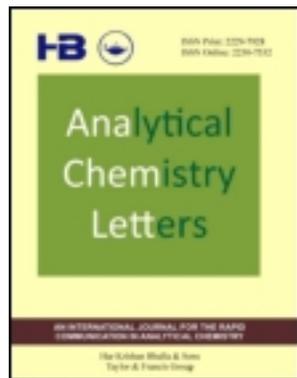


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Phenolic Content and Antioxidant Activity of *Falcaria vulgaris* Extracts

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Abstract: The aim of this study was to examine the phenolic contents and antioxidant properties of *Falcaria vulgaris* extracts obtained from microwave-assisted extraction (MAE) and maceration. Our results revealed that extracts obtained from 50 % ethanol, 80 % methanol and 70 % ethanol (as extractants) had the highest contents of total phenolic compounds. The effect of extraction time was also determined for both extraction methods. It was observed that the maximum phenolic concentration was achieved at 24 h (49.302±0.14 mg GAE/g powder dw) in maceration method and at 12 min (64.287 ± 0.32mg GAE/g powder dw) of exposure during MAE by ethanol extracts of *F.vulgaris*. From the antioxidant activity point of view, the lowest IC₅₀ in DPPH (97.36 ± 0.29 µg/ml), reducing power (200.118 ± 0.14 µg/ml) and total antioxidant capacity (281.901±0.12 µg/ml) were associated with the ethanolic extract from MAE. The latter extract showed higher reducing power and total antioxidant capacity than BHA.

Keywords: *Falcaria vulgaris*, phenolic, microwave, maceration, antioxidant activity.

Introduction

Antioxidants are used as food additives to protect food against deterioration. Antioxidants are an important class of preservatives as oxidation reactions, unlike bacterial or fungal spoilage, occur relatively rapidly even in frozen, refrigerated and oily foods ³⁰.

The most common molecules attacked by oxidation are unsaturated fats; oxidation causes them to turn rancid. Since oxidized lipids are often discolored and usually have unpleasant tastes such as metallic or sulfurous flavors, it is important to avoid oxidation in fat-rich foods. Antioxidant preservatives are also added to fat-based cosmetics such as lipstick and moisturizers to prevent rancidity.

Phenolic compounds are secondary metabolites in the plant kingdom that are derived from pentose phosphate, shikimate and phenylpropanoid pathways. These compounds exhibit a wide range of beneficial effects due to their antioxidant activity. The antioxidant activity of phenols is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers. The food-processing industry is moving away from the use of synthetic antioxidants because of their toxic and carcinogenic effects. Natural antioxidants are more desirable as food additives, not only for their free-radical-scavenging properties, but also because they are healthier and safer than synthetic ones; this also makes them more readily acceptable to modern consumers ⁴.

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Phenolic compounds have received much attention as natural antioxidants due to their ability to act as both efficient radical scavengers and metal chelators. Numerous studies have shown that phenolic compounds have high antioxidant activity. Ethanol extract of *Cortex fraxinifolia* exhibits excellent scavenging activity on DPPH¹ and hydroxyl radicals and reduced lipid oxidation in peanut oil¹⁷. Salta *et al.*²² reported that antioxidant capacity and oxidative stability were substantially improved for commercially available oils (olive oil, sunflower oil, palm oil, and a vegetable shortening) enriched with polyphenols of olive-leaf extract. Extracts from *Guierasene galensis* leaves and roots and *Combretum hartmannianum* leaves were markedly effective in DPPH scavenging. Plant extracts (500 mg) effectively inhibited the formation of peroxides in sunflower oil and showed higher antioxidant activity than 20 mg BHA¹⁴.

Extraction is the first essential step for the recovery and purification of many bioactive components such as phenolic compounds from plant materials. The conventional extraction methods used for many decades are very time-consuming and require relatively large quantities of solvents, and are mostly based on the choice of solvents, heat or blending for increasing the analysis solubility and the rate of mass transfer. Conventional methods can also lead to thermal decomposition of some bioactive compounds at high temperatures³¹. Thus, it is necessary to develop an optimal extraction method. One of the current methods for extraction processes is microwave-assisted extraction. The main advantage of MAE² is the considerable reduction of extraction time and solvent consumption. In this method, microwaves irradiate the mixture of solvent and sample and are converted into heat, causing increase in target molecules' solubility and yield-extraction efficiency¹⁵.

