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Brocchia cinerea essential oil from Brezina (Algerian Sahara): Chemical characterization and antibacterial activity

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Abstract

The essential oil of wild-growing medicinal specie *Brocchia cinerea* (Asteraceae) from Brezina (Algerian Sahara) was studied for chemical composition by GC/MS analysis and antibacterial activity. Twenty-nine identified components constituted 94.07% of the oil. The most important major components in the oil were trans-Thujone (36.11 %), Camphor (12.08 %), Santolina triene (11.25 %), 1,8-Cineol (6.98 %) and Cis-Verbenyl acetate (6.33 %), together reaching 72.75% of the total oil which is characterized by the presence of high content of oxygenated monoterpenes. The antibacterial activity of the essential oil was tested using the disc diffusion assay. The results showed that the essential oil of *Brocchia cinerea* had antibacterial activity against all tested Gram-positive bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli, Klebsiella pneumonia*, and *Pseudomonas aereginosa*).

Keywords: Essential Oil; *Brocchia Cinerea*; GC/MS; Oxygenated Monoterpenes; Antibacterial activity; Sahara

Introduction

Since ancient times, volatile essential oils and plant extracts are used for preserving human health in most ancient civilizations. Thus, the World Health Organization (WHO) has recognized the potential utility of traditional remedies and strives to preserve primary health care involving medicinal plants [1]. The importance of natural products to human health was emphasized by the award of the 2015 Nobel Prize in Physiology or Medicine to Professor Satoshi Omura and Dr. William Campbell for the discovery of avermectin from a *Streptomyces* sp. and its development as a treatment for river blind¬ness and other parasitic diseases, and to Professor Tu Youyou of Beijing for her discoveries leading to the development of the antimalarial drug artemisinin from *Artemisia annua* [2], a substance which, together with chloroquine, constitutes a candidate drug for the treatment of the current SARS-Cov-2 (COVID 19) pandemic [3].

Due to its geographic position, with a varied landscape and diversified climate favorable for rich vegetation, Algeria has a varied flora of about 4450 taxa of which 3950 indigenous with 6.5% endemic [4]. In particular, the Sahara part constitutes an important reservoir of many plants that have not been investigated until today. Furthermore, Ozenda noted that Asteraceae, Fabaceae, and Poaceae are everywhere dominant families in the flora of Sahara. This Asteraceae family represent 13,8%, 11,2%, and 7,9% of the total flora respectively in Sptentrional Sahara, Central Sahara, and Meridional Sahara[5].

Among this flora, *Brocchia cinerea* species from the Asteraceae family have been widely used in the Algerian Sahara ethnopharmacopea for the treatment of various diseases as a medicinal plant [6]. *Brocchia cinerea* is traditionally used as an infusion and decoction to treat digestive troubles, rheumatoid arthritis, fever, inflammation, headaches, migraines, colic, diarrhea, cough, cooling broncho-pulmonary, urinary and pulmonary infections [7, 8].

Some interesting biological studies have been described in the literature that has examined the antibacterial, antiprotozoal, analgesic, and antipyretic activities of essential oils and some extracts from *B. cinerea* [9-13].

However, as far as we know, tracing the current literature,

nothing was found concerning the chemical composition and biological activity of *B. cinerea* essential oil growing wild in the Brezina area (southwest of Algeria, Wilaya El Bayadh). Thus, as a part of our investigation into Algerian medicinal plants [14-19], in this study, we investigate for the first time the chemical composition and antibacterial activity of the essential oils from the Saharan medicinal plant *Brocchia cinerea.*

Materials and Methods

Plant material

Brocchia cinerea Del. (Syn. *Cotula cinerea* Delile, *Tanacetum cinereum* (Delile) DC.) is a small annual herb with discoid capitula and homogamous hermaphrodite florets figure 1. It is widely distributed in the Sahara desert and represents one of the monotypic Anthemideae genera which are characteristic of the North African flora [20].

Figure 1: The medicinal plant *Brocchia cinerea* - Algeria Sahara

- Kingdom : Plantae
- Division : Angiosperm
- Class : Eudicots
- Subclass : Asterids
- Order : Asterales
- Family : Asteraceae
- Subfamily : Asteroideae
- Tribe : Anthemideae
- Sub-tribe : Cotulinae
- Genus : Brocchia
- Specie : *Brocchia cinerea*
- Vernacular name : *Guertofa Beida, Rabrouba, Chouhia*
- N° POSL Herbarium : CA 07/10

Aerial parts of *Brocchia cinerea* were collected in Brezina (Latitude: 33° 5′ 58″ N; Longitude: 1° 15′ 39″ E; Altitude: 1028m)) during the flowering period in February (2018). The plant was identified by Pr A. Marouf (Department of Biology, University Center Naama – Algeria) and a voucher specimen is kept in the Herbarium of POSL Laboratory, (UTMB and Algeria) under N° CA 07/10.

Isolation of the Essential Oil

The *Brocchia cinerea* essential oil (EO) was obtained from dry plant material (100 g) by hydro distillation using the Clevenger apparatus for 3 h, following the 3rd Edition of the European Pharmacopoeia cited by [21]. The obtained oil was dried over anhydrous sodium sulphate and stored in colored glass at 4 °C until analysis.

