Cilt 9, Sayı 2, Sayfa 157-169, 2020 Volume 9, Issue 2, Page 157-169, 2020

Araştırma Makalesi https://doi.org/10.46810/tdfd.823003 Research Article

Türk Doğa ve Fen Dergisi
Turkish Journal of Nature and Science
www.dergipark.gov.tr/tdfd

Alchemilla persica'nın Farklı Polaritelerdeki Ekstraktlarının Antisitotoksik, Antioksidan ve Antimikrobiyal Aktivitelerinin Belirlenmesi

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(Alınış: 07.11.2020, Kabul: 18.12.2020, Online Yayınlanma: 30.12.2020)

Anahtar Kelimeler
Alchemilla persica, Antisitotoksik, Antioksidan, Antimikrobiyal, Bitki Ekstraktları

Öz: *Alchemilla persica*, hekzan, diklorometan, etil asetat, metanol ve su gibi artan polariteye sahip çözücülerde farklı polaritelere sahip bitki özleri elde edildi. Antioksidan aktivitenin belirlenmesinde toplam fenol içeriği, toplam flavonoid içeriği ve likopen içeriği etil asetat, fenolik asit içeriği hegzan ekstraktında; *β*–karoten içeriği metanol ekstraktında diğer ekstraktlara göre yüksek olarak belirlenmiştir. Toplam antioksidan aktivite, DPPH serbest radikal giderme aktivitesi, metal şelatlama kapasitesi, toplam indirgeme gücü, hidrojen peroksit giderme, hidroksil radikal uzaklaştırma aktiviteleri farklı konsantrasyonlarda belirlenmiştir. Antimikrobiyal aktivitenin belirlenmesinde agar disk difüzyon tekniği kullanıldı. Antimikrobiyal testlerde, *Alchemilla persica*'nın diklorometan ekstraktı, en yüksek aktiviteye sahip ekstrakt olarak belirlendi. Etil asetat ekstresinin antimikrobiyal özelliği, diklorometan ekstraktına hemen hemen benzerdi. Bu ekstraktları antimikrobiyal değerlendirmede metanol ve su ekstreleri izledi. *Alchemilla persica*'nın farklı özütleriyle yapılan antioksidan çalışmaları sonucunda, α-tokoferol, BHA, BHT ve Trolox gibi standart antioksidanlardan daha etkili olduğu bulunmuştur. Bitki ekstraktlarından su ve metanol ekstraktları anti-sitotoksik etki için çalışılmış ve bu ekstraktlar farklı konsantrasyonlarda hücre büyümesini engellemiştir. Çalışmada elde edilen sonuçlar bu *Alchemilla persica* türüne ait ekstrelerin antioksidan, antimikrobiyal ve antisitotoksik etki gösterdiği belirlenmiştir.

Determination of Anticytotoxic, Antioxidant and Antimicrobial Activities of *Alchemilla persica* Extracts in Different Polarities

Keywords
Alchemilla persica, Anticytotoxic, Antioxidant, Antimicrobial, Plant Extracts

Abstract: Plant extracts with different polarities were obtained by transferring *Alchemilla persica* into different solvents with an increasing polarity such as hexane, dichloromethane, ethyl acetate, methanol, and water. In determining the antioxidant activity, total phenol content, total flavonoid content, and lycopene content in ethyl acetate, the phenolic acid content in hexane extract; *β*-carotene content in methanol extract was determined to be higher than other extracts. Total antioxidant activity, DPPH free radical removal activity, metal chelating capacity, total reduction power, hydrogen peroxide removal, hydroxyl radical removal activities were identified in different concentrations. Agar disc diffusion was used in the determination of the antimicrobial activity. Dichloromethane extract of *Alchemilla persica* appeared as the extract with the highest activity. The antimicrobial property of the ethyl acetate extract was almost similar to dichloromethane extract. Methanol and water extracts followed these extracts in antimicrobial evaluation. As a result of antioxidant studies with different extracts of *Alchemilla persica*, was found to be more efficient than standard antioxidants such as α-tocopherol, BHA, BHT, and Trolox. Water and methanol extracts from plant extracts worked for the anticytotoxic effect, and these extracts prevented cell growth at different concentrations. The results obtained in the study were determined that the extracts of *Alchemilla* persica have antioxidant, antimicrobial, and anticytotoxic effects.

1. INTRODUCTION

Alchemilla L. species belongs to the Rosaceae family. Rosaceae family is a large and significant family of dicotyledons that includes herbaceous and arboreal plants. This family contains up to 3370 taxa within 122 taxonomies. Several species are grown in parks and gardens as ornamental plants. Most of the *Alchemilla* L. species are observed in the Northern Anatolia Region in Turkey. It was argued that these could be close relatives of the species that grow in Crimea, Caucasia, and Iran [1].

It was observed that vegetable and fruit rich diets prevent diseases such as chronic coronary risks, cardiovascular diseases, arthritis, chronic inflammation, and cancer in epidemiologic and empirical studies [2 – 6].

The physiological functions of vegetables and fruits are related to their phenolic content. Chromatic vegetables and fruits contain phenol sources and carotenoids, including plenty of flavonoids and anthocyanin in their structures [7 – 11]. More than 4000 species of flavonoids that belong to polyphenol plant taxonomy were discovered [11]. Flavonoids and anthocyanidins responsible for the color blue in orange and red vegetables and fruits could be classified as flavanols (catechins), flavones, and flavonols [11]. Lipid peroxidation in biological systems; biological structures and especially membranes contain high rates of unsaturated lipid. These lipids incur oxidation either due to a radical initiator or in the existence of oxygen. Lipid peroxides cause cellular damage, change the metabolism, and reduce the blood flow in tissues due to the use of chemical substances they contain. They also affect amino acids and proteins via free radicals, which could modify unsaturated amino acids that contain sulfur (tryptophan, tyrosine, phenylalanine, histidine, methionine, and cysteine) and proteins that contain these amino acids. Their effects on nucleic acids and DNA could be summarized as base modification in nucleic acids, the formation of mutations due to the breaking of fibrils in DNA, and necrosis. They could cause changes in cell surface receptors and synovial fluid viscosity by adding hyaluronic acid in carbohydrates and reducing the amounts and activities of cofactors that contain nicotinamide and flavin [12].

Phytonutrients are quite significant for health since they fulfill certain physiological functions. When these nutrients are consumed frequently and regularly, they have considerable protective effects on human health. In vitro studies demonstrated that phytochemicals in nutrients have powerful antioxidant potential, reduce the risk of diseases, and protect the body against cancer and cardiovascular diseases. Vitamins, ascorbic acid, α-tocopherol, and β-carotene are among the antioxidants that are found in nutrients. Studies demonstrated that vitamins C and E and carotene antioxidants are more effective than flavonoids, isoflavone, anthocyanin, catechin, and isocatechin [13]. Industrial food producers add high-intensity synthetic antioxidants to products to increase their shelf lives, to protect nutritional value, and to reduce the formation of detrimental substances [14].

The effects of synthetic antioxidants, BHA, and NHT were tested on animals. It was determined that these synthetic antioxidants accumulated in the animals' bodies tested and damaged their livers, having carcinogenic effects [14].

Certain countries have banned the use of synthetic antioxidants. It is considered that many others would prohibit the use of synthetic antioxidants in food products in the future. Studies are conducted to replace synthetic antioxidants with natural substances [15].

There are studies conducted on the effects of natural products obtained from plants on antibacterial and antioxidant agent development to replace synthetic chemicals, and today these studies represent a field called 'functional nutrients' [16].

The widespread and unregulated use of antimicrobial substances, especially in developing nations, causes a selection favouring resistant strains [17]. Thus, antibiotic resistance increased considerably during recent years, and the increase in therapeutic problems is becoming a significant health issue [18, 19].

Studies to discover new antimicrobial agents are obligatory in the fight against infections since pathogen bacteria develop resistance against several commonly used antibiotics and overcome the resistance problem. Due to the side-effects that existing antimicrobial agents cause [20]. Scholars are recently interested in biologically active compounds isolated from plant species and can destroy pathogenic microorganisms. In recent years, medicinal plants' antimicrobial properties have been increasingly reported in different parts of the globe [21, 22].

