Essential Oil-Bearing Plants from Nigeria: Studies on *Vernonia perrottettii* (Leaf and Stem Bark), Young Leaves from *Eucalyptus decaisneana* and Immature Leaves of *Hyptis suaveolens*[#]

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Abstract

The essential oils from four medicinal plants from Nigeria were obtained by hydrodistillation and analyzed by means of GC and GC/MS. The main constituents of the leaf and stem oils of *Vernonia perrottettii* Sch. Bip. (Asteraceae) were sesquiterpene hydrocarbons (92.1%) with abundance of β-caryophyllene (28.1% & 39.8%) and germacrene D (34.5% & 16.0%) in the leaf and stem oils, respectively. On the other hand, the leaf oil of *Eucalyptus decaisneana* Sm. (Myrtaceae) was dominated by the monoterpene hydrocarbons (90.6%) with α-phellandrene (31.1%), p-cymene (20.8%) and γ-terpinene (11.7%) being present in large quantities. The oil composition of immature leaf of *Hyptis suaveolens* L. (Lamiaceae) revealed the abundance of β-caryophyllene (22.3%), α-phellandrene (10.6%) and caryophyllene oxide (10.3%). The compositional pattern may probably lead to the delineation of a new chemotype of this species.

Key Word Index

Vernonia perrottettii, Asteraceae, *Eucalyptus decaisneana,* Myrtaceae, *Hyptis suaveolens,* Lamiaceae, essential oil composition, β-caryophyllene, α-phellandrene, p-cymene, γ-terpinene, caryophyllene oxide, germacrene D.

Introduction

Vernonia is a genus of about 1000 species of forbs and shrubs in the family Asteraceae (1). The plants are annual and herbaceous. Some species are sometimes known as iron-weeds while others are edible and of economic value. They are known for their intense purple flowers. Plants are thornless and vary in height and number of flowers. Several *Vernonia* species, including *V. calvoana* Drake, *V. amygdalina* Delile, and *V. colorata* Drake, are eaten as leaf vegetables. Common names for these species include bitter-leaf, ewuro and ndole. They are common in most West African and Central African countries. They are one of the most widely consumed leaf vegetables of Nigeria, where they are a key ingredient of stew (2). The leaves have both a sweet and bitter taste. They are sold fresh or dried and are a typical ingredient in egusi soup. For example, *V. galamensis* is used as an oilseed plant in East Africa. *Vernonia* species have been used in traditional medicine for the treatment of malaria, bacterial infection, and ulcers, etc. (2). They are used as food plants by the larvae of some *Lepidoptera* species including *Coleophora vernoniaeella*

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and *Schinia regia* (which feeds exclusively on this genus) (3). There are several reports describing the pharmacological potentials of the extracts and compounds isolated from the genus *Vernonia*. These included anthelmintic (3), cathartic (4), analgesic and anti-inflammatory (5,6), anti-plasmodial (7), hypoglycemic (8) and anti-mutagenic (9). *Vernonia* oils have potential in industrial application (10). The nutritive potentials of the plants have also been described (11–13). The genus is a source of biologically active natural compounds with diverse structural features, such as sesquiterpenes lactones (13–16), fatty acids (17), flavonoids (18) and glycosides (19). A review on the chemistry of important molecules and natural products from *Vernoniae* has been published (20).

Eucalyptus (family Myrtaceae) is a large genus of trees and shrubs, which originates from Australia (mainly Australia and Tasmania). It has been reported that about 500 species of *Eucalyptus* produce terpenoid hydrocarbons (21). The leaves of the *Eucalyptus* species have medicinal, pharmaceutical and flavoring properties. The essential oil-bearing *Eucalyptus* plants rank high both in quantity and frequency among the plants that are widely used all over the world. The oils of *Eucalyptus* have been used to relieve cramps, cleanse the blood, heal wounds, disinfect the air, and to treat conditions such as asthma, bronchitis, throat and skin infections, fevers, kidney infections, rheumatism, bladder infections, and sore muscles, and may lower blood sugar levels (22).

