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MICROWAVE-ASSISTED EXTRACTION OF PHENOLIC COMPOUNDS FROM OLIVE LEAVES; A COMPARISON WITH MACERATION

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ABSTRACT

The purpose of this study was to extract phenolic compounds from olive leaves of Koroneiki, Roghani and Mission varieties by maceration and Microwave-assisted extraction (MAE) methods with different solvents. Our results revealed that the extraction method, solvent and variety had a significant effect on the amount of phenolic compounds ($p<0.05$). In both extraction methods, Koroneiki and Mission varieties had the most and least phenolic contents, respectively. In terms of solvents applied, ethanol was the most effective one, producing the highest extraction yield and acetone had the lowest yield. Generally, the highest phenolic concentration was achieved at 24 h (69.027 mg TAE/ g) in maceration method and at the 15 min (88.298 mg TAE/ g) of exposure during MAE by ethanol extract of Koroneiki variety. Furthermore, we found that MAE had a higher extraction yield compared with the maceration method.

Key words, olive leaf, phenolic, extraction, microwave, maceration.

INTRODUCTION

Lipid peroxidation is one of the major agents of deterioration for vegetable oils, fats and other food systems (Iqbal and Bhanger, 2007). In order to inhibit oxidation, synthetic antioxidants, such as BHA (Butylated hydroxyanisole), BHT (Butylated hydroxyanisole) and TBHQ Ter-butyl hydroquinone have been added to foods but there is concern about the use of these compounds due to their reported adverse effects on health. This has led to an increasing trend in the search and replace of these synthetic antioxidants with natural ones such as phenolic compounds (Mariod *et al*., 2006). For using of phenolic compounds, they must be extracted from raw plant materials (Zhang *et al*., 2008). In recent years has observed an increasing attention for new extraction techniques such as Microwave-assisted extraction enabling accelerating and shortening extraction times, efficient extraction, automation, and reduction of organic solvent consumption (Spingo and Faveri., 2009). The accurate choice of solvent is very critical for achieving an optimal extraction in MAE (Microwave assisted extraction). Solvent choice for MAE depends on solubility of the target analyte, the interaction between solvent and plant matrix, microwave absorbing properties of solvent including dielectric constant (the ability of absorption of microwave energy), dielectric loss (the efficiency of converting microwave energy into heat) and dissipation factor (Jain *et al*., 2009).

Efficiency of microwave for extraction has been previously studied. Extraction of polyphenols from fresh tea shoot by Quan *et al* (2006) showed that yield of MAE for 6 minutes radiation was higher than that of ultrasound-assisted extraction in 60 minutes, conventional heating reflex extraction in 60 minutes and extraction at room temperature in 24 hours. Similar results obtained about extraction of polyphenols and caffeine from green tea leaves (Pan *et al*., 2003). Additionally, MAE was the best technique to extract melilotic acid from *Melilotus officinalis* than Soxhlet apparatus and ultrasound-assisted extraction (Martino *et al*., 2006).

Olea europaea L. is an evergreen tree that belongs to the *Oleaceae* family, commonly known as olive tree Olive (Yaseen Khan *et al*., 2007). Olive leaves have the highest content of these compounds among the different parts of the olive tree (Lujan *et al*., 2006). In addition, olive leaf extracts were reported to have antimicrobial (Pereira *et al*., 2007), anti-HIV (Baoa *et al*., 2007; Huang *et al*., 2003), antioxidant (Farag *et al*., 2007) and hypoglycemic activity (Konaki *et al*., 2003). Since, olive leaves are evergreen and easily available in all seasons; the purpose of our study was to investigate the effects of different solvents and variety on total phenols of olive leaves obtained by two extraction procedures, MAE and maceration.

MATERIALS AND METHODS

Materials: Olive leaves of Koroneiki, Roghani and Mission varieties were collected from the Kordkoy (Golestan province, North of Iran), all the chemicals and reagents used were of analytical reagent grade and were purchased from Applichem or Merck Company.

