-14.366**
3	**T3**	**-12.06**	**-13.375**	**-13.375**
4	**T4**	**-13.147**	**-14.43**	**-14.43**
5	**T5**	**-14.858**	**-15.009**	**-15.009**
6	T6	-10.749	-11.297	-11.297
7	T7	-10.839	-13.529	-13.529
8	T8	-6.729	-7.954	-7.954
9	**T9**	**-14.997**	**-16.406**	**-16.406**
10	Tacrine	-11.16	-11.16	-11.16

All the other Tacrine analogues docking results were analyzed and the binding interaction and binding pose were studied (Table 1 and 3), except T8 all the other compounds showed appropriate interaction with the target enzyme AChE needed for the inhibition. Among them T2, T1 and T7 found to show highest binding affinity with Xp score -14.366, -13.631 and -13.529 respectively (Figure 7).

The molecular docking studies on Hesperetin analogues also revealed the binding ability within the active site of target enzyme AChE. Among the analogues tested H1-H9, ligands H9, H1 and H8 has excellent binding with an Xp scores -11.284, -9.186, -9.177 respectively which are greater than the parent Hesperetin (Table 2 and 3). Hence, these ligandscould be considered as potent for the AChE inhibition. However, the remaining ligands found to be weak inhibitors from the docking scores,

nevertheless all the Hesperetin analogues docked conformation mimics the Hesperetin binding to AChE. The Hesperetin cholinesterase activity has been reported in the previous literature, this flavanone partcomprisedofthree phenolic hydroxyl groups. The analysis of the docking pose of Hesperetin in AChE showed a π-π interaction of aryl group on2nd position of flavanone with Trp83, hydroxyl group on this aryl ring also formed a hydrogen bond with Hie480 another hydrogen bond formed between flavone phenolic hydroxyl group and Thr154(figure 6). Hes also produced other favorable interactions with the AChE.

Ligand H9 interacted with highest binding affinity XP score to AChE active site than its parent molecule Hesperetin and even with the standard Tacrine (Figure 8). The aromatic ring of the flavanone formed a π-π interaction with Trp83, and the phenolic hydroxyl group of the same ring formed a hydrogen bond with Hie480, weak interaction were also observed with Dihedron pyridine with Tyr71 and another hydrogen bond is formed between the hydroxyl group of the 2-phenyl ring with Glu435 (figure 8), another hydrogen bond is observed between ring oxygen of the flavonone with Trp472. From the above observations it is clear that presence of three hydrogen bond interactions and the weak aromatic interactions responsible for the highest binding affinity of the H9.

Table 2. Molecular docking results of Hesperetin analogues on Acetylcholine esterase active site

Entry	Compound	Docking Score	Glide Score	XP Score
11	**H1**	**-9.186**	**-9.186**	**-9.186**
12	H2	-4.844	-4.844	-4.844
13	H3	-6.633	-6.633	-6.633
14	**H4**	**-8.866**	**-8.873**	**-8.873**
15	H5	-8.472	-8.479	-8.479
16	H6	-6.957	-6.964	-6.964
17	**H7**	**-8.859**	**-8.859**	**-8.859**
18	**H8**	**-9.177**	**-9.177**	**-9.177**
19	**H9**	**-11.279**	**-11.284**	**-11.284**
20	Hesperetin	-9.026	-9.045	-9.045

The druggability of a new molecule depends on both pharmacodynamics which means how good a molecule interacting with the target protein and pharmacokinetics, the properties of molecules to reach target such as absorption, distribution metabolism and excretion in short ADME. In recent years, medicinal chemists have been trying to predict these pharmacokinetic parameters even for the designed molecules before being synthesized. The development of many computational tools make such predictions possible and aid researchers to assess the druggability and chose only potential compounds

for the synthesis and further studies. Such strategies would reduce the number of drug discovery failures at later stages.

Figure 5. 2D bindinginteractions of Tacrine(a) andT9 (b)

Figure 6. 2D bindinginteractions of Hesperetin**(a)** andH9 **(b)**

Table 3. Binding interactions of docked ligands with the active site of AChE

Entry	Compound	Interacting amino acid residues within the active site of AChE(1DX4)
1	T1	Trp83 (π-π interaction), Tyr370, Hie 480(H bond)
2	T2	Trp83(π-π interaction), Tyr370, Tyr71,
3	T3	Trp472, Trp83
4	T4	Trp83(π-π interaction), Hie480(H bond), Tyr71, Trp472
5	T5	His480, Trp83(π-π interaction), Tyr370(Hbond)
6	T6	Trp83(π-π interaction), Tyr370(π-π interaction), Tyr71,
7	T7	Trp83(π-π interaction), Tyr71, Tyr370(π-π interaction), Hie480(H bond)
8	T8	Trp83, Tyr370, Hie480(H bond)
9	T9	Trp83, Trp472, Tyr370,
10	Tacrine	Trp83, Tyrn370, Hie480(H bond)
11	H1	Tyr71, Thr 84, Tyr372, Hie480(H bond), Trp83
12	H2	Tyr370, Glu485, Trp83, Asp432,
13	H3	Ser470, Asp432, Trp472, Hie480(H bond), Trp83
14	H4	Trp472(π-π interaction), Ser470, Hie480(H bond), Trp83,
15	H5	Trp370, Trp83, Gly149,
16	H6	Trp472, Ser470, Asp482, Tyr370(π-π interaction), Gly237
17	H7	Trp83, Glu80, Tyr370, Trp472
18	H8	Hie480(H bond), Trp85, Thr71, Tyr370
19	H9	Hie480, Glu485, Tyr71, Trp83, Trp472
20	Hesperetin	Thr154, Hie480, Trp83

T1	T2
T3	T4
T5	T6
T7	T8

Figure 7. AChE protein and docked ligand (Tacrine analogues) complexes with 2D binding interactions

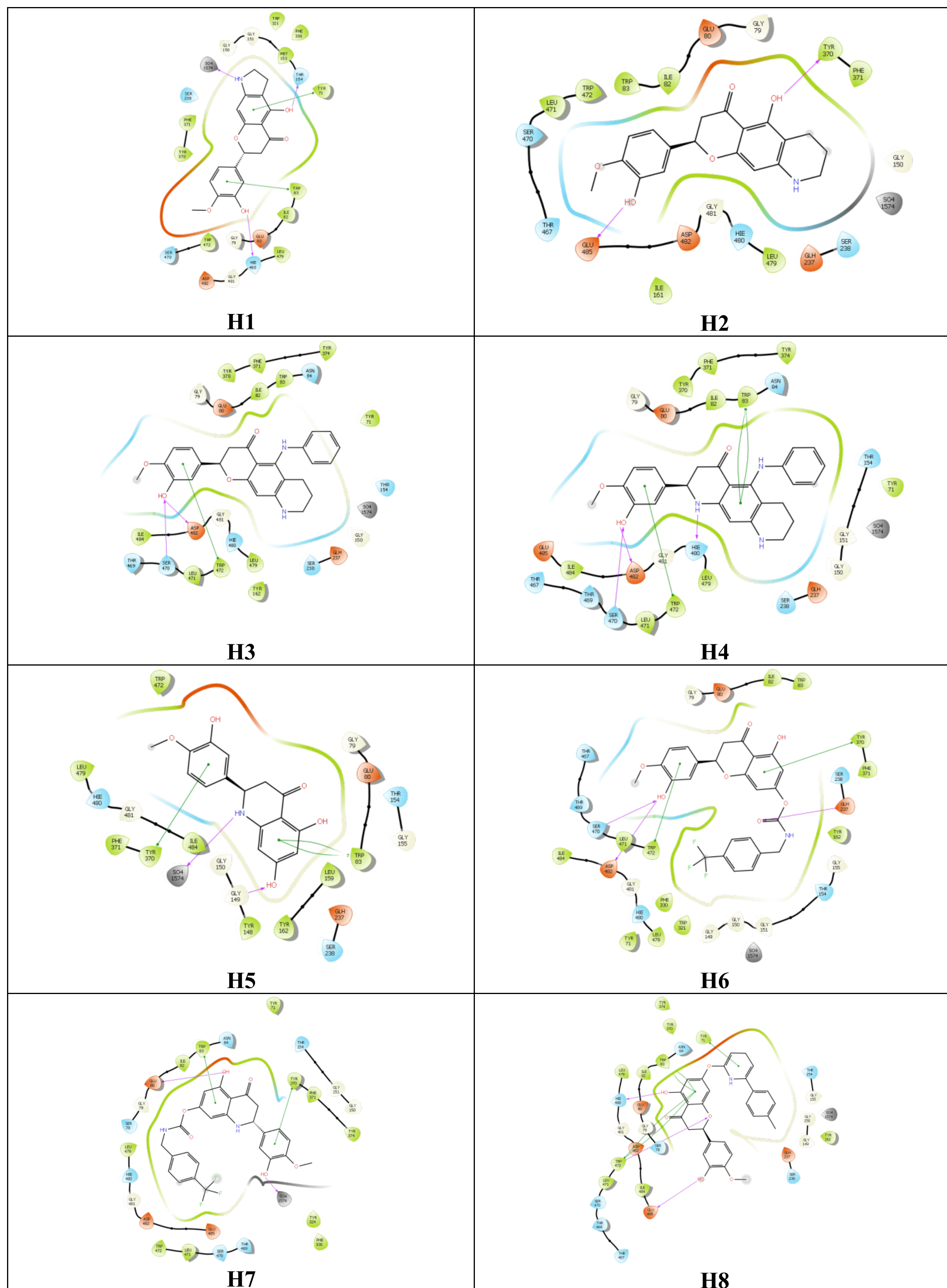

Figure 8. AChE protein and docked ligand (Hesperetin analogues) complexes with 2D binding interactions

In the present investigation the ADME properties of all the designed analogues were predicted using Qikpropapplication in Schrodinger (Table 4), The calculated ADME properties revealed that the logP values are in the range of 1.4 – 6.8, the QPcaco MDCK predict the permeability of the molecule across the human intestine it predicts the mechanism of absorption through passive diffusion or active transport.All the tacrine analogues predicted to exhibit exceptional permeability through human intestinal epithelial cells. WhereastheHesperetin and its analogues exhibited moderate absorption properties. QPlogBB, represents the ability of a molecule to cross BBB, which is an important parameter forassessingCNSactivity, among all the analogues Tacrine analogues T7, T3, T1 has exceptional BBB crossing ability than the parent compound Tacrine.

Table 4. Predicted ADME properties of designed analogues calculated using QikProp

Entry	Compound	MW	CNS	QPlogP	QPPCaco	QPlogBB	QPPMDCK	HOA	%HOA	LogKhsa
1	T1	288.391	1	4.96	6077.657	0.174	3479.173	3	100	0.823
2	T2	340.427	0	4.998	3655.089	-0.01	2008.057	1	100	0.892
3	T3	342.443	1	3.959	638.745	0.222	337.141	3	100	0.713
4	T4	340.427	0	5.136	2879.149	-0.131	1551.53	1	100	0.989
5	T5	278.356	0	3.761	2486.967	-0.155	1324.41	3	100	0.518
6	T6	289.379	0	4.241	3398.901	-0.074	1856.368	3	100	0.597
7	T7	265.357	1	3.345	1112.371	0.387	614.073	3	100	0.343
8	T8	317.393	0	3.302	384.766	-0.141	194.929	3	92.547	0.447
9	T9	314.389	0	4.696	1989.139	-0.372	1040.332	3	100	0.804
10	Tacrine	198.267	1	2.583	2962.313	0.045	1600.027	3	100	0.067
11	H1	327.336	0	1.882	71.465	-0.748	31.597	3	71.152	0.224
12	H2	341.363	-2	2.765	356.23	-1.089	162.115	3	88.808	0.341
13	H3	416.476	-1	4.746	711.443	-0.943	342.398	1	100	0.949
14	H4	415.491	-1	4.415	626.743	-0.997	298.557	1	100	0.824
15	H5	301.298	-2	1.496	115.762	-1.556	48.103	2	72.636	-0.074
16	H6	503.431	-2	4.617	180.962	-1.573	343.95	1	81.428	0.773
17	H7	502.446	-2	4.299	150.011	-1.683	279.7	1	78.106	0.676
18	H8	527.496	-2	6.183	423.122	-1.15	861.848	1	84.239	1.376
19	H9	471.509	-2	5.566	500.46	-1.218	234.102	1	94.889	1.251
20	Hesperetin	302.283	-2	1.802	139.604	-1.459	58.896	3	75.885	0.014

The transportation ability to cross BBB for Hesperetin analogues is poor hence the CNS score is ranging from 0 to – 2 and the percentages human oral absorption(%HOA)found to be in the range of 75-100%,however all the Tacrine analogues except T8, have 100% %HOA which confers the exceptional oral bioavailability of the molecules. The predicted human serum albumin binding (Logkhsa) values were ranging from – 0.067 to 1.376 which are in the recommended range of 95% known drugs. Therefore, these predicted parameterswouldhelpful in identifying druggableand orally active analogues.

This Study further revealed that the designed analogues have appropriate pharmacokinetic properties, especially the Tacrine analogues have more potential to be druggable than the Hesperetin analogues.

In the present work Tacrine and Hesperetin analogues with diverse structures were designed to achieve acetyl choline esterase inhibitor activity to treat Alzheimer's disease. Then the designed analogues were investigated to assess the ability of the molecules to bind and inhibit the AChE, molecular docking approach was used to identify potential ligands. ADME properties also calculated and the druggability of the designed molecules were evaluated. Among the design molecules Tacrine analogues showed a potent binding and inhibition than the Hesperetin analogues, especially ligands T2, T3, T5, T7 and T9 showed highest binding than the standard Tacrine. However, H9 also exhibited highest binding with appropriate interactions necessary for inhibition. From the results of these studies it could be concluded that Tacrine analogues T2, T3, T5, T7 and T9 and Hesperetin analogue H9 have the ability to inhibit AChE and possess good ADME properties, since these molecules are druggable and could be developed as new drugs for the treatment of Alzheimer 's disease.

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J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 44(1): 33-49, 2020
Doi: 10.33483/jfpau.586590

ORIGINAL ARTICLE / ÖZGÜN MAKALE

FORMULATION AND DETAILED CHARACTERIZATION OF VORICONAZOLE LOADED *IN SITU* GELS FOR OCULAR APPLICATION

OKÜLER UYGULAMA İÇİN VORİKONAZOL YÜKLÜ İN SİTU JELLERİN FORMÜLASYONU VE DETAYLI KARAKTERİZASYONU

Neslihan USTUNDAG OKUR[1*], Vildan YOZGATLI[2], Zeynep SENYIGIT[3]

[1]University of Health Sciences, Faculty of Pharmacy, Department of Pharmaceutical Technology, Istanbul, TURKEY

[2]Ege University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Bornova, Izmir, TURKEY

[3]İzmir Kâtip Çelebi University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Izmir, TURKEY

ABSTRACT

Objective: *This study was aimed to prepare, characterize and evaluate in situ gel formulation for a sustained ocular delivery of voriconazole.*

Material and Method: *In situ gels were prepared with three different hydrophilic co-polymers: Poloxamer 188, 407 and 388. The formulations were characterized in terms of their clarity, pH, viscosity drug content uniformity and mechanical/rheological properties. Moreover, in vitro drug release and stabilitystudies were performed.*

Result and Discussion: *The results showed that the optimized in situ gel formulation had desired in vitro properties and a good stability over the period of 3 months. Texture profile analysis presented that formulations offered suitable adhesive and mechanical properties. P2-V formulation exhibited pseudo-plastic flow and typical gel-type mechanical spectra (G' > G") at different frequecy values and at different temperatures. Moreover, all formulations showed a sustained drug release for 24 hours. In conclusion, voriconazole loaded in situ gel could be offered as an encouraging strategy as ocular systems for ocular infections treatment.*

Keywords: *Mechanical properties poloxamer, rheological properties, thermo-sensitive in situ gel, voriconazole*

[*] **Sorumlu Yazar / Corresponding Author:** Neslihan Üstündağ Okur
e-mail / e-posta: neslihanustundag@yahoo.com

Submitted / Gönderilme: 04.07.2019 **Accepted / Kabul:** 30.10.2019

ÖZ

Amaç: *Bu çalışmada vorikonazolün sürekli bir oküler dağılım uygulaması için in situ jel formülasyonu hazırlamak, karakterize etmek ve değerlendirmek amaçlanmıştır.*

Gereç ve Yöntem: *İn situ jeller, üç farklı hidrofilik yardımcı polimer ile hazırlanmıştır: Poloxamer 188, 407 ve 388. Formülasyonlar, berraklık, pH, viskozite ilaç içeriği ve mekanik / reolojik özellikleri bakımından karakterize edilmiştir. Ayrıca, in vitro ilaç salımı ve stabilite çalışmaları yapılmıştır.*

Sonuç ve Tartışma: *Sonuçlar, optimize edilmiş in situ jel formülasyonunun, istenen in vitro özellikler ve 3 ay boyunca iyi bir stabilite göstermiştir. Doku profili analizi, formülasyonların uygun adhezif ve mekanik özellikler sunduğunu göstermiştir. P2-V formülasyonu, farklı frekans değerlerinde ve farklı sıcaklıklarda psödo plastik akışı ve tipik jel tipi mekanik spektrumları (G '> G ") göstermiştir. Ayrıca, tüm formülasyonlar 24 saat boyunca sürekli bir ilaç salımı göstermiştir. Sonuç olarak, vorikonazol yüklü in situ jel, oküler enfeksiyon tedavisi için oküler sistemler olarak teşvik edici bir strateji olarak sunulabilir.*

Anahtar Kelimeler: *mekanik özellikler poloksamer, reolojik özellikler, termo-duyarlı in situ jel, vorikonazol*

INTRODUCTION

Ocular drug delivery is challenging due to the presence of anatomical and physiological barriers. These barriers can affect drug entry into the eye following multiple routes of administration (e.g., topical, systemic, and injectable) [1]. Topical administration is the most common route of ocular drug delivery. This route represents a safer administration, therefore a major challenge to the scientists is to overcome the ocular barriers and reach the tissue target [2].

Although conventional opthalmic dosage forms such as solutions and suspensions are usually preferred to treat disorders of the eye, the biological protecting factors lead to low ocular absorption and poor bioavailability (1– 10%). An efficient ocular drug delivery system, which can provide maximum precorneal residence time, is desirable to overcome ocular barriers and sustain delivery of drugs following topical administration [3].

Bacterial keratitis may arise secondary to corneal epithelial breakdown associated with dry eye, contact lens use, trauma or the presence of a persistent corneal suture. Keratitis is also caused by direct infection or immune-related complications with viruses, bacteria, fungi, yeast and amoeba. Subsequent long term visual loss occurs as a consequence of corneal scarring affecting the visual axis. The extent of scarring may be limited if the infection is identified early and treated adequately [4,5]

A second-generation antifungal agent, voriconazole (VCZ), has exceptional properties such as broad-spectrum activity against resistant fungal species and acceptable tolerability. Besides, studies have demonstrated excellent efficacy of VCZ against ocular mucosa following topical administration [6,7].

The *in situ* thermo-gelling systems are liquid aqueous solutions at room temperature, however they undergo sol-gel transition on the ocular surface at physiological temperature hence they prolong ocular residence time. Various *in situ* gel systems have been developed to prolong the precorneal duration of the drug and to increase ocular bioavailability [8,9]. Among commonly used *in situ* gel

polymers, Poloxamers are well-known thermo-responsive copolymers that exist liquid state at low temperature (4-5°C) while converting into a gel upon increasing temperature. They have been widely used in nasal, ophthalmic, vaginal and topical formulations. However, they represent weak mechanical strength leading to rapid erosion of the polymer.

Therefore; in this study, it was aimed to develop VCZ loaded *in situ* gel formulation with suitable gelation temperature and mechanical properties for ocular drug delivery. In accordance with this purpose, the *in situ* gels were prepared by using different poloxamer types (Poloxamer 188, 407, 388) and ratios. Finally, the gels were characterized in terms of their physicochemical parameters, drug content, mechanical/rheological properties, in vitro drug release and stability.

MATERIAL AND METHOD

Materials

VCZ was purchased from Sigma-Aldrich, Germany. Poloxamer 407, 188 and 338 were kindly gifted from BASF, Turkey. Benzalkonium chloride (BZC) was supplied from Sigma-Aldrich, Germany. Dialysis membrane (Spectra/por 4, diameter 16 mm, the molecular weight of 12–14 kDa) was purchased from Spectrum Chemical Mfg. Corp. (USA). Distilled water was used throughout the study. All the other solvents and chemicals were of analytical or HPLC grade.

Preparation of *in situ* gel formulations

The *in situ* gels were prepared according to cold technique [10,11]. The polymeric solutions were prepared by dispersing the required quantity of Poloxamer 407 and Poloxamer 188 in water using a magnetic stirrer until the poloxamers completely dissolve. Aqueous solutions were stirred for about two hours by using magnetic stirrer [12].

For the preparation of ocular *in situ* gel; VCZ, BZC as well as sodium chloride were incorporated in aqueous solutions containing P407, P188, P388 and distilled water. BZC (0.02% w/w) was added as a preservative to the solutions. Sufficient amount of sodium chloride (0.9% w/w) was added to the mixture to maintain the isotonicity.

Characterization of *in situ* gels

Appearance

The developed formulations were inspected visually for their clarity, colour and particle content both in their sol state and gel state.

Determination of sol-gel temperature ($T_{sol-gel}$)

20 g of cold formulation was put into a beaker and placed in a temperature-controlled magnetic stirrer. A thermometer (JG-220 Digital Thermometer, Turkey, −50+260°C±°1C accuracy) was

immersed in the sample solution for constant monitoring. The solution was heated at the rate of 2°C/min with the continuous with stirring at 200 rpm. The temperature at which the magnetic bar stopped moving due to gelation was reported as the gelation temperature. The maximum limit for gelation was checked up to 60°C and the study was repeated at least 3 times [9].

Gelling capacity

The gelling capacity of the prepared formulation was determined by placing a drop of the formulation in a beaker at 32 ± 0.5 °C and it was visually observed for gelling time [12].

Determination of pH

The pH of the gel was measured using calibrated pH meter (Mettler Toledo, Switzerland). pH measurement was repeated at least 3 times and the average pH values of the formulations were calculated.

Viscosity

The viscosity studies of *in situ* gels was carried out using a Brookfield viscometer (LVDV-E, USA). The *in situ* gel formulations were analyzed with probe 27 at 200 rpm and probe 07 at 20 rpm. Temperature was set to +4±0.5°C and 25±0.5°C by a circulating bath.

Drug content uniformity

0.125 g of the developed formulations was dissolved in 25 mL mobile phase and drug concentration was analyzed by high-performance liquid chromatography (HPLC).

HLPC analysis

The VCZ amount was determined with a HPLC system consisted of a gradient pump, a UV detector (Agilent 1100, Thermo Scientific, Germany) and C18 column (5µm, 150 × 4.6 mm). The samples were analyzed at 256 nm with 1mL/min flow rate at 25°C. The mobile phase was a mixture of acetonitrile: ultrapure water (50:50). The retention time of VCZ was 4.098 min [7]. The method was validated for linearity, limit of detection (LOD) and limit of quantitation (LOQ), precision, accuracy and specificity, selectivity and stability. The linearity between peak area and concentration was analyzed using calibration curve obtained from standard solutions of VCZ (1–30 µg/ mL). The accuracy of an analytical method is the closeness of test results obtained by the method to the true value and is defined recovery. The prepared standard solutions were injected five times at different levels as a test sample. 8 µg/mL solution was injected ten times in order to evaluate method precision, standard deviation (SD) and coefficient of variation.

Spreadability of VCZ loaded *in situ* gels

To determine spreadability of VCZ loaded *in situ* gels, 0.1 g of VCZ loaded *in situ* gels were transferred to the center of a glass plate (10 cm × 10 cm), which this glass plate had temperature 32 ± 0.5 °C and was compressed under another glass plate of the same size. Thus, the gel was spread out in between the plates. After one minute, the weight was removed and the diameter of the spread area (cm) was measured. The measurement was performed in triplicate [9].

Determination of Mechanical Properties

Mechanical properties of gels were determined using a software-controlled penetrometer (TA-XT Plus Texture Analyser Stable Micro Systems, UK) equipped with a 0.5 kg load cell. An analytical probe (10 mm diameter) was twice compressed into gels to a defined depth (15 mm) with a constant rate (test speed: 2 mm/s) at both 25 and 32°C. Mechanical parameters (hardness, adhesiveness, compressibility, cohesiveness and elasticity) were calculated from the obtained force–time curves. Experiments were carried out at least six times [13].

Rheological Measurements

The rheological analysis of the formulations was performed with a controlled stress/controlled rate rheometer (TA Instruments, Discovery HR-1, Hybrid Rheometer, UK) both at 25°C ± 0.1°C and 32°C ± 0.1°C.

Continuous shear analysis was performed in flow mode, in conjunction with parallel steel plate geometry (40 mm diameter) and gap of 1000 µm. Briefly, formulation sample was carefully applied to the lower plate of instrument, ensuring that formulation shearing was minimized and allowed to equilibrate for at least 1 min prior to analysis. Upward and downward flow curves were measured over a range of shear rates (0 - 1000 s-1).

Oscillatory analysis was performed after determination of its linear viscoelastic region at 25°C and 32°C, where stress was directly proportional to strain and the storage modulus remained constant. Frequency sweep analysis was performed over the frequency range of 0.1 - 10 Hz following application of a constant stress and standard gap size was 1000 µm.

Storage modulus (G') and loss modulus (G''), the loss tangent (tanδ) and the dynamic viscosity (η') and were determined. All rheological properties were examined with at least three replicates [14–16]

***In vitro* drug release studies**

In vitro release studies were carried out in simulated tear fluid (composition: sodium chloride 0.68 g, sodium bicarbonate 0.22 g, calcium chloride dihydrate 0.008 g, potassium chloride 0.14 g, and

distilled deionized water to 100 mL [17] to mimic ocular conditions. 5 g of formulations were put into dialysis membrane (Spectra/Por Regenerated Cellulose, Molecular weight cut off 12–14 kDa) and capped with closures. Dialysis membranes were placed into 200 mL simulated tear fluid and stirred at 50 rpm (32±0.1°C). 1 mL of sample was withdrawn at a predetermined time intervals of 30 min to during 12 h and the same volume of fresh medium was replaced. The samples were analyzed with HPLC for determination of the drug content.

Stability of VCZ loaded *in situ* gels

In order to check physical stability, VCZ *in situ* gels were stored at 4 ± 1 °C in the refrigerator and 25±1°C (relative humidity 60%) for 3 months. After storage visual appearance, clarity, pH, gelling capacity and VCZ content of *in situ* gels were investigated. The experiments were repeated three times [18].

Statistical data analysis

Statistical data analysis was performed using the Student's t-test with $P<0.05$ as the minimal level of significance.

RESULT AND DISCUSSION

Preparation of *In Situ* Gel Formulations

Poloxamers represent a class of amphiphilic triblock copolymers comprising a hydrophobic propylene oxide (PPO) block and two hydrophilic ethylene oxide (PEO) blocks, which can undergo a reversible sol-to-gel transition upon heating, as a function of their PEO:PPO ratio. Poloxamer 407 and poloxamer 188 are the two most commonly used poloxamer types for thermosensitive *in situ* gelling systems an d they are approved by US Food and Drug Administration (FDA) [19]. Poloxamer 338 is a new nonionic surface-active agent. The block copolymer poloxamer 338 in aqueous media exhibits micellar structures which can convert into gel like structures based on their length, concentration and temperature [20].

The *in situ* VCZ gels were prepared according to cold technique. Briefly, VCZ, BZC as well as sodium chloride were incorporated in aqueous solutions containing P407, P188, P338 and distilled water. BZC (0.02% w/w) was added as a preservative to the solutions. Sufficient amount of sodium chloride (0.9% w/w) was added to the mixture to maintain the isotonicity. VCZ concentration was 0.1% (w/w) in all formulations (Table 1).

Different Poloxamer 407, 188 and 338 combinations were tried and evaluated according to their physical appearance and gelation temperature properties. Topical drug administration is the simplest and easiest route for localized drug delivery [21]. Topical administration of antifungal agents could have

an increased impact on the antifungal therapy, given that current formulations present lack of efficacy due to the rising antifungal drug resistance [22]. For topical ocular formulations, the carriers are desired to gel at 32ºC, which is the eye surface temperature [23]. Among the tried formulations, the poloxamer ratios given in Table 1 were identified as the most appropriate *in situ* gelling system for ocular administration. Therefore, they were chosen as *in situ* ocular carrier system candidates for VCZ and evaluated for their properties.

Table 1. Formulation codes (FC) and components of *in situ* gels

FC	Poloxamer 407 (%)	Poloxamer 188 (%)	Poloxamer 338 (%)	VCZ (%)	BZC (%)	Physiological saline (0.9% w/w) (q.s) (g)
P1-V	20	5	-	0.1	0.02	100
P2-V	20	8	-	0.1	0.02	100
P3-V	20	18	0.5	0.1	0.02	100

Characterization of *in situ* gel formulations

Psysicochemical parameters of *in situ* gel formulations are important factors to be considered in the formulation development phase especially for ocular application. Firstly, the formulations were inspected visually for organoleptic properties. Clarity is a quality control test to reduce number of the large particles in the formulation which may cause irritation and tear flow and hence the loss of drug from ocular surface [24]. Therefore; first of all, formulations were visually inspected for their clarity, color and particle content. Visual observation showed that all of the *in situ* gels were found to be clear, colorless and free of foreign particles.

In previous studies, the corneal contact time has been increased to varying degrees by different ophthalmic dosage forms. However, most of these carriers (e.g. ointments) have not been fully accepted, because of blurred vision [25]. In this respect; *in situ* gels are advantageous because of their transparent structure. In addition, they extend the corneal contact time and, in this way, they also increase patient compliance.

pH is one of the most important parameters involved in ophthalmic formulations and it was measured using a pre-calibrated pH-meter. The normal physiological pH of the ocular mucosa ranges from 6.5 - 8.5 [9]. pH value of all formulations was found to be between 7.1 – 7.5 and they are within the range of ocular mucosa (Table 2).

Table 2. Physicochemical properties of *in situ* gels

FC	Clarity	pH	Gelling temperature (oC)	Gelling Capacity (sec)	Spreadability (cm)	Viscosity (cP)	
						+4°C	25°C
P1	+++	7.375 ±0.009	30.733 ±0.231	2.100 ±0.1	1.575 ±0.035	13.467±0.141*	255.00±1.838*
P1-V	+++	6.327 ±0.006	30.500 ±0.500	1.800 ±0.100	1.300 ±0.082	15.697±0.135*	275.10±2.796*
P2	+++	7.168 ±0.009	32.233 ±0.115	1.300 ±0.100	1.625 ±0.035	110.000±3.270*	276.15±0.212*
P2-V	+++	6.357 ±0.006	32.200 ±0.265	1.233 ±0.058	1.575 ±0.096	125.50±4.270*	285.26±0.314*
P3	+++	7.553 ±0.019	27.300 ±0.200	1.800 ±0.100	1.487 ±0.052	432.933±0.751*	550.00±4.142**
P3-V	+++	6.617 ±0.006	27.767 ±0.306	1.500 ±0.100	1.325 ±0.096	445.266±0.642*	575.20±3.213**

(*: probe 27 200 rpm; **: probe 07 20 rpm)

An ideal *in situ* forming gel should be free flowing at a low temperature, transform into a semisolid after contacting the ocular surface, and remain in the gel form under conditions of maximum lacrimal fluid dilution [26]. The *in situ* gels developed in this study showed a gelation temperature around 32°C. At this temperature, the administered formulations are expected to transform from sol to gel state and prolong the ocular drug release. It can also be seen from Table 2 that, incorporation of %0.1 VRC didn't significantly affect the gelling temperature and gelling capacity of the formulations.

The gelling capacity is defined as the time taken for the transition of liquid phase to a gel. In this experiment, the gelling capacities of in situ gels were found to be within 0.5 - 2.1 sec. As demonstrated in Table 2, the gelling capacity increased when the concentration of P188 increased. For example, formulation P1 has longer gelation time (2.1 sec) than P2 formulation (1.3 sec).

The results of viscosity were shown in Table 2. The viscosity results of the formulations were different under 4 and 25 °C temperature conditions which are storage conditions of the *in situ* gel formulations. As the collected results showed, the increasing concentration of Poloxamer 188 increased the viscosity of the *in situ* gel. Poloxamer188 is a more hydrophilic poloxamer and is used as an auxiliary gelling agent for modification of Tsol-gel. P188 consists of higher PEO: PPO ratio (79:28) compared to P407 (100:65) and usually incorporated in the P407 thermogels to increase the Tsol-gel [19].

The spreadability results showed that the formulated *in situ* gels (P2 and P2-V) were most effective i.e. they showed best results for spreadability. The results of spreadability were shown in Table 2. Spreading diameter of the P1, P1-V, P3 and P3-V formulations demonstrated that is similar for all formulations.

The analytical method was developed and validation studies were carried out for VRZ. If the standard deviation less than the acceptance criteria which is 2%, the analysis system for the determination of assay is to verify [27]. The LOD and LOQ tests for the procedure were performed on

samples containing very low concentrations of analyses [28]. The LOD and LOQ were determined as 0.022 µg/mL and 0.065 µg/mL, respectively. The used method for VCZ analysis was found to be linear. Finally, the drug content uniformity of P1-V, P2-V and P3-V were found to be 93.622±1.157, 92.625±0.609 and 98.288±0.630, respectively.

Determination of Mechanical Properties

Ocular *in situ* gel formulations should have suitable mechanical properties for easy administration, high spreadability on the ocular mucosa and strong adhesion. Texture profile analyses (TPA) were performed to gather information about the gel structure and to determine the resistance of formulations to compressive stresses and subsequent relaxation. The mechanical properties of the formulations were characterized in terms of hardness, compressibility, adhesiveness, elasticity and cohesiveness. The obtained results and force-time curves were given in Table 3, Figure 1 and Figure 2.

Briefly, hardness expresses the applicability of the gel to ocular surface and it should be low to allow easy administration and good spreadability. Compressibility value determines sample deformation under compression. It should be low to remove the formulation easily from the container during administration. This value also shows high spreadability at the application site. It can be seen that, depending on the increase in the temperature; hardness and compressibility values were significantly increased, which indicates, improved gel strength. This increase was in accordance with oscillatory rheology results, i.e. increased elastic behavior (represented by G′) was exhibited with increasing temperature.

Table 3. Mechanical properties of the formulations

Formulation code	Temperature (°C)	Hardness (g) ± SD	Compressibility (g·sec) ± SD	Adhesiveness (g·sec) ± SD	Cohesiveness ± SD	Elasticity ± SD
P1	25°C	0.588±0.025	1.087±0.013	0.594±0.017	1.057±0.027	0.968±0.010
	32°C	8.167±0.904	12.852±2.395	12.885±2.913	1.033±0.083	1.532±0.189
P1-V	25°C	0.603±0.015	1.121±0.047	0.608±0.020	1.052±0.008	1.018±0.061
	32°C	12.508±0.715	19.939±2.914	18.838±2.354	1.016±0.031	1.504±0.462
P2	25°C	0.231±0.015	0.161±0.017	0.581±0.010	0.976±0.025	1.013±0.169
	32°C	10.255±2.261	13.947±2.059	11.367±0.459	1.111±0.088	1.299±0.247
P2-V	25°C	0.312±0.007	0.337±0.010	0.558±0.005	1.011±0.015	1.021±0.032
	32°C	8.213±0.839	6.863±0.660	5.600±1.024	1.085±0.243	0.990±0.036
P3	25°C	52.476±4.793	95.827±10.097	91.426±7.964	0.971±0.045	1.271±0.177
	32°C	62.471±2.984	132.936±9.109	121.771±6.999	1.027±0.049	1.181±0.056
P3-V	25°C	39.301±2.757	66.951±7.110	60.291±5.153	0.941±0.107	1.252±0.116
	32°C	79.018±0.714	103.778±4.057	79.218±6.072	1.154±0.059	1.062±0.165

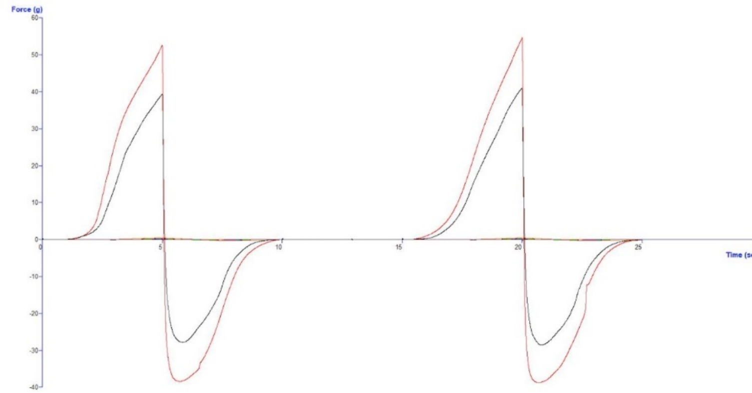

Figure 1. Force-time curves of the gel formulations at 25°C (Blue line: P1, Yellow line: P1V, Green line: P2, Purple line: P2V, Red line: P3, Black line: P3V)

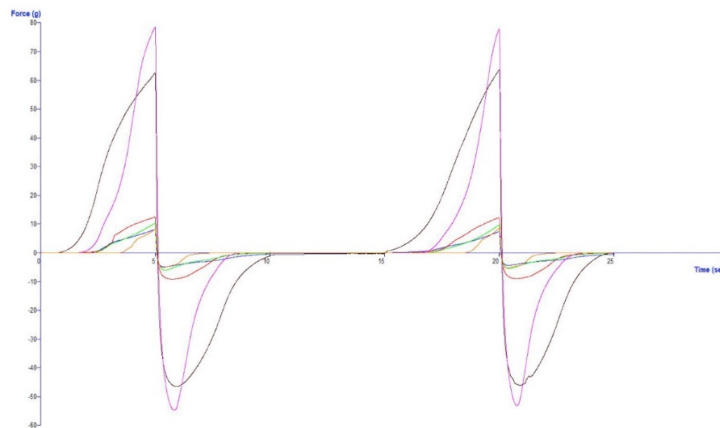

Figure 2. Force-time curves of the gel formulations at 32°C (Blue line: P1, Red line: P1V, Green line: P2, Yellow line: P2V, Brown line: P3, Purple line: P3V)

Adhesiveness value defines the work required to detach the probe from the formulation and it can be related to adhesive properties. Higher adhesiveness value indicates greater adhesion at the tissue surface and it is a desired characteristic to increase the drug retention time. Based on the results it can be seen that, highest adhesiveness value was obtained with P3 formulations, which also shows high gel strength properties. Also in all of the formulations, the increased temperature caused higher adhesive properties.

Cohesiveness shows the effect of repeated shearing stresses on the formulations. Elasticity represents the return rate of the deformed sample to its beginning condition. Lower numerical value in the elasticity indicates greater product elasticity. As it can be seen from Table 4; cohesiveness and elasticity values are nearly 1 as expected and they did not significantly change with the addition of VRC or increasing temperature ($P>0.05$).

Rheological Measurements

The evaluation of rheological properties for *in situ* gels is one of the most important parameters for predicting their in vivo behavior. The rheological properties especially affect both ease of application and retention within the application area. Therefore; P2-V formulation, which showed gelling temperature at 32°C, was selected and evaluated for its rheological behavior. The rheological properties were determined both at room temperature (25°C) and at eye temperature (32°C) to observe the changes in the gel structure.

First of all, the shear stress changes upon shear rates have been observed to determine whether the rheological behavior of the formulation is Newtonian or non-Newtonian. Obtained results showed that in continuous shear rheometry, P2-V formulation showed a non-Newtonian pseudo-plastic flow, showing decreasing viscosity with progressive increases in the shear rate both at 25°C and 32°C (Figure 3). In accordance with this results, it was previously reported in the literature that at temperatures especially higher than the sol-gel transition temperature, non-Newtonian flow is typical for poloxamer solutions [29,30]. Also, it can be seen that higher viscosity and shear stress values were obtained at higher temperature values which indicates the temperature-dependent gellation. This result is also compatible with the results of mechanical analysis where significantly higher hardness values are observed at higher temperature values.

Furthermore; P2-V formulation was subjected to a sinusoidal shear stress and oscillatory rheology studies were performed. In this way, both elastic-like and viscous-like properties were determined. The structural and dynamical properties were elucidated and two dynamic modui were obtained: 1) the storage modulus (G′, a measure of the elasticity); and 2) the loss modulus (G′′, representing viscous components at given frequency).

It was stated in the literature that a strong gel should exhibit a solid-like mechanical spectrum and the storage modulus should be higher than the loss modulus ($G' > G''$) [31]. Figure 4 shows the plots of G′ and G′′ as a function of frequency at two different temperature values. It can be seen that, at both temperature values, G′ dominated G′′ for all frequency ranges, which indicates a strong gel structure. The gap between the two moduli is wider at 32°C indicating stronger gel strength ($G' >> G''$) [32].

The loss tangent is the value of phase angle ($\tan\delta = G'/G''$) and it is a measure of the relative contribution of viscous components to the mechanical properties of the materials. As it can be seen from Figure 4, it was <1 both at 25°C and 32°C which shows solid gel response. As tanδ becomes smaller, the elasticity of the formulation increases, while the viscous behavior is reduced. As expected, tanδ value of P2-V was found to be higher at 25°C than 32°C which indicates that the formulation showed more elastic property at higher temperature value and this result is in accordance with the results of oscillatory measurements [33].

Dynamic viscosity (η´) is described as the flow resistance of the formulation in the structure state to oscillating movement. The higher dynamic viscosity value means the greater the resistance to flow. In our study, η´ value was found to be significantly higher as the temperature increases and it indicates more consistent gel structure. This result is also in accordance with other mechanical and rheological studies.

In vitro drug release studies

In vitro drug release of P1-V, P2-V and P3-V formulations was evaluated by dialysis bag method and the results were given at Figure 5. The results showed that the Poloxamer type or ratio did not significantly affect the release rate of VRC from the *in situ* gel formulations. In all of the formulations, sustained drug releases were obtained up to 24 hours.

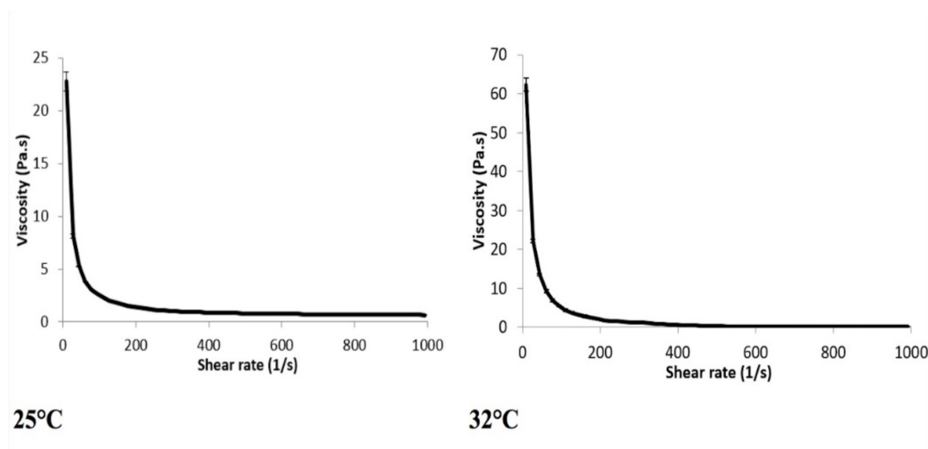

Figure 3. Viscosity versus shear rate graphs of the formulations

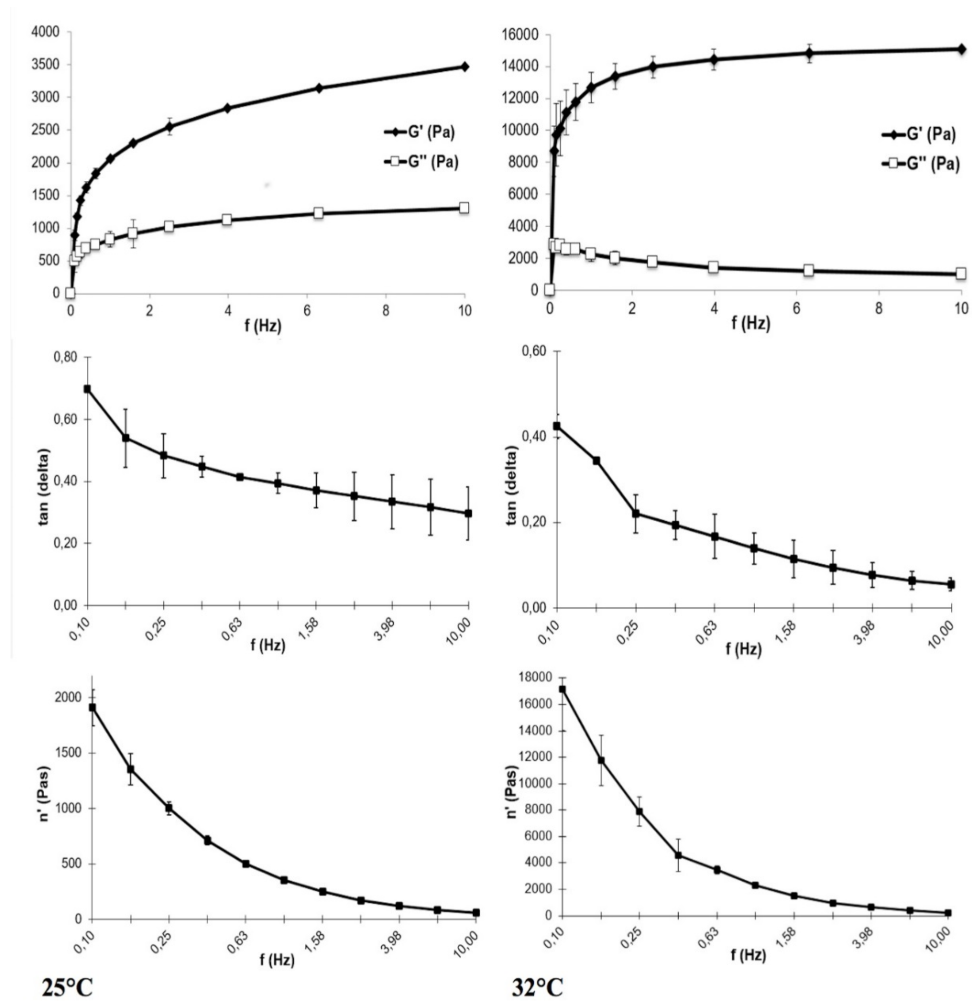

Figure 4. Frequency-dependent changes of viscoelastic properties of P2-V formulation

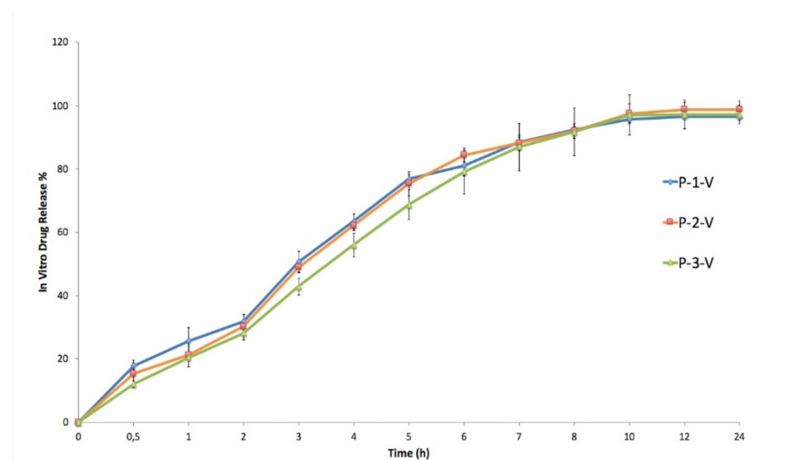

Figure 5. *In vitro* drug release results of VCZ loaded in situ gels (n:3, ±STD)

Stability

According to the detailed characterization studies, the optimized VCZ loaded *in situ* gel P2-V was chosen as optimum formulation and was subjected to stability study for three months at 4 ± 1 °C in the refrigerator and 25±1°C (relative humidity 60%). Based on research, testing of stability aimed to know the time of storage and the use of a material. The stability study revealed no significant change in visual appearance, clarity, pH, gelling capacity and drug content of the formulation (Table 4). Thus, in can be concluded that VCZ *in situ* gel formulated with 20% (w/w) P 407 and 8% (w/w) P 188 was successfully formulated for ocular administration.

Table 4. Stability studies results of VCZ loaded formulations (P2-V)

Parameters	t=0	t=3	
	4 and 25 ºC	4 ºC	25 ºC
pH	6.357±0.006	6.697±0.006	6.987±0.006
Drug content (%)	92.6255±0.609	92.487±1.844	92.657±0.276

The generally poor bioavailability of ophthalmic formulations can be improved by new formulations with a prolonged residence time. In this study, the potential of VCZ loaded thermosensitive *in situ* gels as drug carriers for ocular delivery was evaluated. The optimized VCZ loaded *in situ* gel formulation obtained from this study was composed of 20% (w/w) P 407 and 8% (w/w) P 188 as the gelling matrix. This will ensure that the patient could be treated at much longer time points, meaning that patients could be treated as outpatients, reducing hospital admissions.

DECLARATION OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors would like to thank to the BASF for providing the poloxamers.

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J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 44(1): 50-60, 2020
Doi: 10.33483/jfpau.644407

ORIGINAL ARTICLE / ÖZGÜN MAKALE

EVALUATION ON GALLIC ACID, EGCG CONTENTS AND ANTIRADICAL ACTIVITY OF GREEN TEA AND BLACK TEA EXTRACTS

YEŞİL ÇAY VE SİYAH ÇAY EKSTRELERİNDEKİ GALLİK ASİT, EGCG İÇERİĞİ VE ANTİRADİKAL AKTİVİTESİNİN DEĞERLENDİRİLMESİ

Aslı CAN AGCA[1*], Kadir BATCIOGLU[2], Engin SARER[3]

[1]Ankara Yıldırım Beyazıt University, Institute of Public Health, Department of Traditional, Complementary and Integrative Medicine, Ankara, TURKEY

[2]İnönü University, Faculty of Pharmacy, Department of Biochemistry, Malatya, TURKEY

[3]Ankara University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, TURKEY

ABSTRACT

Objective: *Tea is very well known and consumed beverage worldwide also cultivated widely. It is one of phytonutrients that has a protective role associated with its antioxidant activity. The aim of this article is to study the gallic acid, epigallocatechin gallate contents and antiradical activity of green tea and black tea extracts from Camellia sinensis cultivated in North Anatolia.*

Material and Method: *Gallic acid and epigallocatechin gallate contents were investigated in ethanol, methanol and water extracts of green tea and black tea by HPLC analysis and the antiradical activities were also examined for scavenging effect on DPPH and ABTS free radicals.*

Result and Discussion: *In total 6 extracts, gallic acid contents were determined in the range of 0.052-1.341 mg/100 ml and the value of EGCG (epigallocatechin gallate) were found between 0-19.54 mg/100ml. Water extract of green tea exhibited the best antiradical activity on both DPPH and ABTS radicals. Green tea could be evaluated as a good candidate for health prevention but it should be noted that the harvesting method and manufacturing process, optimum conditions on brewing time, the solvent used, chopping grade of tea leaves should also be taken into consideration during formulating both phytonutrient and pharmaceutical grade products.*

Keywords: *Antiradical activity, black tea, camellia sinensis, egcg, gallic acid, green tea*

* **Corresponding Author / Sorumlu Yazar:** Asli Can Agca
e-mail / e-posta: aslicanagca@yahoo.com

Submitted / Gönderilme: 08.11.2019 **Accepted / Kabul:** 25.11.2019

ÖZ

Amaç: *Çay, dünyada çok iyi bilinen ve tüketilen bir içecek olup antioksidan aktivitesine bağlı koruyucu etkileri bilinen önemli bir fitobesindir. Bu çalışmanın amacı, Kuzey Anadolu kaynaklı Camellia sinensis bitkisinden elde edilen yeşil çay ve siyah çaydan hareketle elde edilen ekstrelerdeki gallik asit ve epigallokateşin gallat miktarı ile ekstrelerin antiradikal aktivitesinin tespit edilmesidir.*

Gereç ve Yöntem: *Yeşil çay ve siyah çayın etanol, metanol ve sulu ekstrelerindeki gallik asit ve epigallokateşin gallat miktar tayini YPSK yöntemi ile tespit edilmiş ve ekstrelerin antiradikal aktivite tayini için DPPH ve ABTS yöntemleri kullanılmıştır.*

Sonuç ve Tartışma: *Toplam 6 ekstredeki gallik asit içeriği 0.052-1.341 mg/100 ml aralığında bulunmuş ve EGCG (epigallokateşin gallat) için bu değer aralığı 0-19.54 mg/100ml olarak tespit edilmiştir. Hem DPPH hem de ABTS radikali üzerinde en iyi etkiyi yeşil çayın sulu ekstresinin sağladığı belirlenmiştir. Yeşil çayın, koruyucu sağlıkta değerlendirilebilecek iyi bir aday olduğu ancak fitobesin ve farmasötik kalitede ürünün formülasyon çalışmalarında, bitkinin hasat yöntemi, bitkiden hareketle çay üretim prosesi, optimum karıştırma zamanı, kullanılan çözücü ve yaprakların parçalanma derecesinin kayıt altına alınması gerekmektedir.*

Anahtar Kelimeler: *Antiradikal aktivite, camellia sinensis, egcg, gallik asit, siyah çay, yeşil çay*

INTRODUCTION

Polyphenols are naturally occured in foods of plant origin and also play an important role as preventing medicine against chronic disorders. They are produced naturally in plants to protect themselves against viruses, bacteria and also linked to oxidative stability in plants. These defense mechanisms of plants could also be helpful for optimizing the human body functions [1-3].

