Essential oil of *Brachylaena hutchinsii* Hutch. from Tanzania: composition and antimicrobial activity

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ABSTRACT: The hydrodistilled essential oil of fresh aerial parts of *Brachylaena hutchinsii* was analyzed by GC/MS. Thirteen compounds representing 94.7 % of the oil were identified. The main components of the oil were sesquiterpene hydrocarbons, caryophyllene (19.1 %), β-cubebene (15.5 %), cis-calamenene (10.5 %) and α-copaene (9.0 %). The oil exhibited antimicrobial activity which was as much as that of gentamycin against *Proteus mirabilis.* It also showed some activity against *Bacillus cereus, Staphylococcus aureus, S. epidermidis, Micrococcus luteus* and *Enterococcus faecalis.*

KEY WORD INDEX: *Brachylaena hutchinsii*, essential oil composition, sesquiterpenes, antimicrobial activity.

INTRODUCTION: *Brachylaena hutchinsii* Hutch. (*B. huillensis* O. Hoffm.), Compositae 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-accuminate, 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 semidecidous forest and lowland dry forest or thicket [1]. It can be found in the north eastern part of Tanzania. The local people, the Sambaa, call it ‘Muhungwe’ or ‘Mhungwe’, while the Swahili name is ‘Muhuhu’ or ‘Mhuhu’. It is used for timber, woodcarving and firewood. Previous phytochemical reports on *Brachylaena hutchisii* were made [2]. However, There are not reports on the chemical composition and antimicrobial activity of the essential oil of *Brachylaena hutchisii.*

EXPERIMENTAL: **Plant material and oil isolation.** Leaves of *Brachylaena hutchisii* were collected on 10th March 1999 from Mombo, Korogwe district in the Tanga region of Tanzania. Voucher specimens are kept in the Herbarium of Institute of Traditional Medicine, Muhimbili University College of Health Sciences (Herbarium No. ITM

1426). Fresh leaves of *Brachylaena hutchisii* were hydrodistilled to yield 5.0 % oil. The oil obtained was dried over anhydrous sodium sulphate and stored in a refrigerator until analysis.

Gas Chromatography- The essential oils were analyzed with a Shimadzu GC-R1A gas chromatograph equipped with a fused silica column (30 m x 0.25 mm) coated with DB-5 (J&W). The temperature of the column was programmed from 60 ^{0}C to 240 ^{0}C at 4 ^{0}C/min. The injector and detector temperatures were at 250 ^{0}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 are based on the peak areas obtained, without FID response factor correction. Programmed temperature retention indices of the compounds were determined relative to n-alkanes [3].

Gas Chromatography-Mass Spectrometry- GC-MS analyses were 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 of 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, mass spectra with those of authentic samples, by peak enrichment, with published data [4], mass spectra library of National Institute of Standards and Technology (NIST 3.0) and our mass spectra library which contains references mass spectra and retention indices of volatile compounds.

Antimicrobial assay- A collection of 8 microrganisms 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 *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 Tripsein-Soy Agar and were subcultured every month. The fungus was stored at the same temperature as bacteria in Sabourand Agar and subcultured every month. The paper disc diffusion method was used to test antibacterial activity. It was performed using an 18 hr culture,

growth at 37 °C and adjusted to approximately 10^6 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 oil was placed on the surface of the media. A gentamycin disc (Brittania Co.) containing 10 μg was used as a reference. The plates were left for 30 minutes at room temperature to allow the diffusion of the oil and then incubated at 37 °C for 24 hrs. After this the inhibition zone around the disc was measured with a calliper.

Antifungal experiments were carried out in the same way using Extracto de Malta Broth for the culture and Sabouraud Agar for the plates.

RESULTS AND DISCUSSION: Twenty-six constituents, representing 94.7 % of *B. hutchinsii* leaf oil were identified by GC and GC/MS. As can be seen from the results in Table 1, the oil is rich in caryophyllene (19.1 %), β-cubebene (15.5 %), *cis* calamenene (10.5 %) and α-copaene (9.0 %). Table 2 shows the oil is as active as gentamycin against *Proteus mirabilis.* It also showed some activity against *Bacillus cereus, Staphylococcus aureus, S. epidermidis, Micrococcus luteus* and *Enterococcus faecalis.* However, it showed no activity against *Escherichia coli, Klebsiela* spp. and *Candida albicans.*

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Table 1. Chemical composition of the essential oil of *B. hutchisii* leaves

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

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

Table 2. Antimicrobial activity (inhibition zone in mm) of the leaf oil (10 μL) of *Brachylaena hutchisii* compared to that of 10 μg gentamicin and anfotericine

Microorganism	Oil inhibition zone (mm)	Positive control inhibition zone (mm)	
		Anfotericine	Gentamicin
B. cereus	10.5	-	25.0
S. aureus	8.5	-	20.0
S. epidermidis	7.0	-	30.0
P. mirabilis	22.5	-	23.0
E. coli	NI	-	18.0
Klebsiella spp	NI	-	22.0
Micrococcus luteus	12.0	-	20.0
Enterococcus faecalis	7.0	-	13.0
C. albicans	NI	20.0	-

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