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REGULAR ARTICLE

ANTIOXIDANT ACTIVITIES AND PHENOLIC PROFILE OF SIX MOROCCAN SELECTED HERBS

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ABSTRACT

The present work evaluated the antioxidant capacity of six plants commonly used in traditional Moroccan medicine. The antioxidant capacity was estimated by DPPH test, ferrous ion chelating activity and ABTS test. As results, the highest antioxidant activities were found in *Mentha suaveolens*, *Salvia officinalis* and *Mentha viridis*. Different species showed significant differences in their total phenolic content (TPC). The highest level of phenolics was found in *Salvia officinalis* and the lowest in *Pelargonium roseum*. Linear correlation was found between TPC, especially the non-flavonoid content (NFC) and the antioxidant activity. Qualitative and quantitative analyzes of major phenolics by reverse-phase high-performance liquid chromatography (RP-HPLC) were also performed. On the basis of the obtained results, these studied medicinal herbs were found to serve as a potential source of natural antioxidants due to their richness in phenolic compounds and marked antioxidant activity.

Keywords: Medicinal herbs, antioxidant activity, phenolic compounds, flavonoid.

INTRODUCTION

The oxidation reactions observed in food products, whether or not catalyzed by enzymes, frequently interfere their nutritional and organoleptic qualities. Processing operations such as peeling, cutting and shredding induce enzymatic browning and enhance the ethylene synthesis, respiration, softening and microbial contamination, consequently decreasing the nutritional quality and safety of foods due to the formation of secondary, potentially toxic compounds (**Zainol *et al*., 2003**).

Recently, there is an increased consumer demand to reduce biological toxicity as well as food deterioration. Several synthetic antioxidants have been used to reduce the biological toxicity or food deterioration as food additives or food supplements. However, they have been shown to exert several deleterious effects in human beings. Therefore, there is a great consumer demand of replacing synthetic antioxidants by using natural oxidizing agents. Medicinal herbs may contain a wide variety of free radical scavenging molecules, such as phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, which are rich in antioxidant activity (**Cai *et al*., 2003**). The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity (**Heim *et al*., 2002**). Phenolic compounds could be a major determinant of antioxidant potentials of foods (**Parr and Bolwell, 2000**), and could therefore be a natural source of antioxidants. Many Lamiaceae extracts are of commercial interest to the food industry as a source of natural antioxidants. The quality of antioxidant activity is highly correlated with phenolic compounds (**Thorsen and Hildebrandt, 2003**).

The action of polyphenols is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (**Itagaki *et al*., 2009**). Polyphenolic compounds like flavonoids have been labelled as ‘‘high level” natural antioxidants based on their abilities to scavenge free radicals and active oxygen species (**Birt *et al*., 2001**).

The objective of this study was to evaluate selected species belonging to three families Lamiacees, Asteraceae and Geraniaceae that are of great importance to the Moroccan food industry, for the presence of phytochemicals important to human health. These species *Mentha suaveolens* (M.S), *Salvia officinalis* (S.O), *Origanum majorana* (O.M), *Mentha viridis* (M.V), *Tanacetum vulgare* (T.V) and *Pelargonium roseum* (P.R) are popular kitchen herbs, which has been used in a variety of food preparations since ancient times (**Durling *et***

***al.*, 2007**). In South Africa a tea is prepared from these herbs to treat coughs, colds, bronchitis and female ailments (**Watt and Breyer-Brandwijk, 1962**). All of the above mentioned species are currently in commercial production in Morocco. However, to date the knowledge about their phytochemical composition and antioxidant capacity is limited.

MATERIAL AND METHODS

Plant material and extraction

Based on traditional use, the species, *Mentha suaveolens, Mentha viridis, Pelargonium roseum, Tanacetum vulgare, Origanum majorana* and *Salvia officinalis* were harvested in April from the Ourika region (Marrakesh, south of Morocco). The plants were identified in the Laboratory of Ecology (Faculty of Science-Semlalia, Cadi Ayyad University) and deposited in the regional herbarium of the same faculty under numbers 6590, 6591, 6595, 6594, 6593 and 6592, respectively. The plant materials were air dried at room temperature in the shade and ground to a powder. The antioxidant activity was evaluated in the phenolic and aqueous extracts of the different species. For aqueous extraction, the dried aerial part of each species was dipping under agitation in distilled water for 8h. The homogenates were centrifuged at 5000 g for 15 min and the supernatant was collected.

