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Research Article

IDENTIFICATION OF POTENTIAL ANTIOXIDANT AND HEPATOPROTECTIVE CONSTITUENTS OF *VITEX DONIANA* BY UHPLC/+ESI-QQTOF-MS/MS ANALYSIS

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ABSTRACT

Objective: This study evaluated the antioxidant and hepatoprotective activities of the dichloromethane (DCM-F) and *n*-butanol (BUT) fractions of *Vitex doniana* and identified the bioactive constituents using liquid chromatography-mass spectrometry (LC-MS) analysis.

Methods: The methanol extract of the leaves was subjected to liquid-liquid fractionation and the BUT soluble separated by vacuum LC (VLC) using binary mixtures of DCM in methanol (1 L) sequentially in the ratios of 9:1 BF1–1:9 BF5. The antioxidant activity was investigated *in vitro* using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging, phosphomolybdenum total antioxidant capacity, and ferric ion reducing antioxidant power assay methods. The *in vivo* antioxidant potential was performed by measuring the concentrations of both enzymatic and non-enzymatic parameters in carbon tetrachloride (CCl_4)-induced hepatic injury.

Results: The extract, DCM-F, and the VLC sub-fractions showed good antioxidant activity which is comparable with ascorbic acid (53.6%). DCM-F showed the highest antioxidant activity (71.6%), while the least activity was shown by BF5 (15.0%). Peak dereplication of LC-MS chromatograms identified several putative compounds in the BUT soluble. DCM-F caused a significant increase ($p<0.05$) in superoxide dismutase, catalase, glutathione peroxidase, and Vitamins C and E in CCl_4-induced rats compared to standard. The levels of serum alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase, and malondialdehyde that were elevated in CCl_4-induced rats were also significantly reduced ($p<0.05$) by the DCM-F compared to standard.

Conclusion: The ability of *V. doniana* leaves to mop up free radicals and reverse the CCl_4-induced hepatic injury in rats suggests antioxidant potentials. The bioactive compounds identified in BUT fraction could support its ethnomedicinal uses in the treatment of illnesses associated with radical scavenging activity.

Keywords: *Vitex doniana*, Dereplication, Antioxidant, Phytochemical constituents, Liquid chromatography-mass spectrometry.

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INTRODUCTION

Man has depended on plants and plant products for food and medicine [1]. The use of herbs to cure ailments in the olden days, even with little knowledge of their active principles, formed the backbone of current research in phytomedicine. All parts of medicinal plants are useful in the cure of disease and this forms the basis for the discovery of new drugs [2]. Their availability has caused an increased interest in their supplements and substance composition such as nutrients, mineral components, and bioactive compounds that are essential for drug discovery and development [3]. Plant products are known to be present in more than 50% of western drugs and have provided defense against pathogens or template for synthetic drugs [2].

Vitex doniana (Family Verbenaceae), commonly called black plum, is a Savanna plant species in wooded grasslands and can also be found along forest edges [4]. It is locally known in Nigeria as uchakiri (Igbo), tunci (Fulani), dinya (Hausa), and oori-nla (Yoruba) [5]. *V. doniana* possesses great potential in the management and prevention of a wide range of human diseases [6]. It is consumed in Nigeria as sauce and vegetables in meals [7]. In ethnomedicine, it is employed in the management of diarrhea, rheumatic and stomach pains, inflammatory disorders, and dysentery [8]. It also has antioxidant properties and anti-hyperlipidemic activities [4,6], all of which could be attributed to the phenolic and alkaloidal constituents of the extract.

Therefore, it was imperative to identify potential bioactive fingerprints from *V. doniana* for eventual isolation, characterization, and for the evaluation of its antioxidant potential using *in vitro* and *in vivo* models.

METHODS

Plant material

The leaves were collected from farmland in Ibagwa, Enugu State, Nigeria and dried under shade. Identification was done by Prof. Charles N. Mba of the Department of Soil Science, School of Agriculture and Agricultural Technology of Federal University of Technology, Owerri. A voucher specimen (Number UUH 998) of the sample was subsequently deposited at the herbarium of the department.

Preparation of extract

The dried leaves were ground into a fine powder. A 250 g of powdered material was cold macerated in 1.5 L 95%v/v methanol for 48 h and filtered using Whatman filter paper #1 (Whatman Int. Ltd., Maidstone). The filtrate was concentrated to dryness in vacuum at room temperature.

Liquid-liquid fractionation

The dried extract was redissolved in aqueous methanol (10%v/v) and successively subjected to liquid-liquid fractionation, in equal volumes (500 ml) of *n*-hexane, dichloromethane (DCM), ethyl acetate, and *n*-butanol (BUT) in increasing order of polarity. The extracts were separated with separating funnel and the filtrates of respective fractionating solvent were concentrated using a rotary evaporator under reduced pressure.

