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Bioactive constituents, hepatoprotective and antioxidant activity of the Sub-fractions of *Fadogia cienkowskii* leaves Schweinf (Rubiaceae)

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Abstract

Background: *Fadogia cienkowskii* is claimed to ethnomedicinally cure general body debility, inflammation, liver diseases, oxidative stress-related diseases, diarrhoea, fever, and other ailments. **Aim and Objectives:** The aim of this study is to determine the bioactive compounds using UV-Vis and FTIR spectroscopic techniques and also to investigate the hepatoprotective and antioxidant activities of the VLC sub-fractions of *Fadogia cienkowskii* leaves. **Methodology:** The ethyl acetate fraction was fractionated using vacuum liquid chromatography (VLC) (n-Hexane/ ethyl acetate and ethyl acetate/ methanol) and bulked using thin layer chromatography to obtain the VLC sub-fractions. The hepatoprotection was determined using paracetamol-induced liver damage model in rat, and antioxidant screening evaluated using both *in vivo* and *in vitro* models. The UV-VIS and FTIR analysis were carried out using standard methods. **Results:** The administration of the VLC sub-fractions of the leaves of *F. cienkowskii* at the different doses, led to significant reduction ($P \leq 0.05$) in the liver and antioxidant enzymes and also the DPPH free radicals. The FTIR analysis identified the presence of the following functional groups, chloro, ether, ethene, amine, ester, carboxylic, nitriles, thiocyanate, methylene and alcohol. **Conclusion:** The effect of the eleven (11) VLC sub-fractions led to a significant reduction ($P < 0.05$) in the liver and antioxidant enzymes and also the DPPH free radicals. The presence of various functional groups and phytocompounds confirm the claims by the herbalists that *F. cienkowskii* leaf is used in the management of liver diseases and oxidative stress related diseases.

Keywords: Hepatoprotective, Antioxidant, Phytochemical, Histopathology, *Fadogia cienkowskii*, Ultra Violet-Visible Spectroscopy, Fourier Transform Infrared Spectroscopic (FTIR).

INTRODUCTION

Medicinal plant is used to maintain health and administered for a specific disease condition, whether in modern medicine or traditional medicine [1, 2]. Medicinal plants, also called Herbs, have been recommended and used in traditional medicine practices since prehistoric times. In modern medicine, a quarter of the drugs prescribed to patients are derived from herbs, and they are rigorously tested [2]. The use of herbs or natural health products with health benefits is increasing in developed countries [3, 4]. The World Health Organization formulated a policy on traditional medicine in 1991, and since then has published guidelines for them, with a series of monographs on widely used herbal medicines [5, 6]. Herbs may serve as a feasible therapy for the prevailing liver problems and oxidative stress induce diseases because of their cost-effectiveness, safety, availability, and environment-friendliness [7].

Liver disease has been classified as the high-priority areas of health care. According to World Health Organization, approximately 500 million people of the world are suffering from a severe form of liver disease [8]. Liver diseases are usually caused by exposure to agents like drugs, viruses, parasites, and toxins. These substances result in degeneration and inflammation of the liver, which may further result in fibrosis and cirrhosis. The main causes of liver cirrhosis are alcoholic liver disease, non-alcoholic fatty liver disease, and chronic viral hepatitis (B and C). Hepatic inflammation is caused by factors such as oxidative stress, reactive oxygen species (ROS), and DNA methylation [9, 10]. Oxidative stress is an instability status where the formation of reactive oxygen species exceeds the cellular antioxidant capacity. This phenomenon has turned to concern and represented the topic of plenty of researches [11, 12, 13].

Fadogia cienkowskii belongs to the Rubiaceae family, provincially called 'Ogwu-agu' in Igbo and 'Ufu-ewureje' in Benue State, Nigeria. The leaves were recognized ethno medicinally for their wide treatment efficacy in the relief of general body debility, headache, hepatoprotective and antioxidant activity, inflammation, and diarrhea, especially in infants. Previous literature also mentions herbal therapy for central and peripheral effect, local anesthetic and analgesic, of which no hepatoprotective, antioxidant therapy, and complete pharmacognostic properties [14, 15, 16].

