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Research Article

GC-MS analyses of four essential oils with antioxidant activity of extracts of *Rytigynia umbellulata* (Hiern) Robyns

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Abstract

Essential oil compositions of leaf, leaf stalk, stem and fruit with phytochemical screening and antioxidant evaluation of extracts obtained from *Rytigynia umbellulata* (Rubiaceae) were determined in this study. The essential oils were extracted using hydro-distillation method and analysed by gas chromatography coupled with mass spectrometry (GC-MS), while crude extraction was done in methanol, subjected to standard phytochemical tests and antioxidant potential of extracts was done using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The numbers of identified compounds in leaf, leaf stalk, stem and fruit essential oils were 15, 19, 27 and 11, respectively. Saturated hydrocarbons (70.95%) and acyclic diterpene (16.91%) dominate the leaf essential oil, with the most abundant being: cyclohexane (65.22%) and phytol (16.91%). Saturated hydrocarbons were also prominent in essential oils procured from the leaf stalk (67.05%), stem (37.86%) and fruit (40.32%). Carboxylic acids (11.84%) were exclusive to stalk oil, while sesquiterpenes were only identified in the stem oil. Methanol extracts from leaves (66.88 g) and stem (22.68 g) gave extractive yields of 19.11% and 4.73% respectively. Flavonoids, phenolic compounds, tannins, saponins, terpenoids and coumarins were the classes of secondary metabolites revealed in both extracts. Alkaloid was exclusive to the stem extract, while quinone was only in the leaf extract. The antioxidant evaluation showed that the stem extract exhibited better activity (IC_{50}=0.30 mg/mL) compared to the leaf extract (IC_{50}=0.41 mg/mL), while the control, butylated hydroxyanisole had IC_{50}=0.19 mg/mL. The chemical compositions of the essential oils, phytochemical screening and antioxidant activity of the extracts being reported for the first time support the uses of *R. umbellulata* in ethno-medicine.

1. Introduction

The use of aromatic plants in traditional medicine and their potential for commercial exploitation as fragrance and flavour enhancers, cosmetics and medications have significantly increased in recent years. The anti-inflammatory, anti-tumor, and antioxidant properties of these fragrant plants have been used for centuries, according to recent studies [1]. Many living things depend on oxidation, for their metabolic activities. However, many diseases, including rheumatoid arthritis, arteriosclerosis, inflammations, cancer, and others are brought on by the unchecked creation of free radicals derived from oxygen. Even in low concentrations in comparison to the oxidant, antioxidants are molecules that prevent the oxidation of the substrate. These antioxidants contain usually high levels of phenolic

compounds that are found in a wide variety of plants and herbs [2, 3]. Among these plants is the *R. umbellulata* (Hiern) Robyns, a species of the Rubiaceae family and is known as the "umbrella tree". The Rubiaceae family is a large and diverse group of flowering plants comprising of 620 generas and over 13,500 species [4]. All members of this family are characterized by their opposite or whorled leaves, interpetiolar stipules and sympetalous actinomorphic flowers. Their ovaries are inferior and the fruit is usually a berry, capsule or drupe [5].

R. umbellulata is native to tropical and southern Africa. It has been used in the treatment of a variety of ailments like malaria, fever, skin conditions such as eczema, diarrhea and treating wounds. It has also been used in cuisine as dried leaves, not only to improve the flavor of the food but also to avoid its deterioration because of its antioxidant activities [6]. Industrially, the flowers have been used as dyes for fabrics as they are non-toxic and biodegradable making them eco-friendly. Despite the aforementioned ethno-medicinal uses of this plant, neither report of its essential oil constituents, nor their antioxidant potential of extracts has been the subjects of scientific investigations to date. Hence, the essential oil compositions of leaf, leaf stalk, stem and fruit with phytochemical screening and antioxidant evaluation of extracts obtained from *R. umbellulata* were carried out in this study.

2. Materials and methods

2.1 Plant Materials and Apparatus

Fresh samples of the leaves and stem of *R. umbellulata* were collected from Agbegi-odofin village, Ikire, Osun state, Nigeria, latitude - 7°23'15.40"N and longitude - 4°13'5.46" E. The plant was identified and authenticated by a taxonomist, with voucher specimen (FHI 113856) deposited in the herbarium section of the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan, Oyo state, Nigeria. Plant samples were sorted into leaf, leaf stalk, stem and fruit parts. Fresh samples were used for essential oil extractions while air-dried, pulverized samples were used for crude extracts; they were dried in air cool environment for two weeks. Clevenger apparatus, sterile syringe, round bottom flask, temperature regulated heating mantle, Gas Chromatography- Mass Spectrophotometer (7890B GC with 5977A MSD, Agilent Technologies, USA), weighing balance, water bath, filter paper, air-tight sample vials, were used in this investigation.