Vacuum liquid chromatography (VLC) separation of extract

VLC technique was adopted to further separate the BUT soluble. A 2 g portion was dissolved in 5 ml methanol and introduced to the silica gel ($G_{100-200}$ mesh size) slurry packed 40 × 3 cm column (sample to silica gel ratio 1:40) at room temperature. The applied fraction was eluted with a gradient of mobile phase consisting of DCM in methanol (1 L), each starting with 90 % DCM and gradually increasing the amount of methanol to 30, 50, 70, and 90% to obtain VLC fractions BF1-BF5, respectively.

Qualitative phytochemical analysis

Freshly prepared extract and the BUT fractions were subjected to qualitative phytochemical analysis to ascertain their constituents using standard procedures [9,10].

Determination of antioxidant property

1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The scavenging activity of extract, fractions, and VLC sub-fractions on DPPH was measured colorimetrically at 517 nm using ascorbic acid as standard [11]. The sample was dissolved in methanol to make a 1000 ppm stock. A 5 ml of DPPH solution was put into a test tube containing 1 ml of each concentration and the solution was evenly mixed. The mixture was incubated for 3 min and observed for color change (deep violet to yellow). The absorbance was measured at 517 nm for the extract and fractions. The concentration of the extract and ascorbic acid used was 10, 20, 30, 40, and 50 ppm. The free radical scavenging activity was obtained from:

$$\% \text{ inhibition} = [(A_0 - A_1)/A_0] \times 100; \text{ where } A_0 = \text{absorbance of blank and } A_1 = \text{absorbance of test sample}$$

Phosphomolybdenum total antioxidant capacity (TAC) assay

Phosphomolybdenum reagent was prepared by reacting 100 ml each of 0.6 M H_2SO_4, 28 mM disodium phosphate and 4 mM ammonium molybdate. A 3 ml of the reagent was added to 1 ml each of the different concentrations of the extract and fractions in methanol. The mixture was incubated at 95°C for 90 min and allowed to cool at room temperature for 15 min. The absorbance was determined at 695 nm. A blank was made by adding methanol to the reagent solution (1:3) and subjected to similar treatments as the test samples. Five different concentrations of the standard ascorbic acid were prepared and used for calibration. The concentrations of the extract, fractions, and ascorbic acid used were 100–6.25 µg/ml. The TAC of the samples was measured as the number of ascorbic acid per gram of the sample [11,12].

Ferric ion reducing antioxidant power (FRAP) assay

Different concentrations (100–6.25 µg/ml) of the extract and fractions were added to 2.5 ml of 20 mM phosphate buffer and 2.5 ml %w/v potassium ferricyanide. The mixture was incubated at 50% for 30 min. Then, 2.5 ml of 10% w/v trichloroacetic acid and 0.5 ml 0.1% w/v ferric chloride were added to the mixture. This was kept aside for 10 min and absorbance determined at 700 nm using ascorbic acid as standard [11].

UHPLC-MS analysis

The BUT soluble was dissolved in 80%v/v aqueous methanol at a 5 mg/ml concentration and analyzed with UHPLC/ESI-QTOF MS/MS [13]. Chromatographic separations were performed on a Dionex Ultimate 3000 RS Liquid Chromatography System with Dionex Acclaim RSLC 120, C18 column (2.1×100 mm, 2.2 µm) using binary gradient (A: Water with 0.1% v/v formic acid; B: Acetonitrile with 0.1% v/v formic acid) at 0.8 ml/min: 0 to 9.5 min: Linear from 5% B to 100% B; 9.5–12.5 min: Isocratic 100% B; 12.5–12.6 min: Linear from 100% B to 5% B; 12.6–15 min: Isocratic 5% B. The injection volume was 5 µl. Eluted compounds were detected over a wavelength range of 200–400 nm and a Bruker Daltonics micrOTOF-QII quadrupole/time-of-flight mass spectrometer. UPLC-MS chromatograms and mass spectra were calibrated with Bruker Daltonics Profile, Analysis 2.0 (Bruker Daltonik GmbH, Germany, 2010. Peak alignment (Join aligner) was used to match relevant peaks across multiple samples under m/z and retention time (tR) tolerances to generate a list of peaks and their partial identification was performed by querying automatically the exact masses of molecular ions $[M+H]^+$ and common adducts ($[M+Na]^+$, $[M+NH_4]^+$, $[M+K]^+$) (error = 0.005 Dalton). The molecular formulae of possible compound(s) from each de-convoluted peaks with their corresponding tR are shown in Table 1.

Identification of constituents

Interpretation of data was based upon the database of the National Institute Standard and Technology and the dictionary of natural products (https://www.dnp.chemnetbase.com/). The spectra of unknown compounds and that of the known compounds in the repositories were compared. The tR, molecular weight, and molecular formula of the sample material were obtained.