The side effects of orthodox drugs, and development of resistance to currently used drugs, has led to an increased importance on the use of herbs as a source of medicines for human diseases. In developing countries, the use of herbal medicine is increasing because modern life-saving drugs are beyond the reach of three quarters of the world's population although many of such countries still spend 40-50% of their total wealth on drugs [17]. This study focuses on the identification of various functional groups and the evaluation of hepatoprotective and antioxidant activities of the VLC sub-fractions of the leaves of *Fadogia cienkowskii*.

MATERIALS AND METHODS

Plant materials: *F. cienkowskii* leaves were collected in January 2021 from Nsukka, Enugu State, Nigeria. The plant was collected in January 2021 and authenticated by a taxonomist (Nwafor Felix I.), and deposited in the Pharmacognosy and Traditional Medicine Department of Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka, Nigeria.

Animals: The Adult wistar rats (weighing 150 – 200 g) were obtained from the facility of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka. The Adult wistar rats were housed in steel cages within the facility under standard conditions and allowed free access to water and standard pellets, which conformed to the ethical guidelines of the National Code of Conduct for Animal Research Ethics (NCARE) [18].

Ethical Approval: Ethical approval is obtained from Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Health Research Ethical Committee with approval number COOUTH/CMAC/ETH.C/VOL.1/FN:04/0057.

Chromatography techniques

Vacuum liquid chromatography (VLC)

VLC was carried out using dry method according to the standard method (AOAC, 2005) [25].

Thin Layer Chromatography (TLC)

TLC was carried out according to standard procedures [25, 26] and the retention factors of the various spots were calculated.

The retention factor of the various components were calculated as shown below

$$\text{Retention factor} = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent}} \quad \text{Equation 6}$$

Ultra Violet-Visible Spectroscopy

A Perkin-Elmer Lambda 19 spectrometer was used in recording the absorption spectra of the study sample. *F. cienkowskii* leaves extract was centrifuged at 3000 rpm for 10mins and filtered through Whatman No. 1 filter paper using high pressure vacuum pump. The sample was diluted to a concentration of 1:10 with the extraction solvent. The study compound was measured in the UV-visible spectrum at a range of 240-900nm with a spectral band width of 2nm and scan speed of 240nm min^{-1}. Prominent characteristic peaks were detected and their absorbance noted [27, 28].

Fourier Transform Infrared Spectroscopic (FTIR) Analysis

Buck scientificM530 USA FTIR was used for the analysis. The instrument was equipped with a beam splitter of potassium bromide and deuterated triglycinesulphate. The Gram A1 software was used to obtain the spectra and to manipulate them. An approximately of 1.0g of samples, 0.5mi of nujol was added, mixed properly and placed on the salt pallet. FTIR spectra was obtained at frequency regions of 4,000-600cm^{-1} at 32 scans and at 4cm^1 resolution and displayed as transmitter values [29, 30, 31].

METHODS

Extraction and fractionation of *F. cienkowskii* leaves

The method of cold maceration was used for extraction of *F. cienkowskii* leaves 2 kg in 6 Liters of ethanol for 48 hr. The extract was subjected to fractionation using liquid-liquid partitioning using n-Hexane, ethyl acetate, butanol and aqueous [19, 20].

Evaluation of hepatoprotective activity of the VLC sub-fractions of *F. cienkowskii* leaves

The hepatoprotective activity of the 11 VLC sub-fractions of *F. cienkowskii* leaves was studied using a method by Olatosin *et al.,* 2014. Thirteen groups of five rats each of albino rats were fasted overnight but allowed free access to water.

The animals received treatment as follows:

Group 1: Treatment with 400 mg/kg of Sample 1 VLC sub-fraction of *F. cienkowskii*

Group 2: Treatment with 400 mg/kg of Sample 2 VLC sub-fraction of *F. cienkowskii*

Group 3: Treatment with 400 mg/kg of Sample 3 VLC sub-fraction of *F. cienkowskii*

Group 4: Treatment with 400 mg/kg of Sample 4 VLC sub-fraction of *F. cienkowskii*

Group 5: Treatment with 400 mg/kg of Sample 5 VLC sub-fraction of *F. cienkowskii*

Group 6: Treatment with 400 mg/kg of Sample 6 VLC sub-fraction of *F. cienkowskii*

Group 7: Treatment with 400 mg/kg of Sample 7 VLC sub-fraction of *F. cienkowskii*

Group 8: Treatment with 400 mg/kg of Sample 8 VLC sub-fraction of *F. cienkowskii*

