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Food, medicine or a poisonous plant: *Arum maculatum* L.

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ABSTRACT: *Arum maculatum* L., which is known with various names such as cuckoo pint and sneakshead, is a perennial, deciduous woodland plant distributed in Europe, East Ukraine and Anatolia. The phytochemical studies revealed that the plant contains different groups of bioactive components, such as alkaloid, saponin, cyanogenic glycosides, terpenic compounds, proanthocyanidins, carotenoids, volatile amines, lectin, mucilage, gum and starch. Various traditional uses are recorded such as antihypertensive, antirheumatic, antihemorrhoidal, analgesic, antidiabetic as well as its use against burns and wounds, *Fasciola hepatica*, sore throat and cold. It is also consumed as vegetable in different cultures. In the Southeastern region of Turkey, a traditional soup called "tirşik soup" is prepared from the leaves of *A. maculatum* and used for the treatment of hemorrhoids, respiratory and gastrointestinal disorders and cancer. It is very crucial to apply a specific process for the preparation of the dish or medicinal remedy, since all parts of the fresh plant are known to be toxic. The toxic effect majorly rises from the oxalate crystals found in the content of plants, along with some other components, such as cyanogenic glycosides, volatile amins, alkaloids etc. Although the bioactivity studies showed antioxidant, antimicrobial, antiinflammatory, analgesic, antiangiogenic and insecticidal activities as well as its protective activity against hemorrhoids; further research is needed on the bioactivity, cytotoxicity and phytochemistry of the plant in order to clarify the mechanisms of action and provide the safe use of the drug.

KEYWORDS: *Arum maculatum*; bioactivity; cuckoo pint; phytochemistry; traditional medicine.

1. INTRODUCTION

The genus *Arum* L., which grows in Europe, Western and Central Asia and North Africa, comprises 29 species and belongs to Araceae family [1, 2]. *Arum maculatum* L., which is also known as cuckoo pint, lords and ladies, devils and angels, cows and bulls, Adam and Eve, adder's root or sneakshead, is a perennial, deciduous woodland plant, which is distributed in Europe, East Ukraine and Anatolia. [3-7].

The tubers of the plant are irregularly cylindrical on a horizontal axis, at the end of which the shoots arise. The mature leaves are oblong-hastate to oblong-lanceolate and the young leaves are obovate. The colour of the leaves are green. Dark purple spots appear on the leaves of *Arum maculatum* var. *maculatum*, while the leaves of *A. maculatum* var. *immaculatum* do not have such spots. The leaves are arrow-shaped and long-stalked. The scape is shorter than petioles and the spathe is pale green usually narrow lanceolate and acuminate. The lower parts of the spathe overlaps forming a tube. The tube has a purple band passing through the middle. The flowers are protogynus. The shape and the size of the pollens are uniform and the pollens have spice on the surface [6, 8, 9]. The orange-red coloured berries of the plant are maturated in autumn and contain oxalate cyristals which cause the ache and irritation on skin and mucose membrane [10].

A. maculatum has a traditional use for centuries. It is recorded in "De Materia Medica" of Dioscorides, in which the use of the plant against snake bites was mentioned [11].

It is reported that the plant is traditionally used against various disorders, such as hemorrhoids, pain, inflammation, sore throat and mumps [3, 12, 13], and the use of the herbal material as a folk remedy inspired the researchers to reveal the bioactivity of the plant, yielding a number of studies showing different effects of *A. maculatum* (Figure 1).

2. PHYTOCHEMISTRY OF *ARUM MACULATUM* L.

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The plant is known to contain several groups of bioactive compounds, such as alkaloid, saponin, cyanogenic glycosides, terpenic compounds, proanthocyanidins, carotenoids, volatile amines, mucilage, gum and starch along with lectins, which are especially found in the content of the tubers [1, 2, 5, 11, 14-18]. The cyanogenic glycoside, triglochinin was determined in leaves and spathes of the plant [17]. Conisine, nicotine, ethylamine, isoamylamine, isobutylamine, caffeic acid, *p*-coumaric acid and chlorogenic acid were also detected in the content of the leaves [19, 20]. Unsaponifiable lipids including long chain alcohols and hydrocarbons, along with carotenoids were determined in the spadices [18]. The nutritional value of the soup prepared using *A. maculatum* is high, although some bioactive compounds are lost during the cooking process. The raw material is reported to contain protein, fat, carbohydrate, fibers and vitamin C and minerals such as iron, calcium, sodium, phosphorus magnesium, potassium, copper, manganese and zinc [1, 15, 21, 22]. The tubers are rich in the content of starch [16].

In a phytochemical study, glucomannan type polysaccharides were isolated from the aqueous extract of *A. maculatum* tubers [23]. Lectins were also isolated from the roots and tubers of the plants extracted with water and precipitated with ethanol [24]. A mannose-binding lectin, *A. maculatum* tuber lectin [25] and a non-glycosylated lectin, *A. maculatum* agglutinin [24] are among these compounds, which also possess promising biological activities.

The quantification of proteins in the content of 30% and 70% ethanolic extracts of *A. maculatum* tubers showed that the lectin amount of 30% ethanolic extract was about six-fold higher than that of 70% ethanolic extract. In the same study, the volatile components of the extracts were also analysed by (gas chromatography-mass spectrometry) GC-MS, revealing hydrocarbons, thiols, alcohols, terpenoid structures, carbonyl derivatives, esters of fatty acids and a compound containing nitrogen (N-(2-ethyl phenyl) benzamide) in the content of the extracts [26].

In the content of the seed oil, fatty acids with medium chain and acids with aromatic residues were detected [18]. The oil obtained from the seeds of *A. maculatum* by Soxhlet extraction was analysed by GC-MS, yielding the content of 13-phenyltridecanoic acid along with the minor components, such as 11-phenyl undecanoic acid, 13-phenyl tridec-9-enoate, 15-phenyl pentadecanoate and 15-phenyl pentadec-9-enoate [27].

Aqueous extract prepared from the leaves of *A. maculatum* was analysed by GC-MS, showing 9-octadecenoic acid methyl ester, hexadecanoic acid methyl ester, benzenepropanoic acid and 3,5-bis(1,1-dimethylethyl)-4-hydroxy methyl ester as major constituents [28].

In another study, the oil extracted from the leaves of *A. maculatum* using hexane was analysed by GC-MS and the major fatty acids were found to be palmitic acid, linoleic acid, oleic acid and α-linolenic acid among 18 fatty acids determined. These components composed 72.61% of the oil and the rest was comprised of minor fatty acids such as stearic acid, butyric acid, myristic acid, arachidic acid, heneicosanoic acids, behenic acid, lignoceric acid, myristoleic acid, palmitoleic acid, cis-10-heptanoic acid, γ-linoleic acid, cis-8,11,14-eicosatrienoic acid, cis-5,8,11,14,17-eicasopentanoic acid and cis-4,7,10,13,16,19-docosahexaenoic acid [2].