EXTRACTION

Maceration extraction: The olive leaves were shade dried for one week and powdered (60mesh). 1 g of dried

leaves was blended with 50 ml of different solvents (50% ethanol, 80% methanol, acetone, water) for different period (3, 6, 9, 12, 15, 18, 21, 24h) with agitation at temperature room (250 rpm, IKA, USA).

MAE: A house hold microwave oven (Samsung CF3110N-5, Korea) was modified in our laboratory with addition of a magnetic stirrer, water condenser, temperature measurement and time controlling as shown in Fig 1 (Gharekhani *et al*., 2009). Olive leaf powder mixed with an appropriate each of solvents (50% ethanol, 80% methanol, acetone, water) and were irradiated under microwave in pre-setting procedures (8 s power on, 15 s power off for methanol., 15 s power on, 15 s power off for water., 6s power on, 15 s power off for acetone) three times to the desired temperature and then 3s power on for heating and 15 s power off for cooling, but not allowed to super-boil. Irradiation was performed for different times (2, 4, 6, 8, 10, 12, 15 min).

Estimation of total phenolics: The extracts obtained from both methods were filtered over a Whatman No. 1 paper. Methanol, ethanol and acetone filtrates were concentrated under vaccum below 45°C in a rotary evaporator (Heidolph vv1, Germany) but the water extract was freeze-dried. Total phenolic content of each extract was analyzed by the Folin–Ciocalteu micro-method (Arabshahi and Urooj., 2007). Briefly, 20 µl of extract solution were mixed with 1.16 ml distilled water and 100 µl of Folin–Ciocalteu reagent, followed by addition of 300 µl of Na_2CO_3 solution (20%) after 1 min and before 8 min. Subsequently, the mixture was incubated in a shaking incubator at 40°C for 30 min and its absorbance was measured at 760 nm. Tannic acid was used as a standard for calibration curve. The phenolic content was expressed as tannic acid equivalents per g of powder (dry weight) using the following linear equation based on the calibration curve,

$Y=0.00114X+0.01062$ $R^2=0.9964$ Eq. 1

Y is the absorbance (µg/ml) and X is concentration as tannic acid equivalents (Rafiee *et al*., 2012).

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.

RESULTS AND DISCUSSION

Maceration extraction of phenolic compounds: The total concentration of phenolic compounds extracted from the three Koroneiki, Roghani and Mission varieties with solvents including water, 80% methanol, 50% ethanol and acetone were compared in a 24 hour period in order to optimize the extraction conditions. The ANOVA results and comparison of the mean values of total phenolic concentrations revealed that the type of variety, solvent, extraction time and interaction of these factors had a statistically significant effect ($P<0.05$) on the yield of extraction in maceration method.

Effect of variety: Concentration of phenolic compounds of different olive leaf extracts in maceration method is summarized in Tables 1, 2 and 3.

As seen in Table 1, the highest phenolic content in the Koroneiki variety with ethanol, methanol and acetone was achieved after 24 h, whereas water gave this maximum value after 18 hours (42.229 mg TAE/g).

Due to finding no significant difference between the concentrations of the Koroneiki ethanol extract achieved at 3, 18, 21 and 24 h and in order to reduce the extraction time, the optimal extraction time was determined to be at the 3 h (68.428 mg TAE/g), for these reasons, this optimal condition is recommended to be considered at the 9 h (40.988 mg TAE/g) and 18 h (17.352 mg TAE/g) in case of using water and acetone, respectively (Table 1).

For Roghani variety, 15 h with ethanol (66.259 mg TAE/g) were deemed to be the optimal extraction time. For water in Roghani variety, the highest phenolic content was observed at 3 h (42.368 mg TAE/g). Additionally, the phenolic extract of Roghani was optimally achieved with acetone after 21 hours (22.922 mg TAE/g) and with methanol at 15 h (60.104 mg TAE/g). Hence, in case of using methanol, water and acetone this would be recommended to be chosen at 15, 3 and 21 hr, respectively (Table 2).