Animal studies

Acute toxicity test

Samples of extract and fractions of the plants were made in 3% aqueous tween 80. Sixty mice of either sex weighing 24.6±7.6 g were used in the study. Three groups of mice containing five mice each were given 10, 100, and 1000 mg/kg, respectively, of the methanol extract and DCM fractions intraperitoneally (i.p). They were observed closely for 24 h for acute toxicity signs and behavioral responses. Further elevated doses of 1900, 2600, and 5000 mg/kg were given i.p to five other mice, respectively, based on the previous observation. They were observed individually for 1 h after the last dosing and once daily for 14 days, for acute toxicity signs and behavioral changes. The maximum test dose of 5000 mg/kg was used as stipulated in organization for economic cooperation development guidelines. Median lethal dose was determined as the geometric mean of the maximum dose of the extract/fraction that resulted in 0 lethality and the maximum dose that resulted in 100% death [14].

Animals

Six groups (A-F) of male albino rats weighing 163.6±2.8 g were housed in cages of five rats each. They were kept to acclimatize for 10 days and were allowed free access to food and water. Liver damage in animals was

Table 1: Constituents of the *n*-butanol sub-fractions of *V. doniana* leaves

Fraction	Mass	t_R (min)	Intensity	Adduct	Fig. 4	Formula	Class
BF2	441.3	6.4	5.00×10^3	$[M+K]^+$	**9**	$C_{27}H_{44}O_7$	Lipid
BF2	361.3	8.9	0.75×10^4	$[M+H]^+$	**10**	$C_{22}H_{34}O_4$	Diterpene
BF2	373.3	8.9	0.60×10^4	$[M+H]^+$	**6**	$C_{19}H_{18}O_8$	Flavonoid
BF2	373.3	8.9	0.60×10^4	$[M+H]^+$	**8**	$C_{19}H_{18}O_8$	Flavonoid
BF2	304.3	9.4	2.90×10^4	$[M+NH_4]^+$	**1**	$C_{19}H_{30}O_4$	Lignan
BF2	333.3	10.3	6.50×10^4	$[M+H]^+$	**7**	$C_{18}H_{38}NO_4$	Alkaloid
BF2	301.1	10.5	1.05×10^5	$[M+H]^+$	**2**	$C_{15}H_{10}O_8$	Flavonoid
BF2	376.3	11.2	0.70×10^4	$[M+2H]^+$	**11**	$C_{22}H_{34}O_5$	Diterpene
BF2	338.3	13.8	2.80×10^4	$[M+NH_4]^+$	**3**	$C_{20}H_{20}O_6$	Lignan
BF3	457.4	8.9	3.25×10^4	$[M+H]^+$	**5**	$C_{30}H_{48}O_3$	Triterpene
BF3	317.1	10.5	0.10×10^5	$[M+H]^+$	**4**	$C_{15}H_{10}O_8$	Flavonoid
BF3	374.3	11.5	1.50×10^4	$[M+2H]^+$	**12**	$C_{16}H_{24}O_{10}$	Monoterpene iridoid

V. doniana: Vitex doniana

induced with carbon tetrachloride (CCl_4) in paraffin oil (1:1) on the 1st and 8th days. Permission to use animals for this study was obtained from the University of Nigeria Ethics Committee guidelines on the handling of laboratory animals in accordance with "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985) and/or the declaration of Helsinki promulgated in 1964 as amended in 1996

Treatments

Rats in Group A (normal control) were given distilled water (0.5 ml) orally once daily. Group B (untreated) received CCl_4 solution (0.5 ml). Group C (standard control) animals were given CCl_4 (0.5 ml) and 200 mg/kg bodyweight of butylated hydroxytoluene (BHT) simultaneously. Groups D, E, and F received simultaneously 0.5 ml of CCl_4 each and 200, 400, and 600 mg/kg of fractions, respectively. Treatments with doses of extract, fractions, and the standard lasted for 14 days [15].

Serum and tissue preparation

The rats were sacrificed under CCl_4 anesthesia, 24 h after the last daily doses, and were made to bleed through the ocular puncture into centrifuge tubes. The sera were obtained by allowing the blood sample to clot for 15 min and centrifuging at 3500 rpm at 40°C × 10 min. Biochemical assay was carried out within 12 h of preparation of sera. Liver removed from the rat was cut thinly and then homogenized in ice-cold 1.25 M sucrose solution (1:5 w/v). Centrifugation of the homogenate was done at 3500 rpm × 10 min at 40°C to obtain the supernatant which was preserved at −20°C for various biochemical assays [15].

Assay of biochemical parameters

The alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were determined as outlined in the Randox kit while total and conjugated bilirubin were assayed following previously described protocols [16]. Other parameters such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) [17,18], Vitamin C [19], Vitamin E [20], and lipid peroxidation (malondialdehyde [MDA]) were determined by standard method [17, 21].

