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Composition of volatile oils from leaf, stem, root, fruit, and flower of *Ruellia tuberosa* L. (Acanthaceae) from Nigeria

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We report composition of *Ruellia tuberosa* L. (Acanthaceae) leaf, stem, root, fruit, and flower volatile oils from Nigeria. The five volatile oils were obtained by hydro-distillation using all-glass Clevenger apparatus designed to British Pharmacoepia specifications and were procured in 0.09 to 0.36% yields. Each was separately examined using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis. Our results revealed leaf oil contain 24 compounds, which make-up 86.95% of it; stem oil has 15 compounds (accounting for 93.96%); root oil with 42 compounds being 91.49%; fruit oil contain 60 compounds which amount to 89.68% and flower oil has 6 compounds representing 95.06% of the oil. Dominant compounds in each essential oil are (%): leaf (E-phytol 21.06, tributylacetyl citrate 19.44, heptacosane 7.55); stem (m-xylene 33.83, heptacosane 16.57, p-xylene 9.67); root (heptane 22.25, heptacosane 12.89, borneol 12.48); fruit (hexacosane 15.43, sextone 13.12, heneicosane 11.14) and flower (tributylacetyl citrate 67.78, 2-methyl-2-pentanol 10.15, 1-methyl-1-cyclopentanol 6.90). Important classes of compounds in Nigerian *R. tuberosa* volatiles are monoterpenes, monoterpenoids, sesquiterpenes, sesquiterpenoids, hydrocarbons, aromatics, esters, alcohols, sulphur compounds, ketones and aldehydes. A total of 109 compounds were identified in the five essential oils of *R. tuberosa*. These compounds have high therapeutic effects and are characteristic of *R. tuberosa*. The oils are good sources of sextone (methylcyclohexane), β-linalool and alcohols. We present the volatile constituents in the leaf, stem, root, fruit, and flower of *R. tuberosa* which have not been earlier reported in literature.

Key words: *Ruellia tuberosa*, Acanthaceae, essential oil, gas chromatography-mass spectrometry (GC-MS), sextone, monoterpenoids, sesquiterpenoids, linalool, alcohol.

INTRODUCTION

Ruellia tuberosa L. (herb) commonly called 'cracker plant', often grown for ornamental purposes is an Acanthaceae: the 3rd largest tropical family of dicotyledonous plants with about 2500 species, most of which have medicinal values (Burkill, 1985). It has tuberous roots with leaf, stem, fruit and flower parts, is native to America, but widely spread in dry and hilly regions. *R. tuberosa* is traditionally used as diuretic, antipyretic, analgesic, anti-hypertensive, anthelmentic, abortifacient, emetic, in curing bladder disease, kidney disorder, bronchitis, gonorrhoea and syphilis (De Fillpps et al., 2004). There are reports that it possess anti-oxidant, anti-microbial, anti-cancer, anti-nociceptive, anti-inflammatory and gastro-protective activities (De Fillpps et al., 2004; Chothani et al., 2010; Agnihotri et al., 2012; Andhiwal et al., 1985; Alam et al., 2009). The plant has emetic properties and serves as substitute for *ipecacuanha* plants, in the treatment of bladder stones. Its leaf decoction is used in treatment of bronchitis, and its tuberous roots are ingredient in health tonic (De Fillpps et al., 2004; Agnihotri et al., 2012; Samy et al., 2015). It displayed ulcer protective activity in male Wistar rats (Sri Kumar and Pardhasaradhi, 2013). Leaf extracts of *R. tuberosa* L. were reported to control lipid peroxide level and help strengthen antioxidant potential in diabetic rats (Manikandan et al., 2010). *R. tuberosa* root extracts displayed anti-oxidant activity, which were comparable with standards (Chothani and Mishra, 2012).

Preliminary phytochemical screening of ethyl acetate extract of *R. tuberosa* reveals presence of saponin, tannins, and flavonoids (SriKumar and Pardhasaradhi, 2013). Five bioactive flavonoids cirsimaritin, cirsiliol 4′-glucoside, cirsimarin, sorbifolin, and pedalitin, with three other metabolites betulin, vanillic acid, and indole-3-carboxaldehyde were isolated and reported from ethylacetate extracts of *Ruellia tuberosa.* First two flavonoids showed cytotoxicity against KB cell line with the IC_{50} values of 30.05 and 17.91 µg/mL, and the third flavonoid was cytotoxic against HepG2 cell line with an IC_{50} value of 38.83 µg/mL (Lin et al., 2006). Apigeninglucuronides have also been isolated from *Ruellia* (Subramanian and Nair, 1972, 1974). Subramanian and Nair reported that *R. tuberosa* leaves have only traces of apigenin and luteolin, but its flowers contain malvidin-3,5-diglucoside in appreciable amount (Subramanian and Nair, 1974).