As seen in the Table 3, the most phenolic content of Mission was optimally obtained from ethanol after 3 h (56.326 mg TAE/g). With methanol, water and acetone as extracting solvents, the highest extraction yield was detected at 24 h (55.973 mg TAE/g), 6 h (31.753 mg TAE/g) and 24 h (19.055 mg TAE/g), respectively. Hence, as for Mission variety, 3 h extraction with ethanol and 24 h with methanol were deemed to be the optimal extraction times. In case of using water and acetone, this would be recommended to be chosen at 3 h and 24 h, respectively.

As shown in Tables 1, 2 and 3, total phenolic contents of varieties were totally different. Genotype and growth conditions (soil, water availability, light quality and temperature) by effecting on quantity and/or types of phenolics can contribute to these differences between varieties. The data obtained from some researches were similar to our results (Faller and Fialho., 2009; Deepa *et al*., 2009).

Effect of solvent type: In case of applying water for extracting the phenolic compounds of olive leaves, the highest concentration was detected at 3 h with Roghani (42.368 mg TAE/g) which was found no significantly different with that of Koroneiki at 9, 18, 21 h. Thus, in order to achieve a rich extract of phenolic compounds,

the 3 and 9 h extraction times will bear the most efficient yield in Roghani and Koroneiki varieties, respectively (Tables 1, 2, and 3). Methanol was the most efficient solvent in extracting the highest phenolics of the Koroneiki variety within 24 hours (67.43 mg TAE/g) compared with the other two varieties.

Table 1. Total phenolic contents of Koroneiki variety extracts in maceration method.

Solvent[a], [b] Time (h)	water	Methanol 80%	Ethanol 50%	acetone
3	38.585 ±0.22 ^{h}	58.119 ± 0.61 ^{e}	68.428 ± 0.44 ^{ab}	13.613 ± 0.62 ^{n}
6	37.383 ± 0.4 ^{hi}	56.088 ± 0.65 ^{f}	65.428 ± 0.39 ^{c}	14.489 ± 0.44 ^{mn}
9	40.988 ± 0.5 ^{g}	58.305 ± 0.61 ^{e}	66.865 ± 0.05 ^{b}	14.3 ± 0.14 ^{mn}
12	36.45 ± 0.62 ^{i}	60.61 ± 0.8 ^{d}	67.246 ± 0.4 ^{b}	16.13 ± 0.4 ^{kl}
15	37.943 ± 0.31 ^{hi}	61.668 ± 0.25 ^{d}	64.264 ± 0.14 ^{c}	15.465 ± 0.1 ^{lm}
18	42.229 ± 0.5 ^{g}	58.107 ± 0.47 ^{e}	67.666 ± 0.54 ^{ab}	17.352 ± 0.77 ^{jk}
21	41.336 ± 0.61 ^{g}	57.709 ± 0.23 ^{e}	68.005 ± 0.43 ^{ab}	16.808 ± 0.65 ^{jkl}
24	37.673 ± 0.71 ^{hi}	67.43 ± 0.48 ^{b}	69.027 ± 0.59 ^{a}	17.762 ± 0.31 ^{j}

[a] Values followed by different letters are significantly different ($p < 0.05$).
[b] Total phenolic content expressed as mg TAE/g power (dw).

Table 2. Total phenolic contents of Roghani variety extracts in maceration method.

