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Chemical Profile and Toxicity of Fresh and Dry Leaves of the Essential Oils of *Vetiveria zizanioides* (L.) Nash. From Southwest Nigeria

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Abstract

The fresh and dry leaves of Vetiveria zizanioides (L.) Nash (Gramineae) plant was collected, air dried and authenticated at the University of Ibadan Herbarium with voucher number: UIH-23237. The plant's dried, fresh leaves were hydro distilled in a Clevenger device to extract the essential oils. The oils were characterized by Gas Chromatography-Mass Spectrometry (GC – MS) while the toxicity of the essential oils was tested using brine shrimps' lethality assay. The percentage yields of fresh and dry leaf oils were 0.045%v/w and 0.42%v/w respectively. Thirty-eight (38) chemical compounds were identified in the fresh leaf sample of V. zizanioides. The major components were 8-Cedren-13-ol (12.24%), Spathulenol (7.01%), 6-(3-acetyl-2-methylcyclopropen-1-yl)-6-methylheptan-2-one (4.95%), β-Patchoulene (3.68%), 9H-Cycloisolongifolene, 8-oxo- (3.65%). Thirty-four (34) compounds were found in all. Thirty-four (34) chemicals were found in the essential oil of V. zizanioides dry leaf. The major components were 6-(3-Acetyl-2-methyl-1-cyclopropen-1-yl)-6-methyl-2-heptanone (13.73%), (1aR,4aR,7S,7aR,7bR)-1,1,7-Trimethyl-4-methylenedecahydro-1H-cyclopropa[e]azulen-7-ol (12.07%), (-)-globulol (9.05%), 1,1,4,7 tetramethyldecahydro1Hcyclopropa[e]azulen4ol, [1aR(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha) (7.90%), hibaene (6.26%), hexahydrofarnesylacetone (5.59%), bicyclo [9.3.1] pentadeca-3,7-dien-12-ol (2.59%), β-Ionone (2.41%). Copaene, α-muurolene, δ-cadinol, phytol and bicyclo [9.3.1] pentadeca-3, 7-dien-12-ol were found to be the common constituents in both fresh and dry leaf essential oils. The cytotoxicity results of both essential oils showed that both oils are extremely toxic with LC_{50} of 10 ppm. The identified compounds from the dry and the fresh samples of V. zizanioides may be responsible for the cytotoxic activities of the plant while the variations in the constituents of the two oils are largely due to different sample preparation conditions.

Keywords: *Vetiveria zizanioides*, Volatile oil, Cytotoxicity, Gas Chromatography-Mass Spectrometry, 8-Cedren-13-ol

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INTRODUCTION

Vetiver (*Vetiveria zizanioides* (L.) Nash) is also known as the *Chrysopogon zizanioides* (Roberty) and it is a perennial grass of the *Gramineae* or *Poaceae* family [1]. The plant is cultivated widely in tropical regions such as Asia, India, Africa, Oceania, and Central and South America. It has also been reported to be cultivated in countries like Indonesia, Malaysia, Japan, China, Brazil, Guatemala, Jamaica, Philippines, Angola, Belgian Congo, Dominican Republic, Argentina, British Guiana, Mauritius, and Honduras [2]. Vetiver grass is native to India, found in plains, lower hills of India particularly in rich marshy areas or river

banks.

Vetiveria zizanioides is a unique grass characterized by long, narrow leaves and deep-reaching roots, which can extend vertically down to the depths of 3 meters to 4 meters. Its visible height can be about 1.5 meters to 2 meters (5 feet to 6.5 feet) [3].

It is commonly called *Khas-Khas*, *Khus* or *Khas* grass in India. Its roots are aromatic, sturdy and compact. Different parts of the grass are attributed to many medicinal importance as well as soil erosion control [4].

Ethno botanical uses of different parts of the plants in India include treatment of ailments like mouth ulcers, rheumatism, fever, headaches, boils, epilepsy, burns, snakebites, and scorpion stings among others [4].