Absorption of microwave energy depends on the nature of the solvent (especially its dielectric properties) and sample matrix. The dielectric constant and dielectric loss factor are used to express the dielectric response of materials in an applied microwave field. The dielectric constant is defined as the ability of a solvent to absorb

microwave energy. The loss factor indicates the ability of a solvent to dissipate the absorbed energy into heat. Moreover, the dissipation factor measures the ratio of the dielectric loss to the dielectric constant of the solvent¹. Recently, MAE has been used to extract active compounds. Kwon *et al.*¹³ found that MAE required 12 min to extract 92.1 % of artemisinin from *Artemisia annua* L; whereas Soxhlet extraction could only recover 60 % of this compound after several hours⁹. MAE required 15 min to recover approximately 95 % of the total capsaicinoid fraction from capsicum fruit, whereas extraction time for the reflux and shaken-flask methods were two and 24 hours, respectively. Shu, Ko and Chang (2003) reported that 15 min of MAE (ethanol-water) of ginsenosides from ginseng root obtained a higher extraction yield than extraction (ethanol-water) at room temperature for 10 hours.

F. vulgaris (known locally as ghazzyaghi or poghazeh) is a member of the *Umbelliferae* or *Apiaceae* family of usually aromatic plants. They are adapted to conditions that encourage heavy concentrations of essential oils; some are used as flavorful or aromatic herbs. This plant grows near farmlands and is consumed as a vegetable in some mountain regions of Iran. In folk medicines, this herb is used for curing skin ulcers, stomach disorders including peptic ulcers, liver diseases and kidney and bladder¹². The optimal conditions for extraction of phenolic compounds from *Falcaria vulgaris* have been not investigated.

Thus, the objectives of this study were (1) to determine the influence of extraction method (MAE and maceration), solvent type and different extraction times on the amount of total phenolic content for *F. vulgaris*; and (2) to evaluate the antioxidant activity of different extracts by three assays: DPPH scavenging assay, reducing power and total antioxidant capacity.

Materials and methods

Falcaria vulgaris was collected from the Arak region (in Markazi province, Iran). The leaves were separated, shade dried for 7 days, and finely powdered (40 mesh) using a grinder. Arak city is located at longitude 49°42'0''E and latitude 34°5'0''N. The maximum temperature of Arak

city may rise to 35 degrees Celsius in summer and may fall below -25 degrees Celsius in winter. The average rainfall is around 300 mm and the annual relative humidity is 50% (<http://www.markazi.rmtto.ir/NewSite/English>).

All the chemicals and reagents used were of analytical reagent grade and were purchased from Applichem or Merck Company.

Extraction MAE

A household microwave oven (Samsung CF3110N-5, Korea) was modified in our laboratory by including a magnetic stirrer, water condenser, temperature sensor and time-controlling device, as shown in Figure 1⁸. Samples (40 mesh) were mixed with different solvents (50 % ethanol, 80 % methanol, 70 % acetone and water) and were irradiated by microwave in pre-setting procedures (15 seconds power on, 15 seconds power off) three times to the desired temperature, and then 3 seconds power on for heating and 15 seconds power off for cooling. The samples were not allowed to super-boil. Irradiation was performed for different times (2, 4, 6, 8, 10 and 12 min). The extracts were filtered through What man No.1 filter paper to

remove fine particles. The filtrate obtained from methanol and acetone was evaporated to dryness at 40°C in a rotary evaporator (IKA RV 05 basic, Germany) and the water extract was freeze-dried (Epron FDV5503, South Korea). The dried sample of each extract was stored at 4°C until use. As for preleaching, there was no microwave energy radiation by magnetic stirring (250 rpm, IKA, USA) for 0-90 min at room temperature and then MAE for 15 min.

Maceration extraction

One g of grounded leaves was mixed with 50 ml of different solvents (50 % ethanol, 80 % methanol, 70 % acetone and water) for different times (1, 2, 3, 4, 5, 6, 7 and 8h) under agitation conditions (250 rpm, IKA, USA) at room temperature. The resulting extracts from maceration were filtered and purified as described for MAE.

Determination of total phenolic content

The total phenolic content of each extract was determined by the Folin-Ciocalteu micro-method (Slinkard & Singleton, 1977). Briefly, 20 µl of each extract solution was mixed with 1.16 ml

