Communication in Physical Sciences, 2020, 6(2):827-834

Volatile Constituents of the Leaves and Stem of *Justicia secunda Vahl*

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Received 19 December 2020/Accepted 27 December 2020/Published online: 30 December 2020

Abstract: Volatile constituents of leaves and stem of *Justicia secunda Vahl* have been investigated. The respective plant parts were extracted using hydrodistillation techniques and the volatile constituents were quantitatively analysed with Gas chromatography coupled to a mass spectrophotometer (GCMS). The results deduced from the spectra of the plant stem and leaf indicated the presence of twenty-four volatile compounds consisting of two (2) monoterpenoids, four (4) sesquiterpenes, one (1) sesquiterpenoid and seventeen (17) non-terpenoids. The isolated compounds and their concentrations were diethyl phthalate (28.71%), bis(2-ethylhexyl) phthalate (52.52%), (3aR, 4R, 7R)- 1,4,9,9-tetramethyl -3,4,5,6,7,8-hexahydro-2H-3a,7-methanoazulen-2-one (6.71%), 13-methyl-Z-14-nonacosene (1.08), 5(1H)-azulenone (34.67%), guaia-1(10), 11-diene (9.54%), γ-elemene (6.59%), 11-octadecenoic acid methyl ester (5.97%), hexadecanoic acid methyl ester (5.95%), trans-13-octadecenoic acid (5.01%), 1-ethoxy-2,2-dimethyl-3-(2-phenyl ethynyl)-cyclopropane (4.10%), 9(1H)-phenanthrone (3.28%), longifolene (2.93%), 2-hydroxycyclopentadecanone (2.92%), patchoulene (2.59%), 9-octadecenoic acid methyl ester (1.72%), oleic acid (1.26%) and 2-octylcyclopropaneoctanal (1.10%). The present study indicates that the stem and leaf of *J. secunda Vahl* is rich in volatile compounds that can provide useful medicinal or other industrial purposes.

Keywords*: *Volatile constituents, Justicia secunda Vahl, GCMS, applications

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1.0 Introduction

Plant materials have been widely acknowledged and reported as significant resources to animals and man because of their applications as food, industrial raw materials and for medicinal and pharmaceutical purposes, among others (Musa, 2020a' Okwunodulu, and Eddy, 2014). However, most of the application areas known for pant and plant materials is based on their phytochemical constituents (Eddy, 2010), which may include their proximate, mineral, vitamins and phytochemical constituents. Also, these constituents have been deeply investigated but studies on the volatile components of plants are still receiving serious research attention because of the future hope especially in medicinal and pharmaceutical applications.

Justicia secunda Vahl is a perennial herbal plant, which belongs to the *Acanthaceae* family (Arogbodo, 2020; Onyeabo *et al*., 2017; Corrêa and Alcântara, 2012). The plant is widely found in Africa, Asia and the West Indians. Due to their numerous applications, the plants have different names in various regions. For example, it is called *Hounsimanî*", "*Sanguinaria* or blood root in Benin, Venezuela and Barbados respectively (Osioma and Hamilton-Amachree, 2017; Carrington *et al*., 2012; Onoja *et al*., 2017). There are several documented reports on the medicinal values of this plant and they are traditionally employed in the treatment of anemic circumstance, wound healing, abdominal pain and fertility issues (Onoja *et al*., 2017; Koné, 2012; N'gussan, 2010; Moswa *et al*., 2005; Chifundera, 2001; Lans 2007). Biochemical profiling of the plant revealed the presence of antioxidant (Osioma and Hamilton-Amachree, 2017), antimicrobial (Carrington *et al*., 2012; Rojas *et al*., 2006; Herrera *et al*., 2002), anti-sickling, haematonic, antihypertensive, anti-inflammatory and other biological activities (Onoja *et al*., 2017; Abo *et al*., 2016; Manda *et al*., 2011; Mpiana *et al*., 2010; Mpiana *et al*., 2010) activities.

Some secondary metabolites have also been identified in the plant including saponins, tannins,

glycosides, terpenoids (steroids) and flavanoids (Arogbodo, 2020). Osioma and Hamilton-Amachree (2017); Theiler *et al*., (2014) respectively isolated alkaloids, phenols, quinines and anthocyanins. luteolin, aurantamide acetate, auranamide, quinoline and pyrrolidine derivatives from various parts of the plant (Theiler *et al*., 2014; Koffi *et al*., 2013; Calderon *et al*., 2013). The presence of polyenoic fatty acid methyl esters in the plant leaves have also been reported (Hamilton-Amachree and Uzoekwe, 2017).

