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RESEARCH ARTICLE

Comparison of Phenolic Contents and Biological Potential of Different Polar Extracts of *Micromeria graeca*, from Algeria

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ARTICLE HISTORY

Received: May 17, 2017
Revised: July 19, 2017
Accepted: July 31, 2017

DOI:
10.2174/1573401313666170807155426

Abstract: ***Background***: *Micromeria* species are rich in bioactive compounds such as phenolics which have several medicinal properties. Different solvents are used for extraction of these substances from natural products and solvent type has a major importance in extraction efficiency. In this context, three solvent systems of methanol, ethanol and acetone and their mixture at 50% were used to extract the phenolics with biological effects from *Micromeria graeca* (L.) Benth. ex Rchb.

Methods: The total phenolic and flavonoid contents were determined by the Folin-Ciocalteu and aluminium chloride methods, respectively. The antioxidant capacity of the extracts was evaluated using three different assays. The antifungal capacity against the two strains *Candida albicans* and *Aspergillus niger* by using the method of diffusion on disc was also carried out.

Results: The 50% acetonic extract gave a better rate of extraction which is 14.4% and presents the highest contents in total phenolics and flavonoids which are of 46.7 ± 2.3 mg gallic acid equivalent /g dry matter and 2.4 ± 0.1 mg quercetin equivalent/g dry matter respectively. A better antioxidant activity for the various tests was observed for 50% methanolic and acetonic extracts. The extract of 50% acetone recorded the most important antifungal activity.

Conclusion: The findings of this study showed that the extract prepared with the 50% acetone from *Micromeria graeca* has the highest phenolics content and revealed the best biological activities. So, this study has allowed us to find the suitable solvent for the preparation of the *M. graeca* extract for several uses.

Keywords: Antifungal activity, *Micromeria graeca* (L.) Benth., phenolic compounds, polar extracts, radical scavenging activity, reducing power, total antioxidant activity.

1. INTRODUCTION

Secondary metabolites of herbal origin have been found to be very potential for the prophylactic against different ailments due to their capacity of activity in numerous biological mechanisms [1]. The natural source, the likelihood to be consumed through the diet, ease of acquirement and reduced adverse effects encouraged increased interest in the use of phytochemicals [2].

A very important medicinal plant family is the Lamiaceae, where it stands for about more than 3000 species of plants dispersed in the warm and temperate region such as the Mediterranean countries. Plants of this family are widely cultivated for perfumery, culinary and ornamental purposes. Besides, they are very rich in medicinal properties,they have a great worth in natural medicine and pharmacopoeia [3]. Generally, several *Micromeria* species are used against heart disorders, headache, wounds and skin infections, antispasmodic, stimulant, and expectorant. Besides, the most usage of *Micromeria* species are in colds as herbal teas [4-6]. *Micromeria graeca* is a widespread herb in Algeria and is frequently used for medicinal purposes and as a condiment. Studies on this species are infrequent and mainly concerned about essential oils [7, 8]. However, there is little data about the medicinal constituents of *M. graeca* such as the nonvolatile phenolic compounds [9].

Different solvent systems have been used for extraction of polyphenols from plant materials. Extraction yield is dependent on the solvent and method of extraction. Water, hydroalcoholic mixtures of ethanol, methanol and acetone are commonly used to extract phenolic compounds from plants [10]. Researchers generally use water [11], ethanol [9], methanol [5] or acetone [12] for the extraction of phenolics from *Micromeria* species. So far, there is no reports dealing

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1573-4013/18 $58.00+.00 © 2018 Bentham Science Publishers

with the comparison of the efficiency of various solvents to extract phenolic compounds.

The aim of the current study was to determine the most suitable solvent system using ethanol, methanol, acetone and their mixtures with water at 50% for extraction of biologically active substances (phenolic compounds) from *M. graeca* (L.) Benth. ex Rchb aerial parts collected in Bejaia (Algeria). Additionally, the antifungel and the antioxidant activities were described; total phenol and flavonoid contents of different extracts were also evaluated.

2. MATERIALS AND METHODS

2.1. Plant Material

Aerial parts of the investigated medicinal plant were collected in August 2009 from Bejaia Algerian locality. Voucher specimen (BR 0000005333899) was authenticated botanically by J. Lejoly (Laboratory of Systematic Botany and Phytosociology, Free University of Brussels (ULB), Belgium) and was deposited in the Herbarium of the National Botanical Garden of Meise (Belgium).