Solvent [a], [b] Time (h)	water	Methanol 80%	Ethanol 50%	acetone
3	42.368 ± 0.7 ^{l}	51.882 ± 0.52 ^{j}	64.599 ± 0.45 ^{b}	17.567 ± 0.53 ^{t}
6	41.155 ± 0.32 ^{lm}	55.562 ± 1.19 ^{i}	60.922 ± 0.25 ^{cd}	15.72 ± 0.56 ^{u}
9	38.976 ± 0.11 ^{no}	57.196 ± 0.7 ^{h}	63.779 ± 0.67 ^{b}	18.472 ± 0.33 st
12	39.039 ± 0.47 ^{no}	57.695 ± 0.48 ^{gh}	58.92± 0.05 ^{fg}	19.5 ± 0.31 ^{s}
15	37.882 ± 0.4 ^{o}	60.104 ±0.5 ^{cdef}	66.259 ± 0.74 ^{a}	19.802 ± 0.82 ^{rs}
18	40.883 ± 0.46 ^{lm}	61.511 ± 0.86 ^{c}	59.035 ± 0.7 ^{efg}	21.206 ± 0.12 ^{qr}
21	42.166 ± 0.07 ^{l}	48.969 ± 0.47 ^{k}	58.791 ± 0.5 ^{fgh}	22.922 ± 0.82 ^{p}
24	40.335 ±0.26 ^{mn}	60.685 ± 0.3 ^{cde}	59.25 ± 0.02 ^{defg}	22.31 ± 0.18 ^{pq}

[a] Values followed by different letters are significantly different ($p < 0.05$).
[b] Total phenolic content expressed as mg TAE/g power (dw).

Table 3. Total phenolic contents of Mission variety extracts in maceration method.

Solvent [a], [b] Time(h)	water	Methanol 80%	Ethanol 50%	acetone
3	30.827 ± 0.8 ^{ij}	50.13 ± 0.62 ^{ef}	56.326 ± 0.17 ^{a}	16.076 ± 0.28 ^{o}
6	31.753 ± 0.14 ^{i}	46.48 ± 0.34 ^{g}	51.606 ± 0.59 ^{d}	14.346 ± 0.39 ^{p}
9	30.223 ± 0.48 ^{j}	50.792 ± 0.54 ^{de}	51.485 ± 0.5 ^{d}	15.105 ± 0.25 ^{op}
12	25.773 ± 0.34 ^{l}	45.719 ± 0.56 ^{gh}	54.444 ± 0.41 ^{bc}	18.08 ± 0.46 ^{mn}
15	28.842 ± 0.47 ^{k}	45.829 ± 0.22 ^{gh}	55.381 ± 0.29 ^{ab}	15.54 ± 0.09 ^{op}
18	28.725 ± 0.74 ^{k}	44.72 ± 0.18 ^{h}	53.657 ± 0.62 ^{c}	17.676 ± 0.14 ^{n}
21	30.125 ± 0.5 ^{j}	49.089 ± 0.31 ^{f}	55.12 ± 0.23 ^{ab}	18.78 ± 0.14 ^{mn}
24	30.465 ± 0.21 ^{j}	55.973 ± 0.35 ^{a}	52.086 ± 0.7 ^{d}	19.055 ± 0.22 ^{m}

[a] Values followed by different letters are significantly different ($p < 0.05$).
[b] Total phenolic content expressed as mg TAE/g power (dw).

Ethanol gave the best extraction yield in Koroneiki after 24 h (69.027 mg TAE/g); however, owing to the fact that reducing the extraction time is a crucial parameter in industry and due to detection of no significant difference in phenolic concentration between 3, 18 and 21 h in 24 hours, 3 h (68.428 mg TAE/g) is presented to be the optimal time of extracting phenolic compounds from olive leaves with ethanol. Based on the information given in the Tables, acetone demonstrated the highest yield of extraction at 21 h in Roghani variety (22.922 mg TAE/g) followed by Mission as the second proper one.

