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Original research

Investigating the antioxidant properties of *Achillea wilhelmsii*

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ABSTRACT

Antioxidant compounds of medicinal plants are one of the valuable and important resources in medical sciences and pharmaceutical, and food industries. *Achillea wilhelmsii* is a plant of the dark_Compositae family, which has anti-inflammatory properties and is used to treat surface scratches. This plant also has antioxidant properties due to the presence of flavonoid compounds. This study aimed to investigate the antioxidant power of ethanoic, methanolic, and hydroalcoholic extracts of *Achillea wilhelmsii* plant aerial parts**.** Then the extracts of *Achillea wilhelmsii* plant in different concentrations of 1, 2, 5, and 10 mg/liter were measured using 2 (DPPH and 2-diphenyl-1-picrylhydrazine) tests and FRAP (antioxidant power evaluation method). The results showed that the type and amount of solvent have an effect on the concentration of phenolic and antioxidant compounds, and a significant relationship between the amount of phenolic compounds and inhibitory properties was observed. The hydroalcoholic extract of *Achillea wilhelmsii* plant at a concentration of 10 PPM has more antioxidant power than other extracts and concentrations. All three extracts had antioxidant properties, but according to the results, it seems that the hydroalcoholic extract of *Achillea wilhelmsii* plant with strong antioxidant properties can be used in the treatment of diseases.

Keywords: antioxidant, flavonoids, hydroalcoholic extract, phenolic compounds, *Achillea wilhelmsii*.

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1. Introduction

Due to the increase in infectious diseases and the resistance that pathogenic microorganisms have shown over time to chemical drugs, and on the other hand, due to the side effects and high treatment costs that chemical and synthetic drugs impose on human societies. In recent decades, the use of medicinal plants of natural origin has become common (Raissy et al., 2014). The importance of using medicinal and natural plants in disease prevention, treatment and preventing the growth of pathogenic bacteria is well known. Despite the great diversity and expansion of medicinal plants in Iran and other countries of the world, many studies on the antioxidant and antimicrobial effects of these plants are still ongoing (Shahidi et al., 2019).

Antioxidant compounds of medicinal plants are one of the valuable and important resources in medical science and pharmaceutical, and food industries. Medicinal plants are valuable sources of natural antioxidants, such as some terpenoids and phenolic compounds, and have high potential as a suitable substitute for synthetic antioxidants in reducing oxidative stress (Hyldgaard et al., 2012). Evidence shows that natural antioxidants in plants reduce the risk of degenerative diseases and also have a protective effect against oxidative stress. These effects may be due to the nature of antioxidants, including phenolic acids, flavonoids, terpenoids, vitamin E, etc., which can inhibit free radicals and protect cells from damage caused by free radicals. The quantity and quality of effective medicinal substances as well as their antioxidant performance under different ecological conditions, as a valuable and natural source of

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new antioxidants, are very significant in the discussion of disease prevention and treatment (Hosni et al., 2010).

Yarrow or Achilles wilhelmsii has the local name of Brynjasaf flower. This plant belongs to the Asteraceae family and is native to Europe, North America and Asia (Turkey, Iran, Caucasus, Central Asia, Afghanistan, Pakistan, Iraq and Syria) (Garcia-Oliveira et al. , 2021). 19 species of Achillea wilhelmsii grow in Iran. This genus is one of the oldest and most indigenous plants in Europe and the Middle East, and has a historical and mythological background. Achillea wilhelmsii is a herbaceous, stable, auto, rhizome plant that has straight stems up to one meter in height. Its flowering time is from the end of May to the end of July, and the best time to harvest it is the first half of July. This medicinal plant has been used for thousands of years as a traditional medicine in the treatment and repair of surface scratches (Sökmen et al., 2004). In addition, this plant is used to treat infectious diseases, treat digestive complications, liver problems, gall bladder problems, repair and stop bleeding, abdominal pain, muscle cramps, and treat colds. Achillea millefolium L., the best known plant in this genus, is traditionally used in folk medicine to treat lung (asthma and bronchitis), indigestion, skin inflammation, and headaches and liver and biliary disorders (Barda et al., 2021). The parts of the plant used are its flowering branches, which have a bitter taste and a strong smell. Achillea wilhelmsii flowers and leaves have medicinal properties. Its effective ingredients are appetizing and cause food digestion and heartache treatment. Using its decoction reduces blood pressure, relieves acute and chronic gastritis, relieves bloating and sourness of food, and it is used to treat gallbladder insufficiency. Achillea wilhelmsii plant is full of flavonoids, sesquiterpenelectone and monoterpenoids that have antioxidant activity (Potrich et al., 2010). The use of plant extracts and essential oils as food preservatives has received special attention. Bagheri and Babazadeh (2022) stated in a research, achillea wilhelmsii,plant extract at a concentration of 1% had a significant effect on fish samples in maintaining their optimal quality and increasing the duration of storage. Considering the wide use of this plant in traditional medicine and its beneficial properties, we decided to investigate the antioxidant power of different extracts of the aerial parts of this plant.