The phenolic extracts were obtained by the following procedure: 5 g of each plant powder was extracted by a water-methanol mixture (4:1 v/v) for 15 minutes stirring at 4°C in the dark. After centrifugation, the hydro-alcoholic extract was evaporated under vacuum to obtain a concentrated aqueous extract. The aqueous phase of each extract was depleted by petroleum ether to remove all traces of apolar compounds. The aqueous phase thus obtained was then extracted three times with ethyl acetate (1:1 v/v). The resulting organic phase was dried by sodium sulphate anhydride Na_2SO_4 to eliminate all traces of water. After filtration the solvent was evaporated at 60°C. The residue was taken in 2 ml of methanol and stored at 4°C until tested.

Antioxidant activity

DPPH method: A DPPH assay was employed to investigate the antioxidant activity of different plant extracts (**Lourens *et al.*, 2004**). Briefly, 900 µl of a 0.004% methanol solution of DPPH was added to 100µl of different concentrations of extract (0.05 to 5 mg/ml). After 30

min of incubation in dark, the absorbance was recorded at 517 nm. The inhibition of free radical DPPH in percent (I %) was calculated as:

$$I\% = \left(\frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100$$

The percentage inhibition was plotted against the samples extracts concentrations in order to calculate the EC_{50} values, which is the concentration of extract that causes 50% loss of DPPH activity. The butylhydroxyltoluene (BHT) was used as a positive control.

Ferrous ion chelating activity: The chelation of ferrous ions by studied extracts was estimated by method of **Dinis *et al.*** (**1994**). Briefly, 40 µl of 2 mM $FeCl_2$ was added to 0.1 ml of different concentrations of the extracts. The reaction was initiated by the addition of 0.1 ml of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm. The inhibition percentage of ferrozine–Fe^{2+} complex formation was calculated using the same equation as for the DPPH inhibition. The ascorbic acid was used as positive control.

ABTS assay: The antioxidant activity was evaluated using the $ABTS^+$ method (**Moolla *et al.*, 2007**), with some modifications. Briefly, the pre-formed radical monocation of ABTS was generated by reacting ABTS solution (7 mM) with 2.45 mM $K_2S_2O_8$. The mixture was allowed to stand for 15 h in the dark at room temperature. Stock solutions of the extracts were diluted with dimethyl sulfoxide (DMSO). To a sample volume of 50 µl of each concentration, in a cuvette, 1 ml of the ABTS was added and kept at 30°C for 4 min in a water bath before the absorbance was recorded at 734 nm. Each sample was tested in triplicate. The percentage of decolourisation was calculated as:

$$Decolorisation(\%) = \left(\frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100$$

where A_{sample} is the absorbance of sample and $A_{control}$ is the absorbance of the control (BHT) at 734 nm.

Determination of total phenol content

The total phenolic content (TPC) of the extracts was determined by the method using Folin–Ciocalteu reagent and gallic acid as standard to produce the calibration curve (**Singleton and Rossi, 1965**). 10 µl of extract solution were mixed with 1.745 ml distilled water and 250 µl of Folin–Ciocalteu reagent, followed by addition of 500 µl of Na_2CO_3

solution (20%) after 3 min. The mixture was incubated in a shaking incubator at 40°C for 30 min. The absorbance was then measured at 760 nm and the total phenols were expressed as gallic acid equivalents/ g of dry weight (mg GAE/ g DW).

Determination of total flavonoid content in phenol and aqueous extracts

Total flavonoid content was measured using a modified colorimetric method of **Kim *et al.*** (**2003**). The extract (1 ml) was added to a test tube containing 4 ml of distilled water. Sodium nitrite solution (5%, 0.3 ml) was added to the mixture followed by 10% aluminium chloride solution (0.3 ml). Test tubes were incubated at ambient temperature for 5 min. After, 2 ml of 1M sodium hydroxide were added. The absorbance of the mixture was measured at 510 nm. Catechin was used as the standard. The flavonoid content was expressed as mg catechin equivalent/ g of dry weight (CE/ g DW).

Determination of non-flavonoid content

1 ml of the extract was mixed with 1 ml of diluted HCl (1:3) and 0.5 ml of 8 mg/ ml of formaldehyde solution and incubated 24 h at room temperature in order to precipitate the flavonoid fraction (**Zoecklein *et al.*, 1995**). The non-flavonoid contents were determined in the filtrate using the procedure of **Singleton and Rossi** (**1965**). Results are expressed as mg/g of gallic acid equivalents (GAE).

HPLC analysis of individual phenol compounds

Twenty-microliter samples of each extract were filtered through syringe filters (Sartorius, Germany) prior to HPLC analysis and analyzed using an HPLC apparatus equipped with a pump, photodiode array detector (Waters 2996), a column ALTIMA reversed phase C18 (250 x 4 mm, 5 µm) under a flow rate of 1 ml/ min. The mobile phase was composed of solvent A (acetic acid-water (2: 98, v / v)) and solvent B (acetonitrile). The elution gradient is shown in Table 1. Triplicate analyses have been performed for each sample. The runs were integrated at 280 and 320, 350 nm for benzoic acid, flavonoids and hydroxycinnamic acid derivatives, respectively. Phenolic compounds were identified by comparing retention times and UV–VIS spectra with those of pure standards analysed in the same conditions.