Both the extracts and oils of *Eucalyptus* species harvested from Nigeria and elsewhere are known to be rich in terpenoid compounds (23–25). One important criterion for studying the oils of this genus is the fact that they contain mono- and sesquiterpenoids and phenylpropanoids with a unique structure and their biological activities may have potential for developing new agents for use in industry and medicine (22). These compounds have been reported to possess various bioactivities including cytotoxicity and antimicrobial (23). In addition to the many species, there are chemovars (chemical varieties) or chemotypes (chemical types) among the species of *Eucalyptus*.

Hyptis suaveolens (L.) Poit (Lamiaceae) is widespread in tropical America and Northeast India. The plant is used as an appetizing agent, to combat indigestion, stomach pain, nausea, flatulence, colds, and infection of the gall bladder (26). The oil of *H. suaveolens* has been the subject of previous work. However, the oils from *H. suaveolens* differ in composition according to the geographic origin (genotype) of the plants (27–30).

In this paper, we report on the composition of the oils of *Vernonia perrottettii*, *Eucalyptus decaisneana* and *Hyptis suaveolens*. This is part of an extensive research program aimed at the characterization of the constituents of the poorly studied species of Nigeria flora.

Experimental

Plant materials: The leaves (256 g) and stem bark (320 g) of *V. perrottettii* were collected along the hill slopes of Isanlu-Isin, Kwara State, Nigeria, while the young leaves of *Eucalyptus decaisneana* (150 g) and immature leaves of *Hyptis suaveolens* (300 g) were harvested from trees and shrubs growing at a Forest Reserve along Ile-Ogbo Road, Iwo, Osun State, Nigeria. All collections were done during the rainy period, July and August, 2006. The plants were authenticated by Mr. T.K. Odewo of the Herbarium Headquarters, Forestry Research Institute of Nigeria (FRIN), Ibadan, where voucher specimens were deposited.

Isolation of the oils: Essential oils were obtained from the air-dried and pulverized plant samples by hydrodistillation in all glass Clevenger-type apparatus. The oils collected over water were dried over anhydrous sodium sulphate and preserved under refrigeration till the moment of analysis.

Analysis of the oils: GC analysis was accomplished with a HP-5890 Series II instrument equipped with a HP-Wax and HP-5 capillary columns (both 30 m x 0.25 mm, 0.25 μm film thickness), working with the following temperature program: 60°C for 10 min, rising at 5°C/ min to 220°C. The injector and detector temperatures were maintained at 250°C; carrier gas N (2 mL/min); detector dual, FID; split ratio 1:30. The volume injected was 0.5 μL. The identification of the components was performed by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (LRI) relative to the series of n-hydrocarbons. The relative proportions of the oil constituents were percentages obtained by FID peak-area normalization without the use of response factor.

GC-EIMS analysis was performed with a Varian CP-3800 gas-chromatograph equipped with a HP-5 capillary column (30 m x 0.25 mm, film thickness 0.25 mm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperature 220°C and 240°C, respectively; oven temperature programmed from 60–240°C at 3°C/min; carrier gas was He at a flow rate of 1 mL/min; injection of 0.2 μL (10% hexane solution); split ratio 1:30. Mass spectra were recorded at 70 eV. The acquisition mass range was 30–300 *m/z* at a scan rate of 1 scan/s.

Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear indices relative to the series of n-hydrocarbons, and on computer matching against commercially available spectral (NIST 98 and Adams) (31,32). Further identifications were also made possible by the use of homemade library mass spectra built up from pure substances and components of known oils and MS literature data (33–36). Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas.

Results and Discussion

The oils, isolated by separate hydrodistillation were obtained in the yields of 1.59% and 1.23% v/w, respectively for the leaf and stem of *V. perrottettii*. The oils were both deep yellow and light-yellow in coloration, respectively. The compositions of the leaf and stem oils determined by us can be seen in Table I.