The plant extract of *V. zizanioides* has been confirmed to show a wide range of medicinal activities such as anti-inflammatory, insecticidal [5], antioxidant [6], hepatoprotective, anti-tubercular [7, 8], anti- hyperglycaemic [9], anthelmintic, antipyretic activity, antibacterial, anti-malaria, and antidepressant [10, 5].

Several studies have revealed that *V. zizanioides* can help improve breathing in humans, acts as a relaxant for the nervous system and also lower their heart rate [11]. It has also been confirmed to cure skin diseases, treat gastrointestinal diseases like flatulence and indigestion, control diabetes and exhibits anticancer activity [2, 5, 11,12].

Chahal [6] reported that the essential oil of *Vetiveria zizanioides* root was found to contain alpha and beta vetivones, zizanal, epizizanal, as well as other sesquiterpene compounds that confer biological and herbicidal activities to the plant [5, 12] and [8]. Chahal [6] explored the therapeutic potentials of essential oil from the root of *Vetiveria zizanioides* while Amarasiri [12] investigated the larvicidal, anticancer, sedative, antidiuretic and as well as the nephroprotective activity. Jayashree [13] also studied the anti-venom potentials of the plant. Other medicinal uses of Khas-Khas include ringworm treatment, cure to indigestion and loss of appetite, treatment of fever and dysuria, muscular and joint pains, obstinate vomiting, cures insomnia, trauma, and other emotional problems [2].

This work sets to characterise and compare the essential oils from the fresh and dry leaves of *Vetiveria zizanioides* (L.) Nash and also ascertain the level of toxicity of the oils as it has been established that the roots oil of the plant possess herbicidal activities.

MATERIALS AND METHODS

Collection and Preparation of Samples

Fresh sample of a whole plant of *V. zizanioides* was collected within the premises of the plantation of the Botany Department, University of Ibadan, Oyo state, Nigeria and identified by Mr. Donatus of the Botanical Gardens Herbarium. The fresh plant sample was separated into leaf and root. Some fresh leaves were separated and pulverized in order to increase the surface area as well as aid effective extraction of essential oil from the fresh leaf while the remaining fresh leaves were air dried for about two weeks.

The fresh and dry leaves were cut into smaller sizes to increase their surface areas and for effective hydrodistillation. Hydrodistillation was carried out in an all glass Clevenger apparatus according to standard procedure.

Essential Oil Analysis and identification of Compounds

The GC-MS analyses of the essential oils were performed using GCMS-QP2010 Plus [14] with HP-5MS as the capillary column, having a length of 30 m, 0.25 mm internal diameter and 0.25 µm film thickness. The GC-MS detector was operated in the EI mode of electron energy 70 eV, with a scan range of 45-700 amu. Helium served as the carrier gas, flowing at a steady 1.61 mL/min rate. The GC oven program was initially at 60°C, followed by 60-180°C at a rate of 10°C/min, then held at 180°C for 2 min, followed by 180-280°C at a rate of 15°C/min, then again held at 280°C for 2 min. At 70 eV, the samples' ionisation mode was EI. Samples were injected into the port at a temperature of 250°C; the interface was kept at 200°C, and the ion source at 200°C. The material was diluted in n-hexane at a ratio of 1:1 (v/v), and precisely 1.0 µl was injected using an auto sampler with a split ratio of 25:1. By first comparing the retention periods of each component with those of the genuine samples and then their retention indices (RI) with the n-hydrocarbon series, the components in the samples were identified. To determine the components, the mass spectra of the constituents were cross-referenced with published spectra and the 2017 NIST database. The true concentration of each component in the sample was determined by integrating the separate peaks [15].

Toxicity Assay

The essential oils of *V. zizanioides* were prepared at 1000 ppm, 100 ppm and 10 ppm in a dimethyl sulphoxide (DMSO) in triplicate. Ten *Artemia salina* (brine shrimp) larvae were then placed in each of the triplicate vials in a total volume of 5 ml. A reference standard was also made consisting of DMSO and 10 brine shrimps in sea water which serves as the negative control. The total number of dead shrimps was counted and the lethal concentration at 50% level was determined after 24 hours.