Table 1 Elution gradient for HPLC analysis

Temps (min)	A (%)	B (%)
0-10	0	100
10-20	30	70
20-35	100	0
35-45	0	100

Data processing and statistical analysis

All data on all antioxidant activity tests are mean values of triplicate analyses. Analysis of variance was performed by ANOVA and follow-up test LSD, using SPSS. The level of signification was set at 5%. The main variance in the data set was detected using principal component analysis (PCA). Full cross-validation was used in the validation models. Partial least square regression (PLSR) was used to test the quantitative correlation between total phenol content (TPC), flavonoid content (CF), non-flavonoid content (CNF) and antioxidant properties of all the aqueous and phenolic extracts evaluated by three systems: DPPH, Fe-chelating and ABTS.

RESULTS AND DISCUSSION

Antioxidant activity

Measuring the antioxidant activity of food products such as natural compounds began to present a great interest in recent years. There are several methods to determine the antioxidant capacity of plant extracts. However, the chemical complexity of extracts could lead to scattered results obtained from different techniques, depending on the test employed. Therefore, an approach with multiple assays in the screening work is highly advisable.

The antioxidant activity of our extracts was evaluated by three methods: free radical scavenging activity using DPPH, ABTS method and metal chelating activity. The systems DPPH and ABTS are excellent tools for determining the antioxidant activity of hydrogen donating and chain breaking antioxidants (**Thaipong *et al.*, 2006**). To compare the antioxidant activity between various aqueous and phenolic extracts, we used the 1/ EC_{50} (Fig 1). It's related to the antioxidant capacity of a compound, as it expresses the amount of antioxidant needed to decrease the radical concentration by 50%. All aqueous extracts showed moderate

antioxidant activity compared to phenolic extracts. A total six plant species evaluated by three systems indicated large variation in antioxidant activity.

The results of DPPH radical scavenging activity of phenolic extracts showed (Fig 1a) that the greatest activity was found with *Mentha suaveolens* (1/ EC_{50}= 0.4) followed by *Mentha viridis* (1/ EC_{50}= 0.37), *Salvia officinalis* (1/ EC_{50} = 0.35), *Tanacetum vulgare* (1/ EC_{50}= 0.24), *Origanum majorana* (1/ EC_{50}= 0.12) and *Pelargonium roseum* (1/ EC_{50}= 0.08). Several lines of evidence suggest that the mint is endowed with antioxidant properties (**Triantaphyllou *et al.*, 2001**; **Dorman *et al.*, 2003**). However, the strongest antioxidant activities may be associated with the total phenolic content (**Arumugam *et al.*, 2006**). The antioxidant activity measured by DPPH showed the same relationships as Ferrozine method (Fig 1a, 1b). On the other side, the extracts possessed a low inhibition effect on the ABTS radical cation (Fig 1c) than the two others systems.

The difference in the behaviour of the extracts in the three assays may be explained by the different chemical mechanisms involved in the tests and the different chemical properties of the radicals. The measurement of antioxidant activity by the ABTS system did not show much difference between the species except the phenolic extract of *Salvia officinalis* that has a marked antioxidant activity (1/ EC_{50}= 0.28).

If we make comparison with synthetic inhibitors, BHT (1/ EC_{50}= 0.3), ascorbic acid (1/ EC_{50}= 0.35) and plants studied, the extracts of *Mentha suaveolens*, *Mentha viridis* and *Salvia officinalis* showed an important antioxidant activity which can be related to the phenolic compounds in these species. Both phenolic and aqueous extracts of *Pelargonium roseum* displayed a low antioxidant activity than the others extracts.

These results are in disagree with a study conducted by **Latté and Kolodziej** (**2004**) which established that the phenols isolated from Pelargonium produced higher anti-oxidant activity than synthetic antioxidants and the flavonoid derivatives detected in the tested Pelargonium extracts) may contribute to their observed in vitro anti-oxidant activities (**Lalli, 2006**).

Generally, free radical scavenging and antioxidant activity of phenolics (e.g. flavonoids, phenolic acids) mainly depends on the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the phenolic molecules, and is also affected by other factors, such as glycosylation of aglycones, other H-donating groups (-NH, -SH), etc. For example, flavonol aglycones such as quercetin, myricetin, and kaemperol, containing multiple hydroxyl groups, had higher antioxidant activity than their glycosides such as rutin, myricitrin and astragalin.