Tea is very well known and consumed beverage worldwide, obtained from the leaves of *Camellia sinensis* (L.) O. Kuntze. According to the processing method, tea can be classified into four types; white tea, green tea, oolong tea and black tea. Fermentation is the corner stone in the process of manufacturing. White and green tea is subjected to a little or no fermentation, oolong tea is a semi- fermented final product. Black tea is the final product of full fermentation [4].

Tea is one of phytonutrients that has a protective role associated with its antioxidant activity. In addition, there are reports on its health benefits for cancer, cardiovascular disease and diabetes mellitus due to the antioxidant effect. Preventive and therapeutic effects of tea products are attributed to phenolic contents [5].

Gallic acid is a common phenolic acid, widely found in plants and tea products. Increasing scientific interest has shown that it plays an important role in the health benefits of food [6,7].

Epigallocatechin gallate (EGCG) is the major catechin and considered as the most active substance among catechins in green tea infusion. According to previous reports EGCG is a promising molecule for both prevention and treatment associated with being antioxidant, antiinflammatory, antibacterial, antiviral agent [8-11].

Especially catechins and theaflavines are major groups of polyphenols in green tea and black tea respectively. Gallic acid has also been reported to be found in both green tea and black tea products and

both epigallocatechin and gallic acid were mentioned to be major indicators of quality during standardizing pharmaceutical green tea samples [12]. There are studies to explain the chemopreventive mechanisms of EGCG, among them, target specific cell signaling pathways draw attention for regulating cellular proliferation and apoptosis [9].

The plant is used in Turkish folk medicine not only as carminative, but also tonic and diuretic. The antidote property of *C. sinensis* for alkaloid poisoning is also mentioned. The dried leaves are known to be used in the treatment of eye infections [13].

It is clear that the composition of tea is depended on geographical location, harvesting time, storage condition and manufacturing process. So, the aim of this article is to study the gallic acid and EGCG content and antiradical activity of green tea and black tea extracts from *C. sinensis* cultivated in North Anatolia and determine whether it could be defined as a source of natural antioxidants.

MATERIAL AND METHOD

Plant Material

All samples of green tea and black tea were supplied from commercial company in North Anatolia location in Turkey.

Preparation of extracts for the determination of antiradical activity

The ethanol and methanol extracts was prepared from 1 g of green tea and black tea products in 100 ml of each solvent by stirring constantly at room temperature for 1 hour and then filtered. The water extracts were prepared from 5 g of each sample by adding 500 ml of boiling water and heating at 100°C temperature for 5 min and then filtered. Total 6 extracts were prepared. Each extract was coded and given in table 1.

Table 1. The samples and extraction solvents.

	EXTRACTION SOLVENTS		
Sample	**Boiled water**	**Ethanol**	**Methanol**
Green tea product	GW	GE	GM
Black tea product	BW	BE	BM

HPLC Conditions

HPLC analysis was conducted on SSI Allience Esence HPLC Workstation System equipped with a SSI Allience Esence Series 4 LC pump, SSI Lab Allience Esence UV-Vis detector. A Shimpack CLC-ODS (M) (25 cm×0.45μm) column was used for seperation in this study. The wavelength was set to 270nm. A gradient elution was performed by varying the proportion of solvent A (acetonitrile) to solvent

B (0.1% phosphoric acid in water) with a flow rate of 1 ml/min. Sample quantity was 25µl. The mobile phase composition started at 8% solvent A and 92% solvent B for 40 min. Then, the mobile phase composition was changed into 11% solvent A and 89% solvent B from 40 min to 62 min. In 62 minute, composition was bring to 18% solvent A and 82% solvent B for 18 minutes. At 80 minute the composition was changed to 23% solvent A and 77% solvent B. All prepared solutions were filtered through 0.45µm membranes before injection onto HPLC.

The linearity was determined from the triplicate analytical curves obtained by gallic acid and EGCG standart solutions. Table 3 presents the correlation coefficient (r^2), limits of quantification (LOQ) and limits of detection (LOD) of both gallic acid and EGCG.

Determination of antiradical activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging test

The solution of DPPH (0.1 mM) was prepared in methanol and 2950 µl of DPPH solution was added to 50 µl of each extract at different concentrations. The mixtures were shaken and allowed to stand in dark at room temperature for 20 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer. Trolox was used as the reference. Lower intense of blue color indicate the higher activity. The percentage radical scavenging activity (RSA) was expressed as the inhibition percentage and was calculated by using the following formula.

% RSA: $[(A_0-A_t) / A_0] \times 100$

A_0 is the absorbance of the control reaction,

A_t is the absorbance in presence of all of the extract samples and reference after 20 min.

All the tests were performed in duplicate and the results were averaged. The radical-scavenging activity was expressed as antiradical activity and trolox equivalent antioxidant capacity (TEAC). The IC_{50} value (µg/ml) is the concentration required to inhibit 50% of the initial DPPH free radical, was calculated from the graph of inhibition curve. Antiradical activity (A_{AR}) was defined as $1/IC_{50}$.

ABTS [2,2 '-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid] radical scavenging test

The radical monocation of $ABTS^+$ was generated by reacting ABTS solution (200 µmol/L) with phosphate buffer solution (pH 7.4) including 4.5 µmol/L myoglobin and also 300 µmol/L H_2O_2 solution was prepared. Then the absorbance was measured at 734 nm by using a UV-VIS spectrophotometer and the change in absorbance was recorded every 30 seconds during 3 minutes to determine the level of spontenous degradation. Trolox was used as the reference. Tea extracts (and trolox solutions at different concentrations) were allowed 1 ml of ABTS solution as described above, and the absorbance was taken at 734 nm during 3 min using a spectrophotometer.

The capability of scavenging the ABTS radical was calculated by using the following formula.

% RSA: $[(A_0\text{-}At) / A_0] \times 100$

A_0 is the absorbance of the control, namely absorbance of ABTS radical in methanol

A_t is the absorbance in presence of all of the extract samples and reference at the end of 3 min.

All the tests were performed in duplicate and the results were averaged. The radical-scavenging activity was expressed as antiradical activity and trolox equivalent antioxidant capacity (TEAC). The concentration that causes a decrease in the absorbance of initial oxidants by 50% (IC_{50}) was determined. Antiradical activity (A_{AR}) was defined as $1/IC_{50}$.

RESULT AND DISCUSSION

In this study, we tested 6 extracts prepared with water, ethanol, methanol from green tea and black tea of *Camellia sinensis* from Turkey for investigating their antiradical activity as well as gallic acid and EGCG contents in extracts.

In our study, gallic acid contents were determined in the ranged from 0.052-1.341 mg/100 ml as shown in Table 4. The highest gallic acid content was found in the water extract of green tea as 1.341 mg/100 ml, on the other hand the ethanolic extract of black tea contents the lowest gallic acid as 0.052 mg/100 ml. The amount of EGCG ranged between 0-19.54 mg/100 ml. EGCG could not detected in water and ethanolic extracts of black tea whereas, methanolic and aqueous extract of green tea samples were found close to each other in a value of 19.54 and 19.53 mg/100 ml, respectively.

The antiradical activities of ethanol, methanol and water extracts of green tea and black tea were also examined for scavenging effect on DPPH and ABTS free radicals.

In present study the water extract of green tea exhibited the best antiradical activity with 126.582 and 65.359 A_{AR} value on DPPH and ABTS radicals, respectively. The antiradical activity values of methanol extracts of black tea and green tea on DPPH radical were found as 59.880 and 81.967, respectively. Metanol extracts of green tea and black tea samples showed moderate scavenging effects on ABTS radical. The A_{AR} values of metanolic extract of black tea was 56.818 and for green tea samples it was determined as 58.139. Under the same experimental condition, the A_{AR} value on ABTS radical of trolox was 114.942.

The results on antiradical activities of test samples by using DPPH and ABTS methods are given in table 2.

TEAC (Trolox equivalent antioxidant capacity) values of all potent extracts obtained by DPPH assay were higher than those obtained by ABTS assay.

Table 2. The results of antiradical effect of green tea and black tea extracts extracts.

Samples	DPPH method		ABTS method	
	A_{AR} (Antiradical activity)	1mg/ml Trolox equivalent extract concentrations (mg/ml)	A_{AR} (Antiradical activity)	1mg/ml Trolox equivalent extract concentrations (mg/ml)
GW extract	126.582	1.837	65.359	3.558
GM extract	81.967	2.791	58.139	4.000
GE extract	52.631	4.418	32.573	7.139
BW extract	62.893	3.697	54.054	4.302
BM extract	59.880	3.883	56.818	4.093
BE extract	-	-	-	-
Trolox	232.558	-------	114.942	------

The codes of extracts were explained in Table 1

Table 3. Linearity results, limit of Detection (LOD) and Limit of Quantification (LOQ) of gallic and EGCG.

Compound	Equation*	r^2	LOQ (µg/ml)	LOD (µg/ml)
Gallic acid	Y=11806x-5302.6	0.9993	0.03	0.010
EGCG	Y=9341,7x-9050.9	0.9997	0.005	0.017

*Linear regression equation y= ax+b, in which x is the concentration as µg/ml and y is the peak area at the 270 nm wavelength.

Table 4. Gallic acid and EGCG content in terms of mg/100ml of methanol, ethanol and water extracts of green tea and black tea.

Samples	Water extracts		Methanolic extracts		Ethanolic extracts	
	Gallic acid	EGCG	Gallic acid	EGCG	Gallic acid	EGCG
Green tea product	1.341	19.53	0.145	19.54	0.384	7.702
Black tea product	0.216	-	0.093	0.358	0.052	-

Chronic diseases have been increasing year by year. And it is very clear that reactive oxygen species (ROS) play a critical role in chronic diseases leading to oxidation of lipids and proteins which ultimately induces all inflammatory diseases, atherosclerosis, neurological disorder and cancer [14].

Having ability to protect the damages caused by free radicals and showing low toxicity makes natural antioxidants valuable for medicine, cosmetic and food industry. Tea products are proved to be strong antioxidants and important dietary source due to polyphenol content. On the other hand, the phytochemical profile and antioxidant activity of these products can vary strongly on the basis of different parameters.

Antioxidant activity encloses different pathways; prevention of radical formation, scavenging the radicals and reparing the damage occured by radicals. So *in-vitro* assays on determination of scavenging radical potential is an important indicator for antioxidant activity.

In this study, we have applied two different *in-vitro* assays to test 6 extracts prepared with water, ethanol, methanol from green tea and black tea from *Camellia sinensis* growing in Turkey for their antiradical activity as well as determined the gallic acid and EGCG contents in these extracts by HPLC. As shown in table 2, the highest activity against DPPH and ABTS was exerted by water extracts of green tea with a 126.582 and 65.359 A_{AR} value and 1.837 and 3.558 mg/ml of trolox equivalent concentrations, respectively. Methanolic extract of green tea exhibited moderate radical scavenging activity against DPPH and ABTS comparing with trolox. The ethanolic extract of green tea revealed a weaker antiradical activity and besides, black tea ethanol extract did not show inhibitory effect on both DPPH and ABTS radicals.

The chemical profile of tea includes polyphenols, alkaloids, amino acids, volatile compounds and minerals. It was mentioned that polyphenols are the most abundant group attributed the health benefits of tea products. EGCG is the major catechin in green tea and shown to have benefical therapeutic effects. Potential benefits of EGCG against cancer have been demonstrated both *in-vitro* and *in-vivo*. Scientists have been studying on proving the poor bioavailability of EGCG by nanotechnology approach. Additionally, recent studies have been focused on combination therapy with other dietary (6-gingerol, curcumin, quercetin) or pharmaceutical agents (5-fluorouracil, cisplatin, docetaxel) to adopt the synergistic effects [10].

On the basis of fermentation process, catechins are inverted to polymerized products named as theaflavin. Theaflavins are another polyphenolic group responsible for antiradical activity of black tea. Previous reports mentioned the strong correlation between phenolic compounds contents in tea and leaf age, plucking time, extraction conditions and manufacturing process [15-17]. It was also reported that antiradical activity of old leaf was higher than in young leaf of the plant [16].

The antiradical activity of tea extracts have been studied widely. In general, according to previous reports, green tea extracts were found to have higher antiradical activity due to higher content of

phenolic compounds in particular flavonoids comparing with black tea samples [18, 19] and Rusak et al [17] have been reported that 40 % ethanol was the most effective solvent in the prolonged extraction of EGCG in tea leaves. In the meanwhile, Zuo et al. [20] reported that fermentation process increases the liberation of gallic acid and resulted in high levels of this acid in black tea samples. Liebert et al. [21] observed that black and green tea extracts showed increasing antiradical activity due to the increased total phenolic content with brewing time. Significant i*n-vivo* antioxidant activity was also reported after ingesting 300 and 450 ml of green tea [22].

There are different reports on gallic acid content in different type of tea samples. Fernandez et al. [23] determined the gallic acid content in 45 commercial tea including non fermented and fermented samples from different location; gallic acid contents were reported in a range of 0.004-2.537% and the lower percentages belong to non fermented tea. Hilal and Engelhardt [24] analyzed teas from German market and gallic acid content in green tea and black tea was reported in a range of 0.01-0.19 g/kg and 0.16-0.60 g/kg, respectively. On the other hand, Zuo et al. [20] determined the gallic acid content of 8 types of tea including green, oolong, puerh and black tea, in the range of 0.37-5.53 mg/g and the high level of gallic acid was reported in full fermented puerh and black tea.

As EGCG is the major tea catechin, its content in different type of tea samples were also studied. Fernandez et al. [25] determined the EGCG content in 37 commercial tea including non fermented and fermented samples from different location; EGCG contents were reported in a range of 0-5.675% w/w surprisingly, in one green tea sample, EGCG was not detected. Wang et al. [26] analyzed green tea catechins and EGCG content in green tea samples was reported in a range of 0.95-32.6 mg/100 ml. On the other hand, Zuo et al. [20] analyzed total eight tea samples, four of tea samples were green tea and EGCG content was determined the in the range of 51.1-62.4 mg/g in green tea samples, in that study black tea sample was found to contain 3.79 mg/100ml EGCG. Ozturk et al studied on quality parameters of Turkish green tea and found EGCG content between 6.10-6.74 g/100g [27].

In this study, the result obtained for gallic acid content was not fully in agreement of previous reports. On the other hand, our experimental data which showed that green tea extracts contained the highest EGCG content and had the best antiradical activity complied with earlier studies. The reasons behind the variability of results could be due to environmental factors, harvesting conditons, storage, leaf age, extraction solvent, extraction time, degree of fermentation. Furthermore, earlier reports pointed out the strong influence of extraction time, drug particle size, solvent used, infusion time, leaf age and temperature factors on chemical composition of the tea products.

Using herbal products is a global trend and becoming popular for health prevention because of having protective effects against dieases. Since preventing is easier and cheaper method when compared with treatment and to hospitalise the patients, scientists have been dealing with protection.

Tea is consumed all over the world and has so many health benefits. As a natural antioxidant being cheap and supplied easily makes the tea product valuable in preventing health. But it is needed to underlined that countries have their own tea brewing and consumption culture that totally affect the ingredients of tea product. So the products obtained from tea should be designed by considering not only cultivation, manufacturing process but also the social habits.

Our results support that different location and manufacturing process form different tea products with different phytochemical profiles and also the variability of compositon of tea products deeply effect the power of antiradical activity. It should be noted that besides the variations on manufacturing process, there is a correlation between brewing conditions and phenolic content of tea beverage. The harvesting method and manufacturing process, optimum conditions on brewing time, the solvent used, chopping grade of tea leaves should also be taken into consideration during formulating both phytonutrient and pharmaceutical grade products.

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J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 44(1): 61-69, 2020
Doi: 10.33483/jfpau.645467

ORIGINAL ARTICLE / ÖZGÜN MAKALE

CHEMICAL CHARACTERIZATION OF THE FATTY ACID COMPOSITIONS AND ANTIMICROBIAL ACTIVITY OF SUMAC (*RHUS CORIARIA* L.) FRUITS, GROWING NATURALLY IN TURKEY AND SOLD IN HERBALIST MARKETS

TÜRKİYE'DE DOĞAL OLARAK YETİŞEN VE AKTARLARDA SATILAN SUMAK (RHUS CORIARIA L.) MEYVELERİNİN YAĞ ASİDİ KOMPOZİSYONLARININ KİMYASAL KARAKTERİZASYONU VE ANTİMİKROBİYAL AKTİVİTESİ

Gülderen YILMAZ[1,*], Gülnur EKŞİ[1], Betül DEMİRCİ[2], Fatih DEMİRCİ[2]

[1] Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Ankara, Turkey

[2] Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, Eskişehir, Turkey

ABSTRACT

Objective: *The aim of this study was to compare the fatty acid components and antimicrobial properties of R. coriaria L. samples which were sold as powder and grains that show natural distribution in Tunceli and Siirt.*

Material and Method: *After the seeds were weighed and powdered, fixed oils were obtained by using soxhalet apparatus. Obtained fixed oils were analyzed by GC and GC / MS methods after methylation process. In vitro antimicrobial activity studies of the samples were performed using six different Gram negative and Gram positive bacteria and Candida albicans using EUCAST disc diffusion and CLSI microdilution methods.*

Result and Discussion: *The main fatty acid components of all samples were determined as oleic acid (42.2 - 43.3%), linoleic acid (25.2 - 28.5%) and palmitic acid (18.4-221.5%), respectively. In vitro antimicrobial activity of fixed oils, such as Escherichia coli NRRL B-3008, Staphylococcus aureus ATCC 6538, S. epidermidis ATCC 14990, Pseudomonas aeruginosa ATCC 27853, Bacillus subtilis NRRL B-A78 A78. Results were compared with standard antimicrobial agents and MIC values> 2.5 - 0.22 mg/ml. The results were found to be significant in terms of antimicrobial efficacy.*

Keywords: *Antimicrobial, CLSI, EUCAST, GC-FID, GC-MS, Rhus coriaria*

* **Corresponding Author / Sorumlu Yazar:** Gülderen Yılmaz
e-mail / e-posta: gulderen_yilmaz@yahoo.com

Submitted / Gönderilme: 12.11.2019 **Accepted / Kabul:** 13.12.2019

ÖZ

Amaç: *Bu çalışma Tunceli ve Siirt ilerinde doğal olarak yayılış gösteren örnekler ile aktarlarda toz ve tane olarak satılan R. coriaria L. örneklerinin yağ asiti bileşenleri ve antimikrobiyal özelliklerini karşılaştırmak amacı ile yapılmıştır.*

Gereç ve Yöntem: *Tohumlar tartıldıktan ve toz haline getirildikten sonra soksalet kullanarak sabit yağları elde edilmiştir. Elde edilen sabit yağların, metilleme işlemi yapıldıktan sonra, GC ve GC/MS yöntemleriyle eş zamanlı olarak analizleri gerçekleştirilmiştir. Sabit yağ numunelerinin in vitro antimikrobiyal aktivite çalışmaları altı farklı Gram negatif ve Gram pozitif bakteri ve Candida albicans'a karşı EUCAST disk difüzyon ve CLSI mikrodilüsyon yöntemleri kullanılarak gerçekleştirilmiştir.*

Sonuç ve Tartışma: *Tüm numunelerin ana yağ asidi bileşenleri, sırasıyla oleik asit (%42.2 - 43.3), linoleik asit (%25.2 - 28.5) ve palmitik asit (%18.4-221.5) olarak tanımlandı. Sabit yağların in vitro antimikrobiyal aktivitesi, Escherichia coli NRRL B-3008, Staphylococcus aureus ATCC 6538, S. epidermidis ATCC 14990, Pseudomonas aeruginosa ATCC 27853, Bacillus subtilis NRRL B-A78 A78 gibi insan patojenik mikroorganizmalarına karşı gerçekleştirildi. Sonuçlar standart antimikrobiyal maddeler ile karşılaştırılmış ve MIC değerleri > 2,5 - 0.22 mg/ml belirlenmiştir. Sonuçların antimikrobiyal etkinlik açından kayda değer olduğu görülmüştür.*

Anahtar Kelimeler: *Antimikrobial, CLSI, EUCAST, GC-FID, GC-MS, Rhus coriaria*

INTRODUCTION

The genus *Rhus* L. contains more than 130 species belong to Anacardiaceae [1]. *Rhus coriaria* L. (sumac) is a wild edible species mainly grown in temperate and subtropical regions; in Mediterranean countries, Turkey and near East. Plant grows up to 3–4 m in height. Mature fruits are reddish-brown in color and have one seed [2]. *R. coriaria* was used both for its nutritional and medicinal values for centuries by crushing the dried fruits in the Mediterranean and Middle East. About 2000 years ago, the Greek physician Pedanius Dioscorides wrote in the "*De Materia Medica*" on the therapeutical traits of sumac [3]. In Arabic Palestinian herbal medicine, it was used for cancer, heart diseases, diarrhoea, blood pressure management, intestinal diseases, eye infections, stomachache, kidney and liver diseases, blood sugar management, respiratory infections, smallpox, oral diseases, headache, bites, dermatitides [4], in Turkish traditional medicine for ulcer, blood sugar management, respiratory infections, smallpox, oral diseases, diarrhoea, bleeding and intestinal diseases. *R. coriaria* has increasing economic importance around the world in terms of pharmacology and food preservation. *R. coriaria* was reported as cytotoxic, antimutagenic, antioxidant, antimalarial, antithrombin, antifibrogenic, and antitumorigenic. Beside, sumac is most notable for its potential antimicrobial, antifungal, and antiviral effects [5]. Different parts of *R. coriaria* plant are an abundant source of tannins, phenolic acids, anthocyanins, gallic acid derivatives, flavonoid glycosides such as methyl gallate, kaempferol, and quercetin [4]. It also contains fatty acids, volatiles, organic acids proteins, fibers, vitamins, and minerals. The fruits encompass phenolic compounds like tannins, volatiles, and organic acids, anthocyanins, and fixed oil [2].

In this study, *in vitro* antimicrobial activity of different fruit fixed oils were performed against five different Gram-negative and Gram-positive human pathogenic bacteria (*Escherichia coli* NRRL B-3008, *Staphylococcus aureus* ATCC 6538, *S. epidermidis* ATCC 14990, *Pseudomonas aeruginosa*

ATCC 27853, *Bacillus subtilis* NRRL B-4378) and *Candida albicans* ATCC 90028 using European Committee on Antimicrobial Susceptibility Testing (EUCAST) disc diffusion and The Clinical and Laboratory Standards Institute (CLSI) microdilution methods.

MATERIAL AND METHOD

Plant materials

Fruits of *Rhus coriaria* were collected from Tunceli and Siirt provinces (Figure 1). Voucher specimens were deposited at the Herbarium of the Faculty of Pharmacy in Anadolu University, Eskisehir, Turkey (ESSE). Plant materials were listed in Table 1.

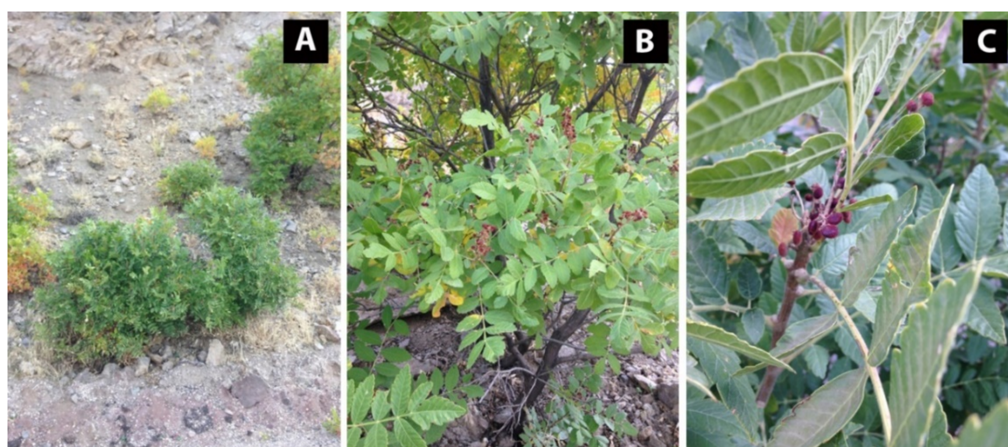

Figure 1. A, B, C Habitus of *Rhus coriaria* L.

Table 1. Plant samples used in the study.

Code	Locality and date	ESSE Number	Local Code
A (Grain)	Tunceli, Batman Village, 1077 m, 2015	34	DT
B (Grain)	Siirt, 895 m, 2015	33	D_S
C (Grain)	Ankara, Commercial, 2015	32	A_1
D (Pulverised)	Ankara, Commercial, 2015	31	A_2

Extraction

The air-dried plant material (10 gr) was crushed and extracted using a Soxhlet apparatus. The resulting oils (25± 5%) were dried over anhydrous sodium sulfate prior to further derivatization and bioactivity evaluations.

Preparation of Fatty Acid Methyl Esters

The residue was refluxed with 0.5 N NaOH solution (5 ml) for 10 min in methanol. Afterward, 14–20% BF_3 (5 ml) in methanol solution was added via the condenser. Then, the solution boiled for an additional 2 min. 5 ml *n*-hexane was added and boiled for a further 1 min. After the solution cooled an additional 5 ml of saturated NaCl solution was added and the flask was rotated gently quite a few times. Finally, saturated NaCl solution was added to float the *n*-hexane solution into the neck of a 1 ml flask and the solution was transferred into a vial [6].

Analyses

Gas Chromatography (GC)

Agilent 6890N GC system was used for the GC analysis. The temperature was set to 300 °C for a flame ionization detector (FID). The simultaneous auto-injection implemented column (Innowax FSC column, 60 m x 0.25 mm, 0.25 µm film thickness) was used in an attempt to achieve the same elution order on the GC-MS to fulfill the identical working conditions. FID chromatograms were used to calculate the relative percentage amounts (%) of the separated compounds.

Gas Chromatography-Mass Spectrometry (GC-MS)

Helium gas was used as carrier (0.8 ml/min gas flow) and the temperature was set for 10 min at 60 °C and calibrated to 220 °C at a ratio of 4 °C/min, and then set permanently at 220 °C for 10 min and at the end conditioned to 240 °C at a ratio of 1 °C/min then set for 20 min at 240 °C. The split rate was kept at 40:1. The port temperature of injection was kept at 250 °C. MS was kept at 70 eV and the designated mass range was between *m/z* 35 and 450. The components of the essential oil were identified by using their relative retention times or by assessment of their relative retention index (RRI) to a series of *n*-alkanes. For the identification of essential oil, Wiley and MassFinder 3, Baser Library of Essential Oil Constituents and also MS literature data was also used [7-11].

Antimicrobial Assay

Disc diffusion is one of the oldest approaches to antimicrobial susceptibility testing (AST) and remains one of the most widely used AST methods in routine clinical microbiology laboratories. The method is appropriate for testing the majority of bacterial pathogens [12,13]. EUCAST initiated the development of a standardized disk diffusion method calibrated to the harmonized MIC breakpoints. The method comprises the use of Mueller–Hinton agar without supplements for non-fastidious organisms and with 5% mechanically defibrinated horse blood and 20 mg/L b-NAD for fastidious organisms, a standardized inoculum resulting in confluent growth, an incubation time of 16–20 h, a

reading guide on how to read zone diameters on individual species-agent combinations and zone diameter breakpoints calibrated to the EUCAST clinical MIC breakpoints [13].

Escherichia coli (NRRL B-3008), *Staphylococcus aureus* (ATCC 6538), *S. epidermidis* ATCC 14990, *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter aerogenes* (NRRL 3567), *Bacillus subtilis* (NRRL B-4378), were used as test bacteria, whereas the human pathogenic yeast *Candida albicans* (ATCC 90028) was also used in the panel. 15% glycerol at –85 °C was used to store the microorganisms. The Mueller Hinton Broth (Merck, Germany) was used to revitalize the bacteria and yeast at 35–37 °C. For purity check, the bacteria and yeast inoculated on the plates of Mueller Hinton agar (MHA, Mast Diagnostics, U.K.). In this study, a microdilution broth susceptibility assay (REF) was used [14]. Dimethylsulfoxide 25% (v/v) (DMSO, Carlo Erba) was used to prepare the stock solutions. Dilutions of samples were prepared in a 96-well microtiter plate by using distilled water. The microbial suspensions were standardized in double strength Mueller Hinton Broth (MHB) and Mueller Hinton Agar (MHA) and then each microbial suspension (100 µL) was added to the appropriate well. The serial dilutions of the essential oil were used as a negative control. The minimum inhibition concentration (MIC, mg/ml) was determined after incubation at 37 °C for 24 h. Chloramphenicol was used as standard antibacterial and as *Candida* positive control Ketoconazole was used. Entire assays were repeated at least 3 times [15-19]. The MIC results are given in Table 3.

RESULT AND DISCUSSION

From the different fruit samples obtained sumac oils were analysed by using the GC and GC-MS (Table 2) to detect the major fatty acid components, and four bioassays assessed in combination in order to release their antimicrobial effects towards human pathogenic microorganisms (Table 3). The composition and comparative proportions of the sumac fatty acids were clarified with the help of GC and GC-MS analyses. Results of the simultaneous analysis with GC and GC-MS method are given in Table 2. The oleic acid contents changed between 42.2 and 43.3%, the linoleic acid contents were changed between 25.2 and 28.5% and finally, the palmitic acid content ranged from 18.04 to 21.5% as main constituents. Oleic ($C_{18:1}$), linoleic ($C_{18:2}$), and palmitic ($C_{16:0}$) acids were the main fatty acids in the sumac oil, respectively. The polyunsaturated fatty acid contents (18:2 +18:3) of the total fatty acids changed between 25.9 and 29.9%.

The antibacterial effects of the sumac oils with the major components of oleic acid, linoleic acid, and palmitic acid opposed to the standard antimicrobial agent chloramphenicol were given in Table 2. The oleic acid contents of the oils changed between 42.2 and 43.3%, the linoleic acid between 25.2 and 28.5% and the palmitic acid ranged from 18.4% to 21.5%. In this study, oleic ($C_{18:1}$), linoleic ($C_{18:2}$), and palmitic ($C_{16:0}$) acids were found the main fatty acids in the sumac oil. Polyunsaturated fatty acid contents (18:2 +18:3) were changed between 25.9 and 29.9%. *Staphylococcus aureus* was inhibited

with an intermediate MIC value of 2.5 mg/ml. *S. epidermidis* was inhibited with a low MIC value of 2.5 mg/ml, *Escherichia coli* was inhibited by sumac oils except C with a MIC value of 2.5 mg/ml while C inhibited the *E. coli* with a strong MIC value of 0.62 mg/ml. *Pseudomonas aeruginosa* was inhibited by the sumac oils with a MIC value of 2.5 mg/ml, having much stronger activity than antimicrobial agent chloramphenicol, while *Bacillus subtilis* was inhibited with a moderate MIC value of 1.25–2.5 mg/ml.

Table 2. Fatty Acid Compositions of *Rhus coriaria* L. Fruits identified by GC and GC-MS.

Compound	A (%)	B (%)	C (%)	D (%)
Palmitic acid (16:0)	20.7	20.0	21.5	18.4
Palmitoleic acid (16:1)	tr	tr	0.4	0.3
Stearic acid (18:0)	2.9	2.7	2.1	3.3
Oleic acid (18:1)	42.5	42.4	42.2	43.3
Elaidic acid (18:1)	6.5	5.0	7.2	5.1
Linoleic acid (18:2)	26.2	28.5	25.2	27.6
Linolenic acid (18:3)	1.0	1.4	0.7	1.2
Arachidic acid (20:0)	tr	tr	0.7	0.8
Saturated	23.6	22.7	24.3	22.5
Unsaturated	76.2	77.3	75.7	77.5
Unsaturated/Saturated	3.23	3.41	3.12	3.44
Total	**99.8**	**100.0**	**100.0**	**100.0**

A: Tunceli, B: Siirt, C: Commercial (grain), D: Commercial (pulverised), %: calculated from FID data, tr: Trace (< 0.1%)

Table 3. Minimum inhibitory concentrations of samples (mg/ml) and antimicrobials (μg/ml) by the microdilution method.

Test samples	***E. coli*** **NRRL B-3008**	***S. aureus*** **ATCC 6538**	***S. epidermidis*** **ATCC 14990**	***P. aeruginosa*** **ATCC 27853**	***B. subtilis*** **NRRL B-4378**	***C. albicans*** **ATCC 90028**
A	> 2.5	> 2.5	> 2.5	> 2.5	> 2.5	0.62
B	> 2.5	> 2.5	> 2.5	> 2.5	> 2.5	0.31
C	0.62	> 2.5	> 2.5	> 2.5	1.25	1.25
D	> 2.5	> 2.5	> 2.5	> 2.5	> 2.5	1.25
Chloramphenicol	8	4	2	>64	4	
Ketoconazole						0.25

A: Tunceli, B: Siirt, C: Commercial (grain), D: Commercial (pulverised).

Nevertheless, all the sumac oils exhibited a strong inhibition with a MIC value of 0.62–2.5 mg/ml against plant pathogens with the exception of sample *S. epidermidis.* Antifungal effects of the *R. coriaria* oils were also examined against the standard antifungal agent Ketoconazole. All examined sumac oils

displayed weak antifungal effect with a concentration of 1.25 mg/ml, which recommended a strong resistance of a pathogenic fungus, *Candida albicans* (Table 3). *In vitro* antimicrobial activity studies of fixed oil samples were performed using six different Gram-negative (*E. coli*, *P. aeruginosa*) and Gram-positive (*S. aureus, S. epidermidis*, *B. subtilis*) bacteria and a fungus *C. albicans* by using EUCAST disk diffusion and CLSI microdilution methods. The results were compared with standard antimicrobial agents and MIC values > 2.5–0.22 mg/ml were determined. The results were found to be noteworthy for antimicrobial efficacy (Table 3).

Doğan and Akgül (2005) were extracted the sumac oil by using the cold ether extraction method. Accordingly, oleic acid (34.00 to 40.35%), linoleic and linolenic acid (33.31 to 35.83%) and palmitic acid (20.75 to 25.60%) was shown as the main fatty acids in sumac oil. Polyunsaturated fatty acid (18:2 +18:3) contents of the total fatty acids were changed between 34.84 and 37.36% [20]. Our results were confirmed the previous studies that oleic, linoleic, and palmitic acids are the major fatty acids in sumac fruits [21]. Besides, the oleic acid contents (42.2 to 43.3 %) is higher, the linoleic acid contents (25.2 to 28.5 %) is lower, and the palmitic acid content (18.4 % to 21.5 %) is lower than Doğan and Akgül (2005). The polyunsaturated fatty acid content (25.9 to 29.9 %) is also lower than the results in Doğan and Akgül (2005). To storage capabilities and for longer shelf life polyunsaturated fatty acid levels are very important, since the polyunsaturated fatty acids are more vulnerable to oxidative degradation and a lower polyunsaturated fatty acid content can probably extend the shelf life [20]. Nimri et al., (1999) have been extracted the *R. coriaria* fruits by using ethanol and the results indicated a wide-ranging of antimicrobial activity which was dedicated to the tannins with a MIC value of 10 to 26 mg/ml against numerous bacteria [22]. The following work examined the inhibitory effect of ripened and unripened *R. coriaria* fruits against six Gram-positive and six Gram-negative bacteria suggested the extracts were found to be effective against all tested bacteria, especially against Gram positives and the ripened fruits were found to have a stronger antimicrobial activity [23]. In contrast, in our study, the extracts were found to be more sensitive against Gram-negative bacteria. In another work suggested that the dry *R. coriaria* seed found to have an antibacterial effect against *Pseudomonas aeruginosa* [24] similar to our results. Fruits of *R. coriaria* hydroalcoholic extract were tested against Gram-positive and negative bacteria, *S. aureus*, *B. cereus*, *E. coli* by using a cool percolation method and extract exhibited antibacterial activity against the species tested likewise our results [25]. Water extracts of dried *R. coriaria* fruits exhibited antimicrobial activity against *B. subtilis, S. aureus*, *S. enteritidis* and *E. coli* [23]. *B. subtilis* was found to be more sensitive among Gram-positive bacteria and *E. coli* was found to be one of the most resistant Gram-negative bacteria. In our study, while *S. epidermidis*, among Gram-positive strain, found to be the most resistant, *E. coli* was found sensitive to *R. coriaria* fruit extract. Nasar-Abbas & Halkman (2004) claimed the water extracts of *R. coriaria* fruits presented quit a strong effect against Gram-positive bacteria. In contrast to alcoholic and aqueous extracts from sumac, a

hydrodistillation extract of dried *R. coriaria* fruits was shown to be ineffective as an antimicrobial agent [26].

As the overall conclusion, the results were found to be noteworthy for antimicrobial efficacy. Results indicate that individual fatty acid contents of *R. coriaria* have been grown in the different provinces of Turkey are variable and potential antimicrobial sources.

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J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 44(1): 70-88, 2020
Doi: 10.33483/jfpau.574001

ORIGINAL ARTICLE / ÖZGÜN MAKALE

DESIGN, SYNTHESIS, ANTIMICROBIAL AND ANTIFUNGAL ACTIVITIES OF NEW 1,2,4-TRIAZOLE DERIVATIVES CONTAINING 1*H*-TETRAZOLE MOIETY

1H-TETRAZOL İÇEREN YENİ 1,2,4-TRİAZOL TÜREVLERİNİN TASARIMI, SENTEZİ, ANTİMİKROBİYAL VE ANTİFUNGAL AKTİVİTELERİ

Yuliia FROLOVA[1,*], Andriy KAPLAUSHENKO[1], Nataliya NAGORNAYA[2]

[1]Zaporizhzhya State Medical University, Faculty of Pharmacy, Department of Physical and Colloidal Chemistry, Zaporizhzhya, Ukraine

[2]Zaporizhzhya State Medical University, Faculty of Pharmacy, Department of Medicinal Preparations Technology, Zaporizhzhya, Ukraine

ABSTRACT

Objective: *Antimicrobials and antifungals are the most acquired drugs in the world. Today, it becomes necessary to create new drugs with antimicrobial and antifungal effect. The purpose of our research is the synthesis of new series of 5-(1H-tetrazole-1-yl)-4H-1,2,4-triazole-3-amine, the establishment of physical-chemical, antimicrobial and antifungal properties for all synthesized compounds.*

Material and Method: *5-(1H-tetrazole-1-yl-methyl)-4H-1,2,4-triazole-3-yl-1-(alkyl-, aryl-)-methanimines were synthesized by reacting of 5-(1H-tetrazole-1-yl-methyl)-4H-1,2,4-triazole-3-amine with aldehydes in acetic acid. Then, sodium borohydride was selected as a reducing agent, which allowed for the restoration of the double bond. The structures of synthesized compounds were confirmed by IR, ^{1}H NMR and mass spectra. The synthesized compounds were evaluated for antimicrobial and antifungal activity by "serial dilutions" method.*

Result and Discussion: *During the synthetic studies the new series of 22 compounds were obtained. The 5-(1H-tetrazole-1-yl-methyl)-4H-1,2,4-triazole-3-yl-1-(5-nitrofuran-2-yl)methanimine was observed to be most possessing good antimicrobial and antifungal activity and has exceeded the reference standard for Staphylococcus aureus (12,5/25 mg/ml), Escherichia coli (50/100 mg/ml), Pseudomonas Aeruginosa (100/200 mg/ml), Candida albicans (50/50 mg/ml).*

Keywords: *1,2,4-triazole, 1H-tetrazole, antimicrobial and antifungal activity*

* **Corresponding Author / Sorumlu Yazar:** Yuliia FROLOVA
***e-mail / e-posta*:** yuliia_hulina@ukr.net

Submitted / Gönderilme: 07.06.2019 **Accepted / Kabul:** 12.01.2020

ÖZ

Amaç: *Antimikrobiyal ve antifungal ilaçlar dünyada en yaygın ilaç türlerindendir. Günümüzde mikroplara ve mantarlara karşı yeni ilacın üretilmesine ihtiyaç duyulur. Araştırmamızın amacı, 5-(1H-tetrazol-1-il)-4H-1,2,4-triazol-3-amin türevi yeni bileşiklerin sentezi ile sentez edilmiş bileşiklerin fizikokimyasal, antimikrobiyal ve antifungal özelliklerinin tespit edilmesidir.*

Gereç ve Yöntem: *5-(1H-tetrazol-1-ilmetil)-4H-1,2,4-triazol-3-il-1-(alkil-, aril-)-metanim, 5-(1H-tetrazol-1-ilmetil)-4H-1,2,4-triazol-3-amin'in sirke asidinde aldehitler ile etkileşimi sonucunda sentez edilmiştir. Bir sonraki aşamada indirgen olarak sodyum borohidrür kullanıldığı için ikili bağ yeniden kurulabilmiştir. Sentez edilmiş bileşiklerin yapıları, kızılaltı, nükleer manyetik rezonans ve kütle spektroskopisi ile doğrulanır. Sentez edilmiş bileşikler mikrop ve mantarlara karşı aktivite konusunda serili dilüsyon metodu ile denetlenmiştir.*

Sonuç ve Tartışma: *Sentetik araştırmaların sonucunda 22 bileşikten oluşan yeni seri elde edilmiştir. 5-(1H-tetrazol-1-ilmetil)-4H-1,2,4-triazol-3-il-1-(5-nitrofuran-2-il)metanimin en iyi antimikrobiyal ve antifungal etkisine sahip olup Staphylococcus aureus (12,5/25 mg/ml), Escherichia coli (50/100 mg/ml), Pseudomonas aeruginosa (100/200 mg/ml), Candida albicans (50/50 mg/ml) için karşılaştırmalı ilacı geçtiğini tespit edilmiştir.*

Anahtar Kelimeler: *1,2,4-triazol, 1H-tetrazol, antimikrobiyal ve antifungal aktivite*

INTRODUCTION

Piperidinium 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol)-3-yl) acetate is the active pharmaceutical ingredient of drug "Tryfuzol" (API). It is used in veterinary as an immunomodulatory agent. It increases the resistance of organisms to viral diseases. Forced degradation conditions create the model influence of various environmental factors on the active substance. In these conditions, various impurities may be formed in the decomposition, which may alter or weaken the biological activity of the active compound, as well as increase toxicity. Thus, it is possible to predict which impurities may be generated during the storage or transportation of drugs containing the investigated API. It will also help to offer conditions for the protection of this substance from the influence of harmful factors. Therefore, this study has a significant relevance.

Methods for investigating force degradation effects have been described in a number of publications [1-6]. Regulatory aspects in Development of Stability-Indicating Methods were presented in the review of Renu Sehrawat *et al*. [1]. The condition for stress degradation which usually studied are: acid hydrolysis, base hydrolysis, thermal hydrolysis, oxidation, thermal degradation, photodegradation.

Authors [7] proposed potentiometric titration method for quantitative determination of piperidinium 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol-3-yl)thio)acetate in the 1% and 2.5% solutions. Method is not selective, and it is not applicable for determination of impurities. Method based on adsorbtion of this API in the ultraviolet region of the spectrum was elaborated [8]. Low selectivity and sensitivity of the method are not permitted to measure of impurities.

Our HPLC-DAD method of determination of piperidinium 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol-3-yl)thio)acetate in 1 % solution shown satisfied quality of separation of API from impurities [9]. This work was not contained forced degradation study.

Aim of the research to make forced degradation study of piperidinium 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol)-3-yl) acetate in active pharmaceutical ingredient, 0.1% solution and 1% solution for injection.

MATERIAL AND METHOD

Chemicals and reagents

Piperidinium 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol)-3-yl)acetate was obtained from Toxicological and Inorganic Chemistry Department. Substance was synthesized and its structure was confirmed by the Parchenko V.V. [10,11]. Acetonitrile qualified "HPLC Super Gradient" (Avantor Performance Materials Poland S.A., Poland), methanoic acid was 100% (AppliChem GmbH, Germany), ultra-high pure water (18 MΩ at 25 °C) was prepared by the Direct Q 3UV Millipore (Molsheim, France).

Analytical Instrumentation

Agilent 1260 Infinity HPLC System (degasser, binary pump, autosampler, thermostat column compartment, DAD). Agilent single-quadrupole mass spectrometer 6120 with electrospray ion source (ESI); OpenLAB Software CDS.

Chromatography conditions

The chromatography study was carried out by elution with a water-acetonitrile mixture (70:30) with the addition of 0.1% methanoic acid. Column Zorbax SB-C18, 30 mm x 4.6 mm, 1.8 um. Column Temp. 40 ºC. Flow rate was 0.400 ml/min.

Mass spectrometry conditions

Temperature of drying gas was 100 ºC. Drying gas (nitrogen) flow rate was 10 l/min. Nebulizing gas (N2) pressure was 53 psig. Mass spectra were obtained at m/z 100-2000. Fragmentation of molecular ions was studied at fragmentor voltage: 100, 150, 200 V, positive polarity.

Forced degradation conditions

Samples were taken every day, prepared for injection and injected into HPLC system. Volume of injection for 0.1% solution was 5 uL, for 1% solution was 0.5 uL. Content (%) was taken from the report of OpenLab CDS Software from Signal of the DAD detector at 276 nm.

Laboratory conditions degradation

Substance and solutions (0.1%, 1%) were kept at room temperature in laboratory conditions.

Thermal degradation

Influence of temperature was studied in the thermostat at the 66 ºC for the 0.1%, 1% solutions and substance. The samples were kept at 66 ºC during 5 days.

Oxidative degradation

Hydrogen peroxide (3%) was used for study of the influence of oxidizing agent. About 0.001 g of API was dissolved in the 1 mL of 3% hydrogen peroxide.

Ultraviolet (UV) degradation

The irradiation was carried out by the luminescent UV lamp, YF UV-9W 365 nm, which radiates in the range of long-wavelength ultraviolet with a maximum radiation of 365 nm. The illumination was measured with a luxmeter and was approximately 2000 lux. Solid substance and solutions with concentrations 0.1%, 1% were studied. Maximal period of exposure was 4 days.

Acid hydrolysis

Influence of acid was studied. About 0.001 g of API was mixed with the 1 mL of the 0.1 mole/L of HCl.

Alkaline hydrolysis

About 0.001 g of API was mixed with the 0.1 mole/L sodium hydroxide solution.

Preparation of solutions for laboratory conditions degradation study, thermal decomposition study, UV degradation study

Solution with concentration 0.1% was prepared by dissolution of 0.001 g of API in 1 mL of water. Solution with concentration 1% was prepared according to pharmaceutical preparation "1% solution for injections", viz. 0.01 g of API was dissolved in the 1 mL of water, 0.0059 of sodium chloride was added.

When the solid substance was studied, 0.001 g was dissolved in 1 mL water and 5 uL of solution was injected to the HPLC.

RESULT AND DISCUSSION

Optimized chromatography conditions

2-((5-(Furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol-3-yl)thio)acetic acid was formed in the stream of solvent from the API (salt). Therefore, the detector identified the acid. Thus, API was determined in form of the acid.

Results of the study of the substance decomposition are shown in Table. 1. Mass balance, % (content of the main substance, % plus content of degradation products and impurities, %) in all cases was equaled 100%.

Table 1. Quantitative content of the piperidinium 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol)-3-yl) acetate.

Days / Terms of decomposition	0	1	2	3	4	5	6
Laboratory conditions, 0.1% solution	99.64	99.54	99.53	99.47	99.47	99.46	99.48
Laboratory conditions, 1% solution	99.97	99.97	99.97	99.94	99.94	99.93	
Alkaline hydrolysis. 0.1 M solution of NaOH	99.64	99.58	99.66	99.64	99.64	99.61	99.66
3% H_2O_2	99.63	79.10	73.20	69.54	65.44	61.57	55.49
Thermal effect 66 ºC, 0.1% solution	99.64	99.53	99.35	99.25	99.25	99.24	
Thermal effect 66 ºC, 1%solution	99.97	99.94	99.92	99.87	99.85	99.85	
Thermal effect 66 ºC, substance	99.64	99.80	99.72	99.90	99.80	99.81	
UV light irradiation, solution 0.1%	99.64	97.41	89.31	77.61	56.25		
UV light irradiation, solution 1%	99.97	97.29	93.36	89.68	80.03		
UV light irradiation, substance	99.64	99.80	99.76	99.23	99.76		

Laboratory conditions degradation

During the storage of the 0.1% reference API solution in the laboratory conditions, percentage of the substance was decreased about 0.1% for 6 days. The level of the substance in 1% solution under these conditions was not changed for 5 days.

Thermal degradation

Thermal effect (66 °C) on the 0.1% solution of API leads to its decomposition by approximately 0.4% over 5 days (Fig.1). Substantial degradation products, however, was not identified.

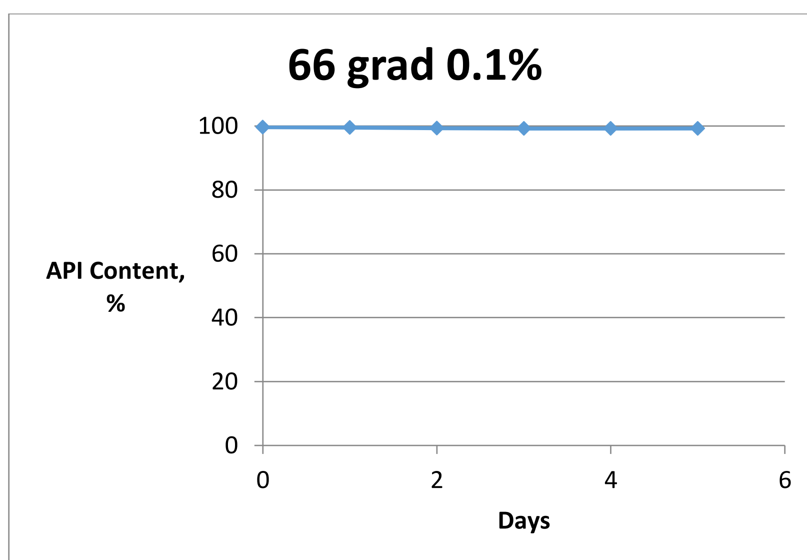

Figure1. The API degradation curve in the 0.1% solution at a temperature 66 °C

At the same time, under the influence of the temperature (66 °C) on the 1% solution decomposition occurs only about 0.1% (Fig. 2). During the study of the thermal effect (66 °C) on the solid substance (API) the content of API in a substance was not changed.

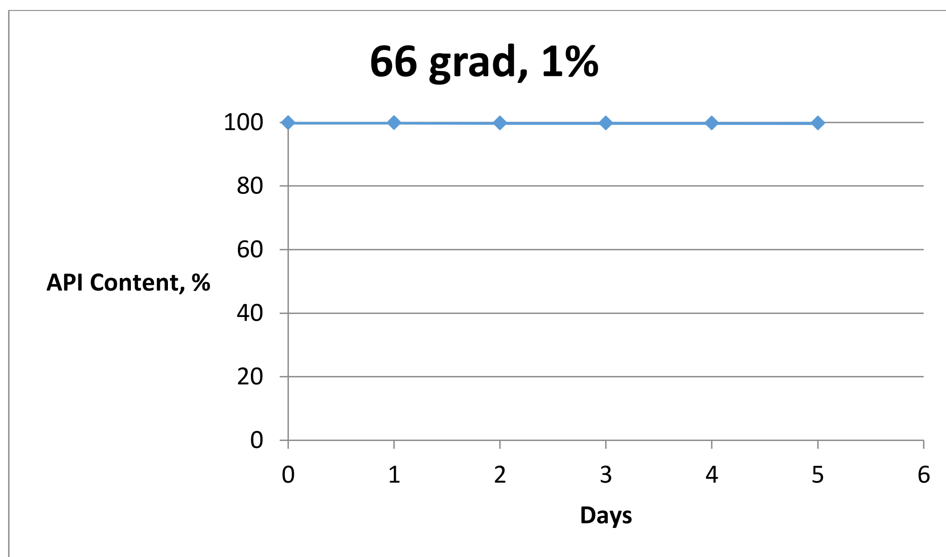

Figure 2. The API degradation curve in the 1% model solution for injection at a temperature 66 °C

Oxidative degradation

The effect of 3% hydrogen peroxide over 6 days results in a decrease in the concentration of API about 2 times (Fig. 3).

