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Ethnopharmacological Study and Evaluation of the Antioxidant Activity of *Ocimum basilicum* L. Extracts

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ABSTRACT

Ocimum basilicum L. (*O. basilicum* L.) with the vernacular name of Reihan or Reyhan is a medicinal plant from the Lamiaceae family, widely used in traditional Algerian medicine. The aim of the present study was to research the traditional uses and the mode of application of *O. basilicum* L. in the M'sila region (northern Algeria), to estimate the content of polyphenols and flavonoids in methanolic and aqueous extracts of this plant and to evaluate the antioxidant activity of these extracts by the 2, 2'-diphenyl-1-picryl hydrazyl (DPPH). The results of ethnopharmacological survey showed that decoction of leaves in fresh or dry form is the most frequent use. The yields of extracts were 28.67% and 15.24% for the methanolic and aqueous extracts, respectively. The phytochemical screening revealed the presence of different compounds such as polyphenols, flavonoids, tannins and essential oils. The results demonstrated that the methanolic extract presented a high polyphenols and flavonoids contents with 225.99 ± 3.13 mg gallic acid equivalent/g dry extract and 83.63 ± 3.48 mg quercetin equivalent/g dry extract, respectively. The evaluation of the antioxidant power is carried out using the method of trapping of the free radical DPPH, with BHT as reference antioxidant. The results showed that the methanolic extract has a significant free radical scavenging capacity with an IC_{50} value of 304.82 ± 24.15 µg/ml; which was greater than the trapping capacity of BHT (IC_{50} = 327.46 ± 13.11 µg/ml). Whereas, the aqueous extract has a weaker effect with an IC_{50} value of 2122.81 ± 107.77 µg/ml.

Keywords: BHT, DPPH, flavonoids, *Ocimum basilicum* L., polyphenols.

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INTRODUCTION

Plants have always occupied a prominent place in human life. Indeed, for thousands of years, man has used various plants found in his environment in order to treat and cure all kinds of diseases. The World Health Organization estimates that around 80% of the population use traditional herbal preparations as primary health care. The plant kingdom represents an important source of an immense variety of bioactive molecules. Among these compounds, we find the secondary metabolites which have especially been illustrated in therapy.

Phenolic compounds are secondary metabolites widely distributed in the plant kingdom. Phenolic acids, tannins and flavonoids are the major classes of polyphenols. These compounds have different biological and pharmacological activities such as antibacterial, antiviral and anti-inflammatory activities [1, 2]. Polyphenols are also known as lipid peroxidation inhibitors [3, 4] and myorelaxant agents [5, 6]. They are involved in the prevention and treatment of diseases linked to oxidative stress such as cancer [2], atherosclerosis [7], diabetes [8] and cardiovascular diseases [9].

Ocimum basilicum L. (*O. basilicum* L. or basil) is a popular culinary herb belonging to the Lamiaceae family. It grows in several regions all over the world [10]. Basil is well-known as a plant of a folk medicinal value. The leaves of basil are used in folk medicine as a tonic and vermifuge, and basil tea taken hot is good for treating nausea, flatulence, and dysentery [11]. The oil of the plant has been found to be beneficial for the alleviation of mental fatigue, colds, spasm, rhinitis, and as a first aid treatment for wasp stings and snakebites [11]. Therefore, the current study was undertaken to elucidate the traditional uses of *O. basilicum* L., to determine the phenolic content and to evaluate the antioxidant activity of the aqueous and methanolic extracts of basil cultivated in M'sila (northern Algeria).

MATERIALS AND METHODS

Ethno-pharmacological survey

The ethno-pharmacological study is carried out in the region of M'sila (northern Algeria). The purpose of this study was to identify the different traditional medicinal uses of *O. basilicum* L. and to document the traditional medicinal knowledge related to the use of this plant. For that, all herbalists were interviewed using Surveys investigating the herbalist as the holder of information (gender, age and level of education) and the traditional uses of this plant and how it is used.

Plant material

The plant *O. basilicum* L. studied was harvested in the Berhoum region (50 km east of M'sila). The freshly harvested leaves were dried and grinded into powder.

Preparation of extracts

The preparation of extracts was carried out according to method used by Markham [12]. 50 g of fine powder were macerated in 500 ml of 80% methanol (v/v) or distilled water for 24 hours at room temperature with permanent stirring. The aqueous solution was then filtered to give aqueous extract and the methanolic solution was filtered and evaporated at 50°C using vacuum rotary evaporator to obtain crude methanolic extract.