2. Material and Methods

In this study, aerial parts of yarrow plant were collected in May 2022 at the time of flowering from Faridan region in the west of esfahan province. After drying in laboratory conditions, the samples were dry, sterile, and subjected to scientific identification. In this study, first, 50 grams of Achillea wilhelmsii were evaporated with 70% ethanol and 30% water in a Soxhlet extraction device, and then its solvent was evaporated with a Rotary device and the crude extract was obtained. After preparing aqueous, ethanolic and methanolic extracts of Achillea wilhelmsii plant, tested in different doses in vitro environment and using FRAP and DPPH tests and PerkinElmer spectrophotometer device, the antioxidant power of the three extracts in concentrations of 1, 2, 5 and 10 mg per litre were investigated.

2.1. Measurement of total antioxidant capacity by FRAP method

This method is based on the ability of plasma to regenerate Fe+3 (ferric) and Fe+2 (ferro) ions in the presence of a substance called tripiridyl-S-TPTZ. The TPTZ+Fe2+ complex is a blue coloured complex with an absorption maximum at 593 nm. The rejuvenating power of plasma or any sample is measured by increasing the concentration of the above complex using a spectrophotometer device (Benzie and Strain, 1996).

2.2. Investigating the antioxidant property by DPPH method (2 and 2 diphenyl 1 picrylhydrazine)

Investigating the antioxidant property with DPPH reagent was used as a stable radical compound. In this way, 50 microliters of different concentrations of the extracts were added to this reagent and then the optical absorption of the samples was read at a wavelength of 515 nanometers against the blank. In this way, the percentage of inhibition of free radicals was calculated using the formula (Villano et al., 2007).

2.3. Determination of the total content of phenolic compounds

Drawing the calibration curve: First, different concentrations of gallic acid were prepared and 0.5 ml was taken from them and mixed with 2.5 ml of 10% (v/v) Folin Ciocaltio reagent and during a period of 0.5 to 8 minutes. 2 ml of sodium carbonate 7.5% (v/v) was added. The samples were kept at room temperature for 30 minutes and then their absorbance was read at 765 nm. Distilled water was used as control (Shahidi and Nackz, 2004).

2.4. Measurement of the phenolic compounds of the extracts

Today, the attention of researchers has been drawn to the use of natural substances instead of chemical compounds in the food and pharmaceutical industries. Plant extracts due to having chemical compounds such as cyanogenic glycosides (oxylein), choline, valeric acid, formic acid, tannin, paracamazolin oil, alkaloid, flavonoid, resin, gum, amino acids, polyphenol compounds, sesquiterpene, alketones, betaines. , achiline, phosphate, nitrate, potassium salts and high acids) have high antioxidant properties. Therefore, in this study, hydroalcoholic plant was used due to the abundance of this plant in Iran and the therapeutic properties and antibiotic effects of the extract of this plant (Bagheri and Babazadeh, 2022).

0.5 ml of saffron petal extracts were mixed with 2.5 ml of folinic acid reagent in test tubes and the rest of the steps were similar to drawing the calibration curve (Kossah et al., 2011).

The difference of means was done with the help of ANOVA method and Duncan's multiple range post hoc test at the probability level of 5% with the help of SPSS and Excel software. The obtained information was expressed as mean ± standard deviation (SD ± Mean).