Group 9: Treatment with 400 mg/kg of Sample 9 VLC sub-fraction of *F. cienkowskii*

Group 10: Treatment with 400 mg/kg of Sample 10 VLC sub-fraction of *F. cienkowskii*

Group 11: Treatment with 400 mg/kg of Sample 11 VLC sub-fraction of *F. cienkowskii*

Group 12: Positive control treatment with 100 mg/kg of Silymarin

The treatment was given daily for 7 days, on the 7^{th} day of treatment, paracetamol (1.8g/kg) was administered after treatment, and then 48 hours after the paracetamol administration the serum liver enzymes ALT (Alanine aminotransferase), AST (Aspartate aminotransferase), ALP (Alkaline phosphatase), Bilirubin levels were analyzed [21, 22].

Evaluation of the *in vivo* antioxidant activity of the VLC sub-fractions of *F. cienkowskii* leaves

The *in vivo* antioxidant activity of the 11 VLC sub-fractions of *F. cienkowskii* leaves were analyzed using a method by Olatosin *et al.,* 2014.

The animals received treatment as follows:

Group 1: Treatment with 300 mg/kg of Sample 1 VLC sub-fraction of *F. cienkowskii*

Group 2: Treatment with 300 mg/kg of Sample 2 VLC sub-fraction of *F. cienkowskii*

Group 3: Treatment with 300 mg/kg of Sample 3 VLC sub-fraction of *F. cienkowskii*

Group 4: Treatment with 300 mg/kg of Sample 4 VLC sub-fraction of *F. cienkowskii*

Group 5: Treatment with 300 mg/kg of Sample 5 VLC sub-fraction of *F. cienkowskii*

Group 6: Treatment with 300 mg/kg of Sample 6 VLC sub-fraction of *F. cienkowskii*

Group 7: Treatment with 300 mg/kg of Sample 7 VLC sub-fraction of *F. cienkowskii*

Group 8: Treatment with 300 mg/kg of Sample 8 VLC sub-fraction of *F. cienkowskii*

Group 9: Treatment with 300 mg/kg of Sample 9 VLC sub-fraction of *F. cienkowskii*

Group 10: Treatment with 300 mg/kg of Sample 10 VLC sub-fraction of *F. cienkowskii*

Group 11: Treatment with 300 mg/kg of Sample 11 VLC sub-fraction of *F. cienkowskii*

Group 12: Positive control treatment with 100 mg/kg of ascorbic acid (positive control).

The treatment was given through the oral route for 10 days. Afterwards, the animals were sacrificed and their livers extracted for the antioxidant evaluation using superoxidase dismutase (SOD) assay, malondialdehyde (MDA) assay and catalase assay (CAT). This procedure was also repeated on the fractions [23].

Evaluation of the *in vitro* antioxidant activity (DPPH free radical scavenging) of the VLC sub-fractions of *F. cienkowskii* leaves

The effect of extract on DPPH radical of the 11 VLC sub-fractions of *F. cienkowskii* leaves were estimated by Liyana-Pathiranan and Shahidi, 2005 [24].

Statistical analysis

The comparisons between the groups were performed using Dunnett's multiple comparison tests using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA). The mean ± standard mean error (SE) values were expressed.

