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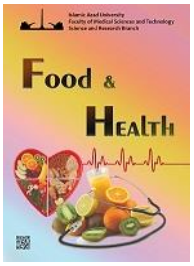

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The effect of extraction method (ultrasonic, maceration, and soxhlet) and solvent type on the extraction rate of phenolic compounds and extraction efficiency of *Arctium lappa* L. roots and *Polygonum aviculare* L. grass

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ABSTRACT

The species of *Arctium lappa* L. roots and *Polygonum aviculare* L. grass have various therapeutic properties in traditional medicine in East Asian countries. The aim of this study was to investigate the effect of three methods of extracting extracts by ultrasonic bath, maceration, and soxhlet to determine phenolic compounds of *Arctium lappa* L. roots and *Polygonum aviculare* L. grass using water and 50% ethanol and the evaluation of extraction efficiency of their extract. The results showed that in the ultrasonic bath extraction method, ethanolic extract of *Arctium lappa* L. root had the highest content of phenolic compounds (976 mg equivalent to Gallic acid per gram of dry sample), and the highest extraction efficiency in this method was related to *Polygonum aviculare* L. grass when using both solvents (14%). In the maceration extraction method, the ethanolic extract of *Arctium lappa* L. root showed the highest content of phenolic compounds (976 mg equivalent to Gallic acid per gram of dry sample), and the highest extraction efficiency in this method was related to the *Polygonum aviculare* L. grass extracted with water (12%). Also, in the Soxhlet extraction method, ethanolic extract of *Arctium lappa* L. root showed the highest content of phenolic compounds (578 mg equivalent of gallic acid per gram of dry sample), and the highest extraction efficiency in this method was related to the *Polygonum aviculare* L. grass extracted with ethanol (18/16%). The content of extraction of bioactive compounds based on the type of solvent, the method, and the type of plant used were significantly different from each other and 50% ethanol solvent was the best solvent for extracting the desired compounds. *Arctium lappa* L. roots also had the highest phenolic composition and extraction by maceration, soxhlet, and ultrasonic bath methods had the most significant effect on the extraction of phenolic compounds, respectively.

1. Introduction

The growth of consumer awareness about the consumption of natural products has led to their attention and demand. Countless compounds in medicinal and medicinal plants can affect health. These substances are rich in polyphenolic compounds, antioxidants, and nutraceutical compounds (1) and have functional properties such as anti-mutagenicity, anti-cancer, anti-inflammatory, and anti-oxidative stress (2, 3). Antioxidants by absorbing free radicals and preventing the oxidation of fats, preventing their rapid (4). Today, the use of synthetic antioxidants is limited due to adverse effects such as toxicity and danger to human health. Therefore, research to identify types of natural antioxidants of plant origin has increased (5). There is a lot of research being done on herbal medicines to replace them with common synthetic compounds found in consumer products (6). In recent years, the use of various species of medicinal plants in Iran and the world has attracted much attention. Some of them are used in a more limited way for medicinal applications. Therefore, due to their efficiency and the potential of their phenolic and antioxidant compounds, these natural compounds can be used in other