The use of GCMS to explore phytochemical and other constituents have been widely reported. For example, constituents of ethanol extracts of *Andrographis paniculate* leaf (Eddy *et al*.,2011), erhanol extract of *Combretum hispidum* (Laws) (*Combretaceae*) root (Ikpeazu *et al*., 2020), *Daniella oliverri* gum (Eddy *et al*., 2012) and ethanol extracts of leaves of *Solanum melongena* leaf (Eddy *et al*.,2010) have been reportedly quantified through GCMS analysis. Some studies to compare phytochemicals in root and stem of some medicinal plants have also been reported (Musa, 2020b; Oruk *et al*., 2020).

Most of the studies conducted on plants' secondary, metabolites are concentrated on their leaves in spite of the fact that volatile compounds have been reported for other plant parts including fruits, flower, stem, root and exudate. Studies have also revealed that phytochemical constituents of plants could vary from geographical regions due to different soil conditions. Therefore, the present study is aimed at investigating some volatile compounds in stem and leaf of *Justicia secunda Vahl* that is native of Bayelsa State of Nigeria.

2.0 Materials and Methods

2.1 Plant Material

Samples of leaf and stem of the plants were harvested from Ogbia Local Government Area of Bayelsa State, Nigeria and was identified in the department of botany, University of Ibadan. The respective samples were reduced to minimal size, dried to constant weight and preserved for extraction.

2.2 Extraction of Volatile Constituents

Volatile constituents were extracted using the hydro-distillation procedure with the aid of a Clavenger fitted round bottom flask apparatus and a regulated heating mantle. Extracted constituents were dried over anhydrous sodium sulphate and stored below 0 ^{o}C in air-tight vials. The percentage yields (w/w) of the extracts were considered using the relationship:

$$\%\, Yield = \frac{weight\ of\ extract}{weight\ of\ plant\ material} \times 100 \qquad (1)$$

2.3 Identification and quantification of volatile constituents

Identification and quantification of volatile constituents were performed with a hyphenated gas-liquid chromatography and mass spectrometry (GC-MS), using Agilent technologies (GC system 7890A coupled with MSD 5975C) equipment with an injection mode 7683B series. Column details were: HP5MS, 30 mm length, 0.320 mm internal diameter and 0.25 μm thickness. The column temperature was programmed within the range, 37 to 320 ^{o}C at an incremental rate of 18-25 ^{o}C/minute. The MS ion source temperature was maintained at 280 ^{o}C with full scan and solvent delay of 0-2.30 mins. MS scan range was m/z 35-500 in 0.10 sec. 1μL of each of the samples was injected per time in Helium carrier gas at split flow of 20 mL/mins. Spectra data matching was done using NIST 14. library.

3.0 Result and Discussion

Percentage yield values of 0.42 and 0.33 were obtained for the leaves and stem extracts respectively. The extracts were volatized, coloured and had characteristic, essential smell. The GCMS spectra of leaf and stem samples of *Justicia secunda Vahl* are presented in Figs. 1 and 2. Volatile compounds identified from the spectra and their relative percentage concentrations are presented in Table 1.

Bis(2-ethylhexyl) phthalate was the most abundant volatile component in the leaf extract of *Justicia secunda Vahl*. This compound was presence at a concentration of 52.22 % and was observed at GC retention time of 31.731 minutes. Bis(2-ethylhexyl) phthalate is the commonest phthalate plasticizer in medical devices such as intravenous tubing and bags, intravenous catheters, nasogastric tubes, dialysis bags and tubing, blood bags and transfusion tubing, and air tubes. It is also use in the production of PVC, vinyl chloride resins and nail polishes. The next volatile compound with highest concentration in the plant leaf was gamma elemene, which was isolated at retention time of 11.269 minutes. Gamma elemene is classified as sesquiterpenes. It has major contribution to the aroma of some plants and is often used as pheromones by some insects. Experiments perform to test the significant of volatile compounds