It is reported that several volatile components were determined in the content of *A. maculatum*. The major compounds were found to be indole, α-pinene, β-pinene, 2-heptanone, nonanal, *p*-cresol, terpinolene, germacrene B and α-copaene [17, 18].

Palmitic acid, methyl 9,12,15-octadecatrienoate, phytol and methyl linolenate were determined as the major components of the essential oil of *A. maculatum* leaves obtained by hydrodistillation, along with 39 minor compounds as the result of GC-MS analysis of the oil [7].

3. THE TRADITIONAL USE OF *ARUM MACULATUM* L.

It is known that the roots of *Arum* species were consumed as nutrient in the archaic ages. In the middle ages, the plant was utilized for its medicinal properties [16]. According to the records of Materia Medica, the plant was then known as a cure for snake bites. In the 17th century, it was used for the treatment of malaria, plague and phlegm in stomach and respiratory system [11, 19]. It was reported that the powdered roots of *A. maculatum* exhibits diaphoretic effect at low doses, but high doses of the herbal drug were toxic. Furthermore, the fruits were also stated to be fatal especially for children [16].

A. maculatum is listed as a medicinal plant in the records of the herbarium of Francesc Bolòs (1773–1844), who is a Catalan pharmacist and naturalist. The medicinal use of *A. maculatum* was documented

as expectorant and antiasthmatic as well as its use against sexually transmitted diseases. The corrosive effect of the plant was also notified as a toxic effect [29].

Various traditional uses are recorded such as antihypertensive, antirheumatic, antihemorrhoidal, analgesic, antidiabetic as well as its use against burns and wounds, *Fasciola hepatica,* sore throat and cold [19]. The plant is reported to be used as an antimalarial agent in Europe. The rhizomes are consumed as food in Czech Republic [18]. In Bulgaria, the tubers of the plant, "Ari Tubera", are used against kidney stones, hemorrhoids, liver diseases, colitis, digestive disorders and gastric hyperacidity. In an ethnopharmacological study on *A. maculatum* in Bulgaria, it was revealed that the plant is mostly used against hemorrhoids. Besides there are less known uses are traumatic pain, inflammation and reducing the formation of adipose tissue. In the same study, the traditional use of the rhizomes macerated in oil against the pain in joints likewise in Sicily [11, 30, 31]. An ethnobotanical study on the plants used for the treatment of cancer; the use of the decoction prepared using the leaves of *A. maculatum* along with some other species (*Arum dioscoridis* Sibth et Am., *Arum hygrophilum* Boiss. and *Arum palaestinum* Boiss.) was listed [32]. It is reported that the plant is consumed as food and is used as a contraceptive in folk medicine, in Jordan [33]. In Northern Morocco, the powdered roots are used as folk medicine against some disorders of the stomach [34]. The washed tubers are cut into small pieces and dried for at least 5 days to be used as folk medicine in Serbia. The recommended use is recorded as one piece 3 times a day for 30 days against hemorrhoids [35]. In Syria, the decoction prepared from the leaves and tubers is consumed against constipation and cancer by local people [36]. The macerate of the leaves in ethanol is used as antirheumatic and antineuralgic in Lebanon [37].

Especially the dried and powdered fruits of the plants are stated to be used for the treatment of hemorrhoids along with some other *Arum* species, such as *Arum balansanum* R. Mill., *Arum detruncatum* C.A. Meyer ex Schott, *Arum elongatum* Steven [10]. The mash or decoction prepared from the roots, leaves and fruits of *A. maculatum* are recorded to be used as folk medicine, which is used as remedy against *Herpes zoster,* sore throat, mumps, headache, abdominal pain, colitis, arterial hypertension, rheumatism, goitre and diabetes as well as hemorrhoids in Turkey [11, 12, 38]. According to the records in old books explaining herbal therapy, *A. maculatum* was also used as abortive [39]. According to the data obtained by ethnobotanical studies, the decoction prepared with tubers was recommended to be used 3 times a day for one week, for the treatment of hemorrhoids [40]. The plant's traditional use with its expectorant, antihelmintic and diaphoretic effects is also reported [15]. Especially the tubers of the plant were utilized with diaphoretic, diuretic, expectorant, purgative, antihelmintic, rubefacient and antirheumatic properties. The slices cut from the leaves or tubers are put on the skin for the purpose of ripening the boils [16, 20]. The dried tubers are recommended 1-2 g/day as an infusion; the fresh tubers and leaves are externally applied on the skin. Dried fruits and tubers are powdered and mixed with cacao butter to prepare a suppository [20]. Antifungal use of mashed roots mixed with flour is also reported [41]. Aphrodisiac properties were also attributed to the plant due to the shape of its flowers, although there is no scientific data showing this effect of the plant. The decoction prepared from the plant is used as mouthwash for the maintanence of the health of gum [16]. According to an ethnobotanical study investigating the medicinal plants in the Eastern region of Turkey, *A. maculatum* leaves were reported to be used as an antiinflammatory and digestive, as well as the use of the plant against gout, gastrointestinal, liver and respiratory tracts disorders, pain in joints, hemorrhoids, and rheumatic pain [42]. In Central Anatolia, the leaves of the plant are consumed as a vegetable and cooked as a meal, filled with wheat or rice, or roasted [43]. The tubers of the plant are reported to be used against hemorrhoids internally, in Southeast of Turkey [44]. A dish called "tirşik soap" is prepared using a traditional process from *A. maculatum* leaves in a specific season, in Kahramanmaraş (Andırın), Adana and Osmaniye in the southern region of Turkey [1, 45]. To prepare this meal, which is "the doctor of Andırın", the leaves collected in winter are cut in small pieces and some nutrients such as split wheat, yoghurt and tarhana, which is a traditional fermented cereal food used for making soup, are added. The surface of the mixture is covered with flour and the pot is closed and left for 8-10 hours. Covering with flour provides an aerobic medium for fermentation. Then it is cooked at least for 1 to 6 hours until the meal does not cause a tingling sense on the tongue, then boiled chickpea, garlic and salt are added into the soup [45-47]. The dish prepared with the leaves of the plant is used as a galactagogue and against cold, hemorrhoids, some gastrointestinal disorders and cancer [16, 45]. The plant is reported to be consumed as soup in Iran, as well [7]. The leaves of the plant are also cooked using a special process to

get rid of the toxic compounds, in Iraq [28] The use of decoction prepared from the leaves against intestinal parasites and rheumatism among the population in Iraq is recorded, as well [18].

