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Compositions of *Harungana madagascariensis* Lam. ex Poiret leaf and stem essential oils

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ABSTRACT

Leaf and stem essential oils of Harungana madagascariensis Lam. ex Poir, [an endangered medicinal Hypericaceae] were obtained in Nigeria by hydro-distillation using the all-glass Clevenger apparatus designed to British Pharmacopeia specifications. It gave good yields of 0.13and0.08 % respectively. The oils were analyzed using gas chromatography [GC], gas chromatography-mass spectrometry [GC-MS] in University of Botswana. Forty-nine compounds were identified in leaf oil, which account for 85.64 % of it; while fifteen identified compounds are responsible for 56.82 % of stem oil. Most abundant compounds in leaf oil are α-caryophyllene (33.41 %); β-caryophyllene (17.78 %) and 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-[1S-(1α,2α,4α)]-cyclohexane (6.21 %). Dominant compounds in stem oil are α-caryophyllene (22.38 %); α-farnesene (13.90 %) andβ-caryophyllene (8.26 %). Leaf oil is dominated by unique classes of compounds (%): like the ubiquitous sesquiterpenes (56.53) and sesquiterpenoids (1.91);naphthalenes (13.33); unsaturated mostly diene hydrocarbons (7.04); aromatics (3.02); azulenes (1.36);alcohols (1.02); esters (0.89); sulfurates (0.19); ketones (0.18); alanine derivative (0.08); phenols (0.05) and squalene (0.04).Classes of compounds in stem oil are (%) the ubiquitous sesquiterpenes (45.62) and sesquiterpenoids (1.05); hydrocarbons (5.00); aromatics (2.21); azulenes (1.42); naphthalenes (1.32) and alcohols (0.20). Our results indicate that Harungana madagascariensis essential oils are terpenoidal, dominated by sesquiterpenes. This study revealed important compounds in the leaf and stem essential oils of Harungana madagascariensis, which have not been reported earlier in literature.

Keywords: *Harungana madagascariensis*, Hypericaceae, essential oil, sesquiterpenoids, GC/ GC-MS.

INTRODUCTION

Harungana madagascariensis Lam. ex Poir belong to the family'Hypericaceae', earlier known as'Guttiferae'. It is a tropical much-branched shrub to small tree growing up to 12 m tall, commonly known as 'Dragon's blood tree'. It is native to Madagascar, Mauritius and tropical Africa growing on margins of wet forests. *H. madagascariensis* possess bright orange bark exudates and distinctive broad egg-shaped opposite leaves which are 10-20cm long and 6-10cm wide. Its fragrant flowers are very small whitish with black glands; its orange-brown fleshy fruits are also small about 2-3mm and contain 2-4 seeds (Figure 1). The wood is orange-red to yellow, and is particularly attractive[1,2,3,4].

H. madagascariensis possess many adaptive features such as forming dense thickets from root suckers for exclusion of all other species around. The plant invades cyclone-damaged and gaps in rainforest. It can re-grow after disturbance and is capable of withstand poor drainage on alluvium. The plant is a vigorous colonizer, hence named '*Harungana madagascariensis*' also referred to as pest plant [2,3,4,5a,5b].*H. madagascariensis* have many social applications and medicinal uses:Its light wood is utilized in construction of hut, store-houses, thwarts and seats in canoes, yam-sticks and hockey-sticks; The wood is utilized as fuel in local metallurgy; Its gummy delicate dye is useful for dying velvet, it is also a good stain for wood and sealing-wax on newly fired pots [2,3,5a,5b].