Statistical analysis

Statistical analyses were carried out with SPSS version 16.0 windows. All the values were expressed as mean±standard deviation and analyzed for analysis of variance and post-hoc Duncan multiple range tests. The difference between groups was considered significant at $p<0.05$ levels [22].

RESULTS

Result of phytochemical test

The methanol extract, DCM, and BUT fractions and the VLC sub-fractions all tested positive for the presence alkaloids, saponins, tannins, terpenoids, flavonoids, lignans, and iridoid glycoside.

Results of antioxidant test

The methanol extract, DCM, and BUT fractions and the VLC sub-fractions tested positive to qualitative antioxidant assay test (i.e., color changed from violet to yellow). The scavenging activity of extract and fractions on DPPH free radicals was expressed as percentage inhibition, and they were compared with standard ascorbic acid (Fig. 1). The DCM-fraction (DCM-F) produced significant inhibition (71.6%) with an IC_{50} of 26.3 mg/g, higher than that of ascorbic acid (53.6%) with an IC_{50} of 20.6 mg/kg. In the phosphomolybdenum TAC and FRAP assays, the reducing potency of the extract and fractions was significant compared with that of ascorbic acid. The DCM-F showed higher reducing potency (1.500±0.004) when compared with the standard (0.30±0.00) (Fig. 2) in the TAC model. Similarly, in the FRAP model, the reducing effect of DCM-F is 1.51±0.001 compared to 0.27±0.002 recorded with the standard. This indicates that DCM-F has greater reduction potency than the standard (Fig. 3).

Result of UHPLC-MS analysis

The LC-MS analysis, including their molecular formula, molecular weight, tR, peak area, and composition of the bioactive components

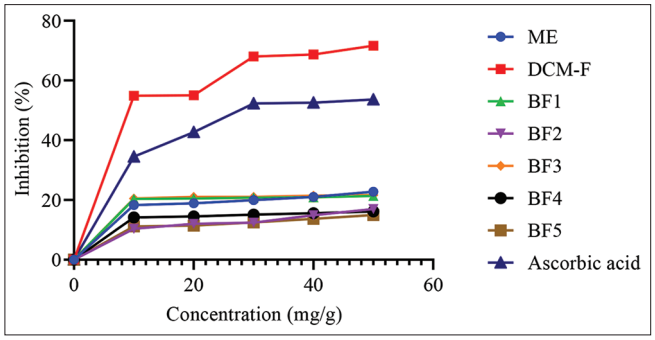

Fig. 1: 1, 1-diphenyl-2-picrylhydrazyl scavenging activity of the extract and fractions of *Vitex doniana* leaves. (ME= methanol extract; DCM-F= dichloromethane fraction; BF1-5= *n*-butanol fractions 1–5)

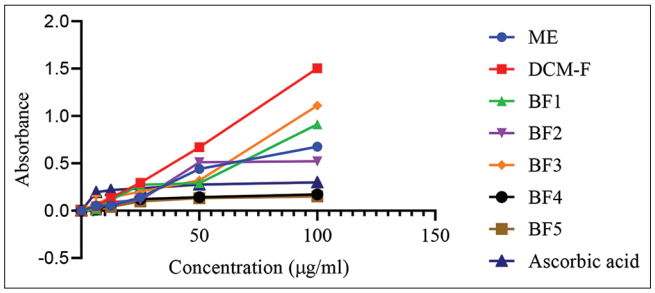

Fig. 2: Phosphomolybdenum total antioxidant capacity assay of *Vitex doniana*. (ME= methanol extract; DCM-F= dichloromethane fraction; BF1-5= *n*-butanol fractions 1–5)

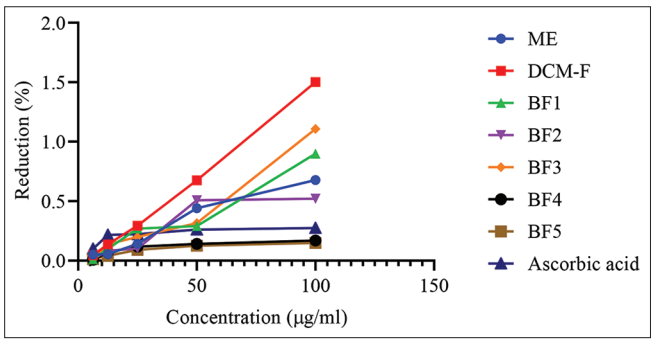

Fig. 3: Reducing effects of *Vitex doniana* leaves. (ME= methanol extract; DCM-F= dichloromethane fraction; BF1-5= *n*-butanol fractions 1–5)

of *V. doniana* leaves, is presented in Table 1 and Fig. 4. The base peak chromatograms of the active sub-factions and mass spectra of the identified constituents are shown in Figs. 5 and 6, respectively.