The yield of extraction expressed as percentage was calculated from the following equation:

Yield (%) = (W1/W2)*100). Where,

W1= weight of the extract residue obtained after solvent removal.

W2= weight of the plant powder.

Qualitative study (Phytochemical screening)

The qualitative assay of the different metabolites such as polyphenols, tannins, flavonoids, saponins, anthocyanins, organic acids, essential oils, coumarins and free quinines was based on the staining reactions and precipitation [13, 14]. All tests were carried out on the leaves of *O. basilicum* L.

Quantitative study

Determination of total polyphenols

The content of total polyphenols was determined using the Folin-Ciocalteu colorimetric method as described by Li et al. [15]. An aliquot of 100 µl of extracts or standard gallic acid solution was added to 500 µl of freshly prepared Folin-Ciocalteu reagent (diluted 10 times). 4 minutes later, 400 µl of 7.5% sodium carbonate solution (Na_2CO_3) was added to the mixture. After incubation period of two hours in the dark and at room temperature, the absorbance was measured at 765 nm against a blank using a UV-Visible spectrophotometer (Schimadzu-UV-2401PC, UV-Vis). The results were expressed in milligrams of gallic acid equivalent per gram of dry extract.

Determination of total flavonoids

The flavonoid content of the extracts is determined using the colorimetric method with aluminum trichloride ($AlCl_3$) [16]. One milliliter of the extracts or quercetin was added to one milliliter of 2% $AlCl_3$. The mixture is left to react for 10 minutes and the absorbance was measured at 430 nm using a UV-Visible spectrophotometer (Schimadzu-UV-2401PC, UV-Vis). The concentration of flavonoids in the extracts was expressed in milligrams of quercetin equivalent per gram of dry extract.

Evaluation of antioxidant activity by the DPPH test

The free radical scavenging activity of the two extracts was measured by the DPPH test according to the method described by Sanchez-Moreno et al. [17]. 50 µl of methanolic, aqueous extracts or butylated hydroxytoluene (BHT as a reference antioxidant) was mixed with 1950 µl of DPPH solution (0.002% in methanol). The reaction mixture is stirred vigorously for 10 seconds and left to stand in the dark for 30 minutes before reading the absorbance at 517 nm (against a blank) by a UV-Visible spectrophotometer (Schimadzu-UV-2401PC, UV-Vis).

The inhibition percentage (I%) of free radical DPPH was calculated according to the following equation: $I\% = [1-(Abs_{sample} / Abs_{Control})] \times 100$, where:

Abs_{sample}: Absorbance of the sample.

$Abs_{Control}$: Absorbance of the control.

The results were expressed as IC_{50} (µg/ml), the concentration of extract required to scavenge 50% of free radicals.

Chemical reagents

The chemical reagents used in this study were purchased from Sigma Chemicals (Germany), Fluka and Prolab.

Statistical analysis

All results are expressed as mean ± SEM. The comparison of the content of polyphenols and flavonoids in the two extracts was carried out by the Student test. The IC_{50} values (inhibitory concentration at 50%) were calculated by the method of linear regression. One-way analysis of variance followed by the Tukey test was performed to assess differences between the two extracts and the reference antioxidant (BHT). The values of $P \leq 0.05$ are considered statistically significant. All statistical analyzes were carried out using GraphPad Prism 7® software.

RESULTS AND DISCUSSION

Ethno-pharmacological survey

In the present study, 24 herbalists were interviewed through face-to-face interviews in the M'sila region. In this investigation 33 therapeutic properties of *O. basilicum* L. have been noted. The results showed that this plant is frequently used as digestive (12.39%), stomachic, sedative (7.08%) agents and as a spice (6.19%). While the decoction (30.56%) and infusion (23.61%) are the most common method of preparation as a fresh or dry leaf (15.28%). The leaves and flowers of basil are used in folk medicine as a tonic and vermifuge, and basil tea is good for treating dysentery, nausea and flatulence. The oil of the plant is beneficial for the alleviation of spasm, rhinitis mental fatigue, cold, and as a first aid treatment for wasp stings and snakebites [11]. It has been used as a folk remedy convulsion [18]. The plant is effective in treatment of stomach problems [19].

Extraction

In this study, methanol (80%) and distilled water have been used to extract the active compounds of *O. basilicum* L. leaves. The colors of aqueous and methanolic extracts were brown and green respectively. It has been also shown that pH of the two extracts was neutral (Table. 1). The UV / Visible absorption spectrum of the solutions of the two

extracts at 0.1% (w/v) indicates the same absorption peak at 307 nm (Table. 1).

