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Chemical Composition and Biological Activities of the Volatile Oils of *Palisota hirsuta* (Thunb) K. Schum and *Trema orientalis* (L) Blume

Sherifat A. Aboaba[1, 2], Iqbal M. Choudhary[2]

[1]Department of Chemistry, University of Ibadan, Ibadan, Nigeria

[2]H.E.J Research Institute of Chemistry, University of Karachi, Karachi, Pakistan

Correspondence: Sherifat A. Aboaba, Department of Chemistry, University of Ibadan, Ibadan, Nigeria. Tel: +2348038011394, E-mail: saboaba@gmail.com

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Abstract

Leaves of *Palisota hirsuta* (Thunb) K. Schum. and *Trema orientalis* (L) Blume. were collected from a farm land in Nigeria. The volatile oils were isolated using hydrodistillation and GC-MS method to determine their yield and composition. Antimicrobial activities of various oils obtained were also evaluated. Thirty-three (33) and thirty-seven (37) compounds were identified representing 98.9% and 99.4% of the entire constituents in the leaf oils of *P. hirsuta* and *T. orientalis* respectively. The main components of *P. hirsuta* oil were nonanal (19.6%), 1-Octen-3-ol (9.4%), hexenal (7.7%) and o-Cymene while those from *T. orientalis* were tetradecanal (33.3%), n-hexadecanoic acid (19.5%), farnesylacetone (5.6%) and linalool (4.3%). Both leaf oils displayed different activity against the tested microorganisms.

Keywords: *Palisota hirsuta*, *Trema orientalis*, tetradecanal, nonanal, antimicrobial activity

1. Introduction

Palisota hirsuta K. Schum belongs to the Commeliaceae family also known as Spiderwort family (Ansah et al., 2008). *P. hirsuta* is a robust plant in forest re-growths about 2 – 4 m high with distinct main stem and large leaves (Inafidon et al., 2010). It is common along road sides and in forest clearing in closed forest in southern Ghana and distributed from Senegal to Cameroon; also in Fernando Po and the Congo while the young leaves are eaten in Nigeria (Dokosi, 1998). The plant is used in traditional medicine in Ghana and Nigeria for treatment of disorders of the respiratory and central nervous system; the dried powdered leaves are used as an anti-dysentery enema (Burkill, 1995). The methanol extract of the lea http://www.ccsenet.org/journal/index.php/ijc/article/view/50880ves has been found to possess antiviral activity (Anani et al., 2000, Hudson et al., 2004), anti-arthritic and anti-pyretic activites (Boakye-Gyasi et al., 2009a) and it also has sexual stimulant effects in rats (Benson et al., 2008). Two rare phytoecdysones (ecdysteroids), ecdysterone and 20, 26-dihydroxyecdysone were extracted from the rhizomes of *P. hirsuta* (Kusamba et al., 1995).

Trema orientalis (L.) Blume (Ulmaceae) is a medium-sized evergreen weedy tree with very long pointed leaves. It is up to 18 m high and 0.6 m in trunk diameter. The tree grows rapidly extending into forest openings in moist lowland area in Hawaii (Orwa et al., 2009). The leaves and stem bark is useful as inhalant, lotion, vapour bath for coughs and also in the treatment of sore throat, diarrhoea, asthma, bronchitis and chicken pox (Oladunmoye and Kehinde, 2011). The chemical investigation of dichloromethane and ethylacetate extracts from the trunk and root barks of *T. orientalis* afforded the isolation of 16 compounds among which is hexacosanoic acid. Six other species of *Trema* have also been the subject of literature discussion (Noungone et al., 2001). The stem bark extract of *T. orientalis* also exhibited hypoglycaemic activity (Dimo et al., 2006).

In this report, we present for the first time, the chemical compositions of the volatile compounds and also evaluate the antimicrobial activities of the leaf essential oils of *P. hirsuta* and *T. orientalis* for the multi-purpose utilization.

2. Materials and Methods

2.1`Sample Collection and Preparation

The samples were collected by Mr. Hezekiah Igumoyi along farm road at Abavo, Ika south local government area, Delta state, Nigeria in August 2014. Identification and authentication of the samples were done by Dr Henry Adebowale Akinnibosun of the Department of Botany, University of Benin, Benin city, Nigeria where herbarium specimens were deposited with voucher numbers UBH_P 0179 and UBH_T 0181 for *P. hirsuta* and *T. orientalis* respectively. The air dried leaves of each plant (250g) were pulverised and hydrodistilled using an all glass-clevenger apparatus for 3.5 h (British Pharmacopoeia, 1980). The oils obtained were stored under refrigeration at 4^{o}C until analyses.