2.2 Methods

2.2.1 Extraction of essential oils

The hydro-distillation method was employed using the Clevenger-type apparatus designed according to British Pharmacopeia specifications described in our earlier reports [7]. Samples of the leaf (69.25 g), leaf-stalk (251.45 g), stem (72.67 g) and fruits (193.24 g) of *R. umbellulata* were carefully weighed and then introduced separately into a 5L round bottom flask until fully immersed in water. The flask with the content was then placed on a temperature regulated heating mantle and the Clevenger apparatus was mounted on the flask; thereafter, the condenser inlet tube was connected to a suction pump. About 1 mL of hexane was introduced to the system through the side-arm of the Clevenger apparatus and a clear layer was formed between the hexane and water. Ice flakes were continuously introduced to the system through the side-arm to ensure a favorable condition for trapping the essential oils. The extraction was carried out for about 3 hours at a regulated temperature. The oil was trapped in hexane which acts as the solvent as it partitions on top of the water layer. After the extraction, the oils were collected with the aid of a sterile syringe and stored in air-tight sample vials. The weights of the oil extracted from the leaf, leaf stalk, stem and fruit of the plant were recorded, their percentage yield was calculated and the physical features of the oils were also observed. The oils were then stored in a refrigerator prior to further analysis.

2.2.2 Crude extraction of leaf and stem samples

The grounded dried samples of the leaf and stem of *R. umbellulata* were separately soaked in methanol (cold extraction). 480 g of stem was soaked in 1.3 litres of methanol, while 500 g of the leaves was soaked in 1.9 litres of methanol for 3 days. After which, it was decanted, filtered using a filter paper, and the filtrate was concentrated using a rotary evaporator. Due to potential difficulties in collecting the extract, total solid concentration was not performed; instead, some solvent was allowed to be present in the extract. To fully concentrate into a solid extract, this was left in desiccator packed with activated drying agent (silica gel), till a dried extract

was obtained. The weight of the extract collected for the stem was 7.56 g, while the leaves were 29.34 g.

2.2.3 Identification of essential oils components

Identification of the essential oil components was based on comparison with their mass spectral fragmentation patterns with in-built computer data and commercial systems, such as the National Institute for Standards and Technology (NIST) database, 02L, 14.L, W9N11, Chemstation data system, courtesy Wiley GC-MS Library.

2.2.4 Phytochemical screening

The phytochemical screening of the crude extracts of the leaves and stem of *R. umbellulata* was carried out using standard qualitative methods of the *International Journal of Chemical Studies*

Test for alkaloids: Dragendroff's Test

A small quantity of the extract was acidified with 1% HCl for 2 minutes and then treated with a few drops of dragendroff's reagent in a test tube. The formation of a reddish brown precipitate indicates the presence of alkaloid [9, 10].

Test for cardiac glycoside: Baljet's Test

A small quantity of extract was treated with a few drop of baljet's reagent in a test tube. The formation of a yellow-orange color indicates the presence of cardiac glycoside [11, 12].

Test for flavonoids: Lead Acetate Test

A small quantity of plant extract was treated with a few drops of 10% lead acetate solution. The formation of a yellow precipitate indicates the presence of flavonoids [9, 10, 13].

Test for phenolic compounds: Lead Acetate Test

A small quantity of extract was dissolved in 5 mL of distilled water and treated with 3 mL of 10% lead acetate solution. The formation of a white precipitate indicates the presence of phenolic compounds [14].

Test for tannins: 10% NaOH Test

A small quantity of plant extract was treated with 4 mL of 10% NaOH and shaken well. The formation of emulsion indicates the presence of tannins [10].

Test for saponins: Foam Test

A small quantity of extract was dissolved in 2 mL of distilled water; the resulting solution was shaken vigorously for a few minutes. Foaming that persisted for 10 minutes, was taken as evidence of the presence of saponins [13].

Test for terpenoids

A small quantity of plant extract, which was evaporated in a water bath, was dissolved in 2 mL chloroform. The resulting mixture was treated with 3 mL conc. H_2SO_4 and boiled in a water bath. The presence of a grey colored solution indicates the presence of terpenoids [15].

Test for quinones: Conc. HCl Test

A small quantity of plant extract was treated with conc. HCl. The formation of a green color indicates the presence of quinone [16].

Test for anthraquinone: ammonium hydroxide test

A small quantity of extract was shaken with 4 mL of benzene. The mixture was filtered and 10 mL of 10% ammonia solution was added to the filtrate. The mixture was shaken vigorously and the formation of pink, red or violet color in the ammoniacal solution (lower phase) indicated the presence of anthraquinone [17].

