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Preliminary analysis on volatile composition of *Chrysopogon zizanioides* (L.) Roberty cultivated in Tunisia

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Abstract – The roots of *Chrysopogon* zizanioides (L.) Roberty, belonging to the Poaceae family, are the main source of volatile that has substantial value in perfume, cosmetics, pharmaceutical and food industries. The study of *C. zizanioides* volatiles, obtained from plant roots cultivated in Tunisia, using GC and GC/MS revealed that more than 50 compounds were detected of which 14 constituents were identified representing 33.87% of the total. Oleic acid (17.09%) and (E) -3-Eicosene (7.77%) were the major components at the species level. However, 10,13 methyl ester octadecadienoic acid (0.01%), (Z) - 9,17-octadecadienal (0.01%), (Dimethylethyl-1,1) Bis-2,6 Methyl- 4 Phenol (0.04%), methyl pentadecanoic acid, 13-methyl-, methyl ester (0.05%), (Z) -13-octadecenal (0.06%) and linoleic acid ethyl ester (0 , 06%) are the constituents which have the lowest contents.

Keywords: Vetiver, Volatile, Perrenial crop, Tunisia

Introduction

Chrysopogon zizanioides (L.) Roberty (Vetiver), (2n=20) is a Poaceae family member (Peng et al. 2014). It is a sterile plant multiplied by burst of stumps (Rao et al. 2000). Native to India, the species was spread worldwide in the last century for many purposes (Lawrence 2016). The species is well known to prevent soil erosion and showing a highly drought-tolerance due to its substantial vertical root system (Weyerstahl et al. 2000). Moreover, the species is used not only as a phytoremediation agent for metal-contaminated soils but also for animal bedding and feed (Slinger 1997; Chiu et al. 2006). In addition, almost all parts of *C. zizonoides*, are exploited in traditional medicines to treat notably flu, colic, nausea pleurisy and to dissolve gallstones (Chomchalow and Chapman 2003).
The multipurpose species is mainly cultivated to produce essential oil (Danh et al. 2010). Its roots are usually steam distilled to obtain essential oil (Maffei 2002). *C. zizonoides* EO has exhibited sweet and persistent notes (Sreenath et al. 1994). Because of EO’s composition intricacy, *C. zizonoides* EO could not be reproduced by chemical synthesis (Chahal and Bhardwaj 2015). In this way, *C. zizonoides* EO has integrated numerous fragrances and widely used in aromatherapy as a result to its regulatory activity on skin and sedative qualities (Filippi et al. 2013).
To the best of our knowledge, this paper constitutes the first survey that investigates the volatile composition obtained from cultivated C. *zizonoides* roots in Tunisia. It is a part of wider study concerning the enhancement of this species in order to elaborate agricultural programs.

2. Materials and methods

C. zizonoides was cultivated in the experimental station of the Higher School of Agriculture of Mateur (37°3'0'' N, 9°37'12'' E). The site is characterized by a loamy-clay soil and a subhumid climate with hot summers and cold winters.

2.1. Volatiles extraction

Roots of *C. zizonoides* are submitted to the analysis of the chemical composition of essential oil. 20 g of root samples were finely ground in liquid N_2 and then macerated in 100 ml of hexane for 24 h. After maceration, extracts were transferred to test tubes and centrifuged at 1300 rpm for 10 min. The supernatant was dried by evaporation using a rotary evaporator (50 ˚C) and then diluted in 1.5 ml of hexane before analysis. Measurements were repeated twice for each sample.

2.2. Gas Chromatography and Gas Chromatography/Mass Spectrometry Analysis

Gas Chromatography analysis was accomplished with a gas chromatograph AGILENT 6980 series II system fitted with a HP-INNOWAX cap. column (30 m-0.25 mm, 0.25 mm film thickness). The oven temperature was programmed from 50 ˚C to 250 ˚C at a rate of 8˚C /min and then held isothermal for 10 min. Temperatures of injector and FID detector were 220˚C and 280˚C, respectively. The carrier gas was He at a rate flow of 2ml/ min. Samples (2 ml) were injected using a split-sampling technique.
GC/MS Analyses were performed on a Hewlett-Packard 5972 mass spectrometer connected to a Hewlett-Packard 5890 series II gas chromatograph, which was equipped with a HP- INNOWAX MS capillary column (30 m x 0.25 mm; film thickness 0.25 mm). He was used as carrier gas at a rate flow of 1.2 ml/min. The oven temp. was programmed from 50 ˚C to 250 ˚C at a rate of 5 ˚C /min and kept at 250 ˚C for 10 min. MS was performed at 1 scan s-1 with ionizing voltage of 70 eV and ion source temp. of 250˚C.