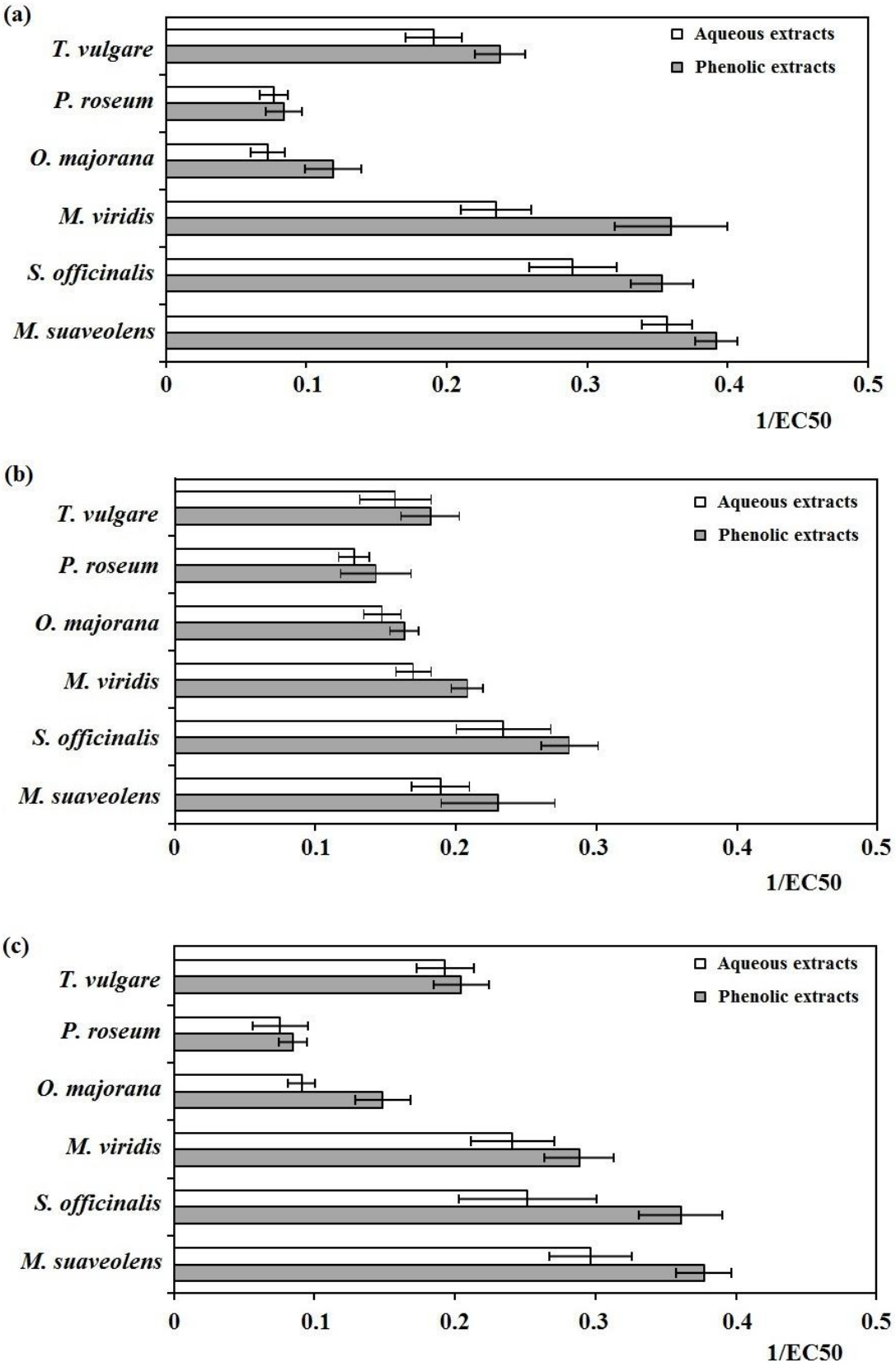

Figure 1 Antioxidant capacity of aqueous and phenolic extracts of the studied species expressed as 1/EC50. (**a**) DPPH radical scavenging activity, (**b**) ABTS activity and (**c**) Ferrous ion chelating activity

Total phenolic content

The total phenolic contents of studied plants extracts are presented in Table 2. The amount of total phenolics, measured by Folin–Ciocalteu method, varied widely according species and extract and ranged from 0.48 ± 0.08 to 4.2 ± 0.6 mg EGA/g DW for phenols extracts and 0.12 ± 0.01 to 1.77± 0.1 mg EGA/g DW for aqueous extracts. The highest level of phenolics was found in phenolic extract of *Salvia officinalis*. It's scientifically known to be rich in phenolics (**Lu and Foo, 2002**). The total phenolic contents of others species decreased in the order of *Salvia officinalis, Mentha suaveolens, Mentha viridis, Tanacetum vulgare, Origanum majorana* and *Pelargonium roseum*.