Test for anthocyanins: HCl Test

A small quantity of plant extract was dissolved in 2 mL of HCl to give a pink-red solution. The mixture was treated with few mLs of ammonia and the formation of a blue-violet color indicates the presence of anthocyanins [18, 19].

2.2.5 Antioxidant potential

The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. The experiment was carried out according to the method of Ojah *et al.*, [8], with a slight modification. Five different concentrations of the sample were prepared and absorbance measurement was taken at 517 nm with the aid of a spectrophotometer. Butylated Hydroxyl Anisole (BHA), which is a standard antioxidant, was used as the positive control. The reduction of the radical is followed by a decrease in the absorbance. In the methanol crude extracts of the leaves and stem, a stock solution of 5.0 mg/mL was prepared by dissolving 20 mg of the extracts in 4 mL of methanol. The 2.5, 1.25, 0.625 and 0.3125 mg/mL concentrations were then prepared from the 5.0 mg/mL stock solution using serial dilution.

The DPPH solution was prepared by dissolving 1.97 mg of DPPH in 50 mL of methanol to give a 0.1 mM concentration. For evaluation, 2 mL of the DPPH solution was then added to all the samples. A blank sample was made with just DPPH and methanol and this served as the negative control.

All samples were kept in a dark room immediately after preparation to incubate for 30 minutes and

Table 1. Essential oil procured from leaf, leaf stalk, stem and fruit of *R. umbellulata*

Part of the plant	Weight of sample (g)	Weight of essential oil procured (g)	Percentage yield of essential oil procured (%)	Physical examination of essential oil
Leaves	69.25	0.90	1.30	Colourless, Spicy odour
Leaf stalk	251.45	1.95	0.78	Yellow-green color, Woody smell
Stem	72.67	2.05	2.82	Light yellow, Woody smell
Fruits	193.24	1.33	0.69	Cream color, Spicy odour

absorbance reading was taken at 517 nm in triplicates. The antioxidant potential of the crude extract was calculated using the formula below:

$$\% \text{ inhibition} = \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}} \times 100$$

Where: Abs (Control) = Absorbance of control (i.e., without sample); Abs (Sample) = Absorbance of sample. The antioxidant activity of Butylated hydroxyanisole (BHA) was used as a standard for comparison.

3. Results and discussion

3.1 Percentage yield of essential oils

Essential oils procured from the fresh leaf, leaf stalk, stem and fruits of *R. umbellulata* by hydrodistillation have a colorless, yellow, light yellow and cream appearance, and in the yield of 1.30, 0.78, 2.82 and 0.69 % respectively (Table 1).

3.2 Percentage yield of extracts

Methanolic extracts obtained from the leaf and stem of *R. umbellulata* gave a yield of 19.11 and 4.73% respectively (Table 2). The high yield obtained for the leaf extract may be a result of the ease with which the solvent used percolates into the mesophyll layers of the leaf cells, compared to that of the stem which may comprise more woody cells.

Table 2. Methanolic extracts obtained from the leaf and stem of *R. umbellulata*

Part of the plant	Weight of the dried plant material soaked (g)	Weight of the extract (g)	Percentage yield (%)
Stem	480	22.68	4.73
Leaves	350	66.88	19.11

3.3 Phytochemical screening of extracts

Results of the secondary metabolites present in leaves and stem methanol extracts (Table 3) of *R. umbellulata* revealed the presence of flavonoids, tannins, saponins,

Table 3. Phytochemical screening of methanolic extracts of leaf and stem

Phytochemicals	Leaves	Stem
Alkaloids	-	+
Cardiac glycosides	-	-
Flavonoids	+	+
Phenolic compounds	+	+
Tannins	+	+
Saponin	+	+
Terpenoid	+	+
Quinone	+	-
Anthraquinone	-	-
Anthocyanin	-	-
Coumarin	+	+

Keys: + Detected; - Not detected

phenolic compounds, terpenoids and coumarin in both plant extracts. Cardiac glycoside, anthraquinone and anthocyanin were not detected in the plant extracts. Alkaloids were detected in the stem extract but absent in the leaf extract, while quinones were present in the leaf extract but absent in the stem extract. The presence of these secondary metabolites is attributed to the medicinal use of the plant. Phenolic compounds exhibit anticarcinogenic effect, which is attributed to their antioxidant properties as well as their ability to modulate enzyme activity and block hormone receptors. Polyphenols also protect blood vessels, reduce the aggregation of blood platelets and lower the LDL-cholesterol level in the blood. Tannins are phenolic compounds and their derivatives are also considered as primary antioxidants or free radical scavengers important in protecting cellular oxidative damage including lipid peroxidation. Alkaloids are one of the most investigated phytochemicals that play some metabolic role and control development in living systems. They are also involved in protective function in animals and are used in medicine, especially the steroidal alkaloids. Saponins are bioactive chemical constituents which are involved in plant disease resistance because of their antimicrobial