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fields as well. These plants are also used to protect against oxidative damage that leads to diseases such as cardiovascular disease, inflammation, and cancer (7). *Polygonum aviculare L* has the ability to inhibit DPPH free radicals and is used in traditional eastern medicine and belongs to the group of herbal medicines (8). This plant is from the genus *Polygonum L.*, which belongs to *Polygonaceae Juss*. It is an annual herbaceous plant with small oval leaves and this family is composed of about 300 different species around the world (9). Plants belonging to this genus include secondary drug metabolites such as tannins, anthraquinones, triterpenoids, phenylpropanoids, coumarin, lignans, flavonoids, etc. (10). This plant is traditionally used in the treatment of upper respiratory disorders and skin diseases (11, 12). It also has anti-inflammatory activity due to the effect of flavonoids (13). *Arctium lappa L*. is a medicinal plant that belongs to the family Compositae (*Asteraceae*). However, it is mainly used in traditional medicine in many Asian countries (Japan, Korea, and Thailand) for treatment (14). Health Benefits *Arctium lappa L.* can be used as an antioxidant, anti-diabetic, anti-inflammatory, anti-cancer, anti-allergy, anti-acne, anti-infertility, gastrointestinal protection, liver protection, and anti-aging (15, 16). Various parameters are effective on the extraction of phenolic compounds and extracts from plants, including extraction method, solvent type, etc. (17). The effect of parameters such as extraction method (basis of different performance) and type of solvent in extraction is due to the different polarity of solvent-forming molecules and the differences in intramolecular and intermolecular bonds and their effect on these compounds (18). Classic extraction methods are based on contact of the plant with the appropriate solvent, which is used by stirring or heating methods to increase the speed of the process. Among the classic methods, we can mention soxhlet and maceration methods. There are various methods for extracting bioactive compounds from plants. One of the common methods is the maceration method, which is widely used to extract solid compounds and causes the extraction of valuable compounds by proper selection of solvent polarity according to the type of plant (19). In this method, more solvent is used to immerse the plant, and also more time is required (17). Other methods include extraction by soxhlet and ultrasound, which is an effective method for extracting bioactive compounds (20). Soxhlet method is used to extract low or medium volatile compounds that are resistant to heat (21). This method has advantages such as simplicity and ease, the fresh solvent is constantly in contact with plant particles, the use of high temperature, which leads to increased solubility of low-soluble compounds at low temperatures. In extraction methods, the choice of solvent is very important, so that the choice of different solvents will cause different extracts and different compounds in that extract (22). Ultrasound has unique benefits, especially in the storage and processing of food and its use is expanding every day. In the process of using ultrasound, the extraction of heat-sensitive compounds can also occur because it uses low temperatures. This performance is the result of mechanical waves and their cavitation causes a flow to increases the rate of solvent permeability into cells and plant tissues and enhances mass transfer (23). Ultrasonic bath has a higher reproducibility than its probe (5, 24). In this study, the effect of solvent type in extraction methods of maceration, soxhlet, and ultrasonic bath on the content of phenolic compounds and the efficiency of extracting extracts from *Arctium lappa L.* roots and *Polygonum aviculare L.* grass were investigated.

2. Materials and methods

The chemicals used to include hydrochloric acid, sodium carbonate, Folin-Ciocalteu reagent, Gallic acid, ethanol and methanol, were prepared by the German (Merck company). Equipment used include mill (IKA, M20), oven (Wiseven), vacuum rotary evaporator (Heidolph, Laborota 4003, Germany), shaker (Heidolph, UNIMAX 2010), spectrophotometer (Cary 300 UV-Vis, Varian, Mulgrave, Victoria, Australia), ultrasound bath (WiseClean) and centrifuge (Sigma, 3-18k**).** *Arctium lappa L.* roots and *Polygonum aviculare L.* grass were obtained from the local market of Tehran. The plants were pulverized by a mill (IKA, model M20) after washing and drying at room temperature. The powdered samples were stored in a dark container and refrigerated at 4 °C until use. All chemicals are of high purity and were purchased from Merck Company.

2.1. Preparation of plants

The plants were washed with distilled water and were dried in a hot oven at 40 °C for 8 h to remove the moisture. Then, they were powdered (with an electric milling machine) and passed through the mesh (25, 26).

2.2. Alcoholic extraction

3 gr plants powder was weighted mixed with 30 ml ethanol/water solvent (50:50). The mixture was shaken at 180 rpm overnight. The extract was then centrifuged at 1000 rpm for 2 minutes at 4 °C and filtered using Watman Paper No. 1 and concentrated under vacuum by a rotary evaporator (Heidolph, Laborota 4003, Germany). The resulting extracts were placed in dark vials and stored in the refrigerator until use (5, 26, 27).

2.3. Aqueous extraction

For this, distilled water was added to plant powder**.** The mixture was shaken gently on a shaker at 180 rpm (20 °C) overnight. Then obtained extracts were filtered by Watmann Paper No.1. The extract was concentrated by a rotary evaporator (Heidolph, Laborota 4003, Germany) under vacuum. Transparent extracts kept at a refrigerated temperature in dark vials until use (5).