of plant origin indicated some anti-fungal activity and the possibility of using such plants for chemotherapy (Li *et al*., 2005; Yao et al., 2008; *Zhu et al*.,2011). This compound was absent in the plant stem and may partly account for the more intense aroma of the plant leaf than that of the stem. 5(1H)-azulenone was the most abundant volatile components in the stem, accounting for 34.67 % and was observed at a retention time of 17.472 minutes. Cyperotundone whose IUPAC name is (3aR, 4R, 7R)- 1,4,9,9-tetramethyl -3,4,5,6,7,8-hexahydro-2H-3a, 7-methanoazulen-2-one was found in only the leaf of the plant at a concentration and retention time of 6.71% and 16.717 minutes respectively. It is a sesquiterpenoid and a major constituent of *Cyperus totundone* plant. Most volatile compounds have been reported to have a wide range of medicinal and pharmaceutical applications including astringent, diaphoretic, diuretic, analgesic, antispasmodic, aromatic, carminative, antitussive, emmenagogue, litholytic, sedative, stimulant, stomachic, vermifuge, tonic and antibacterial (Sivapalan, 2013). 1.08% 13-methyl-Z-14-nonacosene was also identified in the leaf of the plant at a retention time of 42.128 minutes. The compound has been reported to has significant biological activities and has also been isolated from *Rosa roxburghii* and *Rosa sterilis* fruits by Lu *et al.* (2016). Other volatile components were present in concentration less than 1%. The stem extract seems to be richer in volatile components than the leaf extract. Bulnesene was present at 9.54% and was isolated under GC retention time of 13.839 minutes. Sitarek *et al*. (2017) also identified this volatile compound in from hairy and normal roots of *Leonurus sibiricus L* and reported that they have good antibacterial, antiinflammatory, aantioxidant, and aantiproliferative activities. Hexadecanoic acid methyl ester, 11-octadecenoic acid methyl ester, 9-octadecenoic acid methyl ester, N, N-dimethyl-S-1,3-diphenyl-2-butenyl ester and di(4-chloro-2-methoxyphenyl) ester were some of the ester derivatives that were presence in only the roots of the plant. Their biological activities have been reported (Aikoye, 2020; Chibuzo and Aikoye, 2020; Chibuzo and Okop, 2020; Ogoko, 2020). Some useful fatty acids were also identified from the plant stem including trans-13-octadecenoic acid, 11-octadecenoic acid methyl ester and 11-octadecenoic acid methyl ester. Biological activities of some of these fatty acids were also reported by Musa (2020a),

Table 1. Phyto-constituents of the leaves and stem of *Justicia secunda Vahl*

Retention time (minute) ~~RI~~	Identified compound	% concentration ~~Area~~ Leaves	Stem	S.N
11.269	γ-elemene	-	6.59	1
13.753	Diethyl phthalate	28.71	-	2
13.839	Guaia-1(10), 11-diene	-	9.54	3
13.982	Patchoulene	-	2.59	4
14.966	Diethyl phthalate	-	1.74	5
16.162	Longifolene	-	2.93	6
16.310	9(1H)-Phenanthrone	-	3.28	7
16.717	(3aR, 4R, 7R)- 1,4,9,9-tetramethyl -3,4,5,6,7,8-hexahydro-2H-3a, 7-methanoazulen-2-one	6.71	-	8
17.472	5(1H)-azulenone	-	34.67	9
17.684	Hexadecahydropyrene	-	0.23	10
20.819	Hexadecanoic acid methyl ester	-	5.95	11
23.326	1-ethoxy-2,2-dimethyl-3-(2-phenyl ethynyl)-cyclopropane	-	4.10	12
23.657	11-octadecenoic acid methyl ester	-	5.97	13

23.709	9-octadecenoic acid methyl ester	-	1.72	14
23.726	Oleic acid	-	1.26	15
23.749	2-hydroxy cyclopentadecanone	-	2.92	16
24.069	Trans-13-octadecenoic acid	-	5.01	17
25.506	2-octylcyclopropaneoctanal	-	1.10	18
31.731	Bis(2-ethylhexyl) phthalate	52.52	-	19
41.441	3-methyl heneicosane	0.88	-	20
41.521	N,N-dimethyl-S-1,3-diphenyl-2-butenyl ester	0.45	-	21
42.128	13-methyl-Z-14-nonacosene	1.08	-	22
42.311	Methyl-2-hydroxyeicosanoate	0.50	-	23
42.752	Di(4-chloro-2-methoxyphenyl) ester	0.51	-	24
43.044	n-propyl-11-octadecenoate	0.76	-	25
	Total	92.12	89.60	
	Terpenoids	1	1	
	Sesquiterpenes	-	4	
	Sesquiterpenoids	-	1	
	Non-terpenoids	8	10	