Table 2 Total Phenolic Content (TPC, mg/ g of Gallic Acid Equivalent (GAE)), Flavonoid Content (FC, mg/g Catechine Equivalent (CE)) and Non-Flavonoid Content (NFC, mg/g of GAE) in phenolic and aqueous extracts of the six analyzed plants

	Phenolic extracts			Aqueous extracts		
	TPC	FC	NFC	TPC	FC	NFC
M.S.	2.67 ± 0.3	0.3 ± 0.08	2.1 ± 0.1	1.54 ± 0.1	0.1 ± 0.02	1.2 ± 0.02
S.O.	4.2 ± 0.6	1.1 ± 0.02	2.8 ± 0.5	1.77 ± 0.1	0.3 ± 0.01	1.35 ± 0.1
M.V.	2.12 ± 0.12	1.4 ± 0.3	0.6 ± 0.02	0.88 ± 0.05	0.2 ± 0.01	0.48 ± 0.03
O.M.	0.8 ± 0.03	0.5 ± 0.04	0.25 ± 0.03	0.38 ± 0.01	0.12 ± 0.04	0.2 ± 0.02
P.R.	0.48 ± 0.08	0.39 ± 0.1	0.11 ± 0.01	0.12 ± 0.01	0.08 ± 0.01	0.03 ± 0.01
T.V.	1.56 ± 0.08	0.71 ± 0.07	0.6 ± 0.02	0.57 ± 0.03	0.2 ± 0.06	0.3 ± 0.02

In this study, we found an important correlation between total phenolic content and antioxidant capacities. Our result is in agreement with others findings. A linear correlation between the content of total phenolic compounds and their antioxidant capacity have been frequently demonstrated (**Djeridane *et al.*, 2006; Katalinic *et al.*, 2006; Katsube *et al.*, 2004**). Also **Heim *et al.* (2002**) reported a good correlation between the antioxidant activity and total phenolic compounds present in the fruits and grains. The significant relationship between the antioxidant activities and total phenolic compounds suggest that phenolic compounds are the major contributors of antioxidant capacities of these species.

Total flavonoid and non- flavonoid content

In the present study, the total flavonoid content was found to be significantly ($p < 0.05$) higher in the phenolic extracts than in the aqueous extracts (Tab 2), with the highest content being found in the phenolic extract of *Mentha viridis* (1.4± 0, 3 mg CE/ g DW) and the lowest in the aqueous extract of *Pelargonium roseum* (0.08± 0.01 mg CE/ g DW). Flavonoid concentration in phenolic extracts of *Pelargonium roseum*, *Mentha viridis*, *Tanacetum vulgare* and *Origanum majorana* was higher than non flavonoid fraction in these species. In all aqueous extracts, except that of *Pelargonium roseum*, the content of non-flavonoid was greater than flavonoid content.

No clear correlation was found between the total flavonoid content and antioxidant activity. The highest antioxidant activity was found in phenolic extract of *Mentha suaveolens* while its flavonoids concentration was low. Our results are in disagreement with those of **Geetha *et al*.** (**2005**) who reported that flavonoids have been shown to be responsible for the antioxidant activity of many of the plants. On the other hand, we found in most aqueous extracts a high correlation between antioxidant capacity and non-flavonoids content.

These results imply that the antioxidant activity can be due to others non-flavonoid compounds such as phenolic acids. So, *Mentha suaveolens* and *Mentha viridis* which have a high antioxidant activities are rich in rosmarinic acid known for its strong antioxidant activity while it's absent in others species (**Shekarchi *et al*. 2012**).

Principal component analysis study

Principal component analysis (PCA) was conducted to understand the relationships between six parameters, namely, DPPH free radical scavenging ability, ferrous ion chelating activity, ABTS, TPC, CF and CNF. DPPH free radical scavenging ability, chelating, and ABTS were shown to be highly and similarly loaded on PC1 which indicated the three properties are closely related to antioxidant activity (Fig 3). TPC and CNF were also loaded on PC1, which suggests that phenolic compounds and especially CNF contained in studied species are good antioxidants. While CF loaded heavily on the second component, which illustrates well that no clear correlation exists between FC and antioxidant activity evaluated by three systems. Accordingly, three species (*Mentha suaveolens*, *Mentha viridis* and *Salvia officinalis*) with the highest antioxidant activity were located to the right along PC1 (Fig 3). On the other hand, *Pelargonium roseum*, *Origanum majorana* and *Tanacetum vulgare* with

rather low levels of TPC as well as weak antioxidant activity were situated on the opposite side of PC1. In the other hand, the phenol extract of *Tanacetum vulgare* was located some distance away from all of the other samples. It's appeared in the negative part of PC2. This proves that its composition in flavonoids is high.

