IJSM International Journal of Secondary Metabolite

International Journal of Secondary Metabolite
2023, Vol. 10, No. 2, 245–256

https://doi.org/10.21448/ijsm.1139025

Published at https://dergipark.org.tr/en/pub/ijsm **Research Article**

Phytochemical screening and *in vitro* assessments of antioxidant and cytotoxic potentials of extracts from *Aesculus hippocastanum* L. green fruit mesocarps

Tulay Askin Celik[1,*], **Ozlem Sultan Aslanturk**[1]

[1]Aydın Adnan Menderes University, Faculty of Science, Department of Biology, Central Campus, Aydın-Türkiye

Abstract: In this study, the in vitro antioxidant and cytotoxic effects of water and methanol extracts obtained from the green fruit mesocarp of *Aesculus hippocastanum* L. (Hippocastanaceae) were investigated. Phytochemical content of the methanol extract and the water extract were determined by qualitative methods; antioxidant activity was determined by DPPH free radical scavenging test, and total antioxidant capacity was determined by phosphomolybdate test. The effects of the extracts on proliferation and cell viability of BJ normal human foreskin fibroblasts were also evaluated by the WST-8 cell viability test.

Qualitative phytochemical screening results showed that the methanol extract contains phenols, tannins, flavonoids, and saponins, but no alkaloids and anthraquinones. On the other hand, phenols, flavonoids, anthraquinone, and saponins were found in the water extract, tannins and alkaloids could not be detected.

In addition, an increase in antioxidant activity was also observed with each increasing concentration of methanol and water extract. When the antioxidant capacity and free radical scavenging activity of methanol and water extracts were compared, it was determined that the methanol extract was more effective than that the water extract. The WST-8 trial results showed that both water and methanol extracts obtained from the green fruit mesocarp of *A. hippocastanum* did not have cytotoxic effects on BJ cells, on the contrary, treatment concentrations of 10, 20 and, 30 μgmL^{-1} increased cell proliferation significantly at the 24-hour work.

ARTICLE HISTORY

Received: July 01, 2022
Accepted: Apr. 26, 2023

KEYWORDS

Aesculus hippocastanum L.,
Antioxidant capacity,
BJ cells,
Cell viability.

1. INTRODUCTION

Medicinal plants, according to the World Health Organization (WHO), have bioactive chemicals that can be used for therapeutic purposes or synthesize metabolites that can be used to make effective medications (Paul *et al.,* 2018). A number of reports have been published in the literature regarding the antibacterial, anti-inflammatory, and wound healing efficacy of different plants, but the vast majority has yet to be investigated (Thakur *et al.,* 2011). Phytochemicals are naturally occurring chemicals in plants, providing a protective function against bacteria, viruses, fungi, the harms of free radicals, insects, and herbivores that feed on

*CONTACT: Tulay ASKIN CELIK ✉ tcelik@adu.edu.tr Aydın Adnan Menderes University, Faculty of Science, Department of Biology, Central Campus, Aydın-Türkiye

e-ISSN: 2148-6905 / © **IJSM 2023**

them and pose any environmental threat (Molyneux *et al.,* 2007; Curran, 2018). Medicinal plants generally contain phenolic compounds, including flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans, and lignin. Many of the beneficial effects of medicinal plants are attributed to their high polyphenolic content. These molecules are known to reduce cellular oxidative stress, which plays a crucial role in the pathogenesis of a range of diseases including cancer and cardiovascular diseases.

Aesculus hippocastanum L. (Hippocastanaceae), commonly known as Horse chestnut, is native to Western Asia, but today it is widely distributed and cultivated all over Europe. (Zhang *et al.,* 2010; Roy *et al.,* 2011; Geetha *et al.,* 2013). *A. hippocastanum* seeds are used for abdominal pain, stomach ache, cold, hemorrhoids, arterial stiffness, rheumatism, edema, diarrhea, antihemorrhagic, and antipyretic treatment (Baytop, 1999; Comitte on Herbal Medicinal Products, 2011; Tuzlacı, 2016) . The bark of *A. hippocastanum* has been used as a tonic, narcotic, and antipyretic, and also to induce sneezing. The plant constituents utilized medicinally come from the fruits (Roy *et al.,* 2011). In the bark extract of *A. hippocastanum*, coumarin derivatives such as triterpenoid saponins (aescin, prosapogenin), proanthocyanidin A2 and coumarins (esculin and fraxin), scopoline are present.There are flavonoids such as quercetin, kaempferol, astragalin, isoquercitrin, rutin, leucocyanidine and essential oils such as oleic acid, linoleic acid (Sirtori, 2001). Amino acids, allantoin, argyrin, carotin, choline, citric acid, epicatechin, leucodelphinidin, phytosterol, resin, scopoletin, tannin, and uric acid are among the other ingredients. Horse chestnut extract has also been shown to have antioxidant effects (Braga *et al.,* 2012; Vaskova *et al.,* 2015; Kováˇc et al, 2020; Owczarek *et al.,* 2021) and produce contraction force in fibroblasts, which is important for skin regeneration (Wilkinson & Brown, 1999). Due to these effects, horse chestnut extracts are used in skin products (Thakur *et al.,* 2011).