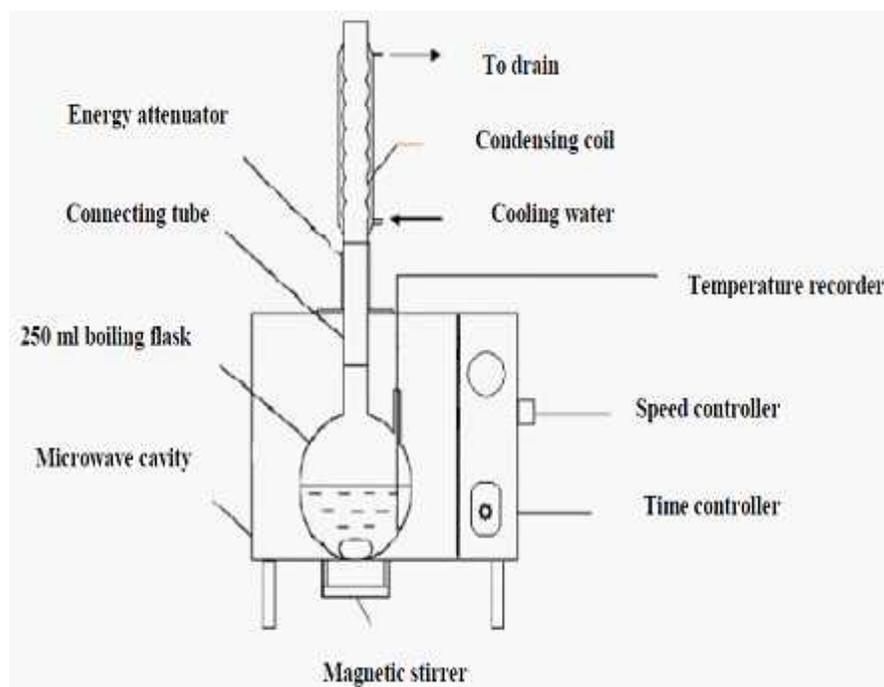


Fig. 1. The modifications done on a house hold microwave oven

distilled water and 100 μ l of Folin-Ciocalteu reagent, followed by the addition of 300 μ l of Na_2CO_3 solution (20 %) after 1 min and before 8 min. The mixture was then incubated in a shaking incubator (Memmert WB14, Germany) at 40°C for 30 min, and its absorbance was measured at 760 nm (PG Instruments T80, UK). Tannic acid was used as a standard for the calibration curve. The phenolic content was expressed as Gallic acid equivalents (GAE) per g of powder dry weight (dw).

DPPH radical scavenging activity

DPPH radical scavenging activities of all samples were evaluated by the method of Arabshahi and Urooj³. In brief, 1 ml of a 1 mM methanol solution of DPPH was mixed with 3 ml of extract solution in methanol (50-1000 μ g/ml). The reaction mixture was incubated for 30 min at room temperature in the darkness. The blank sample contained all reagents without the *F. vulgaris* extract. The absorbance was measured at 517 nm and DPPH radical scavenging activity (%) of the sample was calculated as:

$$(A-B/A) \times 100 \quad (2-2)$$

Where A is the absorbance of control and B is the absorbance of sample.

The DPPH radical scavenging activity was also assayed for BHA and BHT for comparison.

Reducing power assay

The ability of extracts to reduce iron (III) was determined according to the method of Yildirim, Mavi and Kara²⁸. The dried extract (50-1000 μ g) in 1 ml of the corresponding solvent was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$, 10 g l⁻¹). The mixture was incubated at 50°C for 30 min. After incubation, 2.5 ml of trichloroacetic acid (100 g l⁻¹) was added and the mixture was centrifuged (Centurion k2042, USA) at 1650 g for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml of FeCl_3 (1 g l⁻¹), and the absorbance was measured at 700 nm. High absorbance indicates high reducing power. The reducing

power of BHA and BHT was also determined for comparison.

Total antioxidant capacity

This assay is based on the reduction of Mo (VI) to Mo(V) by the sample and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH 1.8. An aliquot of 0.1 ml of sample solution (containing 50-1000 μ g of dried extract in 1 ml corresponding solvent) was mixed in an Eppendorf tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated at 95°C for 90 min. After cooling, the absorbance was measured at 695 nm against a blank sample. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of each solvent, and was incubated under the same conditions as the samples. The extracts' antioxidant activity was expressed as the absorbance of the sample. Higher absorbance values indicate the higher antioxidant activity. BHA and BHT were also assayed for comparison.

Statistical analyses

All experiments were carried out in triplicate. The data were analyzed using analysis of variance (ANOVA) and significant differences among means were determined by Duncan's multiple range test at $P < 0.05$ by the SAS software.

