THE CHEMICAL COMPOSITION AND THE ANTIMICROBIAL PROPERTIES OF THE ESSENTIAL OIL EXTRACTED FROM THE LEAVES OF *TEUCRIUM CAPITATUM* L.

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Received: 04 August 2016, Revised and Accepted: 22 October 2016

ABSTRACT

Objective: In the framework of the search for new biological molecules, an ethnobotanical study has been carried out in the region of El Hajeb in Morocco in the interest of will select the data ethnomedical on medicinal and aromatic plants and on the découvertes new principles assets. On the basis of a questionnaire prepared, a survey was conducted of the local population where we find a close relation which exists between the plant species described in the said region and the different types of diseases affecting the human being; six plants have been chosen to evaluate the antifungal activity of essential oils against the bacterial strain (*Staphylococcus aureus*), and on two yeasts: *Candida albicans* and *Candida glabrata*, which are the most frequently identified in clinical specimens, a mold: *Aspergillus niger*, and 5 dermatophytes: *Microsporum canis*, *Microsporum gypseum*, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Epidermophyton floccosum*.

Methods: The extraction of essential oils was carried out by steam distillation in a clevenger-type apparatus. The antimicrobial activity was determined by the disc diffusion method. The chromatographic analysis of HE five plants was performed with a gas chromatograph (GC) type Hewlett-Packard (6890) coupled to a mass spectrometer (HP5973).

Results: The results show that the essential oil of *Teucrium capitatum* L. has a large antimicrobial activity *vis-a-vis* other plants. Therefore, an identification of their chemical composition is necessary to identify the active molecules based on the analysis GC-only and coupled to mass spectrometry (GC-MS). The essential oil of the plant of *T. capitatum* L. of the region of El Hajeb (Morocco) was extracted to the study its performance, its chemical composition, and its property antibacterial and antifungal. The average content in essential oil of the leaves of this species is approximately 2% (wt) of the total weight of dry matter. The chromatographic analysis showed the profiles, and the rates of the different components compared to the available standards, as function of the retention time by ascending order, four compounds were identified by GC and GC/MS as the main compounds of this oil: Endo-borneol (33%), naphthalene, 1, 2, 3, 5, 6, 8a-hexahydro-4,7-dimethyl-1-(1-methyl ethyl)-, (1s-cis)-(19.63%), bronyle acetate (15.56%), alpha-terpineol (11.96%), bicyclo[3.1.0]hexan-3-ol, 4-methyl-1-(1-methylethyl)-(10.94%) among other 21 compounds.

Conclusion: This study allows, once again, the development of the exploitation of the essential oils in the areas, pharmaceutical and medicines and works the doors of the exploitation of these plants in the pharmaceutical market.

Keywords: *Teucrium capitatum* L., Ethnobotanical study, Antifungal activity, Chromatography.

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INTRODUCTION

The essential oils are complex mixtures consisting of several tens, see more than a hundred compounds, primarily of terpenes. These are constructed from several isoprene entites, constituting a very diverse family both at the structural level that functional [1].

Several studies on the bacteriostatic effect, spasmyltic and anti-inflammatory of *Teucrium* are reported in the literature. This effect is probably due to the presence of several monoterpenes of cyclopentanoide in their essential oil [2].

The genus *Teucrium* originates from the most important genera of the Lamiales family. This genus is distributed in 340 species and varieties surroundings. From the point of view of taxonomic, they are identifiable thanks to the form of the calyx and inflorescence [3]. It is a large genus which differs from the other than in its corollas formed of an upper lip coter and stamens restated above this slot so that the corolla seems only to have a lower lip to five lobes [4].

A large number of works have been published recently on the taxonomy of the genus based on morphological studies (florescences and calyx) [5], micromorphological, (trichomes) [2], but the relations within the group remain confused [6].
The extraction of essential oils has been carried out by hydrodistillation in a device of type Clevenger, 100 g powder of plant material with 500 ml of water has been distilled for 3 hrs. The performance has been determined for each plant. The essential oil has been stored at 4°C in dark in the presence of sodium sulfate anhydrous.

