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CHEMICAL COMPOSITION OF VOLATILE ORGANIC COMPOUNDS OF AN EXTREMELY RARE AND ENDEMIC ALGERIAN APIACEAE SPECIES, *BUNIUM CRASSIFOLIUM* BATT.

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Abstract: *Bunium crassifolium* Batt. (*B. crassifolium*) (Apiaceae) is an extremely rare endemic species from the North East of Algeria. In this study, we extracted the volatile organic compounds (VOC) of *B. crassifolium* Batt. aerial parts using an Agilent G1888 network headspace sampler coupled with an Agilent 7890 GC system. The results revealed the presence of twenty-two (22) compounds, twenty (20) of which were identified as representing 97.48% of the total composition, the major components are: 44.67% of β-Cubebene, 8.82% of β-Caryophyllene, 7.04% of γ-Elemene, 4.70% of δ-Cadinene, 4.11% of γ-Cadinene, 3.77% of Ascaridole and 3.33% of β-Elemene, along with other constituents at a relatively low amount.

Keywords: *Bunium crassifolium*, Apiaceae, volatile organic compounds (VOC), GC-MS and GC-FID

1. Introduction

The genus of *Bunium* belongs to the family of Apiaceae, and represented about fifty species distributed in North Africa and Europe, to the central and southwest Asia. It contains medicinal and aromatic plants such as *B. persicum* (Hajhashemi et al., 2011) and *B. bulbocastanum* (Haroon et al., 2014). In Algeria, genus of *Bunium* contain seven species, four of which are endemic such as *B. crassifolium* Batt., *B. elatum* Batt., *B. chaberti* Batt., and *B. fontanessii* (Pers.) Maire (Quezel and Santa, 1963). *B. crassifolium* Batt. is an endemic and rare extremely species distributed and growing in the North-East of Algeria. It is a perennial plant 30-60 cm in height. The leaves are pinnatisect with long linear divisions of 2-4 cm. Flowers in large umbels 7-10 cm. Fruits are blackish, nearly as wide as long, with very marked primary ribs, keeled on the back and sometimes a little winged (Pottier-Alapetite, 1979).

In Algeria, bulb of the genus *Bunium* were used by some people as a food in colonial period in the nineteenth century. During this period, the Atlas Mountains people use to dry the bulb of *B. incrassatum* in the sun in the form of powder to use it as flour mixed with barley and some wheat to consume it as bread or couscous and sometimes without any other flour (Souilah et al., 2021).

Volatile organic compounds studies previously on species of genus *Bunium* L. reported the presence of a broad range belonging to a lot of classes such as non-terpenoids and terpenoids, which are applied in different medical areas and pharmacological (Mohammadhosseini, 2017a; Mohammadhosseini et al., 2017b; Mohammadhosseini et al., 2021; Zhang et al., 2021).

Different species of the genus *Bunium* usually are potential sources of secondary metabolites and possess a pungent odor, mainly terpenoids, which constitute the volatile organic compounds presented in their secretory glands (Mohammadhosseini et al., 2021), from various organs such as fruit, flowers and leaves (Tholl et al., 2006). A large wide of reports are found in the scientific researchers with the quantitative and qualitative characterizations of several species of *Bunium* (Mohammadhosseini et al., 2021).

Recently, the interesting of scientific researchers in the chemical composition of essential oil of various plants (VOCs) has led the progress of a several systems for the extraction and analysis of volatiles compounds. The developed headspace sampler for Gas Chromatography–Mass Spectrometry (GC-MS) explorers more volatile compounds profile of fresh plants than classic methods by solvent extraction or steam distillation.

In our case, this method is most appropriate because we only have a small amount of a plant endemic and extremely rare (*B. crassifolium* Batt.). To the simplest of our knowledge, the chemical composition of organic volatile compounds (VOCs) of *B. crassifolium* species has not been reported before. For this reason, our study aimed to explore the VOCs for the first time to know their health benefits.

2. Materials and methods

Plant material

Samples of the aerial parts of *B. crassifolium* Batt. were collected during the flowering period in Sérraïdi (Annaba, Algeria), during the month of May (**Fig. 1**). The plant was identified by Dr. Tarek Hamel lecturer in the department of Plant Biology and Environment at University of Annaba (Algeria). A voucher specimen was deposited in the herbarium of the University of Constantine 1. Samples were shade dried, then they were cut into smaller pieces.

