SUPPLEMENTARY MATERIAL

Phytochemical screening and chemical variability in volatile oils of its aerial parts of *Morinda morindoides*

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Abstract

Morinda morindoides is an important Liberian traditional medicine for the treatment of malaria, fever, worms etc. The plant was subjected to integrated approaches including phytochemical screening and gas chromatography mass spectrometry analyses. Phytochemical investigation of the powdered plant revealed the presence of phenolics, tannins, flavonoids, saponins, terpenes, steroidal compounds and volatile oil. Steam distillation followed by GC-MS resulted in the identification of 47 volatiles in its aerial parts: 28 were in common including various bioactive volatiles. Major constituents of leaves were phytol (43.63%), palmitic acid (8.55%) and geranyl linallol (6.95%) and stem were palmitic acid (14.95%), eicosane (9.67%) and phytol (9.31%) hence a significant difference in the percentage composition of aerial

parts was observed. To study seasonal changes, similarity analysis was carried out by calculating correlation coefficient (r) and vector angle cosine (z) that were more than 0.91 for stem-to-stem and leaf-to-leaf batches indicating considerable consistency.

Keywords: Jologbo; aerial parts; volatile constituents, Phytol, similarity analysis

1. Experimental section

The plant was collected from Liberian forest, Grand Cape Mount County, West Tropical Africa and was identified by Mr. David Wah from the Faculty of Forestry, University of Liberia as *Morinda morindoides*. Two batches of plant were collected; first batch in early October of 2010 and the second batch in late February of 2011. The plant was cleaned, ground, dried and passed through a 45 μ m sieve to the needed size for extraction.

1.1 Qualitative phytochemical screening and extraction of volatile oil: Qualitative phytochemical investigation of the powdered sample was carried out by the reported standard screening tests for identification of the plant phytoconstituents (Harbone 1998). On the other hand, 45 grams of the fine powder was prepared to undergo steam distillation for 4 hours in Clevenger-type apparatus and dichloromethane was used as the collecting solvent. The pale yellow oil was collected. Extracts were stored under -4 °C for further use.

1.2 Chemicals and standards: All solvents used in experiments were of high purity analytical grade; whereas, phytol, benzothiazole, α -ionone and β -ionone were purchased from Acros, Shanghai, China. Alkane mixture containing C₈-C₄₀ was also purchased from Sigma Aldrich, Shanghai, China.

1.3 Instrumentation and chromatographic conditions: Agilent 7890A gas chromatography coupled to 6975 mass spectrometer (Santa Clara, USA) was used in EI mode. Volatile components were separated using an HP-5 capillary column with ID 30 m x 0.25 mm. The oven temperature ramp was as follows: 50°C was held for 10 min; increased with the rate of 5°C/min to 280°C. 2 μ L sample was injected in split mode (split ratio 1:30). The flow rate of carrier gas (helium) was 1 mL/min. The ion source temperature was 230 °C and ionization temperature-electron impact was 70eV. Identification of components in the sample was based on both retention time and retention index. Retention Indices were

determined relative to n-alkanes (C₈ - C₄₀) under the identical operating conditions (Van Den Dool & Kratz 1963). Identification of volatiles was carried out by comparing their spectra with those stored in commercial NBS 75k and NIST-08 MS spectral libraries. (Adams 2007).

2. Supplementary results

Table S1: The qualitative phytochemical analysis of the aerial parts of Jologbo

No.	Phytochemical Constituents	Test or Reagent	Leaves	Stems
1	Phenolic compounds	Ferric chloride	++	++
2	Cardenolides	Keller-Killiani	-	-
3	Volatile Oil	Petroleum spirit	++	++
4	Free Anthraquinones	A. Borntrager	-	-
		B. Sulfuric acid	-	-
		C. Potassium hydroxide	-	-
5	Flavonoids	A. Sodium hydroxide	+	+
		B. Shinoda	+	+
		C. Lead acetate test	+	+
		D. NH ₃ , AlCl ₃ and Ethyl acetate	+	+
6	Tannins	A. Ferric chloride	+	~+
		B. Gelatin-salt	+	+
7	Terpenoids / Terpenes	A. Salkowski	++	+
		B. Acetic anhydride	++	+
8	Saponins	Frothing	+	~+
		Emulsion	+	~+
9	Steroidal Compounds	A. Salkowski	+	+
		B. Lieberman	+	+