Table 4. Chemical composition of essential oils from leaves, leaf stalk, stem and fruits of *R. umbellulata*

S/N	Identified Compound	Class of Compound	RT[b]	MS[c]	Leaf (% TIC[d])	Hard Stem (% TIC[e])	Leaf Stalk (% TIC[f])	Fruit % TIC[g]
1	Cyclohexane	Saturated hydrocarbon	2.33	56,84,41,55,69	65.22	29.10	58.99	29.46
2	3-Methyl Hexane	Saturated hydrocarbon	2.38	43,70,71,41,57	-	8.58	-	-
3	UI	Unidentified	2.39	43,70,56,41,71	2.43	-	2.46	1.69
4	1,3-dimethylcyclopentane	Saturated hydrocarbon	2.46	70,56,55,41,71	5.73	-	8.06	10.72
5	UI	Unidentified	2.99	32, 44,45	-	-	-	52.87
6	1,3,3-trimethyl-tricyclo[2.2.1.0(2,6)]heptane	Saturated hydrocarbon	8.75	93,91,119,79,121	-	0.18	-	-
7	3-carene	Bicyclic mono-Terpene	8.76	93,119,91,71,121	0.14	-	-	-
8	Decanal	Aldehyde	10.38	41,57,43,55,82	-	0.20	-	-
9	2,6,6-trimethyl-1-cycohexene-1-carboxaldehyde	Aldehyde	10.68	137,152,109,123,81	0.21	-	-	-
10	α-Copaene	Sesquiterpene	12.89	159,161,119,105,91	-	0.17	-	-
11	Caryophyllene	Bicyclic sesquiterpene	13.49	133,93,91,161,105	-	-	0.63	-
12	α-Ionone	Sesquiterpene	13.54	121,93,136,91,192	1.80	-	-	-
13	Aromadendrin	Sesquiterpenoid	13.75	161,91,105,107,204	-	0.37	-	-
14	Tetrahydrolavandulol	Sesquiterpene alcohol	13.97	94,79,93,135,205	0.14	-	-	-
15	4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	Terpene	14.04	161,105,91,133,204	-	0.24	-	-
16	1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene	Monoterpene	14.18	161,105,119,91,93	-	0.24	-	-
17	Germacrene-D	Sesquiterpene	14.28	161,159,177,105,43	-	0.35	-	-
18	β-Ionone	Sesquiterpene	14.28	177,173,43,178,159	2.15	-	-	-
19	Germacrene-B	Sesquiterpene	14.47	121,161,93,105,119	-	4.35	-	-
20	1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methyl ethyl)-naphthalene	Bicyclic Sesquiterpene	14.68	100,159,105,119,204	-	0.19	-	-
21	1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene	Bicyclic sesquiterpene	14.74	161,204,159,134,19	-	0.58	-	-
22	β-Vatirenene	Sesquiterpene	14.86	119,105,187,93,159	0.35	-	-	-
23	1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene	Bicyclic Sesquiterpene	15.14	161,159,204,105,119	-	0.36	-	-
24	Aromadendrene	Sesquiterpene	15.25	161,105,204,119,91	-	0.20	-	-
25	UI	Unidentified	15.33	69,81,41,79,93	-	-	-	0.07
26	1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methyl-ethyl)-naphthalene	Monocyclic Sesquiterpene	15.36	161,189,204,105,91	-	1.08	-	-
27	8,9-dehydro-cyclo-isolongi-Folene	Bicyclic Sesquiterpene	15.48	159,131,202,91,105	-	0.18	-	-
28	Aroma-Dendrene	Sesquiterpene	15.55	161,105,91,107,119	-	4.52	0.36	-
29	Valerena-4,7(11)-diene	Bicyclic sesquiterpene	15.67	161,105,107,119	-	3.62	-	-

