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Chemical Composition, Hepatoprotective and Antioxidant Activity of the Crude Extract and Fractions of the Leaves of *Fadogia Cienkowskii* Schweinf (Rubiaceae)

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ABSTRACT

Fadogia cienkowskii (wild plant) belongs to the Rubiaceae family. The purpose of this study is to investigate the chemical composition, hepatoprotective and antioxidant activities of the extract and fractions of *Fadogia cienkowskii* leaves. Phytochemical analysis was carried out using standard protocols; the hepatoprotection was determined in paracetamol-induced liver damage model in rat, and the antioxidant potential evaluated using both *in vivo* and *in vitro* antioxidant models. The phytochemical analysis revealed that tannins, flavonoids and phenols are the highest phytochemical constituents. The histopathological study showed reduction in hepatic damage. The hepatoprotective activity at 200-400 mg/kg ethanol extract and 200-400 mg/kg ethyl acetate fraction showed significant ($p < 0.01$) reductions in serum liver enzymes (ALP, AST and ALT)/ bilirubin levels in paracetamol-induced liver injury in rats. The percentage inhibition of the ethanol extract and fractions of the leaves of *F. cienkowskii* at different doses showed that the ethanol extract at 300 mg/kg has the highest percentage inhibition of the antioxidant enzymes SOD (22.2%), CAT (39.3%) and MDA (9.6%); while for the different fractions, the ethyl acetate fraction at 400 mg/kg has the highest percentage inhibition of the antioxidant enzymes SOD (88.83%), CAT (71.43%) and MDA (68.97%) levels. Thus, ethanol extract and ethyl acetate fraction of *F. cienkowskii* exerted significant hepatoprotection at higher doses even more than Silymarin (100mg/kg) against paracetamol-induced toxicity. Hence, this justified its traditional use by herbalists in the South Eastern Nigeria for the treatment of liver disease.

Keywords: Hepatoprotective, Antioxidant, Phytochemical, Histopathology, *Fadogia cienkowskii*, Gas chromatography-mass spectrometry

Introduction

Medicinal plants have acquired importance in the healthcare system throughout the world for their proven and effective therapeutic properties.[1] Approximately 80% of the world's population uses medicines that contain compounds of herbal origin.[2] The International Union for Conservation of Nature has suggested that flowering plants are used for medicinal purposes.[3] Although medicinal plants have been used globally, their wider usage is limited to a few countries like Japan, India, China, Pakistan, Thailand, Iran, and some African countries.[4-7] Other developing countries are also encouraging the use of plant-based medicinal products in their healthcare systems.[8]

A major concern of scientists investigating herbal treatments is that the chemical composition of the plants contributing to their biological effects is mostly undetermined.[9] Herbs have ingredients that are potential sources of medicines for the treatment of liver disorder and diseases having various modes of actions and bioactivities.[10-,12] A liver disorder has high priority areas when classified in health care.

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World Health Organization, estimates that people of the world, suffer from a severe form of liver disorders, such as chronic hepatitis.[13] Hepatic disorders are caused by exposure to agents like drugs, parasites, viruses, and toxins.[14] These substances result in degeneration and inflammation of the liver through a different mechanism of action, which may further result in cirrhosis and fibrosis. 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 generally caused by factors such as oxidative stress, DNA methylation and reactive oxygen species (ROS).[14,15] Medicine of herbal origin may serve as a feasible therapy for the prevailing liver problems because of their safety, easier availability, cost-effectiveness, and environment-friendliness.[16] Oxidative stress is an imbalance status where the formation of reactive oxygen species surpasses the cellular antioxidant capacity. This is a major concern to the topic of plenty of researches in the field of food and nutrition sciences.[17] Plants constantly experiencing oxidative stress due to several abiotic and biotic factors. Consequently, they have an inherited efficient complex response network of enzymatic and non-enzymatic antioxidants to prevent deleterious of effect reactive oxygen species such as hydrogen peroxide, superoxide, and hydroxyl radicals, which are normally produced in excess upon exposure to biotic and abiotic stresses.[18,19] The enzymatic system mainly includes superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase whereas, the non-enzymatic system consists of low and high molecular weight phyto-antioxidants such as ascorbic acid, phenolics, and flavonoids.[20,21] Non-enzymatic phyto-antioxidants play a vital role in plant growth, defense response, and development, together with their main cellular protective function against oxidative stress.[22,23] Due to this

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reason, researchers are trying to enhance the biosynthesis and accumulation of phyto-antioxidants in plants to provide abiotic and biotic stress tolerance using genetic engineering, plant breeding, and seed priming strategies.[24-26]