In the leaf oil of *V. perrottettii,* monoterpenes were scarcely represented, both as number of compounds and total percentage (7.3%). This main monoterpenes were hydrocarbons while the oxygenated monoterpenoids were detected only in trace amounts. The sesquiterpenes (92.1%) were highly represented by the hydrocarbons (84.0 %) as compared to the oxygenated derivatives (8.1%). The main constituents were germacrene D (34.5%), β-caryophyllene (28.1%), caryophyllene oxide (3.7%)

and α-humulene (3.5%). Furthermore, some non-terpenoid aldehydes such as nonanal, pentadecanal, benzaldehyde and some straight chain (C10, C12 and C13) non-terpenoid hydrocarbons have been detected.

Table I. Constituents (%) identified from the leaf and stem oils of *Vernonia perrottetti*

Constituent	LR [a]	Leaf oil (%)	Stem oil (%)
camphene	955	tr	tr
benzaldehyde	963	-	tr
sabinene	978	tr	-
β-pinene	982	1.0	1.9
myrcene	991	0.4	0.6
decane	999	tr	-
α-phellandrene	1007	tr	tr
p-cymene	1028	tr	tr
limonene	1033	3.8	4.1
(Z)-β-ocimene	1040	0.4	0.3
(E)-β-ocimene	1050	1.7	1.4
linalool	1100	tr	0.2
nonanal	1104	tr	tr
terpinen-4-ol	1180	tr	0.2
α-terpineol	1192	tr	0.1
myrtenal	1195	tr	tr
dodecane	1199	tr	-
hexyl isovalerate	1244	tr	-
carvone	1246	tr	-
tridecane	1299	tr	tr
α-cubebene	1351	tr	tr
cyclosativene	1370	0.9	1.8
α-copaene	1376	2.4	2.6
β-bourbonene	1384	0.4	0.2
β-cubebene	1390	0.4	tr
β-elemene	1391	0.7	0.8
α-gurjunene	1409	0.4	0.5
β-caryophyllene	1419	**28.1**	**39.8**
β-gurjunene	1432	0.5	0.3
α-humulene	1456	3.5	4.8
allo-aromadendrene	1461	0.8	0.9
cis-muurola-4(14),5-diene	1463	0.3	tr
γ-muurolene	1477	1.1	0.7
germacrene D	1481	**34.5**	**16.0**
β-selinene	1487	0.7	0.9
valencene	1492	0.7	0.6
bicyclogermacrene	1495	2.5	1.7
α-muurolene	1496	0.8	0.6
δ-amorphene	1499	0.2	-
germacrene A	1505	1.0	1.0
γ-cadinene	1510	0.9	0.6
δ-cadinene	1524	3.2	3.2
β-sesquiphellandrene	1525	-	1.0
(E)-nerolidol	1564	0.6	0.2
spathulenol	1577	0.4	tr
caryophyllene oxide	1583	3.7	3.9
humulene oxide II	1608	0.6	0.2
1-epi-cubenol	1629	0.3	tr
τ-cadinol	1642	1.3	0.7
cubenol	1644	0.8	0.2
α-muurolol	1647	0.4	tr
α-eudesmol	1654	tr	1.0
selin-11-en-4α-ol	1656	tr	0.4
pentadecanal	1717	tr	0.4
hexadecanoic acid	1940	-	1.4
Total		**99.4**	**95.2**

[a] Linear retention indices on HP-5 column; tr = traces < 0.1%; - absent.

The stem bark oil was dominated by sesquiterpenoids over the monoterpenes (85.5% vs. 8.8%), among which hydrocarbons (79. 0%) prevailed over oxygenated constituents (6.5%). The same is true for the monoterpenes, with the hydrocarbons (8.3%) more abundant than the oxygenated derivatives (0.5%). The main constituents were β-caryophyllene (39.8%), germacrene D (16.0%) and caryophyllene oxide (3.9%) among the sesquiterpenes, and limonene (4.1%) among the monoterpenes.

