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Der Pharma Chemica, 2015, 7(12):308-314
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Chemical analysis, antimicrobial and antioxidant activity of the essential oil of *Teucrium poliumsspaurasianum Labiatae*

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ABSTRACT

We have carried out the extraction of the essential oils of the aerial part of the plant using the hydro-distillation technique which enabled us to reachan extraction efficiency of 0.585 %. The chemical composition of the essential oil was analyzed by GC/MS method therefore 35 components have been identified; the limonene (34.72 %), alpha-pinene (25.42 %), the β-pinene(8.59%), the α-Elemol (4.21 %) are the major components. The antibacterial activity of the essential oil has been measured against eight bacteria; the staphylococcus aureus is the most sensitive one; its diameter is 21 mm of inhibition. The antioxidant activity of the essential oil was evaluated using 2,2–diphenyl-1-picrylhydrazyl (DPPH) test which shows (IC_{50}=58.6336 ± 0.7207)and the antioxidant power of reduction of iron (FRAP) shows (EC_{50}=48.1936±0.5628)

Key words: antibacterial activity, antioxidant activity, essential oil, *TeucriumPoliumaurasianum*

INTRODUCTION

*Teucrium*sis one of perennial plants which belongs to the family Labiatae that is represented by over 340 species including 20 found in Algeria [1]

TeucriumPoliuma plant is well known by its significant properties;it is sudorific, antipyretic, antispasmodic, anti-inflammatory, anti-hypertensive medication, anti-nociceptive and hypoglycemic [2, 3]The essential oils of *Teucrium*were have been recently reported as antimicrobial and antispasmodic [4, 5]

In traditional medicine, this species are used in case of stress [6], its anti-stress and antioxidant properties contribute in the fight against the skin aging [7]

Our study is to determine the antibacterial and antioxidant activities of the essential oil of the subject plant; this study was conducted to identify the chemical composition of the essential oil of the aerial part of *TeucriumPoliumaurasianum*and study its antibacterial and antioxidant activity,

This study enters within the context of developingthe biodiversity of the Algerian medicinal and aromatic plants especially the species of*TeucriumPoliumaurasianum*because of their medicinal, alimentary and conservative property.

MATERIALS AND METHODS

1.1. Plant Material

The plant has been harvested in the Wilaya of Batna which is in Aures region in the month May 2013 at an altitude of 1547 m (35°10'0"N; 6°10'0"E). Samples have been deposited in the Herbarium of the National Institute of Agronomy (INA), El-Harrach, Algiers, under reference AC-AS3. The harvested part consistsof leaves, stems and flowers (aerial part). It has been dried in air in theshade at ambient temperature for 7 days

1.2. Extraction of essential oil

The extraction of the essential oil was performed through hydro-distillation process usingan extraction device called Clevenger. 100 g of dried plant has been introduced inside a large flask of 2 liters;then we have added a quantity of distilled water corresponding to 2/3 of the volume of the flask. The extracting operation was carried out three hours after the beginning of boiling. Finally the collected oil has been kept in tinted and well-sealed bottles at a temperature of 5^{O}C.

1.3. Determination of the chemical composition of the essential oil by CPG

The analysis of the essential oil of *TeucriumPoliumaurasianumLabiatae* has been conducted in the INRAP (National Institute for Research and Physicochemical Analysis) Tunisia by gas chromatography, type HP 6890 coupled to a mass spectroscopy, type HP 5975B with ionization by electron impact (70 eV), equipped with a capillary column HP-5MS (5 % -phenyl- SGE's) (30 m x 0.25 mm, film thickness was: 0.25 cm) set for a temperature of 50°C up to 300°C and maintained at 300°C for 10 minutes to 2°C/min. The vector gas was helium with a fixed flow rate of 0.8 ml /min.

The essential oil (1 µl) has been automatically injected via Split Mode. The device is controlled by a computer system managing a library of mass spectra.

1.4. Bacterial Strains

The antimicrobial activity of the essential oil of *TeucriumPoliumaurasianum* has been evaluated over several bacterial strains

These strains are: *E. coli, Leisteria, Salmonella, Staphylococuis aureus, staphylococcus SP, Proleus, Klebsiella*

1.5. Tests of antimicrobial activities

The tests of antimicrobial activities have beenperformed through direct contacttechnique (method of dissemination on agar milieu). [8]

This method consists of putting discs of impregnated filter papers with essential oil on the surface of agar plates that have beeninoculated with the germ to be tested and measure the diameters of inhibition in millimeters (mm) after incubation for 24 h at 37°C.