In general, the antioxidant potential of plant samples is routinely assessed by three approaches such as (a) direct measurement of antioxidant enzyme activity, (b) measuring the protective response of plant samples against chemical-induced oxidative stress and (c) *in vitro* radical scavenging and reducing power assessment. However, each of these approaches has its limitations about applicability.[20]

Fadogia cienkowskii locally called 'Ogwu-agu' in Igbo and 'Ufu-ewureje' in Igede tribe of Benue State within the middle belt of Nigeria, which belongs to Rubiaceae family. The leaves were highly acknowledged for their wide therapeutic efficacy in the relief of headache, general body debility, inflammation, diarrhoea, and other ailments, especially in infants.[27]

The methanol extract of *F. cienkowskii* leaves has been investigated for central and peripherally mediated nervous effects. The oral acute toxicity studies and local anaesthetic effects were carried out following standard models.[28]The pharmacognostic, physicochemical and phytochemical properties were also investigated.[29] Due to the importance of *Fadogia cienkowskii* leaves in phytotherapy, no chemical constituents have been investigated, it is necessary to investigate their chemical constituents and biological activities in detail. In the treatment and management of liver diseases many conventional drugs or pharmaceutically derived drugs used, have serious adverse effects and are not easily accessible, and available. The high incidence and prevalence of oxidative stress-induced diseases such as neurodegenerative disorders, cancer, and chronic illnesses in current times in the world, has led to the search for antioxidants. Therefore, this study focuses on the evaluation of hepatoprotective and antioxidant activities of the leaves of *F. cienkowskii*.

Materials and Methods

Plant materials

Fresh leaves of *F. cienkowskii* were collected in January 2019 from Nsukka, Enugu State, Nigeria. The plant was collected in January 2019 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. Herbarium number PCG474/A/005.

Animals

Adult wistar rats (weighing 150 – 200 g) of either sex were used. The animals were obtained from the Laboratory animal facility of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka. Animals were housed in steel cages within the facility under standard conditions and allowed free access to standard pellets and water. Prior to their use, they were allowed two weeks for acclimatization within the work area environment. All experiments were carried out after approval of the protocol by the ethics committee of Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka and conformed to the ethical guidelines of the National Code of Conduct for Animal Research Ethics (NCARE).[34]

Ethical approval

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

Extraction and fractionation of plant material

The powdered plant material (2.0 kg) was cold macerated in 6 L of ethanol for 72 hours with intermittent shaking. The resulting solution was filtered and the filtrate was concentrated *in vacuo* using a rotary evaporator at 40^{o}C. A solid residue was obtained. Then 40 g of the extract was subjected to liquid-liquid partitioning using n-hexane, ethyl acetate, butanol and distilled water in order of their increasing polarity. The fractions were filtered and concentrated using a rotary evaporator at 40^{o}C.[30,31]

Phytochemical analysis

Qualitative Phytochemical Analysis

The plant crude extracts were tested for the presence of tannins, flavonoids, phenols, reducing sugars, glycosides, terpenoids, alkaloids, hydrogen cyanide, saponins, and steroids using standard methods.[32]

Quantitative phytochemical analysis

The coarse powder of the plant material was tested to determine the quantity of tannins, flavonoids, phenols, reducing sugars, glycosides, terpenoids, alkaloids, hydrogen cyanide, saponins, and steroids present.[33]

Evaluation of hepatoprotective activity of F. cienkowskii

The hepatoprotective activity of ethanol extract and different fractions of *F. cienkowskii* leaves were studied using a reported method.[30,35] The rats were grouped into six groups of five rats per group. The animals were kept fasting overnight but allowed free access to water. All the drugs were administered orally with the help of a feeding tube.

The adult wistar rats (150 – 200 g) were treated as follows: group 1 (Negative control) was treated with 1800 mg/kg Paracetamol (PCM) in water, group 2 (Positive control) was treated with 100mg/kg of Silymarin in water, group 3 was treated with 100mg/kg of ethanol crude extract, group 4 was treated with 200 mg/kg of ethanol crude extract, group 5 was treated with 400 mg/kg of ethanol crude extract, group 6 was treated with 200 mg/kg n-hexane fraction, group 7 was treated with 400 mg/kg n-hexane fraction, group 8 was treated with 200 mg/kg Ethyl acetate fraction, group 9 was treated with 400 mg/kg Ethyl acetate fraction, group 10 was treated with 200 mg/kg Butanol fraction, group 11 was treated with 400 mg/kg Butanol fraction, group 12 was treated with 200 mg/kg H_2O fraction, group 13 was treated with 400 mg/kg H_2O fraction.

These treatments were given once daily for 7 days, then on the 7thday of treatment, paracetamol (1.8 g/kg) was administered to the rats in groups 2-5 after treatment. Then 48 hours after the paracetamol administration the rats were sacrificed for serum enzyme ALT (Alanine aminotransferase), AST (Aspartate aminotransferase), ALP (Alkaline phosphatase), Bilirubin level analysis and histopathological study to determine the degree of hepatic damage.