3. Results and Discussion

The results of variance analysis of the data showed that the type and concentration of *Achillea wilhelmsii* plant extracts had a significant effect on the antioxidant power parameter (P<0.05). The inhibitory activity of free radicals and antioxidant power in all samples were concentration-dependent, so that the highest antioxidant power of the hydroalcoholic extract of the aerial part of the elderberry plant was observed at a concentration of 10 ppm. The results of the graph in Figure 1 showed that the maximum antioxidant power of the ethanolic extract of *Achillea wilhelmsii* plant was observed at a

concentration of 10 ppm. Also, the antioxidant power of the methanol extract of *Achillea wilhelmsii* plant shows the highest antioxidant power at a concentration of 10ppm. Of course, the hydroalcoholic extract of aerial parts at a concentration of 10 ppm had higher antioxidant power than the same concentrations of methanolic and ethanolic extracts of this plant. In general, the hydroalcoholic extract of *Achillea wilhelmsii* shoots has the highest antioxidant power compared to the other two extracts.

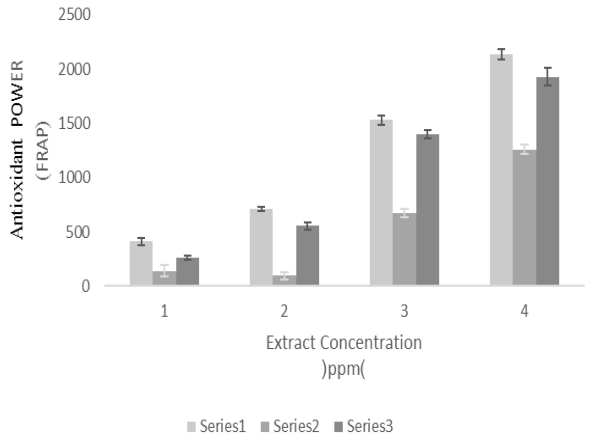

Figure 1- The results of total antioxidant power (FRAP) of hydroalcoholic, ethanolic and methanolic extracts of Achillea wilhelmsii plant aerial parts

The results of the diagram in Figure 2 indicate that the hydroalcoholic extract of Achillea wilhelmsii aerial parts at a concentration of 10 ppm has the highest free radical inhibition percentage.

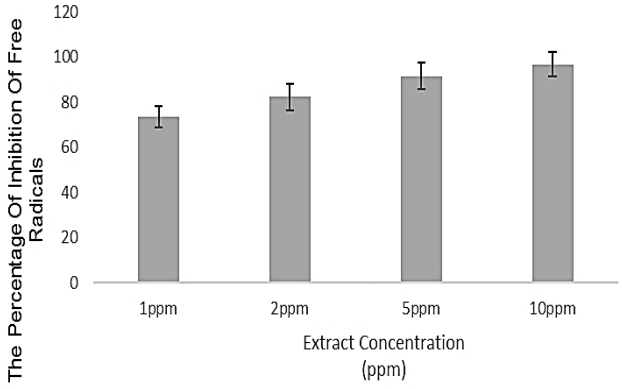

Figure 2- DPPH test results of hydroalcoholic extract of *Achillea wilhelmsii* plant

As can be seen in Figure 3, the highest level of phenolic compounds is at a concentration of 10 ppm of archillea wilhelmsii hydroalcoholic extract (42 mg/g) and then at a concentration of 10 ppm methanolic extract (40 mg/g). Also, the results showed that methanolic extracts have more phenolic compounds than ethanolic extracts at the same concentration.

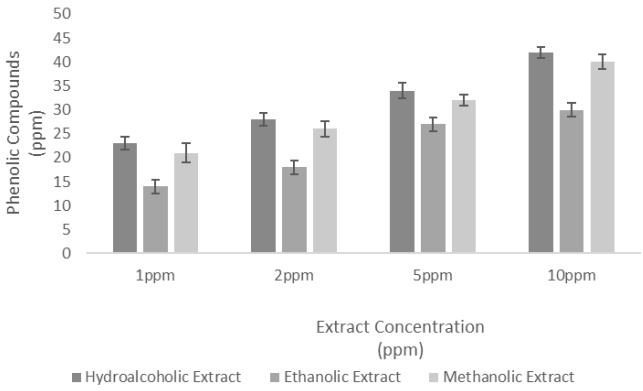

Figure 3- The results of the phenolic compounds of the hydroalcoholic, ethanolic, and methanolic extracts of *Achillea wilhelmsii* plant aerial parts