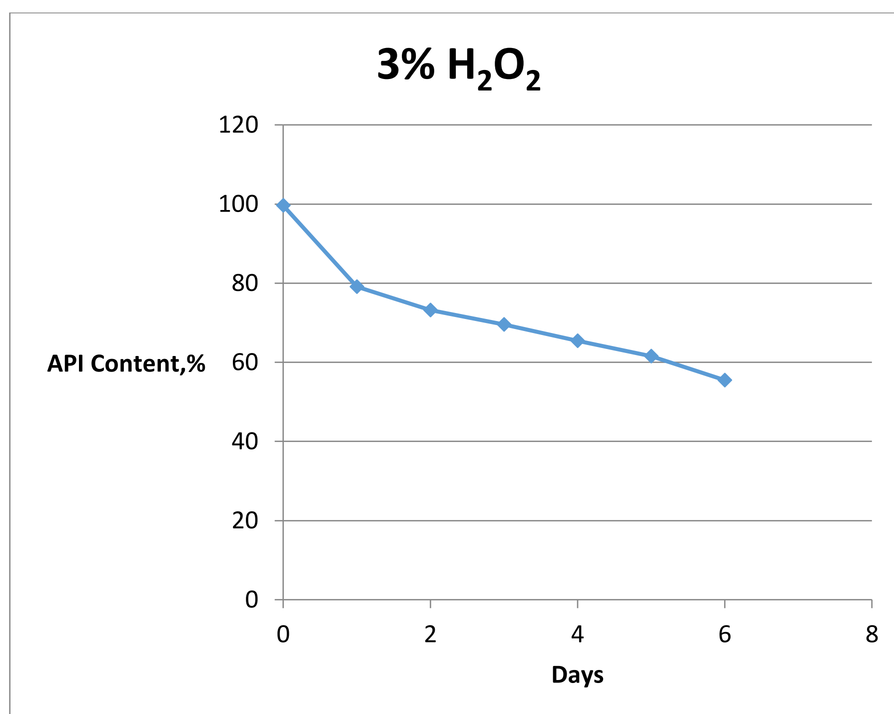

Figure 3. The API degradation curve under action 3% H_2O_2

Ultraviolet (UV) degradation

UV light irradiation causes the decomposition of the 0.1% solution during four days at more than 40% (Fig. 4).

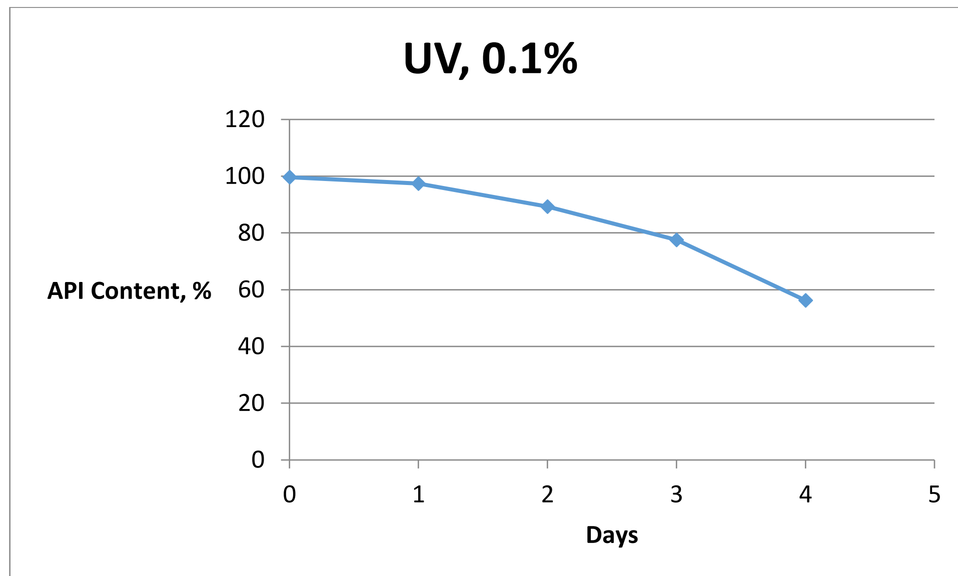

Figure 4. The API degradation curve in 0.1% solution

At the same time, for 1% solution the concentration was decreased about 20% (Fig. 5). The API content was not changed during irradiation of dry substance for 4 days.

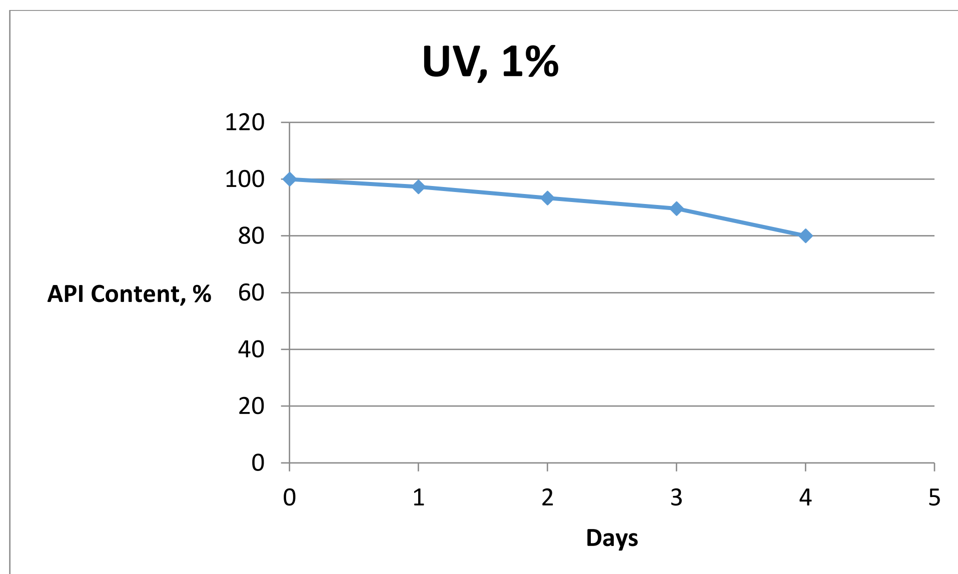

Figure 5. The API decomposition curve in the 1% solution for injection.

Acid hydrolysis

Under the action of 0.1 M solution of chloride acid API immediately decomposes with formation 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol-3-yl)thio)acetic acid, which is insoluble in water. So, the study of exposure of 0.1 M chloride acid was finished at this step.

Alkaline hydrolysis

Under the action of 0.1 M solution of sodium hydroxide, the content of the API was not changed for 6 days.

Determination of the structure of degradation products

Possible structures of compounds formed as a result of API degradation under stress conditions was proposed after study of the mass spectra of the corresponding chromatography peaks.

The structure determination of API degradation products formed by the action of 3% hydrogen peroxide.

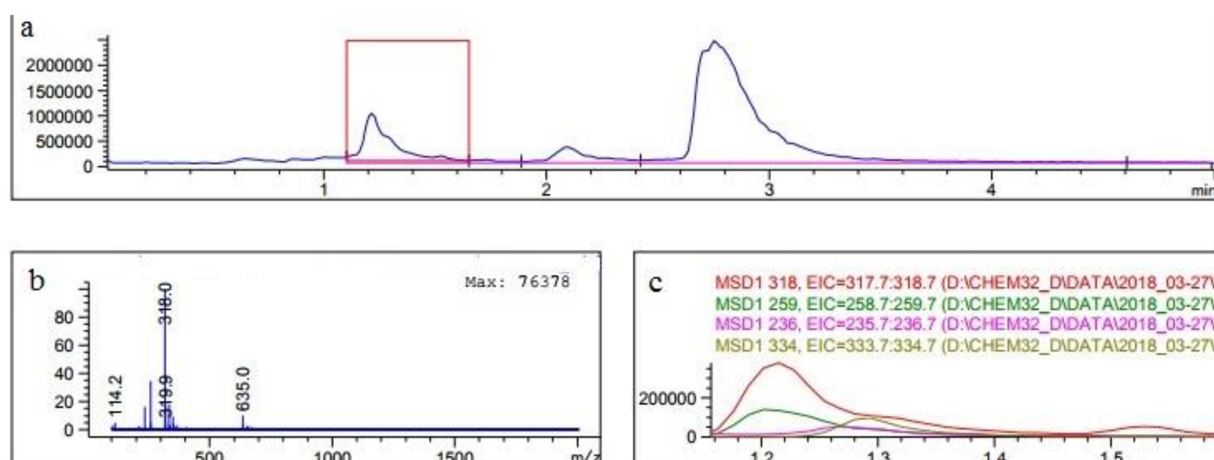

Figure 6. The TIC chromatogram of API degradation products formed by action of 3% H_2O_2 at 150V (a). Mass spectrum of peak at 1.219 min (b). EIC chromatogram (c).

Chromatography of the degradation products appeared after action of 3% H_2O_2 shown two peaks (Fig. 6). First peak (at 1.219 min) was not pure. The most intensive peak in extracted ion chromatogram (EIC) had m/z=318. It corresponded to the sulfoxide (Fig. 7). It is known reaction of sulfoxide formation from organic compounds of sulfur with valence two by the influence of the H_2O_2 solution [12].

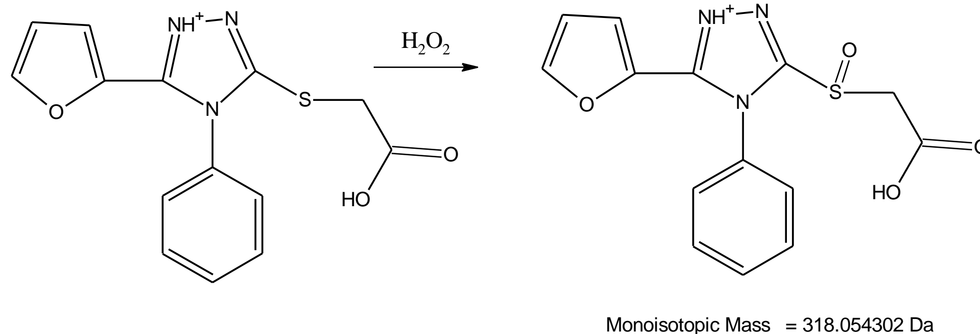

Figure 7. Formaton of 3-[(carboxymethyl)sulfinyl]-5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol-1-ium cation (m/z=318).

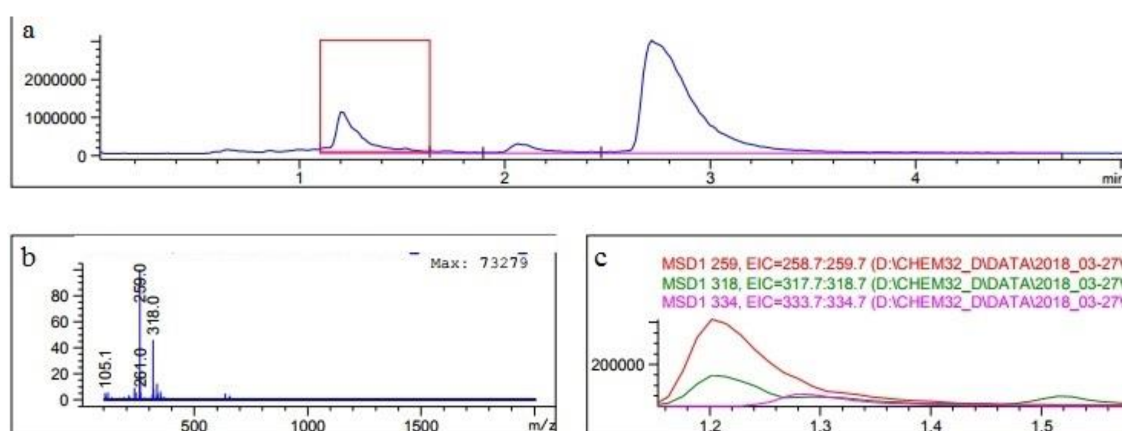

Figure 8. The TIC chromatogram of API degradation products formed by action of 3% H_2O_2 at 200V (a). Mass spectrum of peak at 1.217 min (b). EIC chromatogram (c).

When fragmentation voltage was increased till 200 V the ion with the m/z 259 in the mass spectra of first peak was appeared (Fig.8). The possible structure of this ion is presented at Fig. 9.

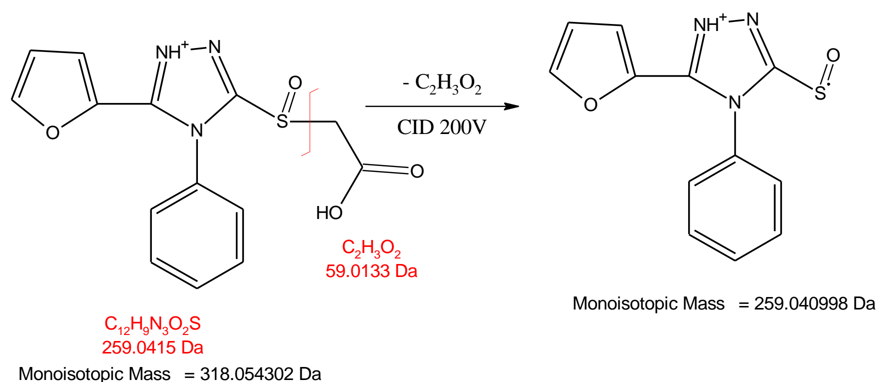

Figure 9. Transformation of cation with m/z 318 during fragmentation in CID at 200V

Second peak of the degradation product was at 2.140 min (Fig. 10).

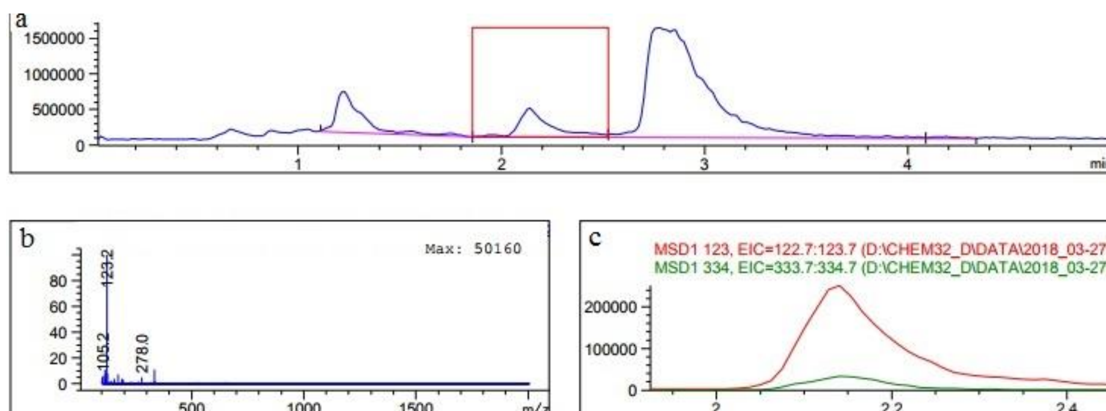

Figure 10. The TIC chromatogram of API degradation products formed by action of 3% H_2O_2 at 100V (a). Mass spectrum of peak at 2.140 min (b).EIC chromatogram (c).

Quazimolecular ion with m/z = 334 correspond to the sulfone which was formed at the second step oxidation by the H_2O_2 (Fig. 11). It is well-known reaction [12].

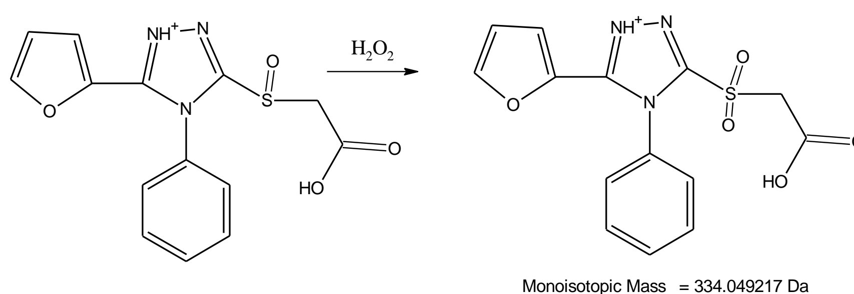

Figure 11. Formation of 3-[(carboxymethyl)sulfonyl]-5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol-1-ium cation (m/z=334).

There are two fragment ions present in mass spectra of second peak at 100 V (m/z =278.0 and m/z=123.2). Possible structure of first ion present at Fig. 12.

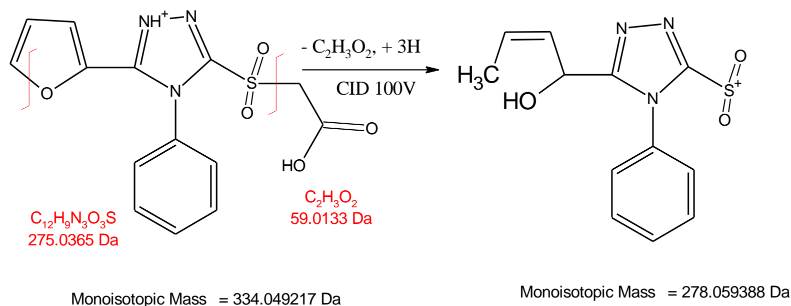

Figure 12. Converting of cation with m/z 344 during fragmentation in CID at 100V to product the cation with m/z 278.

Reaction formation of the ion with m/z 123 present at Fig.13.

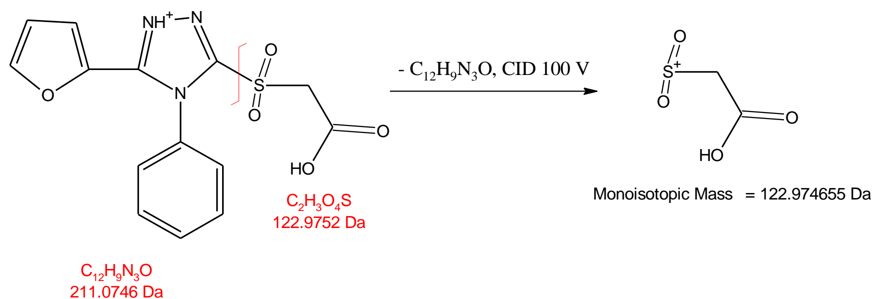

Figure 13. Transformation of cation with m/z 344 during fragmentation in CID at 100V to product the cation with m/z 123.

The structure determination of API degradation products formed by the influence of UV radiation on 0.1% solution.

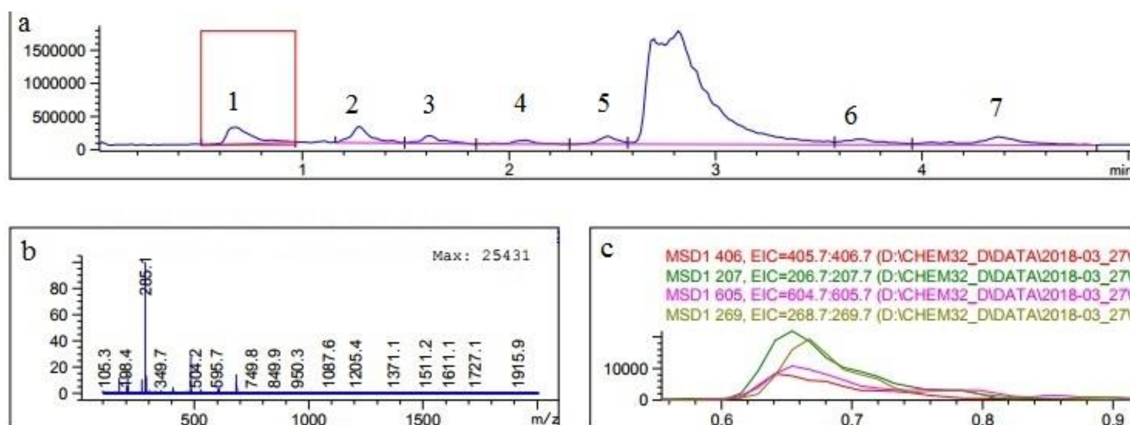

Figure 14. The TIC chromatogram of API degradation products formed by UV radiation (fragmentation voltage 100V) (a). Mass spectrum of peak (1) at 0.675 min (b). EIC chromatogram (c).

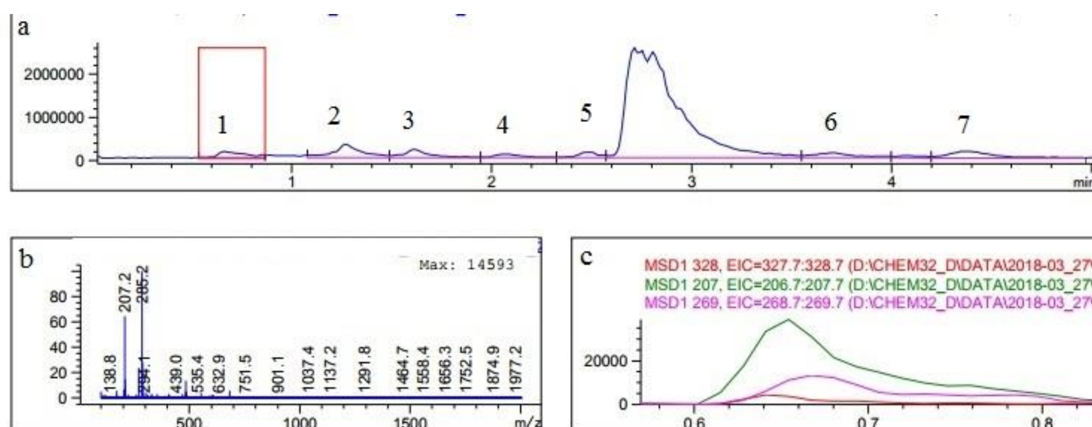

Figure 15. The TIC chromatogram of API degradation products formed by UV radiation (fragmentation voltage 150V) (a). Mass spectrum of peak (1) at 0.670 min (b). EIC chromatogram (c).

The first peak was not identified (Fig. 15). The monoisotope mass m/z = 285.2 and m/z = 207.2 in the mass spectrum of the unidentified peak (1) was observed (Fig.14, 15).

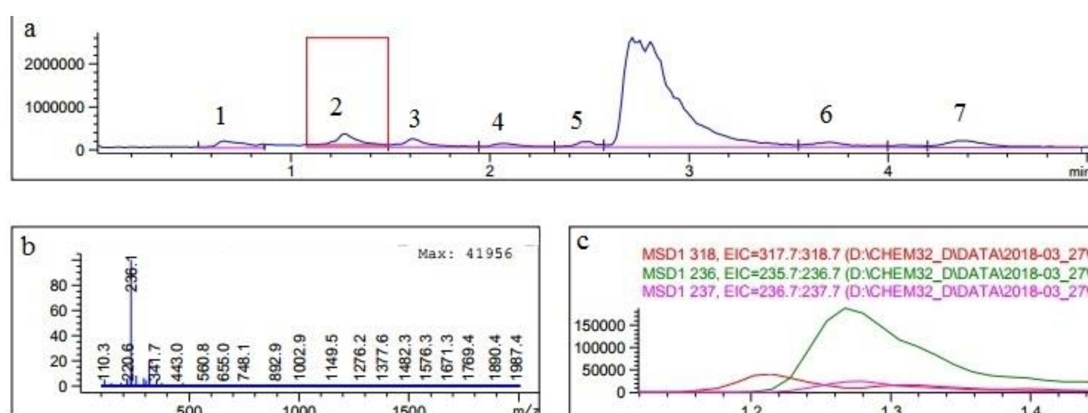

Figure 16. The TIC chromatogram of API degradation products formed by the action of UV radiation (fragmentation voltage 150V) (a). Mass spectrum of peak (2) at 1.275 min (b). EIC chromatogram (c).

Sulfoxide was also observed at 150V in API degradation products formed by the action of UV radiation. The retention time was close to 1.2 (peak 2), m/z 318 (Fig. 16).

There was an impurity that is associated with the cleavage of the furan cycle to form the corresponding structure with m/z 236.1 (Fig.17). On the second day of irradiation there was a peak of dimer ion with m/z 471, which confirms that the quasimolecular ion has a mass 236.

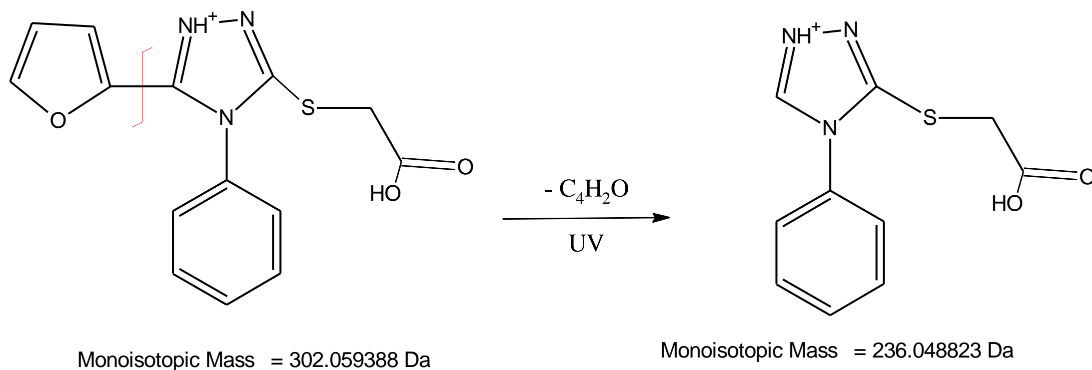

Figure 17. Possible way of degradation of the API at UV radiation influence with cleavage of the furan ring.

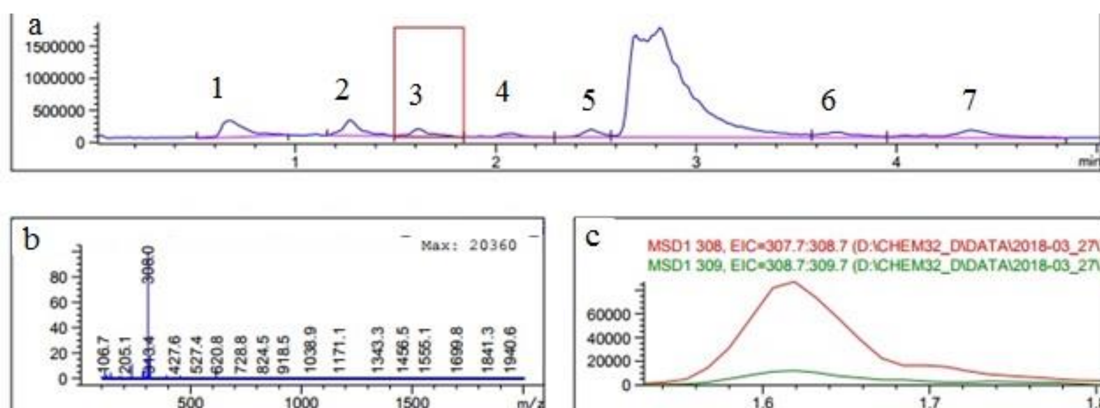

Figure 18. The TIC chromatogram of API degradation products formed by the action of UV radiation (fragmentation voltage 100V) (a). Mass spectrum of peak (3) at 1.616 min (b).EIC chromatogram (c).

Possible structures of the ion with m/z 308 (Fig. 18) ($[M+H]^+$) proposed at Fig. 19. They are formed as a result of the reduction and opening of furan cycle. The dimeric ion with m/z 615 ($[2M+H]^+$) was detected in the mass spectrum on the second day of irradiation. The presence of the corresponding dimer ion confirms that the ion with m/z 308 is a quasimolecular ion.

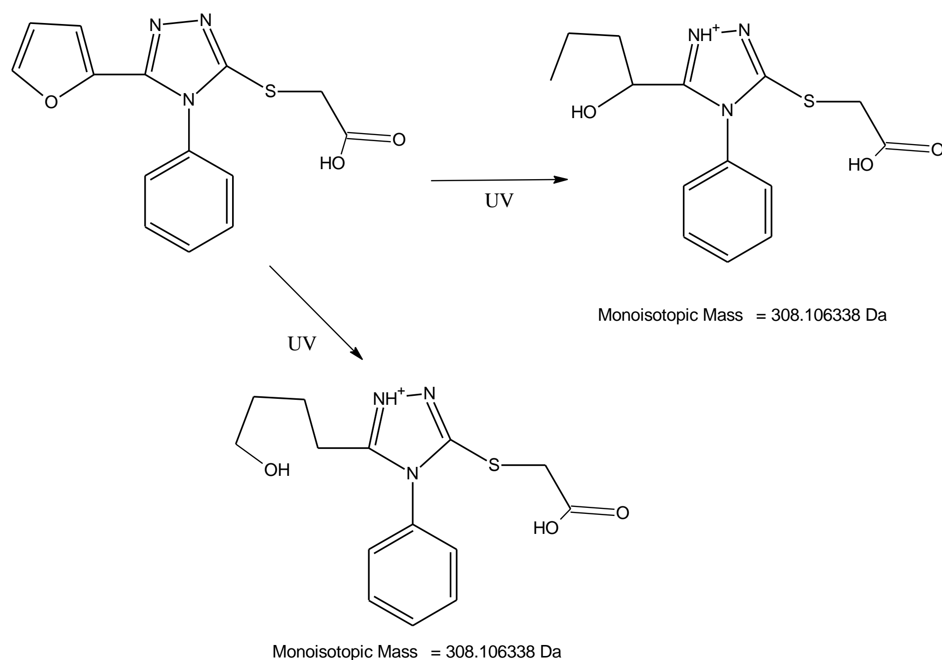

Figure 19. Possible API photodegradation pathway with formation of the product with molecular mass 308.1

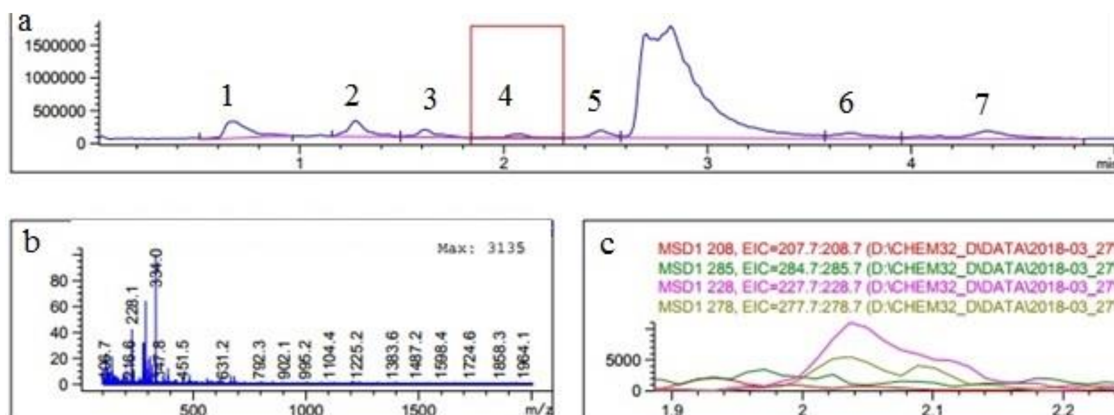

Figure 20. The TIC chromatogram of API degradation products formed by the action of UV radiation (fragmentation voltage 100V) (a). Mass spectrum of peak (4) at 2.070 min (b).EIC chromatogram (c).

There is also sulfone in products of the photodegradation, the retention time is approximately 2.1, m/z 334 (Fig.20).

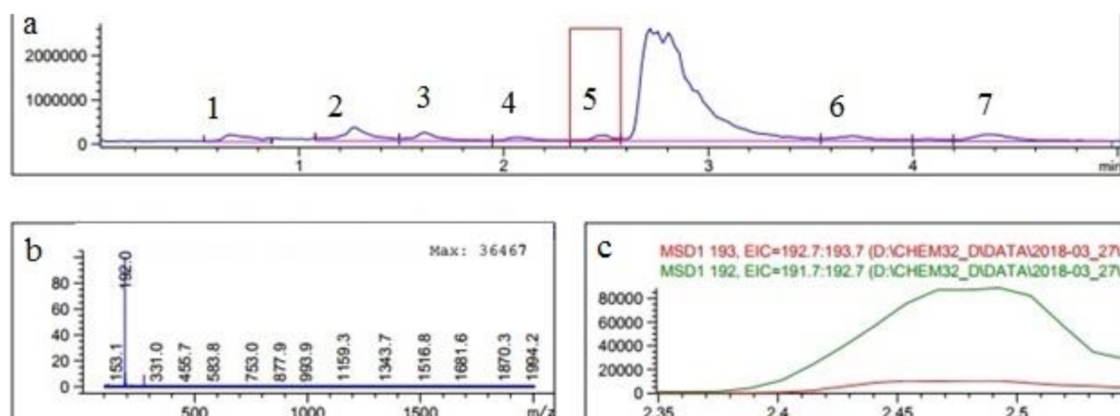

Figure 21. The TIC chromatogram of API degradation products formed by the action of UV radiation (fragmentation voltage 150V) (a). Mass spectrum of peak (5) at 2.478 min (b).EIC chromatogram (c).

An impurity with a retention time approximately 2.5 min and m/z 192 was observed (Fig. 21). It is the product of the breakaway of the furan cycle, as well as carbon dioxide (decarboxylation) from protonated 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol)-3-yl) acetate acid (Fig. 22).

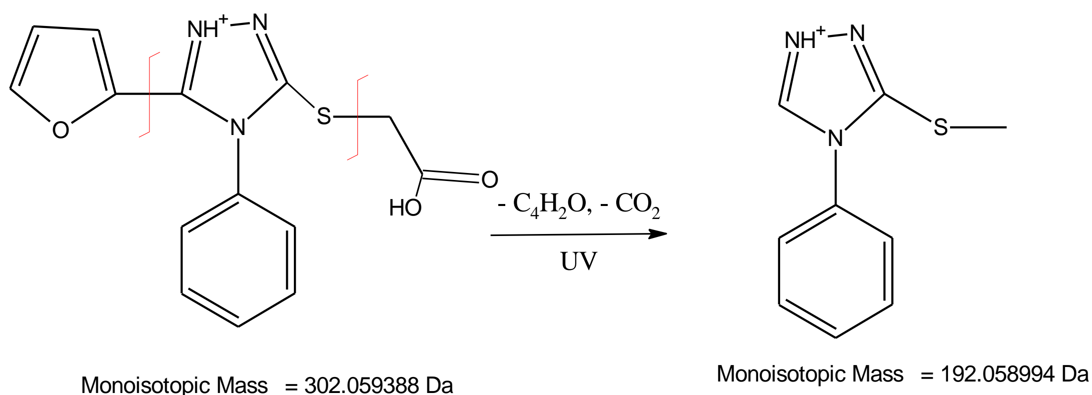

Figure 22. Cleavage of furan cycle and decarboxilation of API

The formation of the thione under the influence of UV radiation was observed. The 5-(furan-2-yl)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione is precursor in the synthesis of API [10, 11]. It was confirmed by the retention time and m/z. The retention time corresponds to the retention time from chromatography of the standard solution of the corresponding thione (3.7 min), m/z of quasimolecular ion equals 244, which corresponds to the molecular weight of the protonated compound (Fig.23, 24).

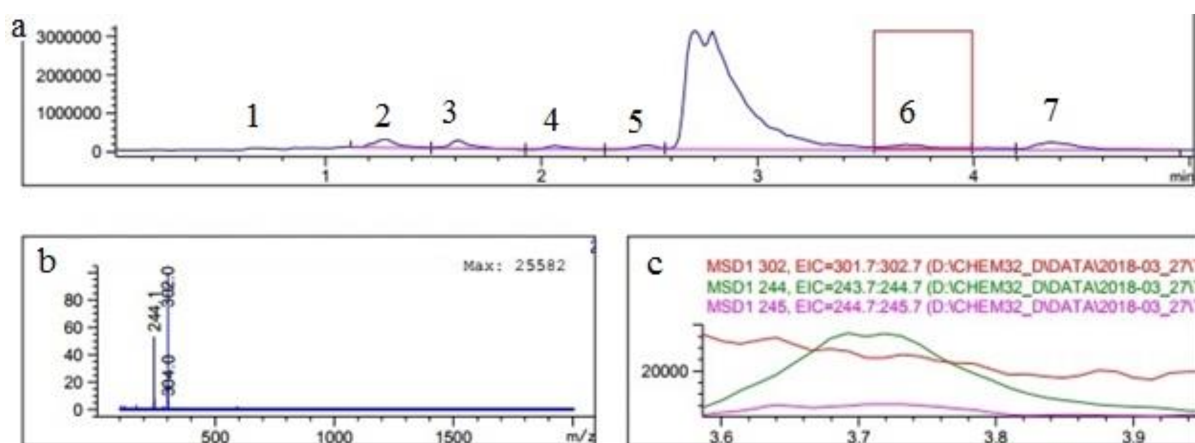

Figure 23. The TIC chromatogram of API degradation products formed by the action of UV radiation (fragmentation voltage 200V) (a). Mass spectrum of peak (6) at 3.695 min (b).EIC chromatogram (c).

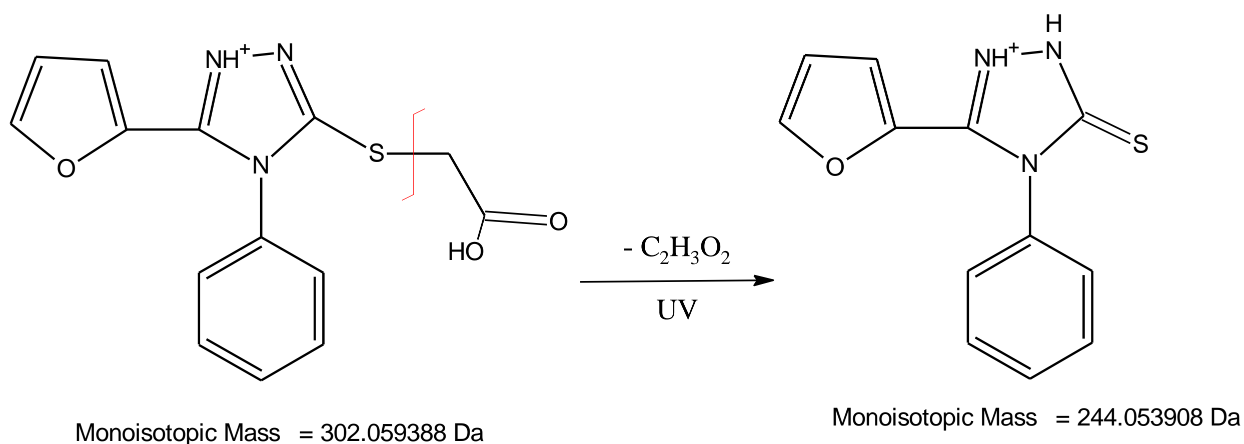

Figure 24. The 5-(furan-2-yl)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione formation.

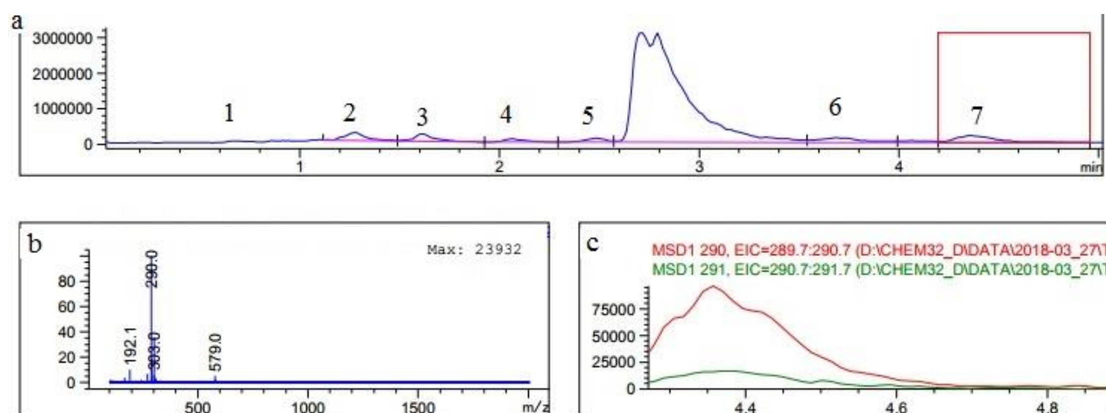

Figure 25. The TIC chromatogram of API degradation products formed by the action of UV radiation (fragmentation voltage 200V) (a). Mass spectrum of peak (7) at 4.363 min (b). EIC chromatogram (c).

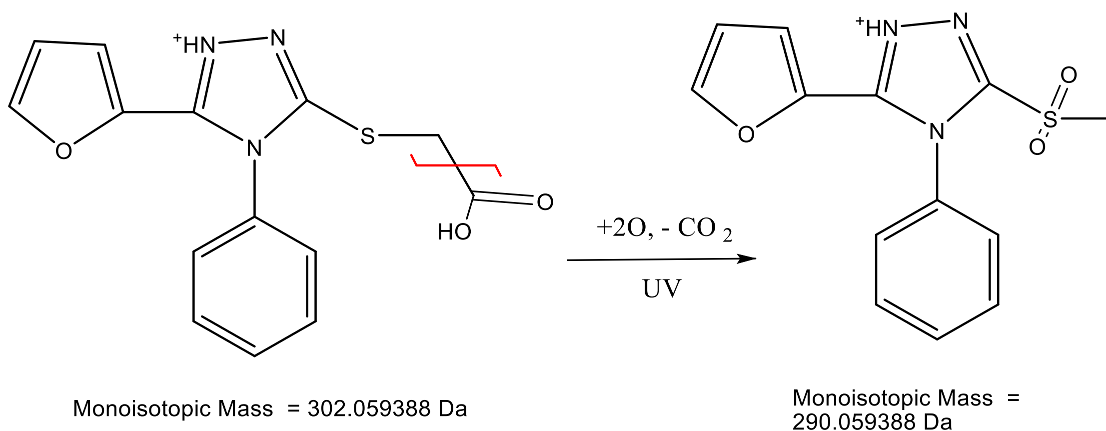

Figure 26. Formation of decarboxilated sulfone.

Anion with m/z = 290.0 was formed in the ion source ([M+H]$^+$), as well as dimer ion with m/z 579.0 ([2M+H]$^+$), which confirms that the ion with m/z 290 is a quasimolecular ion (Fig. 25). The carbon dioxide was eliminated, and the sulfur atom was oxidized under the action of UV light to form the methylsulfone with the monoisotope mass 289.0 (Fig. 26).

Table 2. Impurities were formed in stressful conditions.

#	Compound	3% H_2O_2	UV	Retention time	m/z quasimolecular ion	Monoisotope molecular weight
0	API			2.8	302	301
1	2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol-3-yl)sulfinyl)acetic acid	+*	+	1.2	318	317
2	2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol-3-yl)sulfonyl)acetic acid	+	+	2.1	334	333
4	2-((4-phenyl-4*H*-1,2,4-triazol-3-yl)thio)aceticacid	-**	+	1.3	236	235
5	2-((5-(1-hydroxybutyl)-4-phenyl-4*H*-1,2,4-triazol-3-yl)thio)acetic acid, 2-((5-(4-hydroxybutyl)-4-phenyl-4*H*-1,2,4-triazol-3-yl)thio)acetic acid	-	+	1.6	308	307
6	3-(methylthio)-4-phenyl-4*H*-1,2,4-triazole	-	+	2.5	192	191
7	5-(furan-2-yl)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione	-	+	3.7	244	243
8	3-(furan-2-yl)-5-(methylsulfonyl)-4-phenyl-4*H*-1,2,4-triazole	-	+	4.4	290	289

*Substance was found in degradation products
**Substance was absent in degradation products

Influence of the sodium hydroxide, hydrochloride acid, 3% H_2O_2, temperature, UV radiation on piperidine 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol)-3-yl) acetate substance, 0.1% solution and 1% solution for injections were studied. Dependence of the quantitative content of the piperidinium 2-((5-(furan-2-yl)-4-phenyl-4*H*-1,2,4-triazol)-3-yl) acetate from exposition time was determined. The composition of degradation products formed under the actionof anoxidizer was established (3%H_2O_2). This is sulfoxide and sulfone corresponding to the API. The composition of degradation products which were formed under the influence of UV radiation was proposed.

ACKNOWLEDGEMENTS

We acknowledge complete support of the Zaporozhye State Medical University. We thank rector of Zaporozhye State Medical University Prof. Yu. M. Kolesnyk for his help and support.

There are no conflicts of interest have been declared.

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J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 44(1): 89-98, 2020
Doi: 10.33483/jfpau.620599

ORIGINAL ARTICLE / ÖZGÜN MAKALE

MICROWAVE SYNTHESIS OF NEW 3-(ALKYLTHIO)-5-(THIOPHEN-2-YLMETHYL)-1,2,4-TRIAZOL-4-AMINES

YENİ 3-(ALKİLTİYO)-5-(TİYOFEN-2-İLMETİL)-1,2,4-TRİAZOL-4-AMİNLERİN MİKRODALGA SENTEZİ

Andrii A. SAFONOV*

Department of Toxicological and Inorganic Chemistry, Zaporizhzhya State Medical University, Zaporizhzhya, Ukraine

ABSTRACT

Objective: *The aim of this work is to synthesize 3-(alkylthio)-5-(thiophen-2-ylmethyl)-1,2,4-triazol-4-amines by the Milestone Flexi Wave microwave synthesis system and to prove structure synthesized compounds.*

Material and Method: *The initial compounds 3-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-5-thioles (1-5) were synthesized at the Department of Toxicological and Inorganic Chemistry of the Zaporizhzhya State Medical University (Ukraine). Milestone Flexi Wave microwave synthesis system was used to synthesize 3-(alkylthio)-5-(thiophen-2-ylmethyl)-1,2,4-triazol-4-amines. The elemental analysis of synthesized compounds was established by the universal analyzer Elementar Vario L cube (CHNS). The ^{1}H spectra (at 400 MHz and 100 MHz) were recorded in DMSO-d_6 on a Varian MR-400 spectrometer and analysed with ADVASP™ Analyzer program. The completeness of the reactions and the individuality of the resulting compounds were controlled by the gas chromatograph Agilent 7890B with a 5977B mass spectrometry detector.*

Result and Discussion: *The reaction was carried out in an alcoholic medium by adding a catalytic amount of HCl to 5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiols. Methyl and i-propyl alcohols were used as alcohols. The mixture was heated for 45 minutes at a temperature of 150°C, a pressure 14.4 bar, ΔMW = 200 W.*

The signals of ^{1}H NMR for (4a-b, 6a-j) are consented with the proposed structure.

The elemental analysis (CHNS) was accomplished for synthesized compounds to confirm their basic chemical structures and revealed acceptable agreement with the calculated percentages.

Keywords: *1,2,4-triazole, synthesis, ^{1}H-NMR, gas chromatography, heterocyclic compounds.*

ÖZ

Amaç: *Bu çalışmanın amacı, Milestone Flexi Wave mikrodalga sentez sistemi ile 3-(alkiltiyo)-5-(tiyofen-2-ilmetil)-1,2,4-triazol-4-aminlerin sentezlenmesi ve sentezlenen bileşiklerin yapısının onaylanmasıdır.*

Gereç ve Yöntem: *İlk bileşikler 3-(tiyofen-2-ilmetil)-4H-1,2,4-triazol-5-tiyoller (1-5) Zaporizhzhya State Medical Üniversite Toksikolojik ve İnorganik Kimya Anabilim Dalı'nda sentezlendi (Ukrayna). 3-(alkiltiyo)-5-*

* **Corresponding Author / Sorumlu Yazar:** Andrii A. Safonov
e-mail / e-posta: 8safonov@gmail.com, **Phone / Tel.:** +38 066-177-71-06

Submitted / Gönderilme: 16.09.2019 **Accepted / Kabul:** 18.01.2020

(tiyofen-2-ilmetil)-1,2,4-triazol-4-aminlerin sentezlenmesi için Milestone Flexi Wave mikrodalga sentez sistemi kullanıldı. Sentezlenen bileşiklerin element analizi evrensel analiz Elementar Vario L küpü (CHNS) tarafından yapıldı. ^{1}H spektrumları (400 MHz ve 100 MHz'de), DMSO-d_6'da bir Varian MR-400 spektrometresi üzerinde kaydedildi ve ADVASP ™ Analyzer programı ile analiz edildi. Reaksiyonlar ve elde edilen bileşikler, bir 5977B kütle spektrometre detektörü ile Agilent 7890B gaz kromatografisinde kontrol edildi.

Sonuç ve Tartışma: *Reaksiyon, 5-(tiyofen-2-ilmetil)-4H-1,2,4-triazol-3-tiyollere katalitik miktarda HCI ilave edilerek alkollü bir ortamda gerçekleştirildi. Alkol olarak metil ve i-propil alkoller kullanıldı.*

Karışım, 45 dakika boyunca 150°C sıcaklıkta, 14.4 bar basınçta, ΔMW = 200 W sıcaklıkta ısıtıldı.

Çözücü olarak asetik asit kullanıldı. (4a-b, 6a-j) için önerilen yapı, ^{1}H NMR sinyalleri ile doğrulandı. Temel kimyasal yapılarını doğrulamak için sentezlenen bileşikler üzerinde element analizi (CHNS) yapıldı ve hesaplanan yüzdelerle kabul edilebilir bir uyum sağladığını gösterdi.

Anahtar Kelimeler: *1,2,4-triazol, sentez, ^{1}H-NMR, gaz kromatografisi, heterosiklik bileşikler.*

INTRODUCTION

Heterocyclic compounds have become the most attractive class of organic compounds as a result of the intensive development of science. Studies of the synthetic capabilities of heterocyclic compounds have increased tenfold over the past ten years [1-3]. This tendency has a reasoned explanation due to the many special properties of these substances and the progressive development of organic synthetic chemistry.

1,2,4-Triazoles occupy a worthy place among heterocyclic compounds due to a number of unique properties [4-6]. High reactivity, low toxicity and certainly high biological activity make this class of heterocyclic compounds and its derivatives very attractive for comprehensive study.

The search for biologically active compounds among 1,2,4-triazole derivatives is being carried out by teams of scientists from many countries of the world [7-14]. An interesting fact remains the attempt of many scientists to combine 1,2,4-triazole with various functional substitutes, which in the "complex" may be promising for the detection of new types of pharmacological activity. We have attempted to attach to the 1,2,4-triazole "nuclei" of thiophene, aliphatic and aromatic substituents, each of which is separately a fragment of molecules of biologically active compounds or drugs. Therefore, in our opinion, derivatives of 4-R-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazoles may be interesting and promising in the process of creating new "libraries" of biologically active compounds.

The aim of the work was to synthesize 3-(alkylthio)-5-(thiophen-2-ylmethyl)-1,2,4-triazol-4-amines by the Milestone Flexi Wave microwave synthesis system and to prove structure synthesized compounds.

MATERIAL AND METHOD

Chemicals

The initial compounds 3-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-5-thioles **(1-5)** were synthesized at the Department of Toxicological and Inorganic Chemistry of the Zaporizhzhya State

Medical University (Ukraine) and purified by recrystallization with content of the main component ≥ 98% [15]. The chloride acid (35%), 1-propanol (anhydrous, 99,7%) and methanol (99,5%) were obtained from SIGMA-ALDRICH (Germany).

Equipment

To achieve the purpose, the following devices were used. Milestone Flexi Wave microwave synthesis system (Milestone Srl, Italy) (technical specifications: rotor SK-15, minimum volume - 10 ml, maximum volume - 100 ml, maximum temperature - 300 ° C, maximum working pressure - 100 bar, maximum shutter speed 220 °C - 30 min).

The melting point is defined by the open capillary method on the OptiMelt MPA100 device with platinum RTD sensor and temperature measurements to 400°C with 0.1°C resolution (US production).

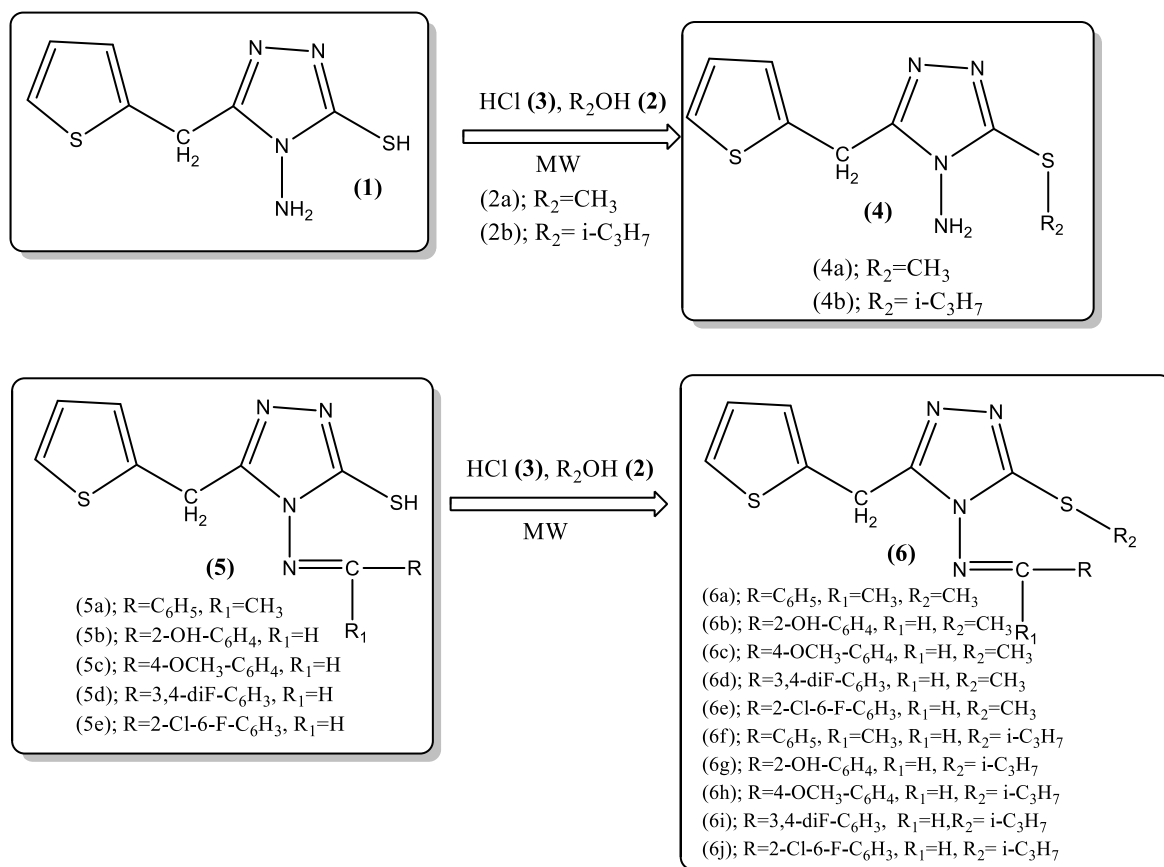
The elemental analysis of synthesized compounds was established by the universal analyzer Elementar Vario L cube (CHNS) (standard - sulfanilamide) (Analysensysteme GmbH, Germany).