The plant material is applied, mainly externally such as bath infusions, creams, ointments, and suppositories, to conditions connected with vascular damage and defective blood clottings, such as venous insufficiency, hemorrhoids, cutaneous capillary fragility, as well as oedemas, small bruises, and limited skin and subcutaneous tissue inflammations (Comitte on Herbal Medicinal Products, 2011). *A. hippocastanum* seeds and their derivates might be thought of as an opencast mine of natural different chemicals that play a variety of roles in various biological activities in several ways.

While there are many reports on the antioxidant and cytotoxic activity of *A.hippocastanum* (AH) leaf, seed, and the seed coat, there are no *in vivo* or *in vitro* research samples about the effects of green fruit-mesocarp extracts. This experimental study was conducted to determine the phytochemical content and antioxidant activity of *A. hippocastanum* green fruit mesocarp methanol and water (decoction) extracts. In addition, the viability and proliferation of BJ normal human foreskin fibroblasts cells were investigated using the *A. hippocastanum* green fruit mesocarp methanol and water extracts.

2. MATERIAL and METHODS

In this study, the in vitro antioxidant and cytotoxic effects of water and methanol extracts of the green fruit mesocarp of *Aesculus hippocastanum* L. (Hippocastanaceae) were investigated. Phytochemical content of the methanol extract and the water extract were determined by qualitative methods. Antioxidant activity was determined by DPPH free radical scavenging test, and total antioxidant capacity was determined by phosphomolybdate test. The effects of the extracts on proliferation and cell viability of BJ normal human foreskin fibroblasts were also evaluated by the WST-8 cell viability test.

2.1. Chemicals and Reagents

The chemicals 1,1-diphenyl -2-picryl-hydrazyl radical (DPPH), sulphuric acid, disodium hydrogen phosphate (Na_2HPO_4), ammonium molybdate, ascorbic acid, dimethyl sulfoxide (DMSO), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and WST-8 cell viability assay kits were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Plant Material and Extracts of *A. hippocastanum* Fruit-Mesocarp (AHFME)

The fresh fruit mesocarp of *A. hippocastanum was* used in this study. The fresh fruit mesocarp of horse-chestnut was collected during the autumn season of 2017 from the Ankara Province of Türkiye. The fruit is a large, round and greenish capsule, covered with small pungent spines, opening into three valves and containing a large shiny brown seed. The fresh fruit shell is a green, spiky capsule that contains one (rarely two or three) nut-like seeds known as conkers or horse-chesnuts. The fresh fruit-mesocarps were washed 2-3 times with water and dried at room temperature.

The dried AHFME was finely powdered with a blender before being used for extraction. 50g of ground dry AHFME was added to 500mL of methanol and the extraction process was conducted. The extraction process was carried out at room temperature until the solvent became colorless (24-48 h). After the methanol extraction, the extract solution was filtered. After the methanol extraction, plant material (AHFME) was dried and subjected to water (decoction) extraction (Domínquez, 1973; Ravishankara *et al.,* 2002; Miliauskas *et al.,* 2004). Then the water extract was filtered as well. The methanol extract was evaporated using a rotary evaporator at 50ºC under decreased pressure. The water extract was lyophilized. The extracts were stored at -20ºC until they were used in experiments.

2.3. Preliminary Phytochemical Screening

The quantitative phytochemical screening tests were performed to determine various active components that are likely to be present in *A. hippocastanum* fruit-mesocarps extracts (AHFME). The test details are as follows:

2.3.1. Detection of phenols

The method of Ravishankara *et al.,* 2002 was used. The methanol and water extracts of AHFME prepared in ethanol were spotted on a filter paper in beakers. After that, the spots were applied to a drop of phosphomolybdic acid reagent and were exposed to ammonia vapors. The presence of polyphenols was indicated by the appearance of fresh radish blue color.

2.3.2. Detection of tannins

10% alcoholic ferric chloride (FeCl3) is added to 2-3mL of the extract of the methanol by doing so, the specific coloring was observed which is the dark blue or greenish-gray color indicative of the presence of tannins in the extracts (Ravishankara *et al.,* 2002).

2.3.3. Detection of alkaloids

A drop of the extracts prepared in methanol was spotted in a small piece of precoated TLC plate and the plate was sprayed with Dragendorff's reagent if the coloring of the solution is orange-reddish it indicates the presence of alkaloids in the extracts (Ravishankara *et al.,* 2002).

2.3.4. Detection of anthraquinones

With 10% ferric chloride solution and 1mL of concentrated hydrochloric acid, about 50mg of the extracts were heated. The extracts were cooled and then filtered, and the filtrates were shaken with diethyl ether. The extracts of ether were further extracted with strong ammonia. If there is aqueous layer coloration in pink or deep red indicates the presence of anthraquinones (Ravishankara *et al.,* 2002).

2.3.5. Detection of saponins

Hot water was added to the test tubes containing 10mg of extract and the tubes were vortexed for 30 seconds. Foamy appearance indicates the presence of saponins in extracts (Ravishankara *et al.,* 2002).

2.3.6. Detection of flavonoids

A piece of magnesium ribbon and 1mL of concentrated hydrochloric acid was added to 2-3 mL of the extracts dissolved in methanol. If pink-red or red coloration is observed it is indicative of flavonoids in the extracts (Ravishankara *et al.,* 2002).