Because polyphenols are mostly polar compounds, highly-polar solvents (e.g. water) and non-

polar ones (e.g. chloroform and hexane) are not appropriate for extracting a high phenolic content. Moreover, the use of water as the only solvent yields an extract with a high content of impurities (e.g. organic acids, sugars, soluble proteins) along with phenolic compounds which could interfere in the phenolic identification and quantification. On the other hand, the absolute alcoholic solvents decrease extraction yield. So, application of water combined with other organic solvents makes it a moderately polar medium ensuring the optimal conditions for extraction of polyphenols. Besides, using water in combination with alcohols leads to an increase in swelling of plant materials and the contact surface area between the plant matrix and the solvent which finally, improves the extraction yield (Chirinos *et al*., 2007).

Acetone could not be a suitable solvent in extracting polar compounds like phenols due to its non-polar entity, and based on what mentioned above, it is understood that methanol and ethanol extracts contain higher phenolics than water. Many studies have confirmed that also in other plant species polar solvents produce a higher yield of phenolic concentration compared with the non-polar ones (Trabelsi *et al*., 2008; Franco *et al*., 2008). Furthermore, the higher level of phenolic content in alcoholic extracts than that in water extracts of *Hieracium pilosella* was proved in the study conducted by Stanojević *et al* (2009).

Effect of extraction time: As seen in Tables 1, 2 and 3, there was no certain correlation between increasing of time and yield extraction. Moreover, the optimal extraction time depended on solvent type and variety. For example, as for Roghani variety, the phenolic contents of water extracts decreased from 42.368 at 3 h to 40.335 mg TAE/g at 24 h, while the highest total phenolic compounds obtained after 24 h for ethanol and methanol extracts.

According to Fick's second law of diffusion, the final equilibrium will be achieved between the solute concentrations in the plant matrix and in the bulk solution (solvent) after a certain time meaning that an excessive extraction time is not useful to extract more phenolic compounds and prolonged extraction process might lead to phenolics oxidation due to light or oxygen exposure (Chan *et al*., 2009). Hence, decrease in the phenolic content in certain extraction times might be due to phenolic oxidation under the medium conditions. However, the significant difference between the optimal extraction times in various tested varieties is due to the differences in polymerization degree, phenolic solubility and reaction of phenols with other food particles leading to a change in the time of solvent-solute equilibrium and eventually optimal phenolic concentration (Thoo *et al*., 2010). It is shown that there is not a certain correlation between phenolic concentration values and the extraction process for extracting polyphenols from grape marc (Spingo *et al*., 2007) and isoflavone compounds from soybean flour (Panizzi *et al*., 2002).

Microwave-assisted extraction (MAE): In MAE as well as the maceration method, the type of variety, solvent, extraction time and interaction of these factors were found to have statistically significant effect on the phenolic extraction ($P<0.05$).

Effect of variety: The phenolic concentrations extracted from various types of varieties through MAE were summarized in the Tables 4, 5, 6. As for the Koroneiki variety, ethanol presented the highest yield at 15 min (88.298 mg TAE/g) and for the methanol extracts 12 min was deemed to be the optimal extraction time with the highest phenolic content of 75.347 mg TAE/g. Water and acetone as extracting solvents gave the highest concentration at 15 min, yet regarding the fact that three highest points at 10, 12 and 15 were found with no significant difference ($P<0.05$), the extraction time of 10 min was considered as optimal for these extracts (Table 4).

For Roghani variety, the most phenolic concentration was related to ethanol extract at 15 min (80.01 mg TAE/g) and in the methanol, water and acetone extracts, the highest content with the respective value of 75.249, 53.29 and 24.023 mg TAE/g was also seen after 15 min of irradiation. With water as the extracting solvent, we found no significant difference between the two higher points of 12 and 15 min ($P<0.05$), hence the optimal extraction time for providing the water extract of Roghani variety was 12 min (52.699 mg TAE/g).

The highest phenolic content (68.833 mg TAE/g) was observed in ethanol extract of Mission variety after 15 min. this value for water, methanol and acetone extract was achieved after 15 min, too. But there was no significant difference between 12 and 15 min in acetone and water extracts. Therefore extraction time can be optimized at 12 min for these extracts (Table 6).