4. Conclusions

This study aimed to investigate the antioxidant power of three ethanolic, methanolic, and hydroalcoholic extracts of *Achillea wilhelmsii* aerial parts. The findings of this research are in agreement with previous reports based on the direct relationship of phenolic components with antioxidant activity and with increasing concentration, the inhibitory percentage also increased. The results show that the hydroalcoholic extract of aerial parts had the highest antioxidant power. Many studies and various methods have been used to determine the antioxidant capacity. These methods provide different results under the influence of different conditions, such as the antioxidant solubility ratio between aqueous and organic phases, temperature, light intensity, oxidation conditions and oxidizing agent, and in certain methods, the end point of the reaction and the amount of oxidation. Therefore, it is not appropriate to use one method to measure antioxidant capacity, and two sensitive and accurate methods, DPPH and FRAP, were used in this study. Bozin and his colleagues in a study in 2008 entitled "Evaluation of phenolic compounds with antioxidant activity of *Achillea wilhelmsii* plant" concluded that two compounds of methylchavicol, orgenol, thymol and cineole in this plant have strong scavenging properties of free radicals. The results of this study also show the antioxidant properties of these plants. In another study, the effect of *Achillea wilhelmsii* hydroalcoholic extract on the severity of convulsions caused by pentylenetetrazole kindling in mice was investigated. The results obtained from this research showed that the hydro-alcoholic extract of *Achillea wilhelmsii* reduces the intensity of epileptic seizures caused by Pentylenetetrazole **(**PTZ) (Beheshti Nasr et al., 2014). The *Achillea wilhelmsii* plant is a rich source of flavonoids and phenolic compounds (Lemmens-Gruber et al.,2006), effective antioxidants in neutralizing oxygen-containing free radicals .This study also shows the antioxidant power of *Achillea wilhelmsii* hydroalcoholic extract. According to studies, the aglycones quercetin, luteolin, and apigenin exhibited the highest antispasmodic activities with IC_{50} values of 7.8 µmol/L, 9.8 µmol/L and 12.5 µmol/L, respectively. Rutin and the flavonoid metabolites homoprotocatechuic acid and homovanillic acid showed no significant effects on the contractility of the terminal ilea. From the results on the spasmolytic activity of the flavonoid fraction, the glycosides, and the respective aglycones it is concluded that in tea prepared from *Achillea wilhelmsii* the concentration of the flavonoids is high enough to exert a spasmolytic effect in the gut,

which is mainly caused by blockade of the calcium inward current, but additionally also by mediator-antagonistic effects (LemmensGruber et al., 2006). High-performance liquid chromatography (HPLC) was also used for chemical analyses. Eight phenolic compounds – chlorogenic acid and flavonoids, namely vicenin-2, luteolin-3',7-di-O-glucoside, luteolin-7-O-glucoside, rutin, apigenin-7-O-glucoside, luteolin, and apigenin – were identified in the extracts from yarrow flowers. Considerable variation in the accumulation of phenolic compounds among the flowers from different locations was observed. The samples were divided into two main groups based on chemical composition: the first group was characterized by lower than the mean total amount of the identified phenolics; the second was formed from samples accumulating higher concentrations of investigated secondary metabolites. The total amount of the identified phenolics in yarrow flowers from different populations varied from 13.290 to 27.947 mg/g (Benetis et al., 2008). The total content of flavonoids ranged between 0.05–0.07%. The highest content of flavonoids was determined in the deep pink morphotype, and the content of essential oil was highest in the white morphotype of *Achillea millefolium* L. The total *content* of flavonoids and the essential oil composition of the white morphotype of *Achillea* millefolium L. were determined at different vegetation period (Bimbiraitė et al., 2008). So plants are one of the important sources of antioxidants due to the presence of polyphenols. Nowadays, studies in this field are increasing, and according to the results of this study, it can be suggested to use the hydroalcoholic extract and parts of its aerial parts to investigate the effect of this plant in the treatment of various diseases in order to understand the effective molecular and cellular mechanisms of this plant. Be more precise.