The ^{1}H spectra (at 400 MHz and 100 MHz) were recorded in DMSO-$_{d6}$ on a Varian MR-400 spectrometer and analysed with ADVASP™ Analyzer program (Umatek International Inc.); chemical shifts are reported in ppm (δ scale) down field with residual protons of the solvent (DMSO-d_6 , δ = 2.49 ppm) as internal standard.

The completeness of the reactions and the individuality of the resulting compounds were controlled by the gas chromatograph Agilent 7890B with a 5977B mass spectrometry detector (US production). The column is DB-5ms 30 m x 250 μm x 0.25 μm with length. The gas-carrier speed (helium) is 1.6 ml / min. Injection volume - 0.5 μl. Separation of the flow is 1:50. The temperature of the sampling unit is 230 ° C → 12 ° C / s → 275 ° C. Thermostat temperature: programmable, 240 ° C (1 minute delay) → 5 ° C / min → 280 ° C. (delay 1 min.). The total time of examination is 10 min. Temperature of interface GS/MS - 280 ºC; ion sources - 230 ºC; quadrupole mass analyzer - 150 ºC. Type of ionization: EI with an electron energy of 70 eV. The range of mass numbers that was scanned: 30-500 m /z.

RESULT AND DISCUSSION

As starting materials were used 4-R-5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazole-3-thiols (**1, 5**) which were synthesized and described by us earlier [15]. The reaction was carried out in an alcoholic medium by adding a catalytic amount of HCl to 4-R-5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazole-3-thiols. Methyl and i-propyl alcohols were used as alcohols.



Scheme 1: Synthesis of 3-(methylthio)-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-4-amines (4a-4b) and N-(2-methoxybenzylidene)-3-(methylthio)-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-4-amines (6a-6j)

To achieve better results, a change in temperature and reaction time was used. The reaction was carried out for 60 minutes (the temperature of the reaction mixture was 110° C), the second series of 50 minutes (temperature of the reaction mixture of 130° C), the third series of 45 minutes (temperature of the reaction mixture 150 ° C). The most technologically optimal method was chosen whereby quantitative outputs were highest.

The mixture was heated for 45 minutes at a temperature of 150 ° C, a pressure 14.4 bar, ΔMW = 200 W (Figure 1).

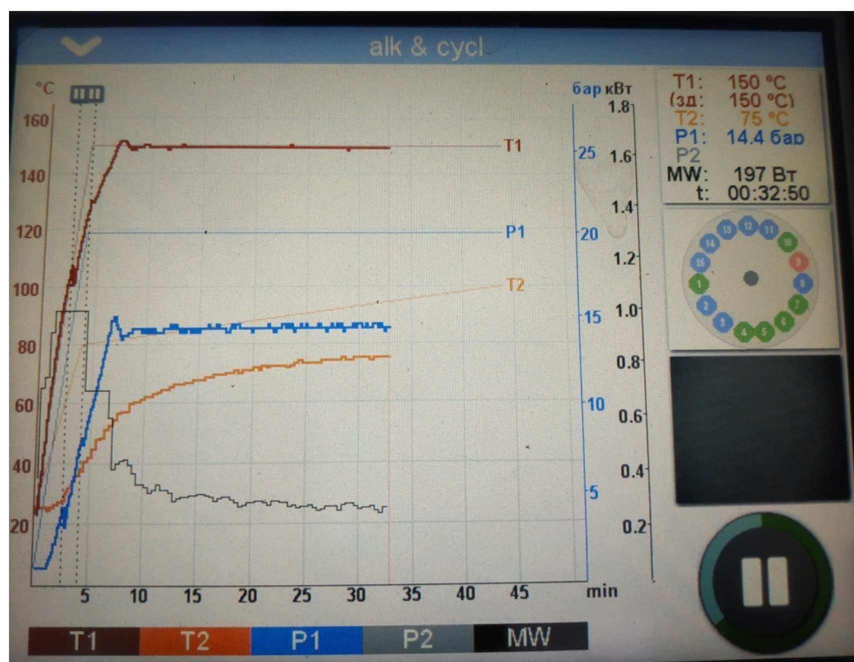


Figure 1. Microwave synthesis of N-R-3-(alkylthio)-5-(thiophen-2-ylmethyl)-1,2,4-triazol-4-amines

The completeness of the reaction was determined using a gas chromatograph Agilent 7890B with a mass spectrometric detector 5977B.

Analyzing the GS/MS chromatogram in the MS spectrum there is a molecular peak with a value of 226.0 (m/z), which corresponds to the calculated theoretical value of 3-(methylthio)-5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazol-4-amine (4a) (Figure 2)

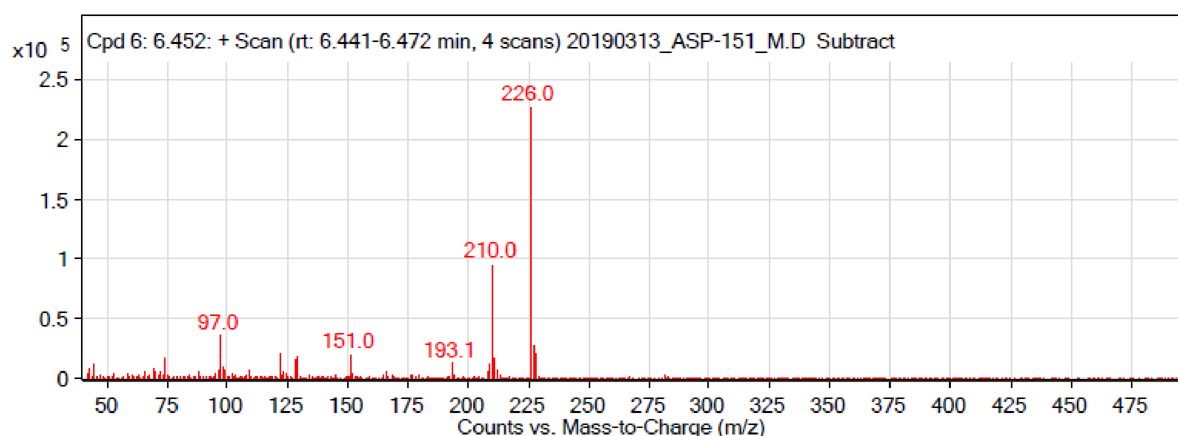


Figure 2. Mass spectrum of 3-(methylthio)-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-4-amine (4a)

In the MS spectrum (Figure 3) there is a molecular peak with a value of 330.1 (m/z), which corresponds to the calculated theoretical value of 2-(((3-(methylthio)-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-4-yl)imino)methyl)phenol (6b).

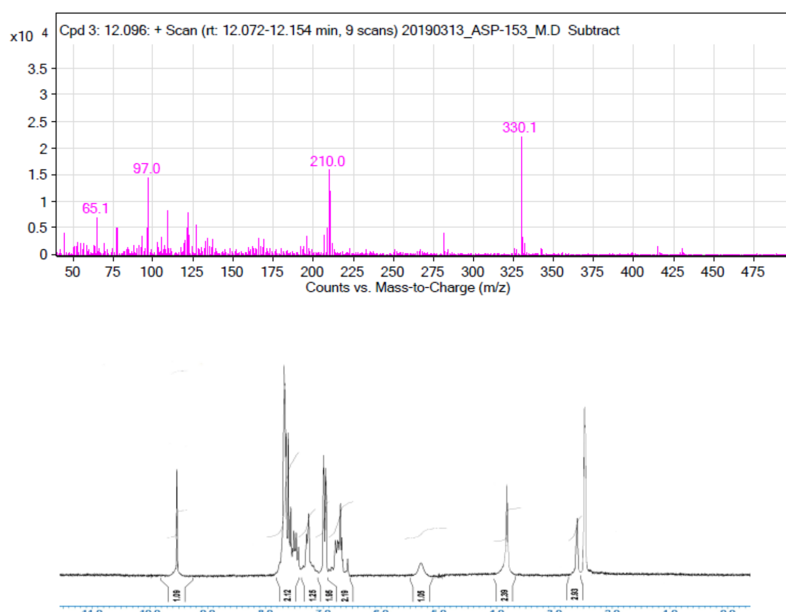


Figure 3. Mass spectrum (left) and ^{1}HNMR spectrum (right) of 2-(((3-(methylthio)-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-4-yl)imino)methyl)phenol (6b)

3-(methylthio)-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-4-amine (4a)

Bright brown powder; yield 89.9%; m.p. 132-134^{0}C ; ^{1}HNMR (400 MHz, DMSO-d6, δ=ppm): 7.32 (1H, d, thiophen-H); 6.86 (1H, t, thiophen-H); 6.68 (1H, d, thiophen-H); 5,82 (2H, S, NH_2); 3.79 (2H, s, CH_2); 2.51 (3H, s, CH_3); CHNS elemental analysis Calcd. for ($C_8H_{10}N_4S_2$) : found C% 43.60, H% 4.41, N% 24.68, S% 28.36; calculated C% 43.46, H% 4.45, N% 24.76, S% 28.34. MS (EI) *m/z* (rel. intensity): 226 (M^+, 100).

3-(isopropylthio)-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-4-amine (4b)

Bright yellow powder; yield 87.8%; m.p. 130-132^{0}C ; ^{1}HNMR (400 MHz, DMSO-d6, δ=ppm): 7.38 (1H, d, thiophen-H); 6.81 (1H, t, thiophen-H); 6.69 (1H, d, thiophen-H); 5,80 (2H, S, NH_2); 3.82 (2H, s, CH_2); 2.90 (1H, m, CH); 1.23 (6H, d, $2CH_3$); CHNS elemental analysis Calcd. for ($C_{10}H_{14}N_4S_2$) : found C% 47.40, H% 5.54, N% 22.07, S% 25.24; calculated C% 47.22, H% 5.55, N% 22.03, S% 25.21. MS (EI) *m/z* (rel. intensity): 254 (M^+, 100).

3-(methylthio)-N-(1-phenylethylidene)-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-4-amine (6a)

Yellow powder; yield 86.7%; m.p. 118-120^{0}C ; ^{1}HNMR (400 MHz, DMSO-d6, δ=ppm): 7.98(2H, d, Ar-H); 7.61(3H, m, Ar-H); 7.42 (1H, d, thiophen-H); 6.76 (1H, t, thiophen-H); 6.68 (1H, d, thiophen-H); 3.80 (2H, s, CH_2); 2.51 (3H, s, CH_3); 1.64 (3H, s, CH_3); CHNS elemental analysis Calcd. for ($C_{16}H_{16}N_4S_2$) : found C% 58.49, H% 4.93, N% 17.10, S% 19.48; calculated C% 58.51, H% 4.91, N% 17.06, S% 19.52. MS (EI) *m/z* (rel. intensity): 328 (M^+, 100).

2-(((3-(methylthio)-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-4-yl)imino)methyl)phenol (6b)

Bright orange powder; yield 86.5%; m.p. 200-202^{0}C ; ^{1}HNMR (400 MHz, DMSO-d6, δ=ppm): 9.56 (1H, s, CH); 7.64 (1H, d, Ar-H); 7.49 (1H, t, Ar-H); 7.38 (1H, d, thiophen-H); 7.02 (2H, d, Ar-H); 6.76 (1H, t, thiophen-H); 6.68 (1H, d, thiophen-H); 5.31(1H, s, OH); 3.82 (2H, s, CH_2); 2.54 (3H, s, CH_3); CHNS elemental analysis Calcd. for ($C_{15}H_{14}N_4OS_2$) : found C% 54.41, H% 4.29, N% 16.99, S% 19.42; calculated C% 54.52, H% 4.27, N% 16.96, S% 19.41. MS (EI) *m/z* (rel. intensity): 330 (M^+, 100).

N-(4-methoxybenzylidene)-3-(methylthio)-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-4-amine (6c)

Yellow powder; yield 89.1%; m.p. 180-182^{0}C ; ^{1}HNMR (400 MHz, DMSO-d6, δ=ppm): 9.96 (1H, s, CH); 7.86 (2H, d, Ar-H); 7.39 (1H, d, thiophen-H); 7.08 (2H, d, Ar-H); 6.79 (1H, t, thiophen-H); 6.65 (1H, d, thiophen-H); 3.96 (3H, s, CH_3); 3.81 (2H, s, CH_2); 2.55 (3H, s, CH_3); CHNS elemental analysis Calcd. for ($C_{16}H_{16}N_4OS_2$) : found C% 55.86, H% 4.70, N% 16.29, S% 18.58; calculated C% 55.79, H% 4.68, N% 16.27, S% 18.62. MS (EI) *m/z* (rel. intensity): 344 (M^+, 100).

N-(3,4-difluorobenzylidene)-3-(methylthio)-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-4-amine (6d)

Bright yellow powder; yield 90.4%; m.p. 168-170^{0}C ; ^{1}HNMR (400 MHz, DMSO-d6, δ=ppm): 9.98 (1H, s, CH); 7.82 (1H, m, Ar-H); 7.54 (1H, m, Ar-H); 7.39 (1H, d, thiophen-H); 7.30 (1H, m, Ar-H); 6.79 (1H, t, thiophen-H); 6.64 (1H, d, thiophen-H); 3.80 (2H, s, CH_2); 2.51 (3H, s, CH_3); CHNS elemental analysis Calcd. for ($C_{15}H_{12}F_2N_4S_2$) : found C% 51.23, H% 3.44, N% 15.96, S% 18.32; calculated C% 51.41, H% 3.45, N% 15.99, S% 18.30. MS (EI) *m/z* (rel. intensity): 350 (M^+, 100).

N-(2-chloro-6-fluorobenzylidene)-3-(methylthio)-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-4-amine (6e)

Brown powder; yield 86.7%; m.p. 149-151^{0}C ; ^{1}HNMR (400 MHz, DMSO-d6, δ=ppm): 9.54 (1H, s, CH); 7.48 (1H, m, Ar-H); 7.39 (1H, d, thiophen-H); 7.24 (2H, m, Ar-H); 6.76 (1H, t, thiophen-H); 6.64 (1H, d, thiophen-H); 3.78 (2H, s, CH_2); 2.57 (3H, s, CH_3); CHNS elemental analysis Calcd. for ($C_{15}H_{12}ClFN_4S_2$) : found C% 49.02, H% 3.32, N% 15.28, S% 17.44; calculated C% 49.11, H% 3.30, N% 15.27, S% 17.48. MS (EI) *m/z* (rel. intensity): 366 (M^+, 100).

3-(isopropylthio)-N-(1-phenylethylidene)-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-4-amine (6f)

Bright yellow powder; yield 91.3%; m.p. 126-128^{0}C ; ^{1}HNMR (400 MHz, DMSO-d6, δ=ppm): 7.91(2H, d, Ar-H); 7.56(3H, m, Ar-H); 7.40 (1H, d, thiophen-H); 6.78 (1H, t, thiophen-H); 6.64 (1H, d, thiophen-H); 3.80 (2H, s, CH_2); 2.89 (1H, m, CH); 1.84 (3H, s, CH_3); 1.24 (6H, d, CH_3); CHNS elemental analysis Calcd. for ($C_{18}H_{20}N_4S_2$) : found C% 49.02, H% 3.32, N% 15.28, S% 17.44; calculated C% 49.11, H% 3.30, N% 15.27, S% 17.48. MS (EI) *m/z* (rel. intensity): 356 (M^+, 100).

2-(((3-(isopropylthio)-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-4-yl)imino)methyl)phenol (6g)

Bright brown powder; yield 88.9%; m.p. 154-156^{0}C ; ^{1}HNMR (400 MHz, DMSO-d6, δ=ppm): 9.54 (1H, s, CH); 7.61 (1H, d, Ar-H); 7.50 (1H, t, Ar-H); 7.39 (1H, d, thiophen-H); 7.04 (2H, d, Ar-H);

6.74 (1H, t, thiophen-H); 6.63 (1H, d, thiophen-H); 5.30(1H, s, OH); 3.84 (2H, s, CH_2); 2.88 (1H, m, CH); 1.24 (6H, d, CH_3); CHNS elemental analysis Calcd. for ($C_{17}H_{18}N_4OS_2$) : found C% 56.91, H% 5.07, N% 15.65, S% 17.92; calculated C% 56.96, H% 5.06, N% 15.63, S% 17.89. MS (EI) *m/z* (rel. intensity): 358 (M^+, 100).

3-(isopropylthio)-N-(4-methoxybenzylidene)-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-4-amine (6h)

Yellow powder; yield 89.9%; m.p. 183-185^{0}C ; ^{1}HNMR (400 MHz, DMSO-d6, δ=ppm): 9.97 (1H, s, CH); 7.85 (2H, d, Ar-H); 7.39 (1H, d, thiophen-H); 7.04 (2H, d, Ar-H); 6.78 (1H, t, thiophen-H); 6.69 (1H, d, thiophen-H); 3.84 (3H, s, CH_3); 3.81 (2H, s, CH_2); 2.88 (1H, m, CH); 1.24 (6H, d, CH_3); CHNS elemental analysis Calcd. for ($C_{18}H_{20}N_4OS_2$) : found C% 57.14, H% 5.44, N% 15.08, S% 17.24; calculated C% 57.08, H% 5.41, N% 15.04, S% 17.22. MS (EI) *m/z* (rel. intensity): 358 (M^+, 100).

N-(3,4-difluorobenzylidene)-3-(isopropylthio)-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-4-amine (6i)

Bright yellow powder; yield 93.5%; m.p. 205-207^{0}C ; ^{1}HNMR (400 MHz, DMSO-d6, δ=ppm): 10.01 (1H, s, CH); 7.81 (1H, m, Ar-H); 7.50 (1H, m, Ar-H); 7.36 (1H, d, thiophen-H); 7.30 (1H, m, Ar-H); 6.74 (1H, t, thiophen-H); 6.63 (1H, d, thiophen-H); 3.82 (2H, s, CH_2); 2.89 (1H, m, CH); 1.24 (6H, d, CH_3); CHNS elemental analysis Calcd. for ($C_{17}H_{16}F_2N_4S_2$) : found C% 53.86, H% 4.27, N% 14.81, S% 16.95; calculated C% 53.95, H% 4.26, N% 14.80, S% 16.94. MS (EI) *m/z* (rel. intensity): 378 (M^+, 100).

N-(2-chloro-6-fluorobenzylidene)-3-(isopropylthio)-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-4-amine (6j)

Orange powder; yield 91.2%; m.p. 217-219^{0}C ; ^{1}HNMR (400 MHz, DMSO-d6, δ=ppm): 9.55 (1H, s, CH); 7.49 (1H, m, Ar-H); 7.36 (1H, d, thiophen-H); 7.21 (2H, m, Ar-H); 6.75 (1H, t, thiophen-H); 6.64 (1H, d, thiophen-H); 3.79 (2H, s, CH_2); 2.88 (1H, m, CH); 1.22 (6H, d, CH_3); CHNS elemental analysis Calcd. for ($C_{17}H_{16}ClFN_4S_2$) : found C% 51.61, H% 4.09, N% 14.20, S% 16.27; calculated C% 51.70, H% 4.08, N% 14.19, S% 16.24. MS (EI) *m/z* (rel. intensity): 394 (M^+, 100).

In conclusion, the novel 3-(methylthio)-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-4-amines (4a-4b) and N-(2-methoxybenzylidene)-3-(methylthio)-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-4-amines (6a-6j) were synthesized and characterized. The structure of synthesized compounds is confirmed using Elemental analysis (CHNS), ^{1}HNMR and Chromatographic mass spectral analysis.

ACKNOWLEDGEMENT

We're grateful to the Zaporizhzhia State Medical University for providing some facilities in carrying out the research.

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Ankara Ecz. Fak. Derg. / J. Fac. Pharm. Ankara, 44(1): 99-111, 2020
Doi: 10.33483/jfpau.561095

DERLEME MAKALE / REVIEW ARTICLE

AMELANCHIER MEDIK. (ROSACEAE) CİNSİNİN FARMASÖTİK BOTANİK AÇISINDAN ÖNEMİ

THE IMPORTANCE OF THE GENUS AMELANCHIER MEDIK. (ROSACEAE) IN TERM OF PHARMACEUTICAL BOTANY

M. Mesud HÜRKUL[1,*], Ayşegül KÖROĞLU[1,2]

[1]Ankara Üniversitesi, Eczacılık Fakültesi, Farmasötik Botanik Anabilim Dalı, 06100, Tandoğan, Ankara, Türkiye

[2]Afyonkarahisar Sağlık Bilimleri Üniversitesi, Eczacılık Fakültesi, Zafer Sağlık Külliyesi, 03200, Afyonkarahisar, Türkiye

ÖZ

Amaç: *Bu derleme çalışması, Rosaceae familyasına ait Amelanchier cinsine ait bitkiler üzerinde dünya genelinde yapılan etnobotanik, fitokimyasal ve biyolojik aktivite çalışmalarının derlenmesini kapsamaktadır. Türkiye'de de 4 takson ile temsil edilen bu cins üzerinde daha sonra yapılacak çalışmalar için katkı sağlanması hedeflenmiştir.*

Gereç ve Yöntem: *1998 ve 2018 yılları arasında periyodik olarak yayınlanan basılı ve elektronik dergiler taranmıştır.*

Sonuç ve Tartışma: *Etnobotanik veriler Amelanchier cinsinin dünya genelinde yaygın kullanımının olmadığını göstermiştir. Amelanchier cinsi üzerinde yapılan fitokimyasal ve in vitro biyoaktivite çalışmaları, A. canadensis, A. arborea, A. ovalis ve A. alnifolia'nın meyveleri üzerinde yoğunlaşmıştır. Meyvelerin flavonoid, karotenoid ve siyanogenetik glikozit yapısında bileşikleri taşıdığı tespit edilmiştir. Meyvelerinin yüksek fenolik madde içeriği nedeniyle biyolojik aktivite çalışmalarının antioksidan etki üzerinde yoğunlaştığı görülmüştür.*

Anahtar Kelimeler: *Amelanchier, Rosaceae, Farmasötik Botanik, Derleme*

ABSTRACT

Objective: *This review includes the compilation of ethnobotany, phytochemical and biological activity studies on the plants of the genus Amelanchier of the Rosaceae family. This genus is represented by four taxa in Turkey, this study is purposed to contribute to further studies.*

Material and Method: *1998-2018 periodically published academic journals of international indexes (PubMed) were scanned.*

* **Sorumlu Yazar / Corresponding Author:** M. Mesud HÜRKUL
***e-posta / e-mail*:** huerkulmm@gmail.com

Gönderilme / Submitted: 06.05.2019 **Kabul / Accepted:** 10.09.2019

Result and Discussion: *Ethnobotanical data showed that the genus Amelanchier was not widely used worldwide. Phytochemical and in vitro bioactivity studies were concentrated on the fruits of A. canadensis, A. arborea, A. ovalis and A. alnifolia. The fruits contain flavonoid, carotenoid and cyanogenic glycoside. Fruits contain phenolic substance, so studies of biological activity are concentrated on antioxidant effect.*
Keywords: *Amelanchier, Rosaceae, Pharmaceutical Botany, Review*

GİRİŞ

Odunsu ağaçlar, çalılar, tırmanıcı ve otsu bitkileri içeren Rosaceae Jussieu familyasının dünyanın her yerine yayılan üyeleri, en fazla gelişmeyi kuzey yarım kürenin ılıman bölgelerinden subtropikal bölgeye kadar olan kısımda gösterir. Dünya genelinde 100 cins ve 2000 civarında türü barındıran familya [1,2], Türkiye'de 37 cins ve 297 türe sahiptir, ayrıca 58 endemik tür ile % 24'lük endemizm oranına ulaşır, bununla birlikte odunlu türler bakımından 218 takson ile ülkemizdeki en zengin familyadır [3].

Amelanchier Medik. yaprak döken, dallanmayan çalılar ya da küçük ağaçlardan oluşur. Yapraklar basit, saplı. Stipula ipliksi, düşücü. Çiçekler beyaz, uçlarda salkım durumunda, nadiren tek başına. Hipantiyum çan şeklinde ve meyve döneminde kalıcı 5 tane üçgen sepal taşır. Petaller dar, obovattan oblanseolata kadar farklı şekillerde. Stamen sayısı 10-20. Ovaryum kısmen alt durumlu; 5 karpelli, birleşik ya da kısmen birleşmiş, meyve döneminde kıkırdaksı bir çeper oluşturur. Stilus 2-5. Meyve mavimsi ya da siyahımsı, 4-10 gözlü, üzümsü, sulu ve tatlı, küçük bir pomdur [4].

Amelanchier cinsinde hibritleşme yaygın olarak görülmektedir. Avrupa ve Kuzey Amerika'da insan etkisiyle *Amelanchier* cinsinde kapsamlı hibritleşme ve ardından morfolojik karmaşıklığın geliştiğini düşündürmektedir. Hibritleşme, bitki yetiştiriciliğinde, arzu edilen özellikleri ekili ve hatta yabani türden başka bir ekili türe kaydırmak için çok önemlidir. Volkanizma ve erozyondan kaynaklanan doğal etkiler de hibritleşmeyi desteklemiştir [5].

Cinse ait dünya genelinde geçerliliği kabul edilmiş 37 takson kayıtlıdır (Tablo 1) [6].

Tablo 1. *Amelanchier* cinsine ait dünya genelinde geçerliliği kabul edilmiş taksonlar

1.	*Amelanchier alnifolia* (Nutt.) Nutt. ex M.Roem.
2.	*A. alnifolia* var. *humptulipensis* (G.N.Jones) C.L.Hitchc.
3.	*A. alnifolia* var. *semi-integrifolia* (Hook.) C.L.Hitchc.
4.	*A. arborea* (F. Michx.) Fernald
5.	*A. arborea* var. *alabamensis* (Britton) G.N.Jones
6.	*A. arborea* var. *austromontana* (Ashe) H.E.Ahles
7.	*A. arborea* f. *nuda* (E.J. Palmer & Steyerm.) Rehder

8.	*A. asiatica* (Siebold & Zucc.) Endl. ex Walp.
9.	*A. australis* Standl.
10.	*A. bakeri* Greene
11.	*A. bartramiana* (Tausch) M.Roem.
12.	*A. canadensis* (L.) Medik.
13.	*A. covillei* Standl.
14.	*A. cretica* (Willd.) DC.
15.	*A. cusickii* Fernald
16.	*Amelanchier* × *grandiflora* Rehder
17.	*A. interior* E.L.Nielsen
18.	*Amelanchier* × *intermedia* Spach
19.	*A. laevis* Wiegand
20.	*A. lamarckii* F.G.Schroed.
21.	*Amelanchier* × *neglecta* Eggl. ex G.N.Jones
22.	*A. obovalis* (Michx.) Ashe
23.	*A. ovalis* Medik.
24.	*A. pallida* Greene
25.	*A. parviflora* Boiss.
26.	*A. pumila* (Nutt. ex Torr. & A.Gray) M.Roem.
27.	*Amelanchier* × *quinti-martii* Louis-Marie
28.	*A. sanguinea* (Pursh) DC.
29.	*A. sanguinea* var. *gaspensis* Wiegand
30.	*A. sanguinea* var. *grandiflora* (Wiegand) Rehder
31.	*A. sinica* (C.K. Schneid.) Chun
32.	*A. spicata* (Lam.) K.Koch
33.	*A. stolonifera* Wiegand

34.	*A. stolonifera* f. *micropetala* (B.L. Rob.) Rehder
35.	*A. turkestanica* Litv.
36.	*A. utahensis* Koehne
37.	*A. utahensis* var. *covillei* (Standl.) N.H.Holmgren

Cins, Türkiye Florası'nda *Amelanchier rotundifolia* (Lam.) Dum.-Courset subsp. *rotundifolia*, *A. rotundifolia* (Lam.) Dum.-Courset subsp. *integrifolia* (Boiss. & Hohen.) Browicz, *A. parviflora* Boiss. var. *parviflora*, *A. parviflora* Boiss. var. *dentata* Browicz olmak üzere dört taksonla temsil edilir (Tablo 2) [4]. *A. rotundifolia* subsp. *integrifolia*, *A. ovalis* Medik. subsp. *integrifolia* (Boiss. & Hohen.) Bornm.'nın, *A. rotundifolia* subsp. *rotundifolia* ise *A. ovalis* Medik. subsp. *ovalis*'in sinonimi olarak kabul edilmektedir [7].

Tablo 2. *Amelanchier* cinsine ait Türkiye Florası'nda kayıtlı taksonlar [4].

1.	*Amelanchier rotundifolia* (Lam.) Dum.-Courset subsp. *rotundifolia*
2.	*A. rotundifolia* (Lam.) Dum.-Courset subsp. *integrifolia* (Boiss. & Hohen.) Browicz
3.	*A. parviflora* Boiss. var. *parviflora*
4.	*A. parviflora* Boiss. var. *dentata* Browicz

Amelanchier türleri yüksek don direnci ve meyvelerinin çekici olması açısından dekoratif olarak Kanada ve İskandinav ülkelerinde yaygın olarak bahçe süslemesinde kullanılmaktadır [8]. *Amelanchier wiegandii* E.L. Nielsen ve *Amelanchier* x *spicata* (Lam.) K. Koch ülkemizde peyzaj düzenlemelerinde kullanılan türlerdir [9].

Amelanchier cinsi hibritleşmeye yatkın olması nedeniyle sistematik olarak sorunludur. Ayrıca üyelerinin dünya genelinde yaygın bir kullanımı olmamasına karşın bazı türlerinin yoğun olarak geleneksel tedavide kullanılması, hem sistematik hem de eczacılık açısından ilginçtir. Türkiye'de dört takson ile temsil edilen bu cinsin, üyeleri üzerinde yapılacak diğer çalışmalara bir temel oluşturabilmesi amacıyla bu cins üzerinde yapılan kimyasal ve biyolojik etki çalışmalarının derlenmesi amaçlanmıştır.

GEREÇ VE YÖNTEM

Bu çalışmada Rosaceae familyasının *Amelanchier* cinsinde yer alan türlerinin dünya genelinde ve ülkemizde halk arasında geleneksel kullanımı, fitokimyası ve biyolojik aktiviteleri üzerinde yapılan

çalışmalar derlenmiştir. Bu amaç çerçevesinde cins, periyodik olarak yayınlanan basılı ve elektronik dergiler, Ankara Üniversitesi e-kütüphanesi, Google Books ve Google akademik veri tabanları ile uluslararası indeksler (PubMed) aracılığıyla, 1998 ve 2018 yılları arasında Türkçe, İngilizce ve Almanca dillerinde taranmıştır.

SONUÇ VE TARTIŞMA

Botanik Çalışmalar

Türkiye'de yetişen *Amelanchier* taksonları (*Amelanchier ovalis* Medik. subsp. *ovalis*, *A. ovalis* Medik. subsp. *integrifolia* (Boiss. & Hohen.) Bornm., *A. parviflora* Boiss. var. *parviflora*, *A. parviflora* Boiss. var. *dentata* Browicz) üzerinde yapılan odun anatomisi çalışmasında, trakelerde az belirgin spiral kalınlaşma ve enine kesitlerinde köşeli yapı gözlenmiştir. Ayrıca enine kesitte öz kollarının varlığı tespit edilmiştir. Bunun yanında incelenen tüm örneklerde parankima hücrelerinde kristallere rastlanmıştır, fakat *A. ovalis* subsp. *ovalis* taksonunda, kristallerin diğer türlere nazaran çok daha yoğun olduğu belirtilmiştir [10].

Geleneksel Kullanımı

Amerika'nın kuzeyinde *Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem. kabukları müshil, yumuşatıcı, ateş düşürücü, gebeliği önleyici, tonik olarak; dekoksiyon şeklinde mide rahatsızlıklarında; dalların dekoksiyonu soğuk algınlığı; meyvelerinin dekoksiyonu kulak ve mide rahatsızlıklarında [11], meyvelerinin infüzyonu mide ağrısı ve karaciğer rahatsızlıklarında; olgun meyvelerinin suyu göz kızarıklıkları ve göze kaçan yabancı maddeleri temizlemek için kullanılır, ayrıca besleyici bir Kuzey Amerika yemeği olan "Pemmican" yapımında *A. alnifolia*'nın meyveleri kurutulmuş et ve yağ ile karıştırılarak kullanılır, yine bu meyvelerden çorba da hazırlanır [12]; bitkinin kökleri dekoksiyon şeklinde öksürük, göğüs ağrıları ve akciğer enfeksiyonu tedavisinde, ayrıca dini rütüellerde ve büyü yapmak için, *A. alnifolia* (Nutt.) Nutt. ex M. Roem. var. *semiintegrifolia* (Hook.) C. L. Hitchc.'nın kabukları dekoksiyon şeklinde belsoğukluğu tedavisinde kullanılır [11]. *A. arborea* (F. Michx.) Fernald ve *A. arborea* (F. Michx.) Fernald var. *arborea* kabukları tonik, antidiyareyik, antihelmintik olarak ayrıca belsoğukluğu tedavisi ve doğum sonrası kadın hastalıklarında; *A. canadensis* (L.) Medik. kabukları antihelmintik, astrenjan, dezenfektan olarak; kökleri dekoksiyon şeklinde dizanteri, kadın hastalıkları tedavisinde ve düşük önleyici olarak, Georgia Eyaleti'nde *A. canadensis*'in meyveleri gıda olarak kullanılmaktadır [13]; *A. humilis* Wiegand kökleri tonik olarak; *A. laevis* Wiegand kabuklarının infüzyonu hamile kadınlar için; *A. pallida* Greene kökleri dekoksiyon şeklinde kadın hastalıkları ve dini ritüellerde; *A. stolonifera* Wiegand kök kabukları tonik olarak; *A. utahensis* Koehne var. *utahensis* yaprakları emetik olarak ayrıca bitki doğum ve doğum sonrası kadın hastalıklarının tedavisinde, dini ritüellerde ve büyü yapmada halk arasında geleneksel olarak kullanılmaktadır [11].

Kanada'da yerel halk *A. alnifolia* meyve suyunu laksatif olarak, ayrıca mide, göz ve kulak rahatsızlıklarında, köklerinin infüzyonunu düşük önlemede, dalları ve gövdesi infüzyon şeklinde doğum sonrasında kadınların banyo suyuna katarak tonik olarak kullanmaktadır. Yine bu bitkinin meyveleri mor renkli boya elde etmek amacıyla da kullanılır [14].

İspanya (Pireneler, Katalonya, İber yarımadası)'da *A. ovalis*'in herba ve çiçekleri infüzyon şeklinde, dahilen körlük tedavisinde [15], sadece herbası ise aynı şekilde nezle tedavisinde [16] geleneksel olarak kullanılmaktadır.

İtalya (Kuzeybatı Alpler)'da *A. ovalis* meyveleri gıda olarak tüketilmektedir [17].

Türkiye'de, *Amelanchier parviflora* Boiss. var. *dentata* Browicz'ın dalları dekoksiyon şeklinde Acıpayam-Denizli'de diüretik olarak kullanılmaktadır [18].

Fitokimyasal Çalışmalar

Amelanchier canadensis meyvelerinin etil asetat ekstresinin kloroform fraksiyonundan 1,3-dilinoleoyl 2-olein ve 1,3-dioleoyl 2-linolein; metanol ekstresinden 5-hidroksimetil-2-furfural, 5-(sorbitoloksimetil)-furan-2-karboksaldehit, 5-(mannitoloksimetil)-furan-2-karboksaldehit, ve 5-(*α*-D-glukopiranosiloksimetil) furan-2- karboksaldehit izole edilmiştir. Ayrıca *A. arborea* (F. Michx.) Fernald meyvelerinin etilasetat ekstresinden oleanolik asit, ursolik asit, kemferol-3-*O*-α-L-ramnopiranosil (1→2) ramnopiranosit ve kemferol-3-*O*-α-L-ramnopiranosit izole edilip yapısı aydınlatılmıştır [19].

A. alnifolia'nın meyveleri ile yapılan YPSK analizinde siyanidin-3-galaktozit, siyanidin-3-glukozit, siyanidin-3-arabinozit, siyanidin-3-ksilozit, kersetin 3-*O*-arabinoglukozit, kersetin 3-*O*-rutinozit, kersetin 3-*O*-galaktozit, kersetin 3-*O*-glukozit, kersetin 3-*O*-ksilozit, kersetin 3-*O*-arabinozit, kersetin 3-*O*-robinobiyozit, 5-*O*-kafeoyilkinik asit ve 3-*O*- kafeoyilkinik asit tespit edilmiştir [20].

A. alnifolia'nın 17 farklı varyetesinin (Success, Lee 3, Martin, Parkhill, Forestburg, Lee 8, Lee 2, Pembina, Honeywood, Northline, Thiessen, Pasture, Nelson, Pearson, Quaker, Smokey ve Regent) meyveleri üzerinde yapılan HPLC-ESI-MS/MS analizinde antosiyaninlerden siyanidin-3-galaktozit, siyanidin-3-glukozit, siyanidin-3-arabinozit ve siyanidin-3-ksilozit; hidroksisinnamik asitlerden 5-*O*-kafeoyilkinik asit, 3-*O*- kafeoyilkinik asit, dikafeoyilkinik asit; flavonollerden ise kersetin-3-galaktozit, kersetin-3- glukozit, kersetin-3-visianozit, kersetin-3-robinobiyozit, kersetin-3-arabinozit ve kersetin-3-ksilozit tespit edilmiştir [21].

A. alnifolia'nın 6 farklı (Honeywood, Northline, Smoky AB, Smoky MB, Martin, Thiessen) kültürünün meyveleri üzerinde karotenoid (lutein, zeaksantin, *β*-karoten) ve siyanogenetik glukozit (amigdalin, prunasin) seviyeleri, meyvelerin olgunlaşma evrelerine (yeşil, kırmızı, kırmızı-pembe, pembe) göre YPSK analizi ile çalışılmıştır. Buna göre lutein miktarı Smoky AB-yeşil'de (14.63 mg/kg FW), zeaksantin Honeywood-yeşil'de (0.77 mg/kg FW), *β*-karoten Smoky AB-yeşil'de (3.03 mg/kg

FW), amigdalin Smoky MB-pembe'de (129.2 mg/kg FW) ve prunasin Smoky AB-yeşil'de (30.39 mg/kg FW) en yüksek miktarda tespit edilmiştir [22].

A. alnifolia'nın meyvelerinden elde edilen etanol ekstresinin YPSK analizinde siyanidin-3-galaktozit, siyanidin-3-glukozit, siyanidin-3-arabinozit ve siyanidin-3-ksilozit tespit edilmiştir [23,24].

A. canadensis taze meyvelerinin suyu ile yapılan YPSK analizinde polifenolik yapıda kafeik, klorojenik, kumarik, ferulik asitler ile hiperozit, izokersitrin, kersetin, kersitrin, rutin, elajik asit, gallik asit, kateşin, epikateşin, kastalagin ve veskalagin maddeleri tespit edilmiştir [25].

Amelanchier alnifolia meyve, yaprak ve dallarından hazırlanan etanol:su:asetik asit (70:30:1) ekstrelerinin total fenol içeriği incelenmiştir. *A. alnifolia* meyvelerinin total fenolik içeriğinin % 50'sini fenolik asitlerin, % 40'ını antosiyaninlerin oluşturduğu belirtilmiştir. Fenolik asitlerden kafeoyil gliserik asit 129 mg/100 g, 3-*O*-kafeoyil kinik asit 113 mg/100 g miktarında olduğu tespit edilmiştir. Bu çalışmanın verilerine göre siyanidin glikozitleri (3-*O*-galaktozit, 3-*O*-glukozit, 3-*O*-arabinozit, 3-*O*-ksilozit) ana antosiyaninlerdir (222 mg/100 g). Flavonol glikozitlerinin total fenol içeriğinin % 10'unu oluşturduğu (56 mg/100 g) ve en yüksek miktarda kersetin 3-*O*-galaktozitin bulunduğu belirtilmiştir. Bunun yanında yaprakların total fenol içeriği 1500 mg/100 g, dalların ise 500 mg/100 g miktarında hesaplanmıştır. UPLC-DAD-ESI-MS yardımıyla, meyve ekstrelerinde 5-*O*-kafeoyilkinik asit, 3-*O*-kafeoyilkinik asit, dikafeoyilkinik asit, kafeoyilmalik asit, kafeoyilgliserik asit, kersetin-arabinoglukozit, kersetin 3-*O*-rutinozit, kersetin 3-*O*-galaktozit, kersetin 3-*O*-glukozit, kersetin 3-*O*-ksilozit, kersetin 3-*O*-arabinozit, kersetin 3-*O*-arabinofuranozit, kersetin 3-*O*-(6′-malonil)-glukozit, kersetin, syanidin 3-*O*-galaktozit, syanidin 3-*O*-glukozit, syanidin 3-*O*-arabinozit, syanidin 3-*O*-ksilozit, yaprak ekstrelerinde (-)-epikateşin, 5-*O*-kafeoyilkinik asit, 3-*O*- kafeoyilkinik asit, dikafeoyilkinik asit, kafeoyilmalik asit, kersetin-arabinoglukozit, kersetin 3-*O*-galaktozit, kersetin 3-*O*-glukozit, kersetin 3-*O*-ksilozit, kersetin 3-*O*-arabinozit, kersetin 3-*O*-arabinofuranozit, kersetin 3-*O*-(6′-malonil)-glukozit, kemferol 3-*O*-glukozit, dal ekstrelerinde ise (+)-kateşin, (-)-epikateşin, 5-*O*-kafeoyilkinik asit, 3-*O*- kafeoyilkinik asit, kersetin-arabinoglukozit, kersetin 3-*O*-rutinozit, kersetin 3-*O*-galaktozit, kersetin 3-*O*-glukozit, kersetin 3-*O*-ksilozit, kersetin 3-*O*-arabinozit, kersetin 3-*O*-(6′-malonil)-glukozit, isoramnetin 3-*O*-rutinozit, isoramnetin 3-*O*- galaktozit, kemferol 3-*O*-rutinozit, eriyodiktiyol 7-*O*- glukozit tespit edilmiştir [26].

Biyolojik Etki Çalışmaları

In Vitro Çalışmalar

Antioksidan Etki

Amelanchier alnifolia'nın kültüre alınan 2 varyetesi (Thiessen ve Smokey) ile yapılan antioksidan kapasite çalışmasında, meyvelerin % 80 etanol ekstresinden elde edilen eter, etil asetat, *n*-butanol ve su fraksiyonları kullanılmıştır. DPPH, ABTS ve demir indirgeme kapasitesi testlerinde Thiessen ve

Smokey meyvelerine ait fraksiyonların % inhibisyon değerleri Thiessen'da sırasıyla 10.9, 25.6, 29.1, 19.2; 8.8, 23.7, 31.7, 18.0; 9.5, 23.6, 26.7, 21.2, Smokey'de ise 8.3, 21.5, 30.8, 15.2; 10.8, 24.5, 32.5, 16.6; 11.7, 22.3, 27.9, 21.9 olarak bulunmuştur [27].

Amelanchier canadensis meyvesinden hazırlanan metanol, etil asetat ekstresi ve meyve suyu ile *A. arborea* meyvelerinden hazırlanan metanol, etil asetat, hekzan ekstreleri ve bu bitkilerin meyvelerinden izole edilen (1,3-dilinoleoyil 2-olein (**1**), 1,3-dioleoyil 2-linolein (**2**), 5-hidroksimetil-2-furfural (**3**), 5-(sorbitoloksimetil)-furan-2-karboksaldehit (**4**), 5-(mannitoloksimetil)-furan-2-karboksaldehit (**5**), 5-(α-D-glukopiranosiloksimetil)furan-2-karboksaldehit (**6**), oleanolik asit (**7**), ursolik asit (**8**), kemferol-3-*O*-α-L-ramnopiranosil (1→2) ramnopiranozit (**9**), kemferol-3-*O*-α-L-ramnopiranozit (**10**)) maddeler ile yapılan lipit peroksidasyon testinde izole edilen maddeler **1** (100 ppm), **2** (100 ppm), **3** (10 ppm), **4**+**5** (karışım) (100 ppm), **6** (100 ppm), **7** (100 ppm), **8** (100 ppm), **9** (100 ppm), **10** (100 ppm), BHA (1.67 ppm; % 89) , BHT (2.2 ppm; % 87), TBHQ (1.67 ppm; % 98)'ya oranla sırasıyla 3.3, 8.7, 8.6, 79, 29, 76, 76, 85, 84 % inhibisyon göstermiştir. Ham ekstrelerden ise etil asetat ekstresinin (100 ppm; % 8.3) en yüksek aktiviteye sahip olduğu görülmüştür [19].

Amelanchier alnifolia'nın 17 varyetesi (Success, Lee 3, Martin, Parkhill, Forestburg, Lee 8, Lee 2, Pembina, Honeywood, Northline, Thiessen, Pasture, Nelson, Pearson, Quaker, Smokey ve Regent) ile yapılan DPPH (2.8 mM/100 g FW) ve ABTS (5.0 mM/100 g FW) testlerinde en yüksek aktiviteyi Nelson varyetesi göstermiştir [21].

A. canadensis taze meyvelerinin suyu ile yapılan antioksidan kapasite çalışmasında demir iyonu indirgeyici güç (FRAP) testinde meyvelerin antioksidan kapasitesi 25.07 ± 0.48 mmol Fe^{+2}/kg olarak tespit edilmiştir. Total fenolik madde miktarı gallik asite eşdeğer olarak 539.24 mg/100kg, total antosiyanin miktarı ise siyanidin-3-*O*-glukozite eşdeğer olarak 220.66 mg/100g olarak hesaplanmıştır [25].

Amelanchier ovalis subsp. *ovalis*'in iki farklı yerden (Gümüşhane ve Rize) toplanan yaprak etanol ekstreleri ile yapılan çalışmada metal iyonlarını şelatlama kapasitesi, demir iyonu indirgeyici güç (FRAP) ve *N*,*N*-dimetil-*p*-fenilendiamin (DMPD) radikal süpürücü etki testleri uygulanmıştır. Antioksidan kapasite sadece Rize'den toplanan örnekde ve metal iyonlarını şelatlama kapasitesi testinde (%27.12) tespit edilmiştir [28].

A. alnifolia'nın meyve, yaprak ve dallarından hazırlanan ekstreler (etanol:su:asetik asit, 70:30:1) ile yapılan antioksidan kapasite tayininde, DPPH testinde % 88.8 inhibisyon, TRAP testinde 424.3 mg/100 mL ve ORAC testinde 1015.23 mg/100 mL değerleri ile en yüksek kapasiteyi yaprak ekstresi göstermiştir. Buna paralel olarak gallik asite eşdeğer total fenolik madde içeriği en yüksek yaprak ekstresinde (227.1 mg/100 mL) tespit edilmiştir [29].

Antibakteriyel Etki

A. alnifolia'nın meyve, yaprak ve dallarından hazırlanan ekstreler (etanol:su:asetik asit, 70:30:1) ile yapılan antibakteriyel aktivite çalışmasında *Escherichia coli* (E-94564), *Staphylococcus aureus* (E-70045), *Listeria monocytogenes* (E-97783), *Bacillus cereus* (E-93143), *Salmonella enterica* sv. Typhimurium (E-95582) mikroorganizmalarına karşı ekstrelerin inhibe edici kapasiteleri test edilmiştir. *S. aureus*, *L. monocytogenes* ve *B. cereus*'a karşı en güçlü etkiyi yaprak ekstresinin gösterdiği tespit edilmiştir [29].

Antienflamatuvar Etki

Amelanchier canadensis meyvesinden hazırlanan metanol, etil asetat ekstresi ve meyve suyu ile *A. arborea* meyvelerinden hazırlanan metanol, etil asetat, hekzan ekstreleri ve bu bitkilerin meyvelerinden izole edilen [1,3-dilinoleoyil 2-olein (**1**), 1,3-dioleoyil 2-linolein (**2**), 5-hidroksimetil-2-furfural (**3**), 5-(sorbitoloksimetil)-furan-2-karboksaldehit (**4**), 5-(mannitoloksimetil)-furan-2-karboksaldehit (**5**), 5-(*α*-D-glukopiranosiloksimetil)furan-2-karboksaldehit (**6**), oleanolik asit (**7**), ursolik asit (**8**), kemferol-3-*O*-α-L-ramnopiranosil (1→2) ramnopiranozit (**9**), kemferol-3-*O*-α-L-ramnopiranozit (**10**)] maddeler ile yapılan siklooksigenaz (COX-1 ve COX-2) inhibitör aktivitesi çalışmasında, **4** ve **5**'in karışımı ile **7** COX-1 testinde en yüksek aktiviteyi % 68 (**4**+**5**) ve % 63 (**7**) inhibisyon ile göstermişlerdir. **7**, **8** ve **9** maddeleri zayıf aktivite göstermiştir. **7**+**8** karışımı ham ekstreler gibi COX inhibisyon aktivitesi göstermemiştir. Standart olarak kullanılan Aspirin (180 ppm), Celebrex (1.67 ppm) ve Vioxx (1.67 ppm), COX-1'de sırasıyla 75, 5, 0 oranlarında % inhibisyon, COX-2'de ise sırasıyla 69, 82, 85 oranında % inhibisyon göstermişlerdir [19].

Antikolinesteraz Etki

Amelanchier ovalis subsp. *ovalis*'in iki farklı yerden (Gümüşhane ve Rize) toplanan yaprak etanol ekstreleri ile ELISA testi yardımıyla yapılan asetilkolinesteraz (AChE) ve bütirilkolinesteraz (BChE) inhibisyon kapasitesi testinde, 200 µg/mL olarak uygulanan estrelerden Gümüşhane ve Rize örnekleri BChE testinde galantamine oranla (% 83.68) sırasıyla % 19.99 ve % 19.58 inhibisyon göstermiştir. AChe testinde Gümüşhane örneği galantamine oranla (% 96.68) % 24.38 inhibisyon gösterirken, Rize örneğinin etki göstermediği belirtilmiştir [28].

Bu çalışmada *Amelanchier* cinsine ait taksonların etnobotanik kullanımları, fitokimyası ve biyolojik aktiviteleri üzerinde 1998-2018 yılları arasında yapılan çalışmalar derlenmiştir.

Etnobotanik veriler *Amelanchier* cinsinin dünya genelinde yaygın kullanımının olmadığını göstermiştir. Fakat Amerika kıtasının kuzeyi (Amerika Birleşik Devletleri ve Kanada)'nde halkın geleneksel olarak birçok rahatsızlıkların tedavisinde (*Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem., *A. alnifolia* (Nutt.) Nutt. ex M. Roem. var. *semiintegrifolia* (Hook.) C. L. Hitchc., *A. arborea* (F. Michx.)

Fernald, *A. arborea* (F. Michx.) Fernald var. *arborea*; *A. canadensis* (L.) Medik., *A. humilis* Wiegand, *A. laevis* Wiegand, *A. pallida* Greene, *A. stolonifera* Wiegand, *A. utahensis* Koehne var. *utahensis*) tıbbi amaçla kullandığı tespit edilmiştir [11,12,14]. Bunun dışında *Amelanchier parviflora* var. *dentata*'ın dallarının dekoksiyon şeklinde Türkiye (Acıpayam-Denizli)'de diüretik olarak [18]; İspanya'da *A. ovalis*'in herba ve çiçeklerinin infüzyon şeklinde, dahilen körlük tedavisinde [15], aynı bitkinin sadece herbasının ise aynı şekilde nezle tedavisinde [16] geleneksel olarak kullanıldığı görülmüştür. Dünya genelinde 37 takson ile temsil edilen cinsin halk arasında kullanılan takson sayısı 12 olarak belirlenmiştir.