The calculated yields of extracts from dry weight of methanolic and aqueous extracts are shown in table 1. The yield of methanolic extract (28.67%) was higher than aqueous extract (15.24%). This can be explained by the ability of methanol to extract the maximum of compounds in comparison with water.

Table 1: Some characteristics of *O. basilicum* L. extracts

Extracts	Yield (%)	Color	pH**	λ_{max} (nm)
Methanolic	28.67	Green	7.67	307
Aqueous	15.24	Brown	7.47	307

** 0.1% extract solution (w/v).

Qualitative study (Phytochemical screening)

The phytochemical screening results indicated that *O. basilicum* L. leaves contains polyphenols, flavonoids, tannins, organic acids, saponins, coumarins, free quinones and polyphenolic derivatives (Table 2). On the other hand, the preliminary analysis showed the absence of anthocyanins and starch. This result is in agreement with those of several studies which showed that the aerial part of *O. basilicum* L. is rich in active compounds [20, 21, 22, 23]. In addition, the phytochemical screening of the present study showed that this plant also contains essential oils. Indeed, Özcan and Chalchat [24] identified 41 essential oils of *O. basilicum* L. from Turkey. Other studies have revealed the presence of essential oils in the aerial part of this plant [25, 26].

Table 2: Phytochemical screening of *O. basilicum* L. leaves.

polyphenols	+++
flavonoids	+++
tannins	++
organic acids	+++
starch	-
saponins	+++
coumarins	+
free quinones	+++
polyphenolic derivatives	+++
anthocyanins	-
essential oils	+++

(+) = Present; (++) = Abundant; (+++) = Very abundant; (-) = Absent.

Quantitative study

Polyphenols and flavonoids content

The determination of total polyphenols by the Folin-Ciocalteu method showed that the methanolic extract contains a high content of total polyphenols (225.99 ± 3.13 mg gallic acid equivalent/g dry extract) compared to that of the aqueous extract (41.20 ± 0.40 mg/g dry extract) (Fig. 1). Statistical analysis demonstrated that there is a significant difference between the two extracts ($P \leq 0.05$).

The quantitative determination of total flavonoids by the $AlCl_3$ method using quercetin as standard showed that the absorbance increases linearly with the concentration of this flavonoid. The results of the total flavonoids assay indicated that the methanolic extract contains the greatest amount of flavonoids. It contains 83.63 ± 3.48 mg quercetin equivalent/g dry extract. However the aqueous extract contains 12.96 ± 0.51 mg quercetin equivalent/g dry extract (Fig. 2). The study of Bougandoura and Bendimerad [27] revealed that methanolic and aqueous extracts of *Satureja calamintha* had a moderate flavonoid contents. Examination of the results of the flavonoid assay showed that there is a significant difference between the amounts of flavonoids in the two extracts.

The difference in the amounts of total polyphenols and flavonoids in the two extracts may be due to the nature of the solvent of extraction (water, methanol). This suggests that methanol is the best solvent for the extraction of polyphenols and flavonoids in comparison with water. This suggestion is in agreement with the result obtained by Telli et al. [28] which showed that the best polyphenol extraction solvent is methanol. This may be due to the better solubility of polyphenols in methanol than in water [29]. The difference in the amount of polyphenols and flavonoids of medicinal plants may be due to the environmental factors such as altitude, temperature, humidity, soils, precipitation, illumination [30, 31]. In addition, depending on the extraction and the quantification methods and the plant growth phase, the content of secondary metabolites polyphenols and flavonoids varies [24]. Indeed, the richness of *O. basilicum* L. in polyphenolic compounds confirms its use in traditional medicine.