4. BIOACTIVITY STUDIES ON *ARUM MACULATUM* L.

4.1. Antimicrobial activity

The roots of *A. maculatum* were extracted using petroleum ether and ethanol, succesively, in order to evaluate the antimicrobial activity of the plant by disc diffusion and microbroth dilution assays. The microbial strains, *Staphylococcus aureus* ATCC 6558, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 1539, *Salmonella typhi*, *Shigella flexneri*, *Proteus mirabilis* and *Candida albicans* ATCC 10231 were used for the tests. The petroleum ether extract was found to be potent against *S. epidermididimis* with the minimal inhibition concentration (MIC) of 39.1 µg/mL. MIC values of this extract against *Staphylococcus aureus* and *S. thypi* were found as 312.5 µg/mL and 625 µg/mL, respectively [41].

The antimicrobial activity of the extracts prepared using different solvents (petroleum ether, ethyl acetate and 70% methanol) using *A. maculatum* leaves. The tests were conducted on 10 bacterial strains (*Bacillus cereus* NRRL 3711, *Escherichia coli* ATCC 25922, *Enterobacter aerogenes* NRRL B-3567, *Enterococcus faecalis* ATCC 29212, M*icrococcus luteus* NRRL B-1018, *Pseudomanas phaseolicola, Proteus vulgaris* NRRL B-123, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 25923 and *Yersinia enterocolitica*), 6 molds (*Aspergillus flavus* NRRL 1957, *Aspergillus fumigatus* NRRL 163, *Aspergillus parasiticus* NRRL 465, *Fusarium graminearum* (wild type), *Fusarium solani* (wild type) and 1 yeast (*Candida albicans* NRRL Y-12983) using disc diffusion test and microbroth dilution assay. Tetracycline and vancomycine were used as a positive control against bacteria, while Amphotericin B is used for fungi and yeast. Among tested microorganisms, all of the samples were found to be effective against *A. niger* and *B. cereus* strains, while *A. fumigatus* and *A. parasiticus* strains were sensitive to none of the tested extracts. As the result of the study, the extracts were found to be more effective on Gram-positive bacteria [15]

The antimicrobial activity of *A. maculatum* against the strains of *S. thypi, P. aeruginos, Shigella boydii, Shigella dysanteriare, B. subtilis, Klebsiella pneumoniae, S. aureus, P. vulgaris, Corynebacterium diphteriae and C. albicans* was tested using agar-well diffusion test. No inhibition zone was observed for the concentration of 72.0 µg/mL [48].

The extracts *A. maculatum* leaves prepared using 80% ethanol were tested against *S. aureus, Propionibacterium acnes, E. coli, Salmonella typhi, Pseudomonas aeruginosa, Acinetobacter baumani, K. pneumoniae, S. dysentriae* by disc diffusion test. MIC values of the extract were found as 3 µg/mL against *P. aeruginosa* and *A. baumani* and 2.5 µg/mL against *S. typhi, S. aureus, P. acne* and *E. coli* [49].

In a study evaluating the antimicrobial activity of *A. maculatum* against *S. aureus* and *E. coli,* the leaves were extracted with petroleum ether, chloroform, ethyl acetate and 70% methanol, succesively. The extracts were tested at concentrations of 20, 50, 200 and 500 mg/mL, and Imepinem was used as a positive control. The methanol extract was found to be effective at all concentrations tested, while none of the strains were sensitive to chloroform extract. The extract prepared by petroleum ether showed antibacterial effect only at the concentration of 500 mg/mL and ethyl acetate extract was active at all concentrations except 20 mg/mL [50].

In a study, the antimicrobial effect of the methanolic extracts prepared from *A. maculatum* leaves and fruits against some microbial strains (*Escherichia coli, Enterobacter aerogenes, Staphylococcus aureus* ATCC 6538, *Lactobacillus casei* ATCC 393, *Pseudomonas aeroginosa* ATCC 9027, *Yarrovia lipolytica, Candida albicans*) was determined using agar-well diffusion test. *P. aeruginosa* was found to be the most sensitive strain to the tested extracts, MIC of fruits and leaves were found as 25.56 mg/mL and 9.44 mg/mL, respectively. The extracts did not show antifungal activity against tested microorganisms, while only the leaf extract was found to be effective against *L. cassei* with a MIC value of 37.49 mg/mL [10].

In another study evaluating the antimicrobial activity of the essential oil of *A. maculatum*; the bacterial strains of *Klebsiella pneumonia* PTCC 1290, *Staphylococcus aureus* PTCC 1431, *Staphylococcus epidermidis* PTCC 1435, *Bacillus cereus* PTCC 1665, *Pseudomonas aeruginosa* PTCC 1707, *Escherichia coli* PTCC 1395 and two strains of fungi (*Penicillium digitatum* PTCC 5251 and *Aspergillus niger* PTCC 5012) were tested using broth microdilution method. The essential oil exhibited mild activity on tested bacteria and fungi compared to the references ampicillin for Gram-positive bacteria, tetracycline for Gram-negative bacteria and fluconazole for fungi [7]

The antibacterial activity of *A. maculatum* leaf extracts were evaluated using microdilution and agar well diffusion assay. Three Gram-negative (*Pseudomonas aeruginosa, Salmonella enteritidis, Escherichia coli*) and two Gram-positive (*Listeria monocytogenes, Staphylococcus aureus*) bacterial strains were used for the assays. In the microdilution assay, MIC and minimum bactericidal concentrations (MBC) values of the extracts on the strains were calculated and it was shown that Gram-positive bacteria were more sensitive to the samples than Gram-negative bacteria. The lowest MIC value (12.5 mg/mL) was found against *S. aureus*. The researchers demonstrated the bacteriostatic effect of the extract at lower doses and bactericidal effect at higher doses by microdilution assay. In the same study, disk diffusion test and well diffusion test were conducted against the same strains. The growth inhibition zones of Gram-positive bacteria were observed greater than that of Gram-negative strains [51].

The antimicrobial activity of *A. maculatum* leaves against *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 6633, *Sarcina lutea* ATCC 9341NA, *Escherichia coli* ATCC 309628, *Enterococcus faecalis*, *Enterococcus faecium*, *Candida parapsilosis* and *Candida albicans* were determined using well diffusion method. The aqueous and methanolic extracts of the leaves were prepared for the tests. The samples were ineffective against the strains of *E. faecium, S. lutea, C. parapsilosis*, while *S. aureus* and *E. coli* strains were sensitive to all of the tested extracts. The methanolic extract obtained by ultrasound extraction was found to possess the highest antibacterial activity against *B. subtilis, S. aureus* and *E. coli* with the MIC of 2 mg/mL, 16 mg/mL and 8 mg/mL, respectively [2].