Also the flower buds have maximum proportion of flavonoids yielding 3% of apigenin-7-O-glucuronide. Other flavones identified were apigenin-7-O-glucoside, apigenin-7-O-rutinoside and luteolin-7-O-glucoside (Subramanian and Nair, 1974). Aerial parts of *R. tuberosa* yielded 21-methyldammar-22-en-3β, 18, 27-triol atriterpenoid (Singh et al., 2002). Identified and reported essential oil components are sources of important data and information on the plant and its family classifications (Baser and Buchbauer, 2010). Twenty-five compounds were reported from GC-MS analysis of ethanol extract from tuber of *R. tuberosa* (%): Lupeol (68.14), stigmasterol (8.89), α-sitosterol (3.99), sucrose (2.24), 3α-bromo-cholest-5-ene (2.24), 2-methyl-octadecane (2.10), 2-methyl-nonadecane (1.93), 2-methyl-eicosane (1.79), hexacosane (1.43) and heptacosane (1.29) as its prominent compounds (Rajendra et al., 2014).

Previously we examined and reported volatile chemical compositions on three Acanthaceae: *Brillantaisia patula* (leaf and stem), *Hypoestes phyllostachya* (leaf and stem), *Asystasia gangetica* (aerial, seed and root) (Moronkola *et al.,* 2009a, 2009b, 2011). This study examines the composition of volatile content of leaf, stem, root, fruit, and flower of *R. tuberosa* L. also an Acanthaceae, from Nigeria, which have not been earlier reported in literature.

MATERIALS AND METHODS

Plant material

Fresh samples of *R. tuberosa* were collected from Ibadan, Oyo State, Nigeria, on 15th October 2014. The plant was authenticated in the herbarium, Department of Botany, University of Ibadan, Ibadan, where some voucher samples have been deposited, with voucher number UIH - 22426. Collection of the sample was done during the day time. The plant was separated into leaf, stem, root, fruit, and flower parts.

Extraction of the essential oils

Each separated parts (leaf, stem, root, fruit, and flower) of *R. tuberosa* was crushed and hydro-distilled for 3 to 3½ h in an all glass Clevenger-type apparatus designed to British Pharmacopeia specifications and the oils refrigerated until analyses. Essential oils from the pulverized air-dried plant materials were procured in 0.09 to 0.36% yields (Table 1). Each of the oils had distinct characteristic pleasant smell.

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analyses

Composition of the essential oils was determined by gas chromatography-mass spectrometry (GC-MS) using an Agilent 7890N gas chromatography hyphenated with an Agilent system mass detector Triple Quad 7000A in EI mode at 70 eV (m/z range 40 - 600 amu) with an ion source temperature of 250°C and an Agilent ChemStation data system. GC column was equipped with an HP-5MS column (30 m × 250 µm × 0.25 µm) a split-split less injector heated at 200°C and a flame ionization detector (FID) at 230°C. Oven temperature was programmed as follows: Initial temperature 40°C for 5 min, increased 5°C/min to 180°C for 6 min and then 10°C/min to 280°C for 12 min. Helium was the carrier gas at flow rate of 1 mL/min. Injection volume was 2.0 µL (split ratio 1:20).

The components were identified by comparison of their mass spectra with NIST 1998 library data of the GC-MS system as well as by comparison of their retention indices (RI) with the relevant literature data (Adams, 2007). The relative amount of each individual component of the essential oil was expressed as the percentage of the peak area relative to the total peak area. RI value

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Table 1. Yields of volatile oils procured from leaf, stem, root, fruit, and flower of *R. tuberosa* L.

S/N	Plant parts	Weight of sample (g)	Weight of essential oil procured (g)	% Yield of essential oil procured	Physical examination (note)
1	Leaf	582	1.78	0.31	Herbal/ leafy
2	Stem	717	2.61	0.36	Slightly choking but pleasant
3	Root	421	0.39	0.09	Woody
4	Fruit	571	0.77	0.14	Fruity
5	Flower	52	0.13	0.25	Floral

of each component was determined relative to the retention times of a homologous n-alkane series with linear interpolation on the HP-5MS column.

