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Essential Oils and Fatty Acids Composition of Dry Fruits of *Tetrapleura tetraptera*

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ABSTRACT: The composition of essential oil and fatty acids of dry fruits of *Tetrapleura tetraptera* were analyzed by GC/MS. Forty-four compounds representing 98.5% of the essential oil were characterized. The essential oil was dominated by acetic acid (34.59%), 2-hydroxy-3-butanone (18.25%), butanoic acid (8.35%), 2-methyl butanoic acid (7.58%), 2-methyl butanol (7.45%), butanol (4.30%), 2-methyl butenoic acid (3.65%) and Nerol (3.25%). The fatty acid was dominated by palmitic acid (49.44%), linoleic acid (26.81%), oleic acid (19.72%) and stearic acid (3.20%). The fatty acid was about 54% saturated and 46% unsaturated with omega-6 and omega-3 constituting 27% and omega-9 (20%). The effectiveness of *T.tetraptera* to the treatment of variety of ailments does not depend largely on the essential oils composition of the plant since the oil is dominated by acetic acid. Any essential oil that is dominated by acid is not a true essential oil.

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Tetrapleura tetraptera, locally known as Uyayak in Annang/Ibibio, Aiden in Yoruba, ubukirihu in Igbo languages of Nigeria, is a deciduous forest plant which belongs to the Mimosaceae family (Abii and Amarachi, 2007; Akin-Idowu et al., 2011). It has a distinctive four winged fruits consisting of woody shell, a fleshy pulp and a small brownish-black seeds with characteristic distinct fragrance. The distinct fragrance is attributed to the essential oils content of the fruit (Akin-Idowu et al., 2011). The dry fruit has a characteristic pleasant aroma which makes it a popular seasoning spice in Southern and Eastern Nigeria (Essien et al., 1994; Adesina, 1982; Okwu, 2003). It is used extensively in soups of nursing mothers to prevent post partum contractions and gas-intestinal disorders especially stomach ulceration (Atawodi et al., 2014; Nwawu and Alah, 1986; Noamesi et al., 1992). In cold weather, it is used to prepare pepper soup and the aroma is believed to drive away snakes (Abii and Amarachi, 2007). The fruit has wide application in Nigeria folk medicine. It is used extensively in the management of an array of human ailments including diabetes mellitus, arthritis, hypertension, epilepsy, asthma, etc. (Abii and Amarachi, 2007; Akin-Idowu et al., 2011). The plant is claimed to be therapeutically useful in the management of convulsion, leprosy, inflammation and/or rheumatoid pains (Adewunmi, 2001). Ghanaians use the fruit as multivitamins while Nigerians and other West African countries use it as spice (Adewunmi, 2001). The potential use of this fruit with some of their corresponding phytochemicals has been identified as molluscidal, antimicrobial, anticonvulsants, toxic and insecticidal (Adewunmi, 2001). The dry powdered fruit has been formulated into soap to increase the antimicrobial activity and improve the foaming and hardness of soaps (Adebayo et al., 2000). The fruit is said to contain caffeic acid which serves as HIV replication inhibitor and also inhibit antitumour and inflammatory characteristic (Adesina, 1982).

Although much researches have been done on this plant *Tetrapleura tetraptera*, particularly on its dry fruits to assess the phytochemicals, minerals and nutrients contents (Abii and Amarachi, 2007; Akin-Idowu et al., 2011; Adewunmi, 2001; Adebayo et al., 2000; Adesina, 1982; Antwi-Boasiko and Animapauh, 2012; Essien et al., 1994; Noamesi et al., 1992), reports on the essential oils and fatty acids composition of the fruit has been scarce. This work examines the essential oils and the fatty acids composition of the dry fruits, to ensure full exploitation of its therapeutic properties and health benefits.

MATERIAL AND METHODS

Sample Preparation: The dry fruits of *Tetrapleura tetraptera* were procured from Choba market and Boundary market, Omouku, Port Harcourt in Rivers State, Nigeria. The sample was freed of dirt by hand picking. The dirt free sample was placed in the mortar and crushed with pestle and stored in airtight plastic.