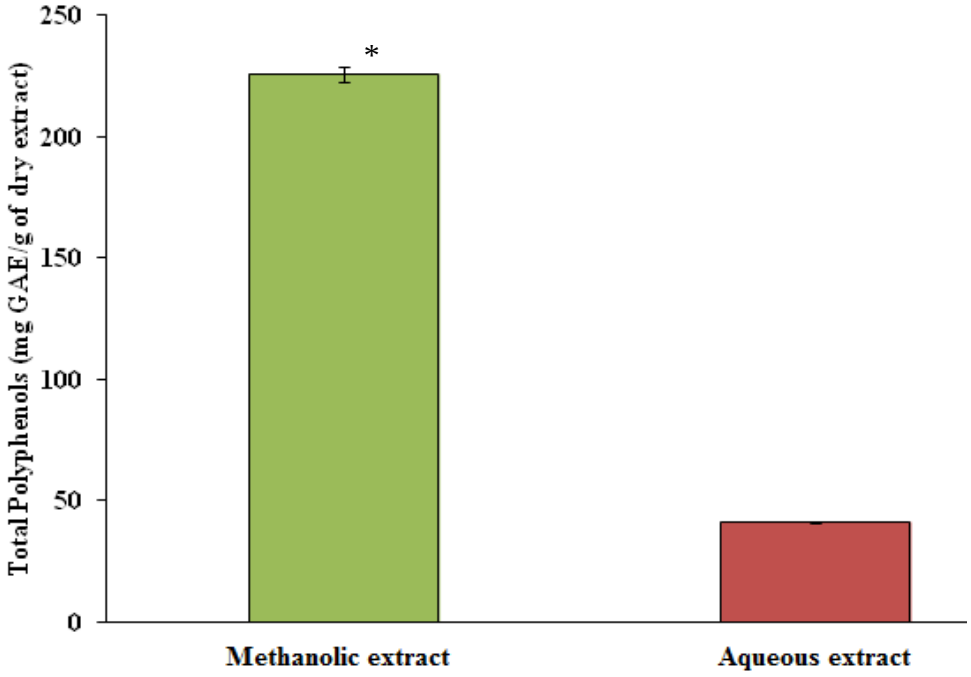

Figure 1: Determination of the level of total polyphenols in *O. basilicum* L. extracts. Values are the mean ± SEM (n=6); *: Comparison between the two extracts, P≤0.05.

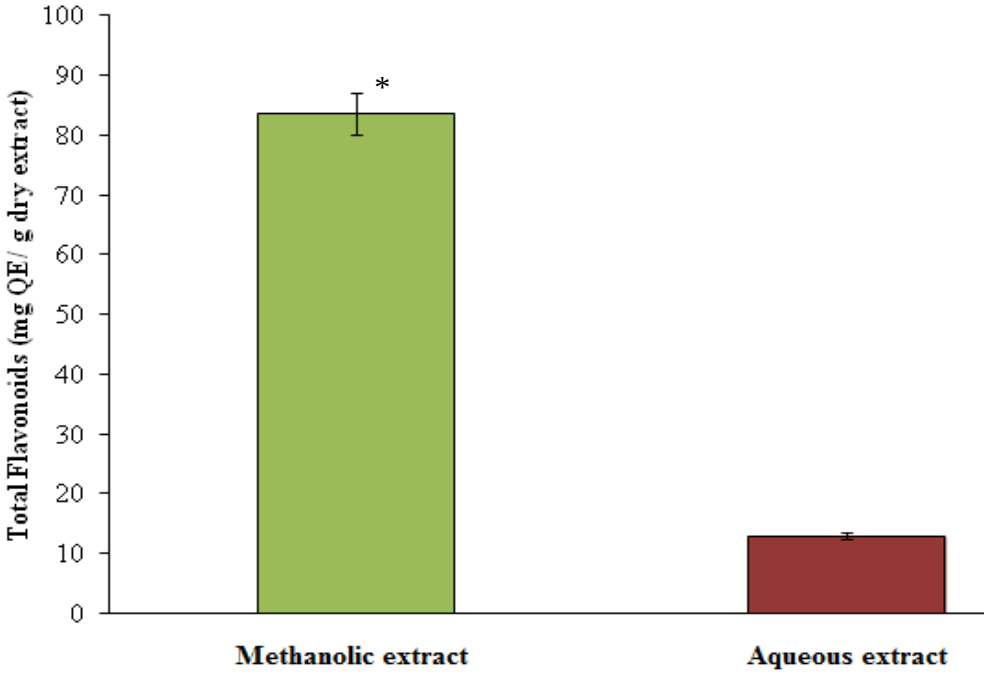

Figure 2: Determination of the level of total flavonoids in *O. basilicum* L. extracts. Values are the mean ± SEM (n=6); *: Comparison between the two extracts, P≤0.05.

Evaluation of antioxidant activity by the DPPH test

The antioxidant activity of *O. basilicum* L. leaves extracts on DPPH radicals was determined *in vitro* using BHT as reference antioxidant. The extracts reduced DPPH radicals significantly as compared to the control (P<0.05). The methanolic extract present the highest antioxidant activity compared with the aqueous extract and BHT. The methanolic extract showed an IC_{50} value of 304.82 ± 24.15 µg/ml compared with the aqueous extract and the BHT which was used as the positive control with an IC_{50} value of 327.46 ± 13.11 µg/ml (Fig. 3). The aqueous extract was significantly (P≤0.05) less active (IC_{50}=2122.81 ± 107.77 µg/ml) than the methanolic extract and BHT (Fig. 3). The antioxidant activity of *O. basilicum* L. extracts is in agreement with the results of several studies [25, 32, 33, 34, 35].

