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Fractionation, dosage and rating of the antioxidant activity of the aqueous extract of *Melissa Officinalis* from northeastern Morocco

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Abstract

So far, many efforts have been made to discover phytochemical alternatives to chemically synthesized antioxidants. The candidate groups of natural molecules have appeared to be polyphenols and flavonoids. In this study, the amount of phenolic and flavonoid compounds in aqueous extracts of *Melissa Officinalis*, from North-East Morocco (TAZA), was explored by using two common approaches (Folin Ciocalteu and Aluminum Chloride). Their antioxidant capacity was assessed by DPPH free radical scavenging assay. The results obtained demonstrated that the two fraction of ethyl acetate and diethyl ether were rich in polyphenols (505 ± 26 and 378 ± 16 µg/mg respectively) and had moderate quantities of flavonoids (8 ± 0.1 and 10 ± 0.2 µg equivalent of routine per mg of extract). At 2 µg/ml the antioxidant activities of two fractions appeared to be a lower (70 and 71%) than that of ascorbic acid (83%).

Keywords: *Aqueous extract, DPPH method, flavonoids, Melissa Officinalis, phenols.*

1. Introduction

Plant secondary metabolites, including polyphenolics and flavonoids, have recently been paid great attention because of their therapeutic potentials. Most popular metabolites have been alpha-tocopherols, beta- ascorbic acid, and carotene [1]. Natural flavonoid and phenolic compounds have been renown by their free radical scavenging properties. Phenolic compounds and some of their derivatives are very effective in preventing oxidation. Reactive oxygen species (ROS) and oxidative stress have been shown to be linked to some of the life-threatening illnesses, especially to the development of cancer. The identification and quantification of natural products extracted from aromatic and medicinal plants have thus been the subject of several scientific researches [2]. However, due to the often encountered toxicity problems, only a few phenolic compounds are currently permitted to be used as food antioxidants. Although the antioxidant properties have been extensively studied, toxicity of their degradation products has mostly remained to be clarified [3]. Given the relationship between the amount of polyphenols and flavonoids and high antioxidant activity, not to mention the importance of finding new natural antioxidants, this paper is coming to cover the assessment of phenolic and flavonoid constituents in aqueous extracts of *Melissa Officinalis* and their antioxidant capacity were determined. In similar studies antioxidant properties of some other plant extracts have previously been demonstrated [1,4-7].

2. Materials and methods

2.1. Plant material

The aerial parts of wild *Melissa Officinalis* were harvested from the regions of Taza (Morocco). Dried leaves of *Melissa Officinalis* were stored in the laboratory at 298 K until extraction. Taxonomic identity of *Melissa Officinalis* [8]:

Scientific name: *Melissa officinalis*
Common name: *Melisse officinale*
Local Name: Mchéchtrou
Order: Lamiales
Family : Lamiaceae
Genre : Melissa
Reign: plantae
Under the reign: Tracheobionta
Division : Magnoliopsida
Subclass: Asteridae
Flowering: June - September.

Figure 1. *Melissa Officinalis.*

2.2. Aqueous extract fractionation

Polyphenols were obtained by filtration of the aqueous extract in two different solvents, ethyl acetate and diethyl ether, having polarity values 0.58 and 0.38, respectively. One hundred milliliter of the aqueous extract was obtained by distillation and filtration. It was mixed with diethyl ether or ethyl acetate in a separation funnel. The organic portion was poured in an Erlenmeyer. This final step repeated thrice. The organic solvent in the third extraction was evaporated with anhydrous sodium sulfate and the extracts were kept in glass bottles at 4°C.

2.3. Determination of the phenolic content

Various methods can be used to estimate the amount of polyphenols [9]. In the study the Folin-Ciocalteu method was preferred. It relies on the reduction of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) mixture by the extracted phenolic compounds [10]. Ten times dilution of the Folin reagent was prepared in water (1mL Folin reagent, 9 ml water). Onto this solution 200 µl of gallic acid solution was added. After the elapse of 4 min, the reaction samples were supplemented with 800µlofsodiumcarbonatesolution (75 mg/mL). The samples were kept for 45 min at room temperature. Absorbance of the samples and standards were read at 760nm. Sample and standard absorbance readings were plotted into two curves and the amount of phenolic compounds was estimated by comparison. Phenolic content was measured as µg gallic acid in per mg of the extract. The reduction reaction was repeated three times and the corresponding gallic acid readings were presented as average (X) ± SD (standard deviation).

