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Original Research Article

Chemical Composition and Antimicrobial Activity of *Geniosporum rotundifolium* Briq and *Haumaniastrum villosum* (Bene) AJ Paton (Lamiaceae) Essential Oils from Tanzania

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Abstract

Purpose: *To determine the chemical composition and antimicrobial potential of essential oils from two aromatic plants of Tanzania, Geniosporum rotundifolium Briq. and Haumaniastrum villosum (Benè) A.J. Paton (Lamiaceae).*
Method: *Essential oils from the aerial parts of the plants were extracted by hydro-distillation for 3 h using a Clevenger type of apparatus. The constituents were analyzed by gas chromatography – mass spectrometry (GC/MS).The minimum inhibitory concentrations of the essential oils were determined for eight bacterial strains and three pathogenic fungi using agar dilution method.*
Results: *The constituents of G. rotundifolium oil were mainly oxygenated derivatives of mono- and sesquiterpenes; spathulenol (12.46 %), α-terpineol (4.65 %) and germacrene-D (3.71 %) were the most abundant. Those of H. villosum oil were predominantly sesquiterpenes (72.61 %) with caryophyllene oxide (19.01 %), humulene epoxide II (11.95 %), β-bourbonene (5.7 %), α-humulene (5.63 %) and β-caryophyllene (5.39 %) being more abundant. The oil of G. rotundifolium exhibited weak to moderate activity against the bacterial species but showed no activity against the test fungi. However, H. villosum oil showed very promising activity against all the test microorganisms (MIC 0.08 – 10.34 mg/mL).*
Conclusion: *The major components of G. rotundifolium essential oil were oxygenated derivatives of mono- and sesquiterpenes whereas those of H. villosum were sesquiterpenes. All tested microorganisms were susceptible to H. villosum oil.*

Keywords: *Geniosporum rotundifolium, Haumaniastrum villosum, Essential oils, Chemical composition, Antimicrobial activity*

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INTRODUCTION

Geniosporum rotundifolium Briq. and *Haumaniastrum villosum* (Benè) A.J. Paton (Lamiaceae) are known as “Nkulilo” in the Nyakyusa dialect of Rungwe District, Mbeya Region, Southwestern Tanzania. *Geniosporum rotundifolium* (syn. *G. paludosum* Bak) [1], is a stout, erect, perennial herb which grows in damp grassland at high altitude [2]. It is confined to

several African countries including Tanzania [3]. Its leaves, stems and essential oils are given in combination with leaves of other plants for a number of medical uses. In Burundi it is used as an enema, cough remedy, laxative and anti-abortion while in Uganda it is used against fungal and bacterial infections [4]. A previous study on *G. rotundifolium* growing in Cameroon indicated that the essential oil from this plant possessed significant antifungal activities against *Fusarium moniliforme* and *Rhizopus stolonifera*. Furthermore, its chemical composition was determined with sesquiterpene hydrocarbons constituting more than 90 % of the oil [5].

Haumaniastrum villosum is an annual or short-lived perennial herb confined to the African continent and Madagascar, in the sub-humid climate [6]. There is scanty information on the medicinal uses and biological activities of *H. villosum* and to our knowledge there is no information on its phytochemical studies. Its synonym *H. galeopsifolium*, has been reported to be used traditionally in Burundi, alone or in combination for a number of health problems including urogenital infections [1]. It has also been reported to be used in controlling crop pests in the Democratic Republic of Congo [7].

In the current study, chemical compositions and antimicrobial activities of the essential oils of *Geniosporum rotundifolium* and *Haumaniastrum villosum* from Tanzania are reported for the first time.

EXPERIMENTAL

Plant material

Aerial parts (leaves and flowering tops) of *G. rotundifolium* and *H. villosum* were collected from the wild, in Rungwe district, Mbeya region, Tanzania in June, 2000. The plants were authenticated by Mr. H. Selemani of the Department of Botany, University of Dar es Salaam. Voucher specimen Nos. ODN/DBR 001 for *G. rotundifolium* and ODN/DBR 002 for *H. villosum*, respectively, were deposited in the herbarium of the Department of Pharmacognosy, School of Pharmacy, Muhimbili University of Health and Allied Sciences.