RESULTS AND DISCUSSION

Table 1: Hepatoprotective activity of the mean values of the VLC sub-fractions

Treatment & Doses	ALT	AST	ALP	BILIRUBIN
Group 1 (400mg/kg 10:0-9:1 nHexane/EtOAc)	51.77±0.098 ^{ns}	73.675±0.5052^{ns}	59.29±7.973**	4.163±0.855^{ns}
Group 2 (400mg/kg 8:2 nHexane/EtOAc)	31.30±3.080 ^{ns}	57.05±5.456 ^{ns}	46.845±1.510 ^{ns}	4.625±0.053 ^{ns}
Group 3 (400mg/kg 7:3 nHexane/EtOAc)	53.06±0.248 ^{ns}	76.65 ± 0.202*	68.66±2.898 ^{ns}	4.903±0.053 ^{ns}
Group 4 (400mg/kg 6:4 nHexane/EtOAc)	52.115±0.396*	73.675±1.111 ^{ns}	51.8±6.403 ^{ns}	4.348±0.107 ^{ns}
Group 5 (400mg/kg 5:5 nHexane/EtOAc)	53.405±0.644 ^{ns}	75.508±0.314 ^{ns}	45.075±8.271 ^{ns}	3.855±0.107 ^{ns}
Group 6 (400mg/kg 4:6-2:8 nHexane/EtOAc)	38.7±8.141*	59.525±10.378^{ns}	50.21±11.836 ^{ns}	4.163±0.160 ^{ns}
Group 7 (400mg/kg 1:9 nHexane/EtOAc -9:1 EtOAc/MeOH)	51.86±0.647 ^{ns}	74.55±0.808 ^{ns}	65.48±0.603 ^{ns}	4.995±0.107 ^{ns}
Group 8 (400mg/kg 8:2 EtOAc/MeOH)	49.275±0.546 ^{ns}	64.925±0.9093^{ns}	52.05±7.852 ^{ns}	4.625±0.107 ^{ns}
Group 9 (400mg/kg 7:3-6:4 EtOAc/MeOH)	54.18±0.098 ^{ns}	74.55±0.6062 ^{ns}	69.68±8.395**	4.718±0.107 ^{ns}
Group 10 (400mg/kg 5:5-4:6EtOAc/MeOH)	50.4±1.490 *	63.175±0.303 ^{ns}	44.58±3.683 ^{ns}	4.625±0.053 ^{ns}
Group 11 (400mg/kg 3:7-1:9EtOAc/MeOH)	52.715±0.546 ^{ns}	65.975±3.334 ^{ns}	41.967±0.482 ^{ns}	4.07±0 ^{ns}
Group 12 (100mg/kg Silymarin (Pos control))	56.72± 9.682 *	79.675±6.769 ^{ns}	68.87±4.469 ^{ns}	4.625±0.214 ^{ns}
Group 13 (1800mg/kg PCM (Neg control)	26.165±1.342**	32.275±0.707 ^{ns}	12.05 ±1.570 ^{ns}	3.07±0.214 ^{ns}

Values are mean ± SEM; n = 3 observations

Table 2: Antioxidant test of the mean values of the VLC sub-fractions

Treatments	SOD	CATALASE	MDA
Group 1 (400mg/kg 10:0-9:1nHexane/EtOAc)	0.475±0.07^{ns}	0.1012±0.077^{ns}	1.596±0.20^{ns}
Group 2 (400mg/kg 8:2 nHexane/EtOAc)	0.355±0.04^{ns}	0.061±0.057^{ns}	1.804±0.19^{ns}
Group 3 (400mg/kg 7:3 nHexane/EtOAc)	0.109±0.03^{ns}	0.044±0.035^{ns}	1.220±0.89^{ns}
Group 4 (400mg/kg 6:4 nHexane/EtOAc)	0.164±0.06^{ns}	0.1153±0.067^{ns}	2.644±0.21^{ns}
Group 5 (400mg/kg 5:5 nHexane/EtOAc)	0.294±0.02^{ns}	0.0855±0.031^{ns}	2.705±0.16^{ns}
Group 6 (400mg/kg 4:6-2:8 nHexane/EtOAc)	0.389±0.06^{ns}	0.1578±0.043^{ns}	2.019±0.75^{ns}
Group 7 (400mg/kg 1:9 nHexane/EtOAc -9:1 EtOAc/MeOH)	0.084±0.02^{ns}	0.0585±0.059^{ns}	1.547±0.77^{ns}
Group 8 (400mg/kg 8:2 EtOAc/MeOH)	0.315±0.01^{ns}	0.0887±0.010^{ns}	2.240±0.002^{ns}

Group 9 (400mg/kg 7:3-6:4 EtOAc/MeOH)	0.109±0.018^{ns}	0.0435±0.047^{ns}	1.811±0.1109^{ns}
Group 10 (400mg/kg 5:5-4:6EtOAc/MeOH)	0.145±0.01^{ns}	0.061±0.047^{ns}	2.644±0.55^{ns}
Group 11 (400mg/kg 3:7-1:9EtOAc/MeOH)	0.197±0.06^{ns}	0.0613±0.020^{ns}	2.811±0.61^{ns}
Group 12 (100mg/kg Ascorbic Acid, Pos control)	0.145±0.06^{ns}	0.005±0.004^{ns}	1.897±0.12^{ns}
Group 13 (10ml/kg Distilled Water, Neg control)	1.478±0.22^{ns}	0.265±0.036^{ns}	4.567±0.18^{ns}