****RI- retention index, CI- compound isolated, S.N- serial number**

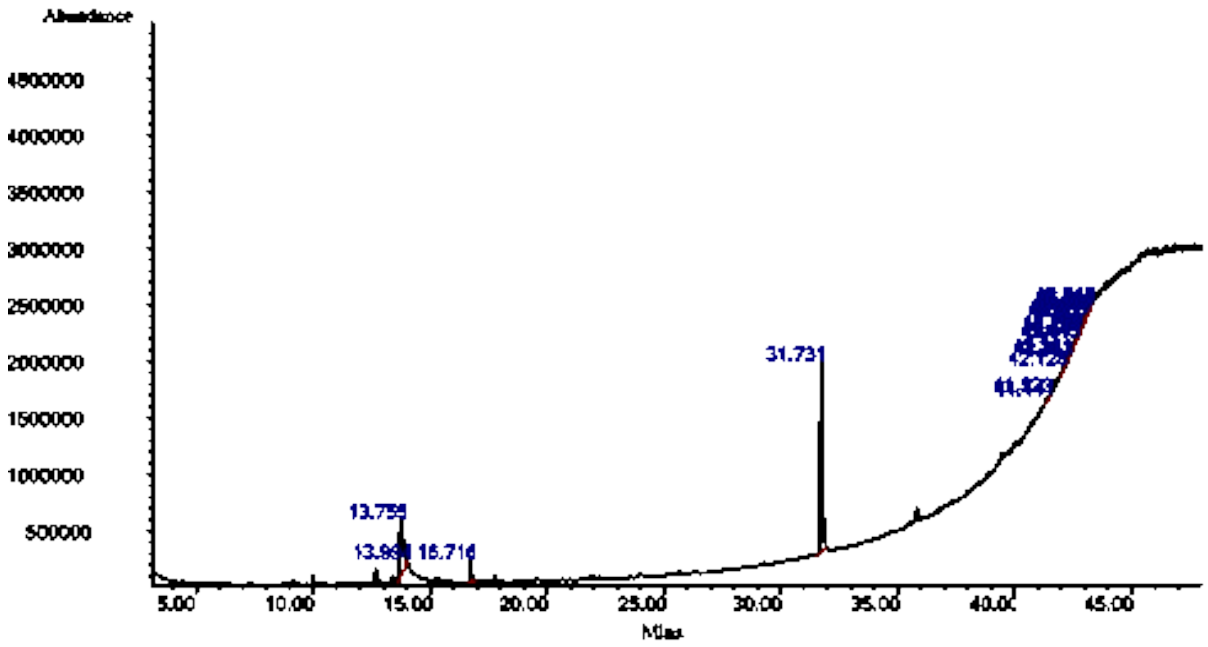

Fig. 1. GC-MS chromatogram of the volatile components of the leaves of *Justicia secunda Vahl*

The non-terpenoids constituents identified in the leaf and stem of the plant were aliphatic methyl esters of hexadecanoic acid, 11-octadecenoic acid and 9-octadecenoic acid; aromatic esters (diethyl phthalate, bis(2-ethylhexyl) phthalate and di(4-chloro-2-methoxyphenyl); otherswere esters (N,N-dimethyl-S-1,3-diphenyl-2-butenyl ester, methyl-2-hydroxyeicosanoate and n-propyl-11-octadecenoate), fatty acids (oleic and trans-13-octadecenoic acids); ketone (2-hydroxy

cyclopentadecanone); aliphatics (3-methyl heneicosane, 13-methyl-Z-14-nonacosene) and cyclics (2,3,4,4a,4b,5,6,7,8,8a,9(1H)-phenanthrone, hexadecahydropyrene, 1-ethoxy-2,2-dimethyl-3-(2-phenyl ethynyl)-cyclopropane and 2-octylcyclopropaneoctanal). Methyl esters polyenoic fatty acids have been reported to be present in the leaves of *Justicia secunda Vahl* (Hamiltons-Amachree and Uzoekwe, 2017).