This study aims to determine the antioxidant and antimicrobial properties specie *Alchemilla persica* of *Alchemilla* L., becoming prevalent in Turkey. Only a few studies in the literature determine whether it could be used in functional nutrient studies.

2. MATERIAL AND METHOD

2.1. Materials

2.1.1. Supply of the *Alchemilla persica*

The plant used were procured from Kırıkkale University ADO Herbarium.
Alchemilla persica samples utilized in this study were collected on the highway that connects Trabzon and Gümüşhane, from Trabzon to the Zigana Pass, and from the road that leads to Gümüşyayla ski area, where it is deployed liberally. The registration numbers and the herbarium that the samples were stored are displayed in Table 1.

Table 1. Gatherer registration number, and the herbarium that the samples were stored.

Plant name	Gatherer Registration No.	Herbarium
A. persica	Yusuf 1508	ADO

2.1.2. Chemical substances used

All chemicals and reagents utilized are procured in analytic or HPLC class purity. Catechin (+), Gallic acid, Folin-Ciocalteu reagent, 1.1-diphenyl-2-picrylhydrazyl (DPPH) radical, ferrozine, trichloroacetic acid (TCA), linoleic acid, α-tocopherol, catechin, quercetin, sodium carbonate, sodium phosphate, sodium nitrite, sodium nitrate, sodium molybdate, ammonium molybdate, aluminum chloride, DMSO, hexane, dichloromethane, ethyl acetate, methanol, ethanol, acetone, ferrous chloride, ferrous cyanide, hydrogen peroxide, 1.10-phenanthroline were purchased from Sigma Aldrich (USA). Mueller Hinton Agar, Dextrose Agar, butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT) were procured from Merck (Darmstadt, Germany). RPMI 1640 medium, Fetal bovine serum was purchased from Biological Industries, Israel. WST-1 cell proliferation assay kit was purchased from Clontech, USA.

2.1.3. Laboratory equipment used

Microbiologic security cabinet (Bilser), UV-VIS Spectrophotometer(Shimadzu/ Jasco V650), UV-Spectrophotometer sink (3 cubic centimeter quartz sink), magnetic stirrer (Ika), heated water bath (Wise Clean), automatic pipettes (Brand), circular shaker (Gerhardt), ultrasonic bath (Wise Clean WUC-D06H), deep-freeze (-86 °C, Hettich/ Nuair), rotary evaporator (Ika RV06-ML), pH-meter (Hanna Instrument), precision balance (Precisa, Denver), incubator (Elektro-Mag 0-300ºC), vortex (Ika MS3 Basic), water purifier (GFL 2004), dispenser (Isopenser), centrifuge (Hettich Universal 320), refrigerator (4°C, Arçelik), autoclave (Hirayama), disinfector (Memmert 100-800) were utilized.

2.2. Methods

2.2.1. Extraction technique

The dry plant sample was pulverized in the mortar. Then the powder was subjected to the extraction process from a nonpolar solvent to the polar solvent. For this purpose, it was mixed in a shaking mixer for 24 hours at 150 rpm in initially hexane fraction and in dichloromethane, ethyl acetate, methanol, and distilled water, respectively. Finally, the extracted liquid was filtered, and the solvent was evaporated in a low-pressure rotary evaporator, and the extract was obtained. The extract was then transferred to a vacuumed desiccator and thoroughly dried and was protected from humidity. The plant residue remaining in the filtrate was transferred into the hexane fraction for the second and then the third time, and the same processes were repeated. These processes were conducted again to obtain the other solvent extracts similarly. Thus, five different fractions were obtained for the plant strain [23].

2.2.2. Antioxidant activity determination techniques

2.2.2.1. Total phenolic substance assay

The extracts' total phenol amount was determined using the Folin-Ciocaltaeu method [24]. Gallic acid and all working samples were prepared in 70% methanol. 0.5 mL sample, 2.5 mL Folin-Ciocaltaeu reagent, and 7.5 mL sodium carbonate solution were mixed in a flask and kept at room temperature for 2 hours. After 2 hours, the absorbance of the solutions was read using a UV spectrophotometer at 760 nm, and total phenol amounts were calculated from the calibration curve plotted with gallic acid, where it would equal the gallic acid per mg [24].

2.2.2.2. Determination of total flavonoid content

To determine the total flavonoid amount, 250 µl of plant extract was transferred into 1.25 mL distilled water. 75 µl 5 % $NaNO_2$ solution was added to this mixture and stirred. After 5 minutes, 10% $AlCl_3.H_2O$ solution was added to this mixture and stirred. On the following 6th minute, 500 µl 1 M NaOH was added, and then 275µl distilled water was added, and the whole mixture was stirred well but gently. Total flavonoid content was calculated in mg per kg plant extract by reading the pink color that appeared at 415 nm based on the previously prepared (+)-catechin standard calibration graph as a (+)-catechin match [25].

2.2.2.3. Total phenolic acid content (TPA)

Freshly prepared plant extract (1.0 mL) was added to the reagent (2.0 mL), which was prepared by dissolving 0.5 M hydrochloric acid (2.0 mL), 10 g sodium nitrite, and 10 g sodium molybdate in 100 m water. Then 2 mL 8.5% sodium hydroxide was added. Then the mixture was filled with distilled water until the volume reached 10 mL. Absorbance was read at 505 nm. For blind tests, 10 mL of water was used for each extract. To determine the total phenolic acid content standard calibration curve was plotted using sinapic acid. Total phenolic acid content was calculated using sinapic acid equivalent in the calibration curve [26].

2.2.2.4. Determination of total B-Carotene and lycopene content

To determine the carotenoid content by a spectrophotometric approach using Barros et al.'s [25] method, 100 mg plant extract was transferred into 10 mL acetone-hexane (4:6) mixture and stirred well for 1 minute and filtered using Whitman No: 4 filter paper. The absorbance values of the filtrate were measured at 543, 505, and 663 nm wavelengths. β-Carotene and lycopene content was evaluated using standard graphs, and carotenoid amounts per kg plant were calculated in mg [25].

β-Carotene and lycopene content was calculated using the formulas 1 and 2:

$$\textbf{β-carotene (mg/100 mL)} = 0{,}216A_{663} - 0{,}304A_{505} + 0{,}452A_{453} \quad (1)$$

$$\textbf{Lycopene (mg/100 mL)} = -0{,}0458A_{663} + 0{,}372A_{505} - 0{,}0806A_{453} \quad (2)$$

Where A_{663}, 663 nm; A_{505}, 505 nm; and A_{453}, 453 nm; indicate absorbance values read in corresponding wavelengths. The results were calculated as mg carotenoid amount per g extract.

2.2.2.5. Removal of the effects of DPPH radical

2.7 mL methanolic solution, including the DPPH radical that was prepared as $6x10^{-5}$ mol per liter, was added into the phenolic extract (0.3 mL) in different polarities and obtained from the plant. This mixture was stirred vigorously and then stored in a dark area for 60 minutes. The reduction of DPPH radical is determined by the Assessment of its absorption at 517 nm. The Removal of the activity of this radical was conducted using the method provided by several researchers [27]. Removal of the DPPH color was indicated by the spectrophotometric method, and DPPH radical removal activity for the extracts was calculated. DPPH radical removal activity was calculated using the formula below:

% Inhibition = $(1-A_{Sample}/A_{Control}) \times 100$ (3)

where $A_{control}$ is the absorbance of the control or the blind, and A_{sample} is the absorbance measured when the extract was present.

2.2.2.6. Determination of the reductive force property

2.5 mL of plant extracts diluted with various concentrations were mixed with 200 mmol/L sodium phosphate buffer with pH:6.6 and 2.5 mL 1% potassium ferrous cyanide and incubated for 20 minutes 50 degrees C. 2.5 mL 10% trichloroacetic acid solution was added. This mixture was centrifuged for 8 minutes at 1000 rpm. After the centrifuge, the upper phase was removed, and 5 mL deionized water and 1 mL 0.1% ferrous chloride was added to the upper phase, and the reading was conducted at 700 nm on the spectrophotometer. High absorbance indicated high reductive property. The calculations were conducted against BHA and α-tocopherol standards [28].