2.2 Gas Chromatography - Mass Spectrometry (GC – MS)

The composition of the essential oils was determined by Gas chromatography-Mass spectrometry (GC/MS) using an Agilent 7890N GC with Agilent mass detector Triple Quad 7000A in EI mode at 70 eV (m/z range 40 – 600 amu) and an Agilent ChemStation data system. The GC column was equipped with an HP-5MS column (30 m x 250 µm x 0.25 µm) a split-split less injector heated at 200^{o}C and a flame ionization detector (FID) at 230^{o}C. The GC oven temperature was programmed as follows: Initial temperature 40^{o}C for 5 min, increased 5^{o}C/min to 180^{o}C for 6 min and then 10^{o}C/min to 280^{o}C for 12 min. Helium was the carrier gas at flow rate of 1 mL/min. The injection volume was 2.0 µL (split ratio 1:20).

The components were identified by comparison of their mass spectra with NIST 1998 library data of the GC-MS system as well as by comparison of their retention indices (RI) with the relevant literature data (Adams 2007). The relative amount of each individual component of the essential oil was expressed as the percentage of the peak area relative to the total peak area. RI value of each component was determined relative to the retention times of a homologous n-alkane series with linear interpolation on the HP-5MS column.

2.3 Screening for Antimicrobial Activity

Tested organisms were as follows: (Bacteria) *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiellae pneumonia*, while (Fungi) *Candida albicans*, *Aspergillus niger*, *Penicillum notatum* and *Rhizopus stolonifer*. The microorganisms were obtained from the stock cultures of the Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria.

Antimicrobial activity was assayed using agar diffusion method. I mL of the essential oils was dissolved in 5 mL of DMSO to give 200 mg/mL. This was serially diluted to give different concentrations. DMSO was used as the negative control. Using a cork borer of 8 mm diameter, the wells were made with already incubated (at 45 ^{o}C) diluted organisms in different concentrations. This was done in triplicates. The plates were incubated for 24 h at 37 ^{o}C for bacteria and 48 h at 28 ^{o}C for fungi (Belboukhari and Cheriti, 2005, Thongson et al., 2004). Zones of inhibition were measured by taking zones greater than 8 mm as inhibition. Gentamicin (5 g/mL) and tioconazide (70%) were used as the positive controls for bacteria and fungi respectively while dimethyl sulfoxide (DMSO) was the negative control.

3. Results and Discussion

From the hydrodistillation, light yellow volatile oils were obtained with yield of 0.77% (w/w) for *P. hirsuta* and 0.41% (w/w) for *T. orientalis* respectively on dried matter basis. A total of 33 compounds were identified in the leaf oil of *P. hirsuta* accounting for 98.9% of the total composition of the essential oil while 37 compounds were identified in *T. orientalis* representing 99.4% of the entire oil. The chemical compositions of the essential oils and their respective Kovat's index (KI) in order of elution on HP-5MS column are compiled in Table 1.

The oil of *P. hirsuta* was made up of largely non-terpene oxygenated compounds (40.8%), non-terpene hydrocarbons (28.7%), monoterpenes hydrocarbon (14.4%) and oxygenated monoterpenes (9.0%) while the remaining composition was characterized by 0.6% sesquiterpene hydrocarbon and 5.4% oxygenated sesquiterpene. The most abundant compounds of the leaf of *P. hirsuta* were nonanal (19.6%), 1-octenol-3-ol (9.4%), hexenal (7.7%), methylcyclohexane (6.4%) and o-cymene (5.3%)

Non-terpene oxygenated compounds (23.5%) and hydrocarbons (50.7%) also dominated *T. orientalis* leaf essential oil. However, the oil was devoid of monoterpenes hydrocarbons but contained oxygenated monoterpenes (4.8%), sesquiterpene hydrocarbons (6.3%) and oxygenated sesquiterpene (14.1%). The prominent constituents in the leaf oil were tetradecanal (33.3%), hexadecanoic acid (19.5%), farnesylacetone (5.6%), heptacosane (4.6%) and linalool (4.3%).