Amelanchier cinsi üzerinde yapılan fitokimyasal ve *in vitro* biyoaktivite çalışmaları da geleneksel kullanımı olan türler ve bu türlerin (*A. canadensis, A. arborea, A. ovalis* ve *A. alnifolia*) özellikle meyveleri üzerinde yoğunlaşmıştır. İncelenen çalışmalarda meyvelerin flavonoid, karotenoid ve siyanogenetik glikozit madde gruplarını taşıdığı tespit edilmiştir [19,20,21,22,23,24,25,26].

Siyanogenetik glikozitler, dünya genelinde 2000 bitki türünde tespit edilen fitotoksinlerdir. Rosaceae familyasında siyanogenetik glikozitlerin varlığı *Malus domestica* Borkh., *M. sylvestris* (L.) Mill., *Prunus armeniaca* L., *Prunus dulcis* (Mill.) D.A. Webb, *Prunus persica* (L.) Batsch gibi türlerde bilinmektedir [30,31]. Bir siyanojenik bitkinin potansiyel toksisitesi, bitki tüketimde maruz kalınan ve toksik olan Hidrojen siyanid (HCN) konsantrasyonuna bağlıdır. Oral uygulamadan sonra hidrojen siyanür kolayca emilir (ayrıca solunmaya maruz kaldıktan sonra, cilt ve gözler yoluyla da kolayca emilir). Emiliminden sonra, siyanid vücutta kan yoluyla hızla dağılır [32]. Siyanogenetik glikozitler içeren bitkilerin kullanımları sırasında bir tehlike oluşturmamaları için bu bitkilerin toksik etkilerinin önceden tespit edilmesi bir zorunluluk arz etmektedir.

Antioksidan etki çalışmalarında *A. canadensis*'in meyvelerinin etil asetat ekstresi lipit peroksidasyon testinde [19] ve meyve suyu FRAP testinde [25], *A. alnifolia*'nın Nelson varyetesinin meyve suyu DPPH ve ABTS testlerinde [21] aktivite göstermiştir. Tian ve arkadaşlarının (2018) yaptığı antibakteriyel etki çalışmasında *A. alnifolia*'nın yaprak ekstresinin aktif olduğu belirtilmiştir. Asetilkolinesteraz (AChE) ve bütirilkolinesteraz (BChE) inhibisyon kapasitesi testinde farklı yerlerden (Gümüşhane ve Rize) toplanan *Amelanchier ovalis* subsp. *ovalis*'in yaprak etanol ekstresi sadece Gümüşhane örneğinde aktivite belirlenmiştir [28].

Antienflamatuvar etki çalışmalarında siklooksigenaz (COX-1 ve COX-2) inhibitör testinde, *Amelanchier canadensis* ve *A. arborea* meyvelerinden izole edilen 5-(sorbitoloksimetil)-furan-2-karboksaldehit ve 5-(mannitoloksimetil)-furan-2-karboksaldehit karışımı karışımı ile oleanolik asit COX-1 testinde en yüksek aktiviteyi göstermişlerdir [19].

A. alnifolia'nın meyve, yaprak ve dallarından hazırlanan ekstreler ile yapılan antibakteriyel aktivite çalışmasında *Staphylococcus aureus*, *Listeria monocytogenes* ve *Bacillus cereus*'a karşı en güçlü etkiyi yaprak ekstresinin gösterdiği tespit edilmiştir [29].

Cins üzerinde yürütülen biyolojik etki çalışmalarının *in vitro* çalışmalarla sınırlı olduğu *in vivo* çalışmaların yapılmadığı görülmüştür. Kimyasal çalışmalarla incelenen taksonların fenolik bileşikler (organik asitler, flavonoitler, antosiyanin, prosiyanin vb.) yönünden zengin bir içeriğe sahip olduğu ve genellikle yaprağın meyveden daha fazla fenolik içeriğe sahip olduğu belirlenmiştir [29].

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Doi: 10.33483/jfpau.559665

DERLEME MAKALE / REVIEW ARTICLE

KENEVİR VE SAĞLIK ALANINDA KULLANIMI

HEMP AND ITS USE IN HEALTH

Seda YILDIRIM, Ufuk KOCA ÇALIŞKAN *

Gazi Üniversitesi Eczacılık Fakültesi Farmakognozi Anabilim Dalı, Ankara

ÖZ

Amaç: *Kenevir, binlerce yıldır özellikle liflerinden ve tohumları olmak üzere tüm kısımlarından çeşitli amaçlarla (yapı-tekstil malzemesi, kağıt, besin, insan ve hayvan sağlığında ilaç vb.) yararlanılan, ilk kültürü yapılan bitkilerdendir. 1930'lu yıllarda öforik amaçla kullanımının suistimal edilmesi ve ticari kaygılardan dolayı uzun yıllar yasaklı/kısıtlı bitki olarak kalan kenevir, yeni nesil kullanım alanları ve özellikle insan sağlığıyla ilgili etkilerinin bilimsel araştırmalarla kanıtlanmasıyla son yıllarda tekrar ön plana çıkmıştır. Günümüzde pek çok ülke kenevirle ilgili yasaların güncellenmesiyle kenevirin hem endüstriyel hem de medikal etkilerinden faydalanmaya başlamıştır. Ülkemizde ise son yıllarda endüstriyel kenevir üretimi ve kullanımı ile ilgili birtakım gelişmeler yaşanmaktadır.*

Gereç ve Yöntem: *Bu çalışmada, kenevirin botanik özellikleri, tarihçesi, fitokimyasal içeriği, terapötik kullanımları ve sağlık alanında yapılan bilimsel çalışmalar derlenmiştir.*

Sonuç ve Tartışma: *Kenevir bitkisinin botanik özellikleri, genel kullanım alanları ile birlikte geçmişten günümüze sağlık alanında kenevirden elde edilen kullanımda olan ilaçlardan örnekler verilerek fitokimyasal içeriği ve etkileri detaylı olarak verilmiştir. Kenevir tohumu yağı ve kökündeki aktif bileşenlere de değinilmiş, kenevirin terapötik kullanımı tartışılmıştır. Hem endüstriyel hem sağlık alanında kullanılan fakat üretimi ve kullanımı ülkemizde kısıtlı olan kenevir ile ilgili yasaların bilimsel destekli olarak yeniden gözden geçirilmesi ülke ekonomisi ve sağlık sektörü için oldukça önemli sonuçlar sağlayacaktır.*

Anahtar kelimeler: *Cannabis; kenevir, kannabinoitler, kenevir tohumu yağı*

ABSTRACT

Objective: *Hemp is one of the first cultivated plants that have been used for thousands of years, specifically on behalf of its fibers and seeds, for various purposes (building-textile material, paper, nutrients, human and animal health). In the 1930s, since the abuse of the euphoric use for abstinence, hemp was banned/restricted and remained restricted for many years, hemp has emerged once again in recent years with the usage of the plant in new generation areas and in particular the impacts of human health. Nowadays, many countries have begun to benefit from the both industrial and medical effects of hemp by updating the laws related to hemp. In our country, there have been some developments regarding the production and use of industrial cannabis in recent years.*

Material and Method: *In this study, the botanical characteristics, history, phytochemical content, therapeutic uses of hemp and scientific studies in the field of health were reviewed.*

* **Sorumlu Yazar / Corresponding Author:** Ufuk Koca Çalışkan
e-posta / e-mail: ukoca@gazi.edu.tr

Gönderilme / Submitted: 01.05.2019 **Kabul / Accepted:** 17.09.2019

Result and Discussion: *The botanical properties of cannabis plants, general usage areas as well as phytochemical content and its utilizations in health from past to the present are given in detail. Cannabis seed oil and active ingredients in its roots are also mentioned and therapeutic use of cannabis was discussed. A review of the cannabis laws with the support of scientific revelations, which are limited production and usage both in industrial and health fields, will provide significant results for the country's economy and health sector.*
Keywords: *Cannabis; hemp, cannabinoids, hemp seed oil*

GİRİŞ

İnsanlar tarafından binlerce yıldır liflerinden, fizyolojik ve psikolojik etkilerinden, besin ve yağ üretiminde ise tohumlarından yararlanılan kenevirin anavatanı Orta Asya olup, insanlık tarihinde ilk kültürü yapılan bitkilerden biridir. 4500 yıldan beri Çin'de ekimi yapılan bu kadim bitkinin tarihte kullanılmaya başlandığı zamandan itibaren kullanımı çok farklı alanlara taşmıştır. Tekstil (iplik, kumaş, çadır bezi, kalın sicim, halat yapımı, Şile bezi ve Rize bezi gibi özgün dokuma tekniklerinde geleneksel kullanımda), kâğıt, inşaat (çatı ve duvar yapı malzemesi vb.), uzay sanayii ve otomotiv sektörleri, biyo-bozunur plastik üretimi, kozmetik, ilaç ve gıda desteği üretiminde kullanılır. Tohumları doğrudan kuşyemi olarak veya insan beslenmesinde, elde edilen yağı ise yine kümes hayvanlarının ve besinsel değeri sebebiyle insan beslenmesinde, ayrıca sabun yapımında, aydınlanmada ve cila, muşamba, tual boyası yapımında yaygın bir şekilde kullanılmaktadır [1].

Kenevirin Botanik Özellikleri

'Kenevir', 'kendir' veya 'esrarotu' yaygın olarak İngilizce'de 'cannabis' ya da 'hemp' olarak da bilinen; Cannabinaceae familyasına ait tek yıllık, otsu, dioik, çiçekli bir bitkidir. Çiçekleri çift evciklidir yani dişi ve erkek çiçekler farklı bireylerde bulunur. Dişi çiçekleri oldukça sık yapraklarla çevrilidir ve çiçeklerin etrafı reçine salgılayan trikomlar açısından zengindir. Erkek çiçekleri ise seyrek salkım şeklindedir. Tohumları 3-6 mm uzunluğunda, 2-4 mm eninde ve türüne bağlı olarak gri, siyah, yeşilimsi esmer renklerde olup ince kabukludur.

Kenevir ilk defa Carlous Linnaeus tarafından 1753 yılında *Cannabis sativa* L. olarak isimlendirilmiştir. 1785'te ise ünlü Fransız biyolog Jean-Baptiste Lamarck Hindistan kökenli ve *C. sativa*'dan morfolojik olarak farklı olan *Cannabis indica* türünü tanımlamış, 1924'te Janischevsky Rusya'da, diğer iki türden farklı özelliklere sahip olan *Cannabis ruderalis* türünü keşfetmiştir [2, 3]. Bazı botanikçiler *C. indica* ve *C. ruderalis'i*n *C. sativa*'nın alttürleri olduğunu düşünse de pek çok botanikçinin ortak görüşüne göre kenevirin üç türü olduğunu söylemek mümkündür. *C. sativa* en yaygın olarak kültürü yapılan, yağ ve lif üretiminde esas olarak kullanılan türüdür. Uzun, ince ve gevşek dallanma gösteren, uzunluğu 6 metreye kadar çıkabilen bir gövdeye sahiptir. *C. indica*; 0,90- 2,5 m arasında, piramit şekilli ve yoğun dallanma gösteren, lif üretimi için uygun olmayan, özellikle psikoaktif içerikler açısından zengin olan ve 'Hint keneviri' adıyla bilinen türüdür. *C. ruderalis* ise 'yabani kenevir'

olarak bilinir, gövdesi diğer iki türden daha kısadır ve seyrek dallanma gösterir. Bunların yanında, bitkinin yüksek derecede polimorfizm göstermesi sebebiyle morfolojik özellikleri ve içerdikleri metabolitler açısından birbirinden farklılıklar gösteren alttürleri de bulunmaktadır.

Kenevirin Genel Kullanım Alanları

Kenevir, tamamından yararlanılabilen ve çok farklı alanlarda ekonomik değere sahip, hemen hemen tüm kullanım alanlarında üstünlükleri söz konusu olan değerli ve ender bir bitkidir. Örneğin, çatı ve duvar malzemesi yapımında lif ve mineral karışımının ağırlık, dayanıklılık ve kalite kriterleri göz önüne alındığında beton kullanımına oranla daha avantajlı olduğu saptanmıştır [3]. Üstelik kenevirden elde edilen bu karışımın kullanıldığı yapılarda nemlenme oranı düşmekte ve ortam sıcaklığının izolasyonu en iyi düzeyde olmaktadır [3]. Tüm bu yapıların geri dönüşüme elverişli olması ve çevre kirliliğine sebep olmaması bitkinin bir diğer önemli üstünlüğüdür [4]. Kök sistemleri sayesinde kuraklığa ve aynı zamanda zararlılara karşı oldukça dayanıklıdır. Diğer lif bitkilerinden daha az su gereksinimine sahiptir, oldukça hızlı büyür ve pek çok coğrafyada yaygın bir şekilde kültürü yapılabilmektedir. Lignoselülozik lifleri en kaliteli lifler arasındadır ve aynı zamanda antibakteriyel özelliğe sahiptir [5, 6]. Bu sebeple antibakteriyel tekstil ürünleri [7] ve cerrahi malzeme üretimine [8] oldukça elverişlidir. Bilindiği gibi günümüzde kâğıt sektörü ağaçların kullanımına bağımlı haldedir. Yapılan araştırmalarda belli bir alandaki kenevir topluluğundan aynı orandaki orman alanından dört kat fazla selüloz elde edilebildiği ortaya konmuştur. Tüm bu üstünlüklerine ek olarak kenevirin; birim alanda bol miktarda yaprak üretmesi sebebiyle bol oksijen üretimi, sürdürülebilir olmayan hammaddelerin- petrol türevlerinden elde edilen plastik, toksik polimerler ve ormanlardan elde edilen kağıt gibi- yerini alabilmesi, yakacak olarak kullanıldığında dahi karbon ayak izi bakımından avantajlı olması, yabancı otlarla mücadelede diğer tarla bitkilerinden üstün olması, üretiminde ekonomik anlamda kayıtlara geçmiş çok az hastalığının bulunması sebebiyle insektisit, fungusit vb. ilaçlara pek ihtiyacının olmaması gibi özellikleri, kenevirin çevre dostu bir bitki olduğunu, ağaç popülasyonlarının korunmasında ve doğaya saygılı tarım uygulamalarında kilit role sahip olabileceğini göstermektedir [4].

Kenevir, pamuk lifi ve sentetik lif üretiminin artması ile ve özellikle de Amerika'da 1937 yılında çıkan "Marihuana Vergi Yasası" ile başlayıp psikoaktif madde içeriğinin suistimal edilmesi sebebiyle pek çok ülkede adım adım yasaklanmasına sebep olan diğer uluslararası yasal düzenlemelerle uzun yıllar boyunca yasaklı bitki olarak kalmış ve kültür alanları önemli ölçüde kısıtlanmıştır. Ancak birbirinden farklı pek çok sektörde başarıyla kullanıma elverişli olması özellikle 2013 yılı ve sonrasında kenevir üretim alanlarının tekrar artmaya başlamasına sebep olmuştur. Bazı ülkeler kenevir ile ilgili yasalarını güncellemekte ve ekim alanlarının artması için çaba göstermektedir. Bu artışa başlıca kenevirin aşağıda bahsedilen üç alandaki kullanımı sebep olmuştur [9]. Bunlar:

1- İnşaat sektörü: Çevre dostu bina üretiminde; tuğla, yalıtım malzemesi vb. alanlarda kenevirden elde edilen ürünlerin üretiminde kullanılmaktadır. Bu alanda Avusturalya ve Fransa başı çekmektedir.
2- Biyopolimer sektörü: Günümüzden yaklaşık 70-80 yıl kadar önce kenevir lif ve saplarından biyopolimer üretilmiş ve araba kaportasında kullanılmıştır. Petrolden elde edilen polimerlerin daha ucuz olmasından dolayı biyopolimerler daha geri planda kaldıysa da petrol türevi polimerlerin ve plastiklerin doğadaki parçalanma sürelerinin çok uzun olması ve çevre kirliliğine neden olmasıyla son yıllarda biyopolimerler yeniden gündeme gelmiştir.
3- İlaç sektörü: Kenevir ekim alanlarının artmasında en önemli paya sahip sektördür. Özellikle İsrail, Kanada ve Çin gibi ülkeler kenevirden ilaç üretimi araştırmalarına önem vermektedirler.

Geçmişten Günümüze Kenevirin Sağlık Alanında Kullanımı

İlk kültürü yapılan bitki olduğu düşünülen kenevirin insanoğlu tarafından kullanımı binlerce yıl öncesine gitmekte, Hindistan ve Tibet'te çiçeklerinin ve reçinesinin meditasyonda ve çeşitli dinsel ritüellerde kullanıldığı bilinmektedir [10]. İmparator Chen Nung tarafından yazılan ilk Çin farmakopesinde (M.Ö.3200) kenevirin yorgunluk, romatizma ve sıtma tedavisinde kullanıldığı yazmakta olup, kaynaklara göre kenevir tohumları yağı ve protein içeriği sebebiyle egzamada, psöriazisde antienflamatuvar olarak Çin tababetinde kullanılmıştır [11]. Yaklaşık 3000 yıl önce yazılan Ebers Papirüsü'nden ve Asur tabletlerinden kenevirin yaygın bir şekilde ilaç olarak kullanıldığı anlaşılmaktadır [12]. Kenevir aynı zamanda Eski Yunan ve Roma dönemlerinde de iyi bilinen ve ilaç olarak kullanılan bir bitki olarak karşımıza çıkmaktadır. Bu döneme ait veriler incelendiğinde kenevirin özellikle ağrı kesmek ve duygu durumunu iyileştirmek amacıyla kullanıldığı gözlenmektedir. Romalı bir hekim olan Galen, medikal keneviri reçete eden hekimlerdendir ve Romalı aristokratların akşam yemeğini kenevir içeren bir tatlıyla sonlandırdıklarını notlarına ilave etmiştir [13]. İbn-i Sina El-Kanun Fi't-Tıb adlı eserinde, kaynatılmış kenevir köküyle yapılan kompresin ateşi düşürdüğünü yazmıştır [14].

Tıbbi amaçlarla kenevir kullanımının önce Arabistan, sonrasında da tüm Ortadoğu'ya yayılması dokuzuncu yüzyıla tekabül etmektedir. Ünlü gezgin Marko Polo'nun 13. yüzyıl sonlarında, doğu seyahati dönüşü seyahatnamesinde bahsetmesiyle kenevir bitkisi Avrupa'nın dikkatini çekmiş ve yoğun bir şekilde kullanılmaya başlanmıştır [15]. Alman bir botanist ve hekim olan Leonhart Fuchs, 1542'de yazdığı bitkisel tedavileri içeren kitabında suda kaynatılarak ilgili bölgeye sarılan kenevir kökünün gut hastalığına iyi geldiğini yazmıştır [16]. Portekizli hekimler Garcia da Orta ve Cristobal Acosta kenevirin öforik, sedatif, iştah açıcı, halüsinojenik ve afrodizyak etkilerinden bahsetmişlerdir [17].

18. yüzyılın sonunda Napolyon'un ordusu Mısır dönüşü Fransa'yı kenevir ile tanıştırmıştır [15]. İrlandalı Dr. O'Shaughnessy, Hindistan'da kenevir bitkisinin analjezik, antispazmodik, antiemetik ve hipnotik amaçlarla kullanıldığını gözlemlemiş ve Hindistan'dan edindiği bu bilgilerle 1840'lı yıllarda Birleşik Krallık'ta kenevir bitkisinin tıbbi kullanımının yayılmasını sağlamıştır [18]. 1890'larda Kraliçe

Victoria'nın menstrüel krampları için de kenevir kullanıldığı belirtilmektedir [15]. Bu yıllarda İngiltere'de kenevir ağrı kesici, antienflamatuvar, antiemetik ve antikonvülsan olarak kullanılmıştır.

Kenevir bitkisi 1850-1942 yılları arasında Amerikan Farmakopesinde yer almış, kullanımı bu dönemlerde doruk noktaya ulaşırken, 1930'lardan itibaren ise düşüşe geçmiştir. Birleşik Krallık'ta 1928 yılında önce rekreasyonel kenevir kullanımı yasaklanmış ve sonrasında kenevir veya cannabis ismi yerine çok tehlikeli olduğuna dair bir algı yaratan marihuana adı kullanılmaya başlanmıştır. Hatta 1936 yılında Amerikan gençliğini kenevir kullanmaktan uzaklaştırmak için "Reefer Madness" adlı bir propaganda filmi yapılmıştır. Bu anti-propagandaların nedenleri arasında tehlikeli sentetik türevlerin bulunması, bitkisel ürünlerin standardize edilmelerindeki güçlükten dolayı etkilerinde görülen değişkenlikler, temin kaynaklarının güvenilir olmaması, ticari baskılar ve Mısır, Güney Afrika ve ABD gibi ülkeler başta olmak üzere öforik etkisinin suistimal edilmesi gösterilebilir [15]. 1937 yılında ABD'de çıkan "Marihuana Vergi Yasası" ile yasağın alanı genişletilmiş ve bunun yansıması olarak Türkiye'de de Türk ilaç kodeksinde "Herba Cannabis Indica" ve "Extra Cannabis Indica" adıyla yer alan ilaçlar 1940 yılından sonra kodeksten çıkarılmış, satışı yasaklanmıştır. ABD'de 1970 yılında çıkarılan "Kontrollü Maddeler Yasası" ile marihuana bulundurmak, kullanmak, satmak, satın almak ve yetiştirmek yasaklanmıştır. Tüm bu denetimler ve çıkarılan yasalar, kenevirin kötüye kullanımını engellemiş fakat bu durum aynı zamanda kenevirin bilimsel çalışmalar için temin edilmesini ve klinikte kullanılışını da sınırlandırmıştır.

Kenevir üzerine yapılan bilimsel araştırmalar tüm olumsuz koşullara rağmen devam etmiştir. Gaoni ve Mechoulam 1964 yılında kenevirin öforik etkisinden sorumlu ana bileşeni olan tetrahidrokannabinol'ün (tetrahydrocannabinol-THC) kimyasal yapısını ortaya çıkarmıştır [19]. 1988 yılında kannabinoit reseptörlerinden CB1 reseptörü ve 1993 yılında CB2 reseptörü bulunmuş ve sonrasında endokannabinoitler (anandamid, 2-araşidonil gliserol, vd.) ile endokannabinoitleri sentezleyen ve degrade eden enzimler (FAAH ve MGL) keşfedilmiştir [20, 21]. Tüm bu keşifler kenevirin tıbbi kullanımına olan ilgiyi artırmış, klinikte kullanımı da yeniden popüler hale gelmiştir. ABD'de 1996 yılında medikal kenevir kullanımını serbest bırakan ilk eyalet yasası çıkarılmıştır. Günümüzde ise ABD'de California, Nevada, Massachusetts ve Colorado gibi 29 eyalette kenevir kullanımı yasal durumdadır. Yakın zamanda, çok sayıda Avrupa ülkesinde de kenevirin medikal kullanımına ilişkin yasalar çıkarılmıştır ve medikal kenevirin yasallaşması sürecine sağlık profesyonelleri ve bilim otoriteleri de destek vermektedir. Medikal kenevir kullanımı ile ilgili yasa çıkaran ilk ülkelerden biri de Kanada'dır ve 2001 yılında medikal kenevir kullanım yasasını uygulamaya geçirmiştir [15]. 2019'da Kanada'da yapılan bir araştırmada, doktor ve eczacıların kenevirin tıbbi kullanımına ilişkin olumlu görüşlere sahip olduğu ve hatta kişisel deneyimlerine göre mevcut pek çok tıbbi tedaviden daha az yan etkiye sahip olduğunu düşündükleri belirtilmiştir [22].

Günümüzde kenevirdeki THC etkin maddesinin prototip olarak kullanıldığı 2 sentetik ilaç ve kenevirin standardize ekstrelerinin kullanıldığı 2 ilaç olmak üzere toplamda kenevir bazlı 4 ilaç bulunmaktadır [23]. Dronabinol ve nabilon, kanser kemoterapisine eşlik eden bulantı ve kusma için kullanılan Amerikan Gıda ve İlaç Dairesi (Food and Drug Administration-FDA) onaylı sentetik kannabinoitlerdir. Nabiksimols ise 100 mikrolitresinde 2,7 mg THC ve 2,5 mg kannabidiol (cannabidiol-CBD) içeren ve oromukozal sprey şeklinde mevcut diğer tedavilere cevap vermeyen multipl skleroza bağlı spastisite tedavisinde kullanılan standardize kenevir ekstresidir. Son olarak 2018 yılında Epidiolex isimli kannabidiol içeren ilaç, epilepsinin ağır seyreden ve nadir gözlenen iki formu olan Lennox-Gastaut sendromu ve Dravet sendromunun tedavisinde kullanılmak üzere FDA tarafından onaylanmıştır. Aşağıda bahsi geçen kenevir bazlı 4 ilaç ile ilgili çeşitli bilgiler tablo halinde sunulmuştur (Tablo 1).

Tablo 1. Günümüzde yasal olarak kullanılan kenevir kökenli ilaçlar, içerikleri, onaylandıkları yıl, ülkeler ve endikasyonları [23].

İlaç	**İçerik**	**Onay yılı**	**Ülke**	**Onaylı endikasyon**
Marinol™, Syndros™	Dronabinol (Sentetik THC)	1985	ABD	-Kanser kemoterapisine bağlı bulantı ve kusma
		1998	Kanada	
		1992	ABD	-AIDS hastalarında görülen kilo kaybı ve anoreksi
		2000	Kanada	
Cesamet™	Nabilon (Sentetik THC)	1981	Kanada	-Kanser kemoterapisine bağlı bulantı ve kusma
		1982	Avusturalya, Birleşik Krallık	
		2006	ABD	
Sativex™	Nabiksimols (THC ve CBD içeren standardize ekstre)	2010	Kanada	-Multipl skleroza bağlı spastisite
			Yeni Zelanda	- Multipl skleroza bağlı nöropatik ağrı
			Avrupa (çoğu)	-Kanser hastalarında ağrı tedavisi
Epidiolex™	CBD içeren standardize ekstre	2018	ABD	-Lennox-Gastaut sendromu -Dravet sendromu

Kenevirin Fitokimyasal İçeriği ve Etkileri

Kenevir sekonder metabolit açısından oldukça zengin bir bitkidir. Günümüzde *C. sativa* türünden 545 adet bileşik izole edilmiştir [24] ve yapılacak yeni çalışmalarla birlikte bu sayının daha da artacağı öngörülmektedir. Bunların 100'den fazlası daha ziyade kenevir türlerine spesifik olan ve kannabinoit aktivitesi gösteren fitokannabinoitlerdir [25]. Fitokannabinoitler bitkilerde bulunan ve vücuttaki CB1 (kannabinoit reseptörü 1) ile CB2 (kannabinoit reseptörü 2) reseptörlerine bağlanıp değişik farmakolojik

etkiler ortaya çıkaran, kenevirle ilgili en çok araştırılmış sekonder metabolit grubudur. Kenevirdeki ana üretim yerleri, bitkinin yapraklarında, çiçeklerinde, braktelerinde ve gövdesinde yer alan epidermal salgı tüyleridir [26]. Trikom adı verilen bu salgı tüylerinden reçine salgılanmaktadır ve bu reçine bitkiyi, zararlılardan ve otçullardan koruyan bir savunma mekanizması olarak rol oynamaktadır. Aynı zamanda bitkiyi nemden ve sıcaklıktan koruma fonksiyonu da bulunmaktadır. Psikoaktif ve tıbbi etkilere sahip reçine, başlıca dişi bitkinin çiçekli dal uçlarında ve braktelerinde bulunmaktadır. Erkek bitkilerin yapraklarında ise reçine salgısı düşük miktardadır. Erkek bitkilerin tepal, stamen ve polenlerinde de kannabinoitler bulunmakla birlikte dişi bitkilerdeki kadar yoğun değildir [27]. Trikomlardan salgılanan reçinedeki THC psikoaktif etkiden sorumlu ana moleküldür ve bitkinin hangi organının kullanıldığına bağlı olarak THC miktarları farklı ürünler elde etmek mümkündür. En bilinen narkotik kullanımlarına örnek olarak ***Bhang*** (erkek ve dişi bitkinin kurutulmuş çiçekli uç kısımları ve yapraklarından elde edilir.), ***Ganja*** (tozlaşmamış dişi bitkilerin kurutulup ezilmesiyle elde edilir.) ve ***Charas*** (bitki uç kısımlarında toplanan ham reçineden organik solvanlarla yapılan ekstraksiyon sonucu elde edilir.) verilebilir.

Kenevirin içerdiği fitokannabinoitlerin oranı, yetiştiği çevresel koşullara göre de (örn: radyasyon, sıcaklık, nem oranı, toprak bileşimi, parazitler vb.) değişmektedir [28]. Yağmurun az olması, düşük nemlilik ve güneş miktarının artması bitkinin sentezlediği psikoaktif maddelerin artışına yol açmaktadır. Yine yapılan çalışmalarda bitkinin UVB maruziyeti sonucunda yapısındaki THC oranının arttığı tespit edilmiştir [29]. Günümüze kadar izole edilmiş olan 104 fitokannabinoit 10 kimyasal alt grupta toplanmıştır. Bunlar: $\boldsymbol{\Delta}^9$-tetrahidrokannabinol, $\boldsymbol{\Delta}^8$-tetrahidrokannabinol, kannabigerol, kannabikromen, kannabidiol, kannabinodiol, kannabielsoin, kannabisiklol, kannabinol, kannabitriol ve diğerleridir [25]. Terpenoitler, flavonoitler, steroitler, fenantrenler, yağ asitleri, nitrojen bileşikleri ve diğer yaygın bitki molekülleri kenevirde kannabinoitlerin dışında tanımlanmış diğer bileşiklerdir [25]. Bu bileşiklerden kenevirdeki majör gruplar olması sebebiyle fitokannabinoitler ve terpenoitler detaylı olarak incelenmiştir.

A) Fitokannabinoitler

Kenevirin spesifik etkilerinin çoğunluğundan kannabinoit cinsi bileşiklerin sorumlu olması sebebiyle araştırmalar da özellikle bu bileşikler üzerinden yürümektedir. Kenevirde bulunan fitokannabinoitlerin sınıflandırılması tablo halinde aşağıda sunulmuştur (Tablo 2).

Tablo 2. Kenevirdeki fitokannabinoitlerin sınıflandırılması [25]

Kimyasal grup	*Bileşik sayısı*
Δ⁹- tetrahidrokannabinol	18
Δ⁸- tetrahidrokannabinol	2
Kannabigerol	17
Kannabikromen	8
Kannabidiol	8
Kannabinodiol	2
Kannabielsoin	5
Kannabisiklol	3
Kannabinol	10
Kannabitriol	9
Diğerleri	22
Toplam	104

Fitokannabinoit sınıfına dahil olan birtakım bileşiklerin özellikleri ve etkileri ile ilgili bilgiler aşağıda açıklanacaktır.

Kannabigerol (Cannabigerol-CBG): Kannabigerol, psikoaktif etkiye sahip olmayan heterojenik yapılı bir bileşiktir [30]. *C. sativa'*nın CBG açısından zengin ekstreleriyle yapılan sıçan çalışmalarında, sıçanların iştahını tetiklediği görülmüştür [31]. Ayrıca sedatif, analjezik ve spazmolitik etkileri de saptanmıştır. 2018'de Smeriglio ve arkadaşları tarafından yapılan araştırmada, CBG ile asit türevlerinin ve CBD ile asit türevlerinin aldoz redüktaz aktivitesini inhibe etmek suretiyle, diyabetik semptomları iyileştirebildiği ortaya çıkarılmıştır [32].

Kannabikromen (Cannabichromene-CBC): CBC kenevirdeki fitokannabinoitlerin en stabil olanları arasında yer almaktadır [33]. Bitkinin vejetatif fazında daha çok bulunur ve CBC miktarları bitkideki Δ^9-THC miktarları ile ilişkilidir, ancak Δ^9-THC gibi CB1 reseptörleri üzerinde etki göstermez [34]. 2013 yılında yapılan araştırmada lipopolisakkarit (LPS) tarafından aktive edilen peritonal makrofajlarda nitrik oksit, IL-10 ve interferon-γ seviyelerini düşürdüğü saptanmıştır. Bu etkiler CBC'nin antienflamatuvar özelliklere sahip olduğunu göstermektedir [35].

Kannabidiol (Cannabidiol-CBD): Kannabidiol'ün yapısı Δ^9-THC'ye benzemekle birlikte, onun gibi kuvvetli CB1 reseptörlerine bağlanma özelliğine sahip değildir [24, 36]. Yapılan bir çalışmada günlük 100-600 mg CBD'nin verildiği hastalarda tremorların %20-50 oranında azaldığı gösterilmiştir [37]. Araştırmalar CBD'nin; antikonvülsan, antispazmodik, anksiyolitik, antiemetik, antiromatizmal ve nöroprotektif özelliklere sahip olduğunu ispatlamıştır [24]. Ayrıca Alzheimer, parkinson, çeşitli kanser türleri ve infertilite üzerinde çalışmalar devam etmektedir [38]. Psikoaktif etki göstermeksizin güçlü

antikonvülsan aktiviteye sahip olmasından dolayı son yıllarda CBD ile yapılan klinik çalışmalar özellikle epilepsi üzerine yoğunlaşmış ve bu çalışmaların sonucunda FDA 2018 yılında "Epidiolex" isimli CBD içeren ilacı, epilepsinin ağır seyreden ve nadir gözlenen iki formu olan Lennox-Gastaut sendromu ve Dravet sendromunun tedavisinde kullanılmak üzere onaylamıştır.

Tetrahidrokannabinol (Tetrahydrocannabinol-THC): Bu gruba dahil olan **Δ^8**-THC ve *cis*-Δ^9-THC gibi farklı türevleri de olmakla birlikte *trans*-Δ^9-THC kenevirdeki psikoaktif etkiden sorumlu olan asıl bileşiktir. **Δ^9**-THC hem psikoaktif etkiyi ortaya çıkaran CB1 ve hem de immünolojik ve antienflamatuvar etkileri ortaya çıkaran CB2 reseptörlerine bağlanabilme özelliğine sahiptir [24]. Kanser kemoterapisine eşlik eden bulantı ve kusmaların tedavisinde kullanılan Dronabinol ve Nabilon, **Δ^9**-THC'nin sentetik türevlerini içermektedir. Yapılan klinik araştırmalar **Δ^9**-THC'nin kanser ağrısı, nöropatik ağrı vb. tedavisi zor ağrı çeşitlerinin giderilmesinde öneminin artacağını göstermektedir. Mevcut araştırmalar **Δ^9**-THC'nin Tourette sendromunda da faydalı olabileceği yönündedir [39, 40]. Suliman ve arkadaşları tarafından 2018'de yapılan çalışmada, düşük doz **Δ^9**-THC'nin yaşlı sıçanların hippokampüsünde anlamlı ölçüde nörogenezisi tetiklediği saptanmıştır [41]. **Δ^9**-THC'nin aynı zamanda intranöronal *β*-amiloid birikimini azalttığı ve antienflamatuvar etki gösterdiği de bulunmuştur [42].

Δ^9-Tetrahidrokannabivarin (Δ^9-Tetrahydrocannabivarin-Δ^9-THCV): **Δ^9**-THCV, THC türevi bir bileşiktir. Düşük dozlarda THC'nin etkisini antagonize ederken [43], yüksek dozlarda ise THC'nin etkilerini agonize ettiği görülmüştür [24]. **Δ^9**-THCV aynı zamanda CB2 reseptörleri üzerinden makrofajlarda LPS tarafından stimüle edilen nitrik oksidi inhibe etmektedir [44, 45].

B) Terpenoitler

Fitokannabinoitler dışında kenevirin çiçeklerinde ve yapraklarında bol miktarda terpen türevi bileşikler sentezlenmektedir ve ayrıca trikomlardan salgılanan reçinenin %10'u da terpenoitlerden oluşmaktadır [46]. Kenevirde 200'den fazla terpen türevi bileşik olduğu düşünülmektedir ve terpenler bitkiye karakteristik kokusunu kazandıran bileşiklerdir. Limonen, mirsen ve pinen en yaygın bulunan ve yüksek uçuculuğa sahip olan terpenlerdir. Tıpkı fitokannabinoitler gibi terpenoitlerin de bitkiyi böceklerden ve otçullardan koruma fonksiyonları vardır ve bu fonksiyonu gerçekleştirmede bitkideki fitokannabinoitlerle sinerjistik etki gösterirler [47].

***D*-Limonen:** Limon vb. narenciye türlerinde yaygın olarak bulunan *d*-limonen, güçlü antioksidan özellikleriyle bilinmektedir [48]. Yapılan fare çalışmalarında *d*-limonen'in hipokampüste ve prefrontal kortekste serotonin ve dopamin aracılığıyla anksiyolitik etki gösterdiği ispatlanmıştır [49]. Buna ek olarak perilik asitin- karaciğerde sentezlenen bir limonen metaboliti- sıçan beyninde stres karşıtı etki

gösterdiği saptanmıştır [50]. Sonraki çalışmalar meme kanseri hücrelerinde apoptozisi tetiklediğini göstermiştir [51]. Ayrıca gastro-özafagal reflüde etkili olduğu kanıtlanmıştır [52].

***β*-Mirsen:** *β*-mirsen kenevirde yaygın olarak bulunan ve sinir sistemi üzerinde çeşitli etkilere sahip bir terpendir. Mirsen fare modellerinde analjezik [53], sedatif, antienflamatuvar etki ile spazmolitik etki göstermiştir [54]. Bu veriler kenevirin analjezik ve antienflamatuvar etkilerine fitokannabinoitlerle birlikte mirsenin de katkı sağladığını göstermektedir [55].

***α*-Pinen:** Kozalaklı bitkilerde ve başka pek çok bitkide bulunabilen bisiklik bir monoterpen olan pinen, böcek kovucu etkileri sayesinde bitkiyi koruyucu bir fonksiyon üstlenmektedir. Yapılan çalışmalarda pek çok modelde pinen; antienflamatuvar, bronkodilatör ve antibakteriyel etkiler göstermiştir [56]. Aynı zamanda asetilkolinesteraz enzimini inhibe edebilme özelliğine de sahiptir [57].

***D*-Linalol:** Linalol, anksiyolitik ve sedatif etkilere sahip bir monoterpen alkoldür. Aynı zamanda lokal anestezik özelliğe de sahiptir. Yapılan fare çalışmalarında yüksek dozlarda antikonvülsan, antiglutamaterjik ve analjezik etkileri ortaya çıkarılmıştır [58].

***β*-Karyofilen:** Karyofilen antienflamatuvar ve antimalaryal etkilere sahip bir seskiterpendir. Antienflamatuvar etkisinden dolayı geçmişte İngiltere'de duodenal ülserlerin tedavisinde kullanılmıştır [59]. Selektif olarak CB2 reseptörlerine bağlanmaktadır.

Karyofilen oksit: Antifungal ve insektisit özelliklere sahip bir seskiterpenoksitidir [60]. Aynı zamanda yapılan *in vitro* deneylerde kan sulandırıcı aktivite de göstermiştir. Kenevirin narkotik köpekleri tarafından koklanıp bulunabilmesine olanak sağlayan karakteristik kokusundan sorumlu esas bileşiktir [61].

Fitol: Kenevirde klorofil ve tokoferollerin degredasyonu ile ortaya çıkan bir diterpen olan fitol, kenevirin rahatlatıcı etkisine neden olan bileşiklerden biridir [62].

Kenevir Tohumu Yağı

Kenevirin fitokannabinoitler ve terpenoitlerce zengin içeriğinin yanı sıra, özellikle son dönemlerde gıda desteği hatta bazı hastalıklarda tedaviye yardımcı olarak kullanılan ve besinsel değeri oldukça yüksek olan kenevir tohumu yağından da bahsetmek yerinde olacaktır.

Bileşimine bakıldığında kenevir tohumu yağı; %25-35 oranında yağ asitleri, %20-25 oranında protein, %20-30 oranında karbonhidrat, %10-15 oranında lif ve çok sayıda eser elementten oluşmaktadır

[63]. Tüm esansiyel amino asitleri ve esansiyel yağ asitlerini içermesi sebebiyle oldukça değerlidir. Yağının büyük çoğunluğunu çoklu doymamış yağ asitleri olan linoleik asit (LA) ve α- linolenik asit (ALA) oluşturmaktadır. Üstelik LA/ALA oranı 3:1'dir ve bu ideal omega6/omega3 oranına tekabül etmektedir [63-65]. Bilindiği gibi omega3 yağ asitlerinin antikanser, antienflamatuvar, antitrombotik, kalp-damar sağlığını koruyucu, antiosteoporotik, antialerjik, sinir sistemi sağlığını koruyucu vb. sayısız faydaları vardır. Bu yağ asitlerinin yanı sıra kenevir tohumu yağı aynı zamanda γ-linolenik asit de içermektedir ve bu besinsel değerini daha da artırmaktadır [65]. Doymamış yağ asiti içeriğinin dışında tıpkı kenevirin kendisi gibi yağı da çeşitli terpenleri ihtiva etmektedir. Yapısındaki bu terpenler yağa antienflamatuvar, antialerjik ve sitoprotektif özellikler de kazandırmaktadır [66]. Bunun dışında kenevir tohumu yağı; β-sitosterol olarak bilinen antiviral, antifungal, antienflamatuvar ve antihiperlipidemik özelliklere sahip bir fitosterol de ihtiva etmektedir [67].

Kenevir tohumu yağı aynı zamanda tokoferoller olarak da bilinen E vitamini türevleri açısından da zengindir. Bu da yağın antioksidan kapasitesini artırmaktadır. Farmakolojik etkileri olan bir diğer bileşeni ise asetilsalisilik asite yapısal olarak oldukça benzeyen metil salisilattır [68]. Metil salisilatın antienflamatuvar, analjezik ve antipiretik etkileri olduğu bilinmektedir. Bunların dışında kenevir tohumu yağı, elde edilmesi esnasında bitkinin trikomlarından salgılanan ve başlıca **Δ**9-THC ile CBD içeren reçine ile de kontamine olması sebebiyle çok az miktarlarda da olsa **Δ**9-THC ve **Δ**9-THC'den daha yüksek miktarlarda CBD içerebilir. Ancak şu ana kadar kenevir tohumu yağı tüketimi ile ilgili istenmeyen bir yan etki rapor edilmemiştir. Hatta özellikle yağdaki CBD'nin farmakolojik etkilerinden dolayı sağlığa olumlu etkileri olacağına ilişkin görüşler de bulunmaktadır [68]. Tüm bu veriler değerlendirildiğinde kenevir tohumu yağının hem besleyici içeriğinin oldukça yüksek olduğunu ve aynı zamanda içerdiği sekonder metabolitlerle sağlığa ekstra katkıları olabileceğini söylemek mümkündür. Aşağıda kenevir tohumu yağının içerdiği bileşik grupları ve bu gruplara ait bileşiklerin miktarları tablo halinde sunulmuştur (Tablo 3).

Kenevir Kökü

Kenevirin özellikle fitokannabinoitleri içeren çiçek, brakte ve yaprak gibi kısımlarıyla yapılan çalışmalar ön plana çıkmasına rağmen, tarihsel kullanımına ve fitokimyasal içeriğine bakıldığında, kenevirin kök kısmının da pek çok farmakolojik etkiye sahip olduğunu ve değerlendirilmesi gerektiğini söylemek mümkündür.

Kenevir kökü; çiçek, brakte ve yaprakların aksine kannabinoitler açısından zengin değildir. Bunun yerine yapısında friedelin ve epifriedelanol gibi triterpenleri, kannabisativin ve anhidrokannabisativin gibi alkaloitleri ve çeşitli sterolleri bulundurmaktadır (Tablo 4).

Yapılan çalışmalarda kenevir kökünde bulunan triterpenik bileşiklerin tarihteki kullanımını doğrulayacak şekilde antienflamatuvar, antipiretik ve analjezik etkileri olduğu saptanmıştır.

Tablo 3. Kenevir Tohum Yağının Makro Kompozisyonu [63, 64, 68]

Bileşenler	**Raporlanan**	**Sonuçlar [68]**
Yağ asitleri		
Linoleik asit	50-70 % a/a	52-62 % a/a
α-Linolenik asit	15-25 % a/a	12-23 % a/a
Oleik asit	10-16 % a/a	8-13 % a/a
Palmitik asit	6-9 % a/a	5-7 % a/a
Stearik asit	2-3 % a/a	1-2 % a/a
γ-Linolenik asit	1-6 % a/a	3-4 % a/a
Eikosanoik asit	0.79-0.81 % a/a	0.39-0.79 % a/a
Eikosenoik asit	0.39-0.41 % a/a	0.51 % a/a
Eikosadienoik asit	0.00-0.09 % a/a	0.00 % a/a
Diğer Bileşenler		
Kannabidiol	-	10 mg/kg
Δ^9-Tetrahidrokannabinol	50 mg/kg	-
Mirsen	-	160 mg/L
β-Karyofillen	-	740 mg/L
β-Sitosterol	-	100-148 g/L
α-Tokoferol	7-80 ppm	Değişken
γ-Tokoferol	710-870 ppm	468 mg/L
Metil salisilat	-	Değişken

Tablo 4. Kenevir Kökündeki Aktif Bileşenler [16]

Kenevir kökündeki aktif bileşenler	**Miktar/Konsantrasyon**
Triterpenoitler	
Friedelin	7.5-12.8 mg/kg
Epifriedelanol	14.5-21.3 mg/kg
N-(*p*-hidroksi-*β*-feniletil)-*p*-hidroksi-*trans*-sinnamamit	1.6 mg/kg
Kolin	-
Alkaloitler	
Kannabisativin	2.5 mg/kg
Anhidrokannabisativin	0.3 mg/kg
Steroller	
Sitostrerol	1.5 % a/a
Kampesterol	0.78 % a/a
Stigmasterol	0.56 % a/a
Monoterpenler	*n*-hekzan ile yapılan kök ekstresinde:
Karvon	77.7 % a/a
Dihidrokarvon	23.3 % a/a

Friedelin ile yapılan çeşitli *in vivo* ve *in vitro* çalışmalarda antienflamatuvar, antipiretik ve analjezik etkilerinin olduğu tespit edilmiştir [69]. Ovariyektomi yapılmış Wistar sıçanlarda ise friedelin östrojenik aktivite göstermiştir [70]. Bunun dışında karaciğer koruyucu ve güçlü antioksidan aktiviteleri de bulunmaktadır [71]. Kenevir kökünde tespit edilen aktif bileşiklerden biri olan N-(*p*-hidroksi-*β*-feniletil)-*p*-hidroksi-*trans*-sinnamamit ise fare deneylerinde analjezik etki göstermiştir [72]. Veriler değerlendirildiğinde kenevir kökünün diğer fitokimyasal bileşenleri ile ilgili fazla çalışma olmadığını ancak farklı farmakolojik aktivitelere sahip olma potansiyeli taşıdığını ve dolayısıyla kenevir kökü ile ilgili ileri çalışmaların yapılması gerektiğini söylemek mümkündür.

Kenevir'in Terapötik Kullanımı

Fitokannabinoitler, terpenler ve burada detayları verilmeyen farklı pek çok etkili bileşiği içermesi sebebiyle, kenevirin pek çok hastalığın tedavisinde kullanılabileceği düşüncesi uzun süredir var olmuştur. Bu konuda yapılan bilimsel çalışmalar özellikle son 5 yılda ciddi oranda artmıştır. Aşağıda kenevirin çeşitli hastalıklar ya da semptomlar üzerindeki iyileştirici etkisiyle ilgili veriler bulunmaktadır.

Alzheimer

Endokannabinoit sistemin Alzheimer gibi yaş ile ilişkili nörodejeneratif hastalıklarda hedef mekanizmalardan biri olduğu düşünülmektedir [73, 74]. Yapılan *in vivo* ve *in vitro* preklinik deneylerde, kannabinoitlerin *β*-amiloid toksisitesini azalttığı ve nöroprotektif etki gösterdiği görülmüştür. Suliman ve arkadaşları tarafından 2018'de yapılan çalışmada, düşük doz **Δ**9-THC'nin yaşlı sıçanların hipokampüsünde anlamlı ölçüde nörogenezisi tetiklediği saptanmıştır [41]. Yapılan *in vitro* deneylerle THC'nin mitokondriyal faaliyetleri düzenlediği tespit edilmiş ve bu sebeple THC'nin Alzheimer hastalığının prognozunu yavaşlatabileceği belirtilmiştir [75]. $\mathbf{\Delta}^9$-THC'nin aynı zamanda intranöronal *β*-amiloid birikimini azalttığı ve antienflamatuvar etki gösterdiği de bulunmuştur [42]. Beyindeki inflamasyon, Alzheimer gibi nörodejeneratif hastalıkların oluşumunda anahtar mekanizmalardan biridir. Bu sebeple $\mathbf{\Delta}^9$-THC gibi hem CB1 hem CB2 reseptör agonistleri dışında selektif CB2 agonistlerinin de nöroinflamasyonu ve oksidatif stres hasarını baskılayabileceği ve azalmış hafıza fonksiyonlarını iyileştirebileceği öngörülmektedir [76, 77].

Anksiyete

Yapılan çalışmalar kenevirin hem anksiyojenik ve hem de anksiyolitik olabileceğini göstermektedir. Düşük dozlarda $\mathbf{\Delta}^9$-THC'nin anksiyolitik etki gösterdiği, buna karşın yüksek dozda $\mathbf{\Delta}^9$-THC'nin ise anksiyojenik etki gösterdiği saptanmıştır. Aynı çalışmada CBD'nin hem hayvanlarda hem de insanlarda limbik ve paralimbik bölgeleri etkileyerek anksiyolitik etki gösterdiği bulunmuştur [78].

Tek doz 600 mg kannabidiol'ün kullanıldığı randomize klinik çalışmada, jeneralize sosyal anksiyete bozukluğu olan 24 katılımcı yer almış ve plasebo ile karşılaştırıldığında kannabidiol'ün bu kişilerin toplum önünde konuşmaya bağlı anksiyetesini giderdiği gösterilmiştir [79]. Bu veriler bize neden dünya genelinde pek çok kişinin anksiyete şikayetlerinden kurtulmak için kenevir kullanımına yöneldiklerini açıklamaktadır [80].

Anoreksiya

Kenevir kullanan kişilerin iştahlarının arttığı bilinen bir gerçektir. Aynı şekilde THC ve diğer CB1 agonistlerinin de iştah artırıcı etkileri olduğu belirtilmektedir [23]. Scherma ve arkadaşlarının 2017 yılında anoreksik sıçan modelinde yaptıkları deneylerde hem doğal **Δ**9-THC ve hem de sentetik formunun leptin sinyalini artırarak ve plazma kortikosteron seviyelerini düşürerek sıçanların kilo kaybını azalttığını ve aktivitelerini artırdığını bulmuşlardır [81]. Pek çok çalışma CB1 stimülasyonunun anoreksik hastalarda ya da kanser kaynaklı ciddi iştahsızlıklarda iştahı artırıp kilo kaybını azaltabileceği yönündedir [82, 83]. Sentetik bir THC türevi olan dronabinol, AIDS hastalarında görülen anoreksi tedavisinde 1992 yılından itibaren kullanılmaktadır [23].

Diyabet

Diyabetik sıçan modelinde kannabidiol'ün makrofajlardan IL-12 salınımını ve plazma IFN-γ seviyelerini düşürmek suretiyle β hücre zedelenmesini yavaşlattığı anlaşılmıştır [84] ve bu sebeple diyabette endokannabinoitlerin önemine ilişkin çalışmalar artmaktadır. Ancak bu ana mekanizmayla ilgili olan kanıtlar henüz yeterli düzeyde değildir. Bununla birlikte diyabette, karaciğerde glutatyon rezervlerinin önemli ölçüde azaldığı [85] ve lipit peroksidasyonunun da arttığı tespit edilmiştir [86]. Diyabetik sıçanlarda yapılan bir çalışmada CBD'nin karaciğerde glutatyon rezervlerini artırdığı ve buna bağlı olarak lipit peroksidasyonunu azalttığı tespit edilmiştir [85]. Bu çalışmada aynı zamanda diyabetik nöropatiden sorumlu olan mekanizmalardan biri olan düşük NGF konsantrasyonlarının da, CBD tedavisiyle arttırılabildiği saptanmıştır. Diğer bir çalışmada CBD ve THC'nin, α-tokoferol ve askorbattan daha yüksek düzeyde antioksidan aktiviteleri olduğu kanıtlanmıştır [87]. Tüm bu veriler değerlendirildiğinde kenevirin özellikle diyabetik nöropati gibi oksidatif mekanizmaların rol aldığı komplikasyonlarda umut verici olduğunu, ancak diğer mekanizmalarla ilgili ileri araştırmalara ihtiyaç duyulduğunu söylemek mümkündür.