Many significant physiological active components were identified from the fractions by LC-MS analysis (Fig. 4) such as vitedoin B **(1)**, quercetin **(2)**, vitedoin A **(3)**, myricetin **(4)**, betulinic acid **(5)**, casticin/vitexicarpin **(6)**, vitexlactam **(7)**, 5,3-dihydroxy-3,6,7,4-tetramethoxylflavone **(8)**, 20-hydroxyecdysone **(9)**, rotundifuran **(10)**, vitexilactone **(11)**, and mussaenosidic acid **(12)**.

Result of acute toxicity studies

Acute toxicity studies showed that both the extract and fractions did not produce lethality (mortality) in the treated mice during the period of the study. There was no significant weight loss and changes in feeding patterns in the mice during the study period. The mice did not experience skin dryness, hair loss, and general weakness during the study period.

Results of *in vivo* study

Treatment with CCl_4 increased significantly ($p<0.05$), the levels of ALP, ALT, and AST in the serum (Table 2). Co-administration of CCl_4 and doses of *V. doniana* resulted in a corresponding decrease of the serum enzymes. There was no significant difference in serum enzyme levels in CCl_4 and 600 mg/kg of the fraction, as shown in 80.4%, 84.3%, and 82.8% reduction, respectively.

The significant reduction ($p<0.05$) in total and conjugated bilirubin levels following CCl_4-induced hepatotoxicity was reversed following treatments (Table 2). At 600 mg/kg, total and conjugated bilirubin was increased by 45% and 94.5%, respectively. Similarly, there was a significant ($p<0.05$) reversal of the reduction in the SOD, GPx, and CAT observed among the CCl_4-induced animals.

The effect of *V. doniana* on non-enzymatic antioxidant parameters of CCl_4-induced hepatotoxic rats is shown in Fig. 7. A 600 mg/kg of the fraction significantly increased Vitamins C and E serum levels by 33.19% and 58.45%, respectively. This compared well ($p<0.05$) with that of the distilled water control group.

The effect of *V. doniana* on peroxidized MDA in rats is shown in Fig. 8. BHT reduced MDA significantly ($p<0.05$) by 51.2% lower than 57.35% reduction caused by a 600 mg/kg of the fraction.

DISCUSSION

Antioxidants prevent, protect, and repair free-radical mediated damage [23]. The DCM fraction of *V. doniana* leaves showed antioxidant activity in this study higher than its BUT fraction when