Table 4. *(Continued)*

S/N	Identified Compound	Class of Compound	RT[b]	MS[c]	Leaf (% TIC[d])	Hard Stem (% TIC[e])	Leaf Stalk (% TIC[f])	Fruit % TIC[g]
30	11-tetra-decyn-1-ol-acetate	Carboxylic ester	15.70	82,57,55,41,96	-	-	0.80	-
31	*Cis*-muurola-3,5-diene	Sesquiterpene	15.86	161,179,204,105,119	-	26.03	-	-
32	α-Gurjenene	Carbotricyclic sesquiterpene	15.99	161,105,91,204,119	-	0.58	-	-
33	UI	Unidentified	16.04	161,119,159,105,204	-	0.94	-	-
34	1,2,3,4,4a,5,6,8a-Octahydro-7-methyl-4-methylene-1-(1-methy-lethyl)-naphthalene	Monocyclic Sesquiterpene	16.22	161,105,119,204,93	-	1.87	-	-
35	(-)-Isoledene	Monocyclic ssquiterpene	16.32	161,105, 204,119,91	-	1.03	-	-
36	Rulepidadiene B	Monocyclic sesquiterpene	16.54	159,145, 202,187,105	-	0.49	-	-
37	Hexadecanal	Fatty aldehyde	16.85	82,57,43,55,96	-	1.05	-	-
38	Pentadecanal	Fatty aldehyde	16.85	82,57,43,55,96	0.44	-	-	-
39	6-acetyl-8-methoxy-2,2-dimethyl-2H-chomen-5-ol	Coumarin	17.21	145,119,233,188,189	-	-	0.30	-
40	Isobornyl formate	Bicyclic monoterpenoid	17.37	131,159, 91,189,119	-	-	0.52	-
41	Hexadecadienal	Fatty aldehyde	17.66	67,81,79,55,41	-	-	0.30	-
42	Hexadecyl oxirane	Cyclic ether	17.95	82,57,96,83,43	-	-	0.33	-
43	6,10,14-trimethyl-2-pentadecanone	Sesquiterpene	18.24	43,58,71,55,109	0.49	0.24	-	-
44	9,12-octadecadienoic acid	Carboxylic acid	18.71	67,81,95,55,79	-	0.37	5.49	-
45	9,12,15-octadecatrienoic acid	Carboxylic acid	18.77	79,67,93,95,80	-	0.72	4.03	-
46	16-octadecenal	Fatty aldehyde	18.99	82,57,96,43,55	-	-	0.55	-
47	Neo-3-thujanol acetate	Ester	19.04	74,43,87,69,57	0.31	-	-	-
48	n-hexadecanoic acid	Carboxylic acid	19.38	73,60,57,43,55	-	0.65	2.32	0.29
49	7-ethenyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,1,4a,7-tetramethylphenanthrene	Polycyclic Aromatic hydrocarbon	19.69	137,257,136,272,91	-	0.57	-	-
50	3,7,11,15-tetramethyl-2-hexadecen-1-ol	Acyclic diterpene	20.86	123,95,82,81,68	16.91	0.71	2.49	3.64
51	Verbanol acetate	Ester	22.08	227,255,135,270,119	-	0.24	-	-
52	2-methyl undecanal	Aldehyde	23.09	93,136,91,251,77	-	-	0.43	-
53	Cyclosativene	Sesquiterpene	23.25	93,136,91,79,207	-	-	0.99	-
54	Eicosane	Saturated Hydrocarbon	23.28	57,71,85,207,43	-	-	-	0.14
55	Trans-menthol acetone	Ketone	23.34	93,329,136,91,207	-	-	0.77	-
56	Ethyl cinnamate (Z)	Ester	23.56	245,69,191,93,41	-	-	0.49	-
57	Allyl nonanoate	Fatty acid ester	23.64	93,329,343,136,91	-	-	1.70	-
58	Dehydroangustione	Furanoid	23.80	93,136,343,91,329	-	-	0.82	-
59	UI	Unidentified	23.95	207,281,208,69,124	-	-	-	0.06
60	Methyldecyl ketone	Ketone	24.09	57,71,85,207,43	-	-	-	0.09
61	Jasmonyl Acetate (E)	Ester	24.54	207,149,55,70,133	0.21	-	-	-
62	Sesquithujene	Sesquiterpene	24.84	207,57,71,85,281	-	-	-	0.26
63	β-Isocomene	Monoterpene	24.93	282,151,121,138,133	-	-	-	0.08
64	UI	Unidentified	25.31	207,97,57,281,209	-	-	-	0.18
65	β-Ylangene	Sesquiterpene	25.45	207,83,44,68,104	-	-	-	0.08
66	UI	Unidentified	25.49	207,281,208,253,282	1.96	-	-	-

Table 4. *(Continued)*

S/N	Identified Compound	Class of Compound	RT[b]	MS[c]	Leaf (% TIC[d])	Hard Stem (% TIC[e])	Leaf Stalk (% TIC[f])	Fruit % TIC[g]
67	UI	Unidentified	25.63	57,281,71,85,43	-	-	-	0.15
68	UI	Unidentified	25.68	207,156,96,107,70	0.66	-	-	-
69	α-Ionone	Sesquiterpene	25.82	208,207,107,133,282	0.19	-	-	-
70	Dihydro-β-Ionone	Sesquiterpene	26.09	207,119,70,112,29	0.50	-	-	-
71	β-Humulene	Sesquiterpene	26.17	208,207,268,357,209	0.17	-	-	-
72	2,6,10,14,18-pentamethyl-2,6,10,14,18-eicosa-pentane	N-3 fatty acid	26.88	69,81,95,123,55	-	-	-	0.07
73	Cholesta-3,5-diene	Cholestane	30.92	368,107,145,353,147	-	-	-	0.15