The disks of chromatographic papers of 6 mm diameter, previously sterilized have been deposited on the surface of the inoculated agar after being filled in with 5 µl of essential oil; we utilized negative witnesses using discs which were placed on inoculated agar without essential oil

The sensitivity of the different strains against the essential oil is classified according to the diameter of inhibition as per the following criteria: non-sensitive (-) for Ø<8 mm; sensitive (+) for 9-14 mm; very sensitive to ++Ø 15-19 mm and extremely sensitive SIC for Ø >20 mm [9]

1.6. Determination of CMI

The bacterial suspension (108 CFU/ml) is seeded on the surface by swabbing on agar milieu(Mueller-Hinton) in a Petri plate. The contact with the solutions to test was done through a disc of paper (6 mm diameter) on which 10 uL of solution (essential oil) at different dilutions, 1, ½, ¼, 1/8, 1/16 etc. .µ a disk containing the DMSO is used as blank. Each test is performed in triplet.

1.7. Antioxidant Activity

1.7.1. DPPH essay

To evaluate the antioxidant activity of the essential oil we have used the method of the DPPH 1,1 -diphenyl-di-picrylhydrazyl) proposed by [10, 11]but with modifications.

The solution of DPPH was obtained by dissolving 4 mg of powder within 100 ml of ethanol. The essential oil was prepared by dissolution in absolute ethanol. The test has been carried out by mixing 1 ml of the previous solution of DPPH with 1ml of the oil to be tested at different concentrations. After an incubation period of 30 minutes at the lab temperature; we noticed that the absorbance was 517 nm

The reference antioxidant or the positive control (Ascorbic acid) was also prepared according to the same method with the same concentrations for the comparison

According to [10, 11]the inhibition of free radical of DPPH in percentage (I%) was calculated using the following method:

$$I\ \% = \left(A_{blank} - A_{sample} \Big/ A_{blank} \right) \times 100$$

With A_{blank} is the absorbance of the witness (containing all reagents without the product to be tested) and

A_{sample} is the absorbance of the test.

The graph of the variation of the percentage of inhibition depending on the concentration of the essential oil allowed to determine the IC 50 corresponding to 50 % of inhibition which constitutes the antioxidant activity of the essential oil. This value was compared to the one found for the reference compound.

1.7.2. Method for the iron reduction FRAP (Ferric Reducing Antioxidant Power)

The reductive activity of iron of our extracts is determined according to the method described by [12]whichis based on the reduction of Fe3+ existing in the complex $K_3Fe(CN)_6$ in Fe^{2+}

One milliliter of the essential oil at different concentrations was mixed with 2.5 ml of a phosphate buffer solution 0.2 M (pH 6.6) and 2.5 ml of a solution of potassium ferricyanide$K_3Fe(CN)_6$ to 1 %. The whole is incubated in a water bath at 50C for 20 minutes, and then 2.5 ml of trichloroacetic acid to 10% was added to stop the reaction. The tubes have been centrifuged at 3000 rpm for 10 minutes

2.5 ML of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of a solution of ferric chloride freshly prepared at 0.1 %.

The reading of the absorbance of the reaction mixture was done at 700nm against a similarly preparedsample by replacing the essential oil by distilled ethanol which allows the UV-VIS device calibration (spectrophotometer). The positive control was represented by a solution of a standard antioxidant; the ascorbic acid which the absorbance was measured in the same conditions as the samples. An increase of the absorbance corresponded to an increase of the reducing power of the tested essential oil [13].

RESULTS AND DISCUSSION

1.8. The chemical composition

The results of the analyzes by GC and GC/MS of the essential oil extracted from the plant *TeucriumPoliumaurasianum*are presented in table 1

The essential oil was obtained by hydro distillation technique, the yieldof extraction of the aerial part of *TeucriumPoliumaurasianum* was 0.585 % (w/w) it is low compared to the obtained results which were 1.7 per cent (w/w) and 0.75 % (w/w) [14, 15]respectively

Compared with other species of the same studied family, the obtained amount is considered higher than the one that is obtained by[16] which is 0.1% (w/w) found