2.2. Chemicals

All chemicals and reagents used in this study are of analytical grade. Methanol, ethanol, acetone and Folin-Ciocalteu phenol reagent were purchased from VWR BDH Prolabo (Madrid, Spain). 1, 1-Diphenyl-2-picryhydrazyl radical ($DPPH^•$), gallic acid and quercetin were obtained from Sigma-Aldrich Chemicals (represented by Algerian Chemical Society, Setif, Algeria). Anhydrous sodium carbonate (Na_2CO_3) and aluminium chloride ($AlCl_3$) were purchased from Biochem, Chemopharma (Montreal, Quebec).

2.3. Extraction Procedure

Dry powder (25 g) of *M. graeca* was extracted by maceration with ethanol, methanol, acetone and their mixture at 50% (500 mL) at room temperature for 24 h. After filtration, the extracts were concentrated under vacuum at 40°C and stored at -4°C until used. The extraction yield was calculated as follows: Yield (%) = $W_1/W_2 \times 100$, where W_1 was the weight of extract after solvent evaporation and W_2 was the weight of the dried powder of plant material.

2.4. Antifungal Activity

The following fungi were used: *Aspergillus niger* (2CA 936) and *Candida albicans* (ATCC 1024). Fungal cultures were grown on PDA at 25°C for 7 days. Suspension of each fungus was prepared in 0.85% normal saline. After that, the turbidity of the fungal suspensions was adjusted at 0.5 McFarland standard. A seeded agar plate was prepared by pouring 20 mL PDA into a sterile plate and uniformly overlaying the solid medium with 5 mL of soft agar, preinoculated with 100 µL fungal suspensions.

The antifungal activity of different *M. graeca* extracts, was investigated using the disc-diffusion technique [13]. Sterile filter discs (diameter 6 mm) were impregnated with 5 µL of samples and placed on the medium. After incubation at 37°C for 24 h, the diameter of the inhibition zone was measured and reported as the mean ± SD of three replicates for each fungel species.

2.5. Antioxidant Activity

2.5.1. $DPPH^•$ Radical Scavenging Activity

The ability of extracts to scavenge the $DPPH^•$ radical was measured using the method of Brand-Williams [14]. Aliquots (100 µL) of samples were added to 2 mL of DPPH solution (1×10^{-4} mol/L) and the absorbance was determined at 517 nm after 30 min. The ability to scavenge the $DPPH^•$ radicals was calculated using the following equation:

$$\text{Scavenging effect} = [(A_0 - A_1)/ A_0] \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

The radical scavenging capacity of the tested extracts was compared to that of quercetin, ascorbic acid, α-tocopherol, gallic acid, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).

2.5.2. Reducing Power Assay

The reducing power of *M. graeca* extracts was determined according to the method of Oyaizu [15]. Various concentrations (1 mL) were mixed with phosphate buffer (2.5 mL, 0.2M, pH 7.0) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$], then the mixture was incubated at 50°C for 30 min. After incubation, 2.5 mL of trichloroacetic acid (10%) were added and the solution was centrifuged for 10 min. Finally, 2.5 mL of the supernatant were mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%) and the absorbance was measured at 700 nm.

The reducing power of quercetin, ascorbic acid, α-tocopherol, BHA and BHT samples were also assayed for a comparative analysis.

2.5.3. Phosphomolybdenum Assay (P-Mo)

The total antioxidant capacity of extracts from *M. graeca* was investigated according to the method of Pan *et al.* [16]. The extract solutions (0.2 mL) at different concentrations were mixed with 2 mL of reagent solution containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The tubes were capped and the mixture was incubated in a water bath at 90°C for 90 min. After cooling at room temperature, the absorbance of each solution was measured at 695 nm against a blank.

The total antioxidant activities of ascorbic acid, α-tocopherol, gallic acid BHA and BHT samples were also assayed for a comparative analysis.

2.6. Determination of Total Phenolics Content

The concentration of total phenolics was measured by the method depicted by Brahmi *et al.* [17]. Briefly, an aliquot (100 µL) of the samples was added to a 10 volumetric flask containing 6 mL of distilled water. 0.5 mL of Folin & Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 1.5 mL of Na_2CO_3 solution (20% w/v) was added with mixing and the solution was diluted to volume with dH_2O. After incubation for 60 min at 25°C, the absor-

bance versus the prepared blank was read at 760 nm. Total phenolics content was expressed as mg gallic acid equivalents (GAE)/1 g of dry matter.