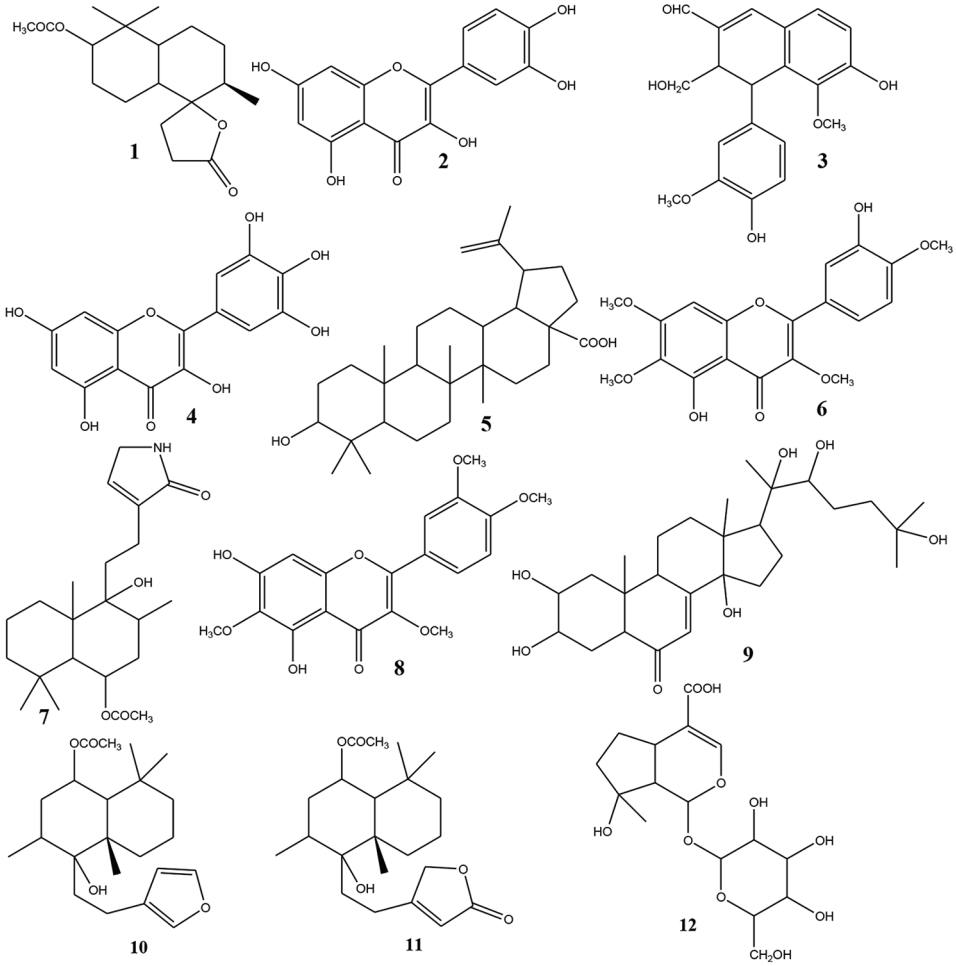

Fig. 4: Structures of some dereplicated compounds in BF2 and BF3

Table 2: Effect of *V. doniana* on serum biochemical markers and antioxidant enzymes of CCl_4-induced hepatotoxic rats

Treatment group	ALP (iu/L)	ALT (iu/L)	AST (iu/L)	BILI (T) (mmol/L)	BILI (D) (mmol/L)	SOD (iu/mg)	CAT (iu/mg)	GPx (iu/mg)
A	75.96±3.45^a	10.78±0.19^a	11.68±0.43^a	0.38±0.02^a	34.42±1.14^a	11.43±0.04^a	1.17±0.08^a	11.14±0.75^a
B	92.61±9.50^b	12.90±0.25^b	14.36±0.34^b	0.18±0.01^b	32.54±0.35^b	10.92±0.04^b	1.12±0.08^a	9.80±0.15^b
C	76.02±4.36^a	10.74±0.10^a	11.76±0.27^a	0.39±0.02^a	35.55±0.24^c	11.43±0.01^a	1.27±0.01^b	11.22±0.01^a
D	56.78±2.60^b	10.22±0.08^b	11.32±0.33^a	0.33±0.07^c	34.61±0.23^a	11.35±0.01^b	1.18±0.04^a	10.77±0.44^a
E	64.34±2.94^c	10.37±0.05^b	11.49±0.36^a	0.37±0.04^a	35.11±0.15^a	11.40±0.01^a	1.28±0.01^c	10.71±0.47^a
F	74.49±2.36^a	10.88±0.04^a	11.90±0.51^a	0.40±0.01^a	34.42±1.17^d	11.45±0.01^a	1.40±0.04^d	11.22±0.01^a

Values are means±SD of five replicates. $^{a\text{-}d}$Values for the treatment groups (for each enzyme) with different superscripts with that of the distilled water control group are significant at $p<0.05$; BILI (T)= total bilirubin; BILI (D) =conjugated bilirubin. CCl_4: carbon tetrachloride, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, ALP: Alkaline phosphatase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, *V. doniana: Vitex doniana*, SD: Standard deviation