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Proximate Analysis: The moisture, ash, lipid, protein, fibre and carbohydrate contents of the pulp of the fruit were determined as described by the Association of Official Analytical Chemists (AOAC). Ash content was determined by furnace method. 3g of the sample was weighed into a porcelain crucible which was previously preheated and weighed. The crucible was inserted into a muffle furnace and regulated to a temperature of 630^{o}C. This was heated for 3 hours and allowed to cool to room temperature then re-weighed. Moisture was determined by drying 1g of sample in an oven at 105^{o}C for 6hours. The difference in weight gave the moisture content. Nitrogen was determined using the Kjeidahl method and the crude protein was calculated by multiplying the percentage Nitrogen content by the conversion factor of 6.25. Lipid was determined using soxhlet extraction method. And the percentage content of each extract was obtained.

Essential oil Extraction and GC/MS Analysis: The essential oil extraction was carried out following the modified method of Jarubol (2009). 100g of pulverized sample was weighed into the 1000 litre round bottom flask. The flask with weighed sample, condenser and other gadgets were connected to complete the hydro-distillation arrangement using Clevenger-type apparatus. The crushed sample in flask was entirely covered with deionized water suspension and placed on the heating mantle. The water was allowed to boil in the flask and the essential oil carried along over to the condenser along with the steam. The essential oil and the steam were separated below the condenser through separator. It was then dried over anhydrous sodium sulphate and stored in a 2ml sealed Agilent vial protected from light at 4^{o}C before chromatographic analysis. The oils were analyzed on an HP6890 GC, powered by HP Chemstation Rev.A09.0 (1206) software. Flame ionization detector (FID) fitted with a fused silica capillary column with dimension 30m x 0.25mm x 0.25µm was used. The oven temperature was programmed from 40^{o}C -200^{o}C at 5^{o}C/min and run at 200^{o}C for two minutes. Split injection temperature of 150^{o}C with split ratio 20:1 was used. The detector temperature was 300^{o}C and the carrier gas was hydrogen at flow rate 1.0ml/min. Hydrogen pressure was 22psi with compressed air of 28psi.

Fatty acid extraction and analysis: The fat was extracted with redistilled n-hexane for the recovery of the undiluted oil. The crude oil extract was made to be free of water by filtering through anhydrous sodium sulphate salt. The hexane was removed from the oil/hexane mixture using rotator evaporator. Fatty acid profile, saturated, mono-and poly-unsaturated, analysis was carried out following the modified AOAC 965.49 and 996.06 official methods. 50mg of the oil sample was saponified (esterified) for 5mins at 95^{o}C with 3.4ml of the 0.5MKOH in dry methanol. The mixture was neutralized by using 0.7MHCl. 3ml of the 14% boron triflouride in methanol was added. The mixture was heated for 5mins at the temperature of 90^{o}C to achieve complete methylation process. The fatty acid methyl esters were thrice extracted from the mixture with redistilled n-hexane.

The fatty acid content was concentrated to 1ml for gas chromatography analysis and 1µl was injected into the injection port of GC-model HP6890, powered with HP chemstation Rev.A09.01 (1206) software. Flame ionization detector (FID) with column dimension 30m x 0.25ml x 0.25µm and type HPIN NOWAX was used. The oven temperature was programmed at 60^{o}C with fist ramping at 10^{o}C/min for 20mins, maintained for 4mins; second ramping at 15^{o}C/mins for 4 mins, maintained for 10mins. Split injection temperature of 250^{o}C with split ratio 20:1 was used. The detector temperature was 320^{o}C and the carrier gas was nitrogen (N_2). The hydrogen pressure was 22psi and compressed air 35psi.

In all, the linear retention indices of the components were determined relative to the retention times of the series of n-alkanes and the percentage compositions were obtained from electronic integration measurements.