2.7. Determination of Total Flavonoids

The Total Flavonoids Content (TFC) was measured by a colorimetric assay as described by Brahmi *et al*. [17]. Aliquots extracts samples solutions (1.5 mL) and calibration solutions of quercetin (20, 40, 60, 80 and 100 µg/mL) were individually introduced in a 5 volumetric flask and immediately 1.5 mL $AlCl_3$ aqueous solution (2 g/100 mL) was added and thoroughly mixed. Absorbance of the mixture, which was yellow in colour, was determined at 430 nm versus the prepared blank. The TFC was expressed as mg of Quercetin Equivalents (QE)/1 g of dry matter.

2.8. Statistics

Triplicate analyses for each measurement were conducted for each sample. Differences between the means were evaluated with one way ANOVA, using the Statistica 5.5 with least significance difference. The significance level was fixed at 0.05 for all the statistical analysis. Correlation coefficients (R^2), to determine the relationship between antioxidant activity and phenolics content, were calculated by using Microsoft Office Excel application.

3. RESULTS AND DISCUSSION

3.1. Antifungal Activity

The antifungal activity of *M. graeca* extracts was manifested by the presence of inhibition zones and the results obtained showed a sensitivity of the two strains towards all the extracts. This explains the use of *Micromeria* plants for the treatment of many infectious diseases [4]. The activity was increased depending on the concentration used and the extracts prepared with 50% solvents had great potential, where aqueous acetone extract exhibited the more potent effect against *Candida albicans*. It was followed respectively by the aqueous ethanolic and methanolic extracts. However, for the 100% solvents, the most important activity towards *C. albicans* was obtained with the methanol extract, while the acetone extract showed the lowest activity. In the activity against *Aspergillus niger*, the most important zone of inhibition was observed with 50% acetonic extract followed by the 100% one, whereas the methanolic extracts revealed the lowest activity (Table **1**).

The most active extract against the two studied strains was the aqueous acetone one. This efficacy may be related to the solvent ability to solubilize chemical compounds contained in this plant. These results may also be explained by the high content of phenolic compounds quantified in this extract.

The other extracts showed no activity or revealed only a weak effect (MIC >2000 µg/mL). It is generally the non polar extracts of aromatic plants which exert a notable antimicrobial activity. Thus, the antifungel activity of *Micromeria* species polar extracts was rarely reported in the literature. However, many studies have been carried out on the antifungal activity of the essential oils of several species belonging to the *Micromeria* genus with respect to different fungal strains [4, 18-20].

The studies carried out on the antifungal activity of the polar extracts of *Micromeria* species showed their inefficiency. The methanolic extract of *M. fruticosa* ssp *serpyllifolia* was inactive against 15 fungi and a yeast tested by Güllüce *et al*. [21]. Furthermore, according to Ali-Shtayeh *et al*. [11] the water extracts of *M. nervosa* were inactive against *C. albicans*. Recently, El Khoury *et al*. [22] demonstrated that an aqueous extract of *M. graeca* completely inhibited aflatoxin production by *A. flavus* but without reducing fungal growth.

3.2. Antioxidant Activity

M. graeca antioxidant activity was rarely reported in the literature. In their review study Charde *et al*. [23] have mentioned that among the medicinal plants shown to have antioxidant activity, *M. graeca* has the capacity to scavenge the hydroxyl radical [24].

Antioxidant activities of different extracts (acetonic, methanolic, ethanolic and their mixture with water) from *M. graeca* were tested by the $DPPH^•$ radical scavenging, the iron(III) to iron(II) reducing activity and the phosphomolybdenum assays.

In the DPPH assay, the scavenging effect of *M. graeca* was increased by increasing the concentration of various extracts and it was varied significantly between them. The 50% acetonic extract recorded the highest scavenging activity, it was followed by methanol 50%, ethanol 50%, methanol 100%, ethanol 100% and acetone 100% was the least active extract (Fig. **1**). However, the reference compounds used demonstrated marked scavenger activity (Table **2**).