Polyphenols and flavonoids were found in the two extracts of *O. basilicum* L. and in the following order: methanolic extract > aqueous extract. The obtained results for DPPH are

in correlation with the phenol contents determined for each extract. The antioxidant activity of *O. basilicum* L. may be due to the presence of polyphenols. This suggestion is in agreement with the study by Ćetković et al. [36] which shows that the phenolic compounds present in the species *Satureja montana* L. from the region of Siberia are responsible for numerous biological activities in particular the antioxidant activity. The phenolic compounds may contribute directly to antioxidative effect. The presence of 3-OH group as well as hydroxyl groups in ring B is related to the superoxide scavenging activity of flavonoids. According to Bougandoura and Bendimerad [27], the reducing power of polyphenols is due to the presence of hydroxyl groups in these compounds which can serve as an electron donor. Likewise, antioxidant molecules such as ascorbic acid, α-tocopherol, flavonoids and tannins have been shown to reduce and discolor DPPH due to their ability to yield a hydrogen atom [27]. The phytochemical screening carried out in this study showed that *O. basilicum* L. contains, in addition to flavonoids, other active compounds such as tannins and essential oils which can participate in the anti-free radical power of its extracts. Indeed, the essential oil of *Lavandula officinalis* is responsible for its antioxidant activity [37].

The comparison of the antioxidant activity of the two extracts of *O. basilicum* L. by the DPPH test showed that the methanolic extract is the most effective. This may be due to the richness of this extract in polyphenols and particularly in flavonoids. Bougandoura and Bendimerad [27] have shown that the methanolic extract of *Satureja calamintha* has a higher reducing power than that of the aqueous extract and that the polyphenols contained in these extracts are responsible for the antioxidant activity of this plant.

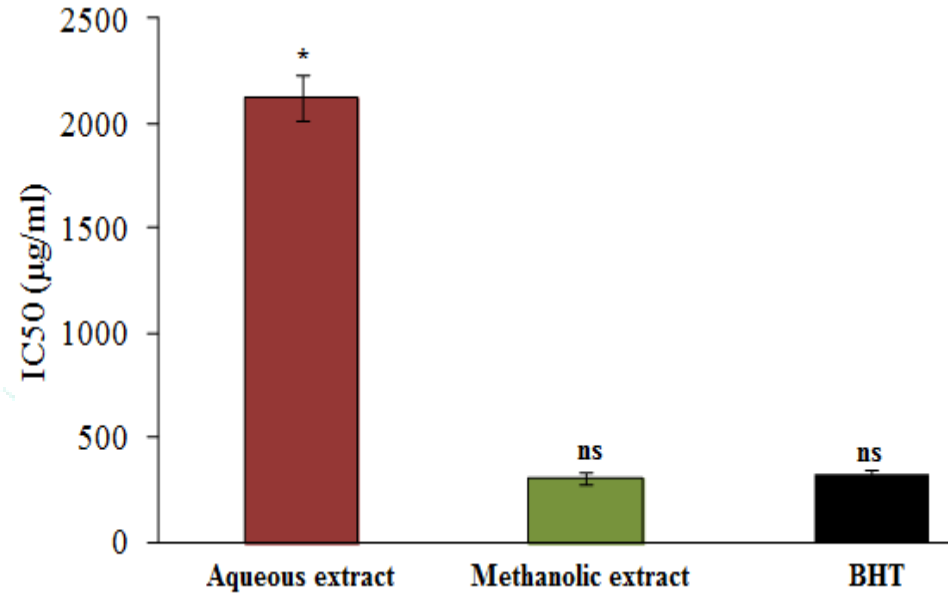

Figure 3: Comparison of IC_{50} of methanolic extract, aqueous extract and BHT. Values are mean ± SEM (n = 6); *: P≤0.05. ns (not significant): P>0.05.

CONCLUSION

This study showed that *O. basilicum* L. is widely used in traditional Algerian medicine as a digestive agent. In addition, the methanolic and aqueous extracts of *O. basilicum* L. leaves possess substantial antioxidant activities. The antioxidant potential of these extracts may be attributed to their phenolic content as well as the presence of the flavonoids, tannins and essential oils. Thus, the free radical scavenging ability of *O. basilicum* L. could provide health benefits to humans by protection against oxidative stress.

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