**RESULTS AND DISCUSSION**

The composition of the essential oils of the Lamiaceae is characterized by a large diversity between species. Each plant has its own fingerprints [22]. Hence, we can say that the composition of the essential oil of *T. capitatum* L. is characterized by the chemotype of the plant as in the case of other species aromatic. It also depends on the part of the plant used and its stage of growth as well as the nature of the soil and of the conditions of culture. The chemical composition of the essential oils of *T. capitatum* the samples studied is different from that of the other species, therefore, present a chemical polymorphism very important.

| Table 1: Chromatographic assay protocol |
|----------------------------------------|
| **Oven** | Injection temperature | 25°C  |
|          | Temperature of the interface | 25°C  |
|          | Initial temperature | 70°C  |
|          | Rise in temperature | 20°C/min  |
|          | Final temperature | 230°C  |
| **Column** | Characteristics of the column | VB-Wax (length 30 m, ID 0.25 mm, temperature limits 20-230°C)  |
|          | Vector gas | Helium  |
|          | Split | Split flow (1.0 ml/minute)  |
|          |       | Split ratio 10  |
| **Spectrometre de masse** | Temperature characteristics of the source | 200°C  |
|          | Temperature of the quadrupole | 200°C  |
|          | Mode of ionization | By electrons  |
|          | Energy of collision | 70 eV  |
|          | Mass range (UMA) | 50-650  |
|          | The ignition delay of the filament | 3 minutes  |
|          | Temperature characteristics of the source | Hexane  |
|          | Solvent | 1/100  |
|          | Dilution factor | 1 µl  |

According to these results, we note that the plant *T. capitatum* L., as other species of families Lamiaceae, is rich in various secondary metabolites.

**Extraction of essential oils**

The essential oil of yellowish color, of a very strong and persistent odor, has been obtained by hydrodistillation with a yield of extraction of 2%. The methods of analysis and identification employed during this study have helped to identify compounds (Table 3).

From the essential oil obtained from the plant *T. capitatum* L., 21 products have been able to be identified, which represents the essential oil. The results are shown on Table 1. The majority compounds are: Endo-borneol (33%), napththalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-{(1-m ethyl ethyl}-(1s-cis)- (19.63%), bronyle acetate (15.56%), alpha-terpineol (11.96%), bicyckl[3.1.0]hexan-3-ol,4-methyl-1-{(1-m ethy lethyl}- (10.94%).

**DISCUSSION**

The literature has shown a variation between the chemical compositions according to the geographical origin [16,17] of the species. For example, myrcene (15.3%), the germacrène D (9.0%), the α-pinene (6.6%), and the α-cadinol (5.1%) were the main components of the essential oil of *T. polium* L. Tunisia [16]. The main compounds reported for *T. polium* of Iran have been the α-pinene (12.52%), the linalool (10.63%), the oxide of caryophyllene (9.69%), the β-pinene (7.09%), and β-caryophyllene (6.98%) [18]. The major compounds of *T. polium* of Algerian Northwest were the germacrène D (25.81%), the bicyclogermacrène (13%), the β-pinene (11.69%), and carvacrol (8.93%) [19]. The major compound of the essential oil *T. polium* Jordan being the β-cedren-13-ol (24.8%) [20]. In the case of the essential oil of *T. polium* also investigated by Hussain et al, 2013 [21], the percentages of majority compounds are the following: The lendenoxyl (II) (20.47%), acetate of linalylene (11.16%), the β-udesmol (11.59%), and α-trans-bergamatene (6.81%).

Table 1: Chromatographic assay protocol
Table 2: Result of the chemical tests of the plant *Teucrium capitatum* L.

| Nom des composés | Nom de test | *Teucrium capitatum* L. |
|------------------|-------------------|-------------------------|
| The alkaloids     | Mayer dragendorff | +                       |
| Tannins          | Diluted solution of ferric chloride | + |
| Catechins tannins| Concentrated HCl  | +                       |
| Flavonoids:      | Reaction of stiasny | +                       |
| Anthocyanins     | H$_2$SO$_4$/NH$_3$OH | +                       |
| Flavones and flavonoids free (genine) | Reaction to the cyanidin | + |
| Leucoanthocyanes  | Reaction to the cyanidin without mg | - |
| Sterol and terpenes| Chloroform/acetic anhydride/H$_2$SO$_4$ | + |
| Compounds reducers| Reagent of fehling | +                       |
| Oses and holosides| H$_2$SO$_4$/ethanol/thymol | -                       |
| Cynogenetique glycosides | Toluene | -                       |
| Anthraquinone free| Chloroform/NH$_3$OH | +                       |
| Anthraquinone combined: |          |                         |
| 0-glycosides     | HCl/NH$_3$OH      | -                       |
| C-glycosides     | FeCl$_3$/NH$_3$OH | -                       |
| Saponins         | Index of foam*    |                         |

Table 3: Chemical composition of essential oil of the *Teucrium capitatum* L.

