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Research Article

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Chemical Composition and Biological Activity of *Balansaea glaberrima* Desf. and Lange, Leaf and Stem Essential Oils

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Abstract Objective: The biological importance of Apiacea promoted us to investigate the leaves and stem of *Balansaea glaberrima* (endemic plant) previously not investigate. The study aimed to investigate the antimicrobial, antioxidant and chemical composition of the essential oils
Results: The major components of the oils of *B. glaberrima* were found to be valencene, apiole and γ-murolene, representing 36.65, 34.87 and 9.83 %, respectively. Antimicrobial activity revealed an important effect against *E. coli* and *A. flavus* whereas antioxidant activity showed a low antiradical effect in comparison with BHT. The IC_{50} of *B. glaberrimais* 9136.00 µg/ml, while IC_{50} of BHT is 41.35 µg/ml.
Conclusion: The results presented here can be considered as the first information on the antibacterial and antioxidant properties of *B. glaberrima* essential oils.

Keywords GC/MS, antimicrobial, antioxidant, activities

Introduction

Because of the side effects of some antimicrobial drugs and the resistance that pathogenic microorganisms build against the antibiotics, much recent attention has been paid to extract and biologically active compounds isolated from plant species used in herbal medicine [1]
Numerous researchers have shown interest in the biologically active components isolated from plants and for their influence on the elimination of pathogenic microorganisms [2]
B. glaberrima (Apiaceae) is also known as *Conopodium glabrerrimum* (Desf.) Engstr was found in the east of Algeria and not known in the folk medicine. Essential oils of this family have been widely used as antibacterial, antifungal, antiviral, anti-parasitic, insecticidal and antispasmodic proprieties [3]. Nowadays, consumer's preference to use natural products resulted in the exploitation of essential oils and/or extracts of many plants, as potent ingredients pharmaceutical, cosmetic and food industries [4]. Excessive production of reactive oxygen species (ROS), beyond body's antioxidant defense capacity induces oxidative stress [5]. Several studies have shown that plants contain a large variety of phytochemicals with antioxidant properties which minimize ROS effects in biological systems [6-7]. Essential oils, extracted from different parts of aromatic plants, contain secondary

metabolites, with potent antimicrobial and antioxidant properties, exploited in phytotherapy [8-9]. The aim of this study is to investigate the chemical composition and the biological activity of essential oils extracted from leaf and stem of *B. glaberrima* Desf and Lange Plant. Our investigation carried out on *B. glaberrima* the essential oils of the leaves and steam of *B. glaberrima* led to the identification of 21 compounds some other compounds were only present in minor amounts. Moreover, the evaluation of the antibacterial activity of essential oils revealed a very important effect against some bacteria strains time the biological activity of essential oil has not been documented.

Materials and Methods

Plant material

The leaves and stems and flowers aerial parts of *B. glaberrima* Desf and Lange, were collected at the flowering stage, on May 2013 from Azazgua area (Tizi Ouzou, Algeria), at 737 meters of altitude (N 36 73046, E 004450 65). The air-drying of the plants was performed in the shade at room temperature. Plant was identified by Errol Vela from Montpellier University and staff of the Natural Biological Resources Valorization Laboratory, Setif-1 University Voucher specimens of the plant material is deposited in the Herbarium at the department of biology and ecology.

Extraction

Essential oils were obtained by hydro distillation of 500 g of dried leaves and stems aerial plant parts, using a Clevenger-type apparatus, for 3h. Diethyl ether was used as the collector solvent. After evaporation of the solvent, the oil was dried over anhydrous sodium sulfate and stored at 0 °C, in tightly sealed vials, under obscurity, later uses.

Microorganisms

B. glaberrima essential oils were screened for antimicrobial activity against six ATCC bacteria; *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *S. aureus* (SARM, 43300), *Bacillus subtilis* (ATCC 6633) and *Listeria inocula* (CIP 74915), three clinical strains. *E. coli*, *P. aeruginosa*, and *S. aureus*, and three fungi: *Candida albicans* (ATCC 1024), *Aspergillus niger* (2CA 936), and *Aspergillus flavus* (NRRL). Microorganisms were supplied by the applied microbiology laboratory of Bejaia University.

Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH) and butylatedhydroxyltoluene (BHT) were purchase from Sigma-aldrich; ethanol from Fluka chemicals; Mueller Hinton (MH) and nutritive gelose (GN), from Chapman, Ektoen. BGT were purchase from Oxide; l'amphotéricine B (AB), from Bristol-Myers-Squibb, Clotrimazol 1%, from ARAC-Syria, and Nystatine, from Bio-Rad. All chemicals were of the finest grade available.