The nature of the solvent used plays an important role in extracting the antioxidants such as phenolics. $DPPH^•$ react with phenols *via* two different mechanisms: direct abstraction of phenol H-atoms and electron transfer processes. The contribution of one or the other pathway depends on the nature of solvent [25].

According to the obtained results, the solvent-water mixtures were efficient for the extraction of compounds that are effective donors of hydrogen atoms or electrons to the $DPPH^•$. Similarly, in the literature, the aqeuous methanolic extract (70%) of *Satureja montana* (116.36 ± 12.83 µg/mL) [26] was potent to scavenge the $DPPH^•$ radical.

These results are in contrary to other reports where a strong $DPPH^•$ scavenging was established for the 100% acetonic extract from *M. cilicica* [12] and for the 100% methanolic extracts of S*atureja cuneifolia* [27] and *M. fruticosa* [21]. Besides, among the species studied by Vladimir-Knežević *et al*. [9], the ethanolic extracts of *M. croatica* (29.48 ± 0.51 µg/mL), *M. graeca* (8.79 ± 0.01 µg/mL), *M. juliana* (17.52 ± 0.33 µg/mL) and *M. thymifolia* (6.53 ± 0.13 µg/mL) showed potent $DPPH^•$ scavenging ability.

This difference is probably related to the procedure of extraction used. Other factors may also affect the scavenging effect such as the harvest season, the age and the origin of the species.

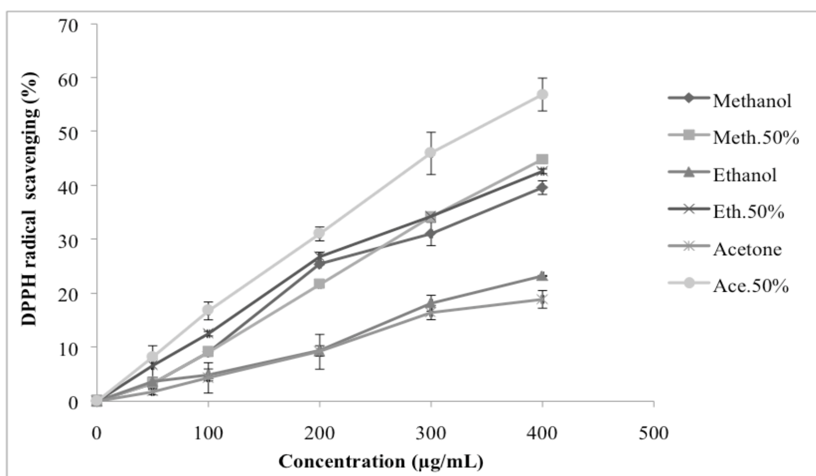

Fig. (1). DPPH$^{\bullet}$ radical scavenging activity of different extracts of *Micromeria graeca* at different concentrations. Each value represents a mean ± SD (n = 3). Meth.50% : methanol at 50 (v/v), Eth.50% : ethanol at 50% (v/v), Ace.50% : acetone at 50% (v/v).

Table 1. Zones of growth inhibition (mm) and the minimal inhibitory concentrations MICs (µg/mL) and minimal bactericide concentrations MBCs (µg/mL) in the antifungal activity for *M. graeca* different extracts against *Candida albicans* and *Aspergilus flavus.*

	***C. albicans* ATCC 1024**	***A. flavus* 2CA 936**	***C. albicans* ATCC 1024**		***A. flavus* 2CA 936**	
Extracts	**Inhibition Zone (mm)[1]**		**MIC (µg/mL)**	**MBC (µg/mL)**	**MIC (µg/mL)**	**MBC (µg/mL)**
Methanol	11±1	8±0	>2000	>2000	>2000	>2000
Meth.50%	12.6±0.6	8±0	1000	>2000	>2000	>2000
Ethanol	10±1	10±1	>2000	>2000	>2000	>2000
Eth.50%	14±0	9±0	1000	>2000	>2000	>2000
Acetone	9±0	11±1	>2000	>2000	>2000	>2000
Ace.50%	16±0	12.3±0.2	500	>2000	1000	>2000
Gallic acid	15±0	12±0	500	1000	1000	>2000

[1]Values of diameter of inhibition zone (mm) including disc diameter (6 mm) are mean of two different experiments ± SD, Meth.50% : methanol at 50 (v/v), Eth.50% : ethanol at 50% (v/v), Ace.50% : acetone at 50% (v/v).

