CHEMICAL CONSTITUENTS AND RADICAL SCAVENGING ACTIVITY OF METHANOL LEAF EXTRACT OF *Vitex doniana*

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Abstract: This study was aimed at investigating the chemical constituents and radical scavenging activity of methanol leaf extract of *Vitex doniana*. The 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, trace metal contents and phytochemical constituents of *V. doniana* leaf extract were carried out. The results demonstrated that the leaf extract possess a high radical scavenging activity with an IC_{50} value of 198.53 µg/ml and 139.52 µg/ml for quercetin which was used as standard. Forty three phytochemicals were identified in the leaf extract of *V. doniana*, major among them include linalool (7.89%), β-Bisabolene (7.22%), erucic acid (7.11%), D-Nerolidol (6.22%), α-Farnesene (5.57%), α-Caryophyllene (5.28%), Calarene (1.02%), α-Copaene (0.80%). The metal element composition showed that the leaf extract is rich in some trace elements such as Mn, Fe and Zn. The presence of the phytochemicals and trace metals (iron, zinc, selenium, manganese and copper) identified in the leaf extract of *V. doniana* could be responsible for its high 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity.

Keywords: Leaf extract, phytochemicals, radical scavenging, *Vitex doniana*

Introduction

The use of plants as medicine is as old as the existence of man on earth. Plants are known to possess secondary metabolites with pharmacological activities. Most of these phytochemicals are antioxidants (Lee *et al*., 2017). Vegetables, fruits and other edible plants are vital dietary sources of vitamins and essential metals. (Zhai *et al*., 2015). Medicinal plants have been explored and utilized as herbal medicine in the treatment of several diseases (Petrovska, 2012). Owing to intensified research, it is been reported that a lot of plants actually possess medicinal worth (Sofowora, 1993). The use of nutritional antioxidants in combating pathological disorders related to oxidative stress has attracted attention in recent years. Products of plant origin are known to exert their ameliorative effects by cleaning up free radicals and adjusting antioxidant defense to the system (Tarasub *et al*., 2011). Plants of tropical and sub-tropical climate are known to possess large amount of phytochemical antioxidant compounds as secondary metabolites which is a natural adaptive way of surviving in a harsh environments. In recent decades, evidences abound from epidemiological and laboratory studies to show that some edible plants possess great potential in the prevention, management and remedy of a wide range of human diseases (Agbafor and Nwachukwu, 2011).

Vitex doniana (*V. doniana*) of the Verbanaceae family is a perenial shrub with wide distribution in tropical West Africa such as Nigeria, and extends to some East African countries and in the savannah and high rainfall areas (Atawodi *et al*., 2003). Vitex doniana is a deciduous tree, usually 4-8m high, occasionally up to 15m, with a heavy rounded crown (Burkill, 2000). *V. doniana* is an edible plant. In the middle belt of Nigeria, like Kogi state, the tender leaves are utilized as vegetables for sauces and porridge for meals (Yakubu *et al*., 2013). *V. doniana* leaves is consumed in Nigeria as vegetables and valued sources of protective foods that is of great benefits for the maintenance of good health and disease prevention (Nnamani *et al*., 2007). In ethno-medicine, *V. doniana* leaf is utilized in the management and treatment of many diseases. It is employed in the treatment of stomach and rheumatic pains, inflammatory disorders, diarrhoea and dysentery (Iwueke *et al*., 2006). According to Iwu (1993), the leaves and roots of *V. doniana* are also utilized in the treatment of nausea, colic and epilepsy. *Vitex doniana* is widely used in traditional system of medicine. The fruit of *V. doniana* is sweet and tastes like prunes; it is at times sold and eaten as food. It contains vitamins A and B and can be made into a jam. The leaves are commonly used as herb and for food (Katende, 1995). The fruit can be prepared into wine, and pounded leaves can also be added to warm filtered grain beer and then drunk. The bark yields a dye that can be used for cloth (Katende, 1995).

V. doniana has been utilized as medication for liver disease, stiffness, leprosy, backache, hemiplegia, conjunctivitis, rash, measles, rachitis, febrifuge, as tonic galactagogue to aid milk production in nursing mothers, sedative, digestive regulator and treatment of eye problems, kidney problems and as supplement for lack of vitamin A and B (Burkill, 2000). This study is therefore aimed at investigating the chemical constituents and radical scavenging activity of the methanolic leaf extract of *V. doniana*

Materials and Methods

Collection and extraction of Vitex doniana leaves

Fresh leaves of *Vitex doniana* were collected within the premises of Kogi State University Anyigba, Nigeria. They were thereafter confirmed in the Department of Biological Sciences, Kogi State University, Anyigba, Nigeria. The plant leaves were rinsed in distilled water, air dried and pulverized. To obtain the methanol leaf extract of *Vitex doniana* (MEVd), 1000 g of the plant material were extracted in 3 litres of methanol for 48 h. The filteration process was done using Whatman grade 1 filter paper and vacuum pump. The extract was concentrated in vacuum at 40^{o}C with a rotary evaporator (Model Modulyo 4K, Edward England), and water bath to dryness. The percentage (%) yield of the methanol leaf extract of *Vitex doniana* was 8.25% and the crude extracts obtained was stored in capped vials at 4^{o}C.