2.4. Ultrasonic Extraction

Extraction operations were performed with two solvents to investigate the effect of the solvent. In this method, water and 50% ethanol solvents were mixed separately with 3 g of dried plants powder in a ratio of 1: 10, and then, the obtained plant mixtures were placed on a shaker for 1 hour (180 rpm). They were then exposed to an ultrasound bath (Wise Clean) for 40 minutes at 60 °C. The extracted extracts were separated from the plant solids using centrifugation (4000 rpm, 10 minutes) (28). The resulting extracts were concentrated using a vacuum rotary evaporator (IKA-RV05 Basic) at 40 ° C. The extracts were stored in a dark container at refrigerator temperature until use (5).

2.5. Soxhlet Extraction

In this method, 3 g of each plant was wrapped in filter paper and placed in a Soxhlet device. The extraction operation was continued separately with 50% ethanol and water solvents for several hours. The solvents containing the extract were then evaporated by a rotary evaporator (29).

2.6. Extraction efficiency

The extract efficiency of extracts was measured using the following Eq. 1 (30).

$$\text{Efficiency of extract} = \frac{\text{Extract Weight}}{\text{Initial Sample Weight (Dried Fruit)}} \times 100 \quad \text{Eq. (1)}$$

2.7. Measurement of total phenolic compounds

Total phenol was obtained by Folin-Ciocalteu method (31). The sample (20 µl) was mixed with distilled water (1.6 ml) and 100 ml folin solution (1:10 v/v). 300 µl of 7.5% sodium carbonate solution was added after 5 minutes. Also, 10 µl of distilled water was added instead of 20 µl sample as a control. Then, all treatments were stored for 2 hours at 25 °C. The absorbance was read at 765 nm by a Cary 300 spectrophotometer (Varian, Mulgrave, Victoria, Australia). The standard curve of Gallic acid was used to determine the actual amount of total phenol. The total phenol of extract was calculated in terms of Gallic acid equivalent and using the regression equation (Eq. 2) obtained from the standard curve and the results were expressed in terms of mg of Gallic acid per hundred grams of extract.

$$Y = 22.553 X + 0.0714 \quad \text{Eq. (2)}$$

2.8. Statistical Analysis

Analysis of variance software (SPSS 26) (SPSS Inc., Chicago, IL, USA) was used to analyze the data and compare the obtained means (three replications). The results were analyzed by the Duncan test ($p<0.05$) based on a completely randomized design.

3. Results and discussion

3.1. Extraction efficiency and phenolic compounds of the extract obtained from the maceration method

The weight of the extracts was calculated after extraction and their efficiency was calculated. As shown in Table 1, two solvents of water and 50% ethanol were used to extract phenolic compounds with different polarities. There was a statistically significant difference ($p<0.05$) between the extraction efficiencies of extracts (maceration method) of different solvents of *Arctium lappa L.* roots, but there was no significant difference between the extraction efficiencies of *Polygonum aviculare L.* grass extracts. Extraction with different solvents yielded different efficiencies. The extract of both solvents of *Polygonum aviculare L.* grass with extraction efficiency of 14% had the highest rate and the aqueous extract of *Arctium lappa L.* roots with an extraction efficiency of 5.33% had the highest content. High water polarity in some plants is not able to extract all phenolic compounds, but organic solvents are usually more efficient in extracting phenolic compounds, and these results were in agreement with the findings of other researchers (32, 33). The content of phenolic compounds of all extracts was expressed as mg equivalent of Gallic acid per 100 g of primary sample of plants (Table 1). The highest content of phenolic compounds was observed in 50% ethanolic extract (*Arctium lappa L.* roots with total phenol 976±0.1 GAE per 100 g of primary powder and *Polygonum aviculare L.* grass with total phenol 156±0.07 GAE per 100 g of primary powder) of both plants. Accordingly, there was a significant difference in the phenol content of all extracts. The antioxidant activity of plants is related to the content of phenolic compounds in them, which allows them to lose hydrogen and trap free radicals (34,35). Phenolic compounds have an aromatic ring and are present in almost all parts of the plant (35). The antioxidant properties of the extract are related to the extraction of phenolic compounds. Factors affecting its content include solvent, extraction method, temperature, and extraction time. The polarity of the solvent and the type of plant compounds also affect the extraction rate. Phenolic compounds can be polar or non-polar and are extracted with different solvents depending on the type of plant and its constituent compounds. Some researchers have extracted phenolic compounds using various solvents (36). In a study, Cucumismelo leaf extract was extracted by soxhlet, ultrasonic, and maceration methods. Soxhlet extract obtained more phenolic compounds than other extracts which were in agreement with the present study (37). In the study of bioactive compounds of *Lepidium sativum*, ethanol and water solvents had different effects on the extracted compounds in different extraction methods (38). The content of phenolic compounds and the extraction efficiency of jujube fruit were determined by ultrasonic and maceration methods by Khoshdouni Farahani et al. (5). The results showed that the ultrasonic bath extraction method, 80% ethanol solvent, extracted the highest content of phenolic compounds from jujube fruit and its extraction efficiency was 66.95%. In the maceration extraction method, 80% ethanol solvent extracted the highest content of phenolic compounds and its extraction efficiency was 77%. In a study on the phytochemical composition and antioxidant capacity of beans was done, the polarity of the extracted solvents was effective on the extraction efficiency of these compounds. Therefore, the use of both polar and non-polar solvents was effective in this regard (39).