Figure 1: *Harungana madagascariensis* [Foliage, fruits and Leaves]

Ethno-medicinal applications include its utilization in the cure for leprosy, jaundice, ulcers, asthma, and prevention for poultry illnesses. Its gum is styptic and haemostatic, arresting flow of blood and is applied to cuts and to dress wounds. The bark and root decoctions are remedy for dysentery and piles, also acts as placental embolic and emmenagogue. It relief stomach-ache, painful menstruation and menstrual problems, dysmenorrheal, menstrual irregularity, miscarriage, sterility and haematuria [6,7,8,9,10]. It is a remedy for cough with bloody sputum and dysentery. It arrest hemorrhage, diarrhea, gonorrhea, sore-throat and fevers[6,7,8,9,10,11,12]. The leaf and roots of *H. madagascariensis*are considered febrifugal and anti-malarial [9,12,13,14]. Leaf-sap is prepared as a remedy for amenorrhea and heart-troubles, as laxative for stomachic and intestinal troubles [15,16]. Ointment made from *H.madagascariensis* fruit in animal fat is used on inflamed ganglia [15,16]. *H.madagascariensis* has been shown to have hypoglycemic effects, lowering blood glucose levels in diabetes mellitus, and others like anti-inflammation, antioxidant, antihepatotoxicity and antimicrobial activities [10,12,17,18,19,20].

Harunganin has been reported as an important constituent of *H.madagascariensis* with its thermal rearrangement and allylations of some of these compounds from it [21,22,23]. The anthranoidsharonginanthrone, harunganol B, kenganthranol A and 1,7-dihydroxyxanthone, with the *O*- and *C*-bisallylation products have also been isolated from it [21,22].

There are recent reports on screening phytochemicals in *Harungana madagascariensis*- methanol and aqueous ethanol stem bark extracts showed presence of phytochemicals like phenols, tannin, alkaloids, anthraquinone and saponin [24,25]; Methanol extract of seed have anthraquinones, flavonoids and aglycones, triterpenoids and terpenoids [25].

The phytochemicals were proposed as bioactive compounds with potential for exploitation in drug development [23,24]. Their isolations and characterizations still remain a challenge.

We report the leaf and stem essential oil compositions of *Harungana madagascanensis*, which is scarce in literature.

EXPERIMENTAL SECTION

Plant material

Fresh samples of leaves and stem of *Harungana madagascariensis* were collected from forest reserved area, Ibadan, Nigeria for extraction of their volatile oils on 24/02/2015. Plant was authenticated at the Herbarium, Department of Botany, University of Ibadan, where voucher samples have been deposited, with voucher number UIH – 22455.

Isolation of essential oils

Sample of *Harungana madagascariensis* was collected in large quantities, which was separated into leaf and stem parts giving 1.5 Kg of leaf and 500 g of stem. They were crushed separately and hydro distilled for 2.5 to 3 hours in an all glass Clevenger-type apparatus designed to British Pharmacoepia specifications. Oils were collected under iced condition with very small quantity of distilled n-hexane, which the analyzing GC corrected for by accounting for [RT 5.00 to 15.00 mins]. Leaf and stem essential oils were procured in 0.13 % and 0.08 % yields respectively (Table 1), each with distinctive characteristics. Leaf gave cream to white oil with leafy choking but pleasant odour, while stem gave light yellow oil with herbal peppery choking but acceptable odour.

Gas Chromatography

Leaf and stem essential oils were analyzed using an Agilent 5975C series Gas Chromatograph - Mass Spectrometer (GC-MS) system. Data analysis was done using Productivity Chemstation Version E.02.01.1177 in conjunction with the Automated Mass Spectral Deconvolution and Identification System (AMDIS). The oven program for the column was as follows: The equilibration time was set at 0.25 min, the initial temperature was set at 70 °C, held there for 4 minutes, and then ramped at 6 °C/min to 140 °C for 6 min. It was further ramped at 15 °C/min to 300 °C for 4 minutes. The total run time was 36.333 minutes. 1 µL of sample extract was injected in the splitless mode. The injector temperature was set at 280 °C and the column head pressure was 56.756 kPa yielding a total flow of 64 mL/min. The transfer line temperature was 280 °C. A HP-5MS 5% Phenyl Methyl Silox column with dimensions of 30 m x 250 µm x 0.25 µm and maximum recommended temperature (MRT) of 325 °C was used throughout. Helium was used as a carrier gas at a flow rate of 1 mL/min.