The dominant constituents in the two oils have been reported from different literatures of their diverse uses. Examining the most abundant constituents in *P. hirsuta*, Nonanal is a food additive permitted for direct addition to food for human consumption as a synthetic flavouring substance but in minimal quantity (Lewis, 1997). It is also very useful in perfumery and as a flavouring agent and has been identified as a common plant volatile.

1-octenol-3-ol is the main component of mushroom flavour and many insects use the compound as an alarm pheromone and it is also believed to be an antifeedant (Combet et al., 2006 and Kline, 1994). Hexenal, another constituent found in significant quantity, has had significant inhibitory effect against pathogen microorganisms frequently isolated from raw materials (*E. coli, S. enteridis* and *L. monocytogenes*) when inoculated in both model and real systems (Lanciotti et al., 2003).

Tetradecanal, which is the most prominent compound in *T. orientalis*, is naturally produced by bioluminescent bacteria of the *vibrio* genus and is one of two substrates produced and consumed by *vibrio fischeri* luciferase light emission system; it is a major component of the essential oil found in the leaves of *Azadirachta indica* (Dastan et al., 2013). Hexadecanoic acid, also called palmitic acid, possesses anti-inflammatory properties and studies validate the rigorous use of medicated oils rich in hexadecanoic acid for the treatment of rheumatic symptoms in traditional medical system of India (Aparna et al., 2012). The compound was also one of the 7 methyl esters of fatty acids detected in the ethylacetate fraction of the stem bark of *T. orientalis* (Abd Malik et al., 2014).

However, the minor constituents could also contribute to the bioactivity or flavouring properties of the oils, as activities observed from essential oils are not only contributed by major constituents.

The results of the antimicrobial activities of the volatile oils from *P. hirsuta* and *T. orientalis* by the agar diffusion method are shown in Table 2. In general, the oils were more active in Gram-positive bacteria. The leaf oil of *P. hirsuta* did not show any activity against *P. aeruginosa* and also no activity at 25 mg/mL and 12.5 mg/mL against *S. typhi*, *A. niger* and *R. stolonifer* while *T. orientalis* did not show activity against *S. typhi*, *K. pneumonia*, and *P. notatum*; in addition there was no activity at the lowest concentration of 12.5 mg/mL against *E. coli*, *S. typhi*, *K. pneumonia*, *A. niger*, *P. notatum* and *R. stolonifer*. Both oils showed excellent activity at the highest concentration of 200 mg/mL against *C. albicans* when compared to the standard drug gentamicin.

The observed activity from this study was better compared to activity from the solvent extracts from the same plant. Chowdhury and Islam, 2004 from their antimicrobial study on the hexane, ethylacetate and methanol extracts of the roots of *T. orientalis* discovered that the extracts were not active gram-positive bacteria and fungus but show only moderate activity against gram-negative bacteria at a dose of 500 mg/mL. In contrast to the findings of Chowdhury and Islam, 2004, the best activity was observed in gram-positive bacteria from our study (Table 2). However, the result of Jayashree et al., 2012 on the extracts from bark of *T. orientalis* were comparable to the result from this study; their result reveals that the plant is a potentially good source of antibacterial agent.

4. Conclusions

In conclusion, we have characterized the volatile oils from leaves of *P. hirsuta* and *T. orientalis*, which to our knowledge have not been reported in literature. The various compounds characterized in the volatile oils contribute to medicinal, organoleptic and biological activities of the plants. Generally, the complex composition of essential oils and the variety of chemical structures of their constituents are responsible for the wide range of biological activities exhibited by essential oils. Particularly, many essential oils and their constituents have traditionally been used for their antimicrobial activity which has long been recognized; the activity displayed by the oils under study also supports the various uses of these plants in traditional medicine.

Table 1. Chemical composition of the volatile oils from the leaves of *Palisota hirsuta* and *Trema orientalis*.