DPPH radical scavenging activity assay

The stable 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radical was used for the determination of free radical scavenging activity of the plant extract. The method as modified by Arogba and Omede (2012) was used for the determination of free radical scavenging activity of the plant extract. To a portion (1 ml) each of the different concentrations of the extract or standard (Quercetin) in test tubes was added 1 ml of 0.3 mM DPPH in methanol. The mixture was vortexed and then incubated in a dark chamber for 30 min after which the absorbance were read at 517 nm against a DPPH control containing only 1 ml of methanol in place of the extract. The experiment was carried out in triplicate and the percentage scavenging activity was calculated using the expression:

$$\% \text{ Scavenging Activity} = [(Ac - As)/Ac] \times 100$$

Where: Ac = Absorbance of control; As = Absorbance of sample

Identification of the chemical constituents of the extract

Gas chromatography-mass spectrometry (GC-MS) analysis was conducted using GCMS –QP 2010 Plus Shimadzu Japan with a GC 2010 injector temperature of 250ºC, column oven temperature of 80ºC and carrier gas pressure of 108.0 kPa. The column length was 30 m with a diameter of 0.25 mm. The elutes were automatically passed into a mass spectrometer. The GC-MS-QP2010 Plus ion source temperature was 230^0C; interface temperature of 250ºC. Comparison of the GC-MS spectrograph of the sample with the instrument's data bank together with computer matching with the database of National Institute of Standard Technology (NIST) library revealed that the methanol leaf extract of *V. doniana* contained various bioactive compounds that eluted at different retention times depending on the boiling point of the eluted constituent. The plant extract sample was injected by splitting with the split ratio 1:60.

Determination of trace metal contents of the extract

The metal content of the leaf extract was determined using atomic absorption spectrometry (AAS). The plant extract (1 g) was weighed into a digestion flask and 20 ml of acid mixture (650 ml conc. HNO_3; 80 ml Perchloric acid (PCA); 20 ml conc. H_2SO_4) was added. The flask was heated until a clear digest was obtained. The digest was diluted with distilled water to the 500ml mark, and aliquots of the diluted clear digest were used for AAS using filters that match the different elements. The method was optimized based on the proper wavelengths for each element: Fe (248.3 nm), Mn (279.5 nm), Zn (213.9 nm), Se (196.0 nm), Cu (324.754 nm), Cr (357.869 nm), Pb (283.754 nm), Cd (228.802 nm), As (189.0 nm) and Hg (253.7 nm).

Results and Discussion

Free radical scavenging activity of the leaf extract

The methanol leaf extract of *V. doniana* showed a very high (94.03 ± 0.44%) DPPH radical scavenging activity which is slightly higher than that of Quercetin (92.97 ± 0.49) at the same concentration (1000 µg/ml). Also at lower concentration, the extract exhibited higher radical scavenging activity than Quercetin. However, the result of this study revealed that quercetin has a lower IC_{50} value (139.52µg/ml) compared to the methanol extract of *V. doniana* leaves which has an IC_{50} value of 198.53 µg/ml (Table 1). Free radicals have become popular in biological research as a result of their pivotal role in physiological processes and in addition to their involvement in various pathological disorders. Free radicals affect several significant biomolecules, and as a result disturb the normal redox balance leading to elevated oxidative stress (Phaniendra *et al*., 2015). This study revealed that the methanol leaf extract of *Vitex doniana* (MEVd) exhibited a high DPPH radical scavenging activity with an IC_{50} of 198.53 µg/ml which is, however, not as high as that of quercetin (139.52 µg/ml). This is an indication that MEVd may be an efficient free radicals scavenger. This is in agreement with the work of Agbafor and Nwachukwu (2011) which revealed that the leaf extracts of *Vitex doniana* possess antioxidant property.