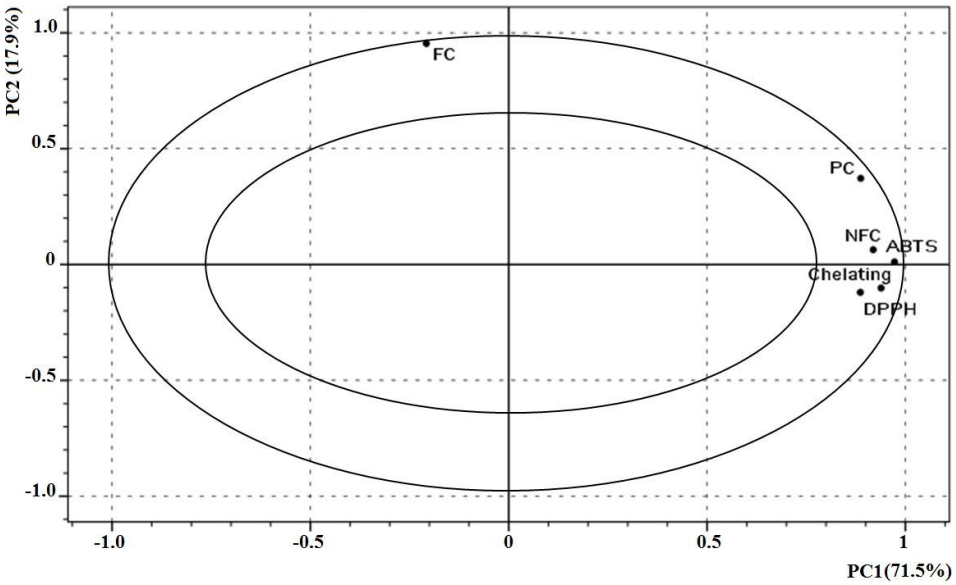

Figure 2 Principal component analysis of PC, FC, NFC, DPPH, ABTS and chelating activity

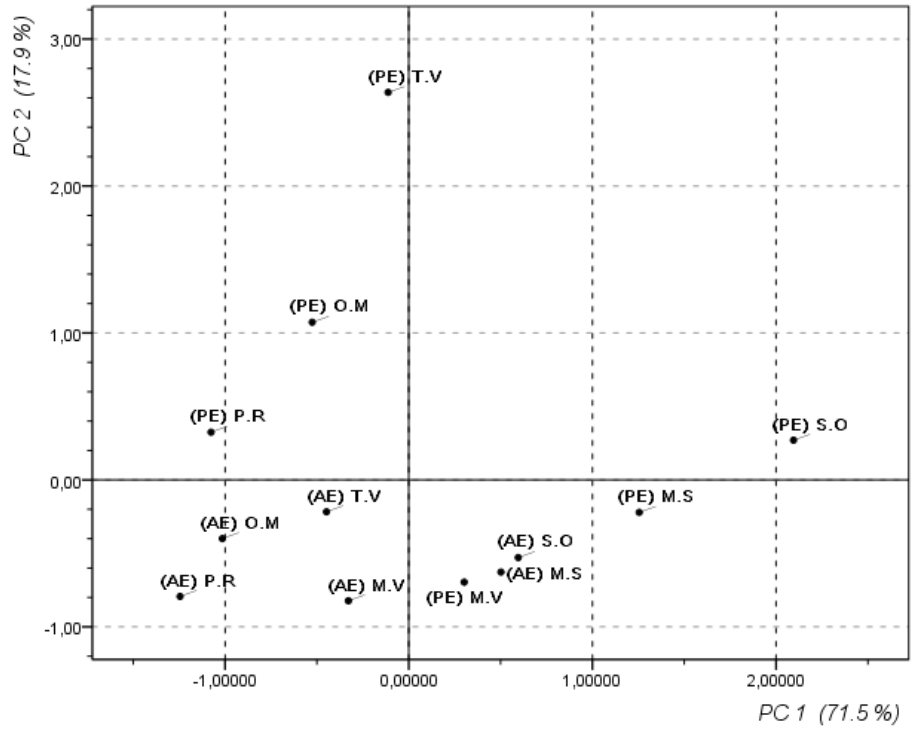

Figure 3 PCA score plot of phenolic and aqueous extracts of the analysed species (T.V, *Tanacetum vulgare*; O.M, *Origanum majorana*; P.R, *Pelargonium roseum*; M.V, *Mentha viridis*; M.S, *Mentha suaveolens*; S.O, *Salvia officinalis*).

