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Assessment of polyphenols contents, antibacterial and antioxidant activities of *Origanum majorana* extracts

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ABSTRACT: The Algerian flora contains many species of vascular plants, including aromatic and medicinal plants, which can be very used for the treatment of diseases and health care. *Origanum majorana* is an Algerian medicinal plant used in the traditional pharmacopoeia. This work was conducted to evaluate the total polyphenolic content, antibacterial effect, and antioxidant capacity of *O. majorana* extracts. The extraction was carried out using the aerial parts of *O. majorana* with water and methanol to produce the aqueous extract (Aq.E) and the methanolic extract (Met.E). The total polyphenolic and flavonoids contents of the extracts were estimated using colorimetric method. The antibacterial effect was evaluated by the method of disc diffusion. ABTS, DPPH radical scavenging, and reducing power were used to determine the antioxidant capacity of the extracts. So, the results showed that the highest concentrations of polyphenolic amounts and flavonoids were recorded in the Met.E with values of 68.66 µg EAG/mg E and 11.71 µg EQ/mg E, respectively. Moreover, all extracts showed a good antibacterial effect against *B. cereus* with inhibition zones ranging from 9 to 13 mm, and moderate activity against *S. aureus* and *P. aeruginosa*. In addition, the Met.E showed the highest effect in case of DPPH and ABTS free radical (EC_{50}=16.15±0.2 µg/ml and 19.66±0.56 µg/ml, respectively). This study demonstrated that the Met.E of *O. majorana* contains bioactive compounds that are related to potential biological activities, such as antioxidant and antibacterial effect.

Keywords: Antibacterial effect; Antioxidant activity; Polyphenols; *Origanum majorana*.

1. INTRODUCTION

Medicinal and aromatic plants have always been considered a basic source of the human health, and many traditional cultures still value plant medicinal prescriptions and their preventive and curative importance and other benefits. Plants contain a large number of effective compounds that reflect the therapeutic potential of these plants, it is known that some plant drugs have a therapeutic capacity greater than that of manufactured medicines in treating some diseases, and the use of these drugs is devoid of the harmful side effects that accompany the use of manufactured medicines sometimes. And among the other characteristic that led to the increase in the use of medicinal plants and other natural products, the emergence of new diseases accompanied by severe complications for which no suitable treatment has yet been found [1].

The functions of the body are related to the oxidation and return reactions that lead to the production of the reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) hydroxyl radicals ($OH^{\cdot}$) and superoxide anion ($O_2^{\cdot}$-), during normal metabolism or upon exposure to an injury. So, the balance between the production of these molecules and their disposal ensures the preservation of the normal physiological functions of the body. The overproduction of oxygen species can lead to radical chain reactions that damages vital biological molecules such as proteins, DNA and lipids in the body, which have etiologic and pathophysiological roles [2]. In recent years, multidrug resistance has developed in human disease-causing microorganisms due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation allowed scientists to search for new antimicrobial compounds from various sources, such as medicinal plants, which are a good source of new antimicrobial chemotherapeutic agents [3]. The secondary metabolite diversity of the medicinal plants explains their multiple pharmacological activities, and as a result, many species of this family are used in traditional medicine. Flavonoids, anthocyanins, tannins, phenols, and other plant constituents are potential antioxidants [4]. Foods's rich in antioxidants play an essential part in the disease's prevention, such as diabetic, cancer, neurodegenerative, cardiovascular disorders, inflammation, and problems caused by cell and cutaneous aging [5,6]. The use of isolated natural products from medicinal and aromatic plants is a good source of novel and clinically important antibacterial agents that are capable of treating some diseases caused by pathogenic bacterial strains [7]. The aim of this research was the valorization of an Algerian medicinal plant, namely *Origanum majorana*, by the assessment of total polyphenolic content, antibacterial effect, and antioxidant activity of their extracts.

