

Essential Oil of *Brachylaena hutchinsii* Hutch from Tanzania: Antimicrobial Activity and Composition

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The hydrodistilled essential oil of saw powder of *Brachylaena hutchinsii* Hutch was analyzed by GC/MS. Twenty six compounds representing 94.7% of the oil were identified. The main components of the oil were hydrocarbons sesquiterpenes, caryophyllene (19.1%), β -cubebene (15.5%), *cis*-calamenene (10.5%) and α -copaene (9.0%). The oil exhibited antimicrobial activity, which was comparable to that of gentamycin against *Proteus mirabilis*. It also showed some activity against *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus* and *Enterococcus faecalis*.

Key Words: *Brachylaena hutchinsii*, essential oil, sesquiterpenes, antimicrobial activity.

INTRODUCTION

Brachylaena hutchinsii Hutch. (*B. huillensis* O. Hoffm.), Asteraceae family, is a tree 10-18 m, evergreen; bark rough, grey, peeling longitudinally. Leaves are narrowly elliptic to slightly obovate, base cuneate or attenuate, apex acute or short acuminate, margin entire or serrate in young plants, 3-12 by 1-4 cm, revolute, densely greywhite-tomentose beneath. Flowers white or greenish yellow, heads in 2-3 cm long erect axillary panicles. It grows in upland semideciduous forest and lowland dry forest or thicket [1]. It is found in the north eastern part of Tanzania. The local people, the Sambia, call it 'Muhungwe' or 'Mhungwe', while the Swahili name is 'Muhuhu' or 'Mhuhu'. It is used for timber, woodcarving and firewood. Screening of *B. hutchinsii* leaves from Tanzania showed that it contains coumarins, essential oil, sterols and triterpenes and tannins [2]. Several sesquiterpenes have been isolated from the methanol extract of the stem bark of *B. hutchinsii* from Kenya [3]. However, there are no reports on the essential oil constituents. This paper reports the chemical composition and

antimicrobial activity of the essential oil of *Brachylaena hutchinsii*.

EXPERIMENTAL

Plant material and oil isolation

Brachylaena hutchinsii was collected on 10th March 1999 from Mombo, Korogwe district in the Tanga region of Tanzania. Voucher specimens were deposited in the Herbarium of Institute of Traditional Medicine, Muhimbili University College of Health Sciences, University of Dar es Salaam (Herbarium No. ITM 1426). Saw powder was hydrodistilled to yield 5.0% oil, which was dried over anhydrous sodium sulphate and stored at 4 °C until analysis.

Antimicrobial assay

A collection of 8 bacteria species were used, including gram-positive bacteria *Bacillus cereus* (from rice), *Enterococcus faecalis* (ATCC 29212), *Micrococcus luteus* (ATCC 9341), *Staphylococcus aureus* (ATCC 25212), and *Staphylococcus epidermidis* (from cow milk), Gram-negative strains *Escherichia coli* (from water) *Klebsiella spp.* (from bird food) and

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Proteus mirabilis (from human urine). All the samples of microorganisms were characterized at the Department of Microbiology, National University of Rio Cuarto, Argentina and voucher specimens were preserved. All the strains tested were maintained at 4 °C in Tryptone Soy Agar and were subcultured every month. The fungus was stored at the same temperature as bacteria in Sabouraud Agar and subcultured every month. The paper disc diffusion method was used to test antibacterial activity. It was performed using an 18 h culture, grown at 37 °C and adjusted to approximately 10⁶ cfu/ml. The inoculum (200 µl) was spread over plates containing Mueller-Hinton Agar and a paper filter disc (4 mm) impregnated with 10 µl of the essential oil placed on the surface of the media. A gentamycin disc (Brittania Co.) containing 10 µg was used as a positive control. The plates were kept for 30 min at room temperature to allow the diffusion of the oil and gentamycin before they were incubated at 37 °C for 24 h. After this time, the inhibition zone around the disc was measured with a calliper. Antifungal experiments were made in the same way using Extracto de Malta Broth for the culture and Sabouraud Agar for the plates, and using Amphotericin B as the positive control.