Mass Spectrometry

The acquisition mode was full scan with a solvent delay of 4.00 minutes. Acquisition mass range was set at *m/z* 50 – 600 and the electron multiplier detector voltage was auto set to 1082 V. The mass spectrometer source and quadrupole mass analyzer temperatures were set to 230 ^{0}C and 150 ^{0}C respectively. The filament emission current was auto set to 34.610 µA.

Identification of components

Identification of the essential oil components were based on their retention indices (determined with reference to a homologous series of n-alkanes), and by comparison of their mass spectral fragmentation patterns with the National Institute for Standards and Technology (NIST) library version 8.0. The AMDIS software version 2.71 build 134.27 was used for deconvolution i.e. to compare the NIST library spectra with component spectra. The minimum match factor was for deconvolution on AMDIS was set to 70 and all identified components had match factors between 70 and 100. Some mass spectral fragmentation patterns with characteristic retention were compared with standard in-built data of Adams (1995) [26].

RESULTS AND DISCUSSION

Volatile oils from leaf and stem parts of *Harungana madagascariensis* Lam. ex Poir, an endangered medicinal Hypericaceae, were obtained in Nigeria by hydro-distillation using the all-glass Clevenger apparatus designed to British Pharmacopeia specifications and gave good yields of 0.13 and 0.08 % respectively. [Table 1]. The oils had characteristic distinctive features: Leaf gave cream to white oil with leafy choking but pleasant odour, while stem gave light yellow oil with herbal peppery choking but acceptable odour.

The oils were analyzed using gas chromatography [GC], gas chromatography-mass spectrometry [GC-MS] in University of Botswana. [Tables 2 and 3; Figures 2 and 3].

Table 1: Yields of essential oils procured from leaf and stem parts of *Harungana madagascariensis*

Plant part	Weight of sample (g)	Weight of essential oil procured (g)	% Yield of essential oil procured	Physical examination
Leaf	1500	1.9	0.13	Cream to white oil with leafy choking but pleasant odour
Stem	500	0.4	0.08	Light yellow oil with herbal peppery choking but acceptable odour

Forty-nine compounds were identified in leaf oil, which account for 85.64 % of it (Table 2 and Figure 2). Most abundant compounds in leaf oil are α-caryophyllene (33.41 %); β-caryophyllene (17.78 %) and 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-[1S-(1α,2α,4α)]-cyclohexane (6.21 %). Leaf oil is dominated by unique and interesting classes of compounds like the ubiquitous sesquiterpenes (56.53 %) and sesquiterpenoids (1.91 %); naphthalenes (13.33 %); unsaturated mostly diene hydrocarbons (7.04 %); aromatics (3.02 %); azulenes (1.36 %); alcohols (1.02 %); esters (0.89 %); sulfurates (0.19 %); ketones (0.18 %); alanine derivative (0.08 %); phenols (0.05 %) and squalene (0.04 %).

Table 2: Chemical Composition of the Leaf Essential oil of *Harungana madagascariensis*