2. MATERIALS AND METHODS

2.1. Materials

The following chemicals and reagents were used: aluminium chloride ($AlCl_3$), Potassium ferricyanide [$K_3Fe(CN)_6$], 1,1'-diphenyl-1-picrylhydrazyl (DPPH), 2-azino-bis(3-ethyl-benzothiazoline-6-sulphonate (ABTS), trichloroacetic acid (TCA), sodium carbonate (Na_2CO_3), Folin-Ciocalteu reagent, ascorbic acid, quercetin, 2,6-Di-tert-butyl-4-methylphenol (BHT), iron chloride ($FeCl_3$), and potassium persulfate ($K_2S_2O_8$).

2.2. Methods

2.2.1. Extraction

The methanolic extraction was performed on the plant material by the maceration method for 24 hours [8]. The 50 g of the powdered plant material were soaked with 500 ml of the methanol. The extracts were filtered with filter paper. The extracts were drained under reduced pressure with a rotary evaporator and oven at 40°C. The aqueous extraction was done following a previous study [9]. The aqueous extracts were produced by decocting 20 g of the powdered plant material in 200 ml of distilled water for 10 minutes. After filtration with filter paper, the recovered extract was drained in an oven at 40°C to produce a powder.

2.2.2. Total phenolic and flavonoids content

The total polyphenolic contents (TPC) of the extracts were estimated by the use of the colorimetric method based on the Folin-Ciocalteu reactant, where the gallic acid used as standard [10]. Basically, 800 µl of Folin-Ciocalteu's phenol reactant (1:10 fold diluted) were mixed with 200 µl of extract. After 4 min, 800 µl of

Na_2CO_3 solution (7.5%) were also added and the mixture was left for 2 h. Absorbance was measured at 765 nm. The quantity of total polyphenols of different extracts was expressed as µg equivalent of gallic acid (EGA)/mg extract. The total content flavonoids (TFC) of extracts were assessed by the use of the aluminum chloride reagent ($AlCl_3$) [11]. Briefly, 450 µl of $AlCl_3$ (2%) was added to 450 µl of extract solution, dissolved in corresponding solvent. The absorbance was taken at 430 nm, after incubation for 10 minutes at room temperature. The quantity of total flavonoids was expressed as µg equivalent of quercetin (EQ)/mg extract.

2.2.3. The antibacterial effect

The antibacterial effect of the various extracts was assessed on six bacteria strains using the disc diffusion method [12], namely *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium ATCC 13311, Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Citrobacter freundii* ATCC 8090 and *Bacillus cereus* ATCC 10876. Whatman® paper discs of 5 mm diameter were imbibed with 20 µl of extract solutions (50 mg of extract were dissolved in 1 ml of DMSO), to get the concentration of 100 µg/disc. The discs were deposited on the surfaces of media that were swabbed with the bacterial suspensions having an optical density of 0.5 McFarland. After an incubation for 24 hours at 37°C, the diameters of the inhibition zones that surround the disc were measured. Gentamicin (25 µg/disc) was employed as a positive control and the antibacterial activity was represented by the inhibition zone diameter in millimeters (mm).

2.2.4. ABTS radical scavenging assay

The ABTS radical scavenging capacity of the extracts was carried according to [13]. The ABTS free radicals were produced by the mixture of the persulfate potassium solution (2.45 mM) with ABTS solution (7 mM) in for 24 hours until obtain a dark solution. The ABTS standard solution was diluted by adding the methanol to obtain an absorbance of 0.700 at 734 nm. A 50 µl aliquot of each extract was mixed with 950 µl of ABTS standard solution and the absorbance was recorded after 30 minutes. Ascorbic acid and BHT were used as standards. Total antioxidant capacity was computed according to the following formulation:

$$\text{Scavenging effect } \% = [(A_C - A_S) / A_C] \times 100$$

where A_C is the absorbance of control and AS is the absorbance of sample. The data were presented as half maximal effective concentrations (EC_{50}), i.e. the concentration of the extract that is necessary to scavenge 50% of the ABTS radicals.