Gas chromatography/Mass Spectrometry

GC/MS analyses were carried out in a gas chromatograph (Agilent, model 6890N, Palo Alto, CA) equipped with a split–splitless injector, an auto-sampler (Agilent, model 7683) and two different Agilent fused silica capillary columns (30 m × 0.25 mm i.d., film thickness 0.25 µm) of different polarities (HP-5, 5% phenyl-methylpolysiloxane, DB-WAX, poly-ethylene glycol). GC conditions used were heating, from 60 to 250 °C, at 3°C/min; followed by 20 minutes, under isothermal conditions. The injector was maintained at 250 °C. Helium was the carrier gas at 1.0 mL/min. Sample (1µL) was injected in the split mode (1:10).

Essential oils compounds were identified by comparison of the mass spectra of the GC results with the NIST02 library data and of the MS results with the Adams libraries spectra (NIST/EPA/NIH Mass Spectral Library data. The chemical composition assessments and the identification of the main components were performed by comparing their retention indices with those of standard compounds.

Antimicrobial Activity

Essential oils antimicrobial activity was evaluated using a modified agar diffusion method [10] as described by Laouer and *al.*[11].Briefly, a dilution of 100 µl of suspension of the tested microorganisms in 10 ml nutrient broth, containing $2.0x10^6$ colony forming units (CFU/ml) for bacteria, 10^7 CFU/ml for yeast, and $2.0x10^5$ spores /ml, for fungi, spread on Mueller-Hinton agar (LB) and Sabouraud dextrose agar (SDA), sterilized in flasks and cooled to 45-50°C, were placed in 9 cm Petri dishes (20 ml). Sterile filter paper discs, 6 mm diameter, were impregnated with 10 µl of dilute essential oils solution (10µl of 1/2, 1/5 and 1/10 v/v) in ethanol and were deposited at equal distances on the surface of the inoculated agar (LB for antibacterial essay and SDA for antifungal essay). Petri dishes were left in at 4°C for 30 minutes before incubation to ensure good oil diffusion into the agar. Inhibition diameter was measured after 24 h at 37°C for bacteria, after 7 days of incubation at 27°C for the fungi and after 48 h of incubation at 37°C for the yeast. Three classes of the inhibition zone diameter were used to determine inhibitory effects of

essential oils. These diameter classes were > 20 mm, >12 mm but<20 mm, and< 12 mm, representing high, medium and no inhibitory effects, respectively [11]. Gentamicin, Nystatine (NY), Amphotericin B (AB), Clotrimazol (CTR) and ethanol were used as positive and negative controls, respectively. The diameters of the inhibitions zones were measured in mm. controls were set up with equivalent quantities of ethanol. Antimicrobial activity was evaluated by measuring the zone of inhibition against each test results were expressed as average values

Antioxidant activity

DPPH scavenging assay

Hydrogen atom or electron-donation ability of the corresponding extracts was measured from the bleaching of the purple-colored methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). This spectrometric assay uses stable DPPH radical600 µL of various concentrations (0,030, 0,021, 0,016, 0,008, 0,004, 0,002, 0,001, 0,0004mg/ml) of the samples in methanol were added to 600µLof a 0,004% methanol solution of DPPH [12]. After a 30 minutes incubation period, at room temperature, absorbance was read against a blank at 517 nm. Inhibition of DPPH free radical in percent (I %) was calculated as follows:

I %=(Abs blank-Abs sample/Abs blank X 100

Where Abs blank is the absorbance of the control (containing all reagents except the test compound), and Abs sample is the absorbance of the compound. Sample concentration, providing 50% inhibition (IC50), was calculated from the graph plotting inhibition percentage against extract concentration (Tests were carried out in triplicate)

Results and Discussion

This study focused essentially on the phytochemical and antibacterial screening of *B. glaberrima*. Essential oils from leaves and stem of *B. glaberrima* have been studied by GC-MS to afford 21 compounds the yield was 0,260% on dry weight basis

The tested oils contained monoterpenes and sesquiterpenes (Table 1). Valencene, Apiole and Murolene were the major components with the following percentages (36.65%, 34.87%, 9, 83%, respectively). *B. glaberrima* yielded 0.26% essential oils, characterized by aromatic odor and a yellow-pale color.