4.2. Antioxidant activity

In a study investigating the antioxidant potential of *A. maculatum* leaves collected from different locations along with the meal prepared using the leaves of the plant, superoxide dismutase (SOD) and catalase (CAT) activities were measured. SOD activity of the fresh material was found in the range of 17.73±7.48-25.05±12.28 U/mg protein, while CAT activity of the leaves was as 13.55±4.30-22.07±4.01 U/mg protein. SOD activity of the meal (62.77 U/mg protein) was significantly higher than that of the leaves and its CAT activity (20.59 U/mg protein) was close to the activity of fresh sample [46].

The polar extract of *A. maculatum* was evaluated in terms of its antioxidant capacity, using phosphomolybdenum, β-carotene/linoleic acid, reducing power, DPPH free radical scavenging and metal chelating effect tests. In β-carotene/linoleic acid test, the inhibitory effect of the extract was found as 88.37%±0.00, where butylated hydroxyanisole (BHA) (86.48%±1.93) and butylated hydroxytoluene (BHT) (92.14%±0.15) were used as references. DPPH radical scavenging activity of the extract was found to be weak (27.54±0.11) in comparison with BHA and BHT. The reducing power seemed to be remarkable at the concentration of 1 mg/mL (1.178±0.020), although the values recorded were lower than BHA (2.303±0.064) and BHT (1.258±0.121). The metal chelating capacity of the extract was found to be moderate with the value of 27.55%±1.58, while the activity of the reference, EDTA (ethylenediaminetetraacetic acid) was 99.74%±0.15. In phosphomolybdenum test, the capacity of the extract was calculated as 54.16±2.09 µg ascorbic acid/mg extract. Total phenol and total flavonoid contents of the extract were found as 24.83±0.82 µg gallic acid equivalent/mg extract and 15.99±0.00 µg quercetin equivalent/mg extract [48].

The antioxidant capacity of 80% methanolic extract of *A. maculatum* was determined by evaluating DPPH free radical scavenging capacity and reductive ability as well as the total flavonoid content of the plant. Total flavonoid content of the extract was found as 535.3±109.9 µg/ml. The extract exhibited the highest reductive at the dose of 0.64 mg/mL, but the capacity of trolox (0.278±0.010), which was used as a positive control, was found to be 3-fold higher than that of the extract at this dose (0.929±0.006). DPPH scavenging capacity of the extract was higher than the reference, trolox. The percentages of inhibitory effect of the extract were found as 72.33%±0.58; 80.00%±1.00; 83.00%±1.00; 93.33%±0.58 and these values for ascorbic acid were 39.00%±1.73; 46.00%±4.58; 50.33%±6.81; 64.67%±5.03 at the doses of 0.062 mg/mL; 0.125 mg/mL; 0.250 mg/mL and 0.500 mg/mL, respectively [30].

Total phenolic content, ascorbic acid content, total anthocyanin content, $ABTS^{+\bullet}$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity tests and ferric reducing ability (FRAP) assay were employed for the evaluation of antioxidant capacities of the methanolic extracts prepared from leaves and berries of *A. maculatum* by Soxhlet extraction. The fruit extracts were found to contain 960.6±7.6 mg/100 g sample of total phenols, 195.7±16.6 mg/100 g total ascorbic acid, while those for leaf extract were calculated as 134.9±2.3 mg/100 g and 230.4±12.7 mg/100 g, respectively. For the extract of leaves; total chlorophyll a (3.901±0.09 µg/g),

chlorophyll b (0.924±0.02 µg/g) and total carotenoid (0.370±0.06 µg/g) contents were also determined, along with total anthocyanin content, which was stated as 41.1±0.7 mg/100 g. In the $ABTS^{+\bullet}$ and DPPH assays; the inhibition values of the fruit extract were found to be 54.8%±0.32 and 55.1%±0.6 and these values for leaf extract were determined as 41.9%±1.79 and 39.5%±2.3, respectively. In FRAP assay; the antioxidant capacity of the berries (884.1±74.5 mmol Fe II/g sample) was found significantly higher than that of the leaves (101.7±2.7 mmol Fe II/g sample) [10].

IC_{50} value of the essential oil distilled from the leaves of *A. maculatum* was calculated as 24.86±21.4 mg/mL in DPPH free radical scavenging activity test [7].

In another study, the bioactivities of some edible plants growing in Ordu province of Turkey was evaluated. Among these plants, the antioxidant capacity of *A. maculatum* was also determined by CUPRAC (cupric ion reducing antioxidant capacity) and $ABTS^{+\bullet}$ cation scavenging assays, showing a slight antioxidant capacity of the extracts prepared using hexane, ethanol and water. IC_{50} values were found in the range of 106.01±0.42-153.99±0.15 µg/mL, while the $A_{0.50}$ values were determined 190.03±0.04 µg/mL for ethanolic extract and >200 µg/mL for aqueous and hexane extracts [38].

DPPH radical scavenging activity was determined as well as the total phenolic and flavonoid content of *A. maculatum.* Methanolic extract was obtained from the aerial parts using Soxhlet extraction. The total phenolic content of the extract was found to be 32.919±1.125 mg gallic acid equivalent/g and total flavonoid content was calculated as 52.045±7.902 µg rutin equivalent/mg. DPPH free radical scavenging test revealed the IC_{50} value of the extract as 105.76 µg/mL [52].

In a study evaluating the effect of extraction method on antioxidant capacity; total phenolic contents of aqueous, aqueous-ethanolic and ethanolic extracts prepared from *A. maculatum* leaves were found between 39.85±0.3 and 55.25±0.96 mg gallic acid in per gram sample; total tannins were in the range of 2.96±0,62-5.33±1.4 mg gallic acid in per gram sample; total tocopherol contents were between 109.62±6.72 and 133.48±3.11 µg α-tocopherol/1 ml sample; total flavonoid contents of the extract were calculated between 1.67±0.65-4.42±0.73 mg cathecin/1 g sample. In the same study; DPPH radical scavenging capacities, β-caroten/linoleic acid bleaching inhibitory effects and oxidative stability indexes of the extracts were investigated. The results of DPPH free radical scavenging capacity test were comparable to the reference butylated hydroxyanisole (76.83%±1.75), especially at the concentrations of 1000 ppm and 1200 ppm with the free radical scavenging capacity values ranging from 42.32%±6.2 to 81.39%±3.86. The results of β-caroten/linoleic acid bleaching assay revealed that the effects of the extracts at the concentrations between 400 and 1200 ppm were comparable (even higher for some samples) with butylated hydroxyanisole (76.83%±1.75), with the antioxidant activity values found between 54.04%±7.82 and 84.14%±3.99. The oxidative stability indexes were also found to be significant. Especially at the concentrations of 1000 ppm and 1200 ppm, the values for the samples (between 5.1±0.145 and 5.84±0.1) were higher than that of butylated hydroxyanisole (5.11±0.16) [51].

