Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(10); 1566-1570

ISSN: 0975-4873

Research Article

Determination of Total Phenol, Flavonoid, Antioxidant and Antimicrobial Activity of Methanolic Extract of *Teucrium polium* L. in Algerian East

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Available Online:15th October, 2016

ABSTRACT
The phenolic compounds are one of the main group of components, which act as antioxidant of the free radicals; it reduces and decrease the accumulation of their products. This article presents the anti-oxidizing activity of *Teucrium polium* L. Methanolic extract which was evaluated by two methods; capacity of reduction of iron and the trapping of the free radical DPPH, which expressed by a remarkable but weak IC_{50} in front of ascorbic acid: 32 mg/ml for the first and 23.09 mg/ml for the reduction of DPPH. Determination of the total polyphenol content in the extracts of *Teucrium polium* L.; has been estimated by the method of Folin-Ciocalteu. The result shows that our plant is rich in polyphenol, which is equal to 288.41±1.83 mg equivalent of gallic acid/gdry material. The total flavonoids were determined by the colorimetry method using $AlCl_3$, and were expressed out of mg equivalent of cathechine/g of dry material, which was estimated at 200.24±2.46 mg EC/gDM. The antimicrobial activity was tested on seven strains of bacteria of ATCC type: *Staphylococus aureus* ATCC25923, *Escherichia coli* ATCC25922, *Salmonella typhimurium* ATCC10428, *Pseudomonas aeroginosa* ATCC27853, *Enterococus faecalis* ATCC29212, *klebsiella pneuminiae* ATCC700603, *Bacillus subtilis* ATCC7033 and one yeast *Candida albicans* ATCC10231. The antibacterial activity was estimated by the test of sensitivity and calculation of the minimal inhibiting concentration (MIC) according to the method of micro dilution. The diameter of inhibition varies between 11.5 mm and 26 mm and resistant for *Salmonella typhimurium* ATCC10428 for a concentration of 600 mg/ml, whereas the values of the MIC are varied between 3.125 mg/ml and 50 mg/ml which showed an important activity for some strains.

Keywords: *Teucrium polium L.*, DPPH, FRAP, antibacterial, polyphenol totals, flavonoid

INTRODUCTION

The medicinal herbs are regarded as an important source of new chemical substances, which have an important therapeutic effect[1] and in the last years there was a crescent interest for the study of these plants. Algeria, offers a rich and various vegetation with a large amount of spontaneous aromatic plants, our research is related to *Teucrium polium* L. East Algerian area of "Meguessemia". *Teucrium polium* L (Germandrée tomenteuse), of the Lamiaceae family is a plant from 10 to 35cm long, perennial, robust stems with branches covered of cottony bristles. The leaves are tight or oval-elongated, the inflorescences -of white aspect- are tight, globular or ovoid at least a centimetre in diameter. The chalice is instituted of 5 unequal teeth whose higher is larger and blunt at the top. The corolla is white, rarely purpurin. Stamens are not rolled up on themselves after flowering[2]. Scientific researchs confirmed the traditional remedies of this plant. According to Alzeweiri et al.[3] infused air part of *Teucrium polium* L. is used for its anti-inflammatory and anti-anorexia effects, it's used also against the jaundice and the spasmodic and gastric colics, and it has an antidiabetic and an antibacterial activity[4,5], it is an antioxidant[6]; We were interested to this plant after noting its interest in local population. Therefore, the aims of the present study were to determine the phenolic compounds (contents of polyphenol on the one hand and flavonoïds on the other hand) in methanolic extract and investigate its antimicrobial and antioxidant activities.

MATERIALS AND METHODS

Plant materiel

The crop of the air part of *Teucrium polium* L. was in the area of "Meguessemia" during 2013. The species was identified in the laboratory of vegetable biology, Department of pharmacy (Faculty of Medical sciences, Badji Mokhtar University–Annaba-Algeria).