Discussion

Effect of the solvent concentrations of on total phenolic content

Aqueous solutions containing different concentrations of ethanol, methanol and acetone were tested using MAE (Figure 2). The extraction yield of phenolics increased sharply with the increase of ethanol concentration up to 50 % (64.287 mg GAE/g). When ethanol concentration increased from 50 to 90 %, extraction yield slightly decreased. For methanol, total phenolic content increased with increments of the methanol concentration up to 80 % (58.026 mg GAE/g), followed by a decrease with further increase in concentration. For acetone, the highest phenolic compounds were obtained at 70 % (51.776 mg

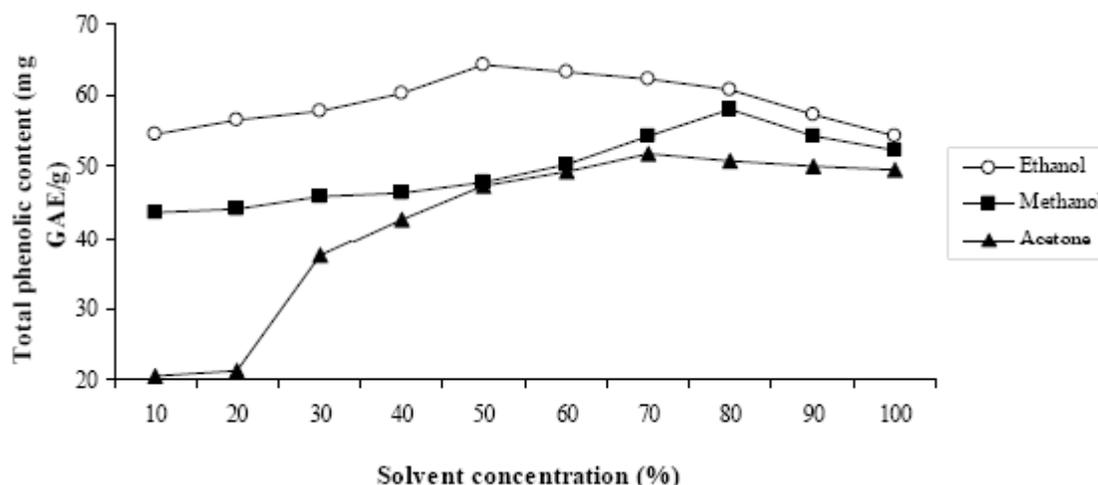


Fig. 2. Effect of concentration of solvents on total phenolic content obtained from *Falcaria vulgaris*

GAE/g), then decreased after reaching maximum recovery. Based on these results, further studies were conducted with 50 % ethanol, 80 % methanol and 70 % acetone.

Because of the polar entity of phenolic compounds, non-polar solvents such as acetone could not be suitable for extracting these compounds. However, water combined with acetone creates a moderately polar medium, ensuring optimal conditions for extraction. This study also confirmed that extraction efficiency for ethanol and methanol was enhanced in the presence of some water. This occurrence can be attributed to increased swelling of plant material, which increases the contact surface area between the plant matrix and the solvent⁶. These findings were

accordance with the study of Turkmen, Sari and Velioglu²⁵.

Maceration extraction of phenolic compounds

Solvent type, extraction time and interaction of these factors had statistically significant effect on amount of phenolic compounds extracted using maceration ($p < 0.05$).

Effect of solvent type

Table 1 illustrates the effect of solvent and time on extraction yield in terms of total phenolic content (TPC). When using water to obtain *F. vulgaris* extracts, the highest phenolic concentration was observed at 6 h (26.280 mg GAE/g). For alcohols, 80 % methanol (43.28 mg GAE/g)

Table 1. effect of solvent and extraction time on TPC in maceration method

Solvent ^{a, b} Time (h)	Water	80 % Methanol	50 % Ethanol	70 % Acetone
2	19.926 ± 0.22qr	32.034 ± 0.37 l	41.07 ± 0.42h	37.45 ± 0.21j
3	20.437 ± 0.26q	35.26 ± 0.21k	42.2 ± 0.51g	40.603 ± 0.28hi
4	22.542 ± 0.25p	37.709 ± 0.33j	44.496 ± 0.24e	43.703 ± 0.43f
5	24.144 ± 0.46o	40.222 ± 0.37i	47.311 ± 0.22c	45.866 ± 0.25d
6	26.280 ± 0.33m	42.226 ± 0.31g	48.286 ± 0.31b	47.483 ± 0.43c
7	26.140 ± 0.32m	43.28 ± 0.25f	49.623 ± 0.22a	47.66 ± 0.22c
8	25.107 ± 0.34n	43.249 ± 0.15f	49.302 ± 0.14a	47.813 ± 0.33c

^a Values followed by different letters are significantly different ($p < 0.05$).

^b Total phenolic content expressed as mg TAE/g power (dw)

and 50 % ethanol (49.623mg GAE/g) had the highest phenolics within 7 h; longer processing times did not significantly ($p>0.05$) improve the recovery of phenolics.

