Chemical Examination of Essential Oil from Stem, Roots and Fruit Peels of Nigerian *Citrus jambhiri*

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Abstract: The chemical profiles of the stem, root and fruit peel essential oils of *Citrus jambhiri* were investigated. Pulverized plant samples (500 g) each was hydrodistilled using all-glass Clevenger-type apparatus to obtain the essential oils. Two-dimensional GC-TOFMS was used for compositional profiling of the extracted oils. Chemical profiling of the stem oil of *C. jambhiri* revealed the presence of 66 components (83.11 %), the root oil of *C. jambhiri* showed 56 components (74.21 %) and the fruit peel oil of *C. jambhiri* revealed 25 components (83.56 %). The major components of the stem oil of *C. jambhiri* is 3,4-dimethyl-1,5-cyclooctadiene (13.43 %); geijerene (14.38 %) and γ-terpinene (8.07 %) were observed as main constituents in *C. jambhiri* root oil whereas *cis*-linalool oxide (19.85 %), *trans*-linalool oxide (furanoid) (14.86 %), terpinen-4-ol (7.37 %) and limonene (4.64 %) were the major components of *C. jambhiri* fruit peel oil. Even though the samples were obtained from the same plant, the compositional profile of the essential oils from various plant parts.

Key words: *Citrus jambhiri*, limonene γ-terpinene, Rutaceae.

Introduction

Citrus jambhiri (Rough lemon) belongs to the Citrus genus, a taxa of flowering plants in the family of *Rutaceae*[1]. Rough lemon is one of the citrus fruits cultivated and consumed in Nigeria[2]. The yield of lemon fruits juice is about half of the fruit weight thereby generating a very high amount of waste annually[3] around 36 metric tons of *citrus* waste produced annually, Florida generates 1.2 million tones which is sold as feed stock for cattle, however, Nigeria generates about 0.3 million tones with potential to generate more annually[4]. This is one of the major agro-waste constituting a health and environmental menace in many streets and market places in Nigeria. The major constituents of Floridian *C. jambhiri* oils were limonene (33.7 %) and other components including sabinene (7.8 %), γ-terpinene (7.4 %), β-ocimene (7.3 %), linalool (5.3 %), cironellal (7.3 %) and (*E*)-β-ocimene (5 %) [5]. Limonene (86 %) was identified as the most abundant component in the fruit peel oil from Pakistan[6]. The leaf oil of *C. jambhiri* from Nigeria contained mainly of limonene (58.1 %), geranial (11.7 %), (*E*)-β- ocimene (2.3 %), and sabinene (1.8 %)[7]. The oil extract from the fruit rinds of *C. jambhiri* Lush (Rough lemon) revealed limonene (98.55 %) as major component[8]. The principal constituent of the leaf oil of *C. jambhiri* grown in Pakistan was limonene while other components in significant proportions were sabinene, β-myrcene, α- terpineol, 1,3-tetradecadiene and linalool[9]. (*E*)-caryophyllene (14.47 %) was reported as the major component in Egyptian *C. jambhiri* leafy oil[10]. It is thus, evident that the leaf oil of this plant shows extremely variable composition by different localities.

Lemon essential oils are complex mixtures of chemical compounds like limonene, γ-terpinene, citral, linalool and β-caryophyllene among others, which can be represented by three main classes, namely hydrocarbon monoterpenes, oxygenates, and sesquiterpenes[11]. Literature reports have shown several studies on the leaf and peel essential oils of the plant without any investigations on the compositional profile of the stem and root essential oils of *C. jambhiri*. Therefore, this study is aimed at investigating the chemical compositions of the stem, root and fruit peel essential oils of *C. jambhiri* grown in Nigeria.

Materials and methods

Plant samples collection

Selected plant parts of *C. jambhiri* (stems, roots and fruit peels) were collected (500 g of each) from National Horticultural Research Institute (NIHORT) Ibadan, Nigeria. *C. jambhiri* was authenticated at the herbarium section of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria with herbarium number FH110391. The taxonomist involved in the identification of the plant was Mr. Obiyemi, S.