2.4. Antioxidant activity of AHFME

2.4.1. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH free radical scavenging potential assay is based on the stable DPPH scavenging activity (Brand-Williams *et al.,* 1995). 1 mL of 0.1 mM DPPH methanol solution was added to 3 mL of different concentrations 10, 20, 30, 50, 75, 100, and 150 μgmL^{-1} of extracts in methanol. The mixture was shaken vigorously and kept at room temperature. Using a microplate reader (Elisa Reader, Biotek Co, USA) the absorbance of the mixture was measured at λ = 517 nm after 30 min. Ascorbic acid was used as the standard. The experiments were carried out three times. DPPH radical scavenging activity of the extracts was calculated using the following equation:

DPPH Scavenging capacity (%) = $[(A_0-A_1)/A_0] \times 100$

[Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the *A. hippocastanum* fruit-mesocarps methanol and water extracts].

The actual decrease in absorption induced by the test was compared with the positive controls. The EC_{50} (concentration providing 50% inhibition) values were calculated by using the dose inhibition curve in the linear range by plotting the extract concentration versus the corresponding scavenging effect.

2.4.2. *Phosphomolybdate assay* (*Total antioxidant capacity*)

The total antioxidant capacity (TAC) of the extracts was carried out with the phosphomolybdenum method (Umamaheswari & Chatterjee, 2008). About 0.1 mL of each plant extract sample was added to 1 mL of reagent solution (0.6 M sulphuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate). Tubes containing the mixture were covered with aluminum foil and incubated for 90minutes in a water bath at 95°C. The mixture was then left to cool at room temperature. The absorbance of the solution was measured at 765 nm against a blank. Ascorbic acid was used as the standard. The higher absorbance values indicated the higher total antioxidant potential of the plant extracts.

All the experiments were carried out in triplicate and were repeated three times. The total antioxidant capacity (TAC) was estimated using the following formula:

Total antioxidant capacity (%) = [(Abs. of control−Abs. of the sample/(Abs. of control] × 100

Abs: absorbance of sample;

Abc: absorbance of control

2.5. Cell Culture and Proliferation Assay (WST-8 assays)

BJ cells (Human normal foreskin fibroblast cell line; ATCC CRL-2522) were obtained from Dr. Mehtap Kılıç Eren, Aydın Adnan Menderes University, Faculty of Medicine, Department of Medical Biology, Aydın/Türkiye and used for *in vitro* studies. WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2Htetrazolium, monosodium salt] is a

water-soluble tetrazolium salt used for assessing cell metabolic activity that produces corresponding formazan dye that absorbs at 460nm. WST-8 is typically used as a cell viability indicator in cell proliferation assays.

The cells were maintained in Eagle's minimal essential medium supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich, Germany), penicillin (100IUmL^{-1}), and streptomycin (100 µgmL^{-1}) under a humidified atmosphere of 5% CO_2 at 37 °C until confluent. The cells were trypsinized and proliferation assays were carried out in 48 well plates. BJ cells were seeded into 48 well plates (5x10^3 cells per well) and incubated to form a partial monolayer for 24 h. After 24 h of incubation, the cells were treated with 10, 20, 30 µgmL^{-1} of AHFME for 24h respectively. The negative control cells were grown in a culture medium only and 10% crystalin has used as the standard. At the end of each of the treatment times, cellular viabilities were determined with WST-8 assay method (Tominaga *et al.,* 1999). The absorbance was read at 450nm for WST-8 assay by using a microplate reader (Elisa Reader, Biotek Co, USA). All of the absorbance values were compared to the control samples (without any compound) which represented 100% viability.

Cell viability (%)= [(As-Ab)/(Ac-Ab)] x100

As: Absorbance of sample

Ab: Absorbance of blank

Ac: Absorbance of control

2.6. Statistical Analysis

The analysis was executed by using the statistical package for the social sciences (SPSS version 20.0 Armonk, NY: IBM Corp) SPSS IBM 20. All measurements were carried out in triplicate and expressed as mean ± standard deviation. Statistical significance was determined using a one-way analysis of variance (ANOVA). The normality of variables was evaluated using the Kolmogorov-Smirnov Z test. The statistical differences between the control and treatment groups were carried out using the non-parametric Mann-Whitney Test (for independent samples). The correlations between different variables were determined using the Spearman Rank Correlation Test. P values less than 0.05 were considered as significantly different ($p \leq 0.05$).

3. RESULTS

3.1. Phytochemical Screening

Phytochemical screening results are presented in Table 1. Phytochemical screening results showed that AHFME methanol extracts contain phenols, tannins, flavonoids, and saponins, and do not contain alkaloids and anthraquinones. It was also determined that flavonoids, anthraquinones, and saponins were present in the water extract and tannins and alkaloids were not present.