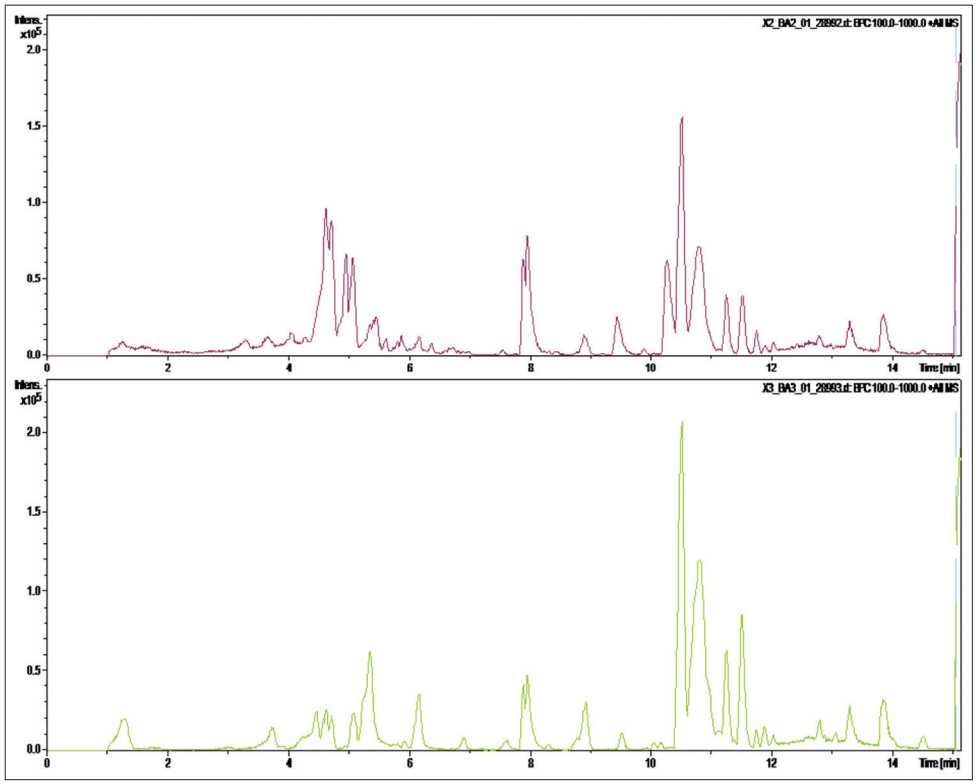

Fig. 5: BPC of BF2 (upper) and BF3 (lower panel), mass 100-1000 Daltons

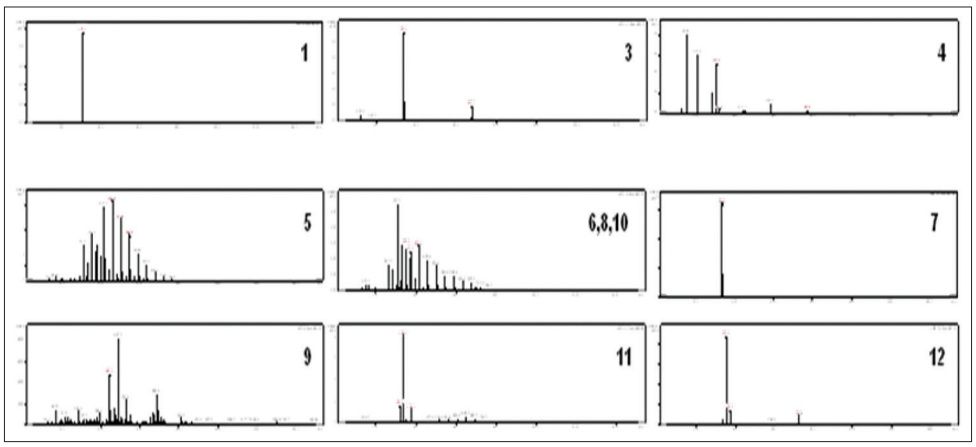

Fig. 6: Compound spectra of some identified constituents of BF2 and BF3

compared with ascorbic acid as well as both the aqueous and ethanol extracts of *V. doniana* leaves as reported by Agbafor and Nwachukwu [6] suggesting the possibility of synergism in antioxidant property of constituents of extract. The scavenging effect of the extracts and fractions of *V. doniana* leaves on DPPH, together with their reducing effect on the ferric ion and phosphate-Mo(IV), may be related to lipid peroxidation, which works mainly in terminating free radical chain reaction. Its DCM fraction displayed the most outstanding scavenging activity more effective than the ascorbic acid, the reference standard used in this assay.

The dereplication analysis indicated that BUT fractions of *V. doniana* leaves contained numerous phytoconstituents such as alkaloids **(7)**, flavonoids **(4)**, lignans **(2)**, terpenoid **(4)**, and lipid **(1)** (Table 1). Importantly, 12 compounds were identified in the most active fractions; 10 were identified in the BF2 and 2 in BF3. Compounds having antioxidant, analgesic, anticancer, anti-inflammatory, antibacterial, antiretroviral, and antimalarial activities were identified. Antioxidants identified included **1**, **2**, **3**, and **4**, while anti-inflammatory agents identified are **5** and **6**. Sheneni *et al.* [4] had earlier reported the use of *V. doniana* leaves as a diuretic, in the control of high blood pressure and in the treatment of swelling, edema, diabetes, and ulcer. The decoction has also been reported useful during labor to quicken delivery time. These might be due to the presence of antioxidant and anti-inflammatory constituents identified. Furthermore, Agbafor and Nwachukwu [6] reported the antioxidant properties of the leaves, root, and stem bark of *V. doniana*. These claims may be due to the presence of an antibacterial compound, **7** identified in BF2 sub-fraction and/or strong antioxidant compounds such as 2, 3, and **4**. Other identified phenolic compounds, **2**, **4**, **6**, and **8** possess significant anticancer, anti-inflammatory, and antioxidant properties. Bioactive alkaloid such as **7** was also identified in both BF2 and BF3, and several alkaloids are known for their use in the cure and treatment of illness such as diarrhea and dysentery [3]. The BUT fraction quantification was found to be rich in phenolic compounds (flavonoids) and therefore exhibited good scavenging activity against DPPH.