Isolation of the essential oils

The plant parts were hydrodistilled separately using all-glass Clevenger-type apparatus according to the method recommended by European Pharmacopoeia[12]. Pulverised 500 g of each sample was soaked in water inside 5 litre round bottom flask and hydrodistilled in thermo regulated heating mantle for 3 h at 60-70^{o}C. The condensed vapour passes through the arm of the Clevenger apparatus, the essential oil was collected and the traces of water were then removed by the addition of anhydrous sodium sulfate (Na_2SO_4). The resulting oils were stored at 4^{o}C before analysis.

Analyses:

Gas chromatography

GC analysis of the essential oils was conducted on LECO Pegasus 2D Gas Chromatography. The GC was equipped with a DB-5 30 m x 0.25 mm x 0.25 µm and 2 m x 0.18 mm x 0.18 µm fused silica column. Nitrogen was used as carrier gas at the constant flow of 1.1 ml min-1 and oven temperature program was 40 ^{0}C (2 min) and then at 8 ^{0}C min -1 to 280 ^{0}C (5 min).

Gas chromatography-Mass spectrometry

GC-MS analysis was carried out on three essential oils obtained from plant parts of *C. jambhiri*. They were all dissolved in n-hexane before the injection. Volatile compounds were identified and quantified using LECO Pegasus 2D GC-TOFMS. The GC was equipped with two columns. The first column was 30 m x 0.25 mm x 0.25 µm and the second column was 2 m x 0.18 mm x 0.18 µm. The flow rate of the helium carrier gas was set up at 1.40 ml min- 1 in the constant flow mode. The injection volume was of 1 µl. The GC inlet was maintained at 250 ^{0}C and was operated in splitting modes (50:1 split ratio). The GC oven temperature program was 40 ^{0}C (2 min) and then at 8 ^{0}C min -1 to 280 ^{0}C (5 min). The MS transfer line temperature was set up at 310 ^{0}C and the ion source temperature at 310 ^{0}C. The electron energy was 70 ev in the electron impact ionization mode (EI+), while the mass acquisition range was 40-450 atomic mass units (amu), and the detector voltage was set at 1650 V.

Identification of volatile organic components was confirmed using their MS data compared to those from the NIST mass spectral library and published mass spectra[13, 14]. The compounds were further confirmed by comparison of their retention indices with those of known compounds[15]. Co-injection with available authentic samples were also made for comparison

The formula used for calculation of KRI is Regression Analysis Method as follows**:**

$$KRI = \frac{100[\log tR\ (A) - \log tR\ (n)]}{[\log tR\ (n+1) - \log tR\ (n)]} + 100Cn$$

Where Cn is the carbon number of the alkane eluting just before the analyte tR(n) is the retention time of the alkane eluting just before the analyte tR(n+1) is the retention time of the alkane eluting just after the analyte tR(A) is the retention time of the analyte. Quantification was done by generating calibration curves of representative compounds from each class and assuming that the response factor of the compounds in the class was the same

Results and discussion

The percentage yields of the essential oils produced by the stem, root and fruit peel of *C. jambhiri* were 1.05 % w/v, 0.90 % w/v and 1.40 % w/v respectively, based on dry weight. The fruit peels of the plant produced more oils compared to the stem and root oils. All of the sampled essential oils gave distinctive aromatic odours.

Table 1 summarizes the result of GCTOFMS analyses of essential oils. The table shows components in order of elution, retention time, calculated kovat index and their percentage. Components with similarity factors of 80 % and above were identified. Sixty-six (66) components were identified in *C. jambhiri* stem oil which represent 83.11 %; in the root essential oil, fifty-six (56) components (74.21 %) were identified; meanwhile twenty-five (25) components were identified which make up of 83.56 %. The results observed were in agreement with the result of Dalila and Assem (2014)[10] where 139 chemical components were identified in the *C. jambhiri* leaf oil, also Wagnet *et al*. (2003)[20] reported that 2D GC-TOFMS improve peak capacity by identifying up to 1200 compounds. The present report is similar to their respective reports.