Histopathological evaluation

After necropsy, the liver was removed. The tissue was fixed in 10% formalin for 24 hours. Formalin-fixed tissue was routinely dehydrated in graded ethanol series, 70% three times for 20 minutes, 80% three times for 20 minutes, 95% three times for 20 minutes, 100% three times for 30 minutes, after dehydration. Tissues were cleared in xylene for 5 minutes each and embedded in paraffin, serial sections of 5 micrometers thick of each tissue were prepared on a slide and stained with Harris's Hematoxylin and Eosin (H&E). Subsequently, the tissues were examined under a light microscope (Labomed, L×100 and L×400) magnification.[36]

Evaluation of the In vivo antioxidant activity of F. cienkowskii

The *In vivo* Antioxidant Activity of ethanol extract and different fractions of *F. cienkowskii* leaves were studied.[37] The rats were grouped into five groups of five rats per group. The animals were kept fasting overnight but allowed free access to water. All the drugs were administered orally with the help of a feeding tube.

The animals were treated as follows: Group 1 received 10 mL/kg of distilled water (negative control), group 2 received 100 mg/kg of ascorbic acid (positive control), group 3 received 50 mg/kg of crude extract, group 4 received 300 mg/kg of crude extract and group 5 received 100 mg/kg of the crude extract,group 6 received 200 mg/kg n-hexane fraction, group 7 received 400 mg/kg n-hexane fraction, group 8received 200 mg/kg Ethyl acetate fraction, group 9 received figures 1-6. Histological examination of the liver sections of control animals revealed the presence of normal hepatocytes with central vein and sinusoidal dilation, as demonstrated by Jeyadevi *et al*.,[36] which states that the histopathological examination of *Ipomoea staphylina*

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Linn leaves, the liver sections of control animals revealed the presence of normal hepatocytes with well-preserved cytoplasm, a prominent nucleus, and distinct sinusoidal spaces. Also, the histopathological observations in paracetamol-treated rats showed severe degeneration of interstitial tissues and hepatocytes. This is due to the formation of

DPPH free radical scavenging activity

The *in vitro* Antioxidant Activity (Effect of extract on DPPH radical) of ethanol crude extract and fractions of *F. cienkowskii* leaves werestudied.[38]A solution of 0.135 mM DPPH in ethanol was prepared and 1 mL of this solution was mixed with AIS (1 mL) of various concentrations ranging from 20 to 100 µg. The reaction mixtures were vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixtures is read spectrophotometrically at 517 nm. BHT was used as the reference standard. The ability to scavenge DPPH radical was calculated by the following equation

DPPH radical scavenging activity (%) = [(Abs control-Abs sample) / (Abs control)] X 100…Equation 1

Where Abs control is the absorbance of DPPH radical + ethanol; Abs sample is the absorbance of DPPH radical + sample extract/standard. All determinations will be carried out in triplicates.

Analysis of the chemical composition of F. cienkowskii leaves by Gas chromatography-mass spectrometry(GC–MS)

The Gas chromatography-mass spectrometry (GC–MS) analyses of *F. cienkowskii* leaf extract and fractions were carried out in Agilent Technologies (Wilmington, Delaware, USA) equipment with a column HP-5MS (30 m × 0.25 mm 1D X 0.25). A sample of 1 µL of each extract was injected. For detection, an ionization system with energy of 70 eV was used. The flow rate of carrier gas was constant and it was maintained at 1.1 mL/min. The injection temperature was 250 °C. The warming program of the oven was isothermal for 5 min at 60 °C followed by a warming of 5 °C/min up to 100 °C/min (2 min), and 10 °C/min up to 250 °C/min (5 min). The interpretation of the mass spectra was made using the National Institute of Standard and Technology (NIST) library.[39]

Statistical analysis

The statistical significance was assessed using one-way analysis of variance (ANOVA) and Dunnett's multiple comparison tests using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA). The values were expressed as mean ± standard mean error (SE). The criterion for statistical significance was *P < 0.05 and **P < 0.01.