Gas chromatography

The essential oils was analyzed with a Shimadzu GC-R1A gas chromatograph equipped with a fused silica column (30 m x 0.25 mm) coated with DB-5. The temperature of the column was programmed from 60 °C to 240 °C at 4 °C/min. The injector and detector temperatures were at 250 °C. The gas carrier was helium, at a flow rate of 1 ml/min. Peak areas were measured by electronic integration. The relative amounts of

the individual components were based on the peak areas obtained, without FID response factor correction. Programmed temperature retention indices of the compounds were determined relative to n-alkanes [4].

Gas chromatography-Mass spectrometry

GC-MS analyses was performed on a Perkin Elmer Q-910 using a 30 m x 0.25 mm capillary column coated with DB-5. The temperature of the column and the injector were the same as those from GC. The carrier gas was helium, at a flow rate of 1 ml/min. Mass spectra were recorded at 70 eV. The oil components were identified by comparison of their retention indices and mass spectra with those of authentic samples, by peak enhancement, with published data [5], mass spectra library of National Institute of Standards and Technology (NIST 3.0) which contains reference mass spectra and retention indices of volatile compounds.

RESULTS AND DISCUSSION

Table 1 shows the antimicrobial activity of the essential oil (10 µl) as comparable to that of gentamycin (10 µg) against *P. mirabilis*. It had about 50% the activity of gentamycin against *E. faecalis* and *M. luteus*. It also showed some activity against *B. cereus*, *S. aureus*, and *S. epidermidis*. However, it showed no activity against *Escherichia coli*, *Klebsiella* spp. and *C. albicans*.

Twenty-six constituents, representing 94.7% of *B. hutchinsii* oil were identified by GC and GC/MS (Table 2). The oil is rich in caryophyllene (19.1%), β-cubebene (15.5%), *cis* calamenene (10.5%) and α-copaene (9.0%).

Table 1. Antimicrobial of the essential oil of *Brachylaena hutchinsii* leaves

Microorganism	Inhibition zone (mm)	Positive control	
		Amphotericin B	Gentamicin
<i>Bacillus cereus</i>	10.5	-	25.0
<i>Staphylococcus aureus</i>	8.5	-	20.0
<i>Staphylococcus epidermidis</i>	7.0	-	30.0
<i>Proteus mirabilis</i>	22.5	-	23.0
<i>Escherichia coli</i>	NI	-	18.0
<i>Klebsiella</i> spp	NI	-	22.0
<i>Micrococcus luteus</i>	12.0	-	20.0
<i>Enterococcus faecalis</i>	7.0	-	13.0
<i>Candida albicans</i>	NI	20.0	-

Inhibition zone diameter measured in mm, the disc diameter of 4 mm being included. NI: No inhibition.

Table 2. Chemical composition of the essential oil of *Brachylaena hutchinsii* leaves

Compounds*	%	Retention Index	Methods of identification
α -thujene	0.5	931	MS-CO
α -pinene	0.2	939	MS-CO
sabinene	0.2	976	MS-CO
β -pinene	0.2	980	MS-CO
myrcene	0.4	991	MS-CO
carene-3-	0.3	1011	MS
δ -elemene	0.1	1339	MS
α -cubebene	0.1	1352	MS
α -ylangene	5.2	1373	MS
α -copaene	9.0	1376	MS
β -bourbonene	0.2	1385	MS
β -cubebene	15.5	1390	MS
β -caryophyllene	19.1	1418	MS-CO
α -cadinene	0.3	1450	MS
germacrene D	0.1	1480	MS
α -muurolene	8.0	1499	MS
γ -cadinene	3.5	1513	MS
<i>cis</i> -calamenene	10.5	1521	MS
δ -cadinene	8.5	1524	MS
α -calacorene	6.0	1542	MS
β -calacorene	1.5	1556	MS
spathulenol	1.2	1576	MS-CO
β -oploponone	1.5	1606	MS
β -eudesmol	0.9	1649	MS
cadalene	1.5	1678	MS
α -santalol	0.2	1684	MS
TOTAL	94.7		

*Compounds are listed in order of their elution from a DB-5 column. CO: peak identifications based on coinjection. MS: peak identifications based on MS comparison with file spectra.

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