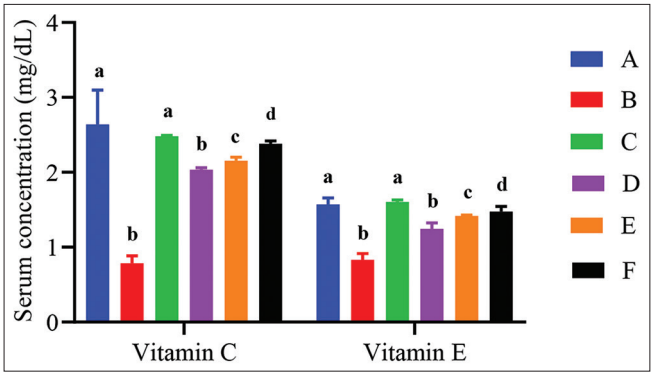

Fig. 7: Effect of *Vitex doniana* on some non-enzymatic antioxidants of rat. (Data are mean±standard deviation n=5. [a-d]Superscripts with that of the distilled water control group are significant at p<0.05)

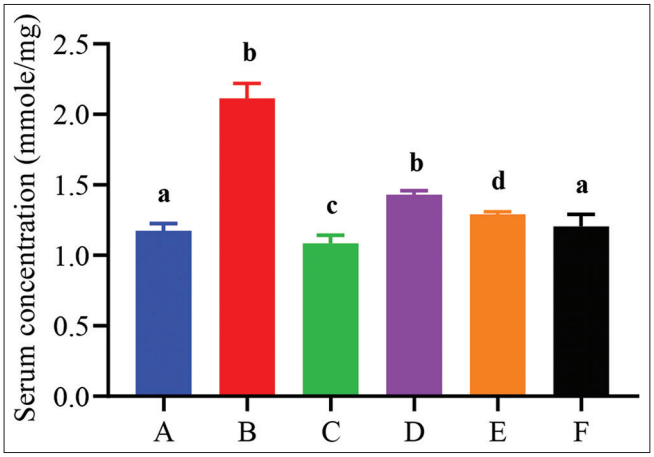

Fig. 8: Effect of *V. doniana* on lipid peroxidized product (malondialdehyde) of rat liver. (Data are mean±standard deviation n=5. [a-d]Superscripts with that of the distilled water control group are significant at p<0.05)

The acute toxicity test performed on both the crude methanol extracts and the DCM fractions of *V. doniana* leaves showed that no death was recorded in the mice following oral administration of both methanol extracts and the DCM fractions. This signifies that both the methanol extracts and DCM fractions of both plants are relatively safe. This may be in agreement to the work of Sha'a *et al.* [14], which evaluated the toxicity of the extracts of *V. doniana*.

The animal study was designed on the rat model to assess the curative effects of *V. doniana* against the CCl_4-induced toxicity in rats. CCl_4 significantly elevated lipid oxidation and affects different blood biochemical parameters such as antioxidant markers and liver enzymes. Treatment with *V. doniana* prevented these effects, indicating that they can reverse the effects caused by CCl_4. The ability of 600 mg/kg DCM fraction to reverse the damage caused by CCl_4 administration in a way similar to BHT indicates the antioxidant potential of the fraction. *V. doniana* can prevent the buildup of free radicals in the animals. The ability of the DCM fraction to significantly lower the levels of ALP, ALT, and AST after CCl_4 intoxication suggests the hepatocurative ability of the fraction. Yakubu *et al.* [7] in their previous work had reported the ameliorating effect of the extracts of *V. doniana* on the biochemical markers following an aluminum-induced injury on the liver.

When there is an obstruction in the excretion of bile due to liver injury, level of serum bilirubin increases [24], the 600 mg/kg fraction was able to normalize this trend following CCl_4 toxicity, indicating it might regenerate hepatocytes and healing of hepatic parenchyma. This is in agreement with the earlier work reported [7]. Liver toxicity also leads to failure or inefficiency of internal enzymatic mechanisms of SOD, CAT, and GPx. The DCM fraction can complement their *in vivo* free radical scavenging activity when taken. This is in agreement with the report of Chinwe [3] suggesting that leaves, root, and stem bark extracts of *V. doniana* showed antioxidant properties comparable to the standard antioxidant drugs. Similarly, the ability of the DCM fraction to reverse the depletion of Vitamins C and E caused by the toxicity of CCl_4 indicates that it can play an important role in protecting the natural defense system of the animals. A 600 mg/kg of DCM fraction was able to preserve the non-enzymatic antioxidant system. MDA in the liver of animals serves as an indicator of lipid peroxidation [25]. The inhibition of elevation of the level of MDA upon CCl_4 administration also suggested the protective effect of the fractions against hepatic lipid oxidation induced by CCl_4.

CONCLUSION

The study revealed the presence of potential bioactive compounds from *V. doniana* leaves. The DCM-F can prevent damage caused by free radicals, improve serum biochemical markers, repair hepatic enzymes and fibrosis, and regulate hepatocyte function. In summary, *V. doniana* could be a natural source of antioxidant molecules. At present, isolation of the bioactive compounds for the development of new agents of biomedical importance is ongoing.

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AUTHORS' CONTRIBUTIONS

The authors contributed equally in both the research and framework

CONFLICTS OF INTEREST

The authors confirm that there were no conflicts of interest.

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