HPLC analysis

In this study, major types and their representative constituents of phenolic compounds in the tested herbs were identified by HPLC analysis with different standard samples and by comparison with literature data. Because of the diversity and complexity of the natural mixtures of phenolic compounds in our herb extracts, it is rather difficult to characterize every compound, but it is not difficult to identify major groups and important types of phenolic compounds.

Table 3 shows the content of the individual phenolic compound determined in phenol and aqueous extracts of the species analyzed and expressed as (µg equivalent gallic acid /g dw). With regard to phenolic acids, rosmarinic acid was the major constituent detected by HPLC. It's present in phenolic and aqueous extracts of *Mentha suaveolens* (1751± 0.9; 336.83± 1.8 µg GAE/g DW) and *Mentha viridis* (506.5± 4.6; 282.5± 0.88µg GAE/g DW), respectively.

The high antioxidant activity of these two species can be due to rosmarinic acid. This phenolic acid has a very high antioxidant activity. Several studies have shown that the antioxidant properties of the genre *Mentha* are due to the presence of rosmarinic acid (**Zheng *et al.*, 2001**). *Salvia officinalis* has also a significant antioxidant activity, major compounds found in this species were p- coumaric acid (1133 ± 8.4µg GAE/g DW) and luteolin 7-glucoside (650 ± 0.45 µg GAE/g DW). The good amounts of luteolin, kaempferol, vanillic acid, caffeic acid and carnosic acid were also detected in the phenolic and aqueous extracts of *Salvia officinalis*. Caffeic and carnosic acids are common in many plants and are strong radical scavengers (**Cuvelier *et al.*, 1996**). The antioxidant activity of these species could be due to their composition of phenolics and synergistic effect of compounds. Generally, Lamiaceae species are rich sources of phenolic compounds (**Ozgen *et al.*, 2006**).

Therefore, it is likely that the phenolic constituents present in the *Mentha* species and *Salvia officinalis* are responsible for the antioxidant and free radical scavenging activities. The major phenolic component in *Tanacetum vulgare* was chlorogenic acid (383.6 ± 0.6µg GAE/g DW) in phenolic exract and (259.8± 5.6µg GAE/g DW) in aqueous extract. It's also present moderately in *Mentha suaveolens* and *Mentha viridis*. Chlorogenic acid could be the main contributor to the high antioxidant activity of the extracts. The commonest individual chlorogenic acid has a chemical structure which combines caffeic acid and quinic acid. It has been shown that both chlorogenic acid and caffeic acid are strong antioxidants *in vitro* (**Rice-Evans *et al.*, 1996**).

Table 3 Quantitative analysis of major phenolic compounds identified in aqueous (AE) and phenolic extracts (PE) of different species (µg equivalent gallic acid/ g DW)

	M. suaveolens		*M. viridis*		*P. roseum*	
Compounds	PE	AE	PE	AE	PE	AE
1 Gallic acid	53.2 ± 0.9	n.d.	37.4 ± 0.8	17 ± 0.6	11.4 ± 0.3	n.d.
2 Chlorogenic acid	31.4 ± 0.5	42.4 ± 0.1	58.3 ± 0.9	30.9 ± 0.1	n.d.	n.d.
3 Eriocitrin	283 ± 5.6	50.5 ± 0.4	900.9 ± 4.4	45.4 ± 0.6	n.d.	n.d.
4 *p*-coumaric acid	274.1 ± 5.2	131 ± 1.6	n.d.	n.d.	n.d.	n.d.
5 Luteolin 7- glucoside	n.d.	n.d.	63 ± 1.8	n.d.	n.d.	n.d.
6 Vanillic acid	48.3 ± 0.6	16.38 ± 0.4	172.3 ± 3.4	131.3 ± 1.7	41.6 ± 0.4	17.4 ± 0.6
7 Ferulic acid	130.6 ± 0.4	98.9 ± 1.4	31.4 ± 0.9	n.d	n.d.	n.d.
8 Rosmarinic acid	1751 ± 2.9	336.83 ± 1.8	506.5 ± 4.6	282.5 ± 1.9	n.d.	n.d.
9 Luteolin	41.9 ± 0.6	n.d.	60.1 ± 0.7	84.5 ± 1.3	n.d.	n.d.
10 Caffeic acid	n.d.	n.d.	n.d.	n.d.	42.1 ± 0.6	3.86 ± 0.65
11 Rutin	n.d.	n.d.	n.d.	n.d.	249.4 ± 4.5	30 ± 0.5
12 Syringic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
13 Quercetin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
14 Apigenin	n.d.	n.d.	n.d.	n.d.	48.6 ± 0.8	n.d.
15 Catechine	n.d.	n.d.	n.d.	n.d.	81.2 ± 0.6	53 ± 0.6
16 Carnosic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
17 Kaempferol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
18 Gentisic acid	n.d.	n.d.	n.d.	n.d.	22.7 ± 0.5	n.d.
19 *p*- hydroxybenzoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table 3 suite