2.2.2.7. Hydrogen peroxide removal activity assay

Hydrogen peroxide removal activities of the solutions prepared by plant extracts utilized in the study and water that contains various concentrations of synthetic antioxidant substances were assayed using the method developed by Ruch et al. [29]. Hydrogen peroxide (H_2O_2) (40 mM, 0.6 mL) prepared in 100 mM pH 7.4 phosphate buffer was added to 0.4 mL plant extract solutions. As soon as H_2O_2 was added, the mixture's absorbance at 230 nm was read against the blind. H_2O_2 removal activity was calculated using the formula below:

H_2O_2 Removal Activity = $(1-A_{Sample}/A_{Control}) \times 100$ (4)

where $A_{Control}$ is the absorbance of the control or the blind, and A_{Sample} is the absorbance measured when the extract was present.

2.2.2.8. Determination of the hydroxyl radical removal property

Hydroxyl radical removal property was analyzed using Fenton reaction as determined by the method developed by Özyürek et al.[30]. Based on this method, and the reaction mixture was prepared by adding 60 µl 1 mM $FeCl_2$; 90µl one mM 1.10-phenanthroline; 2.4 mL 0.2 M phosphate buffer (pH: 7.8); 150 µl 0.17 M H_2O_2; and 1.5 mL extracts in different concentrations. H_2O_2 was added to this mixture to initiate the reaction. At the end of the 5 minutes of incubation at room temperature, the measurements were taken at 560 nm using the spectrophotometer, and the hydroxyl radical removal activity was calculated using the equation below [30]:

Hydroxyl Radical Removal = $(A_1-A_2/A_0) \times 100$ (5)

where A_o is the absorbance of the control (without the extract); A_1 is the absorbance value measured when the extract was present, and A_2 is the absorbance value measured when the 1.10-phenanthroline was not present.

2.2.2.9 Determination of the metal-chelating activity

Metal chelating activity was analyzed via iron chelation property. It was conducted using the method developed by Dinis et al. [31]. Determining this property in different plant extracts was initiated by adding 1.6 mL deionized water and 0.05 mL 2 mM $FeCl_2$ to each 0.5 mL extract. After 30 seconds 0.1 mL 5 mM ferrozine was added. When ferrozine reacts with two-atomicity iron, it becomes highly soluble in water. The mixture was then stored for 10 minutes at room temperature, and then the absorbance of the mixture of Fe^{+}ferrozine complex was measured at 562 nm. Thus, the iron-chelating activity of the extract was calculated using the formula below [31]:

Chelating Activity = $(1-A_{Sample}/A_{Control}) \times 100$ (6)

where $A_{kontrol}$ is the absorbance of the control or the blind, and A_{numune} is the absorbance measured when the extract was present.

2.2.2.10. Determination of the total antioxidant capacity

The samples' antioxidant activities were evaluated based on the phosphor-molybdenum method developed by Prieto et al. [32] and expressed as ascorbic acid equivalent. In brief, the extract dissolved in 0.4 mL methanol and 4 mL reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) were mixed. For the blind, methanol was used to replace the plant extract. The reaction mixture was stirred with vortex and kept for 90 minutes in a water bath at 95 ^{0}C. Absorbance was measured at 695 nm wavelength. Antioxidant activity was calculated as ascorbic acid equivalent (AAE µg / mL extract) [32].

2.3. Antimicrobial Activity Determination Methods

2.3.1. Selection of test microorganisms

Test microorganisms used in the study were procured from the Refik Saydam Public Hygiene center. Gram (+), gram (-) microorganisms (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae)* and yeasts (*Candida albicans*, *Saccharomyces cerevisiae)* were used.

2.3.2. Agar disc diffusion method

Antimicrobial activity tests were conducted in compliance with the method reported by Berghe and Vlietinck [33]. Plant extracts were dissolved in 100% dimethyl sulfoxide (DMSO) to obtain 100 mg/mL. The extracts that were filtered using 0.22 µm nylon membrane filters, which filter in HPLC purity, were used. 200µl of the culture suspension of the microorganisms (which contains approximately 10^6 colonies according to McFarland 0.5 equation) that would be used in the test were spread over Mueller Hinton agar and Sabouraud dextrose agar, respectively. Later on, 50 µl from each extract kept in a sterile environment after filtered through the 0.22 µm nylon filter was saturated into discs with a 3 mm thickness and 6 mm diameter. Discs saturated with 50 µl DMSO was used as the negative control. Streptomycin was used as the reference antimicrobial agent. Petri dishes were then incubated for 1 hour at 4 degrees and then 24 hours at 37 degrees. Antibacterial activity was determined by the measurement of the zones around the extract saturated discs [33].

2.4. Determination of the Antiproliferative Effect

2.4.1. Cell culture

Human prostate cells (PC-3) were used in this study. Cells were purchased from the American Type Culture Collection (ATCC, USA). The cells were cultured in RPMI 1640 medium (Biological Industries, Israel) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution (Biological Industries, Israel). Cells were grown at 37°C in a humidified incubator with 5% CO_2 and subcultured every two or three days.

2.4.2. Cell proliferation assay

WST-1 cell proliferation assay kit (Clontech, USA) was used for analyzing cell proliferation and viability. Cells were grown in T-25 flasks and harvested. Cells were counted by using a Thoma hemocytometer. $5x10^3$ cells/well in 100 µl medium were seeded in 96 well plates. After 24 h, various concentrations of the extracts of *A. persica* were added to wells in 100 µl medium. Cells were treated with 500 and 250 µg/mL concentrations of the extracts. After 48 h, 5 µl of WST-1 reagent was added to each well, and after four h incubation, absorbance was measured at 450 nm by SpectraMax Plus 384 Microplate Reader (Molecular Devices, USA). Reference absorbance was taken as 630 nm.

2.5. Statistical Analyses

All measurements were repeated three times; the analyses were conducted using students't-test software and $p \leq 0.05$ values were accepted as significant.

3. RESULTS

3.1. Extraction Yields

Table 2 demonstrates the hexane, dichloromethane, ethyl acetate, methanol, and water extract yields obtained by extraction from *Alchemilla persica* strain plants.

Table 2. Solvent extracts of *Alchemilla persica*.

Extraction solvent	Yield (%)
Hexane	0.93
Dichloromethane	0.27
Ethyl acetate	1.50
Methanol	0.34
Water	0.25

3.2. Antioxidant Activity Findings

3.2.1. Total phenolic substance amount assay

Solution absorbance values were calculated after the reading by UV spectrophotometer at 760 nm, where total phenol amounts to be equivalent to mg gallic acid on the calibration curve plotted using gallic acid ($y=0.0024x+0.0313$ $R^2= 0.9981$)

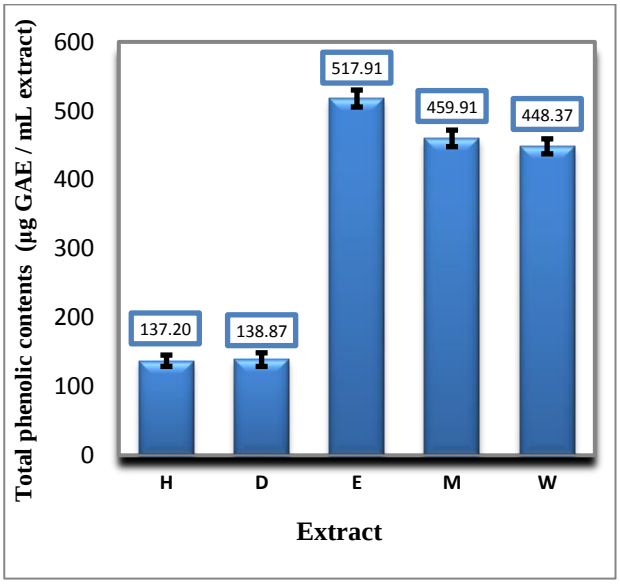

Figure 1. Total phenolic substance content in various solvent extracts for ***Alchemilla persica*** (H: hexane extract D: dichloromethane extract E: ethyl acetate extract M: methanol extract W: water extract) could be ordered as ethyl acetate extract > methanol extract > water extract >dichloromethane extract > hexane extract. ($p \leq 0.05$)

The results of the study on the total phenolic substance were calculated as µg GAE / mL extract. According to the results of the study, the highest was found in Ethyl acetate extract (517.79 ± 12.35 µg GAE / mL extract), followed by methanol extract (459.91 ± 12.01 µg GAE / mL extract) and water extracts (448.37 ± 10.89 µg GAE / mL extract). Following these extracts, the amount of phenolic substance found in the dichloromethane extract was found to be 138.20. Hexane extract (137.20 ± 8.24 µg GAE / mL extract) showed the lowest phenolic substance content. Study findings are shown graphically in Figure 1.