Epilepsi

Preklinik pek çok çalışma kannabinoitlerin epilepside kullanılabileceğini göstermiştir. CBD ve CBD'nin propil türevi olan kannabidivarin ile yapılan preklinik ve klinik çalışmalar, antikonvülsan etkiyi ortaya koymakla birlikte bu etkinin mekanizması tam olarak aydınlatılamamıştır [88]. Szaflarski

ve arkadaşlarının 2018'de 72 çocuk ve 60 yetişkin ile yaptıkları çalışmada CBD tedavisinin anlamlı ölçüde epileptik semptomları giderdiği görülmüştür. Bu çalışmaların ardından FDA,"Epidiolex" isimli CBD içeren ilacı, epilepsinin ağır seyreden ve nadir gözlenen iki formu olan Lennox-Gastaut sendromu ve Dravet sendromunun tedavisinde kullanılmak üzere onaylamıştır. Son olarak Aran ve arkadaşları retrospektif çalışmalarında, CBD'nin ASD (atriyal septal defekt)'li çocuklardaki inatçı davranış problemlerinde güvenilir, tolere edilebilir ve etkili bir tedavi olduğunu belirtmişlerdir [89]. Tüm bu veriler göz önüne alındığında, fitokannabinoitlerden özellikle CBD'nin çeşitli nöbetlerde, epilepside ve diğer nörodejeneratif rahatsızlıklarda tedavi potansiyelinin yüksek olduğu görülmektedir [55].

Kemoterapiye Eşlik Eden Bulantı-Kusma

Bilindiği gibi bulantı ve kusma kanser kemoterapisinde kullanılan ilaçların en sık görülen yan etkileri arasında bulunmaktadır. THC ve kannabidiol'ün CB1 reseptörleri üzerinden ve başka birtakım mekanizmalarla bulantı ve kusmayı önlediği bilinmektedir. Dronabinol ve nabilon özellikle konvansiyonel antiemetiklerle sonuç alınamayan kanserli hastalarda, kemoterapiye bağlı bulantı ve kusmanın giderilmesinde 1980'li yıllardan beri klinikte kullanılmaktadır [23].

Kronik Ağrı

Bilindiği gibi kenevirin insan sağlığına yönelik keşfedilen ilk etkilerinden birisi analjezik aktivitesidir. Medikal kenevir kullanıcıları da keneviri en çok bu etkisi sebebiyle kullanmaktadır [90]. Migren, kemik ve eklem ağrısı, menstrüel kramplar vb. pek çok ağrı tipinde etkili olmakla birlikte, özellikle nöropatik ağrı ve kanser ağrısı gibi konvansiyonel tedavilere cevap vermeyen tedavisi güç ağrı çeşitlerinde etkinliği çok daha yüksektir. Bu amaçla THC ve CBD içeren standardize kenevir ekstresi nabiksimols, 2010 yılından beri klinikte kullanılmaktadır [23].

Kolit

Kenevirin farklı preparatlarının gastrointestinal ağrılar, gastroenterit, diyare vb. pek çok gastrointestinal hastalıkta uzun bir süredir kullanıldığı bilinmektedir [91]. CBC, CBD ve CBG gibi fitokannabinoitlerin çeşitli deney modellerinde inflamatuvar bağırsak rahatsızlıklarında antienflamatuvar olarak rol oynadığı çeşitli araştırmacılar tarafından rapor edilmiştir [92-94]. Nallathambi ve arkadaşlarının yaptıkları araştırmada ise, Δ^9-THCA'nın kolon epitelyal hücrelerinde antienflamatuvar özelliği olduğu tespit edilmiştir [95].

Multipl Skleroza Bağlı Spastisite

Günümüzde kas spastisitesi, nöropatik ağrı, tremor, ataksi ve nörojenik mesane gibi multipl skleroz semptomlarının tedavisinde kullanılan ilaçlar tam olarak tedavi edici değildirler ve yan etkileri

sebebiyle kullanımları kısıtlı olabilmektedir [96]. Bu durum tüm dünyada multipl skleroz hastaları tarafından kenevirin artan oranlarda denenmesinin sebebidir. Nabiksimols 2010 yılından sonra başta Kanada ve çoğu Avrupa ülkesi olmak üzere pek çok ülkede, konvansiyonel tedavilere cevap vermeyen multipl skleroza bağlı spastisite ve ağrı tedavisinde kullanılmaya başlanmıştır. Bu tedaviler sırasında psikoaktif yan etki ve direnç oldukça düşük düzeyde rapor edilmiştir ve hastalar tedaviyi iyi tolere etmişlerdir [97].

Şizofreni ve Diğer Psikozlar

Kannabidiol'ün THC'nin psikoaktif etkilerini inhibe ettiği ve aynı zamanda şizofrenideki metabolik, inflamatuvar ve stresle ilişkili semptomlar üzerinde de olumlu etkileri olduğu gösterilmiştir [98]. Leweke ve arkadaşlarının yaptığı randomize çift-kör klinik çalışmada, 42 şizofreni hastasında kannabidiol ile amisülpirid tedavisi 4 haftanın sonunda karşılaştırılmış ve hem psikotik semptomları daha etkili bir şekilde azaltması hem de yan etkilerinin az olması nedeniyle kannabidiol tedavisi daha üstün bulunmuştur [99].

Uyku Bozuklukları

Nabilon ve dronabinol ile yapılan kısa süreli tedavilerin obstrüktif uyku apnesinde yararlı olabileceği ile ilgili çalışmalar bulunmaktadır [100, 101]. Nabilonun aynı zamanda post-travmatik stres bozukluğuna bağlı kabusları azalttığı ve kronik ağrısı olan hastalarda uyku kalitesini artırdığı yapılan çalışmalarla kanıtlanmıştır [102].

Tourette Sendromu

Tourette Sendromu (TS), en az bir yıl süren motor ve vokal tiklerle karakterize, çocukluk çağında % 0.4-0.6 sıklığında görülen nörogelişimsel bir bozukluktur [103]. Bazı klinik çalışmalarla, kronik kenevir ya da dronabinol tedavisinin tedaviye dirençli tourette sendromunda tikleri azalttığı kanıtlanmıştır [39, 40].

SONUÇ VE TARTIŞMA

Görüldüğü gibi kenevir, insanlık tarafından binlerce yıldır insan ve hayvan sağlığı açısından kullanılmış, Çin tababeti ve Ayurveda gibi kendine has felsefesi ve binlerce yıllık birikimi olan geleneksel tıp sistemlerinde de kendine önemli bir yer edinmiş tıbbi bir bitkidir. İlerleyen yıllarda kenevirin psikoaktif etkilerinden dolayı suistimal edilmesi, onun sayısız alandaki üstün kullanımlarına rağmen yasaklanmasına sebep olmuştur. Oysaki kenevirde, yasaklanmasına sebep olan ana bileşik $\mathbf{\Delta}^9$-THC dışında yaklaşık olarak 550 adet bileşik daha saptanmıştır. Üstelik $\mathbf{\Delta}^9$-THC'nin de alzheimer gibi nörodejeneratif hastalıklar, bulantı, kusma, anoreksiya başta olmak üzere pek çok hastalıkta tedavi edici

etkisi olduğu yapılan bilimsel çalışmalarla ortaya çıkmıştır. Makalede bahsi geçen hastalık ya da semptomlardaki etkileri dışında kenevirin; glokom, post-travmatik stres bozukluğu, depresyon, Huntington hastalığı, Parkinson hastalığı, distoni vb. başka hastalıklarda kullanımı ile ilgili çalışmalar da yapılmaktadır.

Tedavi potansiyeli bu kadar yüksek ve çeşitli olan kenevirin, potansiyel etkilerini tamamen ortaya çıkarabilmek, tedavi mekanizmalarını aydınlatabilmek ve kronik tedaviler sırasında olası yan etkilerini öngörebilmek için etkili bileşiklerin etki mekanizmalarının detaylı araştırılması ve ileri klinik çalışmaların yapılması yerinde olacaktır.

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J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 44(1): 137-166, 2020
Doi: 10.33483/jfpau.586114

REVIEW ARTICLE / DERLEME MAKALE

MEDICINAL PLANTS TRADITIONALLY USED TO TREAT SKIN DISEASES IN TURKEY – ECZEMA, PSORIASIS, VITILIGO

TÜRKİYE'DE HALK ARASINDA CİLT HASTALIKLARININ TEDAVİSİNDE KULLANILAN TIBBİ BİTKİLER – EGZAMA, SEDEF HASTALIĞI, VİTİLİGO

Zeynep Busra ERARSLAN[1], Gulay ECEVIT GENC[1], Sukran KULTUR[1]*

[1]Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 34116, Istanbul, Turkey

ABSTRACT

Objective: *Different types of skin diseases negatively affect the lives of many people, both physically and mentally. Here, we aimed to document medicinal plants used as traditional folk medicine for the treatment of eczema, psoriasis and vitiligo in Turkey.*

Material and Method: *Literature search was conducted by screening ethnobotanical studies. Moreover, pharmacological and phytochemical studies were reviewed to assess the efficacy of the most commonly used plants on skin diseases.*

Result and Discussion: *In total, 191 taxa belonging to 134 genera and 64 families were determined as being traditional herbal medicines used in defined skin diseases. Based on their number of citations, the most frequently used medicinal plant species are Juglans regia L. (19), Urtica dioica L. (18), Juniperus oxycedrus L. (9), Chelidonium majus L. (8), Dracunculus vulgaris Schott (7), Ecballium elaterium (L.) A. Rich. (6), Ficus carica L. (6), Malva sylvestris L. (6) and Rosa canina L. (6), respectively. It is concluded that several pharmacological and phytochemical studies support the traditional usage of plants, but further studies are needed. All findings are expected to be the basis for novel pharmaceutical products and a handbook for healthcare professionals.*

Keywords: *Ethnobotany; medicinal plants; skin diseases; traditional medicine; Turkey*

ÖZ

Amaç: *Farklı tipteki cilt hastalıkları birçok insanın yaşamını hem fiziksel hem de ruhsal olarak olumsuz yönde etkiler. Bu çalışmada, Türkiye'de egzama, sedef hastalığı ve vitiligo tedavisinde geleneksel halk ilacı olarak kullanılan tıbbi bitkilerin derlenmesi amaçlanmıştır.*

Gereç ve Yöntem: *Etnobotanik çalışmalar taranarak literatür araştırması yapılmıştır. Bununla birlikte, en sık kullanılan bitkilerin cilt hastalıkları üzerindeki etkinliğini değerlendirmek amacıyla farmakolojik ve fitokimyasal çalışmalar incelenmiştir.*

* **Corresponding Author / Sorumlu Yazar:** Şükran Kültür
***e-mail / e-posta*:** skultur@istanbul.edu.tr

Submitted / Gönderilme: 03.07.2019 **Accepted / Kabul:** 27.09.2019

Sonuç ve Tartışma: *Toplamda 134 cins ve 64 familyaya ait 191 taksonun geleneksel bitkisel ilaçlar olarak egzama, sedef hastalığı ve vitiligo tedavisinde kullanıldığı saptanmıştır. Aldıkları atıf sayısına göre en sık kullanılan tıbbi bitki türleri sırasıyla Juglans regia L. (19), Urtica dioica L. (18), Juniperus oxycedrus L. (9), Chelidonium majus L. (8), Dracunculus vulgaris Schott (7), Ecballium elaterium (L.) A. Rich. (6), Ficus carica L. (6), Malva sylvestris L. (6) ve Rosa canina L. (6) olarak belirlenmiştir. Birçok farmakolojik ve fitokimyasal çalışmanın bitkilerin geleneksel kullanımını desteklediği, ancak daha fazla çalışmaya ihtiyaç duyulduğu sonucuna varılmıştır. Tüm bulguların yeni farmasötik ürünler için temel oluşturması ve sağlık çalışanları için bir başvuru kitabı olması beklenmektedir.*
Anahtar Kelimeler: *Cilt hastalıkları; etnobotanik; geleneksel tıp; tıbbi bitkiler; Türkiye*

INTRODUCTION

Skin diseases are extremely common worldwide and various types of them affect patients mentally and physically. Skin, the largest organ in the human body, forms a physical barrier against harmful effects of chemical, mechanical, thermal and microbial factors. Most people care about their physical appearance which promote mental health by increasing the self-confidence of the person. Due to their visibility, patients primarily require treatment of skin diseases [1, 2]. Some of them tend to be persistent and have a higher prevalence in public. Especially, eczema, psoriasis and vitiligo are most conspicuous skin diseases among them [3, 4].

Eczema is a non-infective chronic inflammatory skin condition that cause severe pruritus and red rash [5, 6]. About 5 to 20% of people are affected by this disease at some stage in their lifetime (especially in childhood) [5, 7]. The cause of the eczema varies according to the type of the disease. Although there are many theories about the reason of the eczema, it is characterized by majority of activated T lymphocytes [7]. Eczema is often accompanied by atopic diseases such as asthma, allergic rhinitis and more [5]. Treatment of eczema helps to relief both skin inflammation and itching. The most commonly used treatment today is topical steroids due to their immunosuppressive activity [7]. Psoriasis, the most common immune-mediated skin disorder, affects millions of people around the world. Its prevalence in the general population is assumed to be 1.5-2% [8, 9]. It can be recognized by circumscribed, red, thickened plaques with an overlying silver white scale [10,11]. Cause of psoriasis couldn't be enlightened despite recent researches [12]. It is known as an organ-specific autoimmune disease which is triggered by an activated cellular immune system [8]. Psoriasis has several treatment modalities which are topical (corticosteroids, anthralin, retinoids, coal tar, etc.), phototherapy (UVA, PUVA, UVB) or systemic-biological (acitretin, cyclosporine, methotrexate) [13]. Vitiligo, whose prevalence is probably lower than other skin diseases, is a hereditary or acquired disease [14, 15]. It is characterized by destruction of melanocytes in the skin that causes sharply demarcated depigmented lesions of variable size and shape [15]. Although its etiology cannot be clearly explained, genetic predisposition, a number of triggering factors such as stress, systemic diseases and physical trauma are also prominent [15, 16]. The main purpose of the treatment is to obtain skin repigmentation. Topical steroids, calcineurin inhibitors, systemic, physical and surgical therapy are therefore needed [15, 17].

In addition to conventional medicine, traditional medicine also plays an important role in the treatment of skin diseases. The therapeutic use of medicinal plants is still popular today as in the past [18]. Herbal remedies are greatly preferred by patients because of the belief that herbal therapy is less harmful than conventional therapy [18, 19]. So far numerous medicinal plants have been reported to be used in skin diseases and many studies that demonstrate effectiveness of herbal remedies have been carried out [1, 2, 20-23].

It is known that the most proper way to find out the plants used in traditional medicine is ethnobotanical studies. Ethnobotanical studies not only document the interaction of mankind with plants in historical process, but also determine medicinal plants which have an important place in human health [24]. Since ancient times local people have benefited primarily from plants to feed and resolve health issues. This extensive knowledge on traditional medicine has attracted the attention of many researchers. As a result, ethobotanical studies, based largely on observation and documentation of the usage of plants by people, have begun to perform. In recent years, there has been an increasing number of reports on ethnobotanical knowledge throughout the world and many of them have been conducted in Turkey. Furthermore, medicinal plants which take place in ethnobotanical studies become recognised as a valuable source for pharmacological studies and pharmaceutical industry [24, 25].

Thanks to the floristic richness that it has, Turkey is one of the leading countries in terms of the accumulation of ethnobotanical knowledge [24, 26]. According to the latest data, more than 10.000 plant species have been identified within its borders and approximately 31% of them is endemic [27]. Geological and geomorphological diversity, geographical location, various climate types and topographical structure are the main factors of the floristic richness and the high rate of endemism in Turkey [28].

On account of the fact that medicinal plants have a significant position in medical therapy, it is necessary to determine those that are effective in dermatological treatment. The aim of our study is to define medicinal plants traditionally used for the treatment of eczema, psoriasis and vitiligo in Turkey. Moreover, in order to identify gaps in research field, the efficiency of medicinal plants that could be candidate for new pharmaceuticals was researched by screening pharmacological and phytochemical studies.

MATERIAL AND METHOD

A literature search was conducted on medicinal plants used for eczema, psoriasis and vitiligo in Turkey by referencing studies published in journals, reports and books from 1994 to 2019. Detailed information about taxa such as botanical, family and local names, used parts, preparation methods, administration/dosage and duration of the treatment, ailments treated/therapeutic effects were given in Table 1. Based on the data, the most frequently used plant families and taxa for each stated disease are

presented in charts. The scientific names of plants and plant families were verified using The International Plant Names Index (IPNI). Furthermore, pharmacological and phytochemical studies are searched with a view to evaluating efficacy of the most commonly used plants on skin diseases.

RESULT AND DISCUSSION

In this review, a total of 191 taxa belonging to 134 genera and 64 families were determined as being traditionally used for the treatment of eczema, psoriasis and vitiligo in Turkey. These medicinal plants are arranged in alphabetical order of their families and presented in Table 1 with the relevant information. The number of taxa used in each disease category (eczema, psoriasis and vitiligo) was found to be as 176 taxa (59 families), 38 taxa (29 families) and 2 taxa (2 families), respectively. Only a minority of the plants (*Juglans regia* L. and *Gundelia tournefortii* L.) are used to treat vitiligo. It has been observed that some taxa used in both diseases, for example, 15 taxa are used in both eczema and psoriasis, and 1 taxon is used in both eczema and vitiligo. According to the results, most widely used medicinal plant species to treat skin diseases (eczema, psoriasis, vitiligo) are *Juglans regia* L. (19), *Urtica dioica* L. (18), *Juniperus oxycedrus* L. (9), *Chelidonium majus* L. (8), *Dracunculus vulgaris* Schott (7), *Ecballium elaterium* (L.) A.Rich. (6), *Ficus carica* L. (6), *Malva sylvestris* L. (6) and *Rosa canina* L. (6), their usage quantities defined by number of citations for each disease category are given in Figure 1. Moreover, the most commonly used plant families for treatment are listed as follows: Asteraceae (20 taxa), Lamiaceae (11 taxa), Polygonaceae (10 taxa), Euphorbiaceae (9 taxa) and Rosaceae (9 taxa) (Figure 2).

People benefit from various plant parts such as leaf, fruit, aerial parts, flower, root, branch, bark, seed and latex. It was found that the leaves are the most commonly used part of the plant (20%), for the treatment of skin diseases. Aerial parts are the second most commonly used part of the plant, accounting for 16%. Also, in some studies the plant parts were not mentioned and the ratio of these unspecified parts was found to be 7% (Figure 3). As a result of review, different forms of preparation like decoction (30%), infusion (11%), mash (8%), powder (3%) and maceration (2%) were detected (Figure 4). These preparations are applied more externally (39%) than internally (32%), the ratio of unspecified application method is 29%.

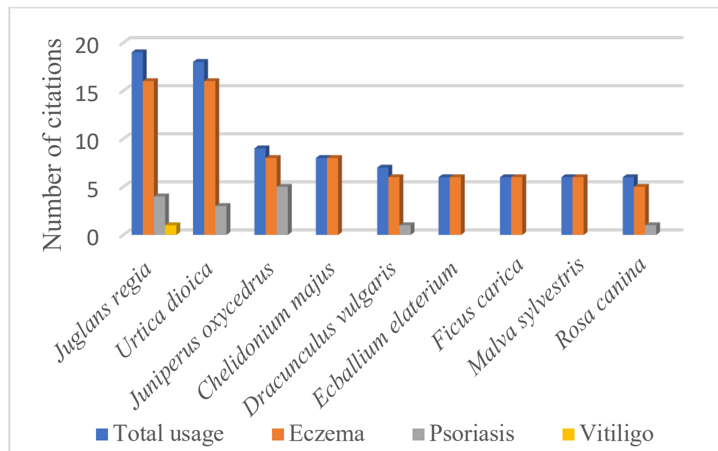

Figure 1. Most frequently used plants in eczema, psoriasis and vitiligo.

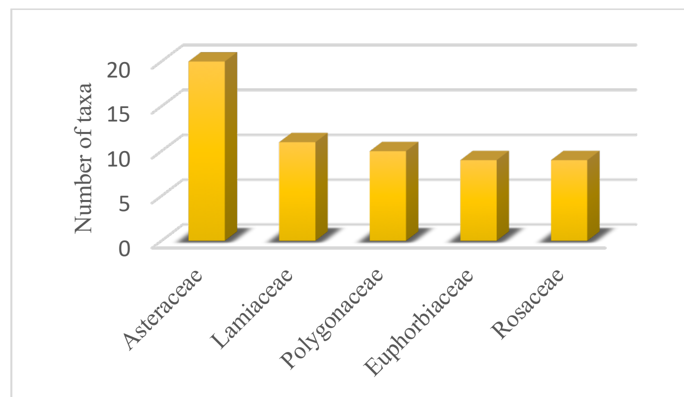

Figure 2. Most frequently used families in eczema, psoriasis and vitiligo.

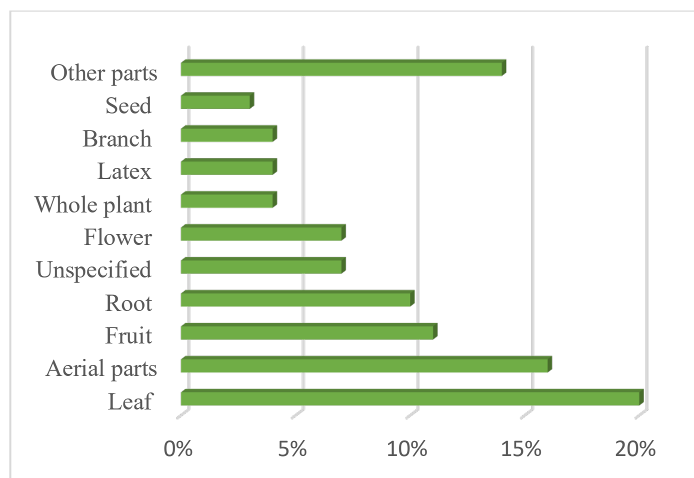

Figure 3. Plant parts used to treat eczema, psoriasis and vitiligo.

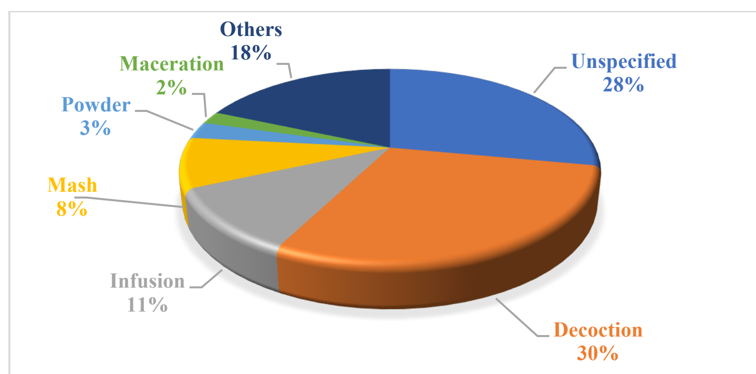

Figure 4. Preparation methods of the plants used to treat eczema, psoriasis and vitiligo.

Although evidence on the use of herbal therapy in dermatologic conditions is relatively sparse, herbal remedies were reported as the most commonly used complementary and alternative medicine method among patients [19,29]. Many studies conducted in the world from past to present have emphasized the usage of plants in skin diseases including eczema, psoriasis and vitiligo. Some commonly used medicinal plants reported in those studies are similar to our findings like *Juglans regia*, *Ecballium elaterium*, *Ficus carica*, *Juniperus oxycedrus*, *Calendula officinalis* L. [2,30-33]. Unlike other countries, only few plant species were determined as a traditional medicine against vitiligo in Turkey [32, 34]. Mabona and Vuuren [20] stated that medicinal plants used for the treatment of skin ailments in South Africa are most commonly prepared by decoction or infusion. As in many other indigenous communities worldwide, the most frequently used part of the plant was determined as leaf. According to our data, while the most widely used part is leaf, the most preferred method of preparation is decoction. Even though plants used in diseases vary according to geography, similarities are observed in their usage patterns.

Knowledge on traditional medicine requires *in vitro*, *in vivo*, and clinical studies to assess and validate the effectiveness and safety of medicinal plants [30]. Recently, many pharmacological studies that confirm the folkloric claim of plants have been carried out. We reported that nine plant species are frequently used in Turkish folk medicine for the treatment of eczema, psoriasis and vitiligo. As these skin diseases are related to inflammation and the most commonly used prescription drugs are corticosteroids, the plants were mainly evaluated for their anti-inflammatory activity. Furthermore, these diseases are immune mediated disorders and dermatologists recommend systemic immunomodulators (methotrexate, tacrolimus, etc.), immunomodulatory activity also can be helpful in treatment [32,35]. Pharmacological and phytochemical studies for this purpose were reviewed and the findings were given below.

Juglans regia L.

Review of ethnobotanical studies revealed that various parts of the *Juglans regia* are traditionally used for the treatment of skin diseases in Turkey. Decoction of branches and fruits are used both externally and internally in psoriasis. Infusion of the leaves is used internally and fruits are eaten to treat vitiligo. Leaf is the most mentioned part which is prepared for eczema. Mainly infusion and decoction of the leaves are applied externally. Also, infusion of fruits is used internally. Moreover, fruits are eaten and its poultice is applied externally to cure eczema. Mashed or boiled fruit pericarp is considered as a herbal medicine. Seeds, leaves and fruits which are prepared with different methods such as maceration, decoction, bathed are used externally or internally for eczema and psoriasis. However, used plant parts, preparation and administration methods were not given in several studies. In some countries, leaves are recommended for the treatment of superficial inflammation of the skin and they are used for relieve the itchiness [2]. It is said to have anti-inflammatory activity owing to the flavonoids such as quercetin, luteolin, hesperidin the plant contains [36,37]. Ethanol extract of leaves showed potent anti-inflammatory activity (as potent as indomethacin) in the carrageenan-induced hind paw edema model in mice without causing any gastric damage [38]. According to Hosseinzadeh et al. [36], the aqueous and ethanol extracts of leaves demonstrated anti-inflammatory activity against acute and especially chronic inflammation. In xylene induced ear edema test in mice, the extracts showed anti-inflammatory effects but the efficacy was decreased in higher doses, especially for the aqueous extract. The extracts also showed anti-inflammatory activity against chronic inflammation in cotton pellet granuloma method in mice [36]. Previous studies reported that *J. regia* can cause irritation and skin hyperpigmentation in topical applications to large areas due to content of Juglone. Nevertheless, contact allergy in humans is very rare [37,39].

Urtica dioica L.

Herbal drugs from *Urtica dioica*, which are formed with diverse preparation methods from various parts of the plant, are traditionally used to treat eczema in Turkey. Decoction of leaves are administered externally and internally. Decoction and infusion of roots are used internally; decoction of sprouts is used externally. While decoction of aerial parts is consumed internally, mashed aerial parts are applied externally to the affected areas. Furthermore, herbal medicines prepared by mashed stem, leaves and seeds are used in both internal and external ways for the treatment of eczema and psoriasis. People also benefit from decoction of whole plant and infusion of leafy branches, seeds and sprouts to treat eczema. Leaves are used externally and decoction of whole plant is used internally to treat psoriasis. Unspecified parts, preparation and administration methods were observed in the ethnobotanical literature review. *U. dioica* is also used to treat skin disorders such as eczema, psoriasis, scabies and pruritus in some countries. Quercetin, which is the most active flavonoid in *U. dioica*, has potent antioxidant and anti-inflammatory activities [40]. It has recently been shown that bioactives such as

adenine, nicotinamide, synephrine and osthole which exhibit anti-inflammatory and anti-allergenic effects are readily absorbed into the body and these compounds cannot to cross the blood-brain barrier [41]. Dar et al. [42] reported that hexane extract of leaves showed a significant inhibition in the rat paw edema assay, comparable to that of indomethacin, though no significant inhibition of inflammation was observed in the groups treated with chloroform, ethyl acetate, methanol and aqueous extracts. Moreover, the major compounds (quercetin, kaempherol and isorhamnetin) isolated from the methanolic extract of the aerial parts have been shown to possess immunomodulatory activities [43]. Recent study reported that *U. dioica* 5% ointment cured all psoriatic lesions with a success rate of 71.693% (38 of 53 patients). The best results were obtained when the lesion is localized [44].

Juniperus oxycedrus L.

Different parts (tar, stem, fruit, branch, gum) of *Juniperus oxycedrus* are traditionally used in the treatment of eczema and psoriasis in Turkey. Tar is applied to the affected skin areas for the treatment of eczema and psoriasis. Tar is mixed with flour to form pills when people prefer the internal usage to treat eczema. Fruits are prepared by infusion or decoction and consumed internally for the treatment of eczema and psoriasis. People also eat raw fruits to cure both diseases. In addition, pitch is administered externally as a plaster to treat psoriasis. Gum is used externally as a salve for eczema and psoriasis. Roots, stem and branches are also used externally to treat eczema and psoriasis but information on preparation method were not given in conducted study. *J. oxycedrus* is also widely used (especially oil of cade, known as juniper tar) as traditional folk remedy for treatment of chronic eczema, psoriasis and other several skin diseases worldwide [33]. Oil of cade has an antiseptic and anti-inflammatory effects owing to high percentage of cadinene (sesquiterpene). Thus, it is usually used to treat psoriasis and other skin dermatitis [45]. Moreno et al. [46] reported that methanol and dichloromethanol extracts of leaves and stems displayed prominent anti-inflammatory activity and inhibition of the rat paw edema induced by carrageenin, flavonoids are believed to be responsible for the pharmacological activity. In another *in vivo* study, the n-butanol subextract prepared from fruit ethanol extract provided a remarkable anti-inflammatory effect [47]. The anti-inflammatory activity was appraised by carrageenan-induced and PGE2-induced hind paw edema models. The methanolic extracts of fruit and leaves showed a significant inhibitory activity in both model at a dose of 100 mg/kg [48].

Chelidonium majus L.

In Turkey, the most frequently used part of the *Chelidonium majus* for the treatment of eczema is latex and it is applied externally. Infusion of aerial parts is consumed internally. Flowering branches of the plant are also traditionally used to treat eczema however, knowledge of preparation and application methods were not addressed in the ethnobotanical study. In some countries, *C. majus* has traditionally been used in the treatment of skin diseases such as eczema and ringworm [49]. In many studies, anti-

inflammatory activity of *C. majus* extracts has been shown using various experimental models of inflammation *in vitro* and *in vivo*. It was highlighted that chemical compounds of alkaloids contained in extracts may be responsible for these anti-inflammatory properties [50-52]. The fraction of quaternary benzophenanthridine alkaloids from roots was evaluated in terms of its anti-inflammatory activity against carrageenan-induced rat paw edema, sanguinarine exhibited a higher anti-inflammatory activity than chelerythrine [53]. Moreover, Yang et al. [52] revealed that the administration of aerial parts inhibited the development of atopic dermatit like symptoms in mice.

Dracunculus vulgaris Schott

While fruits and tubers of *Dracunculus vulgaris* are traditionally used in eczema, only tubers are prepared to treat psoriasis in Turkey. Fruits are eaten and its decoction is applied externally to cure eczema. Tubers are prepared by decoction or they are mashed and cooked for the external usage. Moreover, mashed tubers are swallowed up to treat eczema. In psoriasis treatment, tubers are sliced then consumed. As far as can be determined from a survey of the literature, there are few studies supporting the traditional usage of the plant in skin diseases. In one of these studies, anti-inflammatory activity of petroleum ether, ethyl acetate and methanol extracts of roots were evaluated and petroleum ether extract exhibited the highest activity [54]. In the light of the data obtained from various researches, it is known that the underground parts and aerial parts possess antioxidant activity [55-56].

Ecballium elaterium A. Rich.

Fruits and roots of *Ecballium elaterium* are used as traditional folk medicine for the treatment of eczema. People can benefit from fruits and roots in both internal and external ways for the treatment of eczema. It is known that cutted fruits and roots are used internally. Furthermore, decoction of fruits and roots is mixed with sugar and consumed by local people to treat eczema. Cucurbitacins which is one of the main compounds in *E. elaterium* possess anti-inflammatory activity, among them Cucurbitacin B has the highest effect [57,58]. In study by Yeşilada et al. [58], the anti-inflammatory activities of the fruit juice and its triterpenoid constituent, Cucurbitacin B, were examined in mice against serotonin and bradykinin induced edemas and both exhibited a significant dose-dependent inhibition of edema. According to Bourebaba et al. [59], fruits, flowers and leaves extracts possesses anti-inflammatory effect.

Ficus carica L.

Leaves and latex of *Ficus carica* are traditionally used in the treatment of eczema. While decoction of leaves is used externally, infusion of leaves and latex is consumed in internal usage to treat eczema. The compound responsible for the broad-ranging anti-inflammatory activity of the plant is Luteolin which is the main free flavonoid in *F. carica* [60,61]. Quercetin, another flavonoid exist in

plant, is widely used therapeutically in allergic conditions, including eczema [62]. *In vivo*, petroleum ether, chloroform and ethanol extracts of leaves were investigated for anti-inflammatory activity by carrageenan induced rat paw edema and cotton pellet ranuloma methods. The extracts showed notable anti-inflammatory effect in both acute and chronic inflammation, as compared with the standard drug indomethacin [63]. Furthermore, another study on anti-inflammatory activity of *F. carica* revealed that hydro-alcoholic extract of leaves showed anti-inflammatory effects in the carrageenan induced paw edema in rats [64]. In clinical trial, it has been demostrated that the application of aqueous extract of fruits may provide better treatment results than 1.0% hydrocortisone in mild to moderate atopic dermatitis in pediatric patients [65].

Malva sylvestris L.

Macerations of leaves, flowers, aerial parts and roots of *Malva sylvestris* are used internally for the treatment of eczema in Turkey. Aerial parts and roots are prepared by brewing or mashing to use internal way. Decoction of aerial parts is used internally. Infusion and decoction of leaves are also used to treat eczema. Various parts such as leaves, flowers, aerial parts, buds, shoots are considered as efficient in the treatment of eczema by local people. However, detailed information on preparation and administration of plant parts were not detected in literature review. The consumption of several parts of *M. sylvestris* in the treatment of skin diseases is widespread in many countries as well as Turkey, due to their anti-inflammatory properties [66,67]. In a study which appraised the topical anti-inflammatory effect of the plant, 5, 10 and 20% of *Malva* extract creams were applied on the carragenin-induced edema in rats. A significative inhibition of edema was obtained with the 5% *Malva* cream compared with the placebo. It was demonstrated that this effect was higher than that obtained with a 2% indometacin cream [68]. The topical anti-inflammatory activity was also studied with hydro-alcoholic extract of leaves, it reduced edema by 21% (administered at the dose of 300 g/cm2) on croton oil-induced inflammation in the ears of mice [69]. In a randomized clinical trial, the efficacy of *M. sylvestris* on patients with hand eczema was researched. In group on which *M. sylvestris* 4% ointment was applied twice a day, significant healing was observed without side effects in the treatment of hand eczema in comparison with placebo [70].

Rosa canina L.

In Turkey, several parts (especially aerial parts) of *Rosa canina* are used only internally in the treatment of eczema. Decoctions of fruits, leaves, seeds, roots and galls are traditionally used by local healers, whereas fruit is preferred as infusion. Raw fruits are also eaten to treat eczema. Also, people use decoction of fruits internally. In a previous pharmacological study, the anti-inflammatory activity of the hydro-alcoholic crude extract of fruits was tested on the carrageenin induced rat paw edema assay. It was observed that extract inhibited the development of carrageenin-induced edema, similar to the anti-

inflammatory activity of indomethacin [71]. In another study, the aqueous and ethanol extracts of fruits displayed potent anti-inflammatory activity in several *in vivo* inflammatory models (ethanol extract showed a greater effect than the aqueous extract) [72]. The topical use of *R. canina* seed oil was effective in skin disorders like eczema, as seen in a study including 75 patients using seed oil together with an oral fat-soluble vitamin [73]. It was stated that hydro-alcoholic extract of fruits might have immunomodulator effect *in vivo* [74].

In concluding, even though debate continues as to whether plants are sufficiently effective in the treatment of skin diseases, herbal therapies are still in demand. Their popularity has increased even more in recent years due to belief that medicinal plants are cheaper and safer than allopathic medicines. There is an immense amount of information on herbal therapies which can help researchers, pharmacists and doctors. However, the most important problems encountered in herbal treatment are the lack of standardization of the active substance in the herbal preparations in terms of concentration and purity and the inability to control their side effects. Therefore, the dermatologist who wants to use herbal treatment in practice should know the effects and side effects of the plant.

We have compiled the medicinal plants traditionally used in the treatment of eczema, psoriasis and vitiligo and determined nine of them which are most frequently used. Most considered effects for these plants are anti-inflammatory and immunomodulatory activities. Despite the fact that there have been several pharmacological and phytochemical studies proving the efficacy of the plants in the treatment, more studies are needed for some species. In conclusion, findings reinforce the importance of the ethnobotanical literature as a potential source of pharmaceutical raw materials. It is hoped that this study will lead the way for future studies and guide healthcare professionals.

Table 1. Medicinal plants traditionally used to treat eczema, psoriasis and vitiligo in Turkey.

Botanical name, Family name	Local names	Used parts	Preparation/ Adminstration	Ailments treated (Ref)
Adoxaceae				
Sambucus ebulus L.	Şahmelik, Biza, Sultan otu	R	Dec/Int	E [23]
		F	-/Int	E [75]
Sambucus nigra L.	Melikşah, Piran, Piren, Sultanotu, Sultan, Şahmelek	F	Ma, Ea/Int, Ext	E [76]
		F	-/Int	E [77]
		F	Mix, Ma/Int, Ext	E [78]
Altingiaceae				
Liquidambar orientalis Mill.	Günlük ağacı	Bls	-/-	P [79]
Amaranthaceae				
Beta vulgaris L.	Pancar	L	Dec/-	E [80]
Amaryllidaceae				
Allium sativum L.	Sarımsak	Bl	Spl/Ext	E [81]
Anacardiaceae				
Cotinus coggygria Scop.	Tetre, Tetra otu, Tetere	L	Dec/Ext	E [75, 82, 83, 84]
		L	Inf, Dec/Ext	E [85]
		Leb	Dec/Int	E [75]
Pistacia terebinthus L.	Menengiç ağacı, Kokorağaç	F, Gu	-/-	E [86]
		L	Dec/Int	P [84]

Rhus coriaria L.	Sumak, Sumakotu	-	Spi, Dec, Ma/Int, Ext	E [87]
Apiaceae *Caucalis platycarpos* L.	Pıtırak, Bıtırak	R	-/-	E [88]
Crithmum maritimum L.	Kaya koruğu, Ökseotu	Aer	Pasp/Int, Ext	E [76]
Eryngium campestre var. *virens* (Link) Weins	Gazyağlı diken, Kenger dikeni	Lt Aer	-/Ext Dec/Int, Ext	P [89] E, P [90]
Ferula orientalis L.	Kırkor, Kınkor, Kafkorık	St, R	Dec/Int	P [91]
Malabaila lasiocarpa Boiss.	Bijberhik	Aer, F, L	Dec, Ma/Int, Ext	E [92]
Petroselinum crispum (Mill.) Fuss	Maydanoz	L - Aer	Dec/Int -/- Inf/Int	E [93] E [94] E [81]
Apocynaceae *Nerium oleander* L.	Ay ağacı, Zakkum	Lt Fb	App/Ext Dec/Ext	E [95] E [96]
Araceae *Arum italicum* Mill.	Yılan yastığı, Yılancık, Gabarcık, Yılan kılıcı Domuzyandıran	Tb F Tb - Tb	Co, Dec/- Dec/Int Pla/Int -/- Cut/Int	E [97] E [77] E [98] E [94] E [75]
Arum orientale M. Bieb.	Yılanyastığı	F	-	E [99]
Dracunculus vulgaris Schott	Yılan bıçağı, Yılan yastığı, Kabarcık, Yılankamçısı	F - F Tb Tb Tb Tb F	Dec/Ext -/- -/Int Dec/Ext Ma+Co/Ext Sli/Int Ma/Int Ea/Int	E [100] E [101] E [102] E [102] E [96] P [96] E [103] E [104]
Asparagaceae *Asparagus officinalis* L.	Kuşkonmaz	F, R	Dec/Ext	P [105]
Ruscus aculeatus L.	Tavşan cücüğü, Tavşan göbeği, Tavşan elması	R	Dec/Int	E [106]
Aspleniaceae *Asplenium trichomanes* L. (Syn: *Asplenium trichomanes* subsp. *quadrivalens* D.E.Mey.)	Sıçan otu	Wh	Dec/Ext	E [80]
Asplenium adiantum-nigrum L.	Yar otu	Wh	Dec/Ext	E [80]
Asteraceae *Achillea crithmifolia* Waldst. & Kit.	Mayasıl otu, Güzelnamusotu	Cap	Dec/Int	E [75]
Achillea nobilis subsp. *neilreichii* (A.Kern.) Velen	Mayasıl otu	- Cap	-/- Inf/Int	E [101] E [102]
Achillea setacea Waldst. & Kit.	Ayvadana	Aer	Dec/Int	E [107]
Anthemis tinctoria L. var. *pallida* DC.	Papatya	Fl	Dec/Ext	E [108]
Arctium tomentosum Mill.	Dulavrat otu	L	Ma/Ext	E, P [109]
Artemisia absinthium L.	Tahliş	Aer	Inf/Int, Ext	E [92]
Bellis perennis L.	Koyungözü	-	-/-	E [94]
Calendula officinalis L.	Portakal nergisi, Aynısafa	Fl Aer Aer	Moi/Ext Sal/Ext Sal, Dec/Int, Ext	E, P [88] E, P [109] P [84]
Cichorium intybus L.	Hindiba, Mavihindiba, Ham sütlüvan	Aer Aer Aer	Dec/Int Dec/Int -/-	E, P [77] E [110, 111] E [112, 113]
Cirsium hypoleucum DC.	Vişne kangalı	-	-/-	E [94]
Echinops orientalis Trautv.	Topuz dikeni	Aer	-/-	E [114]
Filago arvensis L.	Paryavşan otu, Çayır güzeli	Aer	Mc, Inf, Ea/Int, Ext	E [115]
Gundelia tournefortii L.	Kenger	S S, Lt, R, St F	Coffee/- Dec, App/Int, Ext Roa/Int	V [116] V [117] E [118]

Matricaria chamomilla L.	Adi papatya, Alman papatyası, Mayıs papatyası	Fl	-/Ext	E [119]
Phagnalon rupestre subsp. *graecum* Batt.	Arı boku	Aer	Dec/Ext	E [120]
Senecio vulgaris L.	Sarı papatya	R R	-/Ext Dec/Ext	E [81] E [110]
Taraxacum campylodes G.E. Haglund (Syn: *Taraxacum officinale* (L.) Weber ex F.H.Wigg.)	Karahindiba, Köpek marulu	L, Sh	Tin/-	E [79]
Taraxacum sp.	Hindiba, Aslan dişi	R, Fl	Tin, Inf/-	E [121]
Tragopogon dubius Scop.	Marşıng, Sıpıng	Wh	Ma/Ext	E [122]
Xanthium strumarium L.	Domuz pıtrağı, Pıtrak, Sıraca otu	R, F	Dec/Int, Ext	E [115]
Berberidaceae *Leontice leontopetalum* L.	Patlangaç, Yer somunu	Tb	Dec/Ext	E [106]
Betulaceae *Alnus glutinosa* (L.) Gaertn.	Kızıl ağaç	Mfl	Dec/Ext	E [98]
Carpinus orientalis Mill.	İstiriç	-	-/-	E [94]
Corylus maxima Mill.	Fındık	L	Dec/Int	E [106]
Boraginaceae *Alkanna megacarpa* A.DC.	Havacıva	R	Inf/Ext	E [123]
Anchusa azurea Mill.	Ballık otu, Sığır dili	Fl, L	Dec/-	E [88]
Heliotropium europaeum L.	Bostanotu, Sirken, Pampul, Siğilotu, Deli sirken	Aer, L	Inf, Lot, Mc, He, Dec/Int, Ext	E [115]
Symphytum officinale L.	Karakafesotu	L	-/Ext	P [23]
Brassicaceae *Lepidium draba* subsp. *chalepense* (L.) P. Fourn. (Syn: *Cardaria draba* subsp. *chalepensis* (L.) O.E.Schulz)	Tahliş	Wh	Ma/Ext	E [92]
Nasturtium officinale R.Br.	Su gedimesi	Aer	Ra/Int	E [124]
Campanulaceae *Campanula involucrata* Aucher ex A.DC.	Sarım çanı	L	-/-	E [125]
Capparaceae *Capparis spinosa* L.	Gebre, Gebere, Kapari, Kedi tırnağı, Kındıra	Bud, F, Fr, R	Ra, Che, Dec, Ma/Int	E [115]
Caryophyllaceae *Gypsophila* sp.	Çöven	R	-/Ext	P [23]
Silene latifolia Poir. [Syn: *Silene alba* subsp. *divaricata* (Rchb.) Walters]	Garagile	Aer	Dec/Ext	E [117]
Stellaria media (L.) Vill.	Kuşotu	Aer	-/-	E, P [126]
Cucurbitaceae *Cucurbita pepo* subsp. *ovifera* (L.) D.S.Decker (Syn: *Cucurbita pepo* L. var. *ovifera* (L.) Bailey)	Sakız kabağı	-	-/-	E [94]
Ecballium elaterium (L.) A. Rich.	Acı kavun, Acıdülek, Şeytan kavunu	F, R R R R, F F R	Dec (+sugar) /Int Dec/Ext -/Int Drug, Dro/Int, Ext Cut/Int Cut/Int	E [75] E [127] E [99] E [128] E [129] E [130]
Momordica charantia L.	Kudretnarı	F F, L F	Rind, Pas/Ext Cut/Int Ma/Int	E, P [120] P [108] E [131]
Cupressaceae *Juniperus oxycedrus* L.	Katran ardıcı	Br (Ta) Ta F Pit	-/Ext + Flour to form pills/Int Inf, Dec, Ra/Int Plas/Ext	E [132] E [111] E, P [133] P [134]

		Ta	-/-	E [88]
		Ta	-/Ext	E, P [135]
		Gu	Sal/Ext	E, P [100]
		Wd	Pix/Ext	E [84]
		R, St, Br, Ta	-/Ext	E, P [110]
Juniperus sabina L.	Kara ardıç	Ta	Mix/Int	E [111]
Juniperus sp.	Ardıç bebesi	F	Dec/Ext	E [136]
Juniperus foetidissima Willd.	Katran ardıcı	Br, F, S	-/-	E, P [112]
Dennstaedtiaceae				
Pteridium aquilinum (L.) Kuhn	Akıllı eğrelti, Eğrelti	-	-/-	E [101]
		L	Inf/Ext	E [102]
Dioscoreaceae				
Dioscorea communis (L.) Caddick & Wilkin (Syn: *Tamus communis* L.)	Sarmaşik	R	Sli/Ext	E [104]
Elaeagnaceae				
Elaeagnus angustifolia L.	İğde	Fl	Dec (+ honey) /-	E [137]
Equisetaceae				
Equisetum arvense L.	Atkuyruğu, Çam otu, Dede sakalı	Wh	Dr/Int	E [80]
		L	-/-	E [127]
		-	-/-	E [79]
		Aer, L, Wh	-/-	E [113]
Equisetum ramosissimum Desf.	At kuyruğu, Tilki kuyruğu, Ulama, Yayılgan otu	Wh	Dec/Ext	E [138]
		L, Sh	Inf	E [79]
Equisetum telmateia Ehrh.	Atkuyruğu, Çam otu, Dede sakalı, Zemberek otu, Tilkikuyruğu	Aer	Dec/Ext	E [81]
		-	-/-	E [94]
Ericaceae				
Arbutus andrachne L.	Sandal ağacı	Wd	He/Ext	E [120]
Calluna vulgaris (L.) Hull	Funda, Süpürge otu	Fl, L	Ol/Ext	E [86]
Erica manipuliflora Salisb.	Funda, Süpürge otu	Fr, Br, Fl	Ol/-	E [112]
Rhododendron ponticum L.	Beyaz kumar	Aer	-/-	P [139]
Vaccinium myrtillus L.	Çoban üzümü	L	-/Ext	E [23]
Euphorbiaceae				
Euphorbia esula subsp. *tommasiniana* (Bertol.) Kuzmanov (Syn: *Euphorbia virgata* Waldst. & Kit.)	Sütlücan, Sütcan	Fl	App/Ext	E [117]
		Fl	-/Ext	E [140]
Euphorbia falcata L.	Sütleğen, Yılan otu	Lt	-/Ext	E [117, 141]
Euphorbia falcata subsp. *falcata* var. *galilaea* (Boiss.) Boiss.	Eğri sütleğen	Lt	-/-	E [126]
Euphorbia helioscopia L.	Feribanotu	-	-/-	E [94]
Euphorbia macroclada Boiss.	Sütleğen, Yılan otu	Lt	-/Ext	E [141, 89]
		Aer	App/Ext	E [117]
Euphorbia marschalliana subsp. *armena* (Prokh.) Oudejans (Syn: *Euphorbia armena* Prokh.)	Dualek, Dıle	Lt	-/Ext	E [91]
Euphorbia orientalis L.	Gezer sütleğen	St, Lt	-/Ext	E [142]
Euphorbia oblongifolia (K. Koch) K. Koch	Haladiza	-	-/-	E [94]
Euphorbia rigida M. Bieb.	Sütlü, Sütlüot, Sütleğen	Lt	-/Ext	E [120]
		Aer	Lot/Ext	E [124]
		Aer, Lt, R	Bat, Lot/Ext	E [115]
Fabaceae				
Lathyrus cicera L.	Deli bakla, Deli bezelye, Keklikbaklası	F	-/Int	P [115]
Ononis spinosa subsp. *leiosperma* (Boiss.) Sirj.	Kayışkıran kökü	Aer	Ma/Ext	E [132]
		Aer	Inf	E, P [143]
Fagaceae				
Quercus cerris L.	Kızılmeşe	F	Dec/Int	E [118]
Gentianaceae				
Centaurium erythraea Rafn	Afyonotu, Kırmızı kantaron	Flb	Inf/Int	E [106]
		Aer	Pow/Int	E [77]
		Wh	Dec/Int	E [93]

Geraniaceae				
Erodium moschatum (L.) L'Hér.	Egzama otu, İğnelik, Leylekayağı, Leylekgagası	Aer	Mix/Ext	E [104]
Hypericaceae				
Hypericum perforatum L.	Sinirotu, Sarı kantaron, Yara otu, Kantaron, Mide otu, Binbirdelik otu	Aer	Dec/Int	E [84]
Hypericum scabrum L.	Kantaron otu, Sarı kantaron, Serkil otu	F	Inf/Ext	E [144]
		Fl, L	Dec, Inf, Pom/Int, Ext	E [145]
Hypericum helianthemoides (Spach) Boiss.	Hoşap kızılotu	Fl	-/-	E [125]
Hypericum montbretii Spach	Mayasıl otu, Çay kanraronu	Aer	Dec/Int	E [77]
		Aer	Dec/-	E [110]
Juglandaceae				
Juglans regia L.	Ceviz	-	-/-	E [146, 101, 94]
		L, F	Inf, Ea/Int	E, V [110]
		L	Inf/Ext	E [109]
		L	-/Ext	E [126, 141]
		L	Inf/-	E [147]
		L	Dec/Ext	E [117]
		L, Frp	Ma/Ext	E [120]
		Frp, L	Dec/-	E [143]
		-	Dec, Dro, Mc/Int	E [87]
		L, F, Frp	-/-	E [113]
		F, L	-/Int, Ext	P [145]
		F	Po/Ext	E [111]
		Imfr	Ma/Ext	E [102]
		S, L, F	Mc, Dec, Bat/Int, Ext	E, P [76]
		Br	Dec/Int, Ext	P [91]
		Br, F	Dec/Int, Ext	P [148]
Lamiaceae				
Ajuga orientalis L.	Mayasıl otu	Fl, St	Dec/-	E [149]
Lavandula stoechas L.	Karabaş otu	Fl, L	Inf/Int	E [150]
Origanum onites L.	Kekik	Aer	Dec, Sal/Ext	E [100]
Teucrium chamaedrys subsp. *lydium* O. Schwarz	Egzama otu, Mahmut otu, Mayasıl otu	L, Fl	-/-	E [151]
		-	-/-	E [101]
		Aer	Inf/Int	E [102]
Teucrium flavum subsp. *hellenicum* Rech.f.	Mayasıl otu	Aer	Dec/Ext	E [106]
Teucrium polium L.	Kısamahmut otu, Mayasılotu	-	-/-	E [101]
		Aer	Inf/-	E [128]
		Aer	Dec/Ext	E [129]
		Aer	Inf/Int	E [102]
Thymbra spicata L.	Zahter, Nuzla kekiği	L	Dec/-	E [147]
		L	Dec/Ext	E [109]
		Aer, Fl	Inf, Lot, Pow, Spi, Che/Int	E [78]
Thymus longicaulis subsp. *chaubardii* (Rchb.f.) Jalas (Syn: *Thymus longicauli* var. *subisophyllus* (Borbás) Jalas)	Kekik otu	Wh	Inf/Int	E [93]
Thymus nummularius M. Bieb. (Syn: *Thymus pseudopulegioides* Klokov & Des.-Shost.)	Limonkekiği	-	-/-	E [94]
Thymus transcaucasicus Ronniger	Kek otu, Catıra	Wh	Inf/-	E [149]
Vitex agnus-castus L.	Ayıt	-	-/-	E [101]
		F	Dec/Int	E [102]
		Sh	Inf/Int	E [102]
Lauraceae				