Table 1. Phytochemical screening of methanol and water *extracts of A. hippocastanum* green fruit mesocarps

Active Compounds	Tests	Methanol Extract	Water Extract
Phenols	Phosphomolybdic acid test	++	+
Tannins	Braemer's test	+	-
Alkaloids	Dragendroff's test	-	-
Flavonoids	Shinoda's test	++	+
Anthraquinones	Bornträger test	-	++
Saponins	Frothing test	+	++

- absent of active compound; + moderate amount; ++ high amount after added reagent

3.2. Antioxidant Activity of *A. hippocastanum* Green Fruit Mesocarps Extracts

3.2.1. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant potential of the methanol and water extracts of *A. hippocastanum* green fruit mesocarp (AHFME) was evaluated based on their ability to scavenge stable free DPPH radicals. This test is based on the change of the color of the DPPH solution from purple to yellow due to the scavenging of stable free DPPH radicals. This change from purple to yellow was measured at 517nm (Brand-Williams *et al.,* 1995). A stronger yellow color indicates the ability of the extract to scavenge free DPPH radicals more and thus a stronger antioxidant potential.

The antioxidant potential of the different extracts from the methanol and water extracts from *A. hippocastanum* fruit mesocarps was assessed using different methods and the results are reported as values of IC_{50} (Table 2). The antioxidant activity and radical scavenging activity of the extracts differed depending on the concentration.

When the free radical scavenging effect of *A. hippocastanum* green fruit mesocarp extracts of methanol and water was compared in terms of EC_{50} values, the EC_{50} value of the water extract was found to be lower than that of the methanol extract. Therefore, it was determined that the antioxidant activity of the water extract was slightly higher. However, it has been determined that the antioxidant activity of both the methanol and water extract is significantly lower than that of ascorbic acid.

3.2.2. Phosphomolybdate assay (Total antioxidant capacity)

The phosphomolybdate assay is used routinely for the evaluation of extracts' antioxidant capacities. In this assay, phosphomolybdate (MoVI) is reduced to phosphate/MoV complex in the presence of an antioxidant (Umamaheswari & Chatterjee, 2008). The total antioxidant capacities o the AHFME methanol and water extracts measured are given in Table 2. The total antioxidant capacity of the AHFME methanol extract started to increase from the concentrations of 50µgmL^{-1} and reached 76.14±0.018% at its highest concentration (150 µgmL^{-1}). The EC_{50} value of the methanol extract is found to be 42.37 µgmL^{-1}, and the EC_{50} value of the water extract is found to be 87.95 µgmL^{-1}. However, the total antioxidant capacity of AHFME water extract could only exceed 50% at concentrations of 100 and 150 µgmL^{-1}.

The total antioxidant capacity of ascorbic acid is determined as 25.00%, 79.46%, and 89.71% at 10, 25, and 50 µgmL^{-1} concentration, respectively and the EC_{50} value is 17.65µgmL^{-1}. Although AHFME water extract also shows antioxidant activity, it has been found to show significantly lower than that of both ascorbic acid and methanol extracts (Table 2).

Table 2. DPPH scavenging activity and total antioxidant capacity of methanol and water extracts of *A. hippocastanum* green fruit mesocarps

Groups	Concentrations	DPPH Scavenging activity (% ± SD)	DPPH Scavenging activity (EC_{50})	Total antioxidant capacity (% ± SD)	Total antioxidant capacity (EC_{50})
Ascorbic acid	10 µgmL^{-1}	47.14 ± 0.021*	12.82 µg/mL	25.00 ± 0.005	17.65 µg/mL
	25 µgmL^{-1}	70.80 ± 0.007*		79.46 ± 0.027	
	50 µgmL^{-1}	73.91 ± 0.001*		89.71 ± 0.021	
ME	10 µgmL^{-1}	11.42 ± 0.061	57.57 µg/mL	36.87 ± 0.017*	42.37 µg/mL
	20 µgmL^{-1}	12.85 ± 0.053		39.85 ± 0.009*	
	30 µgmL^{-1}	15.24 ± 0.047		46.27 ± 0.007*	
	50 µgmL^{-1}	49.06 ± 0.003*		57.72 ± 0.001*	
	75 µgmL^{-1}	63.35 ± 0.002*		72.42 ± 0.030*	

	100 µgmL^{-1}	71.42 ± 0.002*		74.78 ± 0.070*	
	150 µgmL^{-1}	72.67 ± 0.001*		76.14 ± 0.018*	
WE	10 µgmL^{-1}	19.05 ± 0.025	41.85 µg/mL	16.67 ± 0.039	87.95 µg/mL
	20 µgmL^{-1}	25.24 ± 0.096		18.95 ± 0.003	
	30 µgmL^{-1}	26.19 ±0.020		20.55 ± 0.002	
	50 µgmL^{-1}	58.39 ± 0.004*		31.95 ± 0.013	
	75 µgmL^{-1}	66.46 ± 0.001*		40.10 ± 0.004*	
	100 µgmL^{-1}	67.70 ± 0.006*		74.27 ± 0.099*	
	150 µgmL^{-1}	68.94 ± 0.035*		75.94 ± 0.035*	

*p<0.05 ME: Methanol extract; WE: Water extract

3.3. Cell Viability and Proliferation Assay

The effect of different concentrations of the AHFME methanol and water extracts (10, 20, and 30µgmL^{-1}) on the cell viability and proliferation of BJ fibroblasts cells are shown in Table 3. WST-8 assay results showed that BJ cells treated with three different concentrations (10, 20, and 30 µgmL^{-1}) of the AHFME methanol extract for 24 hours, significantly increased cell viability and proliferation of BJ cells compared to control and crystalin (p<0.05). Similar results were observed in BJ cells treated with concentrations of 10 and 30 µgmL^{-1} of AHFME water extract, excluding 20 µgmL^{-1} (p<0.05) for 24h, as they were also statistically significant (Table 3). When the methanol and water extracts were compared for their effects on the cell vitality and proliferation of BJ cells, the methanol extract was found to be more effective. Crystalin (10%) did not have a significant effect on the vitality/proliferation of BJ cells (Table 3).