RESULTS AND DISCUSSION

The chemicals found in the essential oils' fresh and dried leaves are shown in Table 1. A total of 38 and 34 compounds representing 88.9% and 84.23% of the total oil were identified in the essential oils of the fresh and dry leaves of *V. zizanioides* respectively. The essential oil from the fresh leaf consist of oxygenated sesquiterpenes (55.36%), sesquiterpene hydrocarbons (21.49%), oxygenated diterpenes (2.69%), diterpene hydrocarbons (4.24%), oxygenated monoterpene (4.95%) and monoterpene hydrocarbon (0.17%) while the dry leaf essential oil had oxygenated sesquiterpenes (47.24%), sesquiterpene hydrocarbons (8.36%), oxygenated diterpenes (4.38%), diterpene hydrocarbons (6.26%), monoterpene hydrocarbon (0.17%), triterpene (0.31%) and non-terpene derivative (0.17%).

The major components identified in the fresh leaf essential oil were 8-Cedren-13-ol (12.24%), Spathulenol (7.01%), 6-(3-acetyl-2-methylcyclopropen-1-yl)-6-methylheptan-2-one (4.95%), β-Patchoulene (3.68%), 9H-Cycloisolongifolene and 8-oxo- (3.65%). Other minor components present in the fresh leaf essential oil of *V. zizanioides* are 8-Propoxycedrane (3.32%), Humulane-1,6-dien-3-ol (3.29%), Patchoulene (3.20%), β-Eudesmol (3.19%) and (-)-Isolongifolol, acetate (2.90%). The main constituents in the dry leaf essential oil were 6-(3-Acetyl-2-methyl-1-cyclopropen-1-yl)-6-methyl-2-heptanone (13.73%), Espatulenol (12.07%), (-)-Globulol (9.05%), Epiglobulol (7.90%), Hibaene (6.26%), Hexahydrofarnesyl acetone (5.59%) while Verticiol (2.59%), β-Ionone (2.41%), 5-Hydroxymethyl-1,1,4a-trimethyl-6-methylenedecahydronaphthalen-2-ol(1.81%) and Phytol (1.75%) were present as minor compounds 6-(3-Acetyl-2-methyl-1-cyclopropen-1-yl)-6-methyl-2-heptanone copaene, α-muurolene, δ-cadinol, phytol and Bicyclo [9.3.1] pentadeca-3, 7-dien-12-ol were identified as common constituents in fresh and dry the leaves essential oils of *V. zizanioides.*

Table 2 shows result of the brine shrimp bioassay of the essential oils from the two plant samples. The essential oils from the fresh and dry leaves both have lethal concentration at 50% level of toxicity (LC_{50}) of 10.00 ppm. This value indicate that the essential oils from *V. zizanioides* are extremely toxic and contain medicinally active compound(s).

CONCLUSION

This work provided information on the essential oil components obtained from the fresh and dry leaves of *V. zizanioides* (L.) Nash which has not been previously reported in literature from Southwest

of Nigeria. It was observed from the summary in Table 1 that the class of terpene to terpenoid present in both essential oils were comparable though there were a few compound that differ significantly.

Table 1. Chemical Profile of the Essential Oil of *Vetiveria zizanioides* fresh and dry leaves from GC-MS Analysis.