In another study, DPPH free radical scavenging capacity of the aqueous extract prepared from *A. maculatum* leaves was investigated. The tests conducted with the concentrations of the extract at 100 µg/mL, 150 µg/mL, 200 µg/mL, 250 µg/mL and a positive control, L-ascorbic acid. A dose-dependent antioxidant capacity was determined as the result of the study [28].

The $ABTS^{+\bullet}$ and DPPH free radical scavenging capacities of *A. maculatum* leaf extracts prepared using 30% ethanol were investigated along with the ability of the sample to scavenge hypochlorite and superoxide anion radical using luminol-enhanced chemiluminescence model. IC_{50} value for $ABTS^{+\bullet}$ scavenging capacity was found as 0.409±0.025 mg/ml and 0.924±0.018 mg/mL for DPPH radical scavenging ability of the extract. IC_{50} values against hypochlorite and superoxide radical were determined as 0.421±0.023 mg/mL and 4.037±0.121 mg/mL, respectively [26].

Another study evaluating the antioxidant capacity of the aqueous and methanolic extracts prepared from the leaves of *A. maculatum* using different methods revealed that total phenolic content was in the range of 4.83±0.48-12.44±0.02 mg gallic acid equivalent/g dry sample and total flavonoid content was found to be between 1.96±0.09 and 3.03±0.08 µg quercetin equivalent/g dry sample. *In vitro* antioxidant capacities were investigated using DPPH assay, where IC_{50} values were determined between 1.97±0.05 mg/mL and FRAP assays, with the values of 3.82±5.89 mmol ascorbic acid equivalent/g dry sample. The methanolic extract of the leaves was found to be the most potent in terms of antioxidant capacity [2].

DPPH and $ABTS^{+\bullet}$ radical scavenging capacities of *A. maculatum* fruits were investigated. IC_{50} values of methanolic extract was found as 0.75±0.15 mg/mL and 0.52±0.18 mg/mL against $ABTS^{+\bullet}$ and

DPPH radicals, respectively, while the antioxidant capacity of trolox, which is employed as the reference for $ABTS^{+\bullet}$ radical scavenging activity, was 0.013±0.004 mg/mL and IC_{50} value of the reference for DPPH radical scavenging activity, ascorbic acid was 0.004±0.001 mg/mL [53].

4.3. Anticholinesterase activity

Acetylcholinesterase and butyrylcholinesterase inhibitory effects of hexane, ethanol and water extracts of *A. maculatum* were evaluated. IC_{50} values for the reference galanthamine were determine as 0.5±0.01 µg/mL in AChE assay and 17.4±0.03 µg/mL in BChE assay, while these values for the tested extracts were found in the range of 128.01±0.53->200 µg/mL for AChE assay and 23.73±0.09->200 µg/mL for BChE assay. Nonpolar extracts were found to be more potent in terms of cholinesterase inhibitory effect [38].

4.4. Insecticidal activity

A homotetrameric lectin, *A. maculatum* tuber lectin, isolated from the tubers of the plant was tested for its activity against the pests *Aphis craccivora* and *Lipaphis erysimi*, yielding IC_{50} values of 16 µg/mL and 21 µg/mL, respectively [25].

4.5. Antiangiogenic activity

The antiangiogenic activity of 30% and 70% ethanolic extracts of *A. maculatum* tubers were investigated on human EA.hy926 cell line (ATCC: CRL-2922TM) by a modified MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Growth inhibition (GI_{50}), total cell growth inhibition (TGI) and LC_{50} values were determined. An angiostatic compound, namely hydroxyarthemisinine, which is a sesquiterpene lactone was used as reference. *A. maculatum* extracts exhibited strong inhibitory effect on proliferation of EA.hy926 cells in a dose-dependent manner. TGI and GI_{50} values were found as 4.06±0.4 and 0.87±0.1 for the reference, while these values were calculated as 338±19 and 152±21 for 30%ethanolic extract; 489±23 and 277±17 for 70% ethanolic extract, respectively. Even the highest concentrations of the tested extracts did not effect the cell viability [26].

4.6. Immunomodulatory activity

Immunomodulatory effect of 30% and 70% ethanolic extracts of *A. maculatum* tubers was determined by the evaluation of the capacity of the extracts to influence on mitogen activated production of interleukin-2 (IL-2) in Jurkat E6-1 cells. The extract obtained with lower concentration of ethanol was found to possess higher activity; suggesting less denaturation of the lectins in this extract, compared to the lectins exposed to ethanol at higher concentration [26].

4.7. Antiinflammatory activity

Aqueous extract of *A. maculatum* was evaluated in terms of its effects on the levels of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) of mice at the concentrations of 50 µg/mL and 100 µg/mL. The extract significantly influenced IL-1β levels at both doses, while it inhibited TNF-α only at 100 ng/mL concentration in comparison with the control group [28].

The extracts of *A. maculatum* tubers were prepared using 30% and 70% ethanol to investigate the effect of the plant on collagenase and cyclooxygenases (COX-1 and COX-2). The IC_{50} value of *A. maculatum* extracts prepared with 30% ethanol was determined as 318.1±14 µg/mL and that of 70% ethanolic extract was 548.5±27 µg/mL. 1,10-phenylanthroline (IC_{50}=21.17±3.9 µg/mL) was used as the reference inhibitor of bacterial collagenase. IC_{50} values for COX-1 inhibition were found as 589.90±23.4 µg/mL for 30% ethanolic extract; 978.1±47.9 µg/mL for 70% ethanolic extract, 11.36±3.2 µg/mL and 5.6±0.9 µg/mL for the reference compounds nimesulide and indomethacin. For the inhibitory effect on COX-2, the IC_{50} values for nimesulide, indomethacin and 30% ethanolic extract were determined as 0.42±007 µg/mL; 0.74±0.09 µg/mL; 499.1±20.5 µg/mL, respectively, while this value for 70% ethanolic extract was higher than 1000 µg/mL [26].

The clinical use of the tubers due to their antiinflammatory effect in respiratory and intestinal tract was reported in the study of Petkov (1982) [54].

4.8. Pro-inflammatory activity

A. maculatum agglutinin, which is a lectin purified from the tubers of the plant, was tested for inflammatory activity using neutrophil chemotaxis *in vitro* and neutrophil migration model *in vivo*. The compound administered into the peritoneal cavities of the rats at the concentrations of 80, 200, 500 µg per cavity provided an increase in neutrophil migration; but surprisingly, the effect of the lectin decreased at the dose of 500 µg. The migration process of the neutrophils was not effected by peritoneal lavage applied subsequent to the administration or by the treatment with compound 48/40, which decreases the number of mast cells in the peritoneal cavity. The neutrophil migration induced by the

compound was enhanced by 119% by the administration of 3% thioglycolate solution, a component that increases the population of macrophages at the rate of 236% itself, prior to the injection of the test component at the dose of 200 µg/cavity. This result suggested that the lectin enhanced the release of the chemicals inducing the migration of neutrophils from macrophages. In the model used for the evaluation of neutrophil chemotaxis, the promoting effect of the lectin by 368%, at the dose of 300 µg/mL. As the last step of the test, i.p. injection of the supernatants obtained from the macrophage cultures provided an induction of neutrophil migration at the rate of 110%. Eventually, the results of the study showed the pro-inflammatory effect of *A. maculatum* agglutinin [55].