The main compounds in this study have been recently reported to be the major constituents of the oil of *Vernonia scorpioides* (Lam.) Pers (37). These were β-caryophyllene (30.6%), germacrene D (27.3%), and bicyclogermacrene (8.5%). We have also recently shown that the oils of the leaves and flowers of *Tithonia diversifolia* (Hems) Gray, a member of the Asteraceae family, were rich in β-caryophyllene and germacrene D (38). This may lead to further research on the chemotaxonomy of the Nigerian grown Asteraceae family of plants. There is lack of literature information on the oil of this species. As such, this may represent the first comprehensive systematic analysis of the oil constituents of *V. perrottettii*.

Monoterpenes (90.7%)—typical of *Eucalyptus* oils—were the predominant class of compounds identified in the colorless oil of *E. decaisneana* (Table II). The oil yield was 1.22% v/w. The hydrocarbon contents consist mainly of α-phellandrene (31.1%), p-cymene (20.8%) and γ-terpinene (11.7%). In addition, β-phellandrene (6.2%), α-terpinene (3.9%) and α-pinene (2.2%) among the hydrocarbons, and terpinen-4-ol (4.6%) among the oxygenated derivatives, were also present in sizeable proportions. Sixteen sesquiterpenoids have been identified, but γ-eudesmol (2.2%) was the only sesquiterpenoid identified with a percentage above 1%. 1,8-Cineole, the commonly reported compound of some *Eucalyptus* oils, occurred in trace amount in the studied oil. It has been proposed that *Eucalyptus* oil, with high a content of phellandrene, may contain little or no cineole (39). Due to its high α-phellandrene content, the oil of *E. decaisneana* may be classified as belonging to the same group as those of *Eucalyptus obliqua* L' Hérit (39). β-Phellandrene was also among the quantitatively significant constituents of *E. obliqua* described by Li et al. (40). However, it could not be detected in significant quantity in *E. obliqua* analyzed from Australia (3). It should be noted that *E. decaisneana* has once been described as a synonym of *E. obliqua* (41).

The oil of *H. suaveolens* has been the subject of previous works. However, the oils from *H. suaveolens* differ in composition according to the geographic origin (genotype) of the plants. Previous studies on the plant from Nigeria (27) have revealed the abundant of α-pinene (13.6%), sabinene (13.2%), p-cymene (11.7%); and sabinene (30.0%), terpinen-4-ol (11.4%) from two different collections. In another analysis, Asekun and Ekundayo identified sabinene (16.5%) and *trans*-α-bergamotene/β-caryophyllene (19.8%) as the compounds occurring in higher amount (28). Iwu et al. had earlier reported the abundance of limonene, thujene, α-pinene, α-phellandrene, 3-cyclohexanol, 4-methyl-1-isopropy-3-cyclohexenol, 3-cyclohexenal, elemene and β-caryophyllene (42). In this present study on the light yellow oil (0.38% v/w yield) of immature leaves of the plant, β-caryophyllene (22.3%), α-phellandrene (10.6%) and caryophyllene oxide (10.3%) were the compounds identified in higher proportions (Table III). In addition, sabinene and

Table II. Oil composition (%) of *Eucalyptus decaisneana*

Constituent	LRI[a]	(%) Composition
α-thujene	932	0.2
α-pinene	941	2.2
camphene	955	tr
sabinene	978	0.2
β-pinene	982	tr
myrcene	991	1.0
α-phellandrene	**1007**	**31.1**
α-terpinene	1018	3.9
p-cymene	**1028**	**20.8**
limonene	1031	2.8
β-phellandrene	1032	6.2
1,8-cineole	1035	tr
(E)-β-ocimene	1050	tr
γ-terpinene	**1063**	**11.7**
terpinolene	1088	2.7
p-cymenene	1091	tr
linalool	1100	0.3
isoamyl isovalerate	1105	0.1
cis-p-menth-2-en-1-ol	1121	0.6
trans-p-menth-2-en-1-ol	1126	0.5
borneol	1167	tr
terpinen-4-ol	1169	4.5
p-cymen-8-ol	1185	0.3
α-terpineol	1192	0.6
trans-piperitol	1207	0.3
carvotanacetone	1247	0.1
piperitone	1254	tr
carvacrol	1299	0.4
terpinen-4-yl acetate	1340	0.2
longicyclene	1374	tr
(Z)-jasmone	1394	tr
tetradecane	1399	tr
methyl eugenol	1401	tr
α-gurjunene	1409	tr
β-caryophyllene	1419	tr
aromadendrene	1439	0.4
α-humulene	1456	tr
allo-aromadendrene	1461	0.1
β-selinene	1487	tr
valencene	1492	tr
pentadecane	1500	tr
γ-cadinene	1511	tr
δ-cadinene	1524	tr
globulol	1585	0.6
viridiflorol	1592	tr
guaiol	1597	0.1
10-epi-γ-eudesmol	1624	0.5
γ-eudesmol	1632	2.2
bulnesol	1667	tr
Total		**94.6**