3.2.2. Determination of total flavonoid content

The absorbance of the solutions was read with a UV spectrophotometer at 415 nm. ,and total flavonoid amounts were calculated based on (+) Catechin standard calibration curve in µg / mL. (+) Catechin standard calibration graph (y= 0.0014x+0.0334 R^2= 0.9917) is provided.
Figure 2 shows the total flavonoid content study results for *Alchemilla persica* extracts. The total amount of flavonoid substance contained in ethyl acetate extract (176.85 ± 2.50) was higher than other solvent extracts. Methanol extract (124.71 ± 2.35), water extract (94.5 ± 2.50) and dichloromethane extract (82.00 ± 2.00) followed respectively. Hexane extract (57.21 ± 1.20) was seen to show the lowest total flavonoid content.

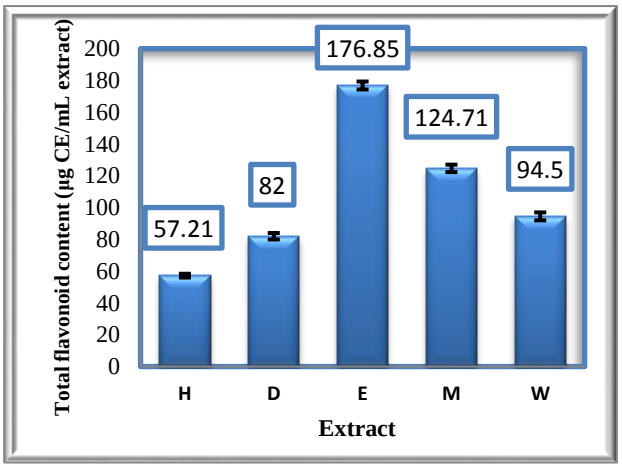

Figure 2. Total flavonoid content in various solvent extracts for ***Alchemilla persica*** (H: hexane extract D: dichloromethane extract E: ethyl acetate extract M: methanol extract W: water extract). ($p \leq 0.05$)

3.2.3. Total phenolic acid content (TPA)

The extract prepared was read at 505 nm wavelength for phenolic acid content of the samples. Total phenolic acid content was calculated based on the standard calibration curve for sinapic acid (y=0.0002x+0.071 R^2=0.9884).

Figure 3 demonstrates the total phenolic content findings for *Alchemilla persica* extracts. Results were determined as the amount of µg per 100 ml. The total phenolic acid substance amount found in hexane extract (5583.65 ± 50.33 µg/100 mL extract) was higher when compared to other solvent extracts. It was observed that methanol (854.50 ± 10.54 µg/100 mL extract) and water extracts (1139.87 ± 10.21 µg/100 mL extract) demonstrated the lowest total phenolic acid content. Dichloromethane (1267.23 ± 20,21 µg/100 mL extract) and ethyl acetate extracts (1451.45 ± 1.71 µg/100 mL extract) displayed similar results.

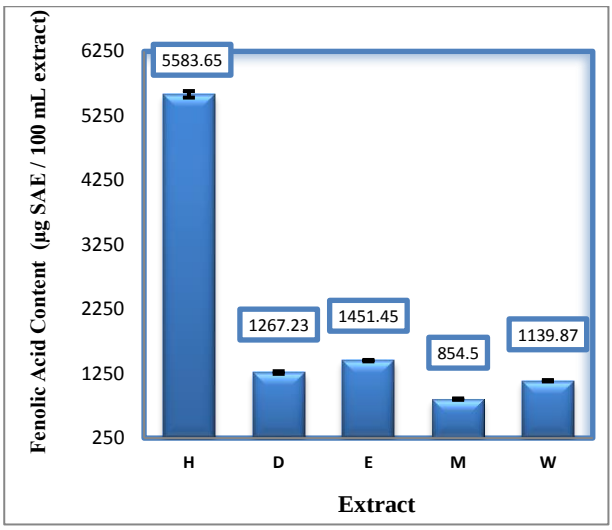

Figure 3. Total phenolic acid content in various solvent extracts for ***Alchemilla persica*** (H: hexane extract D: dichloromethane extract E: ethyl acetate extract M: methanol extract W: water extract). ($p \leq 0.05$)

3.2.4. Determination of total β-Carotene and lycopene content

Absorbance values for the filtrate were measured at 453, 505, and 663 nm wavelengths. β-carotene and lycopene content was calculated using standard graphs. The carotenoid amount in 100 mL plant was calculated in milligrams [25]. Results were calculated in mg per 100 mL extract carotenoid (Figure 4) and lycopene (Figure 5).

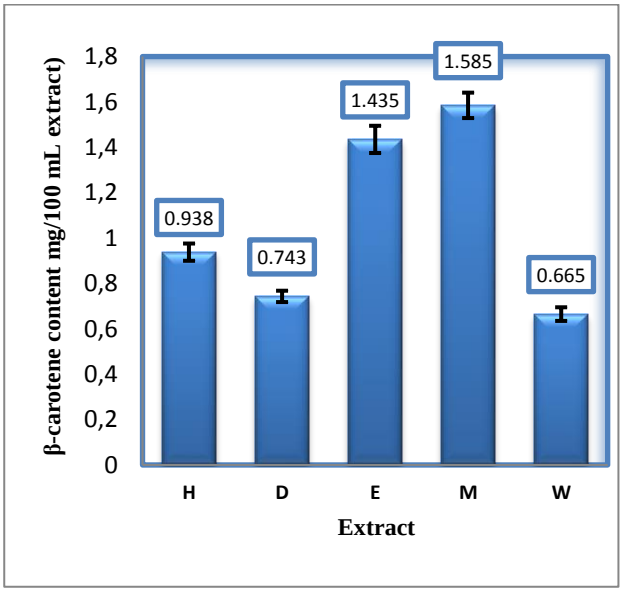

Figure 4. *β*-carotene content for various ***Alchemilla persica*** solvent extracts. (H: hexane extract D: dichloromethane extract E: ethyl acetate extract M: methanol extract W: water extract), ($p \leq 0.05$).

Results, methanol extract (1.585 ± 0.056 mg/100 mL extract)> ethyl acetate extract (1.435 ± 0.06 mg/100 mL extract)> hexane extract (0.938 ± 0.04 mg/100 mL extract)> dichloromethane extract (0.743 ± 0.02 mg/100mL extract) > water extract (0.665 ± 0.03 mg/100 mL extract) in the order found.

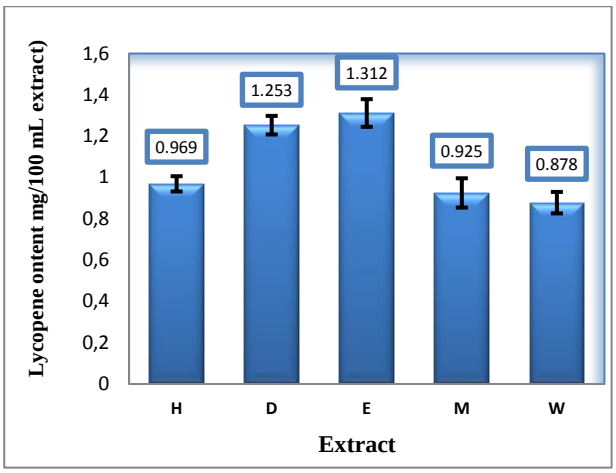

Figure 5. Lycopene content for various ***Alchemilla persica*** solvent extracts. (H: hexane extract D: dichloromethane extract E: ethyl acetate extract M: methanol extract W: water extract) ($p \leq 0.05$)

When ranking for the values determined in figure 5, the order of lycopene content was determined as ethyl acetate extract (1.312 ± 0.07 mg/100 mL extract) > dichloromethane extract (1.253 ± 0.05 mg/100 mL extract) > hexane extract (0.969 ± 0.04 mg/100 mL extract) > methanol extract (0.925 ± 0.07 mg/100 mL extract) > water extract (0.878 ± 0.052 mg/100 mL extract).