RESULTS

Results of the yields (0.09 to 0.36%) of extracted essential oils from five parts of *R. tuberosa* L. (leaf, stem, root, fruit, and flower), with their physical examinations are presented in Table 1. GC and GC-MS analysis of the oils afforded the identification of 24 compounds in leaf, 15 in stem, 42 in root, 60 in fruit and 6 in flower. Results of the 109 identified compounds are presented in Table 2. Table 3 has results of the comparison of the eleven classes of compounds found in the five *R. tuberosa* essential oils studied.

DISCUSSION

Volatile oils from five parts of *R. tuberosa* L. (leaf, stem, root, fruit, and flower) were obtained by hydro-distillation. Oils were procured in 0.09 to 0.36% yields, each having characteristic distinctive notes: Leaf oil (0.31% yield) had a pleasant herbal to leafy note; stem oil (0.36%) possessed slightly choking but pleasant smell; root oil (0.09%) had woody odour; fruit (0.14 %) with fruity note, while the flower oil (0.25%) had a floral note (Table 1).

Compounds identified in each are listed in Table 2. A total of 109 volatile compounds had been identified in the five essential oils from the Nigerian *R. tuberosa*. Our results revealed leaf oil contain 24 compounds, which make-up 86.95 % of it; stem oil has 15 compounds (93.96 % of it); root oil with 42 compounds being 91.49% of it; fruit oil contain 60 compounds (89.68% of it); flower oil has 6 compounds responsible for 95.06%. Dominant compounds (%) in each essential oil are: Leaf (E-phytol 21.06, tributylacetyl citrate 19.44, heptacosane 7.55); stem (m-xylene 33.83, heptacosane 16.57, p-xylene 9.67); root (heptane 22.25, heptacosane 12.89, borneol 12.48); fruit (hexacosane 15.43, sextone 13.12, heneicosane 11.14) and flower (tributylacetyl citrate 67.78, 2-methyl-2-pentanol 10.15, 1-methyl-1-cyclopentanol 6.90).

The oils are generally good sources of sextone, β-linalool and alcohols, with monoterpenoids more abundant than sesquiterpenoids. Important classes of compounds in Nigerian *R. tuberosa* volatiles are monoterpenes, monoterpenoids, sesquiterpenes, sesquiterpenoids, hydrocarbons, aromatics, esters, alcohols, sulphur compounds, ketones and aldehydes. Percentage of each of this is shown in Table 3, for the five *R. tuberosa* essential oils.

Each of the identified 109 compounds plays important role in the vast ethno-medicinal uses and biological activities demonstrated by *R. tuberosa* (De Fillpps et al., 2004; Chothani et al., 2010; Agnihotri et al., 2012; Andhiwal et al., 1985; Alam et al., 2009, Samy et al., 2015). High content of linalool in *R. tuberosa* oils present anticonvulsant and hypnotic activities. This was reported for *Ocimum basilicum* essential oils (Ismail,2006). Limonene, which is in appreciable amount in *R. tuberosa* root and fruit oils, and citral compound (in leaf oil) have sedative and stimulant effects, they may be responsible for *R. tuberosa* application as antinociceptive and anticonvulsant. These activities have been observed and reported in the essential oils of *Lippia alba* and *Satureja hortensis* (Vale et al., 2002; Viana et al., 2000; Hajhashemi et al., 2002). β-caryophyllene and other sesquiterpenes are abundant in *R. tuberosa* leaf, root and fruit essential oils, known to be responsible for high anticancer properties, hence responsible for anti-cancer effects of *R. tuberosa* (Sylvestre et al., 2005, 2006). Limonene (in root and fruit oils), with perillyl compounds and other monoterpenoids in *R. tuberosa* essential oils are agent with special benefit as anti-tumor (Jahangir and Sultana, 2007; Low-Baselli et al., 2000).

Conclusion

Our study on the five volatile oils of *R. tuberosa* L. from Nigeria resulted in the identification of a total of 109 compounds. Monoterpenoids are more abundant than sesquiterpenoids, with the oils rich in esters, alcohols, aromatics and carbonyls. The oils are good sources of sextone, β-linalool and alcohols. We have presented the volatile constituents in *R. tuberosa* (leaf, stem, root, fruit, and flower) which have not been earlier reported in

Table 2. Chemical composition of volatile oils of leaf, stem, root, fruit, and flower of *R. tuberosa* L.