2.2.5. DPPH radical scavenging test

The antioxidant capacity of the extracts was estimated by using the DPPH radical scavenging test according to [14]. 2.9 ml of various concentrations of the extracts were mixed with 800 µl of DPPH methanolic solution (0.1 mM), the absorbance of mixture was taken at 517 nm, after an incubation for 30 minutes in the dark. For the control, the extracts were replaced by the methanol. Quercetin, BHT and gallic acid were used as antioxidant standards. The inhibition percentage was computed using the following formulation:

$$\text{Radical-scavenging activity } (\%) = [(A_C - A_S) / A_C] \times 100$$

where A_C is the absorbance of control and AS is the absorbance of sample. The data were presented as half maximal effective concentrations (EC_{50}), i.e. the concentration of the extract that is necessary to scavenge 50% of the DPPH radicals.

2.2.6. Reducing power

The capacity of the extracts to reducing ferric ions (Fe^{+3}) was determined by the method described by [15]. A volume of 700 µl of the extract at different concentrations was combined with 700 µl of phosphate buffer solution (0.2 M, pH=6.6) and 700 µl of potassium ferricyanide [$K_3Fe(CN)_6$] solution (1%), the mixture was incubated for 20 minutes at 50°C. Then, the process was stopped by the adding of 700 µl (10%) of trichloroacetic to the mixture, and the whole was centrifuged for 10 min at 3000 r/min. Finally, 700 µl of distilled water were mixed with 700 µl of the supernatant solution and 90 µl of $FeCl_3$ (0.1%), then, the absorbance was taken at 700 nm. The increase of the absorbance of the reaction mixture indicates an increment in the reducing power. The positive control was represented by ascorbic acid and the result was represented as µg equivalent of ascorbic acid /mg extract.

2.2.7. Statistical analysis

The results were represented as mean±standard deviation of triplicate. The EC_{50} values were computed from the linear regression. The data were examined by Student's *t*-test to identify the statistical significance. A *p*-value where $p<0.05$ was taken as indicative of significance. All statistical analyses and graphing of the data were done using GraphPad Prism 7 software.

3. RESULTS AND DISCUSSION

3.1. Quantitative analysis of extraction

The plant parts were extracted with a rapport of 1/10 (weight/volume) and after drying the extract different yields were obtained (Table 1). The extraction revealed that the Aq.E and Met.E had a height yield, with 18.24% and 9.06%, respectively.

The total extraction is the main process in the extraction and isolating of the phytochemicals and bioactive coupounds from plant materials. The extraction yield can be affected by the method used, the solvent used, chemical nature of phytochemicals, time of the extraction, as well as the parts used in the extraction. The difference in the polyphenol and flavonoid contents of extracts from the difference in polarity of the organic solvents, the extraction time and temperature, the solid-liquid extraction rate as well as the physical and the chemical characteristics of the samples [15-16]. By comparing the results obtained, the TPC and TFC of the various extracts were analyzed and reported in Table 1.

Table 1. Total polyphenol contents and total flavonoids contents of *O. majorana* extracts.

Yield (%)		**TPC (µg EGA/mg E)**		**TFC (µg EQ/mg E)**	
Met.E	Aq.E	Met.E	Aq.E	Met.E	Aq.E
9.06	18.24	68.66	56.08	11.71	5.43

The results of total polyphenols were obtained by extrapolation the absorbance of the extracts on the calibration curve of gallic acid. The results show that Met.E is the richest polyphenols and flavonoids with content of 68.66±0.15 µg EGA/mg E and 11.71±0.06 µg EQ/mg E. The total polyphenolic contents in the plant extract are depending to the type of extractction, i.e. the time of extraction and the solvent used in the extraction (the polarity of solvent used in extraction). Often the polyphenol concentration in plants extracts is high when using high-polar solvents for extraction, such as water and alcohol (methanol and butanol) [17]. Therefore, several studies reveled that apolar solvents gave less yields than polar solvents, ince these last have

the ability to break down cell walls allowing the exit of secondary metabolites that are trapped inside cells. While non-polar solvents do not have the capacity to extract the maximum amount of bioactive coumpounds, because they are immiscible with water, which it contained in the plant tissue.