Values are Mean ± SEM & n=3 observations

Table 3: DPPH Antioxidant test of the mean values of the VLC sub-fractions

Treatments	**500µg**	**250µg**	**125µg**	**62.5µg**	**31.25µg**
Group 1 (400mg/kg 10:0-9:1nHexane/EtOAc)	0.317±0.0083***	0.310±0.0077***	0.267±0.0085***	0.210±0.0051***	0.156±0.0091***
Group 2 (400mg/kg 8:2 nHexane/EtOAc)	0.337±0.0073***	0.305±0.0067***	0.275±0.0075***	0.165±0.0041***	0.168±0.0081***
Group 3 (400mg/kg 7:3 nHexane/EtOAc)	0.017±0.0063^{ns}	0.006±0.0057^{ns}	0.050±0.0065^{ns}	0.081±0.0031^{ns}	0.099±0.0071^{ns}
Group 4 (400mg/kg 6:4 nHexane/EtOAc)	0.348±0.0053***	0.339±0.0047***	0.291±0.0055***	0.213±0.0021***	0.167±0.0061***
Group 5 (400mg/kg 5:5 nHexane/EtOAc)	0.330±0.0043***	0.316±0.0037***	0.266±0.0045***	0.214±0.0011***	0.175±0.0051***
Group 6 (400mg/kg 4:6-2:8 nHexane/EtOAc)	0.123±0.0033***	0.189±0.0027***	0.185±0.0035***	0.163±0.0015***	0.152±0.0041***
Group 7 (400mg/kg 1:9 nHexane/EtOAc -9:1 EtOAc/MeOH)	0.012±0.0023^{ns}	0.005±0.0017^{ns}	0.062±0.0025^{ns}	0.082±0.0014^{ns}	0.093±0.0031^{ns}
Group 8 (400mg/kg 8:2 EtOAc/MeOH)	0.186±0.0013***	0.220±0.0071***	0.225±0.0015***	0.186±0.0013***	0.138±0.0021***
Group 9 (400mg/kg 7:3-6:4 EtOAc/MeOH)	0.080±0.0003^{ns}	0.039±0.0072^{ns}	0.043±0.0058^{ns}	0.061±0.0003^{ns}	0.016±0.0011^{ns}
Group 10 (400mg/kg 5:5-4:6EtOAc/MeOH)	0.272±0.0038***	0.283±0.0073***	0.281±0.0057***	0.211±0.0015***	0.170±0.0019***
Group 11 (400mg/kg 3:7-1:9EtOAc/MeOH)	0.333±0.0037***	0.347±0.0074***	0.293±0.0056***	0.233±0.0014***	0.179±0.0018***
Group 12 (100mg/kg Ascorbic Acid)	0.013±0.0036^{ns}	0.004±0.0075^{ns}	0.044±0.0055^{ns}	0.062±0.0013^{ns}	0.017±0.0017^{ns}

Values are Mean ± SEM, n=3 observations

Table 4: The UV-VIS Spectrum Peak of *Fadogia cienkowskii* Leaves

S/No.	**Wavelength (nm)**	**Abs.**
1	240	0.023
2	260	0.045
3	280	0.056
4	300	0.076
5	325	0.079
6	340	0.098
7	365	0.099
8	380	0.114
9	395	0.147
10	420	0.366
11	440	2.712
12	460	1.379
13	480	0.691
14	500	0.505
15	520	0.458
16	540	0.464
17	560	0.449
18	580	0.341
19	600	0.274
20	620	0.261
21	640	0.332
22	660	0.336
23	680	0.345
24	700	0.451
25	720	0.185
26	740	0.148

27	760	0.145
28	780	0.149
29	800	0.159
30	820	0.163
31	840	0.162
32	860	0.12
33	880	0.155
34	900	0.126