Table 1: DPPH Radical Scavenging Activity (%) of the methanol leaf extract of *V. doniana*

Concentration	% Radical scavenging.	
	Extract	Quercetin
1000	94.03 ± 0.44	92.97 ± 0.49
500	87.62 ± 0.92	85.29 ± 0.80
250	60.61 ± 1.10	74.28 ± 0.49
125	51.53 ± 0.96	64.85 ± 0.47
62.50	36.50 ± 1.22	38.03 ± 1.09
31.25	22.52 ± 1.14	15.86 ± 0.86
IC_{50} (µg/ml)	198.53 ± 0.96	139.52 ± 0.70

Table 2: Qualitative and quantitative assessment of some metals by AAS

Metal	Mn	Fe	Zn	Se	Cu
ppm × 10^{-3}	95.3	92.1	64.2	35.6	21.5

Concentration of some trace elements in the leaf extract

The analysis on the methanol leaf extract of *V. doniana* for the presence and concentration of some trace elements is shown in Table 2. This result showed that the plant extract contained essential trace elements that may have probably contributed to the radical scavenging activity of the extract. The AAS analysis as carried out in this study showed that the methanol leaf extract of *Vitex doniana* contained essential metallic nutrients such as Iron (92.1 × 10^{-3}), Zinc (64.2 × 10^{-3}), Selenium (35.6 × 10^{-3}) and Corper (21.5 × 10^{-3}).

Zinc is a well recognized essential metal known to alleviate heavy metal toxicity. There are complex inter-relationships between cadmium and some essential trace elements. A number of essential trace elements such as zinc (Zn), iron (Fe), selenium (Se) and copper (Cu) play vital roles in controlling various metabolic and signaling pathways. Among the trace elements Zn and Fe are essential for maintenance of life and health. Zn is an essential trace metal with numerous functions in biological systems. It controls several enzymes of intermediary metabolism, DNA and RNA synthesis, gene expression, immune-competence and plays a significant role in homeostasis of hormones. Zn also takes part in the defense against excessive amounts and following damage of certain metals, and it does so through the interaction with metallothionein. It has been noted that Zn has a relationship with many enzymes in the body and can prevent cell damage through activation of the antioxidant defense system (Jamakala and Rani, 2014). Zn has similar chemical and physical characteristics with Cadmium. As a result it competes with Cadmium for the binding sites of metallic transporters and proteins of enzymatic function (Bridges and Zalups, 2005). Zn reduces the oxidative stress generated by Cadmium. This is as a result of the functional role as a co-factor of copper zinc-superoxide dismutase (Cu/Zn SOD) which is an antioxidant enzyme (Zhai *et al*., 2015). There are reports to show that Selenium (Se) intake provides protection against Cadmium toxicity in several organs including the liver and kidney. Se is a co-factor of glutathione peroxidase (GPx), the antioxidant enzyme which contributes to the antioxidant defense system. This capacity of Se empowers it to decrease Cadmium toxicity by decreasing Cadmium-induced oxidative stress and re-enforcing defense system of Cadmium-exposed host (Liu *et al*., 2013). Iron (Fe) competes with Cadmium for enterance into intestinal metal uptake transporters. This could explain the reduction in intestinal absorption of Cadmium following supplementation. The expression of these metal transporters is usually regulated by essential minerals such as Fe and Zn (Zhai *et al*., 2015). Manganese (Mn) is an essential trace mineral required in biological systems both as an activator and a constituent of several enzyme. Mn is a significant co-factor of mitochondrial superoxide dismutase, an antioxidant enzyme responsible for quenching and mopping out ROS (Eybl and Kotizová, 2010).

Phytochemical constituents of the leaf extract

The GC-MS analysis showed that the methanol leaf extract of *V. doniana* comprised of forty three phytochemicals which eluted at various retention times based on the boiling point of the eluted component (Table 3). The biological activity, according to literature, of the major phytochemical constituents of the methanol leaf extract of *V. doniana* are shown in Table 4.