3.2.5. Removal of the effect of DPPH radical

For the Removal of the radical activity, the extract obtained from the plant was mixed with the radical, and spectrophotometric readings were conducted. Percentage inhibition graphs were plotted for DPPH free radical removal activities of the extracts were plotted against the concentrations.

Percentage DPPH removal activities of standards in various solvent extracts for ***A. persica*** in 0.05 mg/mL concentration could be ordered as methanol extract > water extract > ethyl acetate extract > dichloromethane extract > hexane extract. Corresponding figures for 0.05 mg/mL concentration were 87.46 ± 1.48 > 87.01 ± 0.51 > 86.49 ± 0.41 > 45.41 ± 0.29 > 44.32 ± 0.25 respectively. Percentage DPPH removal activities in 0.1 mg/mL concentration could be ordered as methanol extract > water extract > ethyl acetate extract > hexane extract > dichloromethane extract. Corresponding figures for 0.1 mg/mL concentration were 87.5 ± 0.73 > 87.25 ± 1.19> 87.21 ± 0.68 > 48.47 ± 0.25 > 46.78 ± 1.24 respectively. Percentage DPPH removal activities in 0.15 mg/mL concentration could be ordered as ethyl acetate extract > methanol extract > water extract > dichloromethane extract > hexane extract. Corresponding figures for 0.15 mg/mL concentration were 87.65 ± 1.23 > 87.62 ± 2.01 = 87.62 ± 1.41 > 53.8 ± 0.24 > 48.75 ± 1.25 respectively. Percentage DPPH removal activities in 0.2 mg/mL concentration could be ordered as water extract > methanol extract > ethyl acetate extract > hexane extract > dichloromethane extract. Corresponding figures for 0.2 mg/mL concentration were 88.08 ± 1.42 > 87.98 ± 0.24 > 87.69 ± 0.65 > 78.31 ± 0.91 > 54.04 ± 0.71 respectively.

Percentage DPPH removal activities in the study conducted using several standards in 0.05 mg/mL, 0.1 mg/mL, 0.15 mg/mL and 0.2 mg/mL concentrations could be ordered as α-tocopherol >BHA >BHT. Corresponding figures for 0.05 mg/mL concentration were 85.19 ± 0.05 > 83.81 ± 0.08 > 75.91 ± 0.03; for 0.15 mg/mL concentration, 85.52 ± 0.09> 85.33 ± 0.11 > 82.22 ± 0.15; for 0.2 mg/mL concentration, 85.72 ± 1.32 > 85.66 ± 1.14 > 83.58 ± 1.05, respectively. Percentage DPPH removal activities in the study conducted using several standards in 0.1 mg/mL concentration could be ordered as BHA > α-tocopherol > BHT. Corresponding figures for 0.1 mg/mL concentration were 85.18 ± 0.22 > 83.44 ± 0.18> 79.42 ± 0.29 respectively.

It was observed in studies conducted with both various solvent extracts and standard substances that in increasing concentrations, DPPH removal activity has increased as well. Findings on DPPH removal activity are presented in Figure 6 graphically.

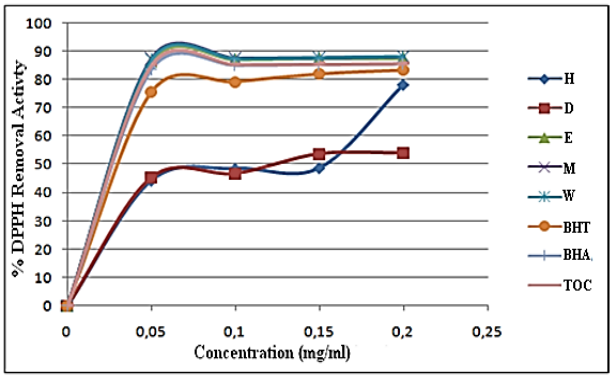

Figure 6. Percentage DPPH removal activities of ***A. persica*** solvent extracts and standard antioxidants in different concentrations
***** H: hexane extract D: dichloromethane extract E: ethyl acetate extract M: methanol extract W: water extract TOC: α-tocopherol BHA: Butylated Hydroxy Anisole BHT: Butylated Hydroxy Toluene

3.2.6. Assessment of the Reducing power

Solutions read at 700 nm on the spectrophotometer demonstrated high absorbance values and high reductive properties. The comparisons were conducted against BHA, BHT, and α-tocopherol standards.

Reductive force activities of the standards used in the study conducted using *A. persica* solvent extracts for several standards in 0.05 mg/mL, 0.1 mg/mL concentrations could be ordered as ethyl acetate extract > water extract > methanol extract > dichloromethane extract > hexane extract. Corresponding figures for 0.05 mg/mL concentration were 2.08 ± 0.76 > 1.86 ± 0.73 > 1.67 ± 0.17 > 0.31 ± 0.02 > 0.28 ± 0.03; for 0.1 mg/mL concentration, 2.97 ± 0.58 > 2.70 ± 1.18 > 2.67 ± 1.14 > 0.45 ± 0.08 > 0.27 ± 0.07, respectively. Reductive force activities in 0.15 mg/mL, 0.2 mg/mL concentrations could be ordered as ethyl acetate extract > methanol extract > water extract > dichloromethane extract > hexane extract. Corresponding figures for 0.15 mg/mL concentration were 3.21 ± 0.75 > 2.99 ± 0.61 > 2.74 ± 0.69 > 0.55 ± 0.04 > 0.28 ± 0.03; for 0.2 mg/mL concentration, 3.33 ± 0.51 > 3.15 ± 0.42 > 3.1 ± 2.01 > 0.68 ± 0.04 > 0.28 ± 0.03, respectively.

In the study conducted using various standards, reductive force activity could be ordered as BHA > α-tocopherol > BHT. Corresponding figures for 0.05 mg/mL concentration were 0.79 ± 0.07 > 0.38 ± 0.05 > 0.25 ± 0.01; for 0.1 mg/mL concentration, 1.43 ± 0.59 > 0.62 ± 0.05 > 0.30 ± 0.07; for 0.15 mg/mL concentration, 2.07 ± 0.06 > 0.94 ± 0.01 > 0.36 ± 0.07; for 0.2 mg/mL concentration, 2.44 ± 0.28 > 0.99 ± 0.11 > 0.39 ± 0.03, respectively.

It was determined that the reductive force properties of ethyl acetate, methanol, and water extracts were higher when compared to dichloromethane and hexane extracts. In studies conducted with both various solvent extracts and standard substances, it was observed that reductive force activity increased with the increasing concentrations. Findings are displayed graphically in Figure 7.

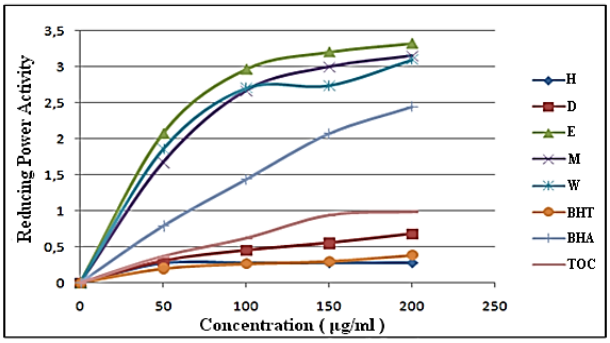

Figure 7. Reductive force activities of ***A. persica*** solvent extracts and standard antioxidants in different concentrations
***** H: hexane extract D: dichloromethane extract E: ethyl acetate extract M: methanol extract W: water extract TOC: α-tocopherol, BHA: Butylated Hydroxy Anisole, BHT: Butylated Hydroxy Toluene

3.2.7. Hydrogen peroxide removal activity assay

The absorbance of the solutions obtained from plant extracts was read at 230 nm against the blind. The percentage of H_2O_2 removal activity for each sample was calculated.