Isolation of essential oil

All materials were air-dried in the shade, prior to hydro-distillation of essential oils for 3 h in a Clevenger-type apparatus. The essential oils collected over water were separated, dried over anhydrous sodium sulfate and stored at 4–6 ^{o}C until chemical analysis and antimicrobial screening.

Gas chromatography

Gas chromatography (GC) analysis was carried out on a Perkin-Elmer 8500 gas chromatograph with a flame ionization detector (FID), fitted with a Supelcowax-10 fused silica capillary column (30 m x 0.32 mm, 0.25 µm film-thickness). The column temperature was programmed from 75 to 200 ^{o}C at a rate of 2.5 ^{o}C/min. The injector and detector temperatures were programmed at 230 ^{o}C and 300 ^{o}C, respectively. Helium was used as the carrier gas, at a flow rate of 1 mL/min.

Gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry (GC-MS) analysis was carried out using a Hewlett Packard 5973-6890 GC-MS system operating on EI mode (equipped with a HP 5MS 30 m x 0.25 mm x 0.25 µm film thickness capillary column). Helium (2 mL/min) was used as the carrier gas. The temperature of the column was programmed from 60to 280 ^{o}C, at a rate of 3 °C/min. Split ratio, 1:10.

Identification of components

The compounds were identified by comparison of their retention indices (RI) [8] retention times (RT) and mass spectra with those of authentic samples, viz, 1,8-cineole, camphor, pulegone, piperitone, bornyl acetate, spathulenol, β-caryophyllene and β-caryophyllene oxide (Extrasynthese), borneol, linalool, limonene (Fluka AG), α –pinene, β –pinene (Aldrich) and/or the NIST/NBS, Wiley libraries spectra and the literature [9]. The percentage composition of the essential oil is based on computer calculated peak areas without correction for FID response factor.

Evaluation of antimicrobial activity

Antimicrobial activity of the essential oils against bacteria and fungi was determined using the agar dilution technique. The microorganisms included four Gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228); *Streptococcus mutans* and *Streptococcus viridian*, with the last two being clinical isolates and oral pathogens; four Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* (ATCC 13883) and *Pseudomonas aeruginosa* (ATCC 227853); and three species of *Candida*, namely, *C. albicans* (ATCC 10231), *C. tropicalis*

(ATCC 13801) and *C. glabrata* (ATCC 28838). Standard antibiotics (netilmicin and amoxicillin) were used as positive controls.

Technical data have been described previously [10]. Briefly, stock solutions of the tested samples were prepared at 10 mg/mL in dichloromethane. Serial dilutions of the stock solutions in broth medium (100 µL of Müller-Hinton broth or on Sabouraud broth for the fungi) were prepared in a microtiter plate (96 wells). Then 1 µL of the microbial suspension (the inoculum, in sterile distilled water) was added to each well. For each strain, the growth conditions and the sterility of the medium were checked and the plates were incubated as referred above. Standard antibiotics, netilmicin and amoxicillin (at concentrations 4-88 µg/ml), were used as positive controls. For each experiment, the pure solvent, dichloromethane, was also applied as negative control. The experiments were repeated three times and the results were expressed as average values. Minimum inhibitory concentrations (MICs) were determined for all the samples and the standard pure compounds, under the same conditions, for comparison purposes. The MICs were taken as the lowest concentrations preventing visible growth.

RESULTS

The oils obtained from both plant species were pale yellow liquids with slight aromatic smell. The yield was 0.06 % v/w for *G. rotundifolium* and 0.12 % v/w for *H. villossum*. A total of 59 components, comprising 91.15 % of the oil got separated in the GC of *G. rotundifolium*, of which 54 constituents were identified (Table 1(a), 1(b) and 1(c). A 44.89 % of the oil was composed of oxygenated derivatives, while mono and sesquiterpene hydrocarbons constituted 36.67 % of the oil. The major compounds identified were spathulenol (12.46 %), α-terpineol (4.65 %) and germacrene-D (3.71 %). In a previous study on plants growing in Cameroon, it was found that sesquiterpene hydrocarbons constituted 90.1 % of the oil with germacrene D, β-caryophyllene and β-gurjunene being the major components [5]. The difference in the composition could be attributed to differences in the geographical location, climate, season and age at which the plants were collected.