For acetone, the yield of phenolics reached the highest level after 8 h (47.813 mg GAE/g). Because reduction of extraction time is a crucial parameter for industrial applications, and due to the detection of no significant difference in phenolic concentration between 7 and 8h, 7h (47.66 mg GAE/g) could be suggest as an optimal time for extracting phenolic compounds from *F.vulgaris* with 70 % acetone. Overall, 50 % ethanol was the most efficient solvent in the recovery of phenolics, followed by 70 % acetone, 80 % methanol and water.

Methanol and ethanol have been demonstrated as effective solvents to extract phenolic compounds especially in presence of water. In the other hand, highly polar solvents (e.g. water) are not appropriate for extracting a high amount of phenolics, and extracts obtained using water alone contain a high content of impurities (e.g. organic acids, sugars and soluble proteins) which can interfere in phenolic identification and quantification. Based on the results above, it was found that the presence of water in the solvents had an important role in allowing more extraction than with organic solvent alone by increasing the diffusion of extractable polyphenols through the plant tissues. The higher level of phenolic content in ethanol extracts than in other extracts, and the beneficial effect of water in the extraction process, have been confirmed by Karadeniz *et al.*¹¹ and Altioik *et al.*², respectively.

Effect of extraction time

As seen in Table 1, there was a correlation between increased time and yield extraction, but in some cases, TPC fell or remained constant. For example, as for extraction with water alone, an increase in extraction time from 7 to 8h was accompanied by a small reduction in TPC (from 26.280 to 25.107 mg GAE/g).

According to Fick's second law of diffusion, final equilibrium will be achieved between the solute concentrations in the plant matrix and the solvent after a certain time, and prolonging the

extraction process after that point might lead to oxidation of the phenolics through exposure to light or oxygen⁵. So, an excessive extraction time is not necessarily profitable for extracting more phenolic compounds. The decreased phenolic content found with higher extraction times in this study might be attributed to phenolic oxidation under the medium conditions. On the other hand, the differences between the optimal extraction times in various extracts could be associated with the differences in polymerization degree, phenolic solubility and reaction of phenols with other food particles²⁴. Similar results have been reported by Yim *et al.*,²⁹ for mushroom.

Microwave-assisted extraction (MAE)

The ANOVA results and comparison of the mean values of TPC revealed that the type of solvent and extraction time and interaction of these factors had a statistically significant effect ($P<0.05$) on the yield of extraction in MAE.

Effect of solvent type

The TPC of various extracts using MAE are summarized in Table 2. For water, the extract obtained at 12 min (43.535 mg GAE/g) contained the highest yield of phenolic content, followed by the extract obtained at 10 min; no significant difference was observed ($p<0.05$). Thus, extraction time of 10 min was considered optimal for providing an extract with the highest TPC yield at the lowest cost.

The methanol extract had the highest TPC (58.125 mg GAE/g) after a microwave radiation time of 10 min.

The ethanol extract showed the highest TPC (64.287 mg GAE/g) at 12 min, which was found not to be significantly different from that of 10 min. Thus, for ethanol, 10 min was designated as preferred due to its shorter radiation time.

Acetone extract showed maximum concentration of phenolic compounds after 10 min (51.88 mg GAE/g).

In overall comparison, TPC decreased in the order: ethanol>methanol>acetone>water.

Non-polar solvents, for example acetone, have lower dielectric constants and dissipation factors in comparison to the polar solvents (ethanol,

Table 2. TPC of *Falcaria vulgaris* extracts produced by microwave irradiation

Solvent ^{a, b} Time (h)	Water	80 % Methanol	50 % Ethanol	70 % Acetone
2	31.3 ± 0.32p	44.376 ± 0.2 j	54.088 ± 0.55e	41.52 ± 0.7m
4	36.45 ± 0.42o	52.98 ± 0.37f	58.108 ± 0.51d	44.094 ± 0.45j
6	38.698 ± 0.22 n	53.465 ± 0.31f	60.603 ± 0.3 c	48.295 ± 0.61i
8	42.106 ± 0.51	57.703 ± 0.24d	61.307 ± 0.45b	50.709 ± 0.63h
10	43.407 ± 0.2k	58.125 ± 0.45d	64.094 ± 0.44a	51.88 ± 0.43g
12	43.535 ± 0.12k	58.026 ± 0.35d	64.287 ± 0.32a	51.776. ± 0.25g

a Values followed by different letters are significantly different ($p < 0.05$).

b Total phenolic content expressed as mg TAE/g power (dw)

methanol and water), producing no heat under microwave³¹. Hence, these solvents are not suitable for extracting phenolic compounds by MAE. In contrast, Pan, Niu and Liu¹⁶ reported that the highest tea polyphenols were extracted by 50 % ethanol.