Analyses of volatile organic compounds

Volatile organic compound (VOCs) analyses of *B. crassifolium* aerial parts were performed using an Agilent G1888 network headspace sampler coupled with an Agilent7890A GC system coupled also with an Agilent 5975C inert triple-axis MS detector.

Fig. 1. *B. crassifolium* Batt. (Aerial part and bulb) (Original)

Results were evaluated as percentage (%) area of VOCs of *B. crassifolium*. The oven temperature program, using an Agilent DB-WAXETR column (60m×0.32mm, 0.25µm), was as follows: between 60 to 150°C at 5°C.min−1, 2min hold; between150 to 220°C at 3°C.min−1, 2min hold. The injection volume was 1µL and the carrier gas was helium at a flow rate of 1.5mL min−1. To perform the analyses, 2g aerial part samples of *B. crassifolium* were placed in 20 mL glass vials and sealed with aluminum seals and polytetrafluoroethylene septa (Koldas et al., 2015).

Components were identified by matching their mass spectra with those in the NIST and Wiley libraries.

3. Results and discussions

The composition and percentages of different compounds are summarized in **Figure 1** and **Tables 1 and 2**. The compounds are classed with their order of retention time. Twenty (20) compounds were identified, corresponding to a total of 97.48%: One (1) oxygenated monoterpene, fifteen (15) sesquiterpene hydrocarbons and four (4) oxygenated sesquiterpenes. Moreover, the results of all scientific literature studied the genus of *Bunium* reported that the number of VOCs were ranged between 9 and 48. Higher values have been recorded for *B. wolffi* Klyuikov from India with the presence of 48 components, *B. ferulaceum* Sm. from Algeria with 40 components and *Bunium* spp from Iran with 37 components. Lowest value has been detected for *Bunium* spp. from India with 9 components (Mohammadhosseini et al., 2021).

The components of essential oils showed several important roles of natural substances for health, such as resistance to diseases and against insects (Gershenzon & Dudareva, 2007), physiological function of growth, development and ecological function (Wink, 2003). They also possess antimicrobial, anti-oxigenic, anti-mycotic, antiviral, anti-parasitic and insecticidal properties (Bishop, 1995; Juglal et al., 2002; Lamiri et al., 2002; Moon et al., 2002; Michaelakis et al., 2007). Regarding phyto-pathogenic viruses, several substances of natural and synthetic origin have been assessed for their anti-phytoviral activity (Yordanova et al., 1996; Rusak et al., 1997; Othman & Shoman, 2004; Krcatović et al., 2008).

The results of VOCs were dominated by a large amount of sesquiterpenes with a 91.58% of the total of compounds identified (97.48%). Divided into 87.87% sesquiterpene hydrocarbons and 3.71% oxygenated sesquiterpenes. Bezic et al., (2011), demonstrated that sesquiterpene are potent inhibitors of Cucumber Mosaic Virus (CMV) infection and bio-substances in the control of plant virus diseases.

The results indicate that the aerial part of *B. crassifolium* is a potential source of producing volatile profiles of sesquiterpene hydrocarbons where the main compounds are: β -cubebene with 44.64%, β -Caryophyllene with 8.82%, β -Elemene with 7.04%, δ-Cadinene with 4.70%, γ-Cadinene with 4.11%, and β -Elemene with 3.33% and oxygenated sesquiterpenes where the majority compounds are: cis-sesquisabinene hydrate with 1.44% and aromadendrene oxide with 1.22%. We notice the conspicuous absence of monoterpene hydrocarbons, products that make up the major part of the essential oils of higher plants. Unfortunately, the only component identified monoterpene oxygenated was ascaridole with 3.77%. This compound with high toxicity that avoids the usage of oils containing it internally or externally as well, except as plants' infusions, given its very low water solubility.

The β -cubebene was recognizable as an induced chemical plant defensive and beneficial attract organisms. The variability of chemical compounds in plants as well as of biotypes or races in insects was considered as a response to selection pressure exercised by insects and flora on each other; flora synthesize new chemicals compound against insects and insects elaborate detoxification systems, preparing each to enter on new zone of adaptation (Murugesan et al., 2012).