Oil of *C. jambhiri* stems represents 83.11 % in which hydrocarbon monoterpenoids (35.74 %) predominate; followed by oxygenated monoterpenoids (22.12 %), non-terpenoid components (11.43 %), hydrocarbon sesquiterpenes (10.98 %), oxygenated sesquiterpenoids (2.78 %) and hydrocarbon diterpenoids (0.06 %). *C. jambhiri* root oil showed 56 components which account for 74.21 %, hydrocarbon monoterpenoids represent 12.20 %, oxygenated monoterpenoids (10.19 %), hydrocarbon sesquiterpenes (26.12 %), oxygenated sesquiterpenoids (2.80 %), hydrocarbon diterpenoid (0.28 %) and non-terpenoid components (22.62 %). Twenty five components (83.56 %) were identified from the essential oil of *C. jambhiri* fruit peel which constitutes hydrocarbon monoterpenoids (12.52 %), oxygenated monoterpenoids (52.30 %); predominant class among others, hydrocarbon sesquiterpenoids (3.47 %), oxygenated sesquiterpenoids (5.04 %) and non-terpenoids (10.23 %) components (figure 1).

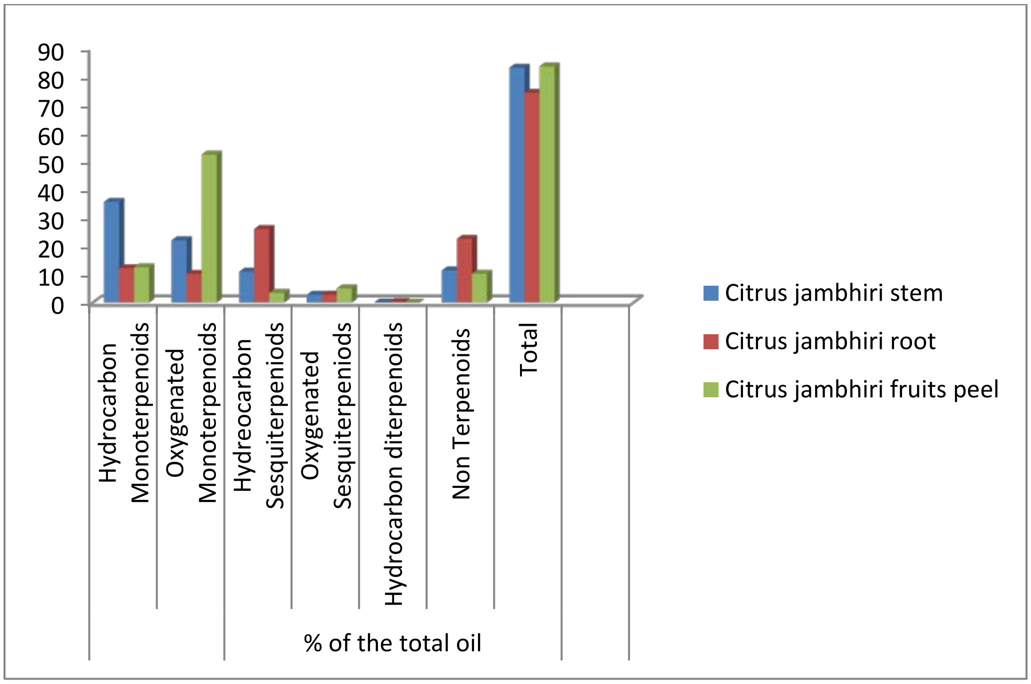
Table I: Chemical comparison of the compounds identified from stem, fruit peel and root Essential Oils obtained from Nigerian *Citrus jambhiri*