		T. vulgare		*O. majorana*		*S. officinalis*	
Compounds		PE	AE	PE	AE	PE	AE
1	Gallic acid	n.d.	n.d.	135 ± 2.2	62 ± 1.2	n.d.	n.d.
2	Chlorogenic acid	384 ± 2.6	260 ± 5.6	n.d.	n.d.	n.d.	n.d.
3	Eriocitrin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4	*p*-coumaric acid	n.d.	n.d.	n.d.	n.d.	1133± 8.4	820.4± 4.3
5	Luteolin 7- glucoside	149 ± 2.9	115 ± 1.4	49.2 ± 0.1	33 ± 0.33	650± 0.45	38.8± 1.2
6	Vanillic acid	n.d.	n.d.	36.3 ± 0.1	23.3 ± 0.1	115± 0.6	97.6± 1.62
7	Ferulic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
8	Rosmarinic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
9	Luteolin	n.d.	n.d.	52.7 ± 0.6	45 ± 0.22	256.5± 2.8	155.6± 1.8
10	Caffeic acid	291 ± 5.1	191 ± 2.4	47 ± 4.6	108 ± 0.7	85.5± 0.9	38± 0.6
11	Rutin	109 ± 4.6	61.3 ± 0.3	208 ± 3.2	82 ± 5.8	n.d.	n.d.
12	Syringic acid	95 ± 0.3	43.8± 0.8	n.d.	n.d.	n.d.	n.d.
13	Quercetin	110 ± 2.2	53.9 ± 0.6	67 ± 0.8	55± 1.2	n.d.	n.d.
14	Apigenin	92 ± 0.5	66.5 ± 1.2	n.d	n.d.	n.d.	n.d.
15	Catechine	n.d.	n.d.	253 ± 4.2	n.d.	n.d.	n.d.
16	Carnosic acid	n.d.	n.d.	n.d.	n.d.	222.4± 8.1	n.d.
17	Kaempferol	n.d.	n.d.	n.d.	n.d.	200.8± 6.4	132± 1.2
18	Gentisic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
19	*p*- hydroxybenzoic acid	n.d.	n.d.	n.d.	n.d.	58.1± 0.4	n.d.

Legend: n.d.: not detected

Significant concentrations of caffeic acid, rutin and luteolin 7-glucoside are as well present in the phenolic and aqueous extracts of *Tanacetum vulgare*. The antioxidant activity of this species is also due to strong radical scavengers of these compounds. *Pelargonium roseum* has a significant amount of rutin, catechin and gentisic acid, whereas others detected components (gallic acid, vanillic acid and caffeic acid) in minor amounts were included. The caffeic acid with others compounds as gallic acid, vanillic acid, rutin, catechine, quercetin and luteolin were also contained in large amount in *Origanum majorana*.

These results are in accordance with those obtained by **Papageorgiou *et al.*** (**2008**) which reported high amounts of flavonoids and phenolic acids in *Origanum majorana*. He is also identified 17 phenolic compounds in this species including gallic, caffeic, dihydroxyphenolic, chlorogenic, syringic, vanillic, rosmarinic, *trans*-2-dihydroxycinnamic, and cinnamic acids as well as rutin, luteolin, coumarin, quercetin, apigenin and amentoflavone.

Although *Origanum majorana* and *Pelargonium roseum* does not have good antioxidant activity measured by three systems comparably to others species. Some authors (**Czapecka *et al.*, 2005**; **Wong *et al.*, 2006**) showed poor linear correlation among total antioxidant activity and phenolic content with no comment. This result is possible owing to the presence of the following factors: the antioxidant activity could possibly due to phenolic compounds contained in our species and also to the presence of some other phytochemicals such as ascorbic acid, tocopherol and pigments as well as the synergistic effects among them, which also contribute to the total antioxidant activity.

CONCLUSION

One of the main findings in this study was that the selected Moroccan medicinal herbs demonstrated good antioxidant activity and contained significantly good amounts of phenolics compounds. A positive and significant correlation existed between antioxidant activity and total phenolics measured by HPLC analysis in some selected herbs, revealing that phenolic compounds such as simple phenols, benzoic acids, hydrolysable tannins, coumarins and secoiridoids were the dominant antioxidant components.

These observations prompt the necessity for further studies of the abovementioned species, focusing on the isolation and structure elucidation of their anti-oxidant compounds, since they have potential use as therapeutic agents in managing diseases associated with free

radicals and also have the potential to be employed as additives in the food and cosmetic industries.

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