[a]Chemical Structure of Identified Compound, [b]Retention Time, [c][*m*/*z*] values of fragment ions with base peak first stated, and other most prominent ions, [d] %TIC (Percentage Total Ion Concentration). HC- Hydrocarbon.

activity. Saponins help reduce congestive heart failure by inhibiting sodium efflux via the blocking of the entrance of the sodium ions into the cell, hence activating sodium-calcium antiporter, producing elevated cytosolic calcium which strengthens the contraction of the heart muscle. The presence of these aforementioned secondary metabolites may contribute to the ethno-medicinal uses of the plant.

3.4 Chemical composition of leaf, leaf stalk, stem and fruit essential oils of R. umbellulata

The constituents in the essential oils of *R. umbellulata.* presented in Table 4 and the chromatogram shown in Figs. 1–4 which showing the chemical identities, retention times and fragment ions from mass spectra. Not less than 15, 11, 19 and 27 compounds made up the essential oils of leaf, fruit, leaf stalk and stem respectively. The most abundant compounds in the leaf essential oil were: Cyclohexane (65.22%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (16.91%), 1,3 -dimethylcyclopentane (5.73%) and β-ionone (2.15%). In the essential oil of the fruit, the most abundant compounds were: cyclohexane (29.46%),1,3-dimethylcyclopentane (10.72%), and 3,7,11,15-tetramethyl-2-hexadecen-1-ol (3.64%).

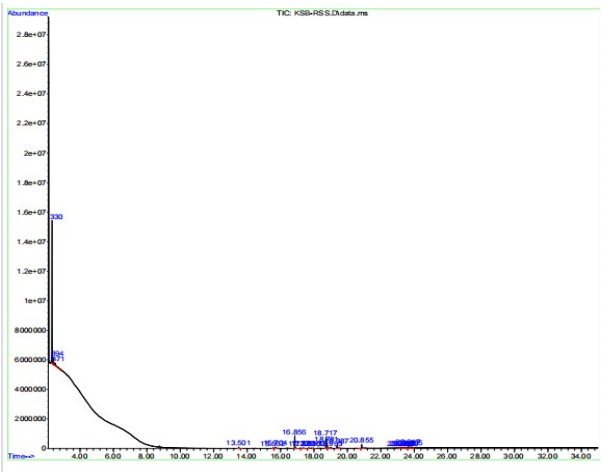

Figure 1. GC-MS result for the analysis of the leaf stalk essential oil

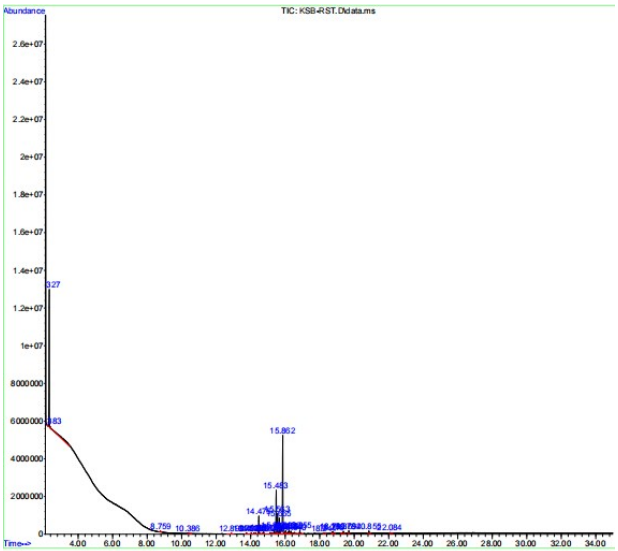

Figure 2. GC-MS result for the analysis of stem essential oil

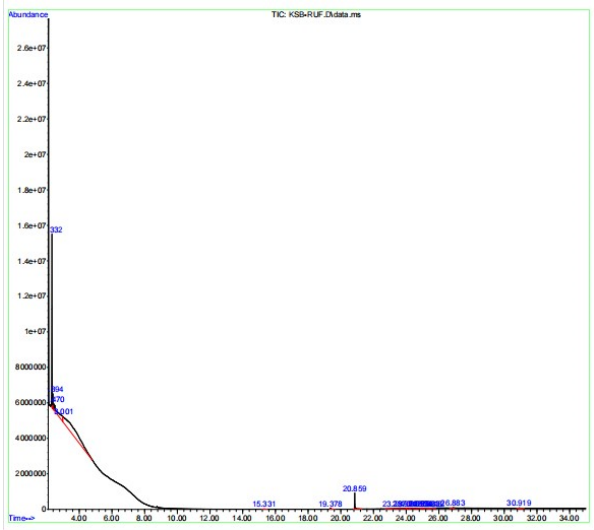

Figure 3. GC-MS result for the analysis of the fruit essential oil

Table 5. Chemical composition of organic compounds in leaf, leaf stalk, stem and fruit essential oils of *R. umbellulata* by class.