Table 3. Effects of methanol and water *extracts of A. hippocastanum* green fruit mesocarps on proliferation and viability of BJ human fibroblast cells.

Groups	Concentrations	Viability/Proliferation WST-8 assay at 24 h (%±SD)
Control	---	100.00±0.00
Crystalin (standart agent)	% 10	97.80±0.300
ME	10 µg/mL	121.22±0.220*
	20 µg/mL	100.14±0.187
	30 µg/mL	112.23±0.130*
WE	10 µg/mL	102.86±0.040
	20 µg/mL	97.70±0.215
	30 µg/mL	105.08±0.537

ME: Methanol extract; WE: Water extract. Data are the mean ± SD of three separate determinations. Values expressed are means ± SD of three parallel measurements ($^*p$<0.05).

4. DISCUSSION and CONCLUSION

This study's aim is to determine the phytochemical content of the methanol and water extracts of *Aesculus hippocastanum* green fruit mesocarps (AHFME), which have not been studied much before, and also to investigate the effects of these extracts on the cell viability and proliferation of BJ cells. The results of the study showed that the AHFME methanol extracts contain phenols, tannins, flavonoids, and saponins, and do not contain alkaloids and anthraquinones. It was determined that phenols, flavonoids, anthraquinones, and saponins were present in the water extract and tannins and alkaloids were not present (Table 1). Other studies on *Aesculus hippocastanum* show that the most commonly found ingredients in the *Aesculus hippocastanum* seed extract are escin and prosapogenin. *A.hippocastanum* seeds also contain

fatty acids such as flavonoids, tannings, amines, amino acids, uric acid, phytosterol, resins, citric acid epicatechin, leukocyanidine, oleic and linoleic acid, and kaempferol derivatives (Makuch & Matlawska, 2013). Leaf extracts include carbohydrates and anthocyanin (Paterska *et al.,* 2017; Idris *et al.,* 2020), while flowers contain coumarins such as esculin, esculetin, scopoletin, and fraxetin. *A. hippocastanum* immature fruit pericarps have been reported to contain saponins, flavonoids, and other phytochemicals (Ertürk, 2017).

Antioxidants inhibit the polymerization chains caused by substances that delay oxidation, free radicals, and other subsequent oxidizing reactions (Halliwell &Aruoma, 1991). When plants are exposed to extreme conditions, they activate the antioxidant mechanisms linked to the synthesis of phenolic compounds such as flavonoids, tannins, and other secondary metabolites (Oscar *et al.,* 2020). These chemicals are produced by plants to defend themselves from microorganisms and oxidative stress. However, nowadays some data indicate that these phytochemicals also often shield humans from different diseases. Some of the medicinal plants' positive effects are due to their high polyphenolic contents. Such compounds minimize oxidative cellular stress, which plays an important role in the pathogenesis of various diseases, including cancer and cardiovascular diseases (Sagdıcoglu Celep *et al.,* 2012).

Low IC_{50} values show a high activity antioxidant which means that the specific dissolvent mixture allowed enormous amounts of metabolites with antioxidant activity (Feghhi-Najafabadi *et al.,* 2019; Aslantürk *et al.,* 2017; Aşkın Çelik & Aslantürk, 2018; Uzunhan & Aşkın Çelik, 2018). The results of the DPPH scavenging assay revealed that the fruit mesocarps methanol extract from *A. hippocastanum* possesses a higher DPPH radical scavenging activity than that of the water extract. In this study, we examined the crude extract of AHFME to identify its antioxidant activity, but the effects of the individual molecules it contains were not examined.

The phosphomolybdate assay provides a method for reducing MoVI to phosphate/MoV complex by electron transport. This reduction will affect many natural products, including phenols and flavonoids (Ahmed *et al.,* 2015). In our analysis, concentrations of the methanol extracts showed a higher overall antioxidant capacity than of the water extract (Table 2). This difference in activity among extracts may be because of the phytochemical variations in the extracts. Additionally, the solvent polarities used for extractions are distinct, since methanol is an organic solvent, and water is an inorganic solvent. Such solvent properties may have contributed to a difference between the extracts in their overall antioxidant efficiencies.

Nowadays *in vitro* cytotoxicity and/or cell viability analyses, have appeared as an alternative to animal experiments and have been more favored because of their ease of use, speed, standardization, low cost, and compatibility with findings of *in vivo* studies (Aslantürk, 2018). Tetrazolium-based analyses (i.e., MTT, MTS, XTT, WST-1, and, WST-8 assays) measuring cytotoxicity via mitochondrial activity are commonly used to evaluate cell proliferation, cell viability (Taşkın *et al.,* 2020), and drug cytotoxicity, especially in cancer cells studies (Berridge *et al.,* 2005). The WST-8 test kit is an easy-to-use tool for evaluating the activation or inhibition of cell proliferation in an *in vitro* model. The test is based on NADH's extracellular reduction of WST-8, provided by transporting trans plasma membrane electron and electron mediator in the mitochondria. WST-8 reduction creates a water-soluble formazan that dissolves directly into the medium of culture, reducing the need for a further stage of the solution. WST-8 is more stable and less cytotoxic than the other salts of tetrazolium, making it particularly useful for longer incubation times, and its sensitivity detection is higher than of other tetrazolium salts (Aslantürk, 2018).