| IR | Constituents                                      | Formula | Percentage |
|----|---------------------------------------------------|---------|------------|
| 4.02 | 2,4-dihydroxycacetophenone                         | C$_4$H$_6$O$_7$ | 0.28 |
| 4.16 | Bicyclo[3.1.0]hexan-3-ol, 4-methyl-1-(1-methylmethyl)- | C$_4$H$_6$O$_7$ | 10.94 |
| 4.40 | Endo-borneol                                        | C$_4$H$_6$O$_7$ | 33.00 |
| 4.57 | Alpha-terpineol                                    | C$_4$H$_6$O$_7$ | 11.96 |
| 4.81 | Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl-[1S-([1a,2a,5a])- | C$_4$H$_6$O$_7$ | 0.26 |
| 5.24 | 4-cyclohexylidene acetate                          | C$_4$H$_6$O$_7$ | 15.56 |
| 5.42 | Phenol, 2-methyl-5- (1-methylmethyl)                | C$_4$H$_6$O$_7$ | 0.88 |
| 5.52 | Fumaric acid, dimyryl ester                        | C$_4$H$_6$O$_7$ | 0.40 |
| 5.93 | Copaene                                            | C$_4$H$_6$O$_7$ | 0.45 |
| 6.00 | Alpha-ylangene                                     | C$_4$H$_6$O$_7$ | 0.32 |
| 6.26 | 15,25,5R-1,4,4-trimethyltricyclo[6.3.1.0 2,6][12]ene | C$_4$H$_6$O$_7$ | 0.42 |
| 6.75 | Longifolene                                        | C$_4$H$_6$O$_7$ | 0.34 |
| 6.18 | Retinoidic acid, methyl ester                      | C$_4$H$_6$O$_7$ | 0.70 |
| 6.90 | Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methyl ethyl)-(1S-cis)- | C$_4$H$_6$O$_7$ | 19.63 |
| 7.12 | Retinol                                            | C$_4$H$_6$O$_7$ | 0.98 |
| 7.86 | 2-[(4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol | C$_4$H$_6$O$_7$ | 0.35 |
| 8.12 | Ethyl5,8,11,14,17-icosapentaenolate                | C$_4$H$_6$O$_7$ | 0.40 |
| 8.26 | 3,5-androstadien-17-one oate                       | C$_4$H$_6$O$_7$ | 0.59 |
| 8.58 | Butyl 4,7,10,13,16,19-docosahexaenolate             | C$_4$H$_6$O$_7$ | 0.51 |
| 8.76 | Pregnenolone                                       | C$_4$H$_6$O$_7$ | 0.40 |
| 8.97 | Aromadendrene, dehydro-                            | C$_4$H$_6$O$_7$ | 0.30 |
| Total |                                                |         | 98.56%      |

Relationship antimicrobial activity/active principles

The great diversity of the structures and functions of terpenoids has caused an interest for their use in traditional medicine and modern.

According to the study antifungal drug [25], it was found that the essential oil of *T. capitatum* has a large antibacterial activity with diameters of inhibition varies between 40 mm and 43 mm. It has exercised a significant inhibitory activity vis-a-vis all fungal, the strains *Trichophyton rubrum* and *Trichophyton mentagrophytes*, and *Epidermophyton floccosum* were inhibited completely from the minimum concentration of 32.26 µL/mL and for strains *Microsporum gypseum* and *Candida glabrata* from 20.41 µL/mL whereas the concentration of 15.87 µL/mL has been sufficient to stop the growth of *Candida albicans*, *Microsporum canis*, and *Aspergillus niger*, the results of each minimum concentration. The report of the minimum inhibitory concentration and the minimum concentration fungicide presented gave as a result that the plant of *T. capitatum* The exerts a fungicidal activity for all strains, after the results were chromatographic was able identified the major compounds that are known by their principles assets; The biological activity of the active principles natural between essential oils is linked to their chemotype, i.e., the or the biologically active molecules and predominantly present; their composition or the functional groups of the compounds in the majority (alcohol, phenols, and terpenic compounds ketone) and to their synergistic actions. This antibacterial activity of natural substances is explained by the lysis of the bacterial membrane; the He, flavonoids, the alkaloids or even the tannins could induce a leak of potassium ions at the membrane level and by way of the consequences of the irreversible lesions at the level of this membrane. This permeability to potassium effect is a precursor of their death [26], therefore, the high content of the essential oil in terpene alcohols, antioxidants, anti-allergens, antispasmodics, antineoplastic, anti-inflammatory, and immunomodulating [24].

According to these results, the essential oil of the plant of *T. capitatum* showed a antimicrobial activity important on the strains studied.
This activity can be attributed to the richness of the chemical composition of phenolic compounds. The results obtained are promising and encouraging because there is a strong correlation: Active compounds/antifungal activity.

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