S/N	Compound	KI	*P.hirsuta* leaves	*T.orientalis* leaves
1	Isobutylacetate	721	1.0	-
2	Methylcyclohexane	781	**6.5**	3.1
3	Butylacetate	785	3.5	-
4	Toluene	794	4.3	1.8
5	Hexenal	806	**7.7**	-
6	E-2-Hexenal	814	1.6	-
7	2-Heptanone	853	0.6	-
8	Ethylbenzene	893	0.4	0.8
9	Heptenal	905	1.8	-
10	p-Xylene	907	2.0	4.4
11	Z-4-Hepten-10-al	913	0.7	-
12	6-Methyl-5-heptene-2-one	938	0.6	-
13	7-Octen-2-one	943	0.9	-
14	α-Pinene	948	0.4	-
15	δ-3-Carene	950	1.0	-
16	1-Octenol-3-ol	969	**9.4**	1.2
17	2-Pentylfuran	987	1.5	0.3
18	Benzaldehyde	982	0.6	-
19	D-Limonene	1018	4.4	-
20	o-Cymene	1042	**5.3**	-
21	Terpinolene	1052	3.3	
22	Linalool	1082	**4.7**	**4.3**
23	Nonanal	1104	**19.6**	0.8
24	E-2-Nonenal	1112	-	0.7
25	α-Terpineol	1143	-	0.7
26	Citral	1174	0.2	-
27	L-isopulegol	1196	0.5	-
28	Lilac-aldehyde-B	1197	0.7	-
29	Lilac-aldehyde-A	1197	0.9	
30	β-Cyclocitral	1204	2.0	0.5
31	E-2-Decenal	1212	0.5	-
32	(E,E)-2,4-Decadienal	1220	-	0.3
33	α-Copaene	1221	-	0.6
34	Naphthalene	1231	-	0.7
35	2,6,6-Trimethyl-1-cyclohexene-1-actaldehyde	1303	0.7	-
36	2,6,6-Trimethyl-1-cyclohexene-1-ethanol	1357	0.6	-
37	(+)-Ledene	1419	-	3.1
38	Z-Geranylacetone	1420	3.0	2.3
39	α-Ionone	1429	-	0.5
40	(E)-β-Damascenone	1440	-	0.4
41	β-Ionone	1457	1.3	0.8
42	Ethylpentamethylbenzene	1459	-	1.2
43	β-Caryophyllene	1494	-	2.3
44	Z-α-Bisabolene	1518	0.6	-
45	2,6,10-Trimethyltetradecane	1519	-	0.5
46	Ledol	1530	0.4	-
47	α-Calacorene	1547	-	0.3
48	Caryophyllene oxide		-	0.6
49	Tetradecanal	1601	-	**33.3**
50	α-Bisabolol	1625	-	0.8
51	Hexahydrofarnesylacetone	1754	0.7	2.3
52	Tetradecanoic acid	1769	-	0.6
53	Pentadecanoic acid	1869	-	0.4
54	Farnesylacetone	1902	-	**5.6**
55	n-Hexadecanoic acid	1968	-	**19.5**
56	Geranylgeraniol	2192	-	0.8
57	Z-9-Octadecanamide	2228	5.5	-
58	Heptacosane	2705	-	4.6
			98.9	99.4

KI = Kovat's index in order of elution on HP-5MS column

Table 2. Antimicrobial Activities of *P. hirsuta* and *T. orientalis* leaf essential oils

Sample	Conc (mg/mL)	Diameter of Inhibition Zone in mm									
		Gram negative				Gram positive		Fungi			
		E.c	S.t	P.a	K.p	B.s	S.a	C.a	A.n	P.n	R.s
P. hirsuta	200	18	14	-	18	20	20	24	14	18	14
	100	16	12	-	14	18	18	20	12	16	12
	50	14	10	-	12	14	14	18	10	12	10
	25	12	-	-	10	12	12	14	-	10	-
	12.5	10	-	-	-	10	10	12	-	-	-
T. orientalis	200	24	-	20	-	24	20	22	16	-	16
	100	14	-	18	-	20	18	18	14	-	14
	50	12	-	14	-	18	16	16	12	-	12
	25	10	-	12	-	14	14	12	-	-	10
	12.5	-	-	10	-	12	12	12	-	-	-
Gentamicin (+ve control)		36	38	40	34	34	38				
Tioconazide (+ve control)								24	26	26	28
DMSO (-ve control)		-	-	-	-	-	-	-	-	-	-

E.c = *Escherichia coli*; S.t = *Salmonella typhi*; P.a = *Pseudomonas aeruginosa;* K.b = *Klebsiellae pneumonia*; B.s = *Bacillus subtilis*; S.a = *Staphylococcus aureus*; C.a = *Candida albicans*; A.n = *Aspergillus niger*; P.n = *Penicillum notatum*; R.s = *Rhizopus stolonifer*; DMSO = Dimethylsulfoxide; (-) = No activity

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