4.9. Wound healing activity

In vivo wound healing activity of *A. maculatum* fruits was investigated on mouse models. The excision wounds which were treated with the methanolic extract of the fruits were photographed and wound contraction was measured for 10 days. At the end of the experiment, there was a significant difference between the contractions measured on days 0 and 10, but no significant difference was observed between the test and the control group at day 10. The integrity of the epidermal tissue, development of granulation tissue and vascularisation were evaluated by histological investigations, yielding a significant effect of *A. maculatum* extract. In the immunohistochemical assessment of the scar tissues, vascular endothelial growth factor staining was found to be increased by the extract, but collagen and platelet-derived growth factor staining did not differentiate significantly in comparison with the control group [53].

4.10. Analgesic activity

In an *in vivo* study conducted on rats, pain was induced by formalin injection and the influence of the aqueous extract prepared from the aerial parts of *Arum maculatum* on the duration of rats' paw licking were recorded in order to evaluate the analgesic activity of the plant. Morphine and diclofenac sodium were employed as positive control, while saline solution was infected as a negative control. The extract was injected at the doses of 250 mg/kg, 500 mg/kg and 750 mg/kg. The activity was assessed in two phases; phase I was the paw licking time right after formalin injection and phase II was 20 minutes after the injection. Morphine injected group for phases I and II were recorded as 30.6 ±1.9 and 37.2±1.1 seconds, while those for diclofenac sodium were 37.8 and 79.5 seconds, respectively. The extract significantly reduced the licking time at all doses tested, in comparison with the negative control group. The licking durations recorded for the test groups were 47.9 seconds in phase I and 81.9 seconds in phase II at 250 mg/kg dose; 30.9 seconds in phase I and 78.9 seconds in phase II at 500 mg/kg dose and 31.03 seconds in phase I and 53.3 seconds in phase II at 750 mg/kg dose. These results showed that the analgesic effect of the extract was comparable with morphine in phase I at the doses of 500 mg/kg and 750 mg/kg. In comparison to diclofenac sodium, the effect of the extracts at 500 mg/kg and 750 mg/kg doses were significant in both phases [56].

The morphine-like activity, due to the capsaicin content of the plant was also reported [16]

4.11. Cytotoxic activity

MTT assay was conducted to evaluate the effect of aqueous extract of *A. maculatum* leaves on the viability of the murine cell line L20B, at the concentrations of 0.4, 4, 40 and 400 µg/ml. The results revealed that the activity was in a concentration-dependent manner. The lowest concentration of the extract showed a growth inhibitory effect of 45%±0.020, while this ratio was found as 69%±0.024 for the highest concentration [28].

4.12. Protective effects against colitis and hemorrhoids

In a study evaluating the protective effect of the methanolic extract prepared using the aerial parts of *A. maculatum* against ulcerative colitis, dextran sulfate sodium was used for inducing colitis in rats. Malondialdehyde (MDA) levels and oxidative stress indexes (OSI) of the colon samples obtained from the feces were analysed. Sulfasalazine was used as positive control. The rats in the test group were treated with the extract at the doses of 50 mg/kg, 100 mg/kg and 150 mg/kg. As the first step of the experiment, macroscobic and microscobic examinations were conducted. Macroscobic examinations revealed the protective effect of the extract against sulfasalazine. The microscobic examination of colon samples showed that the extract was effective at the doses of 100 mg/kg and 150 mg/kg, while 50 mg/kg dose was not protective against the destructive effect of sulfasalazine. According to the results of histological examinations, the inflammatory cell infiltration was decreased by the extracts and the samples exhibited protective effect on the colon epithelium. Afterwards, the biochemical parameters of the colon samples were analysed. The results showed that the extracts reduced the MDA levels, which

was found to increase in the negative control group, in a dose-dependent manner. The MDA levels of the animals in the negative control group, the positive control group and the test groups (50 mg/kg; 100 mg/kg and 15 mg/kg doses) were determined as 9.12±5.49 nmol/mg protein; 1.83±0.43 nmol/mg protein; 4.38±0.64 nmol/mg protein; 1.95±0.32 nmol/mg protein and 1.65±0.28 nmol/mg protein, respectively, while this level of healthy group was 1.42±0.38 nmol/mg protein. The OSI values of negative control group (2.49±0.054) were increased in comparison with the healthy group (1.386±0.073). Expectedly, sulfasalazin reduced OSI levels (1.804±0.038). The OSI levels of the groups treated with 100 mg/kg (1.505±0.048) and 150 mg/kg (1.703±0.043) extract significantly decreased as well. The protective effect of the dose 50 mg/kg (2.198±0.056) was less significant in terms of OSI levels [52].

In a randomized controlled clinical trial investigating the efficacy of *A. maculatum* use for symptomatic treatment of hemorrhoids; patients were treated with *A. maculatum* roots or an antihemorrhoidal cream for 2 weeks. 3 g of *A. maculatum* root was grounded and put into 1 L of milk prior to pasteurization, which provided to get rid of the effects of toxic substances found in the herbal material. 250 mL of this mixture was consumed by the patients in the test group per day. An extensive health survey was used to assess the feed-back of the patients at the beginning and 2 weeks after the treatment. According to this survey, no difference between the test and the control group at the beginning of the trial was determined. A significant improvement was observed in all categories of the survey in the group treated with *A. maculatum* in comparison with the control group [57].

4.13. Other effects

The effect of a purified lectin, *A. maculatum* agglutinin, was tested for its effect on *Chlamydia pneumoniae* strain AR-39, which is an intracellular parasite that causes acute respiratory diseases and chronic obstructive pulmonary disease. It was found that lectin isolated from the roots of *A. maculatum* binded to the sialoglycoprotein receptors, which were *C. pneumoniae* specific, on L-929 fibroblast cells; so that the compound prevented the entry of *C. pneumoniae* into the cell and reduced the replication in the cell at the concentration of 10 µg/mL. The compound caused agglutination of human spermatozoa but not erythrocytes [24].

The antiaflatoxigenic effects of *A. maculatum* extracts prepared with hexane, ethanol and water were evaluated. The inhibitory effect of hexane, ethanolic and aqueous extracts were found to be 19.73%, 28.94% and 21.05%, respectively [38].