Retention time [mins][a]	Compound [b]	% TIC[c]	Retention Index[d]
16.43	β-copaene	1.34	2220
16.94	1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-[1S-(1α,2α,4α)]-cyclohexane	6.21	2279
17.14	1α,2,3,4,4α,5,6,7β-octahydro-1,1,4,7-tetramethyl-,[1αR-(1αα,4α,4αα,7βα)]-1H-cyclopropazulene	1.11	2304
17.87	β-caryophyllene	17.78	2389
17.96	1-methyl-5-methylene-8-(1-methylethyl)-,[s-(E,E)]-1,6-c clodecadiene	0.27	2400
18.16	(trans,trans)-farnesyl acetate	1.60	2419
19.11	α-caryophyllene	33.41	2545
19.15	1,2,3,5,6,7,8,8α-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1α,7α,8αα)]-azulene	0.25	2551
19.49	2-isopropenyl-4α,8-dimethyl-1,2,3,4,4α,5,6, -octahydronaphthalene	1.79	2593
19.58	o-(4-butylbenzoyl)-o'-(2-methylbenzoyl)-1,2-benzenediol	0.97	2595
19.73	1-(1,5-dimethyl-4-hexe yl)-4-methyl-benzene	0.7	2623
19.82	Tridec-2-yn-1-yl,ethyl-terephthalate	<0.02	2634
20.00	Decahydro-4α-methyl-1-methylene-7-(1-methylethenyl)-,[4αR-(4αα,7α,8αα)]-naphthalene	4.20	2658
20.34	1,2,3,4,4α,5,6,8α-octahydro-4α,8-dimethyl-2-(-methylethenyl)-,[2R-(2α,4αα,8αα)]-naphthalene	5.10	2700
20.68	α-farnesene	3.67	2743

20.83	2,4α,5,6,7,8-hexahydro-3,5,5,9-tetramethyl-,(R)-1H-benzocycloheptene	1.27	2762
21.11	(-)-α-panasinsen	0.33	2797
21.32	1,2,3,5,6,8α-hexahydro-4,7-dimethyl-1-(1-methylethenyl)-,(1S-cis)-naphthalene	0.91	2823
22.71	3,7,11-trimethyl-,[E]-1,6,10-dodecatrien-3-ol	0.43	2999
22.91	(Z)-3-hexen-1-ol, benzoate	0.19	3023
23.11	3,6-dimethyl-1,5-heptadiene	0.10	3049
23.30	Veridiflorol	0.11	3073
23.59	1,3-bis(1-methylethenyl) 1,3-cyclopentadiene	0.21	3110
23.89	1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)]-12-oxabicyclo[9.1.0]dodeca-3,7-diene	0.19	3147
23.96	Ethyl tridec-2-ynyl-isophthalate	0.48	3156
24.33	Hexyl, N-(4-butylbenzoate)-, alanine	0.08	3222
24.50	τ-muurolol	0.20	225
24.58	1,2,3,4,4α,7,8,8α-octahydro-1,6-dimethyl-4-(1-methylethyl)-,[1S-(1α,4α,4αα,8αα)]-1-naphthalenol	0.06	3234
23.98	Decahydro-1,4α-dimethyl-7-(1-methylethylidene)-, [1R-(1α,4αα,8αα)]-1-naphthalenol	1.27	3258
25.05	4-(1-phenylethyl)-phenol	.05	3293
25.19	1-methanol, α,4-dimethyl-α-(4-methyl-3-pentenyl)-, [R-(R*,R*)]-3-cyclohexene	0.06	3311
25.33	4-octanone	0.04	3329
26.23	Benzyl benzoate	0.02	3442
26.40	9,10-dihydro-, diethyl, anthracene-9,10-biimine-11,12-dicarboxylate	0.02	3463
26.56	2,2,5-trimethyl-3,4-hexanedione	0.04	3483
26.97	3,7,11,15-tetramethyl-2-hexadecen-1-ol	0.33	3536
27.03	6,10,14-trimethyl-2-pentadecanone	0.04	3543
27.29	Cyclobutyl isobutyl phthalate	0.02	3575
27.56	9-hydroxy-2-nonanone	0.03	3609
27.95	Isophytol	0.06	3659
29.07	2-ethylhexyl isohexylsulfurate	0.09	3800
29.20	Phytol	0.20	3816
29.51	Tributyl,1-propene-1,2,3-tricarboxylate	0.08	3856
30.15	Butylcitrate	0.03	3936
31.79	Bis(2-pentyl)-phthalate	0.05	4143
31.99	2-ethylhexyl hexyl sulfurate	0.05	4167
32.49	2,2,5-trimethylhexan-4-one	0.03	4231
33.26	Squalene	0.04	4327
33.60	Butyldecylsulfurate	0.05	4371
49 Identified compounds (%) TIC	85.64 %		