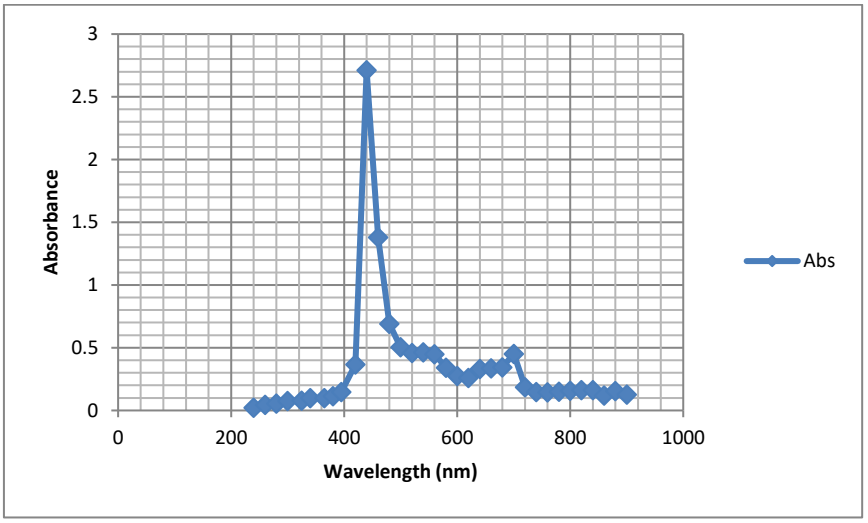

Figure 1: UV-VIS Spectrum of *Fadogia cienkowskii* Leaves

Table 5: Interpretation of FTIR spectra of *Fadogia cienkowskii* leaf (sample FC)

S/N	Wavelength (cm^{-1})	Functional group	Compounds
1	868.4239	C-CI	Chloro C-Cl symmetric stretch
2	1064.754	R-O-R	Ether CO symmetric stretch
3	1274.863	R-O-R	Ether CO symmetric stretch
4	1407.434	H_2C=CH	Ethene C=Canti-symmetric stretch
5	1617.172	RNH_3	1^0 amine NH stretch
6	1853.893	R-COO	Cyclic ester CO stretch
7	2114.577	RCOOH	Carboxylic acid CO stretch
8	2235.904	RC=O	Carbonyl CO antisymmetric stretch
9	2450.340	R-C≡N	Nitriles CN antisymmetric stretch
10	2647.608	CH_2	Methylene CH stretch
11	2750.834	CH_2	Methylene CH stretch
12	2917.153	R-S-C≡N	Thiocyanate SCN antisymmetric stretch
13	3262.097	RCHOH	1^O alcohol OH symmetric stretch
14	3683.564	R_3CHOH	3^O alcohol OH symmetricstretch
15	3825.261	R_3CHOH	3^O alcohol OH symmetricstretch

Hepatoprotective Screening of the VLC sub-fractions

The VLC sub-fractions of *F. cienkowskii* leaves produce a significant reduction in hepatic enzymes. In this study, Group 3 (400mg 7:3 n-Hexane/EtOAc), Group7 (400mg 1:9 n-Hexane/EtOAc -9:1 EtOAc/MeOH) and Group 9 (400mg 7:3-6:4 EtOAc/MeOH) has highest percentage inhibition of the liver enzymes, when compared with the Group 12 (100mg/kg Silymarin).The hepatoprotective activity of the mean values and percentage inhibition of the VLC fractions are shown in Table 1.

The effect of the eleven (11) VLC sub-fractions of *F. cienkowskii* leaves at the dose of 400mg/kg, the VLC sub-fractions of n-Hexane/ethyl acetate, and ethyl acetate/methanol, and Silymarin at 100mg/kg on hepatotoxicity induced by Paracetamol led to the significant reduction ($P \leq 0.05$) in the liver enzymes (AST, ALT, ALP) and bilirubin levels. After treatment, the % inhibition of the eleven VLC sub-fraction groups of the leaves of *F. cienkowskii* gave various values with groups 3, 7 and 9 having the highest percentage inhibition of the liver enzymes ALT, AST, ALP and Bilirubin level. Group 3 had percentage inhibition of the hepatic enzumes ALT (50.68%), AST (57.89%), ALP (82.45%) and Bilirubin

(37.37%). Group 7 had percentage inhibition of liver enzymes ALT (49.53%), AST (56.70%), ALP (81.60%) and Bilirubin (38.54%) while Group 9 has percentage inhibition of liver enzymes ALT (51.70%), AST (56.70%), ALP (82.71%) and Bilirubin (34.93%) levels.

When compared with the effect of the ethanol extract of the leaves of *F. cienkowskii* a different doses (100, 200 and 400 mg/kg), the fractions of n-Hexane, ethyl acetate, butanol and water at 200-400 mg/kg, and Silymarin at100mg/kg (positive control) on hepatotoxicity induced by Paracetamol led to the significant reduction ($_P$ = 0.05) in the liver enzymes (AST, ALT, ALP) and bilirubin levels at 200-400 mg/kg ethanol leaf extract and 400mg/kg ethyl acetate fraction [16].