In the reducing power method all the extracts possessed the ability to reduce iron(III) in a linear concentration-dependent manner. The methanolic and acetonic 50% extracts were a significantly ($p < 0.05$) better iron(III) reducer than the other extracts, followed by the ethanolic 50% and 100% solvent extracts.

The IC_{50} value for the methanolic 50% extract was high as compared to the standards IC_{50} (Fig. **2**; Table **2**). The reducing power of *M. graeca* extracts might be due to their electron-donating ability. Accordingly, the aqeuous methanolic extract might contain higher amounts of reductone, which could react with free radicals to stabilise and block radical chain reactions [25].

However, IC_{50} values for reducing power obtained in this study were comparatively elevated as compared to the previous study realized by Vladimir-Knežević *et al*. [25] for three *Micromeria* species (*M. croatica, M. juliana, M. thymifolia*) ethanolic extracts where IC_{50} ranged between 9.64 ± 0.40 and 17.46 ± 1.41 µg/mL.

The phosphomolybdenum test was also used to determinate the total antioxidant capacity of the different extracts. In this assay, the extracts of *M. graeca* extracts showed a dose response.

The methanolic 50% extract exhibited the highest total antioxidant followed by acetonic 50% and ethanolic 50% extracts. The extracts prepared with 100% solvents showed low activity (Fig. **3**; Table **2**). However, superior IC_{50} values were obtained for all the test samples with respect to positive controls.

The total antioxidant activity of the ethanolic extracts of some *Micromeria* species has been determined [9, 25], but the expression of the results is different for a possible comparison.

In all assays, we recognized that the extracts obtained with aqueous solvents have better antioxidant activity, whereas the lowest activity is obtained with pure solvents. This can be explained by the polar nature of the active substances existing in *M. graeca* extracts. Therefore, the differ-

Table 2. Antioxidant activity of different *M. graeca* extracts and standards expressed as IC_{50} (µg/mL).

Sample	DPPH Test	Reducing Power	Total Antioxidant Activity
Methanol	483.2±19.4[g]	501.5±17.7[f]	483±7.1[g]
Meth.50%	440.4±0.4[g]	242.5±1.4[e]	348±7.1[f]
Ethanol	866.5±57.4[i]	559.5±2.2[f]	547±2.8[h]
Eth.50%	458.1±10.7[g]	493.5±3.5[f]	469.5±21.9[g]
Acetone	993.2±38.1[c]	549±17[f]	475.5±7.8[g]
Ace.50%	340.3±3.6[f]	252.3±5.3[e]	372±5.7[f]
α-tocopherol	276.6±1.4[e]	10.3 ± 0.2[c]	16.6 ± 0.2[c]
Ascorbic acid	82.0 ± 2.9[c]	2.8 ± 0.2[a]	9.6 ± 0.3[a]
BHA	161.9 ± 3.8[d]	2.4 ± 0.1[a]	10.3 ± 0.4[b]
BHT	647.2 ± 18.6[h]	5.9 ± 0.1[b]	27.2 ± 0.6[d]
Quercetin	63.6±0.7[b]	28.5±2.5[d]	nd
Gallic acid	37.2±0.5[a]	nd	292±25.5[e]

The results are expressed as means ± SE of triplicate experiments. For each organic solvent, values in the same column bearing different letters are significantly different at $p < 0.05$, Meth.50% : methanol at 50 (v/v), Eth.50% : ethanol at 50% (v/v), Ace.50% : acetone at 50% (v/v), BHT : Butylated hydroxytoluene, BHA: butylated hydroxyanisole, nd : not determined