Table 3: Phytochemicals detected in MEVd by GC-MS

Peak No.	Name of Compound	R.T.	Molecular Formula	M.W	P.A. (%)
1	Acnetol acetate	4.026	$C_5H_8O_3$	116	0.76
2	β-*cis*-Ocimene	4.791	$C_{10}H_{16}$	136	0.39
3	Propane, 1,1,3-triethoxy-	5.118	$C_9H_{20}O_3$	176	0.42
4	Linalool	5.571	$C_{10}H_{18}O$	154	7.89
5	Phenylethyl Alcohol	5.900	$C_8H_{10}O$	122	1.02
6	Benzeneacetic acid, ethyl ester	7.503	$C_{10}H_{12}O_2$	164	0.95
7	Annulene	8.457	C_8H_8	104	3.67
8	Phenylmethanimine	9.084	C_7H_7N	105	3.55
9	Eugenol acetate	9.396	$C_{12}H_{14}O_3$	206	0.41
10	Lauryl chloride	9.534	$C_{12}H_{25}Cl$	204	1.52
11	Trans-α-Berganotene	9.969	$C_{15}H_{24}$	204	1.19
12	β- Bisabolene	10.144	$C_{15}H_{24}$	204	7.22
13	1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, 1aR-(1a.α.,7.alpha.,7a.α.,7b.α.)]-	10.306	$C_{15}H_{24}$	204	1.02
14	β –Farnesene	10.390	$C_{15}H_{24}$	204	1.46
15	α-Caryophyllene	10.605	$C_{15}H_{24}$	204	5.28
16	α –Farnesene	11.045	$C_{15}H_{24}$	204	5.57
17	α –Cubebene	11.355	$C_{15}H_{24}$	204	1.64
18	Copaene	11.486	$C_{15}H_{24}$	204	0.80
19	D-Nerolidol	11.772	$C_{15}H_{26}O$	222	6.22
20	α - Tetradecene (Neodene)	11.984	$C_{14}H_{28}$	196	0.77
21	Z,Z,Z-4,6,9-Nonadecatriene	12.210	$C_{19}H_{34}$	262	1.08
22	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)]-	12.498	$C_{15}H_{24}O$	220	0.98
23	γ-Eudesmol	12.730	$C_{15}H_{26}O$	222	1.03
24	Eudesm-4(14)-en-11-ol	13.000	$C_{15}H_{26}O$	222	3.04
25	Hexadecyl mercaptan	14.412	$C_{16}H_{34}S$	258	0.65
26	Amyl ketone	15.265	$C_{11}H_{22}O$	170	3.19
27	Palmitic acid, ethyl ester	15.555	$C_{18}H_{36}O_2$	284	1.57
28	Methyl 14-methylpentadecanoate	16.728	$C_{17}H_{34}O_2$	270	1.73
29	Docosanoic acid, ethyl ester	17.371	$C_{24}H_{48}O_2$	368	0.54
30	Stearic acid, ethyl ester	18.095	$C_{20}H_{40}O_2$	312	1.87
31	11-Octadecenoic acid, methyl ester	19.912	$C_{19}H_{36}O_2$	296	2.87
32	Erucic acid	20.971	$C_{22}H_{42}O_2$	338	7.11
33	4-O-decyl-d-glucitol	21.325	$C_{16}H_{34}O_6$	322	5.00
34	Oleic acid amide	21.478	$C_{18}H_{35}NO$	281	1.84
35	Z-11-Hexadecenoic acid	22.170	$C_{16}H_{30}O_2$	254	1.18
36	Olealdehyde	22.649	$C_{18}H_{34}O$	266	0.68
37	Eicosane, 1-cyclohexyl-	22.979	$C_{26}H_{52}$	364	2.19
38	Phthalic acid, dioctyl ester	24.869	$C_{24}H_{38}O_4$	390	0.90
39	Stearic acid, ethyl ester	25.220	$C_{20}H_{40}O_2$	312	1.03
40	Behenic alcohol	25.534	$C_{22}H_{46}O$	326	0.99
41	Ethylamine, 2-((p-bromo . α.-methyl-. α.-phenylbenzyl)oxy)-N,N-dimethyl-	25.927	$C_{16}H_{22}BrNO$	347	1.50
42	Ethylamine, 2-((p-bromo-. α.-methyl-. α.-phenylbenzyl)oxy)-N,N-dimethyl-	26.830	$C_{18}H_{22}Br$	347	3.40
43	Squalene	27.712	$C_{30}H_{30}$	410	3.91

