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PHYTOCHEMICAL CHARACTERIZATION OF THE ESSENTIAL OIL OF *Hyptis martiusii* Benth's (cidreira-brava) LEAVES, BY GAS CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

Isaac Moura Araujo* [1], Paulo Ricardo Batista [1], Daniel Souza Bezerra [2], Francisco Rodolpho Sobreira Dantas Nóbrega de Figueirêdo [3], Gyllyandeson de Araujo Delmondes [3] , Maysa de Oliveira Barbosa [4], Cícero Francisco Bezerra Felipe[5], Marta Regina Kerntopf [6]

[1] Graduation in Biological Sciences at the Regional University of Cariri - URCA; Crato - Ceará, Brazil. E-Mail: pauloricardoadauto@outlook.com;
[2] Postgraduate Program in Biological Chemistry, Regional University of Cariri - URCA; Crato - Ceará, Brazil; E-Mails: danielbezerra02@gmail.com;
[3] Postgraduate Program in Molecular Bioprospecting, Regional University of Cariri - URCA; Crato - Ceará, Brazil; E-Mails: gyllyandesondelmondes@gmail.com; rodolpho@gmail.com
[4] Postgraduate Program in Ethnobiology and Conservation of Nature by Federal Rural University of Pernambuco - UFRPE; Recife – Pernambuco, Brazil E-Mails: maysabarbosa.ce@gmail.com;
[5] Professor at the Federal University of Paraíba - UFPB; João Pessoa – Paraíba, Brazil; E-Mails: cicerof@hotmail.com;
[6] Professor in the Department of Biological Chemistry at the Regional University of Cariri; Crato - Ceará, Brazil; E-Mails: martaluiz@yahoo.com.br;
***** E-Mail: isaac.faculdade@hotmail.com;
Tel.: +55 (88) 997650939.

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Abstract: Among the great Brazilian biodiversity, there are innumerable species of plants that have been traditionally used to treat various diseases. However, great amount of them have not been validated yet, regarding its pharmacological potential, mainly due to the absence of chemical analyses of the plant's components. Therefore, it is emphasized the significance of phytochemistry in the search of knowledge and identification of active chemical compounds which may influence the pharmacological responses of plants with medicinal interest. Thus, the objective of this study was to identify the major compounds present in the essential oil of *Hyptis martiusii* Benth (OEHM). The oil extraction was made using 250g of fresh leaves and 1,5L of water, maintained on ebullition for two hours in a 5L round-bottom flask. The resulting mixture of water and oil was collected in a modified Clavenger apparatus, than it was separated and dried with anhydrous sodium sulfate (Na_2SO_4). The chemical analysis of OEHM was performed by gas chromatography coupled to mass spectrometry (GC/ME) and its compounds identification were performed by comparing the obtained mass spectrum with already existing patterns in literature. Twenty compounds were identified in OEHM,

where mono and sesquiterpenes were the most representative (93,99% of the oil composition), among them, 1,8-cineol was characterized as the major compound. Analysing other studies, it was observed that many representatives of the genus *Hyptis crenata* have presented similarities, regarding chemical composition of the essential oils. For instance, *Hyptis crenata* Pohl has, among others, camphor, 1,8-cineole, α-pinene, β-caryophyllene, which have also been identified in OEHM. Thus, it is emphasized the importance of researching on its isolated compounds, in order to know its possible pharmacological properties.

Keywords: Medicinal plants; Lamiaceae; *Hyptis crenata* Pohl; Phytochemical.

1. Introduction

Amid the great Brazilian biodiversity, it is possible to find the use of innumerable plants for the purpose of healing and application in the therapy of many pathologies. However, many have not yet been validated on their pharmacological potentials, mainly due to the lack of chemical analysis of the elements present. Phytochemical research is especially important when all chemical studies with species of popular interest are not yet ready, aiming to know the chemical compounds of the plant species and to evaluate their presence in them, identifying groups of relevant secondary metabolites (Simões et al., 2004).

In these researches, the selection of the species to be studied should take into account the popular indication of medicinal use and the joint work of a team that can print a common effort to identify the species, isolate compounds, and identify active substances, selection and implementation of pharmacological (BRITO, 1996).

Phytochemical screening is an important procedure for bioprospecting plant species of pharmacological and / or toxicological interest. The chemical composition of an extract can be known through qualitative and rapid chemical tests of low cost, suggesting the possible classes of secondary metabolites of interest (Mattos, 1997).

Therefore, the present work aimed to identify the major components present in the essential oil of Hyptis martiusii Benth (EOHM).