As shown in Tables 4, 5 and 6, total phenolic contents of varieties were totally different which can be justified as mentioned above for maceration method.

Effect of solvent type: The water extracts obtained from the Koroneiki variety at 15 min (62.477 mg TAE/g) contained the highest yield of phenolic content; however, it was sufficient to apply a 10 min irradiation in order to provide a phenolic-enriched of this extract due to finding no significant difference between 10, 12 and 15 min ($P<0.05$). This variety was followed by Roghani and Mission giving extraction yields of 52.699 and 42.199 mg TAE/g respectively after 12 min irradiation (Tables 4, 5, 6).

The methanol extract of Koroneiki showed the highest extraction level of phenolic compounds (76.057

mg TAE/g) after a microwave radiation time of 15 min which was found of no statistically-significant difference with that of 12 min and with Roghani variety at 15 min. Thus, in case of using methanol, Koroneiki is recommended to be the preferred one due to its shorter radiation time. The Koroneiki ethanol extract has higher yield of phenolic content (88.298 mg TAE/g) compared with the two other varieties and the irradiation time of 15 min was reported to be the optimal extraction time in the all three varieties.

In MAE as well as maceration method, acetone extracted showed a higher concentration of phenolic compounds in Roghani variety after 15 min (24.023 mg TAE/g). It was found that Mission gave the same phenolic concentration at 12 and 15 min which was also statistically the same as that of Koroneiki at 15 min; therefore in order to reduce the extraction time the 12 min in Mission (20.778 mg TAE/g) was recommended. In selecting the appropriate solvent type in MAE, several parameters like the target analyte solubility, the solvent-plant matrix interaction and dielectric properties of the solvent should be considered. Non-polar solvents remain transparent to microwave due to their lower dielectric constant and dissipation factor in comparison to the polar solvents, thus producing no heat under microwave and are of no efficiency in extraction with MAE (Mandal *et al*., 2007). That is the reason why in case of using acetone as the extracting solvent, the amount of phenolic compoundes was low Besides, in a similar study it was found that less polar extracting solvents like hexane did not yield a high efficient extraction of ginger with MAE (Mandal *et al*., 2007).

Among the polar solvents water undergoes greater microwave absorption and efficiently converts it into heat due to its high dielectric constant. Both ethanol and methanol have lower values than water, yet show higher heating efficiency when applied in a water mixture combination. In comparison to water, ethanol is preferred due to its greater capability in solving the phenolic compounds and higher heating efficiency (Zhang *et al*., 2008). Ethanol shows also the highest yield in microwave assisted extraction of glycyrrhizic acid from licorice root (Pan *et al*., 2000) and polyphenoles from black wheat (Inglet *et al*., 2010).

Effect of time extraction: There was a positive linear correlation between total phenol content and irradiation time. The longer exposures caused higher values of extraction yield, whereas further increase in irradiation time not only resulted in no improvement in the extraction performance, but sometimes led to a fall in the concentration yield. For example, the highest phenolic content (42.852 mg TAE/g) was observed in water extracts of Mission variety after 15 min, but there was no significant difference between 12 and 15 min in these extracts (Table 6).

The prolonged exposures always involve the risk of degradation by heating. Similarly, in the current study we observed a consistent fall in extraction yield after 15 min of exposure and in most cases no specific increase in this value was detected after 10 min. Also, in extracting polyphenols of flaxseed (Beejmohun *et al*., 2007) and green tea leaves (Pan *et al*., 2003) were seen similar results to our study.

Comparison of maceration method and MAE: According to our results, generally, Koroneiki had the highest and Mission contained the lowest level of phenolic compounds in both methods.