Table 1. Comparison of the effect of solvent and plant types on the extraction rate of phenolic compounds and extraction efficiency using the maceration method.

Plant type	Solvent type	Extraction efficiency (%)	Total phenol (mg gallic acid/100 g)
***Arctium lappa* L. roots**	Water	5.33 ± 0.07^{b}	600± 0.2^{b}
***Arctium lappa* L. roots**	Ethanol 50%	4.33 ± 0.02^{c}	976± 0.1^{a}
***Polygonum aviculare* L. grass**	Water	14± 0.1^{a}	136± 0.05^{d}
***Polygonum aviculare* L. grass**	Ethanol 50%	14± 0.05^{a}	156± 0.07^{c}

Different letters in each column indicate a significant difference ($p<0.05$).

3.2. Extraction efficiency and phenolic compounds of the extract obtained by ultrasonic bathing

According to the results in Table 2, at the temperature and time levels that were considered constant and the type of solvent and plant was different, a significant difference was observed at the level of five percent in terms of extraction efficiency and phenolic compounds. The use of 50% ethanol solvent showed that more phenolic compounds were obtained than water solvents in both plants. On the other hand, water solvent had higher extraction efficiencies in both plants, which could be due to increased solubility and greater solvent penetration, which leads to improved mass transfer. This solvent can increase the extraction of impurities and waste compounds that were observed in aqueous extracts of both plants in a way that the increase in yield was not accompanied by an increase in phenolic compounds. The results of mean extraction efficiency and total content of phenolic compounds of different treatments obtained by ultrasound method showed (Tables 2) aqueous extract of *Arctium lappa L.* roots (2.66%) and *Polygonum aviculare L.* grass (12%) with the highest extraction efficiency were observed which had significantly different from other treatments. 50% ethanolic extract of *Arctium lappa L.* roots with 976 mg equivalent of Gallic acid per 100 g of dry sample and 50% ethanolic extract of *Polygonum aviculare L.* grass, 126 mg equivalent of Gallic acid per 100 g of the dry sample had the highest extraction of phenolic compounds, which had significant difference with other treatments. There was a significant difference between the two plants ($p<0.05$) regarding these evaluations. Therefore, it can be stated that the use of ethanol as an extraction solvent in the extraction of phenolic compounds of *Arctiuml appa L.* roots extract by ultrasonic bath has a significant effect on the extraction of phenolic compounds. This is due to cavitation, which is due to the propagation of ultrasound waves in plant and solvent tissues, which causes contraction and expansion in the medium. These processes lead to the formation of bubbles in two phases and increase their growth over time, causing them to disappear and ultrasound accelerates these oscillations and causes the transfer of compounds from the solid to the solvent. If plant compounds have oxidation-sensitive compounds, prolonged exposure can reduce the rate of extraction (40). Various studies indicate different functions of ultrasound in the extraction efficiency of extracts. Research has shown that the effect of factors such as solvent type, time, and temperature on the extraction of phenolic compounds of wheat bran by ultrasound method was investigated. Solvents of acetone, 80% ethanol, and 70% methanol, temperature range of 25 to 75 °C, and time of 10 to 50 min were used. The results showed that under the same conditions in terms of temperature and time, most extraction of phenolic compounds was done by ethanol and with increasing temperature and time, the extraction rate increased (41). In another study, the effect of different extraction methods on the antioxidant activity of *Crocus caspius* was investigated. The results showed that the extract obtained from the ultrasonic method had a higher phenolic content and the extract obtained from the maceration method was stronger in the inhibitory strength test (42).