In the essential oil of *Haumaniastrum villosum*, a total of 44 components were identified, representing 85.6 % of the oil (Table 2(a) and 2(b)); oxygenated derivatives were again the most abundant chemical category (44.48 % followed by mono- and sesquiterpene hydrocarbons (34.24 %) The most abundant components were caryophyllene oxide (19.01 %), humulene epoxide II (11.95 %), β-bourbonene (5.7 %), α-humulene (5.63 %) and β-caryophyllene (5.39 %).

The oils as well as pure reference compounds were tested for antimicrobial activity against eight bacterial species and three species of *Candida*. The antimicrobial activity as minimum growth inhibitory concentrations of the essential oils, some pure components and the reference antimicrobial agents, are shown in Table 3(a) and (b). Both oils exhibited different levels of antimicrobial activity against the tested microorganisms. The *G. rotundifolium* oil showed moderate activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* and weak activity against *E. coli* and had no activity at tested concentrations against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Enterobacter cloacae*.

On the other hand, *H. villosum* oil showed very promising antimicrobial activity against all the tested microorganisms (bacteria and fungi) with minimum inhibitory concentrations ranging from 0.08 to 10.34 mg/mL. Among the microorganisms, *S. aureus* was the most sensitive (MIC 0.08 mg/mL) and *E. coli* was the least sensitive (MIC 10.34 mg/mL).

DISCUSSION

The major compounds identified for the essential oil of *G. rotundifolium* were different from those identified previously for plants growing in Cameroon in which sesquiterpene hydrocarbons constituted 90.1 % of the oil with germacrene D, β-caryophyllene and β-gurjunene being the major components [5]. The difference in the composition could be attributed to differences in the geographical location, climate, season and age at which the plants were collected.

It would be worth reporting that *H. villosum* oil was strongly active against *S. mutans, S. viridis, Candida albicans, C. tropicalis* and *C. glabrata* (with MIC's 0.14-0.94 mg/mL), which were resistant to oils from *G. rotundifolium* and other plants growing in Tanzania, as reported previously [10-12]. In addition, the essential from *G. rotundifolium* was devoid of antifungal activity against the tested *Candida* species unlike the essential oil growing in Cameroon which was previously reported to have shown significant antifungal activity against *Fusarium moniliforme* and *Rhizopus stolonifera* [15].

Table 1: Chemical composition of the essential oil of *Geniosporum rotundifolium*