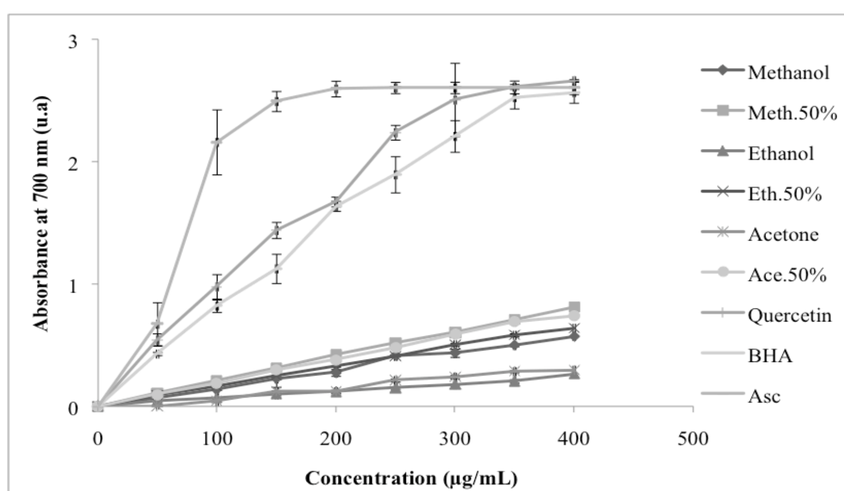

Fig. (2). Reducing power activity of different extracts of *Micromeria graeca* at different concentrations. Each value represents a mean ± SD (n = 3), Meth.50% : methanol at 50 (v/v), Eth.50% : ethanol at 50% (v/v), Ace.50% : acetone at 50% (v/v), BHA: butylated hydroxyanisole, and asc : ascorbic acid.

ence found between different extracts is mainly due to the change in the polarity of the solvents which modifies their ability to dissolve a group of antioxidant compounds and thus influences the estimation of antioxidant activity [10].

Phenolics are one of the most effectual antioxidative components involved in the antioxidant effect of medicinal and aromatic plants. Therefore, it is substantial to determine the phenolic content and to evaluate its involvement to antioxidant capacity.

3.3. Total Phenolics and Flavonoids Contents

The Folin-Ciocalteu method used to determine the Total Phenolics Content (TPC) showed that TPC ranged from 2.6 to 46.7 mg gallic acid equivalent/g dry weight. Whereas, the Total Flavonoids Content (TFC), evaluated by aluminium chloride method, varied between 1.2 and 2.5 mg quercetin equivalent/g dry weight. We noted that TPC and TFC of *M. graeca* extracts are dependent on the solvents and concentrations used (Table **3**). All extracts prepared with 50% solvents contained highest levels of TPC. The lowest amounts of total polyphenols were obtained with 100% ethanol and 100% methanol, respectively. Excluding 100% acetone, our results clearly showed that higher TPC was obtained with an increase in polarity of the solvent used.

The extraction of phenolics is dependent on their solubility in the solvent employed and the increasing of this solubi-

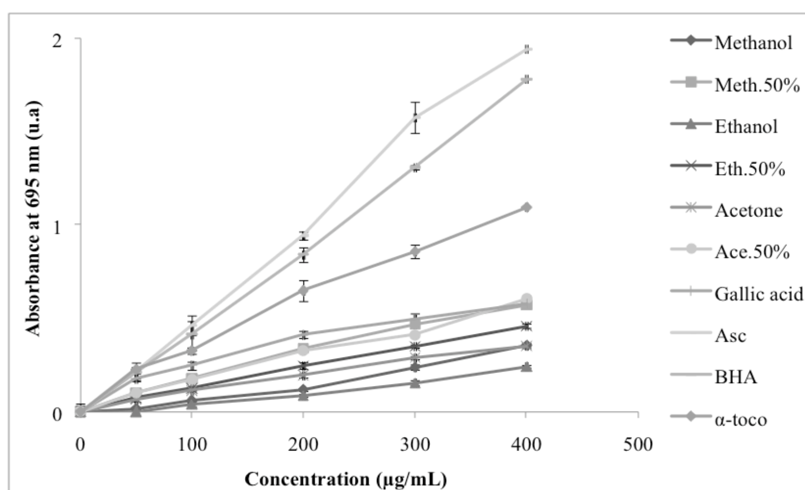

Fig. (3). Total antioxidant activity of different extracts of *Micromeria graeca* at different concentrations. Each value represents a mean ± SD (n = 3), Meth.50% : methanol at 50 (v/v), Eth.50% : ethanol at 50% (v/v), Ace.50% : acetone at 50% (v/v), asc : ascorbic acid, BHA: butylated hydroxyanisole, α-toco : α-tocopherol.

Table 3. Effect of different solvents on polyphenol and flavonoids contents and extraction yield of *M. graeca* extracts.