No	Compounds	RT (min)	KI	% Composition CJS	CJR	CJP
1	Octane	4.54	743	0.24	0.36	2.81
2	Cyclohexane, 1,4-dimethyl-	4.68	774	0.55	1.25	0.22
3	Cyclohexane, ethyl-	5.16	814	0.09	0.28	-
4	Cyclopentanone, 3-methyl-	5.40	832	0.04	-	0.81
5	Ethylbenzene	5.61	857	0.03	0.06	-
6	Octane, 2-methyl-	5.69	865	0.07	0.06	-
7	p-Xylene	5.77	870	0.16	0.19	-
8	Pentalene, octahydro-	5.78	872	0.19	0.16	-
9	o-Xylene	6.18	895	0.04	0.07	-
10	α-Thujene	6.80	928	2.71	0.02	-
11	Camphene	7.23	1002	0.05	0.10	-
12	1-Octyn-3-ol, 4-ethyl-	7.29	1010	-	5.01	-
13	Cyclohexanone, 3-methyl-	7.30	1011	-	-	0.73
14	2H-Pyran, 2-ethenyltetrahydro-2,6,6-trimethyl-	7.59	1029	-	-	1.22
15	Sabinene	7.70	1033	7.19	0.37	-
16	α-Pinene	7.76	1035	3.26	0.13	-
17	5-Hepten-2-one, 6-methyl-	7.81	1040	0.17	-	0.33
18	α-Myrcene	7.91	1045	2.88	0.07	-
19	Furan, 2-pentyl-	7.92	1047	2.90	0.20	-
20	2,3-Dehydro-1,8-cineole	7.94	1048	-	0.09	-
21	Decane	8.10	1055	-	2.07	0.99
22	α-Phellandrene	8.21	1059	0.17	-	-
23	1,4-Cineole	8.37	1067	0.84	-	0.38
24	γ-Terpinene	8.40	1078	1.79	8.07	-
25	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	8.41	1079	1.08	1.02	-
26	Decane, 4-methyl-	8.46	1085	-	1.03	-
27	o-Cymene	8.60	1092	0.06	0.03	-
28	Limonene	8.66	1097	-	0.78	4.64
29	Eucalyptol	8.67	1120	-	0.19	-
30	3,4-dimethyl-1,5-Cyclooctadiene	8.70	1129	13.43	-	-
31	2(3H)-Furanone, 5-ethenyldihydro-5-methyl-	8.74	1130	-	-	2.25
32	α-Ocimene	8.91	1224	3.56	-	-
33	Benzene, 1-methyl-3-propyl-	8.96	1249	-	2.08	-
34	p-Cymene	9.10	1251	0.03	0.06	-
35	2-Furanmethanol, 5-ethenyltetrahydro- α,α,5-trimethyl-, cis-	9.30	1291	-	0.10	19.85
36	trans-Linalool oxide (furanoid)	9.55	1324	0.03	-	14.86
37	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	9.56	1327	0.67	-	-
38	Linalool	9.77	1355	2.26	0.16	6. 19
39	Benzene, 2-ethyl-1,4-dimethyl-	9.84	1360	0.22	0.14	1.24
40	(2R,4R)-4-Methyl-2-(2-methylprop-1-en-1-yl)tetrahydro-2H-pyran	9.93	1363	0.10	-	4.39
41	3-Thujuone	10.08	1345	0.58	0.28	-
42	Limonene oxide	10.31	1354	0.28	-	-
43	6-Octenal, 3,7-dimethyl-	10.58	1370	5.68	-	0.15
44	5-Isopropylbicyclohexan-2-one	10.71	1375	-	-	0.93
45	Isogeranial	11.00	1410	0.06	-	-

46	Terpinen-4-ol	11.06	1423	2.27	-	7.37
47	Benzenemethanol, α,α,4-trimethyl-	11.16	1429	-	-	2.03
48	Naphthalene	11.18	1430	0.29	0.16	-
49	Terpineol	11.28	1435	0.27	-	3.69
50	Undecane, 2,6-dimethyl-	11.50	1450	0.28	1.21	2.19
51	2,6-Octadien-1-ol, 2,7-dimethyl-	11.74	1463	4.30	-	-
52	2,6-Octadienal, 3,7-dimethyl-	11.89	1468	4.60	-	-
53	(-)-Carvone	12.00	1474	0.07	-	1.69
54	Geijerene	12.20	1476	-	14.43	-
55	p-Menthane-3,8-diol, cis-1,3,trans-1,4-	12.60	1490	-	-	2.28
56	4-Isopropylcyclohexa-1,3-dienecarbaldehyde	12.64	1492	0.21	-	-
57	Valeric anhydride	12.88	1503	-	2.36	-
58	2-Methylbutanoic anhydride	12.99	1512	-	2.19	-
59	isocaryophyllene	13.27	1537	0.71	0.67	-
60	bicyclogermacrene	13.28	1542	0.81	-	-
61	6-Octen-1-ol, 3,7-dimethyl-, acetate	13.40	1552	1.27	-	-
62	Tridecane, 4-methyl-	13.56	1558	-	0.07	-
63	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	13.77	1566	4.32	-	-
64	Dodecane, 2,6,10-trimethyl-	13.78	1568	-	0.17	-
65	α-Copaene	13.89	1574	0.02	1.31	-
66	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	14.06	1580	0.44	0.32	-
67	Tricyclo[2.2.1.0(2,6)]heptane, 1,7-dimethyl-7-(4-methyl-3-pentenyl)-, (-)-	14.52	1603	0.07	5.21	-
68	Caryophyllene	14.63	1610	3.19	0.17	3.47
69	trans-α-Bergamotene	14.64	1612	1.43	0.86	-
70	Isosativene	14.80	1623	0.04	-	-
71	Hexadecane	15.18	1642	-	0.16	-
72	Cis-α-Bisabolene	15.22	1644	0.11	-	-
73	α-Farnesene	15.25	1648	0.73	-	-
74	α -Bisabolene	15.32	1660	2.07	0.06	-
75	γ-bisabolene	15.38	1686	0.29	0.35	-
76	Caryophyllene oxide	15.45	1710	2.16	2.19	5.04
77	Humulene	15.52	1730	0.58	0.38	-
78	α-Famesene	15.60	1768	0.04	0.18	-
79	Germacrene D	15.62	1772	0.33	0.19	-
80	α –Muurolene	15.69	1790	-	2.44	
81	γ-Muurolene	15.73	1810	-	7.29	-
82	trans-Calamenene	16.09	1840	0.11	2.24	-
83	α-Calacorene	17.02	1860	0.01	4.28	-
84	2,6,11-Dodecatrienal, 2,6-dimethyl-10-methylene-	17.71	1953	0.28	0.61	-
85	α-Bisabolol	18.02	1955	0.34	-	-
86	Eicosane	20.83	2064	0.06	0.28	-
	Total			83.11	74.21	83.56