S/N	KI	Compound	% Composition in				
			1.Leaf (LF)	2.Stem (ST)	3.Root (RO)	4.Fruit (SD)	5.Flower (FL)
1	681	2-pentanol	-	-	-	-	4.16
2	688	2,5-Dimethylhexane	-	-	-	0.14	-
3	709	3-Methyl-3-pentanol	-	-	-	0.57	-
4	710	2-methyl-2-pentanol	-	-	-	-	10.15
5	717	Heptane	-	-	22.25	-	-
6	722	Dimethyl disulfide	-	-	-	0.16	-
7	752	3-Methylheptane	-	-	-	0.33	-
8	754	3-Hexanone	-	-	0.25	-	-
9	781	Sextone	2.63	7.92	5.14	13.12	-
10	785	n-Butylacetate	-	-	-	8.36	-
11	806	Hexanal	0.30	-	1.12	-	-
12	811	1-methyl-1-cyclopentanol	-	-	-	-	6.90
13	814	(E)-2-hexenal	5.34	0.93		-	-
14	842	(Z)-1,3-Dimethylcyclohexane	-	-	0.23	1.11	-
15	843	1-hepten-3-one	-	1.37	-	-	-
16	853	2-Heptanone	-	-	-	0.14	-
17	860	Amylcarbinol	-	1.50	-	-	-
18	868	(Z)-hex-3-en-1-ol	2.12	-	-	-	-
19	868	(E)-2-hexan-1-ol	1.04	-	-	-	-
20	880	Ethylcyclohexane	-	-	-	0.32	-
21	893	Ethylbenzene	-	7.77	0.35	0.88	-
22	905	Heptenal	-	-	-	0.49	-
23	906	Heptanal	-	-	0.25	-	-
24	907	m-xylene	-	33.83	-	-	-
25	907	p-xylene	2.51	9.67	-	-	3.22
26	916	Nonane	-	0.45	-	-	-
27	919	(E)-2-Hexenal	-	-	-	0.35	-
28	943	1-octen-3-one	1.97	-	-	-	-
29	952	3-octenone	-	-	2.59	-	-
30	953	3-Octanone	1.90	5.04	-	0.94	-
31	969	1-Octen-3-ol	3.31	-	-	0.71	2.85
32	979	3-Octanol	2.56	1.95	0.90	0.42	-
33	982	Benzaldehyde	0.34	-	1.86	0.17	-
34	995	Ethylhexanol	-	0.73	-	-	-
35	1005	Octanal	-	-	-	0.37	-
36	1015	Decane	-	-	-	0.18	-
37	1018	Limonene	-	-	0.34	0.14	-
38	1030	2,4-Dimethylcyclohexanol	-	-	-	0.26	-
39	1040	2-pentylfuran	-	-	0.41	-	-
40	1081	Phenylacetaldehyde	-	-	0.44	0.63	-
41	1082	β-Linalool	5.71	5.32	2.88	1.27	-
42	1088	Borneol	-	-	12.48	-	-
43	1104	Nonanal	-	0.44	4.07	0.69	-
44	1112	(E)-2-Nonenal	-	-	1.57	0.65	-
45	1121	Fenchone	-	-	0.57	-	-
46	1130	Dihydrocitronellol	-	-	-	0.24	-
47	1136	2-methyl-2-nonen-4-one	0.96	-		-	-
48	1138	Exofenchol	-	-	3.58	0.41	-
49	1140	3-methyl-2-(cyclohexen-1-yl)-acetaldehyde	-	-	2.74	-	-

Table 2. Cont'd.