2.3. Compound Identification

Volatile components were identified by comparing their retention times with those of some authentic standards injected under the same chromatographic conditions and by comparison of their retention indices (RI), relative to alkanes, and their mass spectra with the HP Chemstation database HP NBS 75 K. L. Library. The quantification of the compounds was accomplished from their GC peak areas without correction factors.

3. Results and discussion

Our work focused on the preliminary analysis of *C. zizonoides* volatiles obtained from roots using GC and GC/MS. More than 50 compounds were detected of which 14 constituents were identified representing 33.87% of the total oil (Table 1). The analysis of the chemical composition of the extract obtained from the roots is characterized mainly by oleic acid (17.09%) and (E) -3-Eicosene (7.77%). While, 10,13 methyl ester octadecadienoic acid (0.01%), (Z) - 9,17-octadecadienal (0.01%), (Dimethylethyl-1,1) Bis-2,6 Methyl- 4 Phenol (0.04%), methyl pentadecanoic acid, 13-methyl-, methyl ester (0.05%), (Z) -13-octadecenal (0.06%) and linoleic acid ethyl ester (0, 06%) are the constituents which have the lowest contents.
Owing to the many usages of *C. zizonoides* and its products, studies have been conducted on the chemistry of this plant, with emphasis on *C. zizonoides* root volatile (Lavania 2003; Singh et al. 2011; Chou et al. 2012). Champagnat et al. (2006) reported that 110 constituents were identified in the chemical composition of *C. zizonoides* root volatile from nine countries (Brazil, China, Haiti, India, Java, Madagascar, Mexico, Reunion and Salvador). The characteristic constituents were β-vetispirene (1.6–4.5%), khusimol (3.4-13.7%), vetiselinenol (1.3- 7.8%) and α-vetivone (2.5- 6.3%). Whatever the geographical origin, statistical analyses data confirmed the relative homogeneity of chemical composition of *C. zizonoides* root volatile.
In addition, roots of *C. zizonoides* planted in three different cultivation systems (normal soil, normal soil with added microbes and semi- hydroponically) were extracted using a simultaneous steam distillation and solvent extraction (SDE) apparatus. 42 compounds were identified in total. Volatile component profiles of the oils obtained by normal soil and semi-hydroponic cultivation were similar, whereas a quantitative difference was noted in some major volatiles when the cultivation system containing microbes was utilized (Pripdeevech et al. 2006). Moreover, Belhassen et al. (2014) revealed that *C. zizonoides* voatile was very complex and consisted of more than 300 compounds and not all the components have yet been identified.

Table 1. Mean percentage of volatile [%] at Species (P_S) Level of vetiver roots

Constituents		CAS Num	RT	(P_S)
1-	• (Dimethylethyl-1,1) Bis-2,6 Methyll-4 Phenol	128-37-0	15,375	0,04
2-	Pentadecanoic acid, 13-methyl-, methyl ester	5487-50-3	20,811	0,05
3-	10,13-Octadecadienoic acid methyl ester	56554-62-2	23.100	0,01
4-	(Z)-13-octadecenal	58594-45-9	23.666	0.06
5-	Linoleic acid ethyl ester	544-35-4	23.723	0.06
6-	(Z) - 9,17-*Octadecadienal*	56554-35-9	23.826	0,01
7-	(E)-9-Octadecenoic acid	112-79-8	25.697	0.97
8-	Oleic Acid	112-80-1	25.835	17.09
9-	(E)-3-Eicosene	74685-33-9	25.903	7.77
10-	(Z)-9-Octadecenoic acid, 2,3-dihydroxypropyl ester	111-03-5	29.497	0.17
11-	Acetic acid, bicycle-hept-2-en-7-ylidene	126395-65-1	29.909	0.34
12-	1,8,15,22-Tetraoxo-2,7,16,21-tetra-oxocyclo-octacosan	4238-35-1	34.509	2.58
13-	cis-11-Hexadecenal	53939-28-9	34.572	0.96
14		1000130-90-4	36.048	3.81
Total				33.87

CAS Num: It is the unique registration with the chemical abstracts service database. RT: is the time that a solute spends in a column or it can be defined as the time spent in the stationary and mobile phases.

4. Conclusion

C. zizanioides has been utilized traditionally as medicinal and aromatic plants in many countries mainly in Asia. The production of essential oil from *C. zizanioides* roots allowed to feed the perfume industry. However, its abundant use, the molecules responsible for its odor is still a subject of controversy. As a first step, the study of *C. zizonoides* EO roots cultivated in Tunisia has permitted to identify fourteen compounds. In our future work, we will continue to determine the spectrum of essential oil of the species and its biological activities.

5. References

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