In terms of the solvent applied, ethanol was the most effective one, producing the highest extraction yield and acetone gave the lowest yield in extracting polyphenols by these methods. Furthermore, there was a positive linear correlation between extraction time and total phenolic content in MAE but for maceration, increasing of time was not accompanied with high yield extraction. Totally, for MAE and maceration method, the highest phenolic concentration was obtained from ethanol extract of Koroneiki variety after 15 min and 24 h, respectively

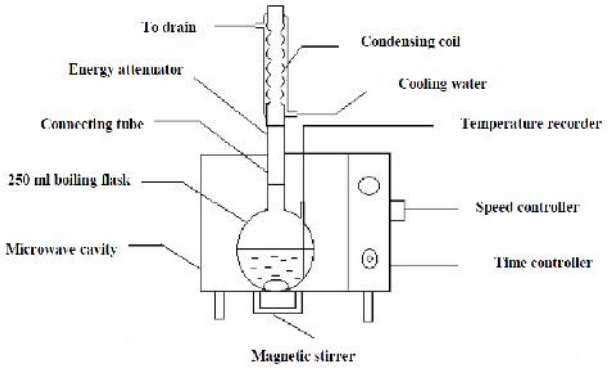

Fig .1. The modifications done on a house hold microwave oven.

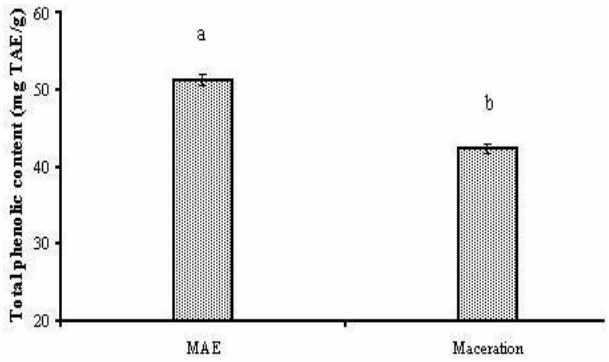

Fig. 2. Comparison of total phenolic contents of extracts obtained from MAE and maceration method. (a) MAE (b) maceration method; Different letters above the bars indicate statistically significant differences (P<0.05).

The ANOVA results showed that the type of method has a significant effect on extracting the phenolic compounds (P<0.05). In comparison of these two methods, MAE had higher extraction efficiency with reduced extraction time leading it to be considered as an appropriate alternative for maceration method (Fig 2). In addition, interaction of microwave with solvent molecules causes an abrupt increase in temperature and internal pressure inside the plant which facilitates the subsequent rupture of cellular wall and the release of active compounds into the solvent (Zhang *et al*., 2008). Its higher efficiency is actually due to the heating produced by microwave which is based on the direct effect of microwaves on molecules by ionic conduction and dipole rotation leading to an increase in solvent temperature and consequently in the solubility of target analytes (Hemwimon *et al*., 2007).

The higher phenolic extraction yield of MAE as compared with the conventional methods has been also proved in extraction of flavonoids from *Radix Astragali* (Xio *et al*., 2008), astragalosides from *Radix Astragali* (Yan *et al*, 2010) and, root polyphenols and antraquinone from *Morinda Citrifoli* (Hemwimon *et al*., 2007).

Table 4. Total phenolic contents of Croniky variety extracts produced by microwave irradiation.

Solvent [a], [b] / Time(min)	water	Methanol 80%	Ethanol 50%	acetone
2	46.862 ± 0.7 [m]	57.964 ±0.6 3 [k]	69.329 ± 0.44 [h]	14.472 ± 0.33 [q]
4	54.082 ± 0.47 [l]	68.405 ± 0.54 [h]	74.566 ± 0.13 [f]	15.842 ± 0.07 [p]
6	54.25 ± 0.61 [l]	69.031 ± 0.07 [h]	76.629 ± 0.22 [d]	18.496 ± 0.28 [o]
8	59.456 ± 0.5 [j]	72.224 ± 0.31 [g]	78.75 ± 0.48 [c]	19.549 ± 0.35 [no]
10	62.111 ± 0.11 [i]	74.255 ± 0.77 [f]	80.022 ± 0.11 [b]	19.86 ± 0.2 [n]
12	62.189 ± 0.07 [i]	75.347 ± 0.76 [ef]	80.801 ± 0.57 [b]	20.362 ± 0.06 [n]
15	62.447 ± 0.5 [i]	76.057 ± 0.42 [de]	88.298 ± 0.25 [a]	20.692 ± 0.26 [n]

[a] Values followed by different letters are significantly different ($p < 0.05$).
[b] Total phenolic content expressed as mg TAE/g power (dw).