Laurus nobilis L.	Defne	Of	-/Ext	E [120]
		L	Dec/Ext	E [152]
		L	Dec/Int	E [153]
		L	Inf/Ext	E, P [154]
Linaceae				
Linum usitatissimum L.	Keten	S	Inf/Int	E [150]
Malvaceae				
Alcea apterocarpa Boiss.	Düğmeli çiçek, Dolik	Fl, Aer	-/-	E [114]
Malva neglecta Wallr.	Doğnuk, Ebe gümeci, Hiru	L	Dec, Ma/-	E [136]
		Aer	Dec, Inf, Mc/Int, Ext	P [145]
Malva sylvestris L.	Ebegümeci	L, Fl, Aer, Bud, Sh	-/-	E [113]
		L, Fl	Mc/Int	E [154]
		L	Inf, Dec/-	E [79]
		Aer, R	Inf, Ma, Mc/Int	E [124]
		Aer	Dec/Int	E [103]
		Aer, L	Dec, Inf, Mix, Mc, He/Int	E [115]
Moraceae				
Ficus carica L.	Deli yemiş, İncir, Yemiş	L	Dec/Ext	E [106, 155]
		L	Inf/Int	E [104]
		-	-/-	E [94, 101]
		Lt	Inf/Int	E [102]
Ficus carica subsp. *rupestris* (Hausskn.) Browicz	Yabani incir	Lt	-/Ext	E [141]
Morus alba L.	Dut	L	Inf/Ext	E [103]
Morus nigra L.	Kara dut	F	Dec, Pas/Int	E [148]
		F	Pas/Int	E [91]
Myrtaceae				
Myrtus communis L.	Mersin, Mersin otu	F	-/-	P [113]
Nitrariaceae				
Peganum harmala L.	Üzerlik, Nazar otu	S	-/-	E [86]
		S	Pow/Ext	E [150]
Oleaceae				
Phillyrea latifolia L.	Pırnal	F	-/Int	E [129]
Oxalidaceae				
Oxalis corniculata L.	Ekşi yonca	Aer	-/-	E [121]
Papaveraceae				
Chelidonium majus L.	Sarılık otu, Sultan otu, Mayasılotu, Yaraotu, Temraotu	Lt	-/Ext	E [156]
		Flb	-/-	E [139]
		Lt	-/-	E [157]
		Lt	-/Ext	E [77, 110]
		Lt	Fresh/Ext	E [84]
		Aer	Inf/Int	E [81]
		-	-/-	E [94]
Fumaria asepala Boiss.	Şahtere otu	-	-/-	E [158]
Fumaria capreolata L.	Şahtere	Lt	-/Ext	E [120]
Fumaria officinalis L.	Şahtere	Aer	Inf/Ext	E [159]
		Fl	Inf/Int	E [160]
		Flb	Inf/Ext	E, P [154]
		Aer	Dec/Ext	P [109]
		Wh	Dec/Int	E [96]
Papaver dubium L.	Gelincik	Aer	Inf/Ext	E [135]
Papaver rhoeas L.	Übük	Lt	-/Ext	E [160]
Pedaliaceae				
Sesamum indicum L.	Susam	Oil	Sal/Ext	E [100]
Pinaceae				
Cedrus libani A. Rich.	Katran ağacı	Res	-/Ext	P [105]
Picea orientalis (L.) Peterm.	Ladin	-	-/-	E [94]
Pinus brutia Ten.	Kızılçam	Br, Ba, C, L, Res	Inf, Oil removed/Int, Ext	P [160]
Pinus nigra J.F. Arnold	Çam	Br, L, Res	Inf/Int, Ext	P [161]

Pinus nigra subsp. *pallasiana* (Lamb.) Holmboe	Karaçam	Ta C, Sh	-/Ext Dec/Int	E [162] E [163]
Plantaginaceae *Digitalis ferruginea* L.	Yabani zambak	Wh	Dec/Ext	E [106]
Linaria genistifolia subsp. *confertiflora* (Boiss.) P.H. Davis	Geyşenik	L, Fl	Dec/Ext	E [118]
Plantago sp.	Damarotu, Sinirli ot, Balyaprağı, Siğil otu, Yedidamar otu	L	He/-	E [136]
Plantago lanceolata L.	Yedidamar otu	L -	Ma/Ext -/-	E [93] E [94]
Plantago major L.	Yara otu, Şimşek otu, Kırksinir otu	Aer L Wh L, S L	Mc, Inf, Dec/Int Dec/Int -/- Ma/Ext	E [124] E [164] P [80] E [139] E [93]
Plantago major subsp. *intermedia* (Gilib.) Lange	Sinirotu, Sinirliot, Kırksinirotu, Kırkdamarotu, Çıbanotu	Aer, L	Mc, Inf, Ra, Cat, Pow/Int	E [115]
Plumbaginaceae *Plumbago europaea* L.	Soyulgan otu, Kuduz otu, Sıtma otu, Döven otu	- L R, L Aer	-/- Ma/Ext Dec/Int Ma/Ext	E [101] E [165, 102] E [166] E [120]
Poaceae *Zea mays* L.	Mısır püskülü	Sty	Dec/Ext	P [82]
Polygonaceae *Persicaria decipiens* (R.Br.) K. L Wilson (Syn: *Polygonum salicifolium* Brouss. ex Willd.)	Bibercik	Wh	Dec/Int	E [93]
Persicaria lapathifolia (L.) Delarbre (Syn: *Polygonum lapathifolium* L.)	Dereotu, Dere biberi, Deve sürdeği	Aer	Dec/Ext	E [84]
Polygonum cognatum Meisn.	Madımak, Kuş ekmeği	Fr, L	-/-	E [112]
Rumex crispus L.	Evelik, Kuzu kulağı	L R	Dec, Ea/Int -/-	P [133] E [167]
Rumex cristatus DC.	Yunan labadası	R	-/-	E [167]
Rumex obtusifolius subsp. *subalpinus* (Schur) Celak.	Yabani labada	R	-/-	E [167]
Rumex patientia L.	Akıllı labada	R L	-/- Ma/Ext	E [167] E [109]
Rumex caucasicus Rech.f.	Trisog, Evelik	R	-/-	E [167]
Rumex conglomeratus Murray	Labada, Kuzukulağı	R Aer	-/- Ma/-	E [167] E [137]
Rumex pulcher L.	Ibıdağ	R	Ma, Dec/Ext	E [96]
Portulacaceae *Portulaca oleracea* L.	Semiz otu	Wh	-/-	P [113]
Ranunculaceae *Ficaria verna subsp. ficariiformis* (Rouy & Foucaud) B.Walln. (Syn: *Ranunculus ficaria* subsp. *ficariiformis* (F.W.Schultz) Rouy & Foucaud)	Basur otu	R	-/-	E [168]
Ranunculus kotschyi Boiss.	Giritlalesi	-	-/-	E [94]
Resedaceae *Reseda lutea* L.	Eşek turpu	Fl	Cat/Ext	P [137]
Rhamnaceae *Paliurus spina-christi* Mill.	Karaçalı	S, L	Dec, Inf/Int	E [110]
Rosaceae *Mespilus germanica* L.	Döngel	L	Po/Ext	E [111]
Prunus spinosa L. (Syn: *Prunus spinosa* subsp. *dasyphylla* (Schur) Domin)	Göğem eriği	F	Dec/Int	E [108]
Prunus persica (L.) Batsch	Şeftali	L	Dec/Int	E [93]
Rosa boissieri Crép. (Syn: *Rosa montana* subsp. *woronowii* (Lonacz.) Ö. Nilsson)	Has gül	-	-/-	E [94]

Rosa canina L.	Kuşburnu, Öküz gözü, Gülbubusu, Yabanigül	F, R	Dec/Int	E [107]
		F	Inf/Int	E [98]
		L	Dec/Int	E [84]
		F, L, S, Gl	Dec, Ra/Int	E [115]
		F	Dec/Int	E, P [90, 93]
Rubus caesius L.	Fuska dikeni, Pamuk dikeni, Handuka, Fiskofi	R	Dec/Int	E, P [80]
Rubus idaeus L.	Diken çileği, Böğürtlen	Wh	-/-	E [139]
		R	Dec/Int	E [82]
Rubus sanctus Schreb.	Böğürtlen, Karantı	L	Dec/Int	E [111]
		R	Dec/Int	E [103]
Sanguisorba minor subsp. *balearica* (Bourg. ex Nyman) Muñoz Garm. & C. Navarro (Syn: *Sanguisorba minor* subsp. *muricata* (Spach ex Bonnier & Layens) Briq.)	Kesmeotu	Aer	Dec/Ext	E [104]
Rubiaceae				
Galium verum subsp. *glabrescens* Ehrend.	Beyazsedef otu	Aer	Inf/Int	P [132]
Plocama calabrica (L.f.) M. Backlund & Thulin (Syn: *Putoria calabrica* (L.f.) DC.)	Yumurta boyası	Aer	Ma/Ext	E [120]
Rubia tinctorum L.	Yapışkan otu	Aer, Sh	-/-	E [113]
Rutaceae				
Ruta graveolens L.	Sedef otu, Biro otu	L	-/-	E, P [86]
		L	Ma/Ext	E [84]
Salicaceae				
Salix alba L.	Söğüt ağacı, Söğüt, Salkımsöğüt	St	Dec/Ext	E [84]
		Ba	-/Ext	P [23]
Sapindaceae				
Acer campestre L.	Akçaağaç	Gl	-/Ext	E [111]
Scrophulariaceae				
Verbascum sp.	Öküz kuyruğu, Sığırkuyruğu	L, Fl	Inf/Ext	E [118]
Verbascum thapsus L.	Burunca	Fl	-/-	E [126]
Smilacaceae				
Smilax aspera L.	Sperne	F, L	Dec/-	E [137]
		Sp	-/Ext	E [120]
Smilax excelsa L.	Öz diken, Diken sarmaşık	F, Sh, Br	Inf, Fr, Co/-	E [97]
		Sp	-/Ext	E [120]
Solanaceae				
Datura stramonium L.	Datura, Tatala	S	-/Int	E [77, 81]
Lycium anatolicum A. Baytop & R.R. Mill	Yapışkan çalı	Sp	-/Ext	E [144]
Mandragora officinarum L.	Adamotu	R	Pow/Int	E [86]
Nicotiana rustica L.	Deli tütün	L	Ma/Ext	E [150]
Solanum tuberosum L.	Patates	Tb	Co, Ma/Ext	E [169]
Urticaceae				
Parietaria judaica L.	Yapışkan otu	Aer	Po/Ext	E [170]
Urtica sp.	Isırgan	L	Inf/-	E [136]
Urtica dioica L.	Isırgan otu	-	-/-	E [94, 146]
		Aer	Ma/Ext	E [120]
		St, L, S	Ma/Int, Ext	E, P [133]
		Aer, L	Dec/Int	E [152]
		Leb, S, Spr	Inf/-	E [171]
		St, L, Fl	-/Int	E [172]
		L, St	Dec/Ext	E [159]
		St, L	-/Int	E [131]
		L, Br	-/-	E [121]
		Aer, S, R	Inf, Dec, Mix, Dec, He/Int	E [115]
		L	Dec/-	E [89]
		Spr	Dec/Int	E [170]
		Wh	Dec/Int	E, P [93]
		Aer	Dec/Int	E [93]

		R	Dec/Int	E [90]
		Aer	-/-	E [168]
		L	Mix/Int	E [111]
		L	-/Ext	P [23]
Urtica urens L.	Isırgan, Dalağan, Dalağazotu	Aer	Dec/Int	E [85]
		R	Dec/Int	E [90]
Violaceae				
Viola tricolor L.	Hercai menekşe	Aer	Inf/-	E [147]
		Aer	Inf/Ext	E [109]
		Wh	Inf/Int, Ext	P [23]
		Aer	Dec/Int	E [172]
Xanthorrhoeaceae				
Asphodeline baytopiae Tuzlaci	İnce çiriş, İnce çiriş otu	R	Ma, Pow/Ext	E [76, 78]
Asphodeline brevicaulis (Bertol.) J. Gay ex Baker	Çiriş, Çiriş otu	R	Ma, Pow/Ext	E [78]
Asphodeline taurica (Pall.) Endl.	Çiriş, Çiriş otu	R	Ma, Pow/Ext	E [78]
Asphodelus aestivus Brot.	Çiriş, Hıdrellez kamçısı	-	-/-	E [101]
		R	-/-	E [168]
		R	-/Int	E [102]
		Tb	Dec/Int	E [103]
Asphodelus ayardii Jahand. & Maire	Çiriş, Çiriş otu	R	Ma, Pow/Ext	E [76, 78]
Asphodelus fistulosus L.	Çiriş, Çiriş otu	R	Ma, Pow/Ext	E [78]
Eremurus spectabilis M. Bieb.	Çiriş, Gulık	L	Fr, Po/Int, Ext	E [117]
		L	Ma/Ext	E [141]
Zygophyllaceae				
Tribulus terrestris L.	Çobançökerten, Demirdikeni, Demir otu, Kızılbacak, Demirpıtrağı	Aer	Dec/Int	E [104, 123]
		Aer	Dec, Inf, Lot/Int	E [124]
		Aer, Fl, Spi	Dec/Int	E [115]

Abbreviations: External use Ext; Internal use Int; Eczema E, Psoriasis P; Vitiligo V; Aerial parts Aer; Balsam Bls; Bark Ba; Branch Br; Bulb Bl; Capitulum Cap; Cone C; Flower Fl; Flowering branch Flb; Flowering bud Fb; Fruit F; Fruit oil Of; Fruit pericarp Frp; Gall Gl; Gum Gu; Immature fruit Imfr; Latex Lt; Leaf L; Leafy branch Leb; Male flower Mfl; Pitch Pit; Raw Fruit Fr; Resin Res; Root R; Sap Sp; Seed S; Shoot Sh; Spicule Spi; Sprout Spr; Stem St; Style Sty; Tar Ta; Tuber Tb; Whole plant Wh; Wood Wd; Cataplasm Cat; Chewing Che; Cooking Co; Direct application App; Dried Dr; Dropped Dro; Eaten Ea; Heated He; Lotion Lot; Maceration Mc; Mash Ma; Mixed Mix; Moisturizer Moi; Ointment with olive oil Ol; Paste Pas; Pickle paste Pasp; Planed Pla; Plaster Plas; Pomade pom; Pounded Po; Powdered Pow; Raw Ra; Roasted Roa; Salve Sal; Skin bath Bat; Sliced Sli; Spice Spi; Split Spl; Tincture Tin.

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Ankara Ecz. Fak. Derg. / J. Fac. Pharm. Ankara, 44(1): 167-187, 2020
Doi: 10.33483/jfpau.523804

DERLEME MAKALE / REVIEW ARTICLE

ALZHEİMER HASTALIĞININ GELİŞİMİNDE BİYOLOJİK AJANLARIN OLASI ETKİLERİ

THE POSSIBLE EFFECTS OF BIOLOGICAL AGENTS ON THE DEVELOPMENT OF ALZHEIMER'S DISEASE

Ayça ADALI[1], Anıl YİRÜN[1,2], Belma KOÇER GÜMÜŞEL[1], Pınar ERKEKOĞLU[1,*]

[1]Hacettepe Üniversitesi Eczacılık Fakültesi Farmasötik Toksikoloji Anabilim Dalı, Sıhhiye, 06100, Ankara, TÜRKİYE

[2]Çukurova Üniversitesi Eczacılık Fakültesi Farmasötik Toksikoloji Anabilim Dalı, Adana, 01250, TÜRKİYE

ÖZ

Amaç: *Dünya çapında artan nüfusa bağlı olarak yaşlı nüfusun artması Alzheimer hastalığı (AH)'nin görülme sıklığının artmasına yol açmıştır. Son yıllarda, farklı demans türleri ve özellikle de AH konusundaki bilimsel araştırmalar hızlanmıştır. Hastalığın multi-genetik nedenlerle ortaya çıkabileceği bilinse de, birçok biyolojik ajana maruziyetin de AH'nin patofizyolojisinde rol oynayabileceği belirtilmektedir. Literatürde Herpes simplex virüs tip-1 (HSV-1), insan immünoyetmezlik virüsü 1 (HIV-1) ve Helicobacter pylori gibi enfeksiyon etkenlerinin ve vücutta endojen olarak bulunan prion proteinlerinin bu hastalığının gelişimine yol açabileceği belirtilmektedir. Diğer taraftan, diyabet gibi hastalıkların da AH'nin gelişimini hızlandırabileceğine dair araştırmalar bulunmaktadır. Bu derlemede, AH'nin genel özelliklerinden bahsedilecek; bu hastalığın patofizyolojinde rol oynayabileceği bildirilen biyolojik ajanlardan ve bu konuda yapılmış çalışmalardan söz edilmesi amaçlanmıştır.*

Sonuç ve Tartışma: *AH'nin ortaya çıkmasında genetik faktörlerin yanı sıra, birçok biyolojik ajanın rolü olabileceği belirtilebilir. Bu konuda daha ileri düzeyde ve mekanistik çalışmalara ihtiyaç duyulmaktadır.*

Anahtar Kelimeler: *Alzheimer hastalığı, Helicobacter pylori, Herpes simplex virüs tip-1 (HSV-1), insan immünoyetmezlik virüsü 1 (HIV-1)*

ABSTRACT

Objective: *The increase in elderly population due to the increasing population worldwide has led to an increase in the prevalance of Alzheimer's disease (AD). In the last years, scientific studies on different types of dementia and particularly on AD have accelerated. It is known that this disease has multi-genetic grounds. However, exposure to several biological agents are also suggested to play roles in the pathophysiology of AD. There are studies in literature suggesting that infections like Herpes simplex virus*

[*] **Sorumlu Yazar / Corresponding Author:** Pınar Erkekoğlu
e-posta / e-mail: erkekp@yahoo.com

Gönderilme / Submitted: 07.02.2019 **Kabul / Accepted:** 05.12.2019

tip-1 (HSV-1), human immunodeficiency virus type 1 (HIV-1), Helicobacter pylori and endogenous prion poteins can contribute to the development of this disease. On the other hand, there are studies suggesting that diseases like diabetes can expedite the development of AD. In this review, we aimed to focus on the the general properties of AD and will also mention the biological agents that are suggested to play roles in the pathophysiology of AD and the studies performed on these agents.

Result and Discussion: *Other than genetic factors, several biological agents may play roles in the emerging of AH. AH'nin ortaya çıkmasında genetik faktörlerin yanı sıra, birçok biyolojik ajanın rolü olabileceği belirtilebilir. Comprehensive and mechanistic studies are needed on this subject.*

Keywords: *Alzheimer's disease, Helicobacter pylori, Herpes simplex virus type-1 (HSV-1), human immunodeficiency virus type 1 (HIV-1)*

GİRİŞ

Sağlık hizmetlerinin yaygınlaşması ve teknolojik ilerlemeler sonucunda dünyada ölüm hızı giderek düşmektedir. Böylece ortalama insan ömrü uzamış ve tüm dünya genelinde yaşlı nüfus artmıştır. Ülkemizde 1960'lı yıllarda yaş ortalaması 48 iken, bu ortalama 2011 yılında 72'ye ulaşmıştır. Dünya Sağlık Örgütü (DSÖ) verilerine göre, ilerleyen yıllarda gelişmekte olan ülkelerin nüfuslarında %95 oranında bir artış beklenirken, yaşlı nüfus için %240 oranında artış olacağı öngörülmektedir [1-3].

Dünyada yaklaşık 30 milyon kişi Alzheimer hastalığı (AH)'den etkilenmektedir [1]. AH, santral sinir sisteminin çeşitli kısımlarında nöron ve sinaps kayıpları sonucu ortaya çıkan, çeşitli davranışsal bozukluklar, bilişsel işlevlerde azalma, öz bakım yetersizlikleri ile karakterize ilerleyici bir nörodejeneratif hastalıktır. AH, beyinde bellekle ilgili yapılardan en önemlisi olan hipokampus bölgesinden başlayarak beynin korteksinde frontal, parietal ve temporal alanlara da yayılarak konuşma, anlama ve plan yapmada bozulmalar, yakın geçmişini unutma ve mekân bilgisinde bozulmalara neden olur [4]. Hastaların çoğunda duygu durumunda bozulmalar, içe kapanma, depresyona eğilim de gözlenebilir [5]. Bu belirtilerin ortaya çıkmasında beyindeki hücrelerin ölümünün yanı sıra, beyinde hücreler arası iletişimde görev alan nörotransmitterlerden biri olan asetilkolinin (ACh) salgılanmasındaki azalmalar da önemli rol oynar. AH geri dönüşümlü bir hastalık olmadığından, sadece hastalığın ilerleyişini durdurmak veya azaltmak için donepezil, rivastigmin gibi asetilkolinesteraz inhibitörü ilaçlar kullanılmasıyla tedavi sağlanır. Hastalığın seyri kişiden kişiye farklılıklar gösterir. Hastalığın erken evresindeki ilk uyarı bulguları genellikle hastalığa spesifik olmadığından, hastada herhangi bir sorunun varlığının anlaşılması zor olabilir ve zaman alabilir [4]. AH'nin tanısı, klinik muayene ve bazı mental durum değerlendirme testleri ile konulabilir [5].

AH'nın sık görülen bir hastalık haline gelmesindeki etmenler kısaca şöyle sıralanabilir:

- Yaşlı nüfustaki artış
- Diyabet, kalp hastalığı gibi hastalıkların sık görülmesi
- Kişilerin bazı enfeksiyonlara maruz kalması gibi faktörler
- Genetik yatkınlık

Genetik yatkınlık, AH gelişimde sözü edilmesi gereken en önemli faktörlerden biridir. AH'ye sahip birinci derece yakın akrabaları olan bireylerde hastalık riskinin arttığı belirlenmiştir [5,6]

Bu derlemenin amacı; AH'nin fizyopatolojisinde rol oynadığı düşünülen prionlar, bakteriyel ve viral enfeksiyonlar ve diğer bazı hastalıklar gibi biyolojik etkenlerin olası rolünü değerlendirmektir.

Alzheimer Hastalığı

Dünyada 30 milyon civarında Alzheimer hastası bulunmaktadır. Türkiye'de, ise bu sayının yaklaşık 350 bin olduğu belirtilmektedir.[1] AH'nin en büyük risk faktörü yaştır ve araştırmalara göre, AH 60 yaş üstü insanlarda daha fazla görülmektedir. Bu nedenle yaşlı popülasyonun fazla olduğu ülkelerde hastalığın görülme oranı daha yüksektir. Günümüzde tüm dünyada, özellikle gelişmiş ülkelerde en hızlı artan yaş grubunu 65 yaş ve üstü kişiler oluşturmaktadır. AH'nin prevalansı 65 yaş üzerinde %6-10, 85 yaş üzerinde %30-47'dir. Prevalans, 60 yaşından sonra her beş senede bir iki katına çıkar. Ayrıca, AH gelişiminin cinsiyete bağlı olarak da değişebileceği ve kadınlarda görülme sıklığının erkeklere oranla daha fazla olduğu belirtilmiştir [7-9].

Türkiye'de 1960'lı yıllarda yaş ortalaması 48 iken, bu rakam 2011 yılında 72'ye ulaşmıştır. DSÖ, 2050'lerde 65 yaş üstü nüfusun %20 civarında olacağını tahmin etmektedir. Türkiye'nin bu yıllarda en çok Alzheimer hastası olacak ülkeler arasında 4. sırada yer alacağı belirtilmektedir. Ülkemizde 65 ve üzerindeki nüfus 2012 yılında 5.682.003 kişi iken, 2016 yılında %17,1 artarak 6.651.503 kişi olmuştur. Yaşlı nüfusun toplam nüfus içindeki oranı ise 2012 yılında %7,5 iken, 2016 yılında %8,3'e yükselmiştir [10]. Türkiye Alzheimer Derneği ve İstanbul Üniversitesinin yaptığı ortak çalışmada, İstanbul'un Kadıköy bölgesi taranmış ve çalışmada 70 yaş üzerinde AH görülme sıklığı %10 oranında bulunmuştur [3].

Ölüm nedeni istatistiklerine göre, AH'den ölen yaşlıların sayısı 2011-2015 yılları arasında yaklaşık 2 kat artmıştır. Dünya genelinde AH'den ölen yaşlıların oranı 2011 yılında %2,9 iken bu oran 2015 yılında %4,3'e yükselmiştir. AH'den ölen yaşlıların oranı cinsiyet bazında incelendiğinde, her iki cinsiyette de artış olduğu görülmüştür. AH'den ölen yaşlıların oranı, 2011 yılında erkeklerde %2,4, kadınlarda %3,4 iken bu oranlar 2015 yılında erkeklerde %3,5'e, kadınlarda ise %5,2'ye yükselmiştir [11].

Risk Faktörleri

Alzheimer hastalığı için bilinen risk faktörleri şunlardır [12]:

- Yaş
- Cinsiyet
- Demans
- Genetik faktörler [Apolipoprotein E (ApoE) e4 geninin varlığı]

- Down sendromu
- Kafa travması
- Majör depresyon öyküsü
- Ateroskleroz
- Hipertansiyon veya hipotansiyon
- Diabetes mellitus (DM)
- Sigara
- İmmünolojik faktörler
- Metabolik faktörler (amiloid-β metabolizması)
- İnflamatuvar faktörler
- Sistemik hastalıklar
- Bulaşıcı faktörler ve bazı zehirli koşullara maruz kalma
- Zehirlenmeler (örneğin toksik metallerle, böcek ilaçlarıyla zehirlenmeler)

Belirtiler

Yaşlanma ile insanlarda unutkanlık, konuşmada yavaşlama, halsizlik, mutsuzluk ve uyku halinin artması görülebilir. Ancak, bu belirtiler AH'nin belirtileri olabilir ve AH gelip geçici bir hastalık değildir. Kişiye göre değişken bir şekilde ilerleme gösterir; hastaların yaşamını oldukça zorlaştırır ve yaşam kalitesini ciddi anlamda azaltır [1].

AH'nin en önemli özelliği spesifik belirtileri olmadan başlaması ve yavaş seyirli olmasıdır. Hastalar ve yakınları yakınmaların başlangıç zamanını kesin olarak söyleyememektedir. Yaşlılıkta unutkanlığın normal olduğu düşüncesi ile AH'nin başlangıcının tespit edilmesi zorlaşır. Bu nedenle hekime başvuru zamanı da gecikir [13].

AH'nin seyrinde yakınma ve bulguların şiddetine göre klinik tablo üçe ayrılır [13-15]:

Erken Evre; Bu evrede bellek bozukluğu ve yeni bir bilginin öğrenilmesinde güçlük görülmesi gözlenir. Soruların tekrar tekrar sorulması, eşyaların yerini karıştırmak, konuşmaların tekrarlanması, isimleri unutmak, anahtarı evde unutup dışarı çıkmak, konuşma esnasında kelime bulmada güçlük çekmek, kullanımı kısmen karmaşık olan cihazları kullanmayı öğrenememek, varsa hobileri gerçekleştirmekte zorlanmalar erken evrenin genel özellikleridir. Genel olarak akıl yürütme becerileri etkilenmiştir. Davranışsal sorunlar fazla görülmez. Bu belirtiler nedeniyle bazı hastalarda reaktif depresyon gelişebilir.

Orta Evre; Başlangıç belirtilerinin ağırlaşmasıyla günlük yaşam aktivitelerindeki çoğu işlevde kayıp görülür. Yeni bir bilgi öğrenme imkânsız hale gelmiştir. Var olan bilgiler de yakın geçmişten başlayarak yavaş yavaş kaybolmaya başlar. Dışarı çıktıklarında kaybolmalar olabilir, ev dışında tek başlarına dolaşamazlar. Parasal ve alışveriş işlerini tek başlarına halledemezler. Karşılıklı sohbet

gerçekleştiremeyecek kadar dilsel işlevlerde bozulmalar görülür. Gece-gündüz ayrımının bozulması, zaman oryantasyon bozuklukları görülür. Bu evrede ajitasyon, yerinde duramama, saldırganlık, suçlayıcı davranışlar, şüphecilik gibi psikiyatrik belirtiler de ortaya çıkabilir.

İleri Evre; Hastanın en temel günlük yaşam aktivitelerini dahi sürdürebilmesi için bir başkasının yardımı gerekmektedir. Giyinme, yıkanma, beslenme gibi ihtiyaçlar tamamen bağımlı hale gelmiştir. Sosyal ortamlara çıkmakta sorunlar olur. Yakın akrabalarını tanıyamama durumu ortaya çıkar; hatta hasta aynada kendisini bile tanımayabilir. Zihinsel işlevler en düşük düzeye inmiştir. Konuşmalarında sadece anlamsız kelimeler veya sesler vardır; bu nedenle hastayı anlamak oldukça zordur. Bu evrenin sonuna doğru hastalar yatağa bağımlı hale gelirler. Yatak yarası enfeksiyonları, akciğer embolisi veya enfeksiyonu, üriner enfeksiyon, beslenme bozukluklarının yarattığı komplikasyonlar başlıca ölüm nedenlerini oluşturur.

Patofizyoloji

Alzheimer hastalığı santral sinir sistemi (SSS)'nin bazı kısımlarında nöron ve sinaps kaybı oluşumuyla ortaya çıkan ilerleyici nörodejeneratif bir hastalıktır. AH'nin gelişiminde en önemli etkenlerden biri genetik yatkınlıktır. Birden fazla yolakta, pek çok seviyede bozukluk ile kendini gösteren kompleks bir hastalıktır. Hastalıkla ilgili iki temel patolojik bulgu tanımlanmıştır. Bunlar, amiloid plaklar ve nörofibriler yumaklardır [5,16]. AH ile ilişkili olduğu saptanmış başlıca genler şunlardır:

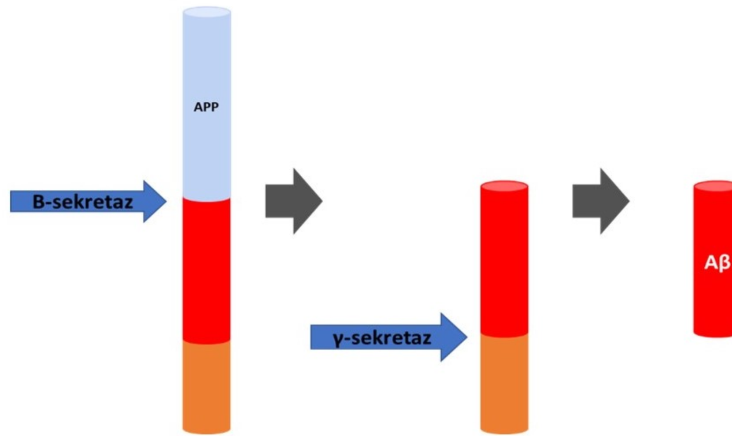
- Amiloid prekürsör protein (APP)
- Presenilin 1 (PS1)
- Presenilin 2 (PS2)
- ApoE

Erken başlangıçlı AH denilen ve 65 yaş öncesi görülen AH'den sorumlu genler APP, PS1, PS2; geç başlangıçlı AH'den (65 yaş sonrası) sorumlu gen ise, ApoE olarak belirlenmiştir. 21. kromozomda yer alan APP ve 1. kromozomda yer alan PS2 genlerinin mutasyonunun, AH'de amiloid-β peptid seviyelerini arttırdığı görülmüştür. Bu mutasyonlar, APP geninin hatalı bölünmesi, toksik amiloid-β üretimine, *Tau* proteinlerinin hiperfosforilasyonuna ve nörofibriler yumak (NFY) oluşumuna neden olur. Erken başlangıçlı ailesel AH'nin %2-3'ü APP, %20'si PS2, %70-80'i PS1 gen mutasyonuna bağlı olarak ortaya çıkmaktadır [5].

ApoE, kandaki lipoproteinlerde bulunur ve yüksek trigliserit içerikli lipoproteinlerin katabolizmasından sorumludur. Esas olarak karaciğer ve makrofajlarda üretilir. Kolesterol metabolizmasında görevlidir. ApoE geninin 3 adet aleli vardır. Bunlar; e2, e3, e4'tür [16].

Geç başlangıçlı AH oluşumunda 19. Kromozomda bulunan ApoE geninin etkili olduğu bildirilmiştir. Bu genin e2 aleli koruyucu aleldir ve AH riskini azaltmaktadır. ApoE e4 aleli ise, amiloid

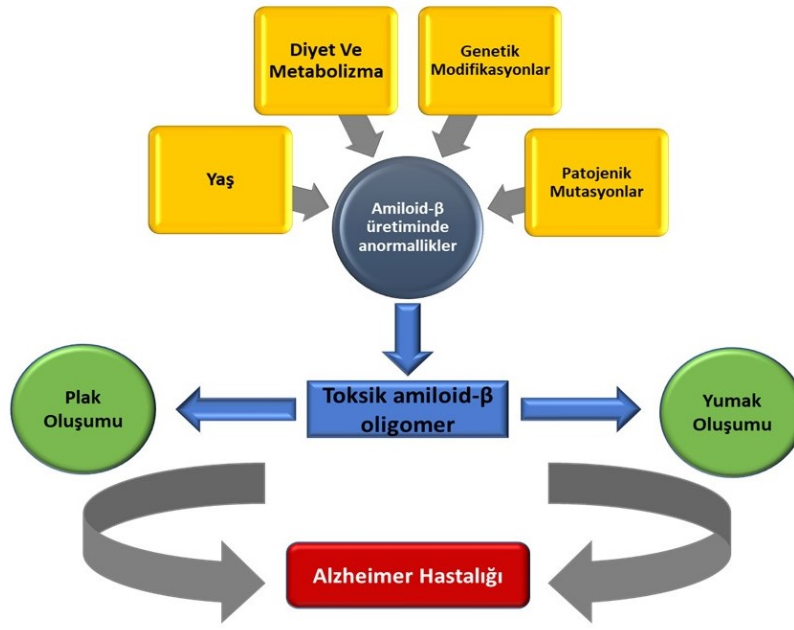
plak ve NFY oluşumuna neden olarak AH riskini arttırdığı bildirilmiştir. ApoE genindeki mutasyonlar geç başlangıçlı AH'nın yaklaşık %50-80'ini oluşturur. ApoE e4, AH'nin ortaya çıkmasında tek başına yeterli bir faktör değildir [5,16]. AH'de en bilinen patolojik bulgu amiloid plaklardır. Amiloid prekürsör proteinin proteolitik yıkımı sonucu oluşan amiloid-β peptidleri hastalık ile ilişkilendirilir. APP proteini, α-, β-, γ- sekretaz adı verilen proteolitik enzimler tarafından metabolize edilir. İlk basamakta APP, α-sekretaz (nörotoksik olmayan, normal kesim) veya β-sekretaz (nörotoksik, anormal kesim) enzimlerinden biri ile kesilir. İkinci basamakta γ-sekretaz, C99'dan amiloid-β ve AICD (APP' nin intraselüler etki alanı) fragmanlarını oluşturur. APP'nin proteolitik yıkımı ve amiloid-β oluşumu Şekil 1'de gösterilmiştir [16].



Şekil 1. APP'nin proteolitik yıkımı ve amiloid-β oluşumu [16]. APP: amiloid prekürsör protein; Aβ: amiloid beta plakları

AH'de senil plaklarda APP'den türeyen nörotoksik amiloid-β'nın ekstraselüler birikimi söz konusudur. Bu durum mitokondriyal ve sinaptik hasara ve *Tau* proteininin hiperfosforilasyonuna neden olarak hücresel işlevlere zarar verir [5].

AH oluşumunda toksik amiloid-β oluşumu ve neden olan faktörler Şekil 2'de gösterilmiştir.



Şekil 2. Alzheimer hastalığı oluşumunda toksik amiloid-β oluşumuna neden olan faktörler [12].

Alzheimer hastalığındaki temel mikroskobik değişiklikler, nöron içerisinde birikim gösteren NFY' ler, ekstraselüler birikim gösteren amiloid plaklar ve nöron kayıplarıdır. AH'de NFY patolojisi beyinde klinik belirtilere paralel bir ilerleme gösterir. NFY'ler, mikrotübül bağlantılı bir protein olan *Tau* proteininin hiperfosforillenmiş şeklini içeren, hücre gövdelerinde ve dendritlerde biriken çift sarmal iplikçik yığınlarından oluşmaktadır. *Tau* proteini hiperfosforile halde mikrotübüllerle etkileşimi azaltır ve hücre işlevlerinde bozukluk meydana getirir [5].

Tau proteininin ana görevi mikrotübüllerin organizasyonu ve stabilizasyonudur. Mikrotübüller ise, akson ve dendritlerdeki transportu sağlamaktadır. Hiperfosforilizasyona uğrayan *Tau* proteinleri mikrotübüllere bağlanamaz, kendilerine bağlanarak agregatlar oluşturur. Bu agregatlar ise, NFY oluşumuna önemli derecede neden olur [17].

Alzheimer hastalığında bazı nöromediyatörlerin ve en belirgin olarak ACh seviyelerinde farklılıklar meydana gelmektedir. ACh sentezinde düşüş görülür. Bunun nedeni asetilkolin transferaz enziminin miktarı ve işlevinin azalması, kolin geri alımının azalması, kolinerjik nöron ve aksonlardaki hasar ve kayıplardır. Öğrenme ve bellek üzerinde etkili olan nikotinik reseptörlerde ve presinaptik M2 muskarinik reseptörlerde de kayıplar gözlenmiştir. AH'de meydana gelen kolinerjik kayıp, hastalardaki depresyon, ajitasyon gibi psikiyatrik rahatsızlıklara neden olur. Bu rahatsızlıkların ortaya çıkmasında serotonerjik ve dopaminerjik nörotransmisyondaki düzensizlikler ve nöron kayıpları da etkilidir. Sinir hücrelerinin, vücudun diğer kısımlarındaki hücrelerden farklı olarak kendilerini yenileme yetenekleri sınırlıdır. Bu nedenle hücre hasarına daha yatkındırlar [5,16].

Alzheimer hastalığında glutaminerjik sistemin sürekli olarak uyarılması sonucunda intraselüler kalsiyum (Ca^{+2}) konsantrasyonu artar; bu durum nöronal eksitotoksisiye, nöronal disfonksiyona ve hücre ölümüne neden olur. Beyinde Ca^{+2} artışı ile bu iyonla aktive edilen nötral proteinazların (kalpainler) aktivitesi artar, bu durum amiloid plak ve NFY oluşumuna neden olur. Ayrıca hastalıkta ortaya çıkan amiloid-β'nın da Ca^{+2} homeostazını bozduğu bilinmektedir [5].

Alzheimer hastalığına neden olan etkenlerden bir diğeri de oksidatif strestir. Beyinde demir, cıva ve alüminyum konsantrasyonlarının artması ve bu maddelerin serbest radikal oluşumunu uyarması ile oksidatif stres oluşur [18].

Tanı

Alzheimer hastalığı tanısı için tek bir test yoktur. Nörolog ve geriatri uzmanı gibi farklı branşlardaki hekimler tanı koymak için çeşitli yaklaşımlar ve tanı koymaya yardımcı araçlar kullanırlar. Bunun için takip edilmesi gerekenler;

- Hastanın psikiyatrik öyküsü, bilişsel ve davranışsal değişim öyküsü dahil olmak üzere bireyden tıbbi ve aile geçmişinin edinilmesi
- Bir aile üyesinden hastanın düşünme becerileri ve davranışlarındaki değişiklikler hakkında bilgi vermesini istemek
- Hastaya bilişsel testler uygulama, fiziksel ve nörolojik muayene
- Bireyin kan testlerinin yapılması, beyin görüntülemesinin yapılması, demans semptomlarının diğer olası nedenlerini (örneğin, tümör oluşumu veya bazı vitamin eksiklikleri gibi) ortadan kaldırır.

Alzheimer hastalığının teşhisi için dikkatli ve kapsamlı bir tıbbi değerlendirme gerekir. Hekimler, bir kişinin demansı olup olmadığını kolayca belirleyebilir; ancak kesin nedeni belirlemek oldukça zordur. Hastanın gerekli testleri ve muayeneleri tamamlaması ve hekimlerin sonuçları yorumlaması ve son olarak da teşhis koyması için birkaç gün veya hafta gerekebilir [19].

Tüm hastalarda kranial beyin tomografisi ya da manyetik rezonans (MR) ile nöroradyolojik inceleme yapılmalıdır. Bu inceleme, diğer demans nedenlerinin dışlanmasına yardımcı olur. Volümetrik çalışmalarla temporal loblarda, hipokampal oluşumda atrofinin görülmesi AH tanısını destekler. Serebrospinal sıvıda artmış *Tau* proteini, azalmış amiloid-β da tanıda kullanılabilecek testlerdir. Bunlara rağmen kesin AH tanısı ancak ölüm sonrası beynin incelenmesiyle konulabilir [20].

Tedavi

AH'nin temel tedavisi semptomatiktir, hastanın hafıza ve bilişsel semptomlarını düzeltmeye yöneliktir. Burada amaç hastanın bilişsel durumunu daha iyi hale getirmek, olmazsa hastalığın ilerlemesini durdurmak veya yavaşlatmaktır. İkincil tedavi olarak ise, hastalığın seyri sırasında ortaya

çıkan depresyon, ajitasyon, uyku bozukluğu gibi bulguların giderilmesini amaçlamaktadır. Hastanın yaşam kalitesini arttırıp bakımını destekleyici niteliktedir [20].

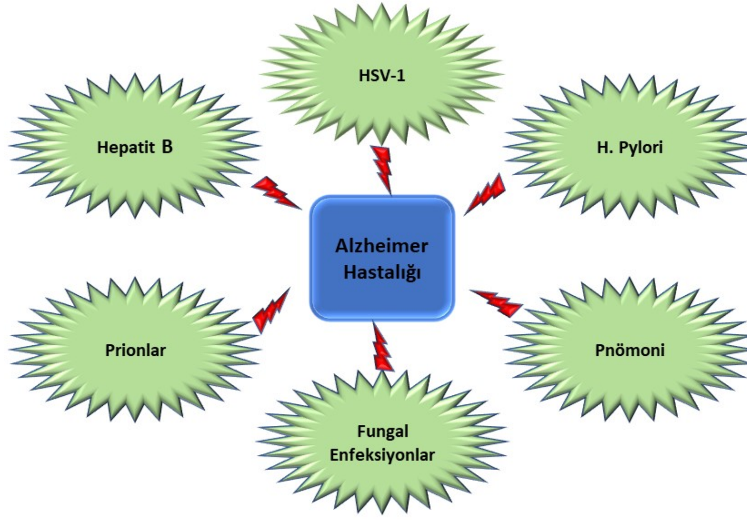
Azhemier Hastalığının Gelişiminde Biyolojik Ajanların Etkileri

- *Enfeksiyonlar*

Yıllar boyunca AH patogenezi ve hastalığın ilerlemesinde etkili faktörlerle ilgili farklı hipotezler ortaya atılmıştır. Viral enfeksiyonların bu konuda önemli bir rol oynadığı düşünülmektedir. Geçmiş yıllardan beri bulaşıcı patojenlerin AH'ye neden olduğu ileri sürülmekte ve bu konuda araştırmalar yürütülmektedir. Farklı klinik ve subklinik enfeksiyonların, AH'nin ilerlemesine neden olabilecek çeşitli mekanizmalarla ilişkisi bulunmuştur. Herpes simplex virüs tip 1 (HSV-1), *Helicobacter pylori, Borrelia burgdorferi* ve *Chamydophila pneumoniae* gibi enfeksiyöz ajanların insanlarda ve deney hayvan modellerinde nörobilişsel azalmalara neden olduğu tespit edilmiştir. Bu patojenler doğrudan SSS enfeksiyonuna ve nöroinflamasyon oluşumuna, dolaylı olarak beyindeki sistemik olayların değişimine neden olabilir. Beyni hedef alan otoimmün bir cevaba yol açarak nöroinflamasyon oluşumuna ve AH'ye neden olabilir. Ancak, insanlarda geç başlangıçlı AH'ye neden olduğu kesin olarak bilinen bir patojen bulunmamaktadır [21,22].

Amiloid-β plakları ve NFY'ler AH'nin histopatolojik bulguları olmasına rağmen bu hastalığa özgü değildir. Kronik enfeksiyonlar ve çeşitli SSS hastalıklarında da bu patolojiler görülür. Örneğin, tüberküloz ve lepra gibi kronik enfeksiyonlarda serum amiloid düzeylerinin artmış olması ve dokulardaki amiloid birikimi sık görülen bir durumdur [22].

Helicobacter pylori, sitomegalovirüs gibi enfeksiyonlar kan-beyin engelini aşan sistemik pro-inflamatuvar sitokinlerin üretilmesine yol açarak nörodejenerasyonu indükler. Patojen kaynaklı inflamasyon ve SSS'de amiloid-β birikmesi AH' nın patofizyolojisine katkıda bulunur ve kan-beyin engeline zarar verir. Herpes Simplex Virüs Tip 1 (HSV-1) ve *Chlamydophila pneumoniae* gibi patojenler ApoE4'ün beyne geçişini arttırabilirler. ApoE4, bağışıklık sistemi tarafından artan pro-inflamatuvar cevap ile de ilişkilidir [23]. Alzheimer hastalağına sebep olabileceği düşünülen enfeksiyonlar Şekil 3'te gösterilmiştir.



Şekil 3. Alzheimer hastalığı oluşumuna etki eden enfeksiyonlar [21-23].

Herpes Simplex Virüs Tip 1

Herpes Simplex Virüs Tip 1 primer enfeksiyonu genellikle çocukluk çağında görülür ve başlangıçta yüz mukozal membranlarının epitel hücrelerini ve ikincil olarak duyusal sinir terminallerini etkiler. Bu bölgelerde virüsün duyu gangliyonlarında gizli halde kalarak sinir sitemini istila edebileceği görülmüştür [24]. İkincil HSV-1 reaktivasyonu sonucu, ensefalit de dahil olmak üzere ciddi nörolojik komplikasyonlar görülebilir. Epidemiyolojik açıdan bakıldığında virüsün kalıcılığının birçok nörolojik kronik patolojiyi tetiklediğinden şüphelenilmektedir. Bu nedenle sadece HSV-1 değil, diğer birçok DNA ve RNA virüsünün (kızamık, HIV vb.) de sinir sisteminin ağır patolojileri ile ilgili olduğu bilinmektedir. Bütün bu hastalıklar birincil enfeksiyondan yıllar sonra gelişebilir [25].

Alzheimer hastalarının beyin otopsilerinde HSV-1 DNA'sının saptanması sonucu bu virüsün AH patogenezinde rol oynayıp oynamadığı araştırılmaya başlanmıştır. Yapılan araştırmalar sonucunda AH olmayan bireylerin de sıklıkla HSV-1 taşıdığı görülmüştür [26]. Moleküler araştırmalar sonucunda HSV-1 enfeksiyonunun nöronal ve glial hücrelerde APP transportunun artmasına, NFY'lerin ana bileşeni olan *Tau* proteininin hiperfosforilasyonuna ve amiloid-β' nın hücre içi düzeylerinde artışına neden olduğu tespit edilmiştir [25]. *İn vitro* çalışmalarda HSV-1 enfeksiyonlarının nöronal hücrelerde β-amiloid plakların birikimine neden olduğu gösterilmiştir [27,28]. Piacentini ve ark. (2015)'nın yaptığı *in vitro* çalışmada ise, HSV-1'in nöron hücrelerinde sinaptik fonksiyonu bozduğu göstermiştir [28]. Ayrıca, hayvan modelleri üzerinde yapılan çalışmalar HSV-1 enfeksiyonunun AH'de etkilenen beyin bölgeleriyle aynı bölgeleri hedef aldığını göstermiştir [29]. Sokolov & Reincke (2012) yaptıkları

çalışmada insan HSV-1 virüsünden kaynaklı ensefalitin ana olarak korteks bölgesinde frontal ve temporal lobları ve hipokampus bölgesini hedef aldığını göstermişlerdir [30].

Herpes Simplex Virüs Tip 1'in AH'nin gelişiminde rol oynayabileceği hipotezi, 1980'lerin başında çeşitli gözlemlere dayanarak Melvyn Ball (1982) tarafından önerilmiştir. Bu gözlemlerden en önemli olanları kızamık virüsünün neden olduğu subakut sklerozan panensefalitte olduğu gibi nörolojik bozukluklara viral tutulumun neden olması; HSV-1'nin bulaşıcı bir hastalık olup AH'nin insidansının yüksek olması ve HSV ensefaliti vakalarında AH'de etkilenen başta limbik yapı beyin bölgelerinin etkilenmesidir. Bunlara ek olarak, bazı akut HSV ensefaliti durumlarında hafıza bozuklukları görülmüştür [31]. Son olarak HSV-1, enfekte olmuş nöronlarda saklanma kapasitesine sahiptir ve virüs daha önce enfekte olmamış bölgelere yayılmasına neden olabilecek çeşitli uyaranlara, özellikle yaşlanmanın doğal bir olayı olan immünosupresyon gibi faktörlere yanıt olarak kendiliğinden yeniden etkinleşebilir. HSV-1'in bu özellikleri AH'nin aşamalı ve kronik olarak ortaya çıkmasıyla uyumludur. Yeniden etkinleştikten sonra virüs nöronlar yoluyla AH'nin neden olduğu ve nörodejenerasyon oluşmuş bölgelerde taşınarak patoloji oluşturabilir ve hastalığın seyrini de hızlandırabilir [32,33].

Beyinde HSV-1'in saptanması için virüsün veya genomunun SSS hücrelerinde bulunması gereklidir. AH'ye sahip kişilerin post-mortem beyin dokularında HSV-1 varlığına dair araştırılmalar yapılmıştır [34]. Beyindeki viral DNA tespiti için *in situ hibridizasyon* tekniği kullanılmıştır. Hibridizasyon çalışmaları insan beyninde HSV-1 DNA'sını başarıyla tespit etmesine rağmen diğer çalışmalar viral genomu tespit etmede başarısız kalmıştır. Çalışmalar sonucunda, Alzheimer hastası bireylerde HSV-1 genomuna rastlanmıştır; ancak hasta olmayan bireylerin beynindeki çalışmalarda da HSV-1 antijenleri tespit edilmiştir [35]. Itzhaki ve ark. (1993) hibridizasyondan daha yüksek hassasiyete sahip polimeraz zincir reaksiyonu (PCR) kullanarak Alzheimer hastaların çoğunun beyinlerinde temporal ve frontal kortekslerinde HSV-1 DNA varlığını göstermişlerdir [36]. Ancak, başka bir çalışmada Alzeheimer hastalarının beyinlerinde nispeten korunan bir bölge olan oksipital kortekste viral genom varlığı bulunamamıştır [37]. Bir diğer çalışmada Itzhaki ve ark. (1997), 46 Alzheimer hastası ile uyumlu yaşlardaki 44 kontrolün post-mortem beyin numunelerinde ApoE genotipi ile HSV-1 DNA varlığı arasındaki ilişkiyi incelemişlerdir [38]. Sonuç olarak, HSV-1 pozitif olan Alzheimer hastalarında ApoE e4 alleli sıklığının belirgin derecede yüksek olduğunu bulmuşlardır. Bir veya daha fazla ApoE e4 aleli taşıyan ve aynı anda beyinlerinde HSV-1 barındıran bireylerde AH teşhisinin daha fazla olduğu bulunmuştur. Dolayısıyla, HSV-1'in AH için tek başına bir risk faktörü olmadığı; ancak diğer risk faktörleriyle birlikte bulunduğunda AH riskini arttırdığı sonucuna varılmıştır [39].

Itzhaki ve ark. (1993) AH'nin patofizyolojisinde rol oynayabilecek virüs spesifik faktörlere de odaklanmıştır. Bu bağlamda, HSV-1 glikoprotein B (gB)'nin bir kısmının amiloid-β'ya çarpıcı benzerlikler sergilediği bulunmuştur. Viral gB *in vitro* koşullarda amiloid-β gibi kendiliğinden amiloid fibriller oluşturur. Ayrıca, amiloid-β'ya türdeş bölümün primer kortikal nöronal kültürlere nörotoksik

olduğu kanıtlanmıştır. gB'nin daha ileri nörodejenerasyona yol açan önemli bir moleküler etkileşimi temsil edebilen lipoproteinlerin, özellikle ApoE ile etkileşime girdiği gösterilmiştir [36].