Percentage H_2O_2 removal activities of the standards used in the study conducted using ***A. persica*** solvent extracts in 0.5 mg/mL concentration could be ordered as hexane extract > water extract > methanol extract > dichloromethane extract >ethyl acetate extract. Corresponding figures for 0.5 mg/mL concentration were 52.92 ± 2.40 > 41.78 ± 0.95 > 36.05 ± 2.12 > 34.07 ± 1.01 > 32.13 ± 2.13, respectively. Percentage H_2O_2 removal activities in 0.1 mg/mL concentration could be ordered as hexane extract > methanol extract > water extract > ethyl acetate extract > dichloromethane extract. Corresponding figures for 0.1 mg/mL concentration were 53.73 ± 2.04 > 45.62 ± 1.03 > 42.42 ± 2.05 > 39.17 ± 0.60 > 34.15 ± 1.06, respectively. Percentage H_2O_2 removal activities in 0.15 mg/mL and 0.2 mg/mL concentrations could be ordered as hexane extract > dichloromethane extract > methanol extract > water extract > ethyl acetate extract. Corresponding figures for 0.15 mg/mL concentration were 53.73 ± 2.04 > 45.62± 1.03 > 42.42 ± 2.05 > 39.17± 0.60 > 34.15± 1.06, for 0.2 mg/mL concentration were 58.89 ± 0.89 > 57.43 ± 0.34 > 56.25 ± 1.26 > 43.73± 0.77 > 42.53 ± 0.24, respectively.

In the studies conducted, it was observed that the percentage of H_2O_2 removal activity increased as the extract concentrations increased. The findings of the study are presented graphically in Figure 8.

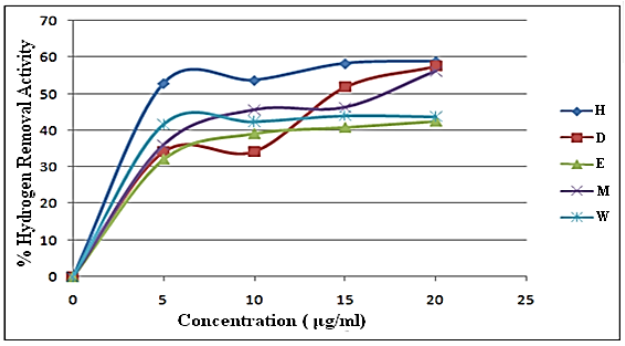

Figure 8. Percentage H_2O_2 activities of ***A. persica*** solvent extracts H: hexane extract D: dichloromethane extract E: ethyl acetate extract M: methanol extract W: water extract

3.2.8. Assessment of the hydroxyl radical removal property

Hydroxyl radical removal activity was calculated based on the equation displayed below, following 5 minutes of incubation of the prepared mixture under room temperature and the measurement conducted at 560 nm with the spectrophotometer. Percentage OH radical removal activity for each sample was calculated and presented.

OH removal activities of the standards used in the study conducted using ***A. persica*** solvent extracts in 0.05 mg/mL concentration could be ordered as ethyl acetate extract > methanol extract > water extract > dichloromethane extract >hexane extract. Corresponding figures for 0.05 mg/mL concentration were 2.58 ± 0.50 > 1.75 ± 0.40 > 0.87 ± 0.23 > 0.44 ± 0.06 > 0.24± 0.06, respectively. OH removal activities in 0.1 mg/mL concentration could be ordered as ethyl acetate extract > methanol extract > dichloromethane extract > water extract > hexane extract. Corresponding figures for 0.1 mg/mL concentration were 2.63 ± 0.13 > 2.52 ± 0.74 > 2.45 ± 0.58 > 0.88 ± 0.04 > 0.64 ± 0.14, respectively. OH removal activities in 0.15 mg/mL concentration could be ordered as ethyl acetate extract > dichloromethane extract > methanol extract > hexane extract > water extract. Corresponding figures for 0.15 mg/mL concentration were 6.84 ± 0.36 > 5.81 ± 0.41 > 3.2 5 ± 0.21 > 1.37 ± 0.45 > 0.89 ± 0.13, respectively. OH removal activities in 0.2 mg/mL concentration could be ordered as dichloromethane extract >ethyl acetate extract > methanol extract > hexane extract > water extract. Corresponding figures for 0.2 mg/mL concentration were 9.27 ± 1.14 > 7.55 ± 2.49 > 3.91± 1.17 > 2.57 ± 1.06 > 0.94 ± 0.17, respectively.

In the study conducted using various standards, OH radical removal activity could be ordered as α-tocopherol >BHA >Trolox. Corresponding figures for 0.05 mg/mL concentration were 0.86 ± 0.05 > 0.43 ± 0.09 > 0.15 ± 0.09; for 0.1 mg/mL concentration, 1.32 ± 0.26 > 0.54 ± 0.09 > 0.16 ± 0.07; for 0.15 mg/mL concentration, 1.41 ±

0.58 > 0.65 ± 0.07 > 0.27 ± 0.04; for 0.2 mg/mL concentration, 2.50 ± 0.21 > 0.86 ± 0.08 > 0.33 ± 0.06, respectively.
It was observed in studies conducted with standard and various solvent extracts that in increasing concentrations, the OH radical removal effect increased as well. Study findings conducted with plant samples extracts are displayed in Figure 9.

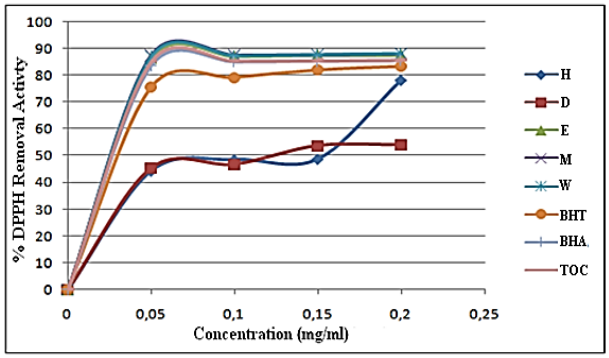

Figure 9. Percentage OH removal activities for ***Alchemilla persica*** solvent extracts and standard antioxidants of BHA, α-Tocopherol and Trolox
***** H: hexane extract D: dichloromethane extract E: ethyl acetate extract M: methanol extract W: water extract TOC: α-tocopherol BHA: Butylated Hydroxy Anisole

3.2.9. Assessment of metal chelating activity

Fe^{+2} Ferrozine complex absorbance of the mixture was measured at 562 nm after the solutions prepared were kept in room temperature for 10 minutes. The percentage of metal-chelating activity for each sample was calculated and presented in Figure 10.

Metal chelating activities measured in the study conducted using ***A. persica*** solvent extracts in 0.05 mg/mL, 0.1 mg/mL and 0.15 mg/mL concentrations could be ordered as hexane extract > dichloromethane extract > methanol extract > ethyl acetate extract > water extract. Corresponding figures for 0.05 mg/mL concentration were 56.38 ± 0.44 > 47.58 ± 0.58 > 39.25± 0.11 > 37.03 ± 0.69 > 31.77 ± 1.21; for 0.1 mg/mL concentration, 59.06 ± 0.27 > 49.29 ± 1.72 > 41.74 ± 1.32 > 39.93 ± 0.83 > 34.37 ± 1.17; for 0.150 mg/mL concentration, 61.58 ± 0.65 > 54.88 ± 0.34 > 41.79 ± 1.69 > 41.14 ± 1.22 > 38.42 ± 2.30, respectively. Metal chelating activities in 0.2 mg/mL concentrations could be ordered as hexane extract > dichloromethane extract > ethyl acetate extract > methanol extract > water extract. Corresponding figures for 0.2 mg/mL concentration were 64.86 ± 2.23 > 56.88 ± 1.36 > 50.83 ± 0.97 > 41.95 ± 0.84 > 39.92 ± 0.14, respectively. In the study conducted using various standards, metal chelating activity could be ordered as Trolox > α-tocopherol > BHA > BHT. Corresponding figures for 0.05 mg/mL concentration were 33.84 ± 0.52 > 33.58 ± 0.21 > 33.28 ± 2.14 > 5.74 ± 0.93; for 0.1 mg/mL concentration, 34.95± 1.18 > 34.32 ± 1.15 > 33.35 ± 0.03 > 13.17 ± 0.05; for 0.15 mg/mL concentration, 38.14 ± 1.61 > 36.78 ± 0.74 > 34.56 ± 2.04 > 16.37 ± 1.41, respectively. Metal chelating activity in 0.2 mg/mL concentration could be ordered as α-tocopherol > Trolox >BHA > BHT. Corresponding figures for 0.2 mg/mL concentration were 43.67 ± 0.65 > 36.94 ± 0.85 > 36.91 ± 1.78 > 36.57 ± 2.08, respectively.
It was observed in studies conducted with various solvent extracts and standard substances that as the concentrations increased, metal chelating activity increased.