Table 1: Chemical composition of the essential oil of *teucriumpoliumaurasianum*

pic	Compound	RI	(%)
1	α-thuiene	742	0.48
2	α-Pinene	782	**25.42**
3	Camphene	822	0.23
4	n-Butylbenzene	846	0.09
5	β-Pinene	953	**8.59**
6	β-Myrcene	1032	5.19
7	D-Limonene	1240	**34.72**
8	β-Ocimene	1323	0.30
9	α-Terpinolene	1621	0.21
10	Isopinocarveol	1832	0.18
11	Myrtenal	2188	0.17
12	Carvone	2502	0.35
13	Acetate	2763	0.25
14	Copaene	3310	0.17
15	Elemene	3421	0.17
16	Caryophyllene	3976	2.66
17	α-Caryophyllene	3954	0.64
18	β-Farnesene	3849	0.89
19	Germacrene D	3775	1.74
20	α –Selinene	3580	0.50
21	1,5-Heptadiene	4038	0.87
22	Amorphene	4145	0.22
23	®–cadinene	4158	0.53
24	Cubenene	4220	2.36
25	α-Elemol	4383	**4.21**
26	Spathylenol	4510	0.42
27	caryophylleneoxide	4530	0.45
28	Cubenene	4792	0.28
29	®-Eudesmol	4817	0.81
30	Naphthalene	4877	2.40
31	β-Eudesmol	4917	0.75
32	α –Eudesmol	4935	0.74
33	α-Cadinol	4961	1.28
34	Ledene	5022	1.08
35	Thujol	5079	0.64
	totale 99,99		

Thirty-five constituents are identified representing a total of 99.99 per cent of this essence. This essential oil presents the majority among the other components such as limonene (34.72 %), alpha-pinene (25.42 %), the β-pinene (8.59 %), the α-Elemol (4.21 %)

The chemical composition of our essential oil extracted from the plant *TeucriumPoliumaurasianum* is different from that of [14] whose content in α-cadinol is much more higher (46.8 %); and different also from [15]which the α-pinene is the major component at (12.52 %) and [17]at (31.24 %) of patchouli alcohol as major component

This is the first time that the oil of *Teucrium Polium* was found to contain limonene with such high percentage, despite [16]found the limonene product as major component but of low percentage (11.18 %) this difference in composition resulted from many extraction operations that were carried on the same is probably due to various conditions including the environment, the genotype, geographic origin, the harvest period, the place of drying, the temperature and duration of drying, the parasites and the extraction method [18]

1.9. Antibacterial activity of the essential oil

Table 2 shows the results which clearly indicates the significant effect of the *TeucriumPoliumaurasianum*on certain studied strains, staphylococcus *aureus* and *staphylococcus SP* are the micro-organisms the most sensitiveto the higher inhibition zone: (21.46 mm) and (20.6 mm) respectively and lower value of MIC (0.33 mg/ml).On the other hand, we see from table (2) that E. *coli* and *Leisteria* were resistant to this essential oil.

According to the available bibliography, there were no studies carried out on the antimicrobial activity of the essential oil of *TeucriumPoliumaurasianum*, for that reason, the results of this study were compared to those obtained for other species of *TeucriumPolium*

The results obtained by [16] show an activity on E.coli and a resistance of the *Pseudomonas* unlike our results that doesn't show an activity on E.coli but active on *Pseudomonas* with a diameter of 10 mm of inhibition

For a same aromatic plants a different composition in the essential oils was found depending on the used parts, the harvest period, the geographic location and even as per the protocol of extraction [19]. This is explained by the concept of chemo-type that shows large quantitative and qualitative variability, which confirms the divergence of results reported for a given plant [20]

Table 2.Antimicrobial activity of the essential oil of*Teucriumpoliumaurasianum*

Microrganism	Inhibition zone (mm)	MIC (mg/ml)
E.coli	0	/
Leisteria	0	/
salmonella	14,76±0,25	0,66
Proleus	11,23±0,25	/
staphylococcus aureus	21,46±0,55	0,33
staphylococcus SP	20,6±0,53	0,33
Pseudomonas aeruginosa	9,73±0,25	/
Klebsiellapneumonia	14,9±0,17	0,5

1.10. Antioxidant Activity

1.10.1. DPPH

The results indicate that the percentage of inhibition of free radical increases with the increase of the concentration either for the ascorbic acid or to the essential oil of *TeucriumPoliumaurasianum*

We noticed that the percentage of inhibition of the free radical to the essential oil is lower than that of ascorbic acid for all used concentrations.

The IC_{50} is inversely related to the antioxidant capacity of a component, since it shows the quantity of antioxidant required to decrease the concentration of the free radical of 50 %. The lower value of IC_{50},means the higher antioxidant activity of a compound.

The essential oil of *TeucriumPoliumaurasianum*could make the free radical stable 2.2 diphenyl-1-picrylhydrazyl (DPPH) to the diphenyl-picrylhydrazine yellow colored with an $IC_{50}$of 58.6336 ± 0.7207 µg/ml showing an antioxidant activity lower than that of the ascorbic acid. It is noticed from this result that the antioxidant is more effective with an IC_{50} of 6.4584 ± 0.4256 µg/ml compared to the studied essential oil.