The depressive effect of the extracts of *A. maculatum* on the division of mouse bone marrow cells were observed with the aim of the investigation of cytogenetic activity of *A. maculatum*. The aqueous extract prepared from the leaves was administered at the doses of 500 mg/mL, 375 mg/mL and 250 mg/mL and 125 mg/mL. Mitotic indexes and mitodepression indexes were calculated for each dose from 8th to 48th hours of the experiment. The mitotic index was found to decrease (from 4.50 to 3.29) and mitodepression in the cells increased proportional to exposure time. The results of the study also revealed the effect of the extract on every stage of mitosis [33].

The protective effect of the polar extract of *A. maculatum* on DNA against oxidative and UV damage was evaluated using pBR322 plasmid DNA. In the presence of the extract, DNA was exposed to hydrogen peroxide and UV. No protective effect was observed as the result of the study [48].

The antimutagenic effect of the methanolic extracts prepared from *A. maculatum* leaves was assessed using the strains of *Salmonella typhimurium* TA98 and TA100. The fruits were found to be ineffective at the doses ranging from 10 to 80 µL/plaque, while leaves exhibited activity only against *S. typhimurium* TA98 strain, at doses of 10 and 80 µL/plaque [10].

In a study investigating α-amylase inhibitory effect of *A. maculatum* leaves, aqueous extracts and methanolic extract were tested. The results showed a slight inhibitory effect upto 30 minutes, with the inhibition ratios found in the range of 4.8%-14.1% [2].

Antiobesity activity of *A. maculatum* was investigated using a rat model in which obesity was induced by high-fat diet. Hexane, ethyl acetate and ethanolic extracts were prepared from the plant. The extract did not provide significant changes in body weight gain and food intake. The effects of the extracts on serum levels of glucose (117.33±10.53 mg/dL), total cholesterol (50.68±2.83 mg/dL), triglycerides (28.88±2.53 mg/dL), VLDL (5.77±0.50 mg/dL), LDL (16.11±1.87 mg/dL) and HDL (28.79±1.66 mg/dL), malondialdehyde (2.816 ± 0.17 µmol/L), aspartate aminotransferase (98.78± 11.06 IU/L) and alanine aminotransferase (37.16 ± 2.99 IU/L) at the dose of 300 mg/kg were found significant compared with the obesity-induced control group (147.03±8.52 mg/dL; 72.66±5.02 mg/dL; 75.20±1.88

mg/dL; 15.04±0.37 mg/dL; 36.30±3.66 mg/dL; 21.31±3.66 mg/dL; 3.411±0.08 µmol/L; 112.41±4.49 IU/L; 54.31±1.98 IU/L) [58].

5. TOXICITY OF *ARUM MACULATUM* L.

It is reported that overconsumption of *A. maculatum* may cause some toxic effects. The adverse effects appeared by eating the tubers, leaves, berries and fresh plants are stomach ache, pupil dilation, swollen tongue and throat, nausea, vomiting, difficulty in breathing, diarrhea, high body temperature, cold sweat, arrhythmia and hallucinations [1, 10, 19]. An ethnobotanical study investigating the toxic plants in Jordan notified that the leaves of *A. maculatum* could cause skin irritation and cardiac problems in humans and cattle, when exposed internally or externally [59]. The oxalate crystals found in the content of the plant are responsible for this effect. The crystals cause edema and pain in mucosal tissue. All parts of the plant are irritant on mucous membranes, but this effect may be reduced by boiling or drying the drug for a long time [19, 20, 60]. Due to the allergy risk, the plant should be handled cautiously [61]. The toxic effect of oxalate crystals found in the content of the plant is reported to vary due to the maturity of the fruits and the localization of the plant [16]. Toxic effects may be also attributed to some volatile amines, saponins, cyanogenic glycosides, lectins and alkaloids, along with the oxalates [18, 28].

Since a tingling sense in the mouth arises as the plant is chewed; excessive consumption resulting the toxication by the plant is rarely encountered. The treatment of *A. maculatum* toxication is based on the precipitation of oxalic acid as its calcium salt in the intestine [60]. It is reported that *A. maculatum* may cause some gastrointestinal and respiratory symptoms such as mucosal irritation, hypersalivation, vomiting, abdominal pain, diarrhea and dehydration in the animals that eat the plant [16].

The daily allowable dose of oxalate exposure for humans is 0.14 mg/kg oxalic acid or oxalate. The value of NOAEL (no observed adverse effect level) for oxalate in mice was found as 162 mg/kg per day. This value gives the permitted daily exposure dose of 0.27 mg/kg [62].

The cyanogenic glycoside called triglochinin is another cause of toxicity of *A. maculatum.* This compound may cause the symptoms of cyanide poisoning, such as cardiac arrhythmia, dyspnea and neurological disorders [63].

In a study evaluating the hepatotoxic effect of *A. maculatum* on mice, 80% methanolic extract prepared from the leaves was used at the dose of 100 mg/kg. Glutamate oxaloacetate transaminase (GOT), glutamic-pyruvic transaminase (GPT) and alkaline phosphatase (ALP) levels of the blood serum were determined as well as the histopathological evaluation of the liver tissue. GPT, GOT and ALP levels determined in positive control group that received CCl_4 (50.00±0.57; 18.00±0.58, 83.00±0.58 U/L, respectively) and the test groups that was treated with *A. maculatum* extract (65.00±0.58; 20.00±0.57, 101.00±0.57 U/L, respectively) and the group received both CCl_4 and the extract (61.00±0.54; 20.00±0.56; 95.00±0.55 U/L, respectively) were found to be significantly higher than the levels of negative control group (37.00±0.57; 15.33±0.88, 57.00±0.58 U/L, respectively) [64].

In a case report, two children at the ages of 11 and 13, who ate the leaves of *A. maculatum* were described. Along with the symptoms caused by oxalate crystals, the damaging effect of these crystals on skin-mucosa barrier resulting in increased the entrance of other toxins into the body was also mentioned in this report [63]. Another case reported that an adult with poisoning symptoms, which were angioedema and obstruction of upper respiratory tract, was hospitalized because of the overconsumption of *A. maculatum* tubers [61].

6. DISCUSSION

Notwithstanding the advancements in synthetic chemistry, there is a vast population relying on the therapeutic effects of herbal preparations. The pharmaceutical industry also utilizes traditional knowledge on herbal therapies, resulting in a wide range of options for herbal supplements/drugs in the pharmacies and markets. [28]. The plants have been a source for pharmaceutical industry due to the vast chemical variety and different biological activities of their metabolites, for centuries. The variety and potential of the synergistic effect of the components stand out as an advantage of phytotherapy, however it is also likely to be exposed to some toxic compounds in such mixtures of natural compounds. At this point, importance of pharmacological and phytochemical evaluation of plants comes forward.