Water extraction had a lower TPC than acetone extraction, but water and acetone extracts obtained from MAE contained on average 1.7 and 1.25 times more phenols, respectively, than extracts achieved by maceration. The reason was water's high ability to absorb microwaves and efficiently convert them into heat.

Effect of time

The yield extraction was very low at shorter times. When the MAE time was longer than 10 min, the yield of TPC did not increase significantly, but for methanol and acetone, TPC declined at 12 min. However, there was no significant difference between these times. Thus, 10 min microwave irradiation was appropriate for achieving a rich extract of phenolic compounds. The low levels of TPC under shorter irradiation time might result from the sample having insufficient time to absorb microwave energy and hence break the analyte-matrix bonds and release analytes to the solvents. The fall in recoveries of these compounds at longer microwave irradiation times could be associated with the adsorption of the effective constituents on the sample particle surface. Furthermore, prolonged exposures always involve the risk of degradation by heating.

Xiao, Han and Shi²⁷ reported similar results for extraction of flavonoids from *Radix astragali*.

Comparison of maceration method and MAE

Generally, in terms of solvent applied, ethanol extracted the highest level of phenolic compounds, and water the lowest (Figure 3).

We observed a positive linear correlation between extraction time and TPC for MAE and maceration. The highest phenolic concentrations were obtained after 10 min (54.377 mg GAE/g) and 7 h (41.6778 mg GAE/g) for MAE and maceration, respectively.

The ANOVA results showed that the type of method has a significant effect on extracting phenolic compounds ($p < 0.05$). MAE had higher extraction efficiency (52.151 GAE/g) with reduced extraction time, leading to its consideration as an appropriate alternative for maceration (Figure 4).

The higher TPC of MAE extracts as compared with the maceration ones could be attributed to the direct effect of microwaves on molecules by ionic conduction and dipole rotation, producing heat and hence, increasing in the solubility of target analytes. Furthermore, the use of microwave transparent solvents would cause a sudden temperature increase inside plant, which may lead to rupturing of the cell walls and the rapid liberation of their constituents into the surrounding medium²⁰. Similarity, Quan, Hang, Ha, De and Tuyen²¹ confirmed higher efficiency of MAE than the conventional method for

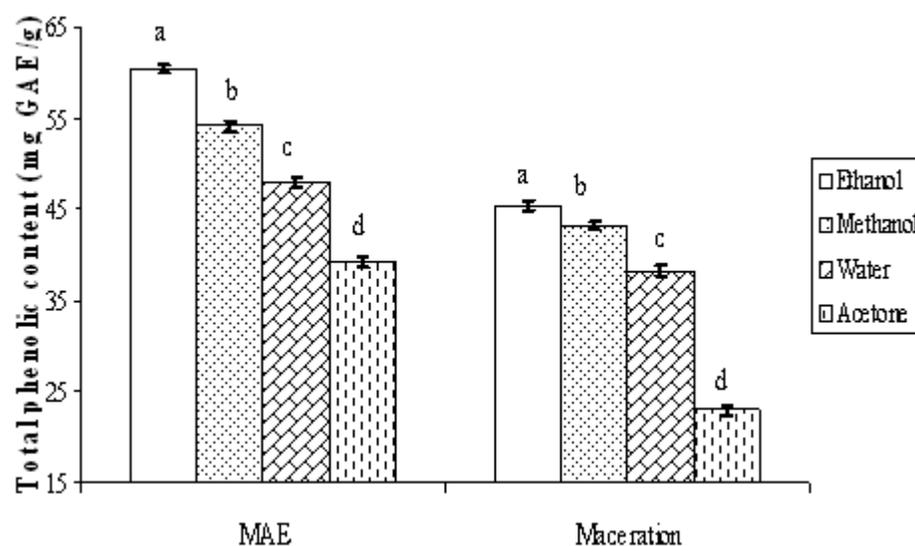


Fig. 3. Effect of solvent type on total phenolic contents of *Falcaria vulgaris* extracts in MAE and maceration method