RESULTS AND DISCUSSION

The results of the analysis are presented in tables 1, 2 and 3. Table 1 shows the result for proximate analysis. Ash content is 3.40%. This value is comparable to earlier report by Akin-Idowu et al. (2011), who worked on the three different sections of the fruits and obtained for the seed 3.48%, pulp 3.17% and woody shell 4.01% with mean value of 3.55%. The value is much lower than 9% obtained earlier for *T. tetraptera* fruit pod as reported by Abii and Amarachi (2007). It is also comparable to the mean value (4.57%) reported for some Nigerian spices (Nwinuka et al., 2005). The value of the crude protein, 9.63% is similar to 7.44% to 17.50% reported for *T.tetraptera* (Okwu, 2003) but greater than 5.6% also reported for *T. tetraptera* (Abii and Amarachi, 2007). It is also similar to 8.65% reported for ginger (Nwinuka et al., 2005). The carbohydrate content 36.86% was comparable to 43.18% to 49.06% for the *T. tetraptera* pod earlier reported by Okwu (2003) and earlier reported values of 34.6% to 71.9% for other spices and herbs (Achinewhu, et al., 1995). The fibre content, 44.81% was compared to 45% earlier reported by Abii and Amarachi (2007).

The recommended daily intake (RDI) of fibre for children, adult and pregnant women are 19-25, 21-38 and 78% respectively (RDI, 2014). Therefore, the fruit could be a good source for dietary fibre. Lipid content was the same percentage with ash while moisture constituted the least percentage of the nutrients.

Essential oils composition: forty-four compounds representing 98.5% of the essential oil were identified. These are listed according to their retention time as shown in table 2. The predominant chemical constituents of the oil were acetic acid (34.59%), 2-hydroxy-3-butanone (18.25%), butanoic acid (8.35%), 2-methyl butanoic acid (7.58%), 2-methyl buatanol (7.45%), butanol (4.30%), 2-methyl butanoic acid (3.65%), nerol (3.25%), 2-methyl butenoic acid ethyl ester (2.70%), 2-methyl butanoic acid ethyl ester (2.09%) and linalool (1.84%). The oil was dominated by carboxylic acid which is quite unusual and completely different from essential oil results obtained from other spices as reported by Onyenekwe et al., (1997), Karioti et al., (2004), Ekwenye and Okorie (2010) and Abugri and Pritchett, (2013). Terpene constituents which often dominate most essential oils as observed in other spices were detected as minor or trace constituents in *T. tetraptera*. For example, β-caryophellene was (0.1%), α-pinene(0.1%), β-pinene (0.2 %), myrcene (0.09 %), γ-terpinene (0.2 %), whereas reports on essential oils of piper guineense showed β-caryophellene (20.8 %), β-pinene (12.15%), α-pinene (10.6%), myrcene (1.8%), γ-terpinene (4.9%), (Karioti et al., 2004; Ekwenye and Okorie ,2010; Abugri and Pritchett, 2013). Linalool constitutes 1.8% of the essential oils and this account for the pepperish nature of the plant.

The characteristic fragrance of *T.tetraptera* is attributed mostly to 2-methyl butanol (7.45%), butanol (4.3%) and nerol (3.25%) constituents of the oil (Stewart, 2004). The fruity flavor is attributed to esters constituents: 2-methyl butenoic acid ethyl ester (2.70%), 2-methyl butanoic acid ethyl ester (2.09%) while the pungent odour is attributed mostly to 2-hydroxy-3-butanone (18.25%) constituent (Stewart, 2004).

Table 3 shows the fatty acids present in the sample. Ten components were more prominent. They are listed in order of their retention time as shown in table 3. The predominant acids were palmitic acid (49.44%), linoleic acid (26.81%), oleic acid (19.72%) and stearic acid (3.20%). The saturated acid made up 54% while 46% was unsaturated. Omega-6 fatty acid constituted about 27% (linoleic acid) while omega-3 (linolenic acid) constitutes 0.1%. Omega-9 fatty acid (oleic acid) constituted about 20%. Frequent used of the fruit *T.tetraptera* as edible spice may lead to increase in blood cholesterol since it is dominated by saturated fatty acid and may result in the development of atherosclerosis, hypertension and eventually heart failure (Atawodi et al., 2014).

However, the present of some essential fatty acids: omega-6 (linoleic acid-26.80%) make the fruit very useful as it could help to retain healthy lipid level in the blood, maintain proper clotting and control inflammation in cases of infection or injury. This may be the reason while it is used by nursing mothers to prevent post partum contraction as reported by Noamesi et al., (1992). The fruit should therefore, be used occasionally and in little quantity in order not to endanger human health.

On a whole, oil from dry fruit of *T. tetraptera* is not considered as true essential oil since it is dominated by acetic acid (34.59%) and as noted by Stewart, (2004), it is capable of reacting with other constituents present in the oils to form other compounds.