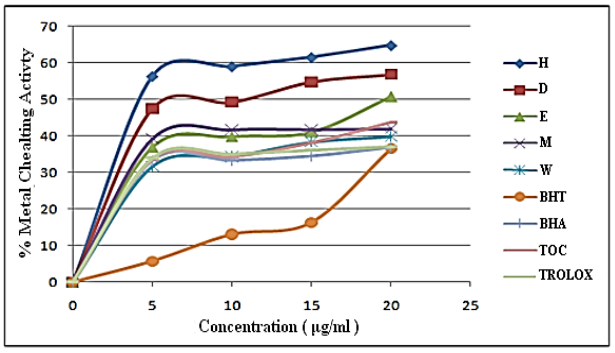

Figure 10. Metal chelating activities in different concentrations of ***A. persica*** solvent extracts and standard antioxidants
***** H: hexane extract D: dichloromethane extract E: ethyl acetate extract M: methanol extract W: water extract TOC: α-tocopherol, BHA: Butylated Hydroxy Anisole, BHT: Butylated Hydroxy Toluene

3.2.10. Determination of total antioxidant capacity

The absorbance of the solution was measured at 695 nm wavelength. Antioxidant activity was calculated as ascorbic acid equivalent (AAE µg / g extract y = 0.0019x, R^2 = 0.9993).

Total antioxidant content found in ***A. persica*** solvent extracts could be ordered as ethyl acetate extract > methanol extract > water extract > dichloromethane extract > hexane extract.

The highest total antioxidant content in ***A. persica*** was found in ethyl acetate extract. Hexane extract displayed the lowest total antioxidant activity. Total antioxidant content findings for the extracts are presented in Figure 11 as a column chart.

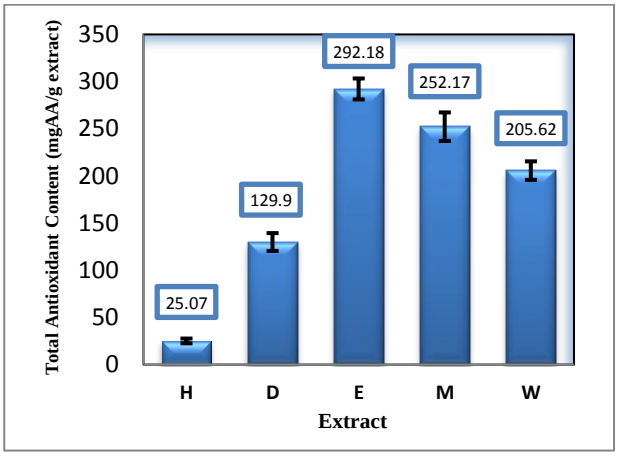

Figure 11. Total antioxidant amount in ***A. persica*** solvent extracts ***** H: hexane extract D: dichloromethane extract E: ethyl acetate extract M: methanol extract W: water extract

3.3. Antimicrobial Activity Findings for Plant Extracts

Antimicrobial activity finding for ***A. persica*** solvent extracts are displayed in Table 3.
While dichloromethane, ethyl acetate, methanol, and water extracts demonstrated effects on microorganisms, hexane extract was not effective on any microorganism.

Table 3. Antimicrobial activities of ***Alchemilla persica*** solvent extracts

Microorganisms	*Alchemilla persica*						
	H	D	E	M	W	Str.	DMSO
	Inhibition zone diameter (mm)						
Bacillus subtilis ATCC 6633	-	11±0.6	12±0.4	10±0.4	10±0.2	26±0.2	-
Staphylococcus aureus ATCC 29213	-	12±0.5	9±0.3	9±0.3	8±0.1	35±0.1	-
Escherichia coli ATCC 25922	-	12±0.4	12±0.5	11±0.3	11±0.1	39±0.8	-
Klebsiella pneumonia ATCC 13883	-	10±0.1	9±0.2	9±0.3	8±0.2	24±0.5	-
Candida albicans ATCC 98268	-	10±0.2	8±0.1	8±0.2	8±0.3	24±0.8	-
Saccharomyces cerevisiae ATCC 76521	-	8±0.2	10±0.1	8±0.2	8±0.1	21±0.7	-

***** H: hexane extract D: dichloromethane extract E: ethyl acetate extract M: methanol extract S: water extract DMSO: Dimethylsulfoxide Str.: Streptomycin
* The figures represent inhibition zone diameters. Each disc had a diameter of 6 mm, and 30 µl extract was saturated to each disc.
*Streptomycin: Positive control, DMSO: Negative control

3.4. Effects of various extracts of *Alchemilla persica* on PC-3 cell proliferation

Cell proliferation was assessed by WST-1 assay. We investigated the cytotoxic effects of methanol and water extracts of leaves of ***Alchemilla persica*** in the PC-3 cell line. Both extracts of ***Alchemilla persica*** decreased PC-3 cell proliferation significantly at concentrations from 250, 500, and 1000 µg/mL when compared to control cells ($p < 0.001$) (Fig. 12). All extracts showed the cytotoxic effect on PC-3 cells.

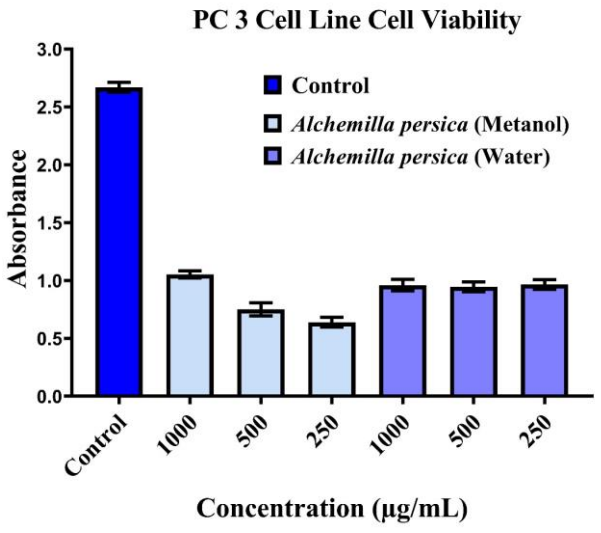

Figure 12. ***Alchemilla persica*** on PC-3 cell proliferation

4. RESULTS AND DISCUSSION

In this study, Hexane, dichloromethane, ethyl acetate, methanol and water extracts of ***Alchemilla persica*** with increasing polarity were used, respectively. Different antioxidant activity measurement analyses were conducted to determine the antioxidant capacity accurately for the extracts prepared with the solvents, as mentioned above. However, according to Frankel and Meyer [34], to measure total antioxidant capacity on products in different times and to determine various oxidative conditions, it must be evaluated in the same molarity with the standard antioxidants and the reductive property and percentage removal IC_{50} values should be similarly compared to standard antioxidant compounds with the same molar concentration.

At the end of the extraction process, the highest amount of extract was obtained with an ethyl acetate solvent system.