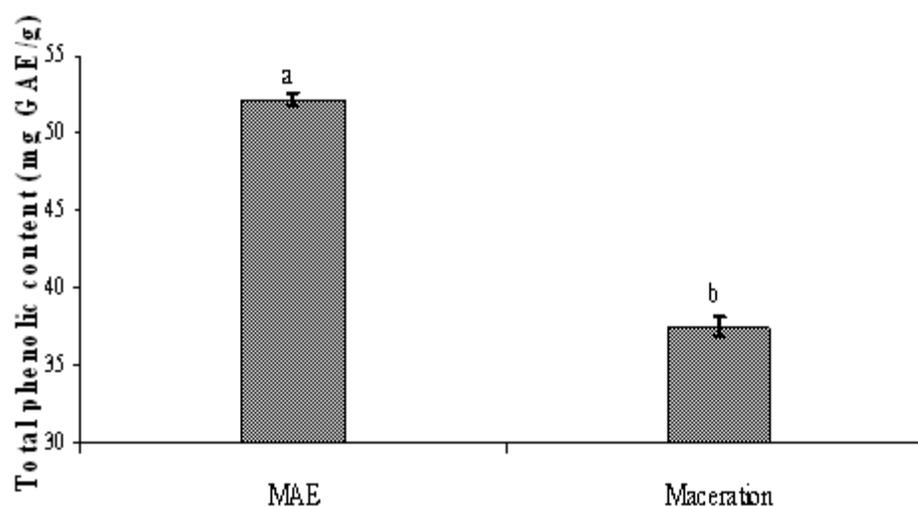


Fig. 4. Comparison of total phenolic contents of *Falcaria vulgaris* extracts produced by MAE and maceration method

extraction of polyphenols from Fresh tea shoots.

Effect of particle size and preleaching time

As the maximum amount of phenolics were obtained from ethanol extract using MAE, effect of particle size and preleaching time on TPC were investigated using this extract (Table 3). Powder mesh size 40 (64.287 mg GAE/g) gave higher amounts of phenolic compounds, followed by 60 and 20. This indicates that powder of mesh size 40 is appropriate for extraction of phenolic compounds. Fine powder can promote the

extraction by enhancing surface area, which increases contact between the plant matrixes. In addition, the use of fine particles allows much deeper penetration of the microwaves, resulting high extraction yield²⁶. Our findings are in agreement with the investigation of Jyothi, Khanam and Sultana¹⁰, who reported that maximum extraction of with an olides from *ashwagandharoots* was obtained with mesh size 44.

As shown in Table 3, the maximum phenolics were obtained after 90 min (69.45 mg GAE/g),

Table 3. Effect of particle size and preleaching time on TPC

Particle size (mesh size)	TPC ^{a,b}	Preleaching time	TPC ^{a,c}
20	58.52 ± 0.41bc	30 min	66.244 ± 0.27b
40	64.287 ± 0.32 a	60 min	69.37 ± 0.12 a
60	61.14 ± 0.29b	90 min	69.45 ± 0.32a

^a Values followed by different letters within each column are significantly different ($p < 0.05$)

^b Total phenolic content expressed as mg GAE/g power (dw); MAE for 12min

^c Total phenolic content expressed as mg GAE/g power (dw); MAE for 12min; mesh size 40

followed by 60 and 30 min. Because the difference in yield between 60 and 90 min were found not to be significant ($p < 0.05$), the preleaching time of 60 min (69.37 mg GAE/g) was considered as optimal for extraction of TPC. It was clear that preleaching before MAE is useful for improving the extraction of phenolic compounds. As efficiency of MAE depends on the dielectric properties of solvent and matrix, better recoveries are achieved by moistening samples with water due to its high dielectric constant²³. These results were agreement with Pan, Niu and Liu¹⁶.

DPPH scavenging activity

Table 4 shows the EC₅₀ for different extracts. In this study, antioxidant activity was evaluated with EC₅₀ values and was expressed as µg of dry extract per 1 ml. Lower values for EC₅₀ indicate higher antioxidant activity. The EC₅₀ value presents the concentration of antioxidant required for 50 % scavenging of DPPH radicals. The samples' EC₅₀ values ranged between 41.566 and 164.67 µg/ml. The lowest EC₅₀ (97.36 µg/ml) among all extracts was obtained for the ethanol extract using MAE. The highest EC₅₀ (164.67 µg/

Table 4. EC50 (µg/ml) of *Falcaria vulgaris* extracts and synthetic antioxidants for different assays

Extraction method	Solvent ^{a, b, c}	DPPH	Reducing power	Total antioxidant capacity
Microwave	Water	135.5 ± 0.23bc	324.5 ± 0.34c	358.14 ± 0.34e
	Methanol	115.533 ± 0.6e	256.71 ± 0.3f	301.89 ± 0.054h
	Ethanol	97.36 ± 0.29f	200.118 ± 0.14h	281.901 ± 0.12i
	Acetone	135.811 ± 0.24bc	265.243 ± 0.25e	312.29 ± 0.63g
	Water	164.67 ± 0.5a	380.8 ± 0.2a	390.567 ± 0.2b
Maceration	Methanol	136.334 ± 0.24b	358.356 ± 0.54b	373.8 ± 0.51c
	Ethanol	134.348 ± 0.44d	303.403 ± 0.29d	346.643 ± 0.62f
	Acetone	135 ± 0.28dc	325.083 ± 0.52c	365.256 ± 0.4d
BHA		89.346 ± 0.43g	205.513 ± 0.65g	422.366 ± 0.5a
BHT		41.566 ± 0.31h	59.418 ± 0.19i	179.61 ± 0.1j

^a Values followed by different letters within each column are significantly different ($p < 0.05$).