GC-MS Analysis

GC/MS analysis was performed on Shimadzu GC-17A gas-chromatograph, interfaced with Shimadzu QP5000 mass spectrometer, operating at an electron impact of 70 eV with an ion source temperature at 250°C, scan a mass range of 40-400 m/z at a sampling rate of 0.5 scans/s. A 60 m ×0.25 mm fused silica non-polar DB-5 capillary column, with a 0.3 µm film thickness was used. The operating conditions were: Temperature programmed as follows: 50°C for 2 min and then up to 240°C at 3°C/min, then to 300°C at 10°C/min, ending with a 10 min at 300°C., and injector and detector temperature were 240°C. The carrier gas was He (1.0 mL/min), injector, and detector temperature 240°C. Samples were injected by splitting and the split ratio of 1:5.

The EO component identification was confirmed by comparison of mass spectral fragmentation patterns with the computer library (NIST MS Library) and verified by comparison of their retention indices (determined relatively to the retention times of an n-alkanes homologous series, C-9 to C-24) of the identified compounds with the literature [15, 22-24]. The relative amounts of the individual components found in the oil are based on the peak areas obtained, without FID response factor corrections.

Antibacterial activity

The antibacterial activity of *Brocchia cinerea* essential oil was determined using the paper disc diffusion method according to our previous studies [15, 25, 26], against three-Gram negative bacteria (*Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (Isolated), *Pseudomonas aereginosa* (ATCC 27853)) and two-Gram positive bacteria (*Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923)) which were obtained from Pasteur Institute (Algiers, Algeria). Antibacterial activities were evaluated by measuring the inhibition zone diameters in mm. Amoxicillin is included in the test as a reference [26, 27].

Results and Discussion

Chemical composition of the essential oil

Yellowish oil with a characteristic artemisias pleasant-smelling odor and sweet taste is obtained with a yield of 1.4% from *Brocchia cinerea*. The results of the chemical composition of the essential oil obtained by GC/MS analysis are presented in Table 1 and components are listed in order of their elution on the DB-5 capillary column. Twenty-nine compounds were identified representing 94.07% of the oil. The most important major components in the oil were trans-Thujone (36.11 %), Camphor (12.08 %), Santolina triene (11.25 %), 1,8 Cineol (6.98 %) and Cis-Verbenyl acetate (6.33 %), together reaching 72.75% of the total oil which is characterized by the presence of high content of oxygenated monoterpenes. Other compounds are present with low percentages between 1.5 and 2.5 % such as Terpinene-4-ol, Santolina alcohol, Camphene, α-Pinene, and β-Pinene.

Brocchia cinerea collected in the region of Oued Souf (Southeast Algeria) during two stages (flowering and fruiting), gave 0.0801% and 0.391% of essential oil (EO) respectively at the flowering and fruiting stage. The chemical study by GC/MS showed the presence of 22 chemical compounds in the flowering period with the dominance of 3-Carène (30.99%), Thujone (21.73%), Santolina triene (18.58%) and Camphor (6.21%). While 21 chemical compounds were obtained during the fruiting period with the dominance: Thujone (28.78%), 3-Carène (15.90%), Eucalyptol (15.13%), Santolina triene (13.38%) and Camphor (7.49%) [28]. The species from Morocco has Trans- Thujone (41.4%), *cis*-verbenyl acetate (24.7%), 1,8-cineole (8.2%) and camphor (5.5%) as the major components [10], Other analyses indicate that EO is dominated by the existence of the iso-3-thujanol with 47.38%, followed by Santolina triene (11.67%) and of camphor (10.95%) [29]. However, the percentage of compounds differed substantially from that reported in *species* collected in Egypt, in which camphor (50%) and *trans-Thujone* (14.4%) were the main oil compounds [30].

Almost all of the components reported in our study were found in *B. cinerea* from Southeast Algeria, Morocco, and Egypt [10, 28-30] and in some species belonging to the tribe Anthemideae, confirming the chemotaxonomic relationships between *Cotula* and Anthemideae genera [10, 31,32]. We observed a qualitatively resembles in the chemical composition of our essential oils and that obtained from *B. cinerea* collected from other regions (Southeast Algeria, Morocco, and Egypt), but with some quantitative divergences, could be attributed to many factors such as vegetative phases of the plant, environmental and growing conditions (e.g. seasonal and geographical variations, soil composition [33].

Antibacterial activity

Microorganisms have the genetic ability to transmit and acquire resistance to antibiotics and have become a major global healthcare problem in the 21st century [25]. Thus, one of the most efficient ways of finding new antibacterial compounds is collecting data on the use of medicinal plants in traditional pharmacopeia [1, 19].