The plant material preparation for the extraction of the active substances is an important step, which is related to the collection, cleaning and drying of the plant. There are several extraction methods to extract the active molecules from plants that using different solvents. The extraction yield varies according to the drying conditions, the plant parts that used in the extraction, plant species, i.e, abundance of each species with secondary metabolites [16,17]. The region and the harvest period are also crucial for the yield. In addition, recovery of polyphenolic compounds is affected by the type/polarity of the used solvent, and the solubility of the secondary metabolites in the solvents of extraction [18,19]. Solvents could substantially affect the total polyphenolics content in plant extracts due to the differences in solvent polarities, which could influence the solubility of various constituents that are present in the parts of plants [20]. The total polyphenolic content in the plant extracts depends on the type of extraction, i.e. the polarity of the solvent used in the extraction. For example, the high solubility of polyphenols in polar solvents leads to a high concentration of these compounds in extracts obtained using polar solvents in the extraction [21].

3.2. Antibacterial effect

The antibacterial activity of the extracts was assessed on six bacteria strains using the disc diffusion method. The obtained capacity was expressed as diameters of the inhibition zones, and the sensitivity was classified with the following parameters:

- Not sensitive or resistant (-): diameter ≤ 8 mm.
- Sensitive (+): 8 mm < diameter ≤ 14 mm.
- Very sensitive (++): 14mm < diameter ≤ 19 mm.
- Extremely sensitive (+++): diameter > 19 mm.

The extracts showed a good antibacterial effect against *B. cereus* with inhibition zones ranged from 12 to 13 mm (Table 2). However, the extracts showed a moderate antibacterial effect against *S. aureus* and *P. aeruginosa*. The antibacterial effect of the extracts was less than the activity of gentamycin antibiotic standard.

Table 2. Antibacterial effect of extracts of *O. majorana* on pathogenic bacteria. Results are presented as inhibition zone (IZ) in mm; R = Resistance, GMN = gentamycin.

	Extracts		**GMN**
	Met.E	**Aq.E**	
S. aureus ATCC 25923	9	11	22
P. aeruginosa ATCC 27853	9	7	16
B. cereus ATCC 10876	12	13	25
E. coli ATCC 25922	R	R	22
C. freundii ATCC 8090	R	R	22
S. typhimurium ATCC 13311	R	R	22

Plant-derived extracts demonstrate antibacterial activity that can be through a variety of mechanisms, that is attributed to bioactive components, including plant-derived polyphenolic compounds. The responsible mechanisms of polyphenols toxicity towards microorganisms involve enzymatic inhibition through oxidized

compounds, probably through a reaction with the non-specific interactions or with sulfhydryl groups [22]. Proanthocyanidins inhibit bacteria by destabilizing the cytoplasmic membrane and make it permeable, inhibition of extracellular enzymes, direct action on bacterial metabolism and deprivation of substrates necessary for bacterial growth, especially essential mineral micronutrients, such as zinc and iron through metal chelation [23].

3.3. Antioxidant activity

3.3.1. ABTS radical scavenging

The ability of the different extracts to scavenging the ABTS radical was assessed. The tested extracts were competent to scavenge the ABTS radical cations with different EC_{50} values. The results are shown in Fig. 1. The results reveal that the *L. sativum* extracts exhibited a high scavenging potential (EC_{50} = 19.66±1.42 µg/ml). The statistical analysis indicated that there is a significant difference between the anti-radical activity of the Met.E and the Aq.E of *L. sativum* (Fig. 1). The activity of the Met.E scavenging $ABTS^{\cdot+}$ can be due to their higher polyphenols contents. The antioxidant capacity of samples correlates with the polyphenols level, which shows a high association between the $ABTS^{\cdot+}$ radical scavenging activity of the extracts and their content of polyphenols. Moreover, polyphenols compounds, undoubtedly, have an influential role in the free radicals scavenging ability, which are considered to be the most effective antioxidant [24].