^b Data are given as means ± SD, n = 3.

^c EC50 values: the effective concentration at which the absorbance was 0.5 for reducing power and total antioxidant capacity; DPPH radicals was scavenged by 50 %.

ml) was observed for the water extract maceration (Table 4). For maceration, the ethanol extract showed the maximum antioxidant activity, with an EC_{50} value of 134.348 $\mu\text{g/ml}$.

In comparison with synthetic antioxidants, none of the extracts could compete with BHT (EC_{50} = 41.566 $\mu\text{g/ml}$) and BHA (EC_{50} = 89.346 $\mu\text{g/ml}$).

Reducing power

For reducing power, the EC_{50} value is defined as the concentration at which the absorbance is 0.5. The extracts' EC_{50} values were ranged from 59.418 to 380.8 $\mu\text{g/mL}$. Significant differences ($P < 0.05$) were observed between solvents. As shown in Table 4, ethanol extract using MAE had the highest reducing power, with an EC_{50} value of 200.118 $\mu\text{g/ml}$, and water extract using maceration gave the lowest, with an EC_{50} value of 380.8 ± 0.2 $\mu\text{g/ml}$ among. Ethanol was the most effective solvent for maceration as well as MAE.

Furthermore, as shown in Table 4, the antioxidant activity of ethanol extract using MAE was superior to BHA and BHT, with EC_{50} values of 205.513 and 59.418 $\mu\text{g/ml}$, respectively.

Total antioxidant capacity

As with reducing power, EC_{50} is the effective concentration at which the absorbance is 0.5. As seen in Table 4, the samples' EC_{50} values varied between 179.61 and 422.366 $\mu\text{g/mL}$. Among all extracts, the ethanol extract using MAE had the lowest EC_{50} (281.901 $\mu\text{g/ml}$), and the water extract using maceration had highest total antioxidant capacity (390.567 $\mu\text{g/ml}$). In comparison with synthetic antioxidants, the total antioxidant capacity of BHT was higher than all extracts, while all extracts had stronger antioxidant activity than BHA. Thus, BHA can be replaced with *F. vulgaris* extracts.

A significantly negative linear correlation was observed between the polyphenol contents and EC_{50} values of extracts in DPPH ($y = -1.4651x + 203.28$ $R^2 = 0.80$), reducing power ($y = -4.6585x + 529$ $R^2 = 0.87$) and total antioxidant capacity (y

$= -3.143x + 488.33$ $R^2 = 0.86$). This negative linear correlation showed that increasing the TPC strengthens the antioxidant activity. These results suggested that 80 % of scavenging activity, 87 % of reducing power and 86 % of the antioxidant capacity of *F. vulgaris* extracts were likely related to phenolics. The antioxidant activity for extracts produced by MAE were higher compared to those produced by maceration in all assays, which can be attributed to their higher TPC. Also, it was demonstrated that plant extracts' antioxidant activity are not only specific to phenolics. Other antioxidant secondary metabolites, such as volatile oils, carotenoids and vitamins, may contribute as much as 20 % of scavenging activity, 13 % of reducing power and 14 % of total antioxidant activity. Moreover, the response of phenolic compounds differed depending on the number of phenolic groups, and the position and degree of hydroxylation of phenolic compounds, especially in the B-ring method used for determination of antioxidant activity¹⁹.

Conclusion

The results showed that of all extracts obtained from different extraction methods, ethanolic extracts had the highest phenolic content and antioxidant activity for DPPH assay, reducing power and total antioxidant activity. Extracts from MAE exhibited lower EC_{50} than those from maceration method in all assays. This result can be attributed to the higher phenolic content in MAE extracts. Preleaching time and particle size had also significant effects on the concentration of phenolic compounds. Moreover, reducing power and antioxidant capacity of ethanolic extract obtained from MAE were found to be stronger than those of BHA. With the results obtained in this study, it can be concluded that optimal conditions for extraction of phenolic compounds from *Falcaria vulgaris* are 50 % ethanol, mesh size 40, 60 min preleaching and MAE. This extract can be recommended as a potential source of natural antioxidants for food-preservation systems.

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