The secondary metabolites, which are the products of the defense mechanism of the organisms, may be used either with their physiological activities or preserving properties, for instance to prolong the shelf life of the products [15]. In this respect, the antioxidant and antibacterial capacity of the plant seem to be promising especially in the field of food industry for preservation of the foods [7].

The phytochemical studies revealed that *A. maculatum* contains saponins which can be suggested as the main group responsible for the antifungal activity. High lectin and flavonoid content of the plant supports the antiinflammatory effect and the flavonoids found in the content of the plant are thought to possess an activity on cardiovascular system [1]. The pro-inflammatory effect of *A. maculatum* agglutinin by the induction of neutrophil migration was also reported [55] and the antiinflammatory activity was shown to be via the inhibition of inflammatory cytokines [28]. The phenolic content of the plant is considered to be responsible for its antioxidant capacity [51]. Besides, the oxygenated terpenes contribute to the antioxidant and antibacterial activities of the essential oil of *A. maculatum* [7].

Lectins are glycoproteins or proteins, which possess various biological activities, such as antioxidant, immunomodulatory, antimicrobial, mitogenic, insecticidal effects and found as storage components in some parts of plants especially in seeds and tubers [55, 65]. Antiparasitic activity of lectin type molecules are proven by different studies, therefore the traditional use of *A. maculatum* as an antiparasitic and antihelmintic drug may be attributed to its high lectin content [16]. Lectins are the compounds of which the effects on immune system are well known. These compounds are considered to be responsible for immune modulating activity of *A. maculatum,* along with mucopolysaccharides found in the content of the plant [26].

Several groups of bioactive compounds, which may be responsible for the cytotoxic effect, were detected in the content of the extract of *A. maculatum.* Fatty acids, also found as major components of *A.* maculatum, are reported to induce apoptosis caused by trauma in nervous system. Besides, flavonoids, saponins, polyphenolic structures are the components, which are known to act against cell death [28].

The hemorrhoidal disease is a condition in which inflammatory process, angiogenesis mechanisms and tissue remodelling are involved, thus the treatment strategies generally fail and palliative therapeutic models are used. In this respect, the antioxidant, antiinflammatory and antiangiogenic properties of *A. maculatum* seem to be promising [26]. The analgesic effect is also important in case of hemorrhoidal problems because pain is a destructive symptom of hemorrhoids. The *in vivo* analgesic activity of *A. maculatum* was also shown and suggested to be via a cholinergic mechanism [56]. Furthermore, there is a clinical study showing that the use of *A. maculatum* improved physical and mental health, herewith the quality of life of the patients suffering from hemorrhoids [57].

Apart from the pharmacological and phytochemical data, *A. maculatum* is considered to be a cultural value due to its special preparation process and use either as a meal or as a folk remedy. In this respect, "Andırın's tirşik" is a geographical indication, which means a product of a specific quality, being synonymous with its origin, for Kahramanmaraş. In the content of this record, there are two specifications of "tirşik"; the specific technique used for its preparation and fermentation, secondly; the biological characteristics of the plant growing in this region [66].

In Turkey, marketing and use of medicinal plants as nutrition, dietary supplement or nutraceutical are regulated by the Ministry of Agriculture, the medicinal plants are listed in two classes; positive and negative lists. According to a report of Ministry and Agriculture General Directorate of Food and Control, the leaves of *Arum* species are clasified as hazardous and can only be consumed after thermal processing. The leaves of *Arum* species are involved in the positive list on condition that the leaves are boiled and the filtrate is discarded. In this report, the regulation of some other countries on *Arum* sp. was mentioned. It is revealed that 9 countries, including Germany, Austria, Belgium, Bulgaria, Switzerland, Lithuania, Hungary, Romania and Russia, listed the plants of this genus in negative lists, which are considered either as a legislation or a recommendation [67].

7. CONCLUSION

The phytochemical and pharmacological studies on *A. maculatum* are very important, since the plant contains some toxic substances and is consumed as food, as well. The preparation process of the meal seems to prevent the toxic effects when it is consumed, but improper methods for cooking and direct consumption of the plant parts are known to cause toxic reactions. It is incontrovertible that the safety and the efficacy of an herbal material, which is widely used as a folk medicine, should be

supported by scientific evidence. Literature research revealed that, although there are plenty of ethnobotanical and ethnopharmacological studies on *A. maculatum*, further research is needed on the bioactivity, cytotoxicity and genotoxicity of the plant in order to clarify the active components, mechanisms of action and safety of the drug.

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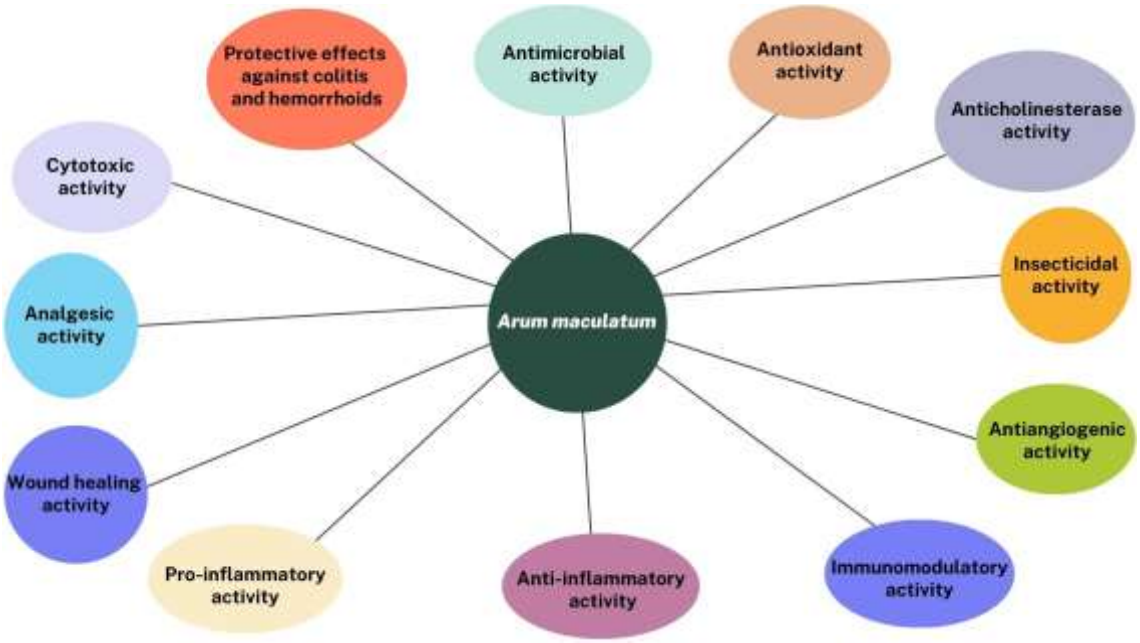

Figure 1. Biological activities of *A. maculatum.*