The major constituents found in the leaf of the plant were diethyl phthalate (28.71%), Bis(2-ethylhexyl) phthalate (52.52%), (3aR, 4R, 7R)- 1,4,9,9-tetramethyl -3,4,5,6,7,8-hexahydro-2H-3a,7-methanoazulen-2-one (6.71%) and 13-methyl-Z-14-nonacosene (1.08) while the ones observed in the stem include: 5(1H)-azulenone (34.67%), guaia-1(10), 11-diene (9.54%), γ-elemene (6.59%), 11-octadecenoic acid methyl ester (5.97%), Hexadecanoic acid methyl ester (5.95%), trans-13-octadecenoic acid (5.01%), 1-ethoxy-2,2-dimethyl-3-(2-phenyl ethynyl)-cyclopropane (4.10%), 9(1H)-Phenanthrone (3.28%), longifolene (2.93%), 2-hydroxy cyclopentadecanone (2.92%), patchoulene (2.59%), 9-octadecenoic acid methyl ester (1.72%), oleic acid (1.26%) and 2-octylcyclopropaneoctanal (1.10%).

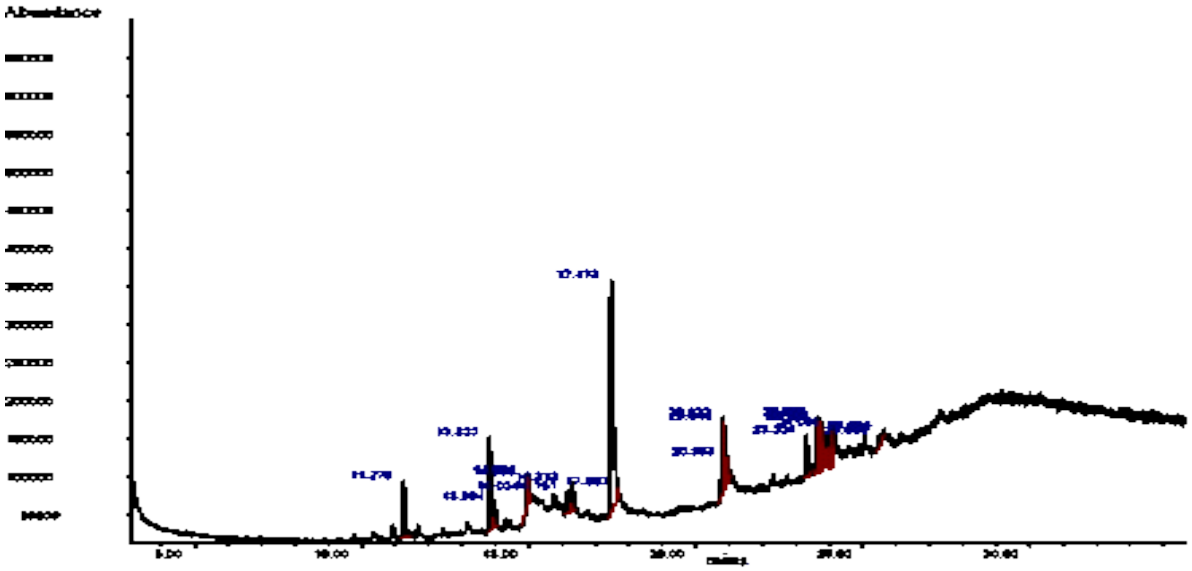

Fig. 2. GC-MS chromatogram of the volatile components of the stem of *Justicia secunda Vahl*

4.0 Conclusion

The volatile phyto-constituents of the leaves and stem of *Justicia secunda Vahl* have been identified in this study. Twenty four volatile constituents have been isolated, characterized and quantified from both the leaves and stem of *J. secunda Vahl.* They consisted of two (2) monoterpenoids, four (4) sesquiterpenes, four (1) sesquiterpenoid and seventeen (17) non-terpenoids. The characteristic oil of *Justicia secunda Vahl* constituents are mainly the C-10 monoterpenoids and the C-15 class (sesquiterpenes) and its derivatives (sesquiterpenoids). The volatile constituents of terpenes and their derivatives included: γ-elemene, guaia-1(10), 11-diene, patchoulene, longifolene, (3aR, 4R, 7R)- 1,4,9,9-tetramethyl -3,4,5,6,7,8-hexahydro-2H-3a, 7-methanoazulen-2-one and 5(1H)-azulenone. The presence of these organic compounds in both the leaves and stem of *Justicia secunda Vahl* has not been reported elsewhere.

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Conflict of Interest

The authors declared no conflict of interest