The signs of hepatic damage are apparently leakage to the cellular enzymes into plasma. In addition, the extent and type of liver injury or damage can be accessed based on the presence or absence of specific enzymes in the blood stream [32]. In general measurement of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are commonly used as marker enzymes in accessing Paracetamol induced hepatotoxicity [33, 34, 35]. The VLC fractions were analyzed for their hepatoprotective activity because recently drugs induced liver damage causing one of the major mortality problems around the world [36, 37]. Therefore, the plant extract has a role in preserving structural integrity of hepatocellular membrane, thus prevented enzymes leakage into circulation.

Antioxidant evaluations of the VLC sub-fractions

The antioxidant enzymes SOD, CAT and MDA levels of the VLC sub-fractions gave the values shown in table 2. In this study, Group 3 (400mg 7:3 n-Hexane/EtOAc), Group7 (400mg 1:9 n-Hexane/EtOAc -9:1 EtOAc/MeOH) and Group 9 (400mg 7:3-6:4 EtOAc/MeOH) has highest percentage inhibition of the antioxidant enzymes, when compared with the Group 12 (100mg/kg Ascorbic Acid, Positive control. The % inhibition of the sub-fractions of the leaves of *F. cienkowskii* at different doses, show that the n-Hexane/ethyl acetate and ethylacetate/methanol fractions of groups 3, 7 and 9 at 400 mg/kg has the highest percentage inhibition of the antioxidant enzymes SOD (92.63%, 94.32%, 92.63%), CAT (83.40%, 77.92%, 83.58%) and MDA (73.29%,66.13%,60.35%), while for the positive control of 100 mg/kg Ascorbic acid also have a high percentage inhibition of the antioxidant enzymes such as SOD (90.18%), CAT (98.11%) and MDA (58.46%) levels. The *in vivo* antioxidant activity of the mean values and percentage inhibition of the VLC fractions are shown in Table 2. Therefore, the VLC fractions can inhibit oxidative stress up to the same level as ascorbic acid, as compared to the study by Bruce *et al*., [16].

The DPPH Antioxidant activity of the mean values of the sub-fractions is shown in table 3. The DPPH values indicate that the eleven (11) VLC sub-fractions of *F. cienkowskii* leaves at different doses possess significant radical quenching property. The n-Hexane/ethyl acetate and ethyl acetate/methanol fractions of groups 3, 7 and 9 at 400 mg/kg has the highest scavenging activity towards DPPH free radicals, Group 3 at 250 µg and 500 µg gave the highest percentage inhibition of 99.23% and 97.83%, Group 7 at 250 µg and 500 µg gave the highest percentage inhibition of 99.36% and 98.47% respectively, and Group 9 at 31.25 µg and 250 µg gave the highest percentage inhibition of 97.96% and 95.03% respectively. Compared with the positive control (Ascorbic acid) at 250 µg and 500 µg gave the highest percentage inhibition of 99.49% and 98.34% respectively as shown in table 3. This study is compared to the crude ethanol extract at 250 µg and 500 µg gave the highest percentage inhibition of 32.9% and 46.61%, while the ethyl acetate fraction at 250 µg and 500 µg gave the highest percentage inhibition of 43.04% and 52.74% [16]. The observed antioxidant of extracts is due to either transfer of hydrogen atom or by transfer of an electron which results in the neutralization of free radicals (DPPH) [38, 39]. The presence of active phytoconstituents can be attributed to scavenging effect. It was observed that the leaf extract contained high level of phenolic and flavonoid content that might have resulted to the strong antioxidant activity observed against the free radicals.