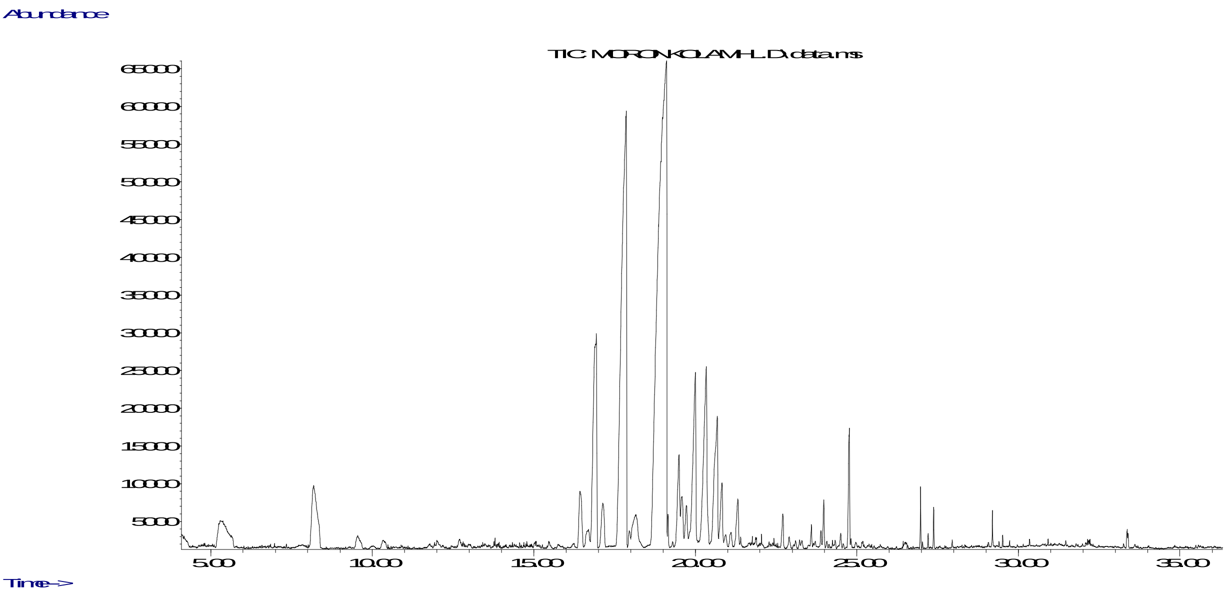

Figure 2:Gas chromatogram of the leaf essential oil of *Harunganamadagascanensis* [see GC conditions under experimental procedures]

Fifteen identified compounds are responsible for 56.82 % of the stem oil (Table 3 and Figure 3). Dominant compounds in stem oil are α-caryophyllene (22.38 %); α-farnesene (13.90 %) and β-caryophyllene (8.26 %). Classes of compounds in stem oil are the ubiquitous sesquiterpenes (45.62 %) and sesquiterpenoids (1.05 %); hydrocarbons (5.00 %); aromatics (2.21 %); azulenes (1.42 %); naphthalenes (1.32 %) and alcohols (0.20 %).

Table 3: Chemical Composition of the Stem Essential oil of *Harungana madagascariensis*