References

Bagheri, F. and Babazadeh, M. 2022. Mehdi Antioxidant effect of yarrow extract (Achillea wilhelmsii) on shelf life of salted rainbow trout (Onchorhynchus my kiss) during the storage period. Journal: Quality and shelf life of agricultural and food products. 2(3):18-26

Barda C., Grafakou M.E., Tomou E.M., Skaltsa H. 2021. Phytochemistry and Evidence-Based Traditional Uses of the Genus *Achillea* L.: An Update. *Sci. Pharm,* 89:50.

Beheshti Nasr, M., Masoud Kushki, A., Taghiabadi, F. and clever Khodada, M. 2014. Investigating the effect of Yarrow hydroalcoholic extract on the severity of convulsions caused by pentylenetetrazol kindling in Syrian rats. Summary of articles of the 12th International Epilepsy Congress.

Benetis, R., Radušienė, J., & Janulis, V. 2008. Variability of phenolic compounds in flowers of Achillea millefolium wild populations in Lithuania. *Medicina*, *44*(10), 775.

Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239(1):70-6.

Bimbiraitė, K., Ragažinskienė, O., Maruška, A., & Kornyšova, O. (2008). Comparison of the chemical composition of four yarrow (Achilea millefolium L.) morphotypes. *biologija*, *54*(3).

Bozin, B., Mimica-Dukic, N,, Bogavac, M., Suvajdzic, L., Simin, N., Samojlik, I. & Couladis, M. (2008). Chemical Composition, Antioxidant and Antibacterial Properties of Achillea collina Becker ex Heimerls.l. and A. pannonica Scheele Essential oils. Molecules, 13: 2058-2068.

Garcia-Oliveira P., Barral M., Carpena M., Gullón P., Fraga-Corral M., Otero P., Prieto M.A., Simal-Gandara J. Traditional plants from *Asteraceae* family as potential candidates for functional food industry. *Food Funct.* 2021;12:2850–2873.

Hosni K, Zahed N, Chrif R, Abid I, Medfei W, Kallel M, et al. Composition of peel essential oils from four selected Tunisian Citrus species: evidence for the genotypic influence. Food Chem 2010;123(4):1098-104.

Hyldgaard M, Mygind T, Meyer RL. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front Microbiol 2012;3(12):1-24.

inflorescences suppresses lipopolysaccharide-induced inflammatory responses in RAW 264.7 murine macrophages. Journal of Medicinal Plants Research 2010;4(3): 225-34.

Kossah, R., Zhang, H., and Chen, W. 2011. Antimicrobial and antioxidant activities of Chinese sumac (Rhus typhina L.) fruit extract.Food Control 22:128-132.

Lemmens-Gruber, R., Marchart, E., Rawnduzi, P., Engel, N., Benedek, B., & Kopp, B. (2006). Investigation of the spasmolytic activity of the flavonoid fraction of Achillea millefolium sl on isolated guinea-pig ilea. *Arzneimittelforschung*, *56*(08), 582-588.

Potrich. F.B., Allemand. A., Mota da Silva. L.M.D., Santos. A.C.D., Baggio. C.H., Freitas. C.S., Mendes. D.A.G.B., Andre. E., Werner. M.F.D.P., Marques. M.C.A. (2010). Antiulcerogenic activity of hydroalcoholic extract of Achillea millefolium L.: Involvement of the antioxidant system. Journal Ethnopharmacology., 130: 85–92.

Raissy M, Khamesipour F, Rahimi E, Khodadoostan A. Occurrence of Vibrio spp., Aeromonas hydrophila, Escherichia coli and Campylobacter spp. in crayfish (Astacus leptodactylus) from Iran. Iran J Fish Sci 2014;13(4):944- 54.

Shahidi F, Tabatabaei Yazdi F, Roshanak S, Alizadeh Behbahani B, Vasiee A, Norouz N. Antimicrobial activity of Taraxacum pseudocalocephalum leaves extract on pathogenic microorganisms and comparison with common therapeutic antibiotics in vitro. Iran J Infect Dis Trop Med 2019;23(83):37-46.

Sökmen A, Sökmen M, Daferera D, Polissiou M, Candan F, Ünlü M, et al. The in vitro antioxidant and antimicrobial activities of the essential oil and methanol extracts of Achillea biebersteini Afan.(Asteraceae). Phytotherapy Research. 2004; 18(6): 451-456.

Villano D, Fernandez-Pachon MS, Moya ML, Troncoso AM, GarciaParrilla MC. 2007. Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta..71(1):230-5.