50	1143	α-terpineol	-	-	0.85	-	-
51	1150	4-Hydroxyl-3-propyl-2-hexanone	-	-	0.38	0.49	-
52	1158	(Z)-β-Terpineol	-	-	-	0.13	-
53	1159	1-nonanol	-	-	0.54	-	-
54	1161	2-Methyl isoborneol	-	-	-	0.45	-
55	1171	p-Anisaldehyde	-	-	-	0.25	-
56	1175	(Z)-piperitol	-	-	0.71	-	-
57	1183	Methyl-nonanoate	-	-	0.60	-	-
58	1186	Safranal	0.53	-	-	-	-
59	1188	2,4-nonadien-1-ol	-	-	0.23	-	-
60	1204	Decanal	-	-	-	0.17	-
61	1205	β-cyclocitral	0.28	-	-	-	-
62	1206	(E)-carveol	-	-	0.50	-	-
63	1207	Perilla aldehyde	-	-	1.23	-	-
64	1215	Decane	-	-	0.82	-	-
65	1218	p-Acetylanisole	-	-	-	0.21	-
66	1218	3-acetylanisole	0.21	-	-	-	-
67	1220	(E,E)-2,4-decadienal	-	-	0.54	-	-
68	1231	Naphthalene	-	0.47	-	-	-
69	1251	2-Undecanone	-	-	-	0.33	-
70	1261	Perilla alcohol	-	-	0.70	-	-
71	1281	Isoaromadendrene epoxide	-	-	1.80	-	-
72	1339	β-Cubebene	-	-	-	0.16	-
73	1371	4,4,7a-trimethyl-2,4,5,6,7,7a-hexahydro-1H-inden-1-one	0.55	-	-	-	-
74	1398	Cedrene	-	-	-	0.59	-
75	1403	β-Gurjunene	-	-	-	3.41	-
76	1407	4-Acetonylcycloheptanone	-	-	-	0.18	-
77	1410	2-Dodecanal	-	-	-	0.80	-
78	1416	(-)-α-Panasinsen	-	-	-	0.49	-
79	1416	Thujospene	-	-	-	0.15	-
80	1419	α-gurjunene	-	-	0.41	-	-
81	1420	Geranylacetone	-	-	0.49	0.44	-
82	1424	(E)-1,10-Dimethyl-(E)-9-decalinol	-	-	-	1.60	-
83	1432	β-Patchoulene	-	-	-	0.39	-
84	1440	Cadinene	-	-	-	0.46	-
85	1457	β-Ionone	4.01	-	-	0.24	-
86	1465	γ-elemene	-	-	0.60	-	-
87	1471	9,9-Dimethyl-9-silafluorene	-	-	-	0.26	-
88	1474	Eremophilene	-	-		0.65	-
89	1475	α-salinene	-	-	0.46	-	-
90	1490	α-Guaiene	-	-	-	0.13	-
91	1494	β-caryophyllene	0.44	-	1.24	-	-
92	1519	2,6,10-Trimethyltetradecane	-	-	-	0.66	-
93	1524	α-curcumene	-	-	0.29	-	-
94	1558	2,2,7,7-Tetramethyltricyclo(6.2.1.0)(1,6)undec-4-en-3-one	-	-	-	3.26	-
95	1564	(+)-(E)-Nerolidol	-	-	-	0.69	-
96	1580	α-Cadinol	-	-	-	0.88	-
97	1593	β-eudesmol	-	-	0.61	-	-
98	1660	Germacrene D-4-ol	-	-	1.19	0.86	-

Table 2. Cont'd.

99	1754	Hexahydrofarnesylacetone	0.84	-	-	1.47	-
100	1890	2-Methylhexadecan-1-ol	-	-	-	0.35	-
101	1910	Nonadecane	-	-	-	1.53	-
102	1999	Octadecanal	-	-	0.39	-	-
103	2046	E-phytol	21.06	-	-	-	-
104	2109	Heneicosane	-	-	-	11.14	-
105	2336	2-(Z)-9-Octadecenyloxyethanol	-	-	-	0.26	-
106	2594	tributylacetyl citrate	19.44	-	-	-	67.78
107	2606	Hexacosane	-	-	-	15.43	-
108	2705	Heptacosane	7.55	16.57	12.89	-	-
109	2804	Octacosane	1.35	-	-	8.12	-
% Identified			86.95	93.96	91.49	89.68	95.06

Table 3. Classes of compounds in the five *R. tuberosa* volatile oils.

S/N	Class of compound	% in each of the *R. tuberosa* essential oils				
		Leaf	Stem	Root	Fruit	Flower
1	Monoterpenes	-	-	0.34	0.14	-
2	Monoterpenoids	10.53	5.32	25.08	3.18	-
3	Sesquiterpenes	0.44	-	3.00	6.57	-
4	Sesquiterpenoids	0.84	-	1.80	3.9	-
5	Hydrocarbons	11.53	24.94	41.33	52.2	-
6	Aromatics	2.72	51.74	0.76	1.09	3.22
7	Esters	19.44	-	0.60	8.36	67.78
8	Alcohols	30.09	4.18	2.38	4.17	24.06
9	sulphur compounds	-	-	-	0.16	-
10	ketones	5.38	6.41	3.22	5.34	-
11	Aldehydes	5.98	1.37	12.98	4.57	-

literature.

Conflict of Interests

The authors have not declared any conflict of interests.

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