Microbial strains

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The tested strains are of type ATCC (American Type Culture Collection), provided by the laboratory of microbiology of the teaching hospital of Annaba: *Bacillus subtilis* ATCC 7033, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 10428, *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATTC 10231.

Preparation of the extract

After drying in a dry and aired place -with the shelter- the plant is crushed. About 100g of the vegetable material was put to maceration during 24 hours in100 ml of methanol. After; a vacuum filtration and evaporation of the solvent using a steam rota at a temperature between 45 and 50 °C, the dry residue is conserved in 3ml of methanol at a temperature of 4 °C[7].

Determination of total polyphenol contents

The proportioning of polyphenols was realized according to the method of Folin-Ciocalteu described by Singleton et al.[8].

0.2 ml of the methanolic extract is mixed with 1 ml of folin diluted at 1/10, after five minutes; we add 0.8 ml of recently prepared sodium carbonate (at 7.5%), the whole is agitated by a vortex. We leave the mixture during 30 minutes in darkness at room temperature; using aspectrophotometer at 700 nm[7]. The calibration curve is realized under the same conditions using the gallic acid. The content of total polyphenol is expressed in milligrams of equivalent of dry material per gram of gallic acid.

Determination of total flavonoid contents

A colorimetric process described by Zhishen et al.[9] determined the content in total flavonoids of methanolic extract of our species. 1 ml of sample was mixed with 4 ml of distilled water, and then we add 0.3 ml of $NaNO_2$ solution. After 6 minutes we add 0.3 of $AlCl_3$ (10%) we let it rest during 6 minutes then we add 2 ml of a NaOH solution (1N) and the total is adjusted at 10 ml with distilled water. We leave the mixture resting for 15 min, the absorbance was determined a length of 510 nm. The results were expressed with reference to the cathechine (mg equivalent cathechine /g dried material)

Antioxidant activity

Capacity of trapping of the free radical "2,2-diphenyl-1-picrylhydrazyl" (DPPH)

The capacity of trapping of free radical DPPH was evaluated according to the method described by Boulila et al.[11].

1 ml of each extract with various concentrations was mixed with 2 ml of a methanolic solution of DPPH (at 0.04 g/L). After 60 minutes of incubation at darkness, the absorbance was measured at 517 nm using a spectrophotometer (JENWAY 6300) against methanol used as a blank. The percentages of inhibition of the DPPH were calculated according to the formula:

$$\% \text{ of inhibition DPPH} = \frac{A0 - Aeq}{A0} x100.$$

Where Aeq is the absorbance of the methanolic solution of DPPH added with the antioxidant at equilibrium and A_0 is the absorbance of the DPPH solution added with only methanol at the same proportions. The curve expressing the percentage of inhibition of the DPPH according to the concentration of the antioxidant in microgram per millilitre (µg/mL) has permitted to deduce the median inhibitory concentration (IC50) defined as the antioxidant concentration necessary to decrease the initial concentration of the DPPH at 50%. The ascorbic acid was used as positive control[12.]

Reduction of iron

The reduction of iron was measured according to the method of Oyaizu et al.[13]. The extracts were diluted in methanol, then 2.5 ml of tri chloracetic acid at 10% (w/v) were added and the whole was centrifuged to 3000 round/min during 10 minutes. To 2.5 ml of each supernatant were added 2.5 ml of distilled water and 0.5 ml of ferric chloride at 0.1% (w/v). The absorbance was measured by a spectrophotometer (Jenway6300) at 700 nm against a blank prepared by replacing the extracts by methanol. The ascorbic acid was used as positive control. The result is expressed by inhibitory concentration IC50.

Antibacterial activity

Disc diffusion assays

The sensitivity of the bacterial strains to the extract was assessed by the method of diffusion on discs[15]. Bacterial suspensions were prepared in a physiological solution starting from young colonies (18-24 hours) by adjusting turbidity with 0.5 McFarland. Using a sterile swab, the bacteria in suspension were sown on a dry agar-agar surface (Mueller-Hinton agar liquefied cooled in Petri dishes). Sterile discs of What man paper (6 mm diameter) were then placed, then impregnated with 10 µL of the extract (concentration: 600 mg/ml). Petri dishes were incubated at 37 °C during 24 hours. The strain is regarded as non-sensitive to the extract for a diameter lower than 8 mm, moderately significant between 8 and 14 mm, significant between 14 and 20 mm and very significant if the diameter is higher than 20 mm[16].