The effect of AHFME of ME and WE extracts on the proliferation and viability of BJ cells are present in Table 3. WST-8 test results showed that the AHFME methanol and water extract were not cytotoxic on BJ cells, except for 20 μgmL^{-1} concentration of the water extracts.

Many phytochemicals show a non-monotonic dose/concentration-response called a biphasic dose-response, with dose and time-dependently. These phytochemicals, which cause biologically opposite effects at different doses and/or durations, are called hormetic compounds (Mohapatra *et al.,* 2015). In cells treated with extracts, the reason for the concentration-independent increase and decrease in proliferation may be the hormetic compounds present in the extracts.

Crystalin (10%) also does not have an important effect on cell viability/proliferation. Interestingly, the ME and WE extract exhibited the highest antiproliferative effect on BJ fibroblast cells depending on the dose. The mitogenic effect is important for fibroblasts, which are important cells involved in wound healing and the production of extracellular matrix components (EMC). A study by Sagdıcoglu Celep *et al.,* (2012) found that the *A. hippocastanum* bark extract (0.01 mg/mL) increased cell proliferation in 3T3 healthy fibroblast cells (120%). The data obtained in our study are similar to the findings of that study. There are study examples showing that *A. hippocastanum* seed extracts have anticancer effects and inhibit proliferation. As the cell proliferation phase progresses, fibroblasts become the predominant cells at the wound site and play an important role in wound contraction to restore the integrity of injured tissue (Aksoy, 2020). Especially, for the wound-healing phase, fibroblast concentration, proliferation and migration are very significant (Addis *et al.,* 2020). Dermal fibroblasts are injury-responsive protective cells. Fibroblast proliferation and movement to the region where significant events occur in the wound healing process (Addis *et al.,* 2020) and stimulation of the development of fibroblast cells are a valuable models for investigating *in vitro* activities of wound healing. The data we obtained as a result of our study showed that the AHFME methanol and water extracts increased proliferation in BJ fibroblast cells. These results suggest that AHFME extracts may have wound-healing effects and it may be important to conduct new studies on this subject.

Aesculus hippocastanum L. seed extracts are known to heal venous ulcers, increase contraction force in fibroblasts, and have antioxidant and anti-aging effects. However, to our knowledge, there are not many *in vitro* studies to evaluate the phytochemical content, antioxidant activity, and effect of *A. hippoastanum* green fruit mesocarps extracts on cell viability and proliferation. The results of this study show that horse chestnut fruit mesocarps extracts have high antioxidant content and increase cell proliferation.

The use of natural antioxidants as a potential preventive for free-radical mediated diseases has become a very important issue for improving the quality of life. Different studies demonstrate the significant antioxidant activity of the ethanolic extract of *A. hippocastanum* in both the models utilized for the free radical scavenging activity. The antioxidant activity of different extracts obtained from *A. hippocastanum* can be attributed to the presence of different phenolic compounds found in extracts and the synergistic effects of other compounds. The results of this study showed that green fruit mesocarps extracts of *A.hippocastanum* have antioxidant activity and may also be useful in evaluating these parts, which are not usually used as waste products. Further studies are required to isolate, identify and elucidate the structure of the bioactive compound particularly responsible for the antioxidant activity of this plant. In addition, the extracts used in our study increased the proliferation of BJ cells *in vitro*. Further *in vitro* and *in vivo* studies are required to determine the effects of these extracts on wound healing and tissue regeneration.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Tulay Askin Celik has designed the study, collected the data, reviewed and edited the manuscript. **Ozlem Sultan Aslanturk** has performed laboratory analysis and statistical analysis of the study.

Orcid

Tulay Askin Celik https://orcid.org/0000-0001-6891-9089
Ozlem Sultan Aslanturk https://orcid.org/0000-0002-2503-0164

REFERENCES

Addis, R. Cruciani,S., Santaniello, S., Bellu, E., Sarais, G., Ventura, C., Maioli,M., Pintore, G. (2020). Fibroblast proliferation and migration in wound healing by phytochemicals: evidence for a novel synergic outcome. *International Journal of Medicinal Sci.*, *17*(8), 1030–1042. https://doi.org/10.7150/ijms.43986

Ahmed, D., Khan, M.M., Saeed, R. (2015). Comparative analysis of phenolics, flavonoids, and antioxidant and antibacterial potential of methanolic, hexanic and aqueous extracts from *Adiantum caudatum* leaves. *Antioxidants*, 4, 394-409. https://doi.org/10.3390/antiox4020394

Aksoy, H., (2020). Evaluation of the biological and wound healing activities of *Centaurea virgata* Lam. *Clin Exp Health Sci*., 10, 304-308. https://doi.org/10.33808/marusbed.789734

Aslantürk, Ö.S. (2018). *In Vitro* Cytotoxicity and Cell Viability Assays: Principles, Advantages, and Disadvantages. *Genotoxicity, A Predictable Risk to Our Actual World*. Chapter 1. Intech-Open Access Publisher, 1-7.