S/N	Class of Organic Compounds	Leaf (%)	Leaf stalk (%)	Stem (%)	Fruit (%)
1	Saturated Hydrocarbon	70.95	67.05	37.86	40.32
2	Monoterpene	0.14		0.24	0.08
3	Aldehyde	0.21	0.43	0.20	
4	Sesquiterpene	4.66	1.35	35.62	0.34
5	Bicyclic Sesquiterpene		0.63	4.93	
6	Sesquiterpenoid	0.50		0.37	
7	Sesquiterpene alcohol	0.14			
8	Terpene			0.24	
9	Monocyclic Sesquiterpene			4.47	
10	Carboxylic ester		0.80		
11	Carbotricyclic Sesquiterpene			0.58	
12	Fatty Aldehyde	0.44	0.85	1.05	
13	Coumarins		0.30		
14	Bicyclic Monoterpenoid		0.52		
15	Cyclic Ether		0.33		
16	Sesquiterpene Ketone	0.49			
17	Carboxylic acid		11.84	0.65	0.29
18	Ester	0.52	0.49	0.24	
19	Polycyclic Aromatic Hydrocarbons			0.57	
20	Acyclic diterpene	16.91	2.49	0.71	3.64

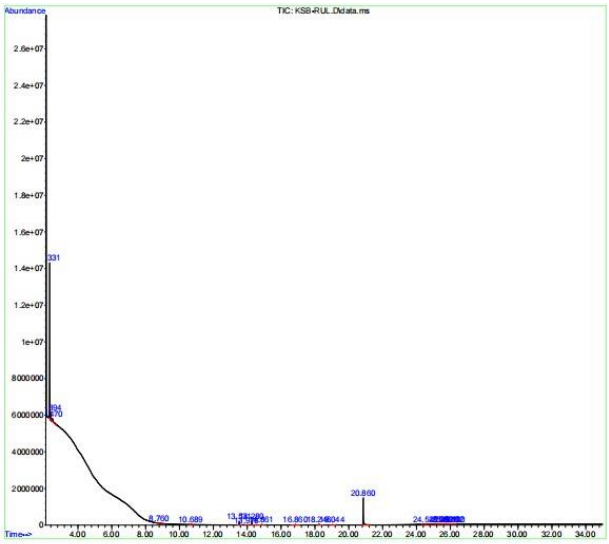

Figure 4. GC-MS result for the analysis of the leaf essential oil

In the leaf stalk essential oil, the most abundant were: Cyclohexane (58.99%), 1,3-dimethylcyclopentane (8.06%),9,12-octadecadienoic acid (5.49%), 9,12,15-octadecatrienoic acid (4.03%), 3,7,11,15-tetramethyl-2-hexadecenoic-1-ol (2.49%) and *n*-hexadecanoic acid (2.32%) (Table 4). In the stem essential oil, the most abundant were: cyclohexane (29.10%), *cis*-muurola-3,5-diene (26.03%), 3-methylhexane (8.58%), aromadendrin (4.52%), germacrene b (4.35%) and valerena-4,7(11)-diene (3.62%) (Table 4).

Leaf essential oil was rich in saturated hydrocarbons (70.95%), acyclic diterpene (16.91%) and sesquiterpene (4.66%). Leaf stalk essential oil was rich in saturated hydrocarbons (67.05%) and carboxylic acid (11.84%); Stem essential oil was rich in saturated hydrocarbon (37.86%), Sesquiterpene (4.66%), bicyclic sesquiterpene (4.93%) and monocyclic sesquiterpene (4.47%) and the fruit essential oil was rich in saturated hydrocarbon (40.32%) and acyclic diterpene (3.64%) (Table 4).

The prominent saturated hydrocarbon found in all parts of the plant's essential oils was cyclohexane. The presence of esters of fatty acids was seen in the essential oils of only the leaf stalk. The stem essential oil also contained: coumarin (0.30%), monoterpenoid (0.52%), esters (0.49%) as well as ketones (0.77%), all in minute quantities.