Minimal Inhibition Concentration (MIC)

The method of minimal inhibition concentration was described according to the method of microdilution[17]. Bacterial suspensions of the young bacterial cells were prepared in sterile tubes containing physiological water at a value of 0.5 McFarland. It must be used in maximum 30 minutes in order to avoid the change in the cellular number. 50 µl of this suspension is completed at 5 ml with liquid MH because the number of bacterial cells for this test is very important and it must be equal to $5x10^5$ Colony Forming Units(CFU)/ml. The 96-well plates were prepared by dispensing into each well 50µl of different methanolic dilutions of the *Teucrium polium* L. extract prepared in DMSO at 10% with 50 µl of inoculated liquid MH. To check that the results of sensitivity are exact, it is necessary to include at least an organization of control with each batch of determinations of MIC. The organizations of control are available starting from different collections of strains. After incubation of 18 hours at a temperature of 37°C, 15µl of nitro blue tetrazolium (NBT) at a concentration of 2 mg/ml were added to the wells and incubated at 37°C for 2 hours. The reading is based on the colouring of the wells in blue, which indicates the presence

of the microorganism growth, however the persistence of the initial yellow colour of NBT implied the total inhibition of bacteria cells.

RESULTS AND DISCUSSION

Determination of total polyphenol and flavonoid contents

The methanolic content of the extract's total polyphenol of *Teucrium polium* L. was given according to the method of Folin Ciocalteu. The results obtained was calculated out of mg of equivalent of gallic acid per gram of dry material (mg EGA/gDM), which produced a colouring proportional to the quantity of polyphenols present in the methanolic extracts.

The results presented in table 1 have shown high percentage of polyphenol (288.41±1.83 mg EGA/gDM) compared to Milan et al.[18] who found a value of 233.68±0.18 for the methanolic extract, whereas the other extracts have lower polyphenol contents: 61.94±0.19 for acetate ethyl, 147.77±0.77 for acetone and 140±0.29 for water. Amin et al.[19] found a total polyphenol contents estimated at 180.2 mg EGA/gDM for the ethanolic extract and less important values were found for other solvents[20]: 169.06±0.75 mg EGA/gDM, 170.62±1.05 mg EGA/gDM, and 50.50±1.26 mg EGA/gDM for dichloromethane, ethanol and cyclohexane, respectively. The variability of the contents of total phenolic compounds in the different extracts can be due to the polarity of solvent[21]. It is well known that methanol is a better extraction solvent for the phenolic compounds than hexane, acetone and ethyl acetate[22]. The total flavonoids were estimated according to the colorimetric method with aluminium tri chloride and sodium hydroxide, by using the cathechine as standard. According to the obtained results, we noticed an important content of flavonoid 200.24±2.46 EC/gDM comparing to Amin et al.[19] who found 135.2 EC/gDM for ethanol extract and 197.4 EC/gDM for the ethyl acid extract. Milan et al.[18] have found a variability of the flavonoids contents in various extracts, acetone and ethyl acetate showed the more important values estimated at 242.95±1.45 EC/gDM and 335.40±0.71 EC/gDM, respectively. However, water and methanol extract presented a less important flavonoide content equal to 195±1.04 EC/gDM and 47.80±0.44 EC/gDM, respectively. The significant difference of the flavonoid content for various solvents depends on the solvent choice.