Table1 Proximate (%) nutrient contents of Tetrapleura tetraptera

Constituents	% Composition
Ash	3.40
Protein	9.63
Carbohydrate	36.86
Fibre	44.81
Moisture	1.90
Lipids	3.40

Table 2: Essential oil constituents of Tetrapleura tetraptera

S/N	Constituents	Retention Time	% Composition
1	acetic acid	4.57	34.59
2	Butanol	4.77	4.30
3	2-methyl butanol	5.51	7.45
4	2-hydroxyl-3 butanone	5.61	18.25
5	butanoic acid	6.55	8.35
6	2- methyl butenoic acid	7.55	3.65
7	2- methyl butanoic acid	7.93	7.58
8	2-methyl butenoic acid ethyl ester	8.76	2.70
9	2-methyl butanoic acid ethyl ester	9.81	2.09
10	α- pinene	9.87	0.14
11	β-pinene	10.71	0.23
12	benzyl alcohol	11.47	0.26
13	cis ocimene	12.14	0.03
14	myrcene	12.92	0.09
15	allo ocimene	13.15	0.11
16	α- thujene	14.22	0.39
17	2,6 dimethyl-5 heptanal	14.48	0.14
18	γ-terpinene	14.70	0.19
19	citral	15.13	0.16
20	geranial	15.45	0.18
21	neral	15.54	0.23
22	isoartemisia	16.35	0.11
23	1,8-cineole	16.88	0.37
24	borneol	17.10	0.26
25	linalool	17.81	1.84
26	nerol	18.46	3.25
27	ascaridole	20.21	0.21
28	linalyl acetate	20.77	0.20
29	α- terpinenyl acetate	21.02	0.14
30	borneol acetate	21.22	0.32
31	ethyl cinnamate	21.41	0.26
32	β-bisabolene	21.97	0.29
33	germacrene b	22.97	0.38
34	β-caryophellene	22.53	0.15
35	cyperene	23.06	0.06
36	α-copane	24.75	0.09
37	β -selinene	28.41	0.11
38	(6)-paradol	28.63	0.03
39	aromadendrene	28.82	0.14
40	γ-muurolene	29.00	0.14
41	aristolone	29.38	0.14
42	viridiflorol	29.61	0.13
43	trans beta farnesene	29.77	0.14
44	(6) gingerol	30.02	0.14

Table 3: Fatty acids compositions of *Tetrapleura Tetraptera*

S/N	Constituents	Retention time	% composition
1	caprylic acid methyl ester (c10:0)	10.71	0.00
2	lauric acid methyl ester (c12:0)	12.33	0.00
3	myristic acid methyl ester (c14:0)	13.81	0.01
4	palmitic acid methyl ester (c16:0)	15.37	49.44
5	palmitoleic acid methylester (c16:1)	16.24	0.00
6	margaric acid methyl ester (c17:0)	17.47	0.00
7	stearic acid methyl ester (18:0)	18.55	3.20
8	oleic acid methyl ester (c18:1)	20.00	19.72
9	linoleic acid methyl ester (c18:2)	21.39	26.81
10	linolenic acid methyl ester (c18:3)	22.00	0.05
11	arachidic acid methyl ester (c20:0)	22.61	0.02
12	arachinic acid methyl ester (c20:4)	23.23	0.08
13	behenic acid methyl ester (c22:0)	24.11	0.44
14	erucic acid methyl ester (c22:1)	24.84	0.24
15	lingnoceric acid methyl ester (c24:0)	25.61	0.00

Conclusion: The effectiveness of the *T.tetraptera* to the treatment of variety of ailments does not depend largely on the essential oils composition of the plant. It was observed that the essential oil of the plant is dominated by acetic acid and from literature; any essential oil dominated by carboxylic acid is not a true essential oil. However, the presence of nerol, linalool, butanol, 2-methyl butanol and some esters in reasonable quantity makes the plant very useful as raw material in perfumes and cosmetic industries. The fatty acids, minerals, nutrients and phytochemical composition of the plant, therefore, play significant roles in its health application. The fruit of *T.tetraptera* is thus a reservoir of medical constituents with a wide range of potential application. It should be used mildly in the formulation of drugs and cosmetic.

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