Table 4: Major phyto-components identified and their biological activities

PA (%)	Name of compound	MF	Biological activity (according to literature)
7.89	Linalool	$C_{10}H_{18}O$	Antioxidant (Seol *et al*., 2016), Anticancer (Sun *et al*., 2015), Anti-inflammatory (Maria *et al*., 2016); Anxiolytic (Cline *et al*., 2008); Antiseptic (Pengelly, 2004); sedative and local anesthetic effects (Cavanagh and Wilkinson, 2002); antinociceptic (Peana *et al*., 2004) activity.
0.39	β–*cis*–Ocimene		Antioxidant and anticancer (Mahmoud, 2013).
7.22	β- Bisabolene	$C_{15}H_{24}$	Anti-oxidant activity (Gholivand *et al., 2010)*
6.22	D-Nerolidol	$C_{15}H_{26}O$	Antioxidant (Vinholes *et al*., 2014); Antiulcer (Klopell *et al.*, 2007); Antinociceptive (Koudou *et al.* 2005); Antimalarial (Lopes *et al*., 1999) activity.
5.57	α –Farnesene	$C_{15}H_{24}$	Antioxidant (Sarikurkcu *et al*., 2013); Anticarcinogenic (Afoulous *et al*., 2013); Antibacterial (Chehregani *et al*., 2010); Antifungal (Al-Maskri *et al*., 2011).
5.28	α-Caryophyllene	$C_{15}H_{24}$	Anti-inflammatory (Fernandes *et al*., 2007); Antitumour (Legault *et al., 2003)*
1.02	Calarene	$C_{15}H_{24}$	Antioxidant and antimicrobial activity (Wang *et al*., 2010).
0.80	α – Copaene	$C_{15}H_{24}$	Antioxidant, anti – inflammatory and anticarcinogenic (Vinholes *et al*., 2013).
3.91	Squalene	$C_{30}H_{30}$	Anti-oxidant and Anticancer (GÜne§ (2013)
7.11	Erucic acid	$C_{22}H_{42}O_2$	Antioxidant, Analgesic and Anti-inflammatory (Ibrahim (2012).
1.02	Phenethyl alcohol	$C_8H_{10}O$	Antioxidant and antibacterial activities (Wang *et al*., 2015).

The GC-MS analysis of this study shows that linalool, a monoterpene, is the major component of the methanol leaf extract of *V. doniana*. A good number of recent studies have focused on the pharmacological activities of linalool. Linalool, a monoterpene alcohol, is a component of many natural aromatic plants. Linalool has been found to have biological activities, including antioxidant (Seol *et al*., 2016), anticancer (Sun *et al*., 2015), anti-inflammatory (Wu *et al*., 2014); anxiolytic (Cline *et al*., 2008); Antiseptic (Pengelly, 2004); sedative and local anesthetic effects (Cavanagh and Wilkinson, 2002); antinociceptic (Peana *et al*., 2004) activity. Sesquiterpenes are 15 – carbon compounds formed from 3

isoprenoid units and are secondary metabolites produced majorly in higher plants. It is one of the most familiar terpenes, are a class of natural products with a wide range of desired properties (Wang *et al*., 2011) Sesquiterpenes have been reported to exhibit antioxidant (Abolaji *et al*., 2013), anticarcinogenic (Afoulous *et al*., 2013), antimicrobial (Wang *et al*., 2013), antifungal (Kundu *et al*., 2013), anti-inflammatory (Wang *et al*., 2013). In recent time, efforts are channeled into research and development of novel drugs from natural products. According to this study, most of the major bioactive components identified in the leaves of *V. doniana* are sesquiterpenes which include β-Bisabolene, D- Nerolidol, α- Farnesene, α-Caryophyllene, and calarene. β-Bisabolene have been reported to have biological activities, including anti-oxidant activity (Gholivand *et al.,* 2010). D-Nerolidol, one of the major sesquiterpenes identified in the extract has been investigated to exhibit myriad of biological activities. There are reports to back-up the antioxidant (Vinholes *et al*., 2014); antiulcer (Klopell *et al.,* 2007); cytotoxic (Sperotto *et al.,* 2013); antinociceptive (Koudou *et al*. 2005), and antimalarial (Lopes *et al*., 1999) activity of Nerolidol. The sesquiterpene α-Caryophyllene has been suggested to show a number of pharmacological and biological activities, including anti-inflammatory (Fernandes *et al*., 2007) and antitumour (Legault *et al.,* 2003). α-Farnesene is found to be associated with many biological activities including antioxidant (Sarikurkcu *et al*., 2013), anticarcinogenic (Afoulous *et al*., 2013), antibacterial (Chehregani *et al*., 2010), and antifungal (Al-Maskri *et al*., 2011) properties. This study has shown that terpenes are the major compounds present in the fresh leaves of *V. doniana*. Owing to the fact that they are the main components, a number of the pharmacological activities and ethomedicinal uses of *V. doniana* can be attributed to the major terpenes present in the plant. Even so, the pharmacological and biological properties of *V. doniana* cannot be ascribed to only a single component, because the components present in the plant may interact in synergy to promote and/or re-enforce the antioxidant effect determined.

Conclusion

From this study, it can be concluded that the methanol extract of *Vitex doniana* possessed a high 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity which could be as a result of the trace elements and phytochemicals identified in the leaf extract. These phytochemicals have been reported to have antioxidant, anti-inflammatory and anticancer activities.

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Conflict of Interest

The authors declare that they have no conflict of interests.

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