	Total phenoli content (mg Gallic Acid Equivalent/g dw)[(a)]	Total Flavonoid Content (mg Quercetin Equivalent/g dw)[(a)]	Percentage Extraction Yield (%)
Acetone	45.0 ± 2.6^{a}	1.2 ± 0.1^{c}	2.4
Acetone 50%	46.7 ± 2.3^{a}	2.4 ± 0.1^{a}	14.4
Methanol	4.3 ± 0.6^{d}	1.3 ± 0.2^{c}	8.8
Methanol 50%	14.9 ± 2.4^{c}	1.2 ± 0.1^{c}	13.7
Ethanol	2.6 ± 0.3^{e}	1.8 ± 0.1^{b}	4.8
Ethanol 50%	37.9 ± 1.6^{b}	1.7 ± 0.1^{b}	13.4

(a) expressed as means ± SE of triplicate experiments. For each organic solvent, values in the same column bearing different letters are significantly different at $p < 0.05$, Meth.50% : methanol at 50 (v/v), Eth.50% : ethanol at 50% (v/v), Ace.50% : acetone at 50% (v/v)

Table 4. Correlation between *M. graeca* different extracts antioxidant activities and total phenolic (TPC) and total flavonoid contents (TFC).

Assays	Correlation R^2	
	TPC	TFC
IC_{50} of DPPH radical scavenging ability	0.666	0.006
IC_{50} of reducing power	0.540	0.477
IC_{50} of total antioxidant capacity	0.490	0.586

lity is relied on the solvent polarity [28]. So, Lopez-Cobo *et al*. [26] used different aqueous mixtures with methanol, ethanol and acetone (80, 70, 60 v/v) to determine the optimal extraction solvent of *S. montana*. They found that methanol/water mixtures, gave the best chromatographic profile. This is consistent with the results obtained by Sultana *et al*. [29] in studying the effect of extraction solvent on the phenolic contents of selected medicinal plant extracts, they showed that higher phenolic contents were obtained using aqueous organic solvents, as compared to the respective absolute organic ones. Nevertheless, Oke *et al*. [27], in their study of a plant from *Satureja* genus found that pure methanol, showed a high TPC (222.5 ± 0.5 µg/mg). The differences might be ascribed to the different availability of extractable components, resulting from the varied chemical composition of plants [29]. Besides, in the case of Folin-Ciocalteu method different reagent concentrations, timing of addition and incubation, and different standards used to express the results may be a source of this difference.

Regarding TFC, low contents were found in all the extracts but it was always the aqueous acetone extract which has the highest content, followed by the ethanolic extracts

(100 and 50%). These results are in agreement with those found by Vladimir-Knežević *et al*. [25]. They noted that the concentrations of TPC in three *Micromeria* species from Croatia were significant, varying from 9.69 to 13.06%. Phenolic acids were the most abundant compounds (5.26-6.84%), followed by tannins (3.07-6.08%), but low percentage of flavonoids were found (0.01-0.09%).

The findings of various antioxidant assays in the analyzed sample were correlated with total phenolics content obtained by Folin-Ciocalteu method (Table **4**); although this correlation was not very high, probably due to the nature of a spectrophotometric method analysis used as that is not as specific [26]. These results were not in line to those of Vladimir-Knežević *et al*. [25], who found a very strong correlation between amounts of TPC and antioxidative activities.

IC_{50} values of reducing power and phosphomolybdate showed an average correlation (R^2; 0.477, 0.586) with total flavonoid content. However, TFC revealed a very weak correlation with the DPPH assay and match with the results described by Vladimir-Knežević *et al*. [25]. An explanation to this could be based on the fact that flavonoids were found in low content, hence the radical scavenging effect could not be linked to the presence of flavonoids in *M. graeca* extracts.

CONCLUSION

In this study, the solvents used influence the total polyphenol and flavonoid contents and the antioxidant and antifungal activities of *Micromeria graeca* extracts. The 50% acetone was the most efficient solvent for polyphenol extraction from this species. *Micromeria* species are used in traditional medicine to treat dermatomucosal and skin infections ; they are also used as a food plants which can be a valuable source of natural antioxidants. So, this study has allowed us to find the suitable solvent for the preparation of the *M. graeca* extract in order to improve its efficiency.

Hence, in future studies it is recommended to choose aqueous acetone for extraction and determination of phenolic compounds of *M. greaca*. However, further studies are necessary to determine what compounds are active in the various extracts.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

We would like to thank everyone who helped in the achievement of this study and in particular Pr. J. Lejoly for his confirmation of the identification of plant studied.

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