Antioxidant results of the studies conducted with different extracts of ***Alchemilla persica*** were observed as follows: Total phenolic substance content indication studies were conducted using Folin-Ciocalteau method. It was found that the ***Alchemilla persica*** extract obtained with ethyl acetate solvent system contained higher total phenolic substance amounts than the extracts obtained using other solvent systems. Quantitatively, the figure was 67.63 ± 1.45 mg GAE/g extract for the ethyl acetate extract. Albayrak et al. [35], in a study conducted with different strains of *Helicrysum* (Asteraceae), reported that total phenolic substance amounts were between 66.74 ± 1.3 and 160.63±1.2 mg GAE/g [35]. Özkan et al. [36] reported a total phenolic extract equivalent to 108.33±0.88 mg in *Helicrysum chasmolycicum* methanolic extracts. It was determined that total phenolic acid content was more in hexane extracts than the extracts obtained with other solvent systems. It was calculated 0.7218 ± 0.33 mg SA/gr as sinapic acid equivalent [36].

There might be a relationship between phenolic substance and flavonoid amounts and antioxidant capacity. The relationship between the DPPH and ABTS methods built on radical capture basis and total phenolic substance and flavonoid amounts could be significant in specific vegetal sources. However, there could also be significant differences between antioxidant capacities and phenolic compounds and flavonoids. It was suggested that these relationships should be indicated when evaluating the antioxidant capacities of vegetal sources [37]. It was observed that β-carotene and lycopene content of solvent systems decreased with the increasing polarity. High amounts of β-carotene and lycopene content were indicated in ***Alchemilla persica*** dichloromethane extract at 1.74 mg/100 mL extract. 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and 2.2-azinobis-(3-ethyl benzothiazoline-6-sulphonic acid) di-ammonium salt (ABTS +) removal properties are commonly used to determine the antioxidant activities of the plants [38]. Huang et al.[39] stated that the plants' antioxidant capacity is mostly related to their phenolic content. A study by Tsai et al. [40], scruti-

nized the total phenolic content and total antioxidant capacity of the vanilla plant and green tea extracts by utilizing DPPH radical scavenger capacity. Thus, the content as mentioned above and analyses were utilized in this study to assess antioxidant activity.

It was determined that the percentage of DPPH removal activity, especially in ethyl acetate and in extracts with higher polarity, was above those of standard antioxidants such as BHT, BHA, and TOC under the same concentrations. As the concentration increased, the DPPH radical removal effect increased as well. Hossain and Rahman [41] determined that the DPPH removal activity in pineapple, which is a tropical fruit, could be ordered as methanol, ethyl acetate, and water extract, respectively. The same study reported that the phenol content could be ordered as methanol, ethyl acetate, and water extract from the highest to the lowest. This study's findings also demonstrated that DPPH removal activity was the highest in the extract with the highest phenolic substance content. In other words, ethyl acetate extract was followed by methanol and water extracts. It was also determined that they demonstrated higher activities than standard antioxidants in this respect.

Comparing all the concentrations of all extracts except for hexane and dichloromethane with synthetic antioxidants showed that the reductive force of ***Alchemilla persica*** was higher than the latter.

Hexane extracts displayed the most hydrogen peroxide removal capacity in the study. Since the hexane fraction is highly nonpolar, it could be argued that this was due to the saturation of fats and fatty acids.

It was observed that the highest OH radical removal activity was seen in dichloromethane and ethyl acetate extracts. Especially dichloromethane displayed a high radical removal activity in all concentrations of all types.

The high metal chelating activity was observed in dichloromethane and hexane extracts as well. In the existence of functional groups in the compounds with lower polarity than ethyl acetate, and especially at the levels of dichloromethane polarity, it could connect to metals better. In all extract, higher activity was observed similarly when compared to standard antioxidants.

Cai et al. [42] examined total antioxidant activities in a study they evaluated the total phenolic compound content of 112 plants known to people with their medical properties and antioxidant capacities of these plants. They have found a significant linear relationship between total phenolic content and antioxidant capacities in sample extracts they tested. The findings of this study also evidenced the existence of this linear relationship. The highest total phenolic content was identified in ethyl acetate, followed by methanol and water. Percentage inhibition activity of the DPPH radical reflected the same order. The phospho-molybdenum method was used, especially in the indication of total antioxidant activity and the findings were parallel to those of Cai et al. [42].

Following the above studies, it was determined by the analysis of all concentrations that as the concentration increased, antioxidant effects of the samples increased as well.

Study findings demonstrated that the differences in phenolic content affected the antioxidant properties of the extracts. It was explicitly observed that high phenolic content or high radical capture activity did not always yield high antioxidant activity. Thus, it could be deduced that deciding on antioxidant activity using only one method was not proper. Therefore, it would be a better approach to use different methods when determining the antioxidant activity, which could simulate biochemical events in living systems, and the activity results obtained should be given based on each property. Support of these results with in-vivo and clinical studies is important in determining bioavailability as well [38].

There are no antimicrobial studies in the literature on *Alchemilla persica* extracts.

During the antimicrobial activity identification study, *Alchemilla persica* extracts effects on the Gram (+) and Gram (-) bacteria and yeasts. *Bacillus subtilis (*ATCC 6633) and *Staphylococcus aureus* (ATCC 29213) were used as gram (+) bacteria, and *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC 13883) were used as gram (-) bacteria, while *Candida albicans* (ATCC 98268) *and Saccharomyces cerevisiae* (ATCC 76521) were used as yeasts.

It was observed that all extracts used in the antimicrobial activity study demonstrated a lower activity when compared to streptomycin used as a positive control. DMSO, used as a negative control had no effects whatsoever. It was indicated that the antimicrobial activity of the hexane extract was lower than other solvent extracts.

Table 3 demonstrates the effects of ***Alchemilla persica*** dichloromethane, ethyl acetate, methanol, and water extracts on microorganisms. The findings presented in the table showed that hexane extract was not effective on any microorganism. DMSO used as a negative control on ***Alchemilla persica*** antimicrobial property's indication using the diffusion technique did not demonstrate any inhibition effect. In antimicrobial effect tests conducted with discs that contained 500 µg/disc extracts, which was obtained with ethyl acetate, the highest inhibition diameter was measured as 12 mm on *Escherichia coli* and *Bacillus subtilis*. This measurement was followed by *Saccharomyces cerevisiae* with approximately 10 mm, *Klebsiella pneumoniae* with a 9 mm inhibition diameter, *Staphylococcus aureus* with a 9 mm inhibition diameter, and *Candida albicans* with 8 mm inhibition diameter. In dichloromethane extract, the highest zone diameter was indicated with *Escherichia coli* and *Staphylococcus aureus*. It reached a zone diameter of 11 mm in *Bacillus subtilis* and prevented its development. Zone diameters of 10 mm were measured in *Klebsiella pneumoniae* and *Candida albicans*. Zone diameter in *Saccharomyces cerevisiae* was measured as 8 mm. The effects of the methanol and water extracts were determined to be similar to each other.

The results of the study for ***Alchemilla persica*** are given in Figure 12. Hexane, Dichloromethane, and Ethyl Acetate extracts could not be resolved in the cell culture medium due to polarity differences. When dissolved in DMSO, the efficiency of the extracts on cell viability could not be determined. For this reason, water-soluble methanol and water extracts were used. When the results were evaluated, it was observed that at concentrations used as 250, 500, and 1000 µg/mL, it stopped cell proliferation and prevented cell growth in the PC-3 (prostate cancer cell line) cell line. However, no difference in concentrations was observed in the water extract. The higher antiproliferative effect was observed when the methanol extract was 250 µg/mL compared to the other two concentrations.

Biological activity studies have not been conducted with ***Alchemilla persica***. With this study, biological activity study was carried out for the first time, and the results were determined. Antioxidant, antimicrobial, and anti cytotoxic effects of ***Alchemilla persica*** have been found.

5. CONCLUSION

The properties of ***Alchemilla persica***, such as higher antioxidant activity than synthetic antioxidants (BHA; BHT; TROLOX), relative efficacy compared to standard antibacterial agent, and high activity for killing cancer cells in methanol and water extract, suggest that it can be used as a food additive. It has shown that an innovative, utterly organic food additive can be created with complementary studies to be done for this.

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