Retention time [mins][a]	Compound [b]	% TIC[c]	Retention Index[d]
16.40	α-copaene	1.08	2216
16.80	1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1α,2α,4α)]-cyclohexane	1.73	2263
17.06	1α,2,3,4,4α,5,6,7β-octahydro-1,1,4,7-tetramethyl-,[1αR-(1αα,4α,4αα,7βα)]-1H-cyclopropazulene	1.42	2294
17.62	β-caryophyllene	8.26	2361
17.99	Cis,trans-farnesyl acetate	0. 6	2368
18.71	α-caryophyllene	22.38	2495
19.42	2,4α,5,6,7,8-hexahydro-3,5,5,9-tetramethyl-,(R)-1H-benzocycloheptene	1.39	2585
19.54	1-(1,5-dimethyl-4-hexenyl)-4-methyl ben ene	0.82	2600
20.09	Decahydro-4α-methyl-1-methylene-7-(1-methylethenyl)-,[4αR-(4αα,7α,8αα)]-naphthalene	1.32	2658
20.54	α-farnesene	13.90	2726
20.68	5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-1,3-cyclohexadiene	1.99	2743
23.57	1,3-bis(1-methylethenyl)-1,3-cyclopentadiene	0.87	3107
24.71	eridiflorol	0.49	3251
26.97	1,1 -tetradecanediol	0.20	3535
27.38	(R)-3,7-dimethyl-1,6-octadiene	0.41	3586
15 Identified compounds (%) TLC	56.82 %		

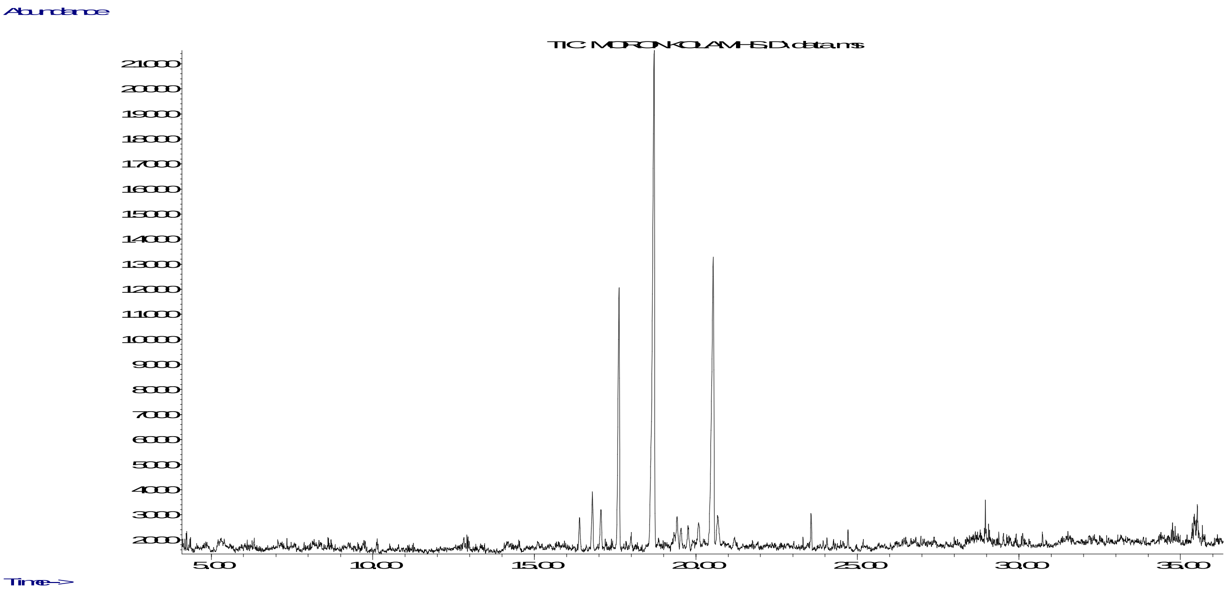

Figure 3:Gas chromatogram of stem essential oil of *Harunganamadagascanensis* [see GC conditions under experimental procedures]

Our results indicate that *Harungana madagascariensis* essential oils are highly terpenoidal, dominated by sesquiterpenes. We suggest the sesquiterpenes are taken as its taxonomic compounds. This study is the first of its kind which revealed important compounds in the leaf and stem essential oils of it, which have not been reported earlier in literature. The identified volatile metabolites may be contributing to the vast uses of *Harungana madagascariensis* in ethno-medicine. It is notable the distinct absence of fatty acids in this two oils. Other essential oil plant parts will be studied in the near future.

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