Several studies have examined the antimicrobial activities of essential oils from *Brocchia cinerea*, Ghanmi et al [29] evaluated the anticandidal and antibacterial activity of the essential oil from Morocco, by using a panel of human pathogenic fungi (*Candida albicans, C. krusei, C. glabrata* and *C. parapsilosis*), authors demonstrate that the oil showed high anticandidal activity against all investigated strains with the inhibition zones against the tested *Candida* species ranging from 19.3 to 25.3 mm. Interesting results obtained of the antibacterial activity of essential oils against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Micrococcus luteus*. All bacterial strains were inhibited at 1/500 v/v concentration. In additional mold (*Penicillium digitatum, Penicillium expansum*, and *Aspergillus niger*) were less sensitive than bacteria and their growth was stopped at 1/250 v/v concentration [29]. The *Escherichia coli* strains and *Staphylococcus aureus* showed high sensitivity to the essential oil of this plant, until the concentration 1/8 (v/v) [28]. El Bouzidi et al. [10] demonstrated that the essential oil of *B. cinerea* presented a great activity against tested yeasts and the specie essences present a wide anticandidal activity spectrum.

We summarized in Table (2), the results of the *in vitro* antibacterial activity of the essential oils against pathogens causing urinary tract, lung, and gastrointestinal infection: *Enterococcus faecalis*, *Escherichia coli, Klebsiella pneumonia, Pseudomonas aereginosa*, and *Staphylococcus aureus*. The inhibition zone, measured in millimeters, including the diameter of the paper disk, was used as the criterion for measuring the antibacterial activity.

The *Brocchia cinerea* essential oil showed the best antibacterial activity, especially against Gram (+) bacteria *Staphylococcus aureus*, with zones of inhibition greater than 21.4 mm. One of the best known resistant Gram (-) bacteria, *Klebsiella pneumonia* seems to be more sensitive to the EO compared to the antibiotic Amoxicillin. Whereas, a moderate antibacterial effect against *Enterococcus faecalis* and *Pseudomonas aereginosa* was observed.

This antibacterial activity of the EO may be explained by its richness in oxygenated monoterpenes such as *trans*-Thujone (36.11%), Camphor (12.08 %) well known for their high and broad-spectrum antimicrobial activity with the appreciable amount of 1,8-Cineol (6.98%) could also contribute to the antibacterial activity.

Table 1: Chemical composition of the *Brocchia cinerea* essential oil

No.	Components	RI*	RI**	Content (%)
1	Santolina triene	910	908	11.25
2	a-Thujene	931	929	0.73
3	a-Pinene	939	934	1.74
4	Camphene	953	944	2.06
5	b-Pinene	980	971	1.64
6	a- Terpinene	1018	1011	0.58
7	Limonene	1031	1025	0.27
8	1,8 Cineol	1033	1032	6.98
9	Santolina alcohol	1035	1034	2.08
10	y-Terpinene	1062	1051	0.56
11	Linalool	1098	1092	0.37
12	cis-Thujone	1102	1107	0.15
13	trans- Thujone	1114	1119	36.11
14	Camphor	1143	1145	12.08
15	Cis-Chrysanthenol	1163	1164	1.13
16	Borneol	1165	1168	1.44
17	Terpinen-4-ol	1177	1179	2.08
18	a-Terpineol	1189	1191	1.82
19	Carvacrol methy ether	1245	1248	0.12
20	Linalyl acetate	1257	1263	0.39
21	Cis-Verbenyl acetate	1280	1285	6.33
22	Bornyl acetate	1287	1289	1.48
23	Carvacrol	1298	1304	0.16
24	Neryl acetate	1365	1374	0.38
25	Geranyl acetate	1383	1386	0.18
26	b-Elemene	1389	1391	0.10
27	Caryophyllene	1418	1420	0.67
28	Germacrene D	1480	1486	1.07
29	Caryophyllene oxide	1583	1587	0.12
Total Identified (%)			94.07	

RI*: Retention indices from the literature [22]
RI**: Retention indices measured (DB-5 column)

Table 2: Antibacterial activity of *Brocchia cinerea* essential oil

		Inhibition Zone Diameters (mm)	
		Essential oil (Volume/disk (25µl))	Amoxicillin (15µg/ml)
Gram (+)	*Enterococcus faecalis*	08.6	18.7
	Staphylococcus aureus	21.4	25.6
Gram (-)	*Escherichia coli*	17.3	25.2
	Klebsiella pneumoniae	10.1	08.3
	Pseudomonas aereginosa	04.8	05.4

Conclusion

In this work, we studied for the first time the chemical composition and antibacterial activity of the essential oil of the medicinal plant *Brocchia cinerea* from Brezina (Algerian Sahara). Chemical analysis of essential oil by GC/MS identified twenty-nine (94.07%) compounds dominated by trans-Thujone (36.11 %), Camphor (12.08 %), Santolina triene (11.25 %), 1,8 Cineol (6.98 %) and Cis-Verbenyl acetate (6.33 %). The result obtained in this study shows that the essential oil has significant activity against *Staphylococcus aureus* and *Klebsiella pneumonia*, this, probably explains the use of this aromatic plant in traditional Saharan ethnopharmacopeae against some human diseases. Further experiments are planned to evaluate other biological activities and to determine the enantiomeric composition of this essential oil by chiral analysis.

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