[a] Retention indices on HP-5 capillary coated column; tr = traces < 0.1%.

Table III. Oil constituents (%) of immature leaves of *Hyptis suaveolens*

Constituent	LRI[a]	(%) Composition
α-thujene	932	0.2
α-pinene	941	1.3
sabinene	978	2.8
β-pinene	982	1.5
myrcene	991	0.4
α-phellandrene	**1007**	**10.6**
α-terpinene	1018	1.5
p-cymene	1028	6.3
limonene	1033	1.3
β-phellandrene	1035	1.6
1,8-cineole	1037	0.6
γ-terpinene	1063	3.9
cis-sabinene hydrate	1070	0.3
terpinolene	1088	2.4
undecane	1099	0.5
linalool	1100	0.2
trans-sabinene hydrate	1102	0.2
β-fenchol	1120	1.1
cis-p-menth-2-en-1-ol	1123	0.2
borneol	1168	0.2
terpinen-4-ol	1179	2.2
dodecane	1199	0.6
tridecane	1299	0.3
β-elemene	1391	0.3
tetradecane	1399	0.1
isocaryophyllene	1404	0.1
β-caryophyllene	**1419**	**22.3**
trans-α-bergamotene	1439	2.2
(E)-β-farnesene	1454	0.2
α-humulene	1456	1.6
germacrene D	1481	0.3
β-selinene	1487	1.5
bicyclogermacrene	1495	4.2
germacrene A	1505	0.3
spathulenol	1577	5.4
caryophyllene oxide	**1583**	**10.3**
globulol	1585	0.1
caryophylla-4(14),8(15)-dien-5-ol	1640	0.6
τ-cadinol	1642	0.3
α-cadinol	1655	0.5
selin-11-en-4α-ol	1658	1.4
trans-α-bergamotol	1691	5.7
Total		**97.6**

[a] Linear retention indices on HP-5 column.

trans-α-bergamotene, two of the major compounds in the earlier report (27, 28), were observed here in lower quantities, while several of the diterpenoids compounds were conspicuously not detected in the present study. Moreover we could not detect any of the cyclo compounds as defined earlier (42). The reason may be attributed to genotype, the age of the analyzed samples or some other ecological and environmental factors.

This result may therefore present a new chemotype of the plant growing in Nigeria. As such, it may be postulated that five chemotypes of the oils of *H. suaveolens* have been found to exist so far in Nigeria; oil with abundance monoterpene and sesquiterpene hydrocarbons in addition to 3-cyclohexanol, 4-methyl-1-isopropy-3-cyclohexenol and 3-cyclohexenal (40); oil dominated by α-pinene, sabinene and p-cymene; those with abundance of sabinene and terpinen-4-ol (27); sabinene and β-caryophyllene/*trans*-α-bergamotene-rich oil (28) and the oil containing higher proportions of β-caryophyllene, α-phellandrene and caryophyllene oxide, as defined in the present study. This result looks similar to those oils from Nigeria (28), Tanzania (29) and Cameroon (30) by their high β-caryophyllene contents. Another study on this species from Cameroon (43) had revealed the abundance of sabinene, β-pinene, limonene, p-cymene and β-caryophyllene. The sample analyzed from Mali was shown to consist mainly of sabinene with β-caryophyllene/*trans*-α-bergamotene (44). However, neither of these mentioned reports nor those from other parts of the world contained high amounts of α-phellandrene and caryophyllene oxide.

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