Table 3: The antioxidant activities of the essential oils of *teucriumpoliumaurasianum*

	DPPH IC_{50} (µg/ml)	FRAP EC_{50} (µg/ml)
Teucriumpoliumaurasianum essential oil	58.6336 ± 0.7207	48.1936±0.5628
Ascorbicacid	6.4584 ± 0.4256	66.3561±0.3471

[21]have found a value of IC_{50} of 95 µg/ml when analyzed the antioxidant effect of the essential oil of *TeucriumPolium*(Bejaia Algeria), which is lower than the antioxidant power of the essential oil of *TeucriumPoliumaurasianum*despite it is same species. On the other hand in comparing with other species *TeucriumPoliummarum*(Lamiaceae) from [22]they found a value of IC_{50} of 13.13 µg/ml which is higher compared to our study

1.10.2. The method of reduction of iron FRAP

These results show that the reducing power depends on the concentration of the essential oil; according to figure 1, it is noticed that the essential oil has a good reaction against Fe^{3+} which is present in the tested solution.

Figure 1 shows that with the concentrations of the essential oil from10 upto 70 µg/ml we recorded an increase of the absorbance from 0.196 to 0.613; however the same concentrations of ascorbic acid increases the absorbance from 0.069 to 0.539 which indicates a significantly greater power reduction than that of ascorbic acid.

The antioxidant activity of the essential oil would probably be related to the major components; the study of [23] has mentioned thatthe two following components: the limonene and the β-pinenerepresented important properties.

Limonene is a monoterpene that exists in citrus and is being used as flavoring agent in foods. It has been shown that the monoterpenes possess an antioxidant activity [24].

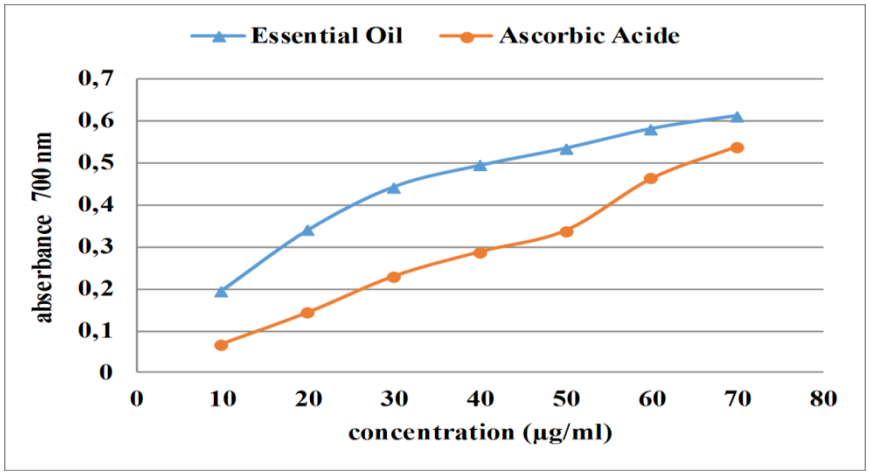

Fig .1. Reducing power of *teucriumpoliumaurasianum* essential oil

CONCLUSION

This contribution consists of the study and highlights the antimicrobial activity and the evaluation of bioactive potential of the essential oil of the medicinal plant *Teucriumpoliumsspaurasianum*that belongs to the family *Labiatae*

The yield of extraction of the essential oil obtained from aerial part of the plant *TeucriumPoliumaurasianum* is 0.585 % (w/w). This value is lower than the quantities obtained in other species of the same kind and higherthanother species

The antimicrobial tests carried out in this work illustrate the antibacterial effect of the essential oil of the plant *TeucriumPoliumaurasianum*on different bacterial strains; Gram-positive andnegative is significant on certain bacterial strains such as *salmonella, staphylococuisaureus, proleus, and pseudomonas;* and inactive on two other bacterial strains such as *E. coli, Leisteria.*

According to the performed activities tests, it can be determined that the essential oil of the plant *TeucriumPoliumaurasianum*represents a very strong antimicrobial power on pathogenic bacterial strains; finally, we found that the essential oil has a significant antioxidant activity with low concentrations; In the light of the acquired results it can be concluded that the essential oil of the plant *TeucriumPoliumaurasianum*represents a very strong antimicrobial power on the pathogenic strains and gives a new alternative within the biological fight using the essential oils.

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