Constituent	Retention Index (LRI)	Fresh leaf	Dry leaf
Copaene	1221	2.29	0.43
β-Elemene	1398	0.14	-
β-Cadinene	1440	0.17	-
β-Damascone	1457	0.17	-
Patchoulene	1386	3.20	-
Longifolene (V4)	1386	1.97	-
γ-Cadinene	1435	1.74	-
α-Muurolene	1440	1.31	1.16
Calamenene	1537	0.64	-
δ-Cadinol	1580	1.79	1.46
β-Eudesmol	1598	3.19	-
Viridiflorene	1419	0.91	-
Spathulenol	1536	7.01	-
Viridiflorol	1530	2.02	-
(-)-Isolongifolol, acetate	1759	2.90	-
6-(3-acetyl-2-methylcyclopropen-1-yl)-6-methylheptan-2-one	1565	4.95	13.73
Humulane-1,6-dien-3-ol	1757	3.29	-
Carotol	1593	0.91	-
(+)-Cycloisosativene	1125	2.86	-
Ledene oxide (II)	1630	2.39	-
Longiverbenone	1574	2.40	-
8-Propoxycedrane	1741	3.32	-
Eudesma-4,11-dien-2-ol	1690	1.98	-
β-Patchoulene	1398	3.68	-
8-Cedren-13-ol	1646	12.24	-
β-Guaiene	1523	2.58	-
Nootkatone	1673	0.73	-
2(1H)Naphthalenone, 3,5,6,7,8,8a-hehydro-4,8a-dimethyl-6-(1-methylethenyl)	1673	1.27	-
9H-Cycloisolongifolene, 8-oxo-	1694	3.65	-
Phthalic acid, 2-(1-adamantyl)ethyl isobutyl ester	2607	1.21	-
Cupressene	1880	2.77	-
Palmitic acid	1968	1.69	-
2,4,7,14-Tetramethyl-4-vinyl-tricyclo[5.4.3.0(1,8)]tetradecane-6-ol	2103	0.43	-
Rimuene	1926	1.47	-
Bicyclo[9.3.1]pentadeca-3,7-dien-12-ol	2190	2.14	2.59
Phytol	2045	2.26	1.75
(Z)-13-Octadecanal	2007	0.89	-
Octadecanoic acid	2167	0.34	-
Nonanal	1104	-	0.17
Decanal	1204	-	0.10
Caryophyllene	1494	-	0.26
Aromadendrene	1386	-	1.40
α-Selinene	1435	-	1.21
Valencene	1580	-	0.57
Calamenene II	1537	-	1.72
4,5,9,10-dehydro-Isolongifolene	1380	-	0.34

(3E)-4-(5-Hydroxy-2,2-dimethyl-6-methylenecyclohexyl)-3-buten-2-one	1368	-	1.27
Aristolene epoxide	1293	-	1.49
Epiglobulol	1530	-	1.29
(1aR,4aR,7S,7aR,7bR)-1,1,7-Trimethyl-4-methylenedecahydro-1H-cyclopropa[e]azulen-7-ol	1536	-	12.07
(-)-Globulol	1530	-	9.05
1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulen-4-ol-, [1aR-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-	1530	-	7.90
Cubenol	1580	-	0.61
β-Ionone	1457	-	2.41
(Z)-7-Hexadecenal	1808	-	1.33
2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol	1745	-	0.87
Hexadecanal	1800	-	0.65
3,7,11,15-Tetramethylhexadecylacetate	2119	-	1.05
1-Bromo-Triacontane	3299	-	0.31
Hexahydrofarnesyl acetone	1754	-	5.59
(3E,12Z)-1,3,12-Nonadecatriene-5,14-diol	2241	-	0.53
(4aS,5S,8aS)-5-Isopentyl-1,1,4a-trimethyl-6-methylenedecahydronaphthalene	1891	-	1.27
Hibaene	1778	-	6.26
5-Hydroxymethyl-1,1,4a-trimethyl-6-methylenedecahydronaphthalen-2-ol	1868	-	1.81
4,8,12,16-Tetramethylheptadecan-4-olide	2258	-	0.46
Hexanedioic acid, bis (2-ethylhexyl)ester	2414	-	1.12
Class of Terpene/Terpenoid Present			
Non-terpene derivative		0.00	0.17
Monoterpene hydrocarbon		0.17	0.00
Oxygenated monoterpenes		4.95	17.51
Sesquiterpene hydrocarbons		21.49	8.36
Oxygenated sesquiterpenes		55.36	47.24
Diterpene hydrocarbon		4.24	6.26
Oxygenated diterpenes		2.69	4.38
Triterpene		0.00	0.31
Total identified		88.9	84.23

LRI = Linear Retention Indices; (-) = Not detected

Table 2. Brine Shrimp lethality test of essential oils from *Vetiveria zizanioides* leaves and stem-bark.

Sample	LC_{50} (ppm)	UCL	LCL
Fresh leaf	10	10.13	9.87
Dry leaf	10	10.13	9.87

UCL: Upper Confidence Limit.
LCL: Lower Confidence Limit.

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