Antioxidant Activity

The antioxidant capacity of the methanolic extracts of *Teucrium polium* L. was evaluated by iron reduction which measures the reducing capacity of an antioxidant by the transformation of Fe^{3+} into Fe^{2+} and the capacity of trapping of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) which depends on the structural formation of the antioxidant. Some compounds react very rapidely with DPPH which reduces a certain number of DPPH molecules[23,24]. *Teucrium polium* L. gave an IC50 equal to 23.09 µg/ml (DPPH) and 32 µg/ml (reduction of iron) however our extract has a less pronounced activity compared to that obtained by Sharififar et al.[25], in the chloroforme, petroleum ether and water extracts which are successively in µg/ml 73.2, 85.4 and 40.6, but our results are more important than methanolic extract obtained by Sharififar et al.[25] (20.1 mg/ml) and Ahmed et al.[26] (18.3±0.8 mg/ml).

Antibacterial activity

The measurement of the diameter of the inhibition zone allows an estimation of the character of sensitivity or resistance of the bacterial strain to the extract. Table 2 shows that all the bacterial strains are sensitive to very sensitive except *Salmonella* which is resistant, these diameters vary between 11.5 mm and 26 mm. The sensitivity of a microorganism to a natural substance depends on the properties of the last[27]. The MIC values (table 2) are about 3,125 mg/ml for *Bacillus subtilis* ATCC7033, 12.5 mg/ml for *Pseudomonas aeroginosa* ATCC 27853, 25 mg/ml in the case *of Escherichia coli* ATCC 25922 and 50 mg/ml for the other three: *Enterococcus feacallis* ATCC 29219 and *Staphylococcus aureus* ATCC 25923.

The antimicrobial activity of the extracts *Teucrium polium* L. is related to the presence of many bioactive secondary metabolites, especially phenolic compounds[28]. The results of several researches showed an antibacterial activity less important than ours. According to Darabpour et al.[29], the methanolic extract of *Teucrium polium* L. was tested on some strains, with a concentration of 600 mg/ml. their results showed that the diameter of inhibition of the strains: *Staphylococus aureus* and *Escherichia coli* are equal to 9 mm, while the strain *Bacillus subtilis* and *Salmonella typhimurium* showed a certain sensitivity since the diameter of inhibition reached 15 mm and 16 mm, respectively. In another study, the methanolic and aqueous extracts of *Teucrium polium* L. at a concentration of 2 mg/ml proved a total growth of the strains which can be explained by a lack of their biological activities[30]. The MIC values obtained by Darabpour et al.[29] were 40 mg/ml for *Staphylococus aureus* and higher than 200 mg/ml for *Salmonella typhimurium*.

CONCLUSION

A large number of aromatic plants contain chemical compounds having antioxidant and antimicrobial

Table 1: The total polyphenol values in (mg EGA/gDM) and the concentration of the flavonoids (mg EC/g DM) and the antioxidant activity (IC50 in µg/ml) of the extract of *Teucrium polium* L.

Methanolic extract of *Teucrium polium* L.	Polyphenol	flavonoid	Antioxidant			
			DPPH		FRAP	
			Methanolic extract	Ascorbic acide	Methanolic extract	Ascorbic acide
	288.41±1.83	200.24±2.46	23.09	0.37	32	2.04

Table 2: Diameter of the inhibition zones (mm) and the minimal inhibition concentration (mg/ml).

Bacterial strain	inhibition zone diameter (mm)	Minimal inhibition concentration (mg/ml)
Bacillus subtilis ATCC 7033	26	3.125
Escherichia coli ATCC 25922	16	25
Enterococcus feacallis ATCC 29219	12	50
Klebsiella pneumoniae ATCC 700603	15.5	50
Pseudomonas aeroginosa ATCC 27853	19.5	12.5
Salmonella typhimurium ATCC10428	-	-
Staphylococcus aureus ATCC 25923	11.5	50

properties. In this context, we tried to evaluate, *in vitro*, biological activities (antibacterial and antioxidant) of the methanolic extract of *Teucrium polium* L., beginning with a quantitative identification of polyphenols and flavonoids in this extract. The methanolic extract exhibited an important antioxidant and antibacterial activities and this is probably due to its high polyphenol and flavonoid contents.

ACKNOWLEDGEMENTS

I thank all the people who helped me to realize this work.

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