The team of Hernandez-Leon (2020) showed that the β-caryophyllene, had a potent agonist selective of subtype 2 receptor of cannabinoid, for that was considered such as a neuropathic pain model in the central nervous system and against nociceptive activity (Segat et al., 2017). Any psychoactive responses are related with central nervous system effects of β-caryophyllene; for that it is reported as a neuroprotective compound (Machado et al., 2018). In addition, it was considered as an anti-inflammatory by oral administration (Klauke et al., 2014). β-elemene was considered as an antitumor drug extracted of *Curcuma wenyujin* by traditional Chinese medicine. The β-elemene exerts its effects by inhibiting and arresting cell proliferation, provoking cell apoptosis and boosting the immune system (Zhai et al., 2018).

Ascaridole has been reported as antifungal, sedative, anti-parasitic and antimalarial (Okuyama et al., 1993). Pollack et al. (1990), reported that ascaridole inhibit the development of some bacteria *in vitro* such as: *Trypanosoma cruziand*, *Plasmodium falciparum* and *Leishmania amazonensis*. Moreover, Valery et al. (2008) reported its exhibition against various tumor cell lines *in vitro* (CCRF-CEM, MDA-MB-231 and HL60).

Volatile organic compounds of *B. crassifolium* also contain a highly oxidative product carbamic acid synonym of butylated hydroxytoluene (BHT). The BHT was considered as a synthetic antioxidant used to prevent oxidative deterioration in fatty foods and fat (Addis, 1986), and applied also in many commercial foods and constitute a little part of many people diet (Hocman, 1988). BHT was reported as anti-carcinogens in several animal models (Wattenberg et al., 1980; Wattenberg, 1986; Williams, 1986; Hocman, 1988; Williams and Iatropoulos, 1996). On the other hand, BHT has carcinogenic effects in experimental animals (Ito et al., 1983; Williams, 1986; Hocman, 1988), especially in the liver of mice and rats (Verhagen et al., 1991; Clayson et al., 1993 and Papas, 1993).