The results of the antimicrobial activity of the essential oil are presented in table 2. Oil of *B. glaberrima* showed high activity, (21 mm), against *E. coli* at the half dilute oil. *S. aureus, B. subtilis* and *A. flavus* showed a moderate sensibility, (16), against the half dilute oil (14) against 1/2 and (14) against 1/2 (v/v) dilute oil much larger inhibition zones. The antimicrobial activities greatly increase with increase of the oil concentrations from 1/10 to 1/2 (Table 2). Strong inhibition zones were observed against *E. coli* ATCC25922, with the highest inhibition zone (26 mm) against the 1/2 (v/v) dilute oil. *S. aureus* ATCC 25923, *B.subtilis*ATCC6633 and *A. flavus* NRLL have a moderately zone of inhibition. The other (*P. aeruginosa* ATCC 27853, *S. aureus* ATCC43300, *L. inocula* CIP 74975, *C. albicans* ATCC 1024 and three clinical strains ; *E. coli, P. aeruginosa, S. aureus* Microorganisms were less sensitive to the two fold oil dilution. This activity is due to the presence of active constituents, mainly attributable to isoprene such as monoterpenes, sesquiterpenes and related alcohols, other hydrocarbons and phenols [13].

Table 1: Chemical composition of *B. glaberrima* essential oils

Compounds	**R**	**%**
alpha-ylangene	21,423	1,320
beta-caryophyllene	23,368	0,398
alpha-humulène	24,736	0,463
Murolene	25,86	**9 ,832**
valencene	25,947	**36,658**
sabinene 9	27,031	0,258
myristicin	27,503	2,052
acétate de linalyle	27,897	0,185
ledene	29,571	0,538
caryophylleneoxide	30,743	0,427
thujopsene	31,639	0,258
alpha-longipinen-7beta-ol	31,997	0,679
E-neroliol	32,172	0,745
quaiacol	32,427	9,423
apiole	33,49	**34,879**
(+) -mayurone	34,341	0,301
alpha Bulnesene	35,355	0 ,847
Total		**99.288**

Major components written in bold

Table 2: Inhibition effects of *B. glaberrima* essential oils

Microorganisms	1/2	1/5	1/10	Gn	AB	CTR	NY	Eth
E. coli ATCC 25922	26 (+)	20 (+)	18 (+)	25				-
P. aeruginosa ATCC 27853	8 (-)	7 (-)	-	24				-
S. aureus ATCC 25923	16 (-)	10 (-)	8 (-)	25				-
S. aureus ATCC 43300	9 (-)	7 (-)	7 (-)	22				-
B. subtilis ATCC 6633	14 (+)	12 (+)	9 (+)	26				-
L. inocula CIP 74975	-	-	-	17				-
E. coli	7 (-)	-	-	25				-
P. aeruginosa	7 (-)	-	-	20				-
S. aureus	8	7	7	22				-
C. albicans ATCC 1024	-	-	-	-	19	33	11	-
A. niger 2CA 936	-	-	-	-	9	20	11	-
A. flavus NRLL	14	-	-	-	8	24	7	-

Numbers indicate the mean diameters (mm) of inhibition of at least triplicate experiments. – indicates no growth inhibition. (-) biocide, (+) biostatic. Gent Gentamicin (Gen) for all bacteria; Amphotericin (AB) and Clotrimazol (CTR) for *A. flavus*, *A. niger* and *C. albicans* were used as the positive controls. Ethanol (Eth) was included as a negative control.

B. glaberrima essential oils showed a low antioxidant activity IC_{50} inferior to those of the standard compounds (BHT). However, the components responsible for the antioxidant activities of the oil were not identified. Jukié and Milos [14] reported that the thymphenolic chemotype possesses stronger antioxidant properties than the non-phenolic one. Essential oil of *B. glaberrima*is not richer in these secondary metabolites.

Conclusion

Our study of the Algerian *B. glaberrima* leaves and stems led to the extraction of 21 compounds followed by the evaluation of antimicrobial and antioxidant activities for the first time the biological activity of essential oil have not been documented, the results of this study demonstrate the antimicrobial potential of *B. glaberrima* essential oils. Essentials oils were extracted by Clevenger type apparatus and their chemical composition was carry out by G-C and GC/MS. γ-himachalene (36, 65%), apiol (34, 87%) and γ-murolene (9, 83%) were the major components of the oil. Antimicrobial activity was determined using nine bacterial strains and three fungi according to the disk diffusion assay. The antioxidant activity was evaluated using the free radical scavenging effects of the DPPH. The *B. glaberrima oil* is active only against *E. coli* ATCC 25922, *A. flavus* NRRL 391 and *C. albicans* ATCC 1024. The oil possesses low antiradical effect in comparison with BHT.

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