2.4. Determination of flavonoid content

Quantification of flavonoids present in the aqueous extracts was performed by the aluminum trichloride method [11,12]. One milliliter of sample or standard solution (dissolved in methanol) was added to 1 ml of the solution of $AlCl_3$ (2% in methanol). After 30 min, the absorbance read at 415 nm. The concentrations of flavonoid compounds were taken as µg routine equivalent per mg of extract. All experiments were performed in triplicate and routine equivalent values were given as X ± SD of triplicates.

2.5 The assessment of antioxidant potential

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was employed to assess the free radical scavenging potential of the two aqueous extract fractions [13]. The antioxidants react with the violate DPPH and change it to yellow 1,1-diphenyl-2-picrylhydrazine [14]. Samples and standards were prepared in ethanol. The concentrations ranged between 0.2 and 2µg/ml. The final mixtures were stirred vigorously and allowed to stand for 30 min at room temperature in the dark. The absorbance was measured at 517 nm. The radical scavenging activity was taken as percent inhibition (I%) [14]:

$$\mathbf{I\ (\%) = 100^* (A_{control} - A_{sample})/A_{control}}$$

$A_{control}$, absorbance of the control reaction; A_{sample}, absorbance of the sample. The sample concentration, yielding 50% inhibition (IC_{50}), was assessed by comparing the control curve with that of the sample. Tests were carried out in triplicate. Positive control was a solution of ascorbic acid.

3. Results and Discussions

3.1. Total flavonoid and phenolic contents

Folin-Ciocalteux and aluminum trichloride ($AlCl_3$) are the two common colorimetric methods that were used to determine the total phenol and flavonoid contents present in the two fractions of aqueous extract of *Melissa Officinalis*. The flavonoids were quantified by the trichloride aluminum method. The flavonoids and phenolics of each of the extract fractions was given as µg equivalent in per mg of extract. Total phenol content was assessed by the Folin-Ciocalteu approach and for each extract fraction it was defined as µg gallic acid equivalent in per mg of extract. The ethyl acetate fraction had a higher total phenol content (505 ± 26µg/mg) compared with that of the diethyl ether fraction (378 ± 16µg/mg, Table 1). The plant had moderate quantities of flavonoids (8 ± 0.1 and 10 ± 0.2 µg equivalent of routine per mg of extract).

Table 1. Total polyphenols and flavonoids content of *Melissa Officinalis.*

	Polyphenols	Flavonoids
Extract	Gallic acid (µg) / extract (mg)	
Diethyl ether fraction	378± 16	8± 0.1
Ethyl acetate fraction	505± 26	10 ± 0.2

3.2. Antioxidant activity

As could be seen (Table 2), the free radical scavenging performances of the extracts were taken as the measure of their antioxidant capacity. Ascorbic acid was employed as the positive control. It can be clearly seen (Fig. 2) that the efficacy of scavenging activity increased significantly at elevated extract concentrations (Fig. 2).

Table 2. Antioxidant potential of the fractions.

Samples	**Antioxidant activity**						
Diethyl ether fraction	Extract (µg/mL)	0,2	0,35	0,5	1	2	
	Scavenging effect on DPPH (%)	9.25	22.53	28.04	45.02	70	
	DPPH IC_{50} (µg/mL)						***1.11***
Ethyl acetate fraction	Extract (µg/mL)	0,2	0,35	0,5	1	2	
	Scavenging effect on DPPH (%)	16.2	26.11	30.6	50	71	
	DPPH IC_{50} (µg/mL)						***1***
Ascorbic acid solution	(µg/mL)	0,2	0,35	0,5	1	2	
	Scavenging effect on DPPH (%)	20	28	32	55	83	
	DPPH IC_{50} (µg/mL)						***0.9***