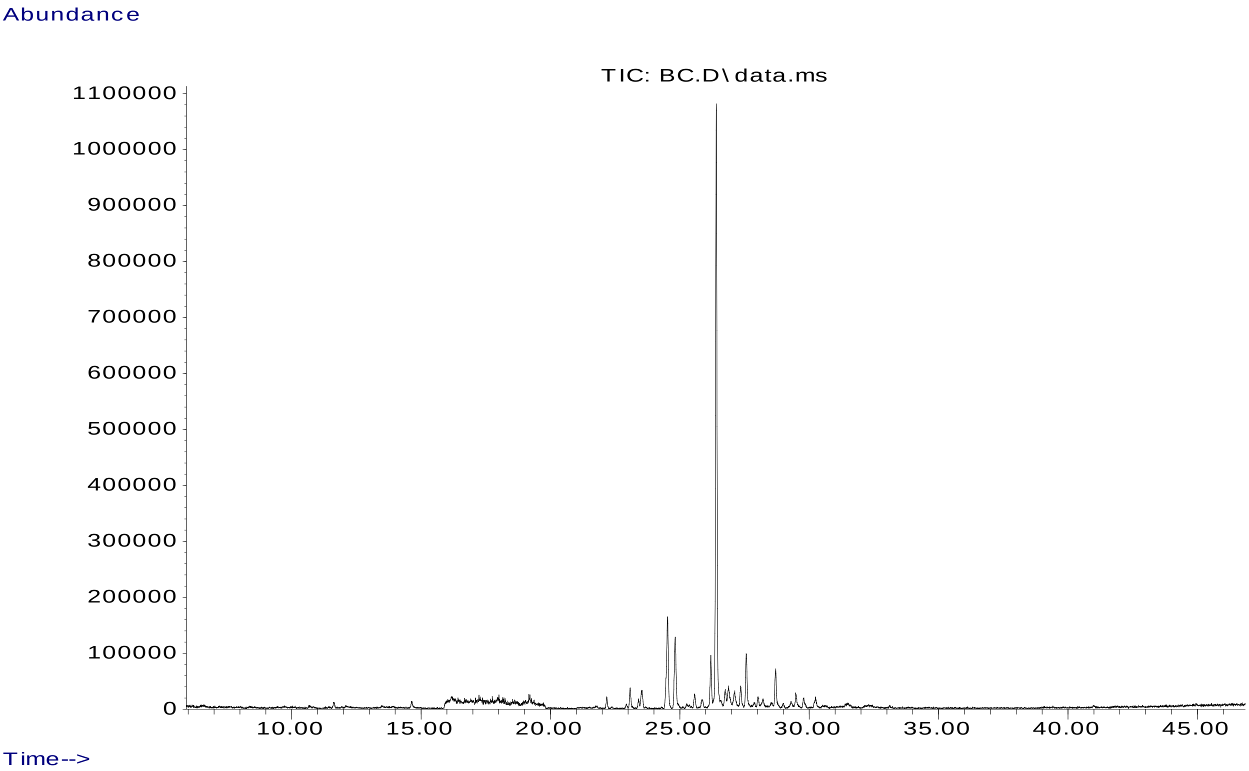

Fig. 1. The spectrum of GC-MS analysis of the oil of *B. crassifolium*

Table.1. Volatile organic compounds of *B. crassifolium* Batt. determined by HS-GC/MS

N°	RT(mn)	Formula	Class	Product	%
1	16.185	$C_{10}H_{16}O_2$	OMT	Ascaridole	3,77
2	17.283	-	N.I	-	1,68
3	22.174	-	N.I	-	0,82
4	23.081	$C_{15}H_{24}$	STH	α-Copaene	1,60
5	23.532	$C_{15}H_{24}$	STH	1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethenyl)	2,52
6	24.525	$C_{15}H_{24}$	STH	β –Caryophyllene	8,82
7	24.819	$C_{15}H_{24}$	STH	γ –Elemene	7,04
8	25.576	$C_{15}H_{24}$	STH	α-Bisabolene	1,23
9	25.865	$C_{15}H_{24}$	STH	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	1,18
10	26.2	$C_{15}H_{24}$	STH	γ –Cadinene	4,11
11	26.408	$C_{15}H_{24}$	STH	β –Cubebene	44,67
12	26.754	$C_{15}H_{24}$	STH	β –ylangene	1,66
13	26.887	$C_{15}H_{24}$	STH	α-Muurolene	2,69
14	27.118	$C_{15}H_{24}O$	PC	2,6-bis (1,1-dimethylethyl)-4-methylphenol (BHT)	2,13
15	27.355	$C_{15}H_{24}$	STH	Germacrene D	2,00
16	27.568	$C_{15}H_{24}$	STH	δ –Cadinene	4,70
17	28.025	$C_{15}H_{24}$	STH	Valencene	0,98
18	28.215	$C_{15}H_{24}$	STH	Selina-3,7(11)-diene	1,34
19	28.7	$C_{15}H_{24}$	STH	β -Elemene	3,33
20	29.492	$C_{15}H_{26}O$	OST	cis-sesquisabinene hydrate	1,44
21	29.786	$C_{14}H_{20}O_2$	OST	3,4,4-Trimethyl-3-(3-oxo-but-1-enyl)-bicyclo[4.1.0]heptan-2-one	1,05
22	30.242	$C_{15}H_{24}O$	OST	Aromadendrene oxide	1,22

Table.2. Percentage of different classes of volatile organic compounds

Class	%	Number of compounds
Oxygenat monoterpene (OMT)	3.77	15
Sesquiterpene hydrocarbon (STH)	87.87	1
Oxygenat sesquiterpenes (OST)	3.71	3
Not idendied (NI)	2.5	2
Phenolic compound (PC)	2.13	1
Total	**99.98**	**22**

Conclusions

This study was performed to investigate the volatile organic compounds (VOCs) of *B. crassifolium* by the headspace sampler with gas chromatography-mass spectroscopy. The result reveals the presence of twenty-two (22) products of which twenty (20) were identified representing 97.48% of the total components. The major component identified were: 44.64% of β-cubebene, 8.82% of β-Caryophyllene, 7.04% of γ-Elemene, 4.70% of δ-Cadinene, 4.11% of γ-Cadinene, 3.77% of Ascaridole and 3.33% of β-Elemene, along with other constituents at relatively low amount. The composition of the (VOCs) is dominated by sesquiterpenes (91.58%). Among these sesquiterpenes, the ones without oxygen are mainly represented (87.87%). The aim of this study based at the research on chemical components useful in food preservation, insecticide and some biological activities *in vitro* and *in vivo*, such as anti-cancer. Finally, we recommended in the future the extracting of essential oil from various plant organs, including flowers, leaves and stems, to find out the chemicals and their concentrations to determine the more active plant organ.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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