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 (Bene) 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

INTRODUCTION

Geniosporum rotundifolium Briq. and Haumaniastrum villosum (Bene) 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 °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 °C at a rate of 2.5 °C/min. The injector and detector temperatures were programmed at 230 °C and 300 °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 60 to 280 °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), bornel, 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 viridans*, 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*.
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. villosum*. 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 Tanzania, as reported previously [10], 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].
| No. | Constituent            | %   | KI(i6) | KI(i5) | KI(i4) |
|-----|------------------------|-----|--------|--------|--------|
| 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-o-cimene           | 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-Campheneal           | 0.39| 1127   | 1125   | 1126   |
| 17  | Trans-pinocarveol      | 1.17| 1139   | 1138   | 1139   |
| 18  | Camphor                | 2.28| 1144   | 1143   | 1146   |
| 19  | α-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   | 1432   |
| 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-bicyclosquiphellandrene | 1.17 | 1488 | 1486 | 1494 |
| 46  | α-Murolene             | 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  | Ceriolide              | 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(a) | KI(b) |
|-----|------------------------------|----|-------|-------|
| 1   | α-Pinene                     | 0.14| 937   | 939   |
| 2   | β-Pinene                     | 0.11| 978   | 979   |
| 3   | 1-Octen-3-ol                 | 0.33| 983   | 979   |
| 4   | α-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-Terpinol                   | 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-Muurolele                  | 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  | Etemol                       | 2.79| 1551  | 1523  |
| 37  | Caryophyllene oxide          | 19.01| 1583  | 1539  |
| 38  | Salvial-4(14)-en-1-one       | 1.24| 1592  | 1550  |
| 39  | Humuleeneoxide 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. cloacae | 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. cloacae | E. coli | S. mutans | S. viridans | C. albicans | C. tropicalis | C. grabrata |
|-----------------------|-----------|----------------|--------------|---------------|-----------|---------|-----------|------------|-------------|--------------|-------------|
| β-Caryophyllene       | >20       | >20            | >20          | >20           | >20       | >20     | -         | -          | -           | -            | -           |
| β-Caryophylleneoxide  | 0.073     | 0.90           | 0.87         | 1.23          | 2.43      | >6.40   | 0.25      | 0.75       | -           | -            | -           |
| Netilmicin            | 4x10^{-3} | 4x10^{-3}      | 8.8x10^{-3}  | 8x10^{-3}     | 8x10^{-3} | 10^{-2} | -         | -          | -           | -            | -           |
| Amoxycillin           | 2x10^{-3} | 2x10^{-3}      | 2.4x10^{-3}  | 2.2x10^{-3}   | 2.8x10^{-3} | 2x10^{-2} | -         | -          | -           | -            | -           |
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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