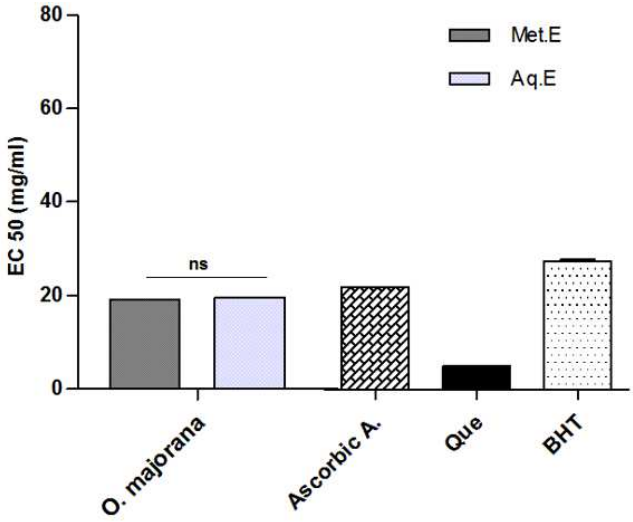

Figure 1. ABTS radical scavenging activity of *L. sativum* extracts (Values were represented as means ± SD of triplicate, ns: p> 0.05; * p: ≤ 0.05; **:p ≤ 0.01; ***:p ≤ 0.001).

Radical scavenging properties are very important due to the harmful effects of free radicals in biological systems and in foods. Various methods are presently used to evaluate the antioxidant capacity of bioactive compounds from medicinal and aromatic plants. Chemical testes are based on the capacity of extract to scavenge the synthetic free radicals by using a variety of systems and methods to generate free radicals [25]. Flavonoids have been reported as effective antioxidants, mainly because they eliminate superoxide and other oxidants. Polyphenols are considered antioxidants due to their ability to scavenge free radicals and active oxygen species, such as single oxygen, superoxide free radicals, and hydroxyl radicals [26]. Flavonoids have been reported to be effective antioxidants, primarily because they scavenge superoxide anions and other

ROS. Polyphenolic compounds are regarded as antioxidants because of their capacity to scavenge the free radicals and the active oxygen species, such as hydroxyl radical, singlet oxygen, and superoxide radicals [26].

3.3.2. DPPH radical scavenging activity

DPPH radical is extensively used as a model system to explore the antioxidant/scavenging activities of several natural antioxidant molecules [27]. The antioxidant activity of the extracts was determined by using the DPPH free radical scavenging test, then, the antiradical effect was expressed as effective concentrations (EC_{50}). The DPPH free radical scavenging activity of the extracts was shown in Fig. 2. The results showed that the extracts of *L. sativum* had the best antiradical activity records in the Met.E (EC_{50} = 16.15±0.2 µg/m).

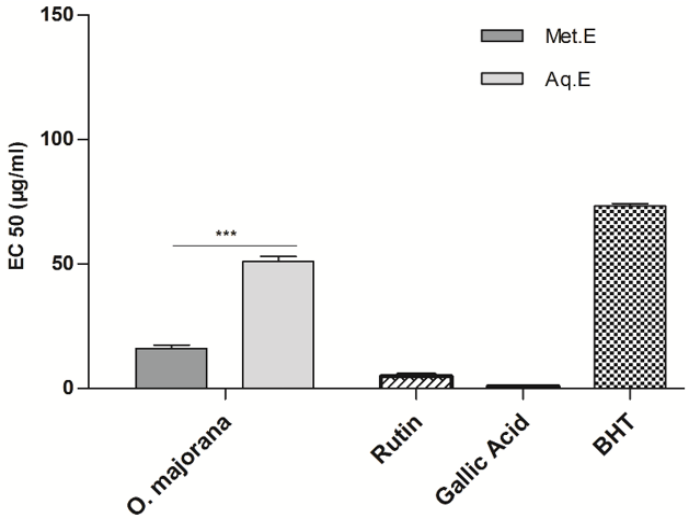

Figure 2. DPPH free radical scavenging effect of *L. sativum* extracts (Values were presented as means ± SD, n=3, ns: p> 0.05; * p: ≤ 0.05; **:p ≤ 0.01; ***:p ≤ 0.001).

Furthermore, the results of the scavenging effect of the extracts were very different from the gallic acid and quercetin standards. It has been indicated that the free radical scavenging capacity of tea extracts was possibly due to its hydrogen donating property, which is attributed to the polyphenolic components [28]. The free radical scavenging activity of the natural antioxidants, such as polyphenolic compounds and flavonoids, can be attributed to their hydroxyl rings. The spatial arrangement and number of OH groups in the flavonoid structures can affect the different antioxidant mechanisms [29].