No.	Constituent	%	$KI_{(\alpha)}$	$KI_{(\alpha 1)}$	$KI_{(\beta)}$
1	*α*-Pinene	2.49	936	935	939
2	Camphene	1.10	951	949	951
3	*β*-Pinene	1.82	978	976	979
4	1-Octen-3-ol	0.73	983	981	979
5	3-Octanol	0.65	988	995	991
6	*p*-Cymene	1.48	1027	1025	1025
7	Limonene	2.65	1031	1029	1029
8	Eucalyptol	1.10	1033	1031	1031
9	*Cis*-ocimene	0.49	1042	1040	1037
10	*Trans*-*β*-Ocimene	0.30	1052		1050
11	*γ*-Terpinene	0.32	1061		1060
12	*cis*-Sabinene hydrate	0.61	1069	1068	1070
13	*α*-Terpinolene	0.28	1088		1089
14	Linalool	2.43	1102		1097
15	*α*-Thujone	0.74	1104	1103	1102
16	*α*-Camphonelal	0.39	1127	1125	1126
17	*Trans*-pinocarveol	1.17	1139	1138	1139
18	Camphor	2.28	1144	1143	1146
19	ε-Myroxide	1.14	1146	1145	1145
20	Borneol	1.26	1166	1169	1165
21	Terpinen-4-ol	2.85	1178	1177	1177
22	***α*-Terpineol**	**4.65**	1191	1190	1189
23	Myrtenal	tr	1193		1196
24	Unknown	2.10	1196	1194	
25	Verbenone	0.40	1206	1206	1205
26	*Trans*-carveol	0.72	1220	1219	1217
27	Carvone	0.42	1244	1244	1243
28	Hexyl tiglate	0.56	1333	1331	1333
29	*α*-Cubebene	0.60	1347	1347	1351
30	Eugenol	2.09	1359	1358	1359
31	*α*-Copaene	2.83	1372	1373	1377
32	*β*-Bourbonene	2.91	1379	1381	1388
33	*trans*-*β*-Damascenone	0.60	1382	1382	1385
34	*β*-Elemene	1.66	1387	1387	1391
35	Methyl eugenol	1.34	1406	1404	1404
36	*β*-Caryophyllene	2.09	1411	1414	1419
37	*β*-Gurjunene	0.91	1423	1425	1434
38	*α*-Bergamotene	0.77	1432	1432	1435
39	*α*-Humulene	0.52	1447	1449	1455
40	Alloaromadendrene	1.15	1453	1456	1460
41	*α*-Amorphene	1.62	1472	1472	1485
42	**Germacrene-d**	**3.71**	1475	1476	1485
43	*Ar*-curcumene	0.31	1479		1481
44	*β*-Ionone	0.74	1482	1482	1489
45	*Epi*-bicyclosesquiphellandrene	1.17	1488	1486	1494
46	*α*-Muurolene	0.75	1494	1490	1500
47	Γ-Cadinene	0.74	1506	1509	1514
48	Δ-Cadinene	2.68	1519	1520	1523
49	*α*-Calacorene	0.54	1537	1539	1546
50	Cerolidol	0.42	1564		1563
51	***Spathulenol***	***12.46***	1574	1576	1578
52	Caryophyllene oxide	2.6	1575	1579	1583
53	Salvial-4(14)-en-1-one	0.85	1585	1588	1595
54	Unknown	1.58	1602	1604	
55	Unknown	1.78	1648	1653	
56	*α*-Cadinol	1.69	1652	1655	1654
57	Cadalene	0.78	1671	1676	1677
58	Unknown	1.25	1686	1692	
59	Unknown	2.88		2168	
	Total	**91.15**			

Table 2: Chemical composition of the essential oil of *Haumaniastrum villosum*

No.	Constituent	%	$KI_{(\alpha)}$	$KI_{(\beta)}$
1	*α*-Pinene	0.14	937	939
2	*β*-Pinene	0.11	978	979
3	1-Octen-3-ol	0.33	983	979
4	p-Cymene	0.14	1027	1025
5	Limonene	0.27	1031	1029
6	Eucalyptol	0.29	1033	1031
7	*Trans*-pinocarveol	0.17	1141	1139
8	Camphor	0.33	1145	1146
9	Menthone	0.63	1156	1163
10	Isomenthone	1.32	1166	1163
11	Neomenthol	1.16	1166	1166
12	*α*-Terpineol	0.10	1192	1189
13	Linalool	1.26	1101	1097
14	Pulegone	0.55	1241	1237
15	Piperitone	0.40	1256	1253
16	*α*-Cubebene	0.45	1350	1351
17	Cycloisosativene	0.70	1366	1364
18	*α*-Ylangene	3.32	1371	1375
19	*α*-Copaene	1.35	1375	1377
20	***β-Bourbonene***	***5.70***	1384	1388
21	*β*-Cubebene	0.81	1389	1388
22	*β*-Elemene	1.00	1391	1391
23	***β-Caryophyllene***	***5.39***	1417	1419
24	*α*- Humulene	**5.63**	1453	1455
25	*Trans*- *β*-Farnesene	**0.32**	1457	1457
26	Alloaromadendrene	0.17	1475	1485
27	*α*-Amorphene	1.34	1475	
28	Germacrene-d	0.70	1479	
29	*β*-Selinene	0.74	1484	1485
30	*α*-Muurolene	0.57	1497	1485
31	*β*-Bisabolene	0.15	1508	1490
32	*γ*-Cadinene	3.61	1512	1500
33	*Trans*-calamenene	0.97	1523	1506
34	*δ*-Cadinene	0.42	1530	1514
35	*α*-Cadinene	0.24	1537	1529
36	Elemol	2.79	1551	1523
37	***Caryophyllene oxide***	***19.01***	1583	1539
38	Salvial-4(14)-en-1-one	1.24	1592	1550
39	***Humuleneepoxide II***	***11.95***	1609	1583
40	Unknown	3.28	1615	1595
41	Unknown	2.60	1622	1608
42	*β*-Eudesmol	1.00	1652	1651
43	*α*-Cadinol	2.95	1656	1654
	Total	**85.60**		