3.5 Class of Compounds in the leaf, leaf stalk, stem and fruit essential oil of R. umbellulata

Sesquiterpene alcohol and sesquiterpene ketone were exclusive to the leaves; Coumarin, bicyclic monoterpenoid, cyclic ether, fatty acid ester, furanoid and carboxylic ester were exclusive to the leaf stalk essential oil; poly-aromatic hydrocarbons (PAHs), terpenes, monocyclic sesquiterpene and carbotricyclic sesquiterpene were exclusive to stem essential oil (Table 5).

Monoterpenes were found in minute quantities in leaf (0.14%), stem (0.24%) and fruit (0.08%). Esters found in leaf (0.52%), leaf stalk (0.49%) and stem (0.24%) and fatty acid esters found only in the leaf stalk (1.70%) (Table 5) are essential constituents in food flavorings, cosmetics, perfumes and surfactants.

3.6 Antioxidant activities of extracts of R. umbellulata

DPPH is a free-radical compound which has been widely used to test the free-radical scavenging ability of various samples. The antioxidant effect of the extract and essential oil on DPPH radical scavenging may be due to their hydrogen donating ability and it reduces the stable violet DPPH radical to the yellow DPPH-H. The effective concentrations of the extract required to scavenge DPPH radical and the scavenging values as inhibition percentage at various concentrations are as depicted in Fig. 5 (include exact values of "effective concentrations" in both your text and in Fig. 5).

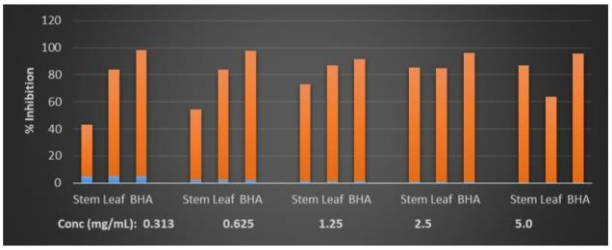

Figure 5. DPPH scavenging potential of methanolic extracts from leaf and stem of *R. umbellulata*

The percentage inhibition and the concentration of sample capable of inhibiting at least 50% of the DPPH radical (IC_{50}) were obtained for each extract (Fig. 6). The assay yielded IC_{50} of 0.409 and 0.299 mg/mL for methanolic extract of leaf and stem respectively. The lower the IC_{50} value, the more potent the antioxidant activity, hence, the stem extract has the highest antioxidant activity, while the leaf gave a moderate antioxidant activity. The percentage inhibition of the leaf increases as the concentration increases up to a concentration of 2.5 mg/mL. At the concentration of 2.5 to 5.0 mg/L, percentage inhibition decreases, and this suggests that the leaf is no longer able to scavenge any free radicals in the solution. This implies that at a certain concentration, the leaf reaches its maximum capacity to scavenge free radicals. The IC_{50} value for the stem extract is 0.299 mg/mL, the antioxidant scavenging ability of the stem extracts is not concentration dependent. The percentage inhibition of BHA is consistently higher than the percentage inhibition of the leaf and stem extracts at all concentrations, The IC_{50} value of BHA is 0.190 mg/mL, indicating that BHA has a stronger antioxidant activity than both extracts. The antioxidant assay of *R. umbellulata* leaf and stem methanolic extract shown in Fig. 5, indicates the activities of the extracts and standard compounds follow the order:

BHA>Stem extract>Leaf extract with the corresponding IC_{50} values of: 0.190>0.299>0.409 mg/mL respectively (Fig. 6).

Figure 6. IC_{50} values of methanolic extracts from leaf and stem of *R. umbellulata*

4. Conclusions

R. umbellulata is one of the plants used in the sub-Saharan traditional medicine system and has been applied in the treatment of malaria, dysentery and skin conditions like eczema. The presence of twenty-five identified classes of organic compounds with the most abundant being saturated hydrocarbon and acyclic diterpene from GC-MS analyses of the essential oils of leaf, leaf stalk, fruit and stem of *R. umbellulata* reported for the first time with the phyto-constituents and antioxidant properties exhibited by the extracts, may explain its ethno-medicinal application in the treatment of dermatological problems such as eczema.

Authors' contributions

Designed the experiment, D.O.M. and C.O.A.; Carried out the sample collection, identification and authentication, S.A.O.; Carried out the extraction and bioactivity aspects, I.I.E., I.V.I. and A..NS.; Carried out the GC-MS analysis, C.O.A. and O.A.A.; Made the first draft of the manuscript, I.I.E.; Prepared the manuscript for submission, C.O.A., A.N.S. and I.I.E.; Revised the manuscript, D.O.M., O.A.A., S.A.O. and I.I.E.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflict of interest.

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