3.3.3. Reducing power

The efficiency of *L. sativum* extracts to reducing Fe^{+3} was assessed using the method outlined by Benslama [15]. In this test, the extracts reduce the complex $iron^{+3}$/ferricyanide to the ferrous form by providing one electron, and then, it was compared with the activity of ascorbic acid (Vit. C), which is known as a potent reducing agent.

The results were represented as µg equivalent of ascorbic acid/mg extract (µg EAA/mg E). As illustrated in Fig. 3, the reducing effect of the Aq.E. was higher than that of the Met.E extract. Moreover, the reducing effect of the extracts was significantly different from the reducing effect of quercetin (1859.29 ± 17.23 µg AA equ/mg E).

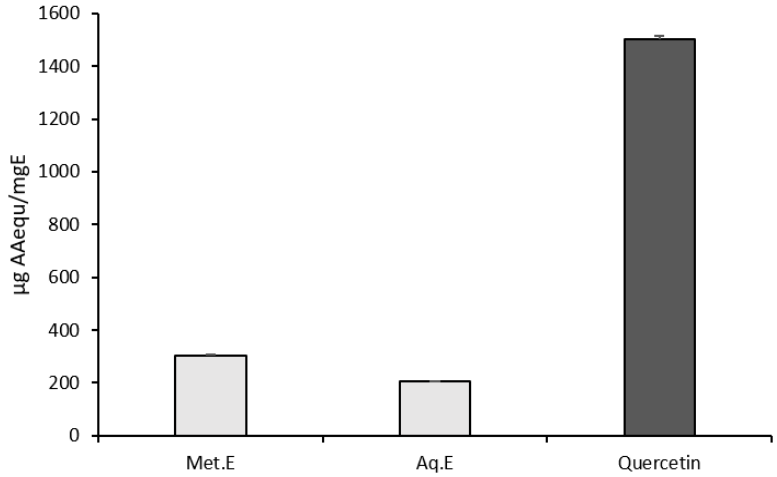

Figure 3. The reducing effect of *L. sativum* extracts (Values were repersented as means ± SD, n=3, ns: p> 0.05; * p: ≤ 0.05; **:p ≤ 0.01; ***:p ≤ 0.001).

The antioxidant activity was cited to be the development that was related to reducing capacity. The antioxidants transform the reactive radicals more stable species by providing electrons. The reducing capacity was a key parameter for estimating of the antioxidant effect. The ferric reducing assay was fast, simple, and responsive for antioxidant screening [30]. The results revealed a high correlation between the reducing capacity of the extracts and their polyphenol contents, thus, proving that the polyphenolic compounds are the powerful elements in these extracts. An additional reaction way in electron donating is the reduction of an oxidized antioxidant compound to restore the active reduced antioxidant. The reducing power was a very significant aspect in the assessment of antioxidant propriety. The reducing effect of a set of compounds refers to its ability to transfer electrons in a redox reaction, which leads to the transformation of free radicals to inert products or less reactive [31]. Compounds having reducing activity reflect that they are electrons donors and are able to decrease the oxidized intermediates in the lipid peroxidation event, so that they are considered as primary and secondary antioxidants [32]. The reducing properties of antioxidants are related to their electron transfer capacity, such as polyphenol and flavonoids. Several studies have shown that plant extracts have a high reducing effect. Moreover, many researchers have referred the association between the structure of polyphenol and their ferric reducing activity [33,34]. Polyphenolic compounds are named antioxidants because of their capacity to scavenge free radicals, enzymes inhibition, reducing effect and lipid peroxidation inhibition [35].

4. CONCLUSION

In conclusion, the results have showed that the extracts of *O. majorana* have an important antioxidant activity and moderate antibacterial effect, which due to their polyphenolic contents. Therefore, the extracts of this plant could be viewed as a natural alternative dietary source, and as a focus for the pharmaceutical and medical sectors. Additional studies are necessary to identify which polyphenolic compounds are responsible for the antioxidant propriety and antibacterial effect of the extracts. Given the potential use of the extract in therapeutic benefits and bioactive compounds justify additional *in vitro* and *in vivo* investigations.

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