Aslantürk, Ö.S., Aşkın Çelik, T., Karabey, B., Karabey, F. (2017) Active phytochemical detecting, antioxidant, cytotoxic, apoptotic activities of ethyl acetate and methanol extracts of *Galium aparine* L. *British Journal of Pharmaceutical Research*, *15*(6), 1-16. https://doi.org/10.9734/BJPR/2017/32762

Aşkın Çelik, T., & Aslantürk, Ö.S., (2018). Phytochemical screening of *Carpobrotus acinaciformis* L. methanol extract and investigation of cytotoxic and apoptotic effect of the extract on the human metastatic breast cancer (MCF-7) and human colon cancer (Caco-2) cells. *Marmara Fen Bilimleri Dergisi*, *2*, 179-195. https://doi.org/10.7240/marufbd.339484

Baytop, T. (1999). *Therapy with Medicinal Plants in Turkey (Past and Present),* Second Edition, Nobel Presshouse, İstanbul, 361-362.

Berridge, M.V., Herst, P.M., & Tan, A.S. (2005). Tetrazolium dyes as tools in cell biology: new insights into their cellular reduction. *Biotechnology Annual Review, 11*, 127–152. https://doi.org/10.1016/S1387-2656(05)11004-7

Braga, P.C., Marabini, L., Wang, Y.Y., Lattuada, N., Calo, R., Bertelli, A., Falchi, M., Dal Sasso, M., & Bianchi, T. (2012). Characterisation of the antioxidant effects of *Aesculus hippocastanum* L. bark extract on the basis of radical scavenging activity, the chemiluminescence of human neutrophil bursts and lipoperoxidation assay. *European Review for Medical and Pharmacological Sciences*, *16*, 1–9. PMID: 22957412.

Brand-Williams, W., Cuvelier, M.E., & Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *Food Science and Technology*, *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Comittee on Herbal Medicinal Products. (2011). Assessment Report on *Aesculus hippocastanum* L. Cortex, European Medicines Agency: London, UK.

Curran, K. (2018). The ethno-herbalist review of phytochemicals. Retrived on August 30, 2018 from www.ethnoherbalist.com/phytochemicals-food-benefits/

Domínguez, X.A. (1973). *Métodos de ınvestigación fitoquímibolivarca*. Limusa, México D. F., México, 39-43.

Ertürk, E. (2017). Antibacterial and antifungal effects of the leaf, seed, seed coat and fruit capsule of *Aesculus hippocastanum* (Sapindaceae) extracts. *Acta Botanica Turcica*, *30*(1), 20-23.

Feghhi-Najafabadi, S., Safaeian, L., & Zolfaghari, B. (2019). *In vitro* antioxidant effects of different extracts obtained from the leaves and seeds of *Allium ampeloprasum* subsp. Persicum. *Journal of Herbmed Pharmacology*, *8*(3), 37. https://doi.org/10.15171/jhp.2019.37

Geetha, R.V., Roy, A., & Sitalakshmi, T. (2013). *In vitro* antioxidant and free radical scavenging activity of the ethanolic extract of *Aesculus hippocastanum*. International *Journal of Drug Development & Research, 5*(3), 403-407.

Halliwell, B., & Aruoma, O.I. (1991). DNA damage by oxygen derived species. Its mechanism and measurement in mammalian systems. *FEBS Letters*, *281*, 9–19. https://doi.org/10.1016/0014-5793(91)80347-6

Idris, S., Mishra, A., & Khushtar, M. (2020). Phytochemical, ethanomedicinal and pharmacological applications of escin from *Aesculus hippocastanum* L. towards future medicine. *Journal of Basic and Clinical Physiology and Pharmacology*, *31*(5), 2- 13. https://doi.org/10.1515/jbcpp-2019-0115

Kováˇc, I., Melegova, N., Coma, M., Takáˇc, P., Kováˇcová, K et al. (2020). *Aesculus hippocastanum* L. extract does not induce fibroblast to myofibroblast conversion but increases extracellular matrix production *in vitro* leading to ıncreased wound tensile strength in rats. *Molecules*, *25*, 917. https://doi.org/10.3390/molecules25081917

Makuch, M.D., & Matlawska, I. (2013). Coumarins in horse chestnut flowers: isolation and quantification by UPLC method. *Acta Poloniae Pharmaceutica*, *70*, 517–522.