Table 2. Comparison of the effect of solvent and plant types on the extraction rate of phenolic compounds and extraction efficiency using ultrasonic bath method.

Plant type	Temperature (ºC)	Time (Min)	Solvent type	Extraction efficiency (%)	Total phenol (mg gallic acid/100 g)
***Arctium lappa L.* roots**	60	40	Water	2.66± 0.03 ^{c}	238± 0.12 ^{b}
***Arctium lappa L.* roots**	60	40	Ethanol 50%	2± 0.1^{d}	976 ± 0.14 ^{a*}
***Polygonum aviculare L.* grass**	60	40	Water	12± 0.04 ^{a}	66± 0.08 ^{d}
***Polygonum aviculare L.* grass**	11.2± 0.06 ^{b}	126 ± 0.11^{c}	Ethanol 50%		

*Different letters in each column indicate a significant difference ($p<0.05$).

3.3. Extraction efficiency and phenolic compounds of the extract obtained by soxhlet method

The results of Table 3 depicted which there was a statistically significant difference of 5% between the extraction efficiencies of extracts (soxhlet method) of different solvents of *Arctium lappa L.* roots and *Polygonum aviculare L.* grass extracts. The highest rate of extraction efficiencies of *Polygonum aviculare L.* grass and *Arctium lappa L.* roots were seen in ethanolic extracts which were 16.18% and 7.33%, respectively. On the other hand, the highest content of phenolic compounds was observed in 50% ethanolic extract (*Arctium lappa L.* roots with total phenol 578 ± 0.03 GAE per 100 g of initial powder and *Polygonum aviculare L.* grass with total phenol 254.76 ± 0.02 GAE per 100 g of initial powder) of both plants. Accordingly, there was a significant difference in the phenol content of all extracts. So, it can be explained that the use of ethanol as an organic solvent in the extraction of phenolic compounds of these plants extract by soxhlet has a significant effect on the extraction of phenolic compounds. This could be due to the long contact of solvent to plant materials and more penetration of ethanol to plants and also, the similar polarity of ethanol with bioactive compounds of these plants which led to more extraction of ethanol rather than water. Research has shown that Dias et al. (43) in a study examined soxhlet and ultrasonic methods with four types of solvents to extract *dedo de moça* pepper extract. The results showed the high effectiveness and efficiency of phenolic and antioxidant compounds obtained by the ultrasonic method.

Table 3. Comparison of the effect of solvent type on the extraction rate of phenolic compounds and extraction efficiency using Soxhlet method.

Plant type	Solvent type	Extraction efficiency (%)	Total phenol (mg gallic acid/100 g)
***Arctium lappa* L. roots**	258± 0.09 [b]	7± 0.02 [d]	258± 0.09 [b]
***Arctium lappa* L. roots**	578± 0.03 [a]	7.33 ± 0.02 [c]	578± 0.03 [a*]
***Polygonum aviculare* L. grass**	60± 0.07 [d]	12.8± 0.03 [b]	60± 0.07 [d]
***Polygonum aviculare* L. grass**	254.76±0.02 [c]	16.18± 0.06 [a]	254.76± 0.02 [c]

*Different letters in each column indicate a significant difference (p<0.05).

Predes et al. (44) investigated the antioxidative activity of *Arctium lappa root* extracts. The outcomes showed the high phenolic contents obtained by soxhlet and maceration extraction and soxhlet extraction was more effective (Table 4).