CJS - *C. jambhiri* stem oil; CJR - *C. jambhiri* root oil; CJP - *C. jambhiri* fruit peel oil

Figure 1: C**lasses of components identified in *Citrus jambhiri* oils**



The major components of *C. jambhiri* stem identified were 3,4-dimethyl-1,5-cyclooctadiene (13.43 %), followed by sabinene (7.19 %), 6-Octenal, 3,7-dimethyl- (5.68 %) and α-ocimene (3.56 %). The principal components of the essential oil obtained from *C. jambhiri* root were geijerene (14.43 %), γ-terpinene (8.07 %), γ-muurolene (7.29 %), tricyclo[2.2.1.0(2,6)]heptane, 1,7-dimethyl- 7-(4-methyl-3-pentenyl)-, (-)- (5.21 %) and 4-ethyl-1-octyn-3-ol (5.01 %). The major components identified from the essential oil obtained from *C. jambhiri* fruit peel were cis-linalool oxide (19.85 %), trans-linalool oxide (furanoid) (14.86 %), terpinen-4-ol (7.37 %), linalool (6.19 %) and limonene (4.64 %). Limonene (84.5 %) was identified in Sudanese *C. jambhiri* fruit peel as major components[9], meanwhile limonene (4.64 %) was observed as a moderate component in Nigeria (present study) *C. jambhiri* fruit peel. In another study of *C. jambhiri* plant part, (*E*)-caryophyllene (14.47 %) was reported as the major constituent in the Egyptian *C. jambhiri* leaf oil [10], this is in agreement with the present study which identified caryopyllene (3.19 % and 3.47 %) in *C. jambhiri* stem oil and *C. jambhiri* fruit peel oil, respectively. Linalool (12.20 %), nerol (7.41%), limonene (7.06 %), γ-elemene (5.11 %), (*Z*)-α-bisabolene (4.14 %), δ-elemene (4.12 %) and citronellal (3.37 %) were also identified [10]. Limonene (58.1 %), geranial (11.7 %) and neral (9.8 %) together with other noticeable constituents viz. (*E*)-β-ocimene (2.3 %), geranial (2.0 %) and sabinene (1.8 %) were observed in the Nigerian *C. jambhiri* leafy oil[7]. The present study agrees with above results which also identified linalool (2.26 and 6.19 %) in the oils of *C. jambhiri* stem and *C. jambhiri* fruit peel. Limonene which has been reported as the principal constituent of the fruit peel oil of *C. jambhiri*[6,8] was found to occur moderately in the fruit peel oil of this study.

Conclusions

This investigation revealed the compositional profile of the stem, root and fruits peel essential oils of *C. jambhiri* grown in Nigeria for the first time. This study showed abundance of hydrocarbon monoterpenoids in the stem oil while the fruits peel oil was predominated by oxygenated monoterpenoids. The root oil was rich in hydrocarbon sesquiterpenoids.

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