Miliauskas, G., Venskutonis, P.R., & van Beek, T.A. (2004). Screening of radical scavenging activity of somemedicinal and aromatic plant extracts. *Food Chemistry*, *85*, 231-237. https://doi.org/10.1016/j.foodchem.2003.05.007

Mohapatra, M.F.D., Kumar, D., Chittoria, R., & Nandhagopal, V. (2015). Current concepts in the physiology of adult wound healing. *Plastic and Aesthetic Research*, *2*, 250–256. https://doi.org/10.4103/2347-9264.158851

Molyneux, R.J., Lee, S.T., Gardner, D.R., Panter, K.E., & James, L.F. (2007). Phytochemicals: The good, the bad and the ugly?. *Phytochemistry*, *68*, 2973-2985. https://doi.org/10.1016/j.phytochem.2007.09.004

Oscar, S.A., Antonio, C.N., Marina, G.V., Elsa, R.S., & Gabriel, V.A. (2020). Phytochemical screening, antioxidant activity and *in vitro* biological evaluation of leave extracts of *Hyptis suaveolens* (L.) from south of Mexico. *South African Journal of Botany*, *128*, 62-66. https://doi.org/10.1016/j.sajb.2019.10.016

Owczarek, A., Kolodziejczyk-Czepas, J., Wo´zniak-Serwata, J., Magiera, A., Kobiela, N.W., Asowicz, K., & Olszewska, M.A. (2021). Potential activity mechanisms of *Aesculus hippocastanum* bark: antioxidant effects in chemical and biological *ın vitro* models. *Antioxidants*, *10*, 9952. https://doi.org/10.3390/antiox10070995

Paterska, M., Bandurska, H., Wysłouch, J., Glura, M.M., & Molinski, K. (2017).Chemical composition of horse-chestnut (*Aesculus* L.) leaves and their susceptibility to chestnut leaf miner *Cameraria ohridella* Deschka&Dimic. *Acta Physiologiae Plantarum*, *39*, 105. https://doi.org/10.1007/s11738-017-2404-y

Paul, A., Barma, K., Roy, K., & Ray, S., (2018). A study of antimicrobial activity and medicinal value of *Adhotoda vasica, Centella asiatica, Paederia foetida, Nyctanthes abhor-tri Ocimum temiflorum. International Journal of Current Microbiology and Applied Sciences*, *7*(5), 1406-1413. https://doi.org/10.20546/ijcmas.2018.705.167

Ravishankara, M.N., Neeta, S., Harish, P., & Rajani, M. (2002). Evaluation of antioxidant properties root bark of *Hemidesmus indicus* R. Br. (Anantmul). *Phytomedicine*, *9*, 153-160. https://doi.org/10.1078/0944-7113-00104

Roy, A., Geetha, R.V., & Lakshmi, T. (2011). Evaluation of the antimycotic activity of aqueous and ethanolic extracts of *Aesculus hippocastanum*- An *in vitro* study. *International Journal of Drug Development & Research*, *3*(3), 335-338.

Sagdıcoglu Celep, A.G., Yılmaz, S., & Coruh, N. (2012). Antioxidant Capacity and cytotoxicity of *Aesculus hippocastanum* on breast cancer MCF-7 cells. *Journal of Food and Drug Analysis*, 20(3), 692-698. https://doi.org/10.6227/jfda.2012200318

Sirtori, C.R. (2001). Aescin: pharmacology, pharmacokinetics and therapeutic profile. *Pharmacological Research*, *44*, 183-193. https://doi.org/10.1006/phrs.2001.0847

Taşkın, A., Ulusal, H., Taşkın, S., & Tarakçıoğlu, M. (2020). Tetrazolium-based cytotoxicity tests may not always reflect accurate results. *Journal of Harran University Medical Faculty*, *17*(1), 6-12. https://doi.org/10.35440/hutfd.600652

Thakur, R., Jain, N., Pathak, R., & Sandhu, S.S. (2011). Practices in wound healing studies of plants. *Evidence-Based Complementary and Alternative Medicine*, Article ID 438056, 17 pages. https://doi.org/10.1155/2011/438056

Tominaga, H., Ishiyama, M., Ohseto, F., Sasamoto, K., Hamamoto, T., Suzuki, K., & Watanabe, M. (1999). A water-soluble tetrazolium salt useful for colorimetric cell viability assay. *Analytical Communications*, *36*, 47-50. https://doi.org/10.1039/A809656B

Tuzlacı, E. (2016). Türkiye Bitkileri Geleneksel İlaç Rehberi. İstanbul Tıp Kitabevleri, İstanbul.

Umamaheswari, M., & Chatterjee, T.K. (2008). *In vitro* antioxidant activities of the fractions of *Coccinnia grandis* L. leaf extract. *African Journal of Traditional Complementary and Alternative Medicine*, *5*(1), 61-73.

Uzunhan, S., & Aşkın Çelik, T. (2018). Phytochemical constituents and *in vitro* antioxidant and cytotoxic activities of different extracts from the aerial parts of *Heliotrophium hirsutissimum* GRAUER. *Jordan Journal of Biological Sciences*, *11*(4), 441-449**.**

Vaskova, J., Fejercakova, A., Mojzisova, G., Vasko, L., & Patlevic, P. (2015). Antioxidant potential of *Aesculus hippocastanum* extract and escin against reactive oxygen and nitrogen species. *European Review for Medical and Pharmacological Sciences, 19*, 879–886.

Wilkinson, J.A., & Brown, A.M.G. (1999). Horse Chestnut-*Aesculus Hippocastanum:* Potential applications in cosmetic skin care products. *International Journal of Cosmetic Science, 21*, 437-447. https://doi.org/10.1046/j.1467-2494.1999.234192.x

Zhang, Z., Li, S., & Lian, X.Y. (2010). An overview of genus *Aesculus* L.: Ethnobotany, phytochemistry, and pharmacological activities. *Pharmaceutical Crops, 1,* 24-51. https://doi.org/10.2174/2210290601001010024