Table 3(a): Antimicrobial activity (MIC, mg/mL) of the essential oils and identified pure compounds

Essential oil/compound	*S. aureus*	*S. epidermidis*	*P. aeruginosa*	*K. pneumoniae*	*E. cloaceae*	*E. coli*	*S. mutans*	*S. viridans*	*C. albicans*	*C. tropicalis*	*C. grabrata*
G. rotundifolium	3.25	3.50	>20	>20	>20	18.50	-	-	-	-	-
H. villosum	0.08	0.95	1.25	1.37	2.50	10.34	0.14	0.39	0.94	0.74	0.82
1,8- Cineole	9.50	9.50	2.75	2.35	3.00	2.00	-	-	-	-	-
Limonene	>20	>20	>25	>25	>25	>20	-	-	-	-	-
Linalool	0.25	0.25	>20	>20	1.75	1.25	0.37	0.45	-	-	-
Camphor	2.70	1.95	2.80	3.24	2.75	1.33	-	-	4.85	3.76	3.56
Pulegone	1.20	0.95	1.45	1.76	1.37	1.45	1.75	1.26	-	-	-
Piperitone	1.50	2.25	0.60	0.80	1.10	0.95	-	-	-	-	-
Bornyl acetate	1.95	1.75	2.30	3.25	3.75	4.88	-	-	-	-	-
Borneol	1.25	1.57	2.50	3.75	4.20	4.50	-	-	-	-	-
Spathulenol	1.35	1.50	>20	>20	>20	8.50	-	-	-	-	-
α-Pinene	7.50	9.50	6.00	15.00	8.00	2.00	-	-	4.00	4.00	2.00
β- Pinene	12.00	16.00	>20	>20	>20	9.75	-	-	-	-	-

Table 3(b): Antimicrobial activity (MIC, mg/mL) of the essential oils and identified pure compounds (*contd*)

Essential oil/compound	S. aureus	S. epidermidis	P. aeruginosa	K. pneumoniae	E. cloaceae	E. coli	S. mutans	S. viridans	C. albicans	C. tropicalis	C. grabrata
β- Caryphylene	>20	>20	>20	>20	>20	>20	-	-	-	-	-
β- Caryphyleneoxide	0.073	0.90	0.87	1.23	2.43	>6.40	0.25	0.75	-	-	-
Netilmicin	$4x10^{-3}$	$4x\ 10^{-3}$	$8.8\ x10_{-3}$	$8x10^{-3}$	$8x10^{-3}$	10-2	-	-	-	-	-
Amoxycillin	$2x10^{-3}$	$2x10^{-3}$	$2.4x10^{-3}$	$2.2x10^{-3}$	$2.8\ x10^{-3}$	$2x10^{-3}$	-	-	-	-	-

The observed antimicrobial activity in the studied essential oils could be attributed to their major components. In the case of *G. rotundifolium*, the activity could be mainly, due to the oxygenated sesquiterpene spathulenol, which showed two to three times more activity than the oil, while the activity of *H. villosum* oil compared well with that of β-caryophyllene oxide. The antimicrobial activity of these oils could also be attributed to the major and minor constituents of the oils, constituents with the known antimicrobial activity such as spathulenol [11], linalool [13] and camphor [14], and their synergistic effects.

CONCLUSION

The composition and antimicrobial potential of two aromatic plants of Tanzania, *Geniosporum rotundifolium* and *Haumaniastrum villosum* have been determined for the first time. *H. villosum* shows good antimicrobial activity and hence should be further evaluated for possible use in preparations of pharmaceuticals for the management of disease conditions caused by these microorganisms, especially the oral and skin infections caused by *Candida* species.

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