Spectrophotometry techniques

Spectrophotometry techniques are those techniques utilized in qualitative and quantitative analysis of pharmaceutical and biological materials [40]. Amongst all spectroscopic techniques, UV-Vis spectroscopy has been considered of great advantage because of its exactness and reproducibility in measuring absorbance against wavelength. It uses light in the visible ranges and the colours of the chemicals present have direct impact on its absorption [41]. The UV-Vis spectrum of ethanolic extract of *F. cienkowskii* displayed absorption bands in the wavelength of 240-900nm, considering the sharpness of the peaks and proper baseline. Varying peaks observed during analysis were indicative of many types of bonds within the molecule and the electronic transitions which in turn signify various chemical constituents responsible for the biological activities. *F. cienkowskii* extracts upon analysis showed peaks at 240nm, 440nm and 900nm with absorption of 0.023, 2.712 and 0.126 respectively. Peaks at 440, 560 and700 show the presence of flavonoids and at 700nm it designates chlorophyll. The UV-Vis spectrum of the ethanolic extract of *F. cienkowskii* as shown in Table 4 and figure 1 respectively. A bathochromic (Red) shift was observed in the violet region as the wavelength increased while the on red region, a Hypsochromic (Blue) shift was equally observed, from a decreased wavelength. Kalsi [42] investigations revealed that for the classification of multiple bonds and aromatic conjugations of organic compounds, UV-Vis spectroscopy might be utilized. The UV-VIS spectroscopy is a simple technique to identify the main phytochemicals, and discriminating between the hydrophilic and lipophilic molecules in relation to the polarity [43, 44].

Fourier Transform Infrared Spectroscopy (FTIR) is an analytical technique with a high-resolution to identify the chemical constituents and elucidate the structural compounds [45, 46, 47].The results (table 5) revealed the presence of alkaloids due to N-H stretching. Polyphenols, and flavonoids due to O-H stretching, terpens due to C-H group [48].

Fourier Transformed Infrared (FTIR) technique is an important tool used to identify the characteristic functional groups, which are instrumental in determination of functional groups and organic compounds inherent in any given sample [47, 48]. From the results above for sample FC as shown in table 5, the peak value 856.5014cm^{-1} was assigned to C-CI stretching vibration of halogen compound. The bands 1063.358cm^{-1} and 1181.225cm^{-1} were both assigned to CO stretching vibration of ether compounds. The peak values 1358.691$cm^{-1}$and 1422.044$cm^{-1}$was assigned to C=C stretching vibration of ethene compound. The medium bands 1613.099cm^{-1}, 1678.575$cm^{-1}$and 3493.264cm^{-1} were both assigned to N-H stretching vibration of 1^o& 2^o amine compounds respectively. The absorption 1851.439cm^{-1} was assigned to CO stretching vibration of cyclic ester compound. The peak values 1976.235$cm^{-1}$and 2930.432$cm^{-1}$was assigned to SCN stretching vibration of thiocyanate compound. The absorbance 2010.909cm^{-1} 2088.957cm^{-1} and 2290.907$cm^{-1}$was assigned to COO stretching vibration of carboxylic acid and carbonyl compound. The absorption 2413.613cm^{-1}, 2466.648cm^{-1} and 2504.406$cm^{-1}$was assigned to C≡N anti-symmetric vibration of nitrile compound. The weak band s 2607.530cm^{-1}, 2757.938cm^{-1} and 2845.291cm^{-1} were assigned to C-H stretching vibration of methylene compound respectively. The strong bands 3073.916cm^{-1}, 3153.201cm^{-1}, 3193.057cm^{-1}, 3302.052cm^{-1}, 3366.968cm^{-1},3715.034cm^{-1}, 3785.655$cm^{-1}$and 3833.126cm^{-1}corresponds to 1^o, 2^o & 3^o alcoholic compound respectively. The FTIR spectrum confirmed the presence of alcohols, phenols, chloro, ether, ethene, amine, ester, carboxylic acid, nitriles, thiocyanate, methylene and amines in the test plant.

CONCLUSION

This study shows that the effects of the VLC sub-fractions of the leaves of *F. cienkowskii* led to a significant reduction ($p< 0.05$) in the liver, antioxidant enzymes and DPPH free radicals which poses reported therapeutic potentials, including hepatoprotective and antioxidant activities. This supports the claims by the herbalists that the plant could be used in the management of liver diseases and oxidative stress-related diseases. The UV-VIS spectroscopy offers a simple technique to identify the presence of phenolic compounds and flavonoids, which has various medicinal properties of the plant. The Fourier Transformed Infrared

(FTIR) technique is an important tool used to identify the characteristic functional groups, which confirms the presence of alcohols, phenols, chloro, ether, ethene, amine, ester, carboxylic acid, nitriles, thiocyanate, methylene and amines in the test plant.

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Conflict of Interests

There is no conflict of interest.

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Authors' Contribution section

The authors hereby declare that the work presented in this article has a collective contribution and it is original.

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