Helicobacter pylori

Helicobacter pylori, spiral şekilli, esas olarak kronik gastrit, peptik ülser ve gastrik kanser gibi üst gastrointestinal bozukluklarla ilişkisi olan gram-negatif bakteridir. Epidemiyolojik çalışmalar sonucunda AH patogenezinde *Helicobacter pylori*'nin rol oynadığı kanıtlanmıştır. Örneğin, Kountouras ve ark. (2009), 27 Alzheimer hastasının ve yaş olarak onlara uyumlu, bilişsel işlevleri normal 27 kişinin serum ve serebrosipinal sıvılarını analiz ederek karşılaştırmış ve AH grubunda *Helicobacter pylori* immünoglobulin G antikorları ve anti-*Helicobacter pylori* konsantrasyonlarını anlamlı derecede yüksek bulmuştur. Ayrıca, Alzheimer hastalarının mide mukozal biyopsilerinde *Helicobacter pylori* görülme sıklığının kontrol grubuna göre çok daha yüksek olduğu bulunmuştur [40]. *Helicobacter pylori*'nin, AH'deki bilişsel bozukluğun artma şiddetiyle ilgili olabileceği de belirtilmektedir. Alzheimer hastalarında yapılan mini-mental durum muayenesinde, *Helicobacter pylori* enfeksiyonu olan hastalar daha kötü performans göstermişlerdir. Ayrıca beyin-omurilik sıvısı (BOS)'daki anti- *Helicobacter pylori* ve immünuglobülin G antikor konsantrasyonları Alzheimer hastaları arasındaki bilişsel bozuklukların şiddetiyle orantılı bulunmuştur [41]. Alzheimerlı hastalarda *Helicobacter pylori* tedavisi yapılması ile enfeksiyonun ortadan kaldırılmasıyla tedaviden 2 yıl sonrasında hastaların bilişsel ve işlevsel durumlarında belirgin iyileşmeler sağlanmıştır ve 5 yıllık sağ kalım oranları anlamlı olarak yükselmiştir [42]. Bütün bu bulgulara rağmen, *Helicobacter pylori* ile AH arasında nedensel ilişki ve olası mekanizmalar konusundaki bilgiler yetersizdir. *Helicobacter pylori*'nın nörotropik etki göstermeksizin, doğrudan nöroinflamasyona yol açarak AH'yi indüklemesi olasılık dahilinde görülmemektedir [22,40].

Chlamydophila pneumoniae

Chlamydophila pneumoniae, kişiden kişiye esas olarak damla teneffüs yoluyla yayılan bir bakteridir. Öncelikli olarak pnömoni ve bronşit gibi alt solunum yolu hastalıklarıyla ilişkilidir; ancak, AH patogenezinde rol alabileceğine dair kanıtlar bulunmaktadır. *Chlamydophila pneumoniae*, postmortem analizlerde Alzheimer hastalarının beyin dokusunda hem doğrudan hem de dolaylı olarak tespit edilmiştir. Örneğin, Balin ve ark. (1998) *Chlamydophila pneumoniae*'nin tespiti için Alzheimer hastası olan 19 vakadan ve 19 kontrol grubundan postmortem beyin dokusu numunelerini test etmek için, elektron ve immünoelektron mikroskobu, kültür ve immünohistokimyayı içeren çeşitli yöntemler kullanmıştır. *Chlamydophila pneumoniae*, 19 vaka numunesinin 17'sinde tespit edilmiştir; ancak 19 kontrol numunesinin sadece 1 tanesinde tespit edilmiştir [43]. Gerard ve ark. (2006) yaptıkları epidemiyolojik bir çalışmada AH'ye sahip hastaların beyinlerinde yapılan Polimeraz zincir reaksiyonu

(PCR) analizi sonucunda *Chlamydophila pneumoniae* DNA'sına rastlanmıştır ve nöronların %20'sinin enfekte olduğu görülmüştür [44]. *İn vitro* ve *in vivo* çalışmalar da AH ve *Chlamydophila pneumoniae* arasında olası bir ilişkiyi ortaya koymaktadır. Postmortem olarak AH'ye sahip bir beyinden izole edilen *Chlamydophila pneumoniae* ile enfekte edilen farelerde AH amiloid-β plaklarına benzer amiloid birikimleri gelişmiştir [45]. Bu birikintilerin yoğunluğu, boyutu ve sayısı enfeksiyon ilerledikçe önemli ölçüde artmıştır. Ayrıca, enfekte hastalarda *Chlamydophila pneumoniae*'nin ortadan kaldırılmasıyla önemli bir klinik iyileşme görülmesi bu ilişkiyi doğrular niteliktedir. Ancak, *Chlamydophila pneumoniae* enfeksiyonu AH arasında bir ilişkinin olmadığına dair de literatürde çalışmalar bulunmaktadır. Bu durum AH patogenezinde *Chlamydophila pneumoniae*'nin rolü konusunda belirsizlik yaratmaktadır [22,45].

Prion

Yapılan birçok çalışma AH, Parkinson hastalığı ve prion hastalıkları gibi nörodejeneratif hastalıkların önemli bir bölümünün patogenezinin temelinde hücre proteinlerinin toplanarak agregatlar oluşturmasının yattığını göstermektedir [46]. Prion hastalıklarından sorumlu olan ajanlar, yanlış katlanmış prion proteinleri olup, normal hücresel prionların (PrPc), anormal bir konformasyon (PrPsc) karakteristiği geliştirmesine neden olur. PrPsc, normal hücre prionu PrPc'nin posttranslasyonel modifikasyonundan ortaya çıkan ve azalmış bir plak yapısıyla karakterize edilen patojenik prion formdur. PrPsc, yeni PrPsc molekülleri oluşmasında ve yanlış katlanmış proteinin büyük fibriler agregatlar oluşturmasında rol oynar. AH'ye neden olan amiloid proteinlerin prion benzeri sekanslar içerdiği düşünülmektedir [47].

Normal hücresel prionların fonksiyonel olarak bakır metabolizması, nöron koruma, sinyal iletimi ve hücrenin gelişiminde etkilidir. Antioksidan PrPc inflamatuvar olayların bastırılmasına katkıda bulunur. PrPc endojen olarak oldukça işlevi olan bir protein olmasına rağmen nörotoksik olma eğilimindedir. AH'de nörodejenerasyon ile ilişkili amiloid-β oligomerinin de PrPc' nin ligandı gibi davrandığı bildirilmiştir [48].

1980'lerden beri PrP ile ilgili çalışmalar, çoğunlukla PrPc'nin PrPsc'ye dönüşmesi ve ölümcül nörodejenerasyon ve agregasyona neden olan bu mekanizmalar üzerine yoğunlaşmıştır. PrPc ekspresyonu prionun neden olduğu nörotoksisitenin gelişmesinde öncü rol oynar. PrPc'nin, APP'nin β-sekretaz bölünmesini düzenlediği ve böylece amiloid-β oluşumunu arttırdığı gözlemlenmiştir. Ayrıca, α-sekretaz N-terminal fragmanı nöroprotektif aktivasyon ile regüle edilerek PrPc'nin bölünmesini düzenler. PrPc'ye amiloid-β oligomerleri bağlanması, Fyn-bağımlı kinaz (FRK) aktivasyonuna yol açar. FRK aktivasyonu, N-metil-D-aspartat reseptörü (NMDAR) ve *Tau* fosforilasyonuna neden olur. Sonuç olarak, bu fosforillenme ile sinaptik bozukluk ve nörodejenerasyon oluşur [49].

PrPC tabanlı lipit raft sinyal platformunda, amiloid-β oligomerine bağlı PrPc' ye sahip reseptörler olan düşük dansiteli lipoprotein reseptörü 1 (LRP1) ve metabotropik glutamat reseptörü (mGluR) kümeleri FRK aktivasyonuna yol açar. FRK, NMDAR ve *Tau* proteinini fosforile eder. Böylece, sinaptik bozukluk ve nörodejenerasyona neden olur [48]. Anormal *Tau* birikimi ve temizlenmesi için ortaya çıkan hücresel mekanizmalar nöronal dengeye zarar verir. İntra-aksonal ve intra-nöronal *Tau* agregatlarının aksonopati ile ilişkili olduğuna dair kanıtlar vardır. Aynı şekilde, AH'de aksoplazmik akış ve aksonal transport engellenir. Prematüre nöron kaybı patolojik sürecin son noktasıdır [50].

Fungal Enfeksiyonlar

Yapılan son çalışmalar fungal enfeksiyonların da AH gelişiminde rol oynayabileceğini göstermektedir. Beyinde enfeksiyonlara karşı üretilen β-amiloidin özellikle *Candida albicans* türü fungal enfeksiyonlarda etkili olduğu gösterilmiştir. Enfeksiyon durumunda β-amiloidin fazla üretilmesinin beyinde plak oluşumununa neden olabileceği bildirilmektedir [51]. Alonso ve ark. (2018) yaptıkları çalışmada AH'ye sahip hastalardan alınan sinir dokularında fungal proteinlerin ve fungal DNA'nın bulunduğunu göstermişlerdir [52]. Ayrıca, Alzheimer hastalarından alınan post-mortem beyin dokusunda yapılan proteomik analizler dokuda fungal proteinlere kesin olarak karşılık gelen birkaç peptiti ortaya çıkarmıştır [53,54]. Bunlara ek olarak Alonso ve ark. (2014) poliglikanlar, proteinler ve DNA gibi fungal makromoleküllerin AH hastalarından alınan periferik kan ve beyin omurilik sıvısı örneklerinde de bulunabileceğini göstrmiştir [55].

- ***Cinsel yolla Bulaşan Hastalıklar***

Hepatit B

Dünya genelinde en yaygın görülen viral enfeksiyonlar hepatit B ve hepatit C'dir. Bu enfeksiyonlara sırasıyla hepatit B virüsü (HBV) ve hepatit C virüsü (HCV) neden olur. HBV pozitif bireylerde AH'de de görülen hafif kognitif bozukluk görülebilir ve HBV enfeksiyonunun AH için yeni bir risk faktörü olduğunu düşündürmektedir. AH'de değişen mikroglial transkriptlerin %6'sının viral süreçlerle ilişkili olduğu görülmüştür. Parkinson hastalığında bu oran %1' dir [56].

HBV pozitif bireylerde, HBV'nin kan-beyin engelini geçme ve beyne ulaşıp ulaşamadığını saptamak için immünohistokimyasal teknikler kullanılmıştır. AH'ye sahip vakalarda beyinde hücre içi nöronal HBV immünreaktivitesi gözlenmiştir. Yapılan araştırmalara göre mikroglial transkript değişikliklerinin en fazla nöronal onarım ile ilgili transkriptlerde olduğu görülmüştür. Bu sonuçlar mikroglianın sinir sisteminde önemli rolleri olduğu bilgisini desteklemektedir. Şimdiye kadar yapılan çalışmaların çoğu Herpes virüsü ve bazı bakteri türlerine odaklanmış olsa da HBV enfeksiyonu da beyinde saptanmıştır. AH vakalarında beyinde artan HBV immünreaktivitesi, AH patogenezinde HBV'nin önemli bir rolü olduğunu gösterir [57].

İnsan İmmünoyetmezlik Virüsü

İnsan İmmünoyetmezlik Virüsü (HIV) ile ilişkili demans, AH'ye benzeyen bir subkortikal nöropatolojidir. Güncel veriler gelecekte AH görülen çok sayıda HIV hastası olabileceğini düşündürmektedir. HIV ile indüklenen amiloid birikimi, glikoprotein ve TAT proteininin eksitotoksik etkilerinden dolayı nörotoksik olması, AH'nin risk faktörleridir. Ayrıca, HIV hastaları, bozulmuş immün işlevlerinden dolayı AH ile ilşkilendirilen diğer patojenlere de duyarlılık göstermektedirler. Antiretroviral ilaçların lipodistrofik ve metabolik etkileri aynı şekilde AH için bir risk faktörüdür. Ayrıca, *Chlamydia* pnömonisi gibi yaygın bulaşıcı ajanlar AH'nin patogenezini şiddetlendirebilir [58].

HIV ile ilişkili demans, AH'den farklı olarak genellikle bilişsel belirtileri giderek değişen ve geri dönüşümlü bir bozuklukken, son zamanlarda AH'de olduğu gibi kalıcı kognitif bozukluklar sıklıkla görülmeye başlanmıştır. Ancak, bu konuda kesin bir yargıya ulaşmak için HIV'li hastalarda AH görülme sıklığıyla ilgili araştırmalar arttırılmalıdır [59].

- ***Diğer Etkenler***

Diyabet

Beyinde insülin sinyalinin azalması AH ile ilişkili davranış bozukluklarının oluşumuna katkıda bulunur. Tip 2 diyabet AH için önemli bir risk faktörüdür. Moleküler seviyede bu durum beyinde insüline yanıtındaki eksiklik sonucunda oluşan nörofizyolojik bozulmalarla ilişkilendirilir. AH ile ilgili davranışsal bozukluklar, belleğe aracılık eden nöronların disfonksiyonu ve ölümünden kaynaklanır. AH'nin patofizyolojisiyle doğrudan ilişkili olan amiloid-β ve *Tau* proteinlerinin hiperfosforilizasyonu da beyinde insülin reseptörlerine erişilebilirliği azaltarak bu döngüyü arttırır. Bu durumun yanı sıra amiloid-β oligomerleri, tümör nekroz faktörü alfa (TNFα) salımını uyarır. TNFα'nın nöronal TNFα reseptörlerine bağlanması nihai olarak insülin etkisini sağlayacak iki protein kinaz olan fosfoinositid 3 kinaz ve serin/treonin kinaz'ın aktivasyonunu önleyerek insülin sinyalini baskılamaktadır. Bu işlemi sağlayan TNFα'nın diğer bir kaynağı mikrogliadır. Mikroglianın amiloid-β oligomerlerine maruz bırakılmasıyla TNFα salımını indüklediği bulunmuştur [60].

Tip 2 diyabet hastalarında beyine giden insülin miktarının ve nöronal insülinin azaldığı gözlenmiştir. Ayrıca diyabet hastalarında gelişen serebrovasküler değişikliklerin de AH gelişiminde rol oynayabileceği bildirilmiştir [61].

SONUÇ VE TARTIŞMA

Dünya genelinde yaşlı nüfus oranının ve kronik hastalıkların giderek artması ve yaşam tarzında doğallıktan uzaklaşma AH görülme sıklığını arttırmaktadır. Bu nedenle, AH'ye yol açması olası

faktörlerin bilinmesi, hastalığa erken dönemde tanı konması ve tedaviye mümkün olduğunca erken başlanması açısından önemlidir.

Birçok kimyasal ve biyolojik ajanın AH'na neden olduğu iddia edilmektedir. Özellikle anestezikler, endokrin bozucular ve alüminyuma temasın AH'ye yol açabileceğine dair literatürde çalışmalar bulunmaktadır. Ancak, hastalığın multifaktöriyel olduğu ve genetik dahil birçok farklı nedenle ortaya çıkabildiği daha yaygın bir kanıdır.

Günümüzde insanların tek tek veya aynı anda birçok biyolojik ajana temas etmesi söz konusudur. Biyolojik ajanların da farklı mekanizmalarla AH gelişimine yol açabileceği belirtilmektedir. Ancak, bu mekanizmalar henüz birçok ajan için tam olarak aydınlatılamamıştır. HSV-1, *Helicobacter pylori*, *Borrelia burgdorferi ve Chamydophila pneumoniae* gibi enfeksiyöz ajanların insanlarda ve deney hayvan modellerinde nörobilişsel azalmalara ve kognitif işlevlede düşüşe neden olduğu tespit edilmiştir.

Epidemiyolojik çalışmalarda AH patogenezinde *Helicobacter pylori*'nin rolünün olabileceğine dair güçlü kanıtlar elde edilmiştir. Alzheimer hastalarında *Helicobacter pylori* immünoglobulin G antikorları plazma ve BOS'ta anlamlı derecede yüksek bulmuştur ve bu yükseklik bozulmuş kognitif işlevlerle ilişkilendirilmiştir. Ayrıca, Alzheimer hastalarından elde edilen mide mukozal biyopsilerinde *Helicobacter pylori* görülme sıklığının da yüksek olduğu belirlenmiştir. Tüm bu verilere rağmen, *Helicobacter pylori* enfeksiyonu ile AH arasındaki ilişkinin mekanizlaları tam aydınlatılamamıştır. Bakterinin doğrudan nöroinflamasyona yol açtabileceği ve AH'ye yol açabileceği en çok üstünde durulan konudur.

Herpes Simplex Virüs Tip 1'in AH'nin gelişiminde rol oynayabileceği Alzheimer hastalarının beyin otopsilerinde HSV-1 DNA'sının saptanması ile gündeme gelmiştir. HSV-1 nöronların içinde saklanabilir ve yıllar sonra aktive olarak da etkisini gösterebilir. HSV-1'in AH patogenezine *Tau* hiperfosforilasyonunu arttırdığı ve NFY benzeri yapılar oluşturduğu bulunmuştur. Anormal Tau birikimi ve takiben bu birikimin temizlenmesi için kullanılan ücresel mekanizmalar nöronal dengeyi bozar. *Tau* agregatlarının aksoplazmik akış ve aksonal transportu engelleyebileceği belitilmektedir. Bu durum aksonopati, prematür nöron kaybı ve AH'nin başlangıcıyla sonuçlanabilir. Yapılan *in vitro* çalışmalarda ise HSV-1'in nöronal hücrelerde β-amiloid plakların birikimine yol açabileceğine dair kanıtlar elde edilmiştir. HSV-1 nedeniyle oluşan ensefalitlerde AH'de etkilenen başta limbik bölgelerin etkilendiğinin belirlenmesi ile de bu konuya yoğunlaşılmıştır.

Prion hastalıklarından sorumlu olan yanlış katlanmış prion proteinleridir. PrPsc'nin yeni PrPsc molekülleri oluşmasına yol açtığı ve yanlış katlanmış proteinin büyük fibriler agregatlar oluşturmasında görev alabileceği belirtilmiştir. Ayrıca, AH'ye yol açtığı bilinen amiloid proteinlerin prion benzeri sekanslar içerebileceği gösterilmiştir ve AH'de ortaya çıkan nörodejenerasyon ile ilişkili olduğu bilinen amiloid-β oligomerinin ise PrPc'nin ligandı şeklinde davrandığı belirlenmiştir.

Sonuç olarak, birçok biyolojik ajanın AH patogenezinde rol alabileceği ifade edilmektedir. Özellikle, bu konudaki post-mortem çalışmaların hız kazanması gerekmektedir. Ayrıca, geniş popülasyonlarda yapılacak epidemiyolojik çalışmalar ile bu ajanlar ile AH arasındaki ilişkinin aydınlatılması, toplumlarda hızla artış gösteren AH ve benzeri nöropatolojik durumların azaltılması ve hatta önlenmesi açısından büyük önem taşımaktadır.

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J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 44(1): 188-203, 2020
Doi: 10.33483/jfpau.599077

REVIEW / DERLEME

APPLICATIONS OF MINIATURIZED AND PORTABLE NEAR INFRARED (NIR), FOURIER TRANSFORM INFRARED (FT-IR) AND RAMAN SPECTROMETERS FOR THE INSPECTION AND CONTROL OF PHARMACEUTICAL PRODUCTS

MİNYATÜRE EDİLMİŞ VE PORTATİF YAKIN KIZIL ÖTESİ (NIR), FOURIER DÖNÜŞÜMLÜ KIZILÖTESİ (FTIR) ve RAMAN SPEKTROMETRELERİNİN FARMASÖTİKLERİN DENETİMİ VE KONTROLÜNDEKİ UYGULAMALARI

Abdullahi Garba USMAN[1], Umar Muhammad GHALI[2], Selin IŞIK[1]*

[1]Department of Analytical Chemistry, Faculty of Pharmacy, Near East University, 99138, Nicosia, North Cyprus, Mersin-10 Turkey

[2]Department of Medical Biochemistry, Faculty of Medicine, Near East University, 99138, Nicosia, North Cyprus, Mersin-10 Turkey

ABSTRACT

Objective: *In this review, the wide range of different applications of portable and miniaturized Fourier transform infrared (FT-IR), near-infrared (NIR), and Raman spectrometers for quality control, assessment and inspection of pharmaceutical products are discussed. In regard to counterfeiting, these portable spectrometers are utilized for vibrational and scattering spectroscopies in the identification of counterfeits, adulterated, fraudulent, falsified and substandard pharmaceutical products which are becoming significant problems and a danger to general well-being, particularly in the developing nations.*

Material and Method: *Different scientific articles and books were researched and reviewed to explain the applications of miniaturized and portable near infrared (NIR), Fourier transform Infrared (FT-IR) and Raman spectrometers for the inspection and control of pharmaceutical products from past to nowadays.*

Result and Discussion: *Adulterated pharmaceutical products have become the greatest threat for developing countries. This problem can reduce the confidence for pharmaceutical products. The application of mentioned portable devices for determinations the quality control of pharmaceutical product, enables the techniques to be more accessible, quick, accurate, simple, precise, robust and more importantly, efficient.*

* **Corresponding Author / Sorumlu Yazar:** Selin Işık
e-mail: selin_isikk@hotmail.com

Submitted/Gönderilme: 18.08.2019 **Accepted/Kabul:** 22.12.2019

Keywords: *Infrared (NIR) and Raman spectrometers, Pharmaceutical products, Portable Fourier Transform Infrared (FT-IR), Quality control.*

ÖZ

Amaç: *Bu derlemede, farmasötik ürünlerin kalite kontrollerinin değerlendirilmesi ve belirlenmesi için portatif ve minyatür Fourier transform infrared (FT-IR), yakın kızıl ötesi (NIR) ve Raman spektrometrelerin geniş uygulama alanları tartışılmıştır. Farmasötik ürünlerde yapılan sahtecilikler sonucunda, genel refah için bir tehlike haline gelen taklit, hileli, tahrif edilmiş ve standart altı farmasötik ürünlerin belirlenmesinde kullanılan bu portatif spektrometreler, titreşimsel ve saçılma spektroskopileri için kullanılmaktadır.*

Gereç ve Yöntem: *Çeşitli bilimsel makaleler ve kitaplar incelenmiş olup, geçmişten günümüze kadar olan süreçte, Fourier transform infrared (FT-IR), yakın kızıl ötesi (NIR) ve Raman spektrometrelerin farmasötik ürünlerin kalite kontrolü ve denetimi ile ilgili uygulamaları derlenmiştir.*

Sonuç ve Tartışma: *Hileli farmasötik ürünler özellikle gelişmekte olan ülkeler için önemli bir tehdit haline gelmiştir. Bu problem, farmasötik ürünlere olan güveni azaltabilmektedir. Bu makalede bahsi geçen portatif cihazların uygulanması, fitöfarmasötiklerin kalite kontrolünde tekniklerin daha erişilebilir, hızlı, doğru, basit, hassas, sağlam ve daha da önemlisi, verimli olmasını sağlamaktadır.*

Anahtar Kelimeler: *Denetim, farmasötik ürünler, kalite kontrol, taşınabilir Fourier dönüşümlü infrared (FTIR) spektroskopisi, Yakın infrared (NIR) ve Raman Spektrometresi.*

INTRODUCTION

Today, some of the greatest dangers and threats encountered by the pharmaceutical industry globally are issues of falsification, counterfeiting, adulteration, incapability and fraudulence. These issues have a negative impact on patients and are not beneficial for curing the target disease due to their reduced efficiency. This threat causes a loss in confidence in pharmaceutical products, health care providers and the health system in general.

According to the World Health Organization (WHO), antibiotics and anti-malarial drugs are the most reported adulterated pharmaceutical products which has led to anti-microbial resistance and likely drug-resistant infections. These falsified and substandard pharmaceutical products can be found in illegal street markets, illegal pharmacies, clinics, hospitals etc. and range from generic innovator medicines to expensive pharmaceutical products for cancer treatment and even includes inexpensive products used as analgesics. WHO defines falsified or substandard pharmaceutical products as medical products that do not contain any active pharmaceutical ingredients (API), while the incorrect ingredients include potato starch, corn starch or chalk etc. or an inadequate proportion of the active pharmaceutical ingredient. Some of these products are found to be toxic in nature, which are mostly produced in substandard and unhygienic environment/conditions, predominantly by unqualified personnel [1].

The usage of portable NIR, FT-IR and Raman spectroscopies are among the most appropriate methods for the detection and identification of organic and inorganic chemical constituents present in any pharmaceutical product. They provide information concerning the vibrational transitions of molecules or crystals with various sensitivities and specificities due to the different selection rules that

govern infrared absorption and Raman scattering [2]. The information obtained from the vibrational spectroscopies is sometimes complementary to a degree depending on the symmetry degree of the molecules investigated [3–5]. Today, commercial handheld and portable FT-IR, NIR and Raman spectrometers are coupled with optical fibers or a proper instrument of measurement that allows spectral acquisition in reflection mode with a distance of about 1 cm and without any contact with the sample surface. Sequentially Shifted Excitation (SSE) is the technology that involves the principle of the Bravo spectrometer to acquire shifted Raman spectra while a suitable or desired algorithm allows the extraction of the spectral data in true Raman space.

This procedure was recently validated extensively for pharmaceutical applications, and is now widely accepted and used in routine analysis [6–15].

This review focuses on the advancement of rapid drug detection techniques using portable, miniaturized and handheld FT-IR, NIR and Raman spectrometers for inspecting the quality and quantity of pharmaceutical products. Moreover, some new characteristics of counterfeit, falsified and adulterated drugs are discussed and the ways of countering such products are given. For example, the usage of NIR models (Universal quantitative models) can be employed to examine pharmaceutical drugs with the same API made by different manufacturers. Finally, these portable spectroscopic techniques have good specificity, robustness, linearity, precision and accuracy [16, 17].

SPECTROSCOPIC METHODS

Near Infrared (NIR) Spectroscopy

The NIR region was first discovered by Herschel over 200 years ago, the region lies between 780-2500 nm, and is located between the red band of the visible light and the mid-infrared region. The obtained NIR spectrum is a result of molecular vibrations which comprise of combinations of fundamental vibrations and overtones of hydrogen bonds such as O-H, C-H and N-H [18]. This technique enables quick analysis for a wide range of materials, which have the possibility of having a large number of physicochemical parameters and chemical composition of many formulations. Many physicochemical parameters of pharmaceutical products can be analyzed quantitatively using NIR spectroscopy such as particle size, hardness, dissolution rate compaction force and water content among others [19–26]. For the last two decades, portable and miniaturized NIR spectroscopy has been utilized to provide numerous possibilities in industrial and pharmaceutical applications. It is a rapid technique, whereby FT-NIR can record a spectrum within only a few seconds. One of the advantages of this technique is the non-destructive method of the analysis, which means that analysis can be conducted with minimal sample preparation or sometimes no sample preparation. This facilitates the avoidance of steps that are responsible for errors. It also enables the measurement of packaged samples via package material [27] .

Bei *et al*. [28] applied an orthogonal approach using handheld NIR, portable FT-IR and portable Raman analyzers which were coupled to offline chemometric data analysis to quickly distinguish different producers of domestic and imported oxcarbazepine drugs found in the Chinese market in a quality controlled laboratory setting. API of the synthesized pharmaceutical product as well as the starting materials, solid dosage forms and coatings were analyzed using an orthogonal approach. The results of the FT-IR, Raman and NIR were found to be in conformity to each other and constitute sources of cross-validation within the scope of this study. All three portable and handheld analyzers employed the advantages of utilized spectroscopic techniques for their potential in assisting and complementing regulatory bodies in adopting efficient, innovative and effective solutions.

Plugge *et al*. [29] reported that NIR spectroscopy coupled with a polar qualification system is a substantial method for differentiating similar pharmaceutical substances which only differ slightly in terms of physical or chemical characteristics. One of the applications of this powerful coupling is the more precise selection and definition of starting materials involved in the synthesis of medicinal products and its application in blending validation.

Vakili *et al*. [30] reported that quality control tools used in assessing the quality of printable orodispersible formulations have yet to be defined. In study, four different orodispersible formulations, consisting of two slightly soluble drugs, prednisolone and levothyroxine, were synthesized by piezoelectric inkjet printing using two different edible substrates. A compact miniaturized and handheld NIR device with the range of 1550-1950 nm was utilized for the quantitative determination of drugs in the printed formulations. The spectral data was further treated with Savitzky-Golay filtering, mean centering and third derivative approach. Orthogonal partial least squares (OPLS) regression and principal component analysis (PCA) were used to create predictive models for the quality control of the printed dosage formulations. The actual dose present in each formulation was confirmed using high performance liquid chromatography (HPLC) for levothyroxine (0.15-0.86 mg with R^2=0.999). It was found that the model has the ability of to predict and cluster the drug formulations using both Q^2 and R^2Y values in the range of 0.94-0.99.

Many other international institutions are aware of the considerable potential of the NIR technique particularly when using handheld and miniaturized devices for process monitoring and quality control in the pharmaceutical industry [31].

In addition, Dreassi *et al* [32] reported the integrated quality control of cefuroxime axetil drug, through the identification of starting materials and the API, and proceeded with controlling the drug ingredients. For the quality control, discriminant analysis (DA) was applied to both the PCA scores and the original spectral data. On the other hand, cluster analysis (CA) was also discussed in the research, which was also applied for the PCA scores. Both methods (DA and CA) gave veritable information

concerning the identification of different intermediates in the production cycle. They can as well be used to differentiate between two similar and related granular types of Cefuroxime axetil.

Fourier Transform Infrared (FT-IR) Spectroscopy

Infrared spectroscopy is a useful tool for the identification of drugs [33]. It is also useful in quantitative analysis as it allows for the continuous monitoring of spectral baseline and aids in the simultaneous analysis of numerous parameters of the same sample [34]. Among its greatest advantages is the ability to study the sample at virtually any given state. As a result of advances in instrumentation, numerous new techniques were designed and introduced in order to test and identify the samples that were formerly considered difficult [35, 36].

The infrared region is at around 700 nm (14.285,71 cm^{-1}), which is immediately beyond the visible region. Wave number is generally used to indicate the infrared spectral region. The radiation energy of this region ranges between 48 $kjmol^{-1}$ at 2,500 nm and 2.4 kj mol^{-1} at 50,000 nm. Two units can be used in such vibrational spectroscopy; cm^{-1} (wave number) and nm. The unit of choice depends on the type of the spectrometer (i.e dispersive vs Fourier Transform) and in order to avoid large numbers whereby nm is used. The mathematical relationship of these two units is given below.

$$[Cm^{-1}] = 1/ [nm] \times 10^{-7} \quad (1)$$

The development of Fourier Transform infrared (FT-IR) has to a true revolution in the area of IR spectrometry, due to its positive impact. It is a fast and accurate analytical technique which provides impressive qualitative information for gaseous, liquid and solid samples. The use of IR has grown immensely in recent years. The importance of FT-IR was originally intended to identify and learn about functional groups present in a particular chemical sample, but in recent years, it has been widely used for the identification, evaluation, quality control and inspection of pharmaceutical products for the purpose of drug design and minimizing the undesired alteration of products [37–42].

The miniaturization of vibrational spectrometers has emerged in the past decade, but the real handheld FT-IR, NIR and Raman scanning spectrometers have only recently become readily and commercially available. This development is due to advances in microelectromechanical systems (MEMS) and recently, they have had a significant impact on the analytical techniques as well as on-site analysis. Their applications have been demonstrated in many fields, such as terror defense, homeland security, forensic investigations and in some drug agencies.

Sorak *et al.* [43] reported that the recent miniaturization of infrared and Raman spectrometers has led to substantial progress in analytical techniques. The usage of handheld and portable infrared spectrometers resulted in a reduction in instrument size and increased measurement performance. These

portable devices have had an impact on quality control as well as process control. They can also be used to calibrate the handheld and portable systems provided, while the qualitative and quantitative results for certain solid and liquid samples of these handheld spectrometers are evaluated according to their comparability with other laboratory instruments and their suitability for field and on-site measurements are also compared. The results showed that the application of these handheld devices for on-site purposes will open up a wide range of new applications of such devices in various industrial branches and hence will revitalize the application of vibrational spectroscopy in quality control, identification, process control and inspection in the near future.

Moreover, FT-IR was designed for quick and direct analysis of acetylsalicylic acid (ASA) in various pharmaceutical drugs [44]. The API were determined and compared using conventional KBr spectra. Two chemometric approaches, principal components regression (PCR+) and partial least squares (PLS), in addition to Beer-Lambert's law were employed in data processing. Additionally, the researchers studied the possibility of applying the Beer-Lambert law to determine the quality of ASA in pharmaceutical products at a wave number of 1,605.49 cm^{-1}, and similarities within the results were observed suggesting the application of PCR+ technique due to a smaller value of relative standard deviation (RSD; <3.0%).

Fourier transform infrared spectroscopy was applied for rapid and direct measurement of vitamin C (ascorbic acid) and Vitamin B_7 (biotin) in various pharmaceutical products. The APIs in drug preparation were best determined by comparing conventional KBr spectra. The data was also processed using chemometric and Beer-Lambert approaches [45]. Vitamin C is an essential nutrient for a wide number of primates [46], which include 20% of the mammalian group, as well as small number of other species such as certain birds, fish and guinea pigs. Biotin is soluble in water and composed of ureido (tetrahydroimidazolone) ring fused with tetrahydrothiophene ring.

Antimalarial drugs such as artesunate tablets were among the most counterfeited pharmaceutical products. A newly introduced analytical technique which comprises the combination of ATR (Attenuated Total Reflection)-FTIR imaging, Raman spectroscopy, and Spatially Raman Spectroscopy (SORS) was developed to screen and characterize genuine and adulterated artesunate tablets.

Vibrational spectroscopy shows the chemical constituents present in the tablets by using the complementary properties of FT-IR imaging and Raman scattering allowing the characterization of the surface and overall compositions of the tablets. The advantages of this analytical technique, point to its potential to be included and used in analytical methods for the investigation of falsified and fake drugs or medicines [47].

The quality control assurance of pharmaceutical products such as ginseng has significant importance for consumer safety [48,49]. Ginseng is an expensive and precious herb normally adulterated with less expensive products. Its quality control and inspection is strongly needed and recommended

due to its high degree of consumption and commercial importance. It can be consumed in powder, capsule, tea, or softgel forms [50]. Extracts of American ginseng (The root of *Panaxquinque folius*), Notoginseng (the root of *Panax notoginseng*) and Asian ginseng (the root of *Panax ginseng*) were distinguished using conventional FTIR spectroscopy (ID-FTIR) and the two dimensional correlation FT-IR using thermal perturbation. Moreover, the differences in their peak intensity were observed at about 1640, 1416, 1372 and 1048 cm^{-1} in the FTIR results of these species to ease their differentiation and identification [51].

A pseudopolymorphic and two polymorphic crystal forms of local anesthetic agent hydrozxyprocaine hydrochloride were characterized and analyzed using spectroscopy (FTIR, SSNMR spectroscopy, FT-Raman), water vapor sorption analysis, thermal analysis (hot stage microscopy, thermogravimetry and differential scanning calorimetry) and powder x-ray diffractometry [52].

Nitrofurantoin and Ampicillin in both the hydrate and hydrous forms were characterized using x-ray diffractometry (XRD), thermogravimetric and differential thermal analysis (TG/DTA) and powder DRIFTs. Among the analytical tools used, only DRIFTs indicate the formation of H-bonds of the anhydrous drug constituents and the presence of crystalline water taken up from the atmospheric moisture, which shows significant absorption from 3,500-3,700 cm^{-1} that corresponds to crystalline water. The FTIR spectral results of the hydrate and anhydrous forms of the nitrofurantoin also show significant differences [53].

FT-IR spectrometry is applied for the analytical quantification of various pharmaceutical formulations with commercial software and chemometric approaches. It is a simple, quick and precise method when compared to chromatographic methods used previously. The quantification is completed within 15-16 minutes, including sample preparation and the spectral result acquisition [54].

Finally, FT-IR is a quick, non-destructive and reliable analytical technique and when coupled with chemometrics, it can be a powerful tool in the pharmaceutical industry. It is also being used in the analysis of herbal medicine and can also be applied in the quality control of pharmaceutical products and to inspect possible counterfeiting [55].

Raman Spectroscopy

The first discovery of a new kind of secondary radiation which called 'Raman' scattering, was by Raman and Krishnan in the year 1928. The instrumentation was further developed when using this principle through advances in optoelectronics and photonics, as well as the rapidly expanding range of usage and applications. Raman spectrometers have evolved continuously over the decades [56]. Based on history, the evolution of Raman tools were also augmented through numerous discoveries of different phenomena rather than the basic Raman Effects such as resonance Raman (RR), surface-enhanced Raman scattering (SERS). It was proven to be a useful instrument in numerous fields such as

pharmaceuticals, process control, in vivo biomedical studies, environmental sciences, catalysts, pigments, archeology, glasses and forensic sciences [57].

The spectral analysis of pharmaceutical products using Raman spectroscopy provides more benefits than mid or NIR spectroscopy (the comparision between Raman and IR spectroscopies are summerized at Table 1). As a result of its scattering technique, it does not require a reference light path as in IR or NIR. This allows remote sampling and is also amenable to fiber optics. A typical Raman measurement for a single scan can give spectral data in the range of 4000-40 cm^{-1} and a finger print range of 4000 and 400 cm^{-1}. Some chemical groups give strong Raman signals such as C=S, which produces a signal in the range of 1000-1250 $cm^{-1}$and C-Cl with a signal around 500-800 cm^{-1}. Certain polar groups such as O-H give a weak signal as opposed to IR, which gives a strong signal. As an example of Raman spectrum, Curcumin shows characteristic groups and is interpreted as having groups such as aromatic C=O stretching signal at 1626 cm^{-1} and C=C stretching signal at 1601 cm^{-1} [58].

Table 1. Comparison between Raman and Infrared (FT-IR and NIR) spectroscopies

RAMAN Spectroscopy	**Infrared Spectroscopy**
It is due to the scattering of light by the vibrating molecules	It is the result of absorption of light by vibrating molecules
The vibration is Raman active if it causes a change in polarizability	Vibration is IR active if there is change in dipole moment
The molecule doesn't have to posses a permanent dipole moment	The vibration concerned should have a change in dipole moment due to that vibration
Water can be used as a solvent	Water can't be used in IR due to its high intense absorption in IR
Sample preparation is not very elaborate it can be in any state	Sample preparation is elaborate gaseous samples can rarely be used
Gives an indication of covalent character in the molecule	Gives an indication of ionic character in the molecule
Cost of instrumentation is very high	Comparatively inexpensive

Without any sample preparation, Raman spectroscopy is able to perform non-destructive and noninvasive screening and analysis into the packaging and hence can keep the sample constituents intact in case of further analysis.

Raman spectrometers are composed of a laser light source, focusing optics and spectrographs that consist of detector and dispersity elements [59].

Nowadays, the Raman-based instruments are portable, handheld, miniaturized, cheaper, smaller and faster. Furthermore, the concept of analyzing real world samples from the production unit through to packaged containers has developed from being merely to a hypothetical proposition to the level of a

well-established concept for the quality control and inspection of various pharmaceutical products [60, 61].

According to a study conducted for the measurement of some pharmaceutical products using portable Raman spectrometer with 785 nm excitation, the sample was further transferred into a small brass cylinder and the head of the spectrometer was set on top of a small brass cylinder to allow for the focusing of the laser beam into the sample. A wave number between 1800-400 cm^{-1} was then chosen to develop PLS-1 calibration for each of the active ingredients. This was done in order to demonstrate the performance of the portable Raman spectrometer for quality control and inspection of the qualitative and quantitative active ingredients [62].

Similar to NIRs, Raman spectroscopy has been tested and shown to be successful in portable and miniaturized units for the identification and detection of counterfeiting and falsification [63]. Raman spectroscopic techniques have become increasingly popular as a result of the invention of portable and miniaturized Raman devices [64].

Noonan *et al*. [65] developed a universal approach for models that can classify and categorize multiple drugs of interest in the presence of three cutting agents, some of them had high similarity to the drugs of interest. Based on the idea of the models developed they were opportune to cluster four drug surrogates successfully, namely lidocaine, benzocaine, norephedrine and isoxsuprine. They further expatiated the spectral acquisition by applying both rugged and home built, handheld and commercial devices as well as the preprocessing of the needed spectra before developing the model.

Loethen *et al* [66] also reported a method which was developed to rapidly screen a bread group of anti-infective agent, as a form of anti-conterfeit screening. They used only Raman spectra of the active pharmaceutical ingredients as reference point.

Rohleder *et al*. [67] demostrated the application of Raman spectroscopy during a study on anti-oxidative stability of cosmetic products formulations [68]. Larmour and Bell utilized the ultraviolet resonance Raman technique to ascertain the stability of some biopharmaceutical protein formulations.

The Raman spectroscopic technique can be applied in testing coatings as well as checking their falsification and counterfeiting. Witkowski *et al*. [69] compared the Raman spectra of an authentic and original coated tablet and that of a coated tablet under suspicion of adulteration. They realized that the major spectral features seen in both the two spectra were due to the presence of titanium oxide. Additionally, upon further inspection on the spectral data, another peak was observed in the Raman spectrum of the suspected adulterated and counterfeited coated tablet, which was not observed on the authentic and original coated tablet's Raman spectrum. Therefore, this study proved the counterfeiting in the sample tablet [70].

Tfayli *et al.* [71] showed the application of confocal Raman microscopy to determine the amount of drugs present in human skin.

Finally, Raman spectroscopy has advantages for the diagnosis of various diseases that have been widely accepted in the health sciences. It has been applied in biomedicine to understand and identify the dynamics of nucleic acids. It can also be applied to detect and test human papilloma virus at its early stage, thereby enabling timely application of the correct medication for curing the disease. It can also help in the development of stroke-preventing medication and in the detection of squamous cell carcinoma, brain tumors, brain metastasis and lymph nodes metastasis through studying the histopathology of tissue [72–74].

RESULT AND DISCUSSION

A wide range of applications, chosen to demonstrate and show the high performance of portable and miniaturized NIR, FT-IR and Raman spectrometers for the inspection and quality control of pharmaceutical products, were explained and summerized in Table 2.

Table 2. Summary of some reviewed studies

Device	Sample	Source
NIF	Four different orodispersible formulations, consisting of two slightly soluble drugs, prednisolone and levothyroxine, were synthesized by piezoelectric inkjet printing using two different edible substrates	[30]
NIF	Quality control of cefuroxime axetil drug, through identifying the starting materials and the API, and proceeded with the drug control	[32]
FTIR	Vitamin C and Biotin	[44]
ID-FTIR and Two dimensional correlation FT-IR	Extracts of American ginseng (The root of Panaxquinque folius), Notoginseng (the root of Panax notoginseng) and Asian ginseng (the root of Panax ginseng)	[49]
FTIR, SSNMR spectroscopy, FT-Raman	A pseudo polymorphic and two polymorphic crystal forms of local anesthetic agent hydrozxyprocaine hydrochloride	[50]
X-ray diffractometry (XRD), Thermogravimetric and Differential Thermal Analysis (TG/DTA) and Powder DRIFT	Nitrofuraintoin and Amphicilline in both the hydrate and hydrous forms	[51]
Raman spectroscopy	Anti-counterfeit rapid screening of some finished pharmaceutical products	[64]
Raman spectroscopy	Anti-oxidative stability of cosmetic products formulations	[67]
Raman microscopy	Determination the amount of drugs present in human skin	[69]

Today, substandard pharmaceutical products have become the greatest threat to the general well-being in developing countries. This can decrease the confidence of the pharmaceutical products and the health care in general. These techniques enable the quick monitoring, inspection and identification of adulteration of different pharmaceutical products.

The application and usage of these portable instruments make the techniques accessible, fast, reagent free (in some cases), simple and more importantly, efficient.

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Yayım Koşulları

1. Ankara Üniversitesi Eczacılık Fakültesi Dergisi (Ankara Ecz. Fak. Derg. - J. Fac. Pharm. Ankara) yılda üç kez (Ocak-Mayıs-Eylül) yayımlanır.
2. Dergiye Eczacılığın her alanında daha önce hiçbir yerde yayınlanmamış, Türkçe veya İngilizce olarak hazırlanmış makaleler kabul edilir. Deneylerde, insan için "the Declaration of Helsinki" ve hayvan için "European Community Guidlines"'a bağlı kalınmalıdır.
3. Yayın Komisyonuna gelen makaleler en az 2 danışmana gönderilir.
4. Makaleler yayına kabul ediliş sırasına göre yayınlanır.
5. Danışmanlar tarafından önerilen düzeltmelerin yapılması için yazar/ yazarlara geri gönderilen makaleler, düzeltilip yayınlanmak üzere 3 ay içinde tekrar yayın kuruluna gönderilmezse, yeni başvuru olarak işlem görür. Makale yayımlanmadan önce yazarların yayımcıya makalenin "Copyright Transfer Form'unu doldurarak telif hakkını göndermesi gerekmektedir.
6. Yayımlarda intihal olup olmadığı kontrol edilmelidir.
7. Dergimize aşağıdaki makale türleri kabul edilir:

   a) **Araştırma makalesi**: Türkçe veya ingilizce hazırlanmış, şekiller ve tablolar dahil tamamı en çok 20 A4 kağıdı sayfası olan, orjinal araştırmaların bulgu ve sonuçlarını açıklayan makalelerdir.
   b) **Derleme:** Türkçe veya ingilizce hazırlanmış, şekil ve tablolar dahil tamamı en çok 25 A4 kağıdı sayfası olan, yeterli sayıda bilimsel makale taranarak, o güne kadarki gelişmeleri özetleyerek ortaya koyan ve sonuçlarını yorumlayarak değerlendiren makalelerdir.
   c) **Önbilgiler:** Devam etmekte olan bir çalışmanın bulgularını zaman kaybetmeden duyurmak için Türkçe veya ingilizce yazılan en çok 5 A4 kağıdı sayfası olan makalelerdir.

Yayım Gönderme

Yazarlar makalelerini https://dergipark.org.tr/tr/pub/jfpanu adresinden online olarak yükleyeceklerdir.

Yazım Kuralları

1. Metinler, A4 normunda (21 x 29,7 cm) yazılmış olmalıdır.
2. Bütün tablo ve şekiller metin içindeki yerlerine yazım alanından taşmadan yerleştirilmiş olmalıdır.
3. Metinler A4 normundaki sayfanın sağ ve sol tarafından 2,5 cm., üst ve alt kenarlarından 3 er cm boşluk bırakılarak (ilk sayfada yukarıdan 5 satır aralığı) 1,5 satır aralıkla yazılmalıdır. Yayımı kabul edilen makaleler doğrudan "Microsoft Word" dosyası halinde online olarak sisteme yüklenecektir (online submission). Ana metin yazı karakteri "Times New Roman" ve 11 punto olmalıdır.
4. Sayfa numaraları makalede belirtilmemelidir.
5. Yazar adı (küçük harf) ve soyadı (büyük harf) koyu olarak başlığın altına üç satır aralık verildikten sonra altına unvan belirtmeden yazılmalıdır. Birden çok yazar varsa virgülle ayrılıp bir boşluk bırakılarak yazılmalıdır. Yazarların soyadları üzerine konulacak rakamlarla hemen isimlerin altındaki satıra kurum adları ve posta adresleri açıkça yazılmalıdır.
6. Başlık sayfasında yayın adı, yazar/yazarların adları ve yazışma yapılacak yazarın açık adresi, telefon ve faks numaraları, varsa e-mail adresi belirtilmelidir. Sorumlu yazarın soyadının üstüne (*) işareti konularak belirtilmelidir. Bu kişinin açık adresi, faks numarası, telefon numarası ve e-mail adresi başlık sayfasının en altında belirtilmelidir.
7. Tablolar üstlerine, şekiller (formül, grafik, şema, spektrum, kromatogram, fotoğraf v.b.) de altlarına arabik rakamlarla (**Şekil 1**., **Tablo 2**.,) numaralandırılmalıdır. "Tablo", "Şekil" sözcükleri ile bunlara ait numaralar koyu yazılmalı ve 11 punto olmalıdır. Şekil/Resim (JPG formatında) makale içinde yerleşmiş olmalıdır.
8. Tablo adları Tabloların üstüne ve şekil adları da Şekillerin altına birer satır aralıkla ve bunların genişliğini aşmayacak şekilde 11 punto yazılmalıdır. Tabloya ait açıklama varsa tablonun altına 1 boşluk bırakılarak 9 punto ile yazılmalıdır. Tablo ve Şekiller metin içine yerleştirilirken metin ile aralarında net ayırımı sağlayacak kadar boşluk bırakılmalıdır.
9. Paragraf başları 1 cm içeriden başlamalıdır.
10. Uluslararası kısaltmalar kullanılabilir. Metin içinde mililitre için ml; dakika için dak. olarak belirtilen şekliyle yazılmalıdır.
11. Makalelerin bölümleri Başlık, Öz, Anahtar kelimeler, Giriş, Gereç ve Yöntem, Sonuç ve Tartışma, Teşekkür ve Kaynaklar sırasına uygun olarak hazırlanmalıdır. Derleme makalelerinde Gereç ve Yöntem bölümü bulunmayabilir. Bu bölümler birbirlerinden 2 satır aralık ile ayrılmalıdır. Bu bölümleri ifade eden başlıklar 12 punto ile koyu olarak büyük harflerle ve sayfanın solundan başlanarak yazılmalıdır. Bölüm başlıkları ile metin arasında ayrıca aralık bırakılmamalıdır.

- **Başlık:** Türkçe ve İngilizce olarak büyük harf ve ilk başlık 14 punto, koyu ve ikinci başlık 12 punto, italik olarak yazılmalıdır. Başlık metine uygun, kısa, çalışmayı tanıtıcı ve açık ifadeli olmalıdır.
- **Öz:** Türkçe ve İngilizce (Abstract) olarak makalelerin başında 200'er kelimeyi geçmeyecek şekilde 10 punto ile, *italik* olarak ve çerçeve içinde yazılmalıdır. Yabancı dilde yazılmış makalelerde mutlaka Türkçe özet bulunmalıdır. Ayrıca öz, kendi içinde amaç, gereç ve yöntem, sonuç ve tartışma olarak alt başlıklar halinde yazılmalıdır.
- **Anahtar kelimeler:** En fazla 5 sözcükten oluşmalı ve özetlerin hemen altına ilgili dilde alfabetik ve italik olarak yazılmalıdır.
- **Giriş:** Araştırmanın amacı ve konuyla ilgili çalışmaların yer aldığı bölüm olmalıdır.
- **Gereç ve Yöntem**: Kullanılan gereç belirtilerek, uygulanan yöntem hakkında gerekli bilgiler açıkça ifade edilmelidir. Deneylerde hayvan kullanılması durumunda lokal etik komiteden veya ilgili düzenleyici makamlardan onay alınmalıdır ve bilgilendirilmiş onam belgelendirilmelidir.
- **Sonuç ve Tartışma:** Bulguların verilerek değerlendirildiği bölümdür.

- **Teşekkür:** Varsa araştırmayı destekleyen kuruluşa ve katkısı olan kişilere kaynaklardan önce yer alan bu bölümde kısaca teşekkür edilebilir.
- **Kaynaklar:** Kaynak yazım stili Amerikan Psikoloji Derneği'ne (APA) göredir. Metinde, geçiş sırasına göre köşeli parantez içinde, örneğin: [1,2,…] gibi numaralandırılmalı ve metin sonunda bu numaralara göre sıralanmalıdır. Kaynaklar aşağıdaki örneklere uygun olarak yazılmalıdır.

  i. **Makale için:** Yazarın soyadı, adının baş harfleri, makalenin tam başlığı derginin adı, cilt no, varsa sayı no (parantez içinde), başlangıç ve bitiş sayfa no, yıl yazar isimlerinden sonra (parantez içinde) olarak yazılmalıdır. Birden fazla yazar varsa hepsi yazılmalıdır. Makalenin adı yazılırken ilk kelimenin ilk harfi büyük diğer kelimelerin ilk harfi küçük yazılmalıdır. Kaynaklarda verilen dergi adları kısaltma yapılmadan açık olarak yazılmalıdır.

  Moncada, S., Palmer, R.M.J., Higgs, E.A. (1989). Biosynthesis of nitric oxide from L-arginine. A pathway for the regulation of cell function and communication. Biochemistry and Pharmacology, 38, 1709 – 1715.

  ii. **Elektronik Makale için:**

  Perneger, T. V. and Giner, F. (1998). Randomized trial of heroin maintenance programme for adults who fail in convential drug treatments. British Medical Journal, 317. Retrieved August 12, 2005, from ttp://www.bmj.com/cgi/content/full/317/7150/

  iii. **Web sitesi için:**

  Clinical Pharmacology Web site. (2001). Retrieved June 16, 2004, from http://cpip.gsm.com/

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  Franke, R. (1984). Theoretical Drug Design Methods, Elsevier, Amsterdam, p.130.

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  Weinberg, E.D. (1979). Antifungal Agents. In: M.E. Wolff and S.E. Smith (Eds.), Burger's Medicinal Chemistry, (pp. 531-537). New York: John Wiley and Sons.

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  Moncada, S., Palmer, R.M.J., Higgs, E.A. (1989). Biosynthesis of nitric oxide from L-arginine. A pathway for the regulation of cell function and communication, Biochemistry and Pharmacology, 38, 1709 – 1715.

  ii. **Electronic Article:**

  Perneger, T. V. and Giner, F. (1998). Randomized trial of heroin maintenance programme for adults who fail in convential drug treatments. British Medical Journal, 317. Retrieved August 12, 2005, from ttp://www.bmj.com/cgi/content/full/317/7150/

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  Clinical Pharmacology Web site. (2001). Retrieved June 16, 2004, from http://cpip.gsm.com/

  iv. **Book:**

  Franke, R. (1984). Theoretical Drug Design Methods, Elsevier, Amsterdam, p.130.

  v. **Chapter in a book:**

  Weinberg, E.D. (1979). Antifungal Agents. In: M.E. Wolff and S.E. Smith (Eds.), Burger's Medicinal Chemistry, (pp. 531-537). New York: John Wiley and Sons.

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JOURNAL OF FACULTY OF PHARMACY OF ANKARA UNIVERSITY

Cilt / Vol : 44
Sayı / No : 1
Yıl / Year : 2020
eISSN : 2564-6524

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