Table 5. Total phenolic contents of Roghani variety extracts produced by microwave irradiation.

Solvent [a], [b] / Time(min)	water	Methanol 80%	Ethanol 50%	acetone
2	40.621 ± 0.25 [p]	56.196 ± 0.45 [j]	66.105 ± 0.18 [h]	15.094 ± 0.76 [v]
4	46.769 ± 0.12 [o]	58.106 ± 0.03 [i]	72.388 ± 0.29 [f]	18.594 ± 0.49 [u]
6	50.201 ± 0.08 [n]	65.114 ± 0.47 [h]	73.95 ± 0.56 [de]	19.701± 0.08 [tu]
8	50.799± 0.36 [mn]	70.637 ± 0.61 [g]	75.13 ± 0.74 [cd]	20.495 ± 0.35 [st]
10	51.768 ± 0.15 [lm]	73.14 ± 0.09 [ef]	75.352 ± 0.1 [c]	21.151 ± 0.13 [s]
12	52.699 ± 0.73 [kl]	73.93 ± 0.25 [de]	76.539 ± 0.57 [b]	22.526 ± 0.05 [r]
15	53.29 ± 0.38 [k]	75.249 ± 0.22 [c]	80.061 ± 0.09 [a]	24.023 ± 0.6 [q]

[a] Values followed by different letters are significantly different ($p < 0.05$).
[b] Total phenolic content expressed as mg TAE/g power (dw).

Table 6. Total phenolic contents of Mission variety extracts produced by microwave irradiation.

Solvent [a], [b] Time (min)	water	Methanol 80%	Ethanol 50%	acetone
2	35.575 ± 0.45 [n]	50.145 ± 0.12 [j]	53.067 ± 0.36 [i]	14.554 ± 0.03 [s]
4	37.263 ± 0.15 [m]	52.667 ± 0.34 [i]	55.602 ± 0.7 [h]	16.734 ± 0.28 [r]
6	40.47 ± 0.6 [l]	57.294 ± 0.58 [g]	59.833 ± 0.28 [f]	18.275 ± 0.5 [q]
8	40.626 ± 0.3 [l]	61.673 ± 0.25 [e]	61.334 ± 0.34 [e]	19.36 ± 0.05 [pq]
10	40.658 ± 0.48 [l]	61.329 ± 0.14 [e]	66.322 ± 0.26 [b]	19.596 ± 0.17 [p]
12	42.199 ± 0.24 [k]	63.967 ± 0.34 [d]	66.388 ± 0.34 [b]	20.778 ± 0.22 [o]
15	42.852 ± 0.17 [k]	65.158± 0.34 [c]	68.833 ± 0.91 [a]	21.504 ± 0.23 [o]

[a] Values followed by different letters are significantly different ($p < 0.05$).
[b] Total phenolic content expressed as mg TAE/g power (dw).

Conclusion: Due to the fact that in maceration method, the polar solvents extracted higher phenolic yield from all extracts of olive leaf than non-polar ones, it can be concluded that the most types of phenolic compounds of olive leaf are polar. Regarding a significant increase in the extraction yield for MAE with polar solvents and especially water as a safe and inexpensive one, we can obtain the same phenolic content as that in maceration method with alcoholic solvents. Hence, the microwave-assisted method has many advantages compared with conventional methods like maceration due to its reduced extraction time, higher extraction efficiency, less labor and high extraction selectivity which makes it a favorable method in extraction of phenolic compounds from olive leaves.

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