On the antioxidant performance the following conclusions could be drawn:

- The two fractions of the aqueous extract of the *Melissa officinalis* exhibited significant antioxidant activities.
- The antioxidant activity rises with the rise of extract concentration.
- The antioxidant activities of the extracts appeared to be a lower (70%) than that of ascorbic acid (83%).
- The ethyl acetate fraction had somewhat lower IC_{50} value (1µg/ml) than that of the diethyl ether fraction (IC_{50} = 1.11µg/mL).

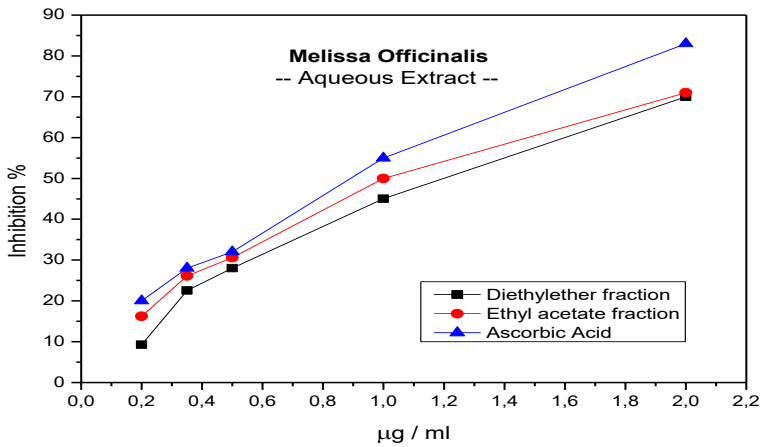

Figure 2. Antioxidant activity of the two aqueous extract fractions.

3.3. Discussion

Medicinal plants, used in traditional/popular medicine in diverse cultures around the world, have also been a good source of natural antioxidants. Currently marketed antioxidants, however, have mostly been produced by chemical means. Beside their beneficial actions, these synthetic antioxidants also harbor many unwanted side effects. Fortunately, however, many governments have recently been encouraging the production of natural antioxidants [1]. Polyphenolics are the most common phytochemical secondary metabolites. These compounds have received considerable attention especially because of their antioxidant activities [15,16]. It is likely that this activity is due to their redox potentials, taking part in the neutralization of free radicals as well as in the decomposition of peroxides [17]. Along the history in some societies the leaves of *Melissa Officinalis* have served as the multipurpose traditional medicinal agent [18-23]. For example, it has been administered as a painkiller, sedative or as an antispasmodic agent. The leaves produce flavonoids (quercitrin, rhamnocitrin, luteolin), polyphenolic compounds (rosmarinic acid, caffeic acid, and protocatechuic acid), monoterpenoid aldehyde, monoterpene glycosides, triterpenes (ursolic and oleanolic acids), sesquiterpenes, tannins, and essential oils [18-24]. It has been shown that some of these compounds exert antiviral activity against Herpes Simplex viruses. The antioxidant potential of the leave extracts has also been found to be comparable to those of the commercialized drugs [18]. In this study it was found that aerial parts of *Melissa Officinalis* were rich in polyphenols and that they contained a meaningful antioxidant capacity (71% at 2µg/ml). This activity could be exerted by its phenolic constituents, having worked synergistically on the substrate. These results also were in good correlation with those of the literature cited above.

4. Conclusion

The results indicated that *Melissa Officinalis* can be exploited as the source for a functional food material with medicinal advantages. Its phenolic constituents could serve an alternative to the synthetic antioxidants available in the market. Both of the aqueous fractions performed a significant natural antioxidant activity. This indicates that the fractions contained some molecules with alternative antioxidant potentials. Although the results hinted that phenolic constituents might have been responsible for the observed antioxidant activity, flavonoids and/or other photochemical constituents might also have played a role in this property. These points can only be clarified by working with pure molecules of the extracts.

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