++: present; +: moderately observed; ~+: slightly observed; -: Undetected

Table S2: Identification of volatiles in leaves and stem batches (October 2010) by GC-MS

Sr. No.	Volatile Constituents	RI	Rel. % in Leaf	Rel. % in Stem
1	Benzaldehyde	959	0.18	-
2	PhenylCarbinol	1036	1.44	0.53
3	Benzeneacetaldehyde	1044	0.17	0.38
4	undecane, 5,7-dimethyl-	1057	0.45	-
5	Linalool	1099	1.27	0.71
6	Pelargonaldehyde	1105	-	0.28
7	Linalool oxide	1176	0.47	0.24
8	α -Terpineol	1195	0.17	0.35
9	Dodecane	1200	0.13	0.52
10	Decanal	1206	-	0.26
11	Benzothiazole	1223	0.27	3.88
12	exo-2-Hydroxycineole	1228	-	0.24
13	4-Methoxybenzaldehyde	1254	-	0.34
14	Nonanoic acid	1275	1.53	-
15	Thymol	1293	-	1.32
16	Naphthalene, 2-methyl-	1310	0.18	0.95
17	Eugenol	1352	0.30	-
18	n-Decanoic acid	1370	-	1.81
19	Vanillin	1394	0.43	0.29
20	Tetradecane	1400	0.36	0.64
21	Naphthalene, 1,3-dimethyl-	1419	0.45	-
22	α-Ionone	1421	0.48	0.25
23	1-Undecanol	1475	0.46	3.30
24	β-Ionone	1479	0.41	0.3*9
25	Acenaphthene	1483	1.57	3.24
26	Pentadecane	1500	0.55	1.20
27	Phenol, 2,4-bis(1,1-dimethylethyl)-	1507	1.76	8.57
28	Dibenzofuran	1518	-	0.94
29	Dihydroactinidiolide	1530	0.92	1.32
30	Mellein	1543	0.15	-

31	Dodecanoic acid	1564	1.02	2.55
32	1-Hexadecene	1593	0.18	-
33	Hexadecane	1601	1.10	2.10
34	Cedrol	1613	1.43	7.77
35	Heptadecane	1701	1.08	2.64
36	Pentadecanal	1716	2.46	-
37	Tetradecanoic Acid	1763	1.74	-
38	Phenanthrene	1784	1.25	4.66
39	Octadecane	1801	2.42	4.41
40	Hexadecane, 2,6,10,14-tetramethyl-	1807	2.36	3.96
41	Phytone	1842	2.00	-
42	Nonadecane	1901	2.96	5.99
43	Farnesyl acetone	1910	1.13	-
44	Palmitic acid	1968	8.55	14.95
45	Eicosane	2001	5.62	9.67
46	Geranyl linallol	2006	6.95	-
47	Phytol	2112	43.63	9.31

-: Not detected; RI: Retention Indices; Highlighted volatiles: confirmed using standards

2.1. Similarity characteristics of different batches of Jologbo:

To evaluate and extract useful information from chromatographic fingerprinting of natural medicines, different computer-based softwares are used for the correction of background, retention times, peak area and peak alignment of unknown constituents present. For this purpose correlation coefficient and vector angle cosine are the most commonly and generally used well-known standards for quality evaluation of multivariate systems (Wold 1995; Du et al. 2011) and these were calculated using Matlab 2009 software in this study. For similarity assessment of different batches of traditional medicines, the analysis was carried out by simply calculating their correlation coefficient (r) and vector angle cosine (z). 37 peaks in leaves and 33 in stem in accordance with their similar retention times, retention indices and spectral matching (same compounds) were analysed. The similarity degrees of different batches were more than 0.91 for both stem-to-stem and leaves-to-

leaves which suggested that the distribution of the components in volatile oil for different batches of Jologbo maintains enough uniformity between the two seasons.

Table S3: The similarity analysis of different seasonal batches of Jologbo leaves-to-leaves and Stem-to-stem

Employed for Leaf calculations	Number of peaks	Correlation coefficient (r)	Vector angle cosine (z)
Whole chromatograms	Whole spectra	0.939	0.959
Peak area	37	0.925	0.931
Peak height	37	0.923	0.934
Employed for Stem Calculations	Number of peaks	Correlation coefficient (r)	Vector angle cosine (z)
Whole chromatograms	Whole spectra	0.943	0.951
Peak area	33	0.918	0.919
Peak height	33	0.936	0.925

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