Petkova et al. (45) surveyed the effect of ultrasonic irradiation frequency on phenolic content and antioxidant activity in *Arctium lappa* L. roots. Ethanol (70%) and water extracts were obtained by ultrasound-assisted technique. Data depicted 10.35 to 18.16 mg Gallic acid equivalent (GAE)/g DW as phenolic compounds content and antioxidant activities of these roots. Ferracane et al. (46) studied bioactive compounds of burdock (*Arctium lappa*) roots, seeds, and leaves. Total phenolic content of hydroalcoholic extracts of burdock ranged from 2.87 to 45 g of Gallic acid equivalent/100 g DW. Some phenolic acids were arctiin, luteolin, and quercetin rhamnoside in burdock roots**.** Antioxidant activity of *Polygonum aviculare* L. extract was investigated by Chin-Yuan Hsu (47). *Polygonum aviculare L*. extract was extracted by ethanol and the total phenol content was 677.4 ± 62.7 mg/g. So, it indicates the high antioxidant effects of *Polygonum aviculare* L. extract. Regarding the comparison of the performance of the three methods of maceration, ultrasound, and soxhlet, the results showed that the soxhlet method with an extraction efficiency of 7.16% and 14.8% for *Arctium lappa L*. roots and *Polygonum aviculare L*. grass, respectively, and maceration method with phenolic content of 788 mg equivalent of Gallic acid per 100 g of primary sample for *Arctium lappa L*. roots and Soxhlet method with phenolic content of 157 mg equivalent of gallic acid per 100 g for *Polygonum aviculare L*. grass are more efficient way to extract phenolic compounds of this plant extracts for industrial and research applications. Universally, the content of phenolic compounds in these plants can vary depending on the species, place of cultivation, place of harvest, and whether it is dry or fresh.

Table 4. Comparison of changes in extraction efficiency and total phenols of extracts under different extraction methods (ultrasound, maceration and soxhlet).

Plant type	Analysis	Extraction method		
		Ultrasound	Maceration	Soxhlet
***Arctium lappa* L. roots**	Extraction efficiency (%)	2.33± 0.07 [f]	4.83± 0.02 [e]	7.16± 0.02 [d]
***Arctium lappa* L. roots**	Total phenol (mg gallic acid/100 g of primary powder)	337± 0.14[c]	788± 0.15 [a*]	418± 0.13 [b]
***Polygonum aviculare* L. grass**	Extraction efficiency (%)	11.6± 0.06 [c]	14± 0.04 [b]	14.8± 0.08 [a]
***Polygonuma viculare* L. grass**	Total phenol (mg gallic acid/100 g of primary powder)	95± 0.09 [f]	146± 0.04 [e]	157.38± 0.02 [d]

*Different letters in each column indicate a significant difference (p<0.05).

4. Conclusion

Extraction and efficiency of bioactive natural plant materials using safe methods to achieve valuable compounds are considered. The type of solvent that can help increase the extraction efficiency of bioactive compounds and is non-toxic is important. The content of phenolic compounds and antioxidant activity of plants is affected by the extraction conditions, including the type of solvent used and the extraction method. Extraction of polyphenols from plant materials is based on the extraction method and the type of solvent used. The degree of polarity of solvent due to the polarity of bioactive compounds of each type of plant can have different effects on the content of extraction. The results of the present study showed that extraction by maceration, soxhlet, and ultrasonic bath methods has a significant effect on the extraction of bioactive compounds of *Arctium lappa L*. roots and *Polygonum aviculare L*. grass. In all methods, the content of extraction of bioactive compounds was different depending on the type of solvent. In all three methods of maceration, soxhlet and ultrasonic bath, *Arctium lappa L*. roots were extracted with 50% ethanol had the highest content of fenolic compounds and *Polygonum aviculare L*. grass had the highest extraction efficiency. Therefore, in all extraction methods, 50% ethanol was the best solvent for extracting the compounds considered by the two plants. The use of selected Soxhlet, maceration, and ultrasound techniques in this study was done to compare the extraction rate and extraction efficiency to show that some plants still show better extraction and efficiency by conventional methods.

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