2. Results and Discussion

The essential oil obtained by hydrodistillation gave a yield of 0.72%, and density of 1.0 g / mL. GC / MS analysis allowed the identification of 20 constituents, exclusively occurring in mono and sesquiterpenes, representing 93.99% of the oil composition (table 1).

With respect to the chemical analysis of the oil of the species Hyptis martiusii Benth, 20 chemical compounds were identified, all of them of the terpene class. Among these compounds, there are the presence of terpenes: α-pinene, β-pinene, limonene, among others mentioned in Table 1. As the major compound, the terpene, 1,8-cineol, was found. It has already been shown that many representatives of the Lamiaceae family show similarity to the chemical composition of their essential oils. Menichini et al. (2009) identified a great richness of terpenes in four species of Teucrium (Lamiaceae), with emphasis on α-cadinene, caryophyllene oxide, α-pinene. Satureja hortensis, another Lamiaceae, presented in its essential oil the α-pinene and β-pinene compounds (HAJHASHEMI *et al.*, 2012).

Among the studies already carried out with some species of the Hyptis genus, some of these chemical components and many others have been identified in their constitution, attributing various pharmacological effects. It is the case of the presence of camphor, 1,8-cineol, α-pinene, β-caryophyllene in Hyptis crenata Pohl (DINIZ et al., 2013); (+) - carene, trans-β-caryophyllene, germanrreno in Hyptis suaveolen (L.) (MOREIRA et al., 2010); of α-pinene, β-pinene, cineol in Hyptis spicigera Lam (TAKAYAMA et al., 2011).

Table 1. Chemical constitution of the essential oil of the leaves of *H. martiusii* Benth. The majority of the compounds present in OEHM are shown in bold.

Compounds	(%)	TR (Min)
α-pinene	2,5	12,5
β-pinene	1,3	14,9
β - ocimene	23,4	16,84
p-cymene	2,5	17,62
limonene	5,3	17,9
1,8-cineole	**25,93**	**18,1**
camphor	3,88	25,04
β - caryophyllene	0,81	41,11
bicyclogermacrene	3,15	45,89
δ-cadinene	1,79	46,65
valencene	1,42	47,17
palustrol	1,39	47,7
espatulenol	2,15	47,86
caryophyllene oxide	6,93	47,98
guaiol	2,1	48,1
β - eudesmol	1,62	48,23
ledol	1,68	48,28
torreiol	1,28	48,55
aromadendrene	2,53	48,7
viridiflorol	3,63	48,91
TOTAL	93,99	

3. Materials and Methods

3.1 – Obtaining essential oil

Extraction of the oil was performed using 250 g of the fresh leaves which were placed in a 5 L glass flask along with 1.5 L of water and kept boiling for a period of two hours. After the boiling period the essential oil was extracted from the vegetable and condensed to form a heterogeneous mixture with water. The mixture consisting of water and oil was collected in a modified Clevenger type apparatus (GOTTLIEB; MAGALHÃES, 1960) then separated and dried with anhydrous sodium sulfate (Na_2SO_4).

3.2 Essential oil chemical analysis (Gas chromatography coupled to Mass Spectrometry)

The chemical composition of the OEHM was performed using a gas chromatography coupled to a mass spectrometer (GC / MS) in SHIMADZU apparatus with a mass selective detector QP5050A, operating under ionization energy of 70 eV. The capillary column used was DB-5HT, in the following specifications: temperatures of 270 ºC in the injector and 290 ºC in the detector with helium as drag gas (1.7 mL/min); linear velocity of 47.3 cm/sec; total flow 24 mL/min; carrier flow 24 mL/min; 107.8 kPa pressure; and the column heating temperature was programmed to 60 ° C (2 min) - 180 °C (1 min) at 4 ° C/min and 180 - 260 °C at 10 °C/min (10 min).

The identification of the components was performed by comparing their respective mass spectrum with those standards recorded in the Wiley library database 229 and among the retention indices calculated with values from the specialized literature (ADAMS, 1991).

4. Conclusions

Analyzing others studies, it was noticed that many representatives of the genus Hyptis have similarities, regarding the chemical composition in essential oils. Hyptis crenata Pohl has, among others, camphor, 1,8-cineol, α-pinene, β-caryophyllene, which were also evidenced in the OEHM. Thus, it is emphasized the importance of research on the components in isolation to know the possible pharmacological properties.

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Author Contributions

The authors contributed to the tests, writing, translation and work orientation.

Conflicts of Interest

The authors declare no conflict of interest.

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