Results and Discussion

Liver diseases are serious health problems; however, no satisfactory liver protective drugs were discovered in allopathic medical practice for serious liver disorders. Herbal drugs play a major role in the management of various liver disorders and diseases which speed up the natural healing processes of the liver.[41]

The qualitative phytochemical analysis of the ethanol extract, ethyl acetate, butanol and aqueous fractions of *Fadogia cienkowskii* leaf revealed the presence of tannins, saponins, glycosides, reducing sugars, alkaloid, steroids, terpenoids, phenols, flavonoids and the absence of hydrogen cyanide while the n-hexane fraction revealed the presence of terpenoids and steroids, and the absence of tannins, saponins, glycosides, reducing sugars, alkaloid, phenols, flavonoids, and hydrogen cyanide (Table 1). In a recent study Bruce *et al*.,[30] it was discovered that the qualitative phytochemical analysis of *F. cienkowskii* leaf extract revealed the presence of tannins, saponins, glycosides, reducing sugars, alkaloid, steroids, terpenoids, phenols, flavonoids and the absence of hydrogen cyanide. Alkaloids are known to be the largest group of secondary metabolites found in plants. They are claimed to have powerful effects on humans and animals and hence can be used as analgesics.[42] Alkaloids are found to have activity by inhibiting DNA topoisomerase.[43]

The quantitative phytochemical test of the ethanol leaf extract of *F. cienkowskii* revealed that tannins (18.61%), flavonoids (16.70%), and phenols (7.80%) had the highest percentage. The fractions also revealed that tannins, flavonoids, and phenol had the highest percentage in ethyl acetate, butanol, and aqueous fractions. For ethyl acetate fraction, tannins (15.71%), flavonoids (14.51%) and phenols (10.72%), butanol fraction, tannins (18.08%), flavonoids (20.67%), and phenols (17.88%), aqueous fraction, tannins (17.91%), flavonoids (18.58%) and phenols (8.41%). The n-Hexane fraction only has the presence of terpenoids (4.36%) and steroids (1.57%) as presented in Table 2. Bruce *et al*.[30] observed a similar trend that the quantitative phytochemical test in *F. cienkowskii* leaf extract, revealed that flavonoids (17.7%) and tannins (17.6%) are the highest phytochemical constituents. Tannins reduce the risk of coronary heart disease.[44]Saponins have been suggested as possible anti-carcinogens. Flavonoids and phenols are good sources of natural antioxidants.[45] Steroids have been reported in clinical studies as anti-inflammatory and analgesic agents and also used in the treatment of congestive heart failure.[46] Tannins also suggest having anticancer activities[47] and could be used for cancer prevention.

Hepatoprotective activity evaluation

Effect of ethanol extract of F. cienkowskii leaves on Enzyme and bilirubin levels

The effect of the ethanol extract of the leaves of *F. cienkowskii* at 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 at 100mg/kg (positive control) on hepatotoxicity induced by Paracetamol led to the significant reduction ($P \leq 0.05$) in the liver enzymes (AST, ALT, ALP) and bilirubin levels at 200-400 mg/kg ethanol leaf extract and 400mg/kg ethyl acetate fraction. The % inhibition of the ethanol crude extract and fractions of the leaves of *F. cienkowskii* at different doses of 200 and 400mg/kg, the ethanol crude extract at 400 mg/kg has the highest percentage inhibition of the liver enzymes ALT (48.21%), AST (41.69%), ALP (67.29%) and bilirubin level (31.26%), while for the different fractions, the ethyl acetate fraction of 400 mg/kg has the highest percentage inhibition of the liver enzymes ALT (83.47%), AST (88.02%), ALP (81.33%) and bilirubin level (7.47%) levels as shown in Table 3 and Figure 1 respectively.

The chloroform extract of *Ichnocarpus frutescents* leaves (Family: Apocynaceae) administered at a dose of 250 mg/kg orally once daily for 7 days, inhibited the PCM mediated increase in the activities of these biomarkers (ALP, AST, ALT, and bilirubin) by 28.91%, 29.4%, 32.02% and 45.81% respectively.[41] The seed extract of *P. nitida* fractions such as ethyl acetate fraction at 400 mg/kg produced a significant ($P \leq 0.05$) inhibition in liver enzymes (ALT (82.97%), AST (71.95 %), ALP (43.04%)) and bilirubin level (67.27%).[31, 48]

Histopathological evaluation

Histopathological studies of rat liver tissue from Group 1 animals (normal control) show normal hepatocytes with central vein and sinusoidal dilation (Figure 1). In the paracetamol treated group (Group 2), hepatotoxicity was observed by the presence of focal degeneration of interstitial tissues and hepatocytes (Figure 2). The liver taken from Group 3 animals treated with standard drug Silymarin showed normal hepatocytes with the central vein (Figure 3). While group 4 animals treated with 100 mg/kg of extract show normal hepatocytes but with distorted endothelial lining of the central vein (Figure 4). Group 5 (200mg/kg extract) animals show normal hepatocytes with moderately hypertrophied central vein (Figure 5), while the animals in group 6 (400mg/kg extract) show normal hepatocytes with central vein and sinusoidal dilation (Figure 6).

The histopathological studies support the results of the biochemical studies and it demonstrates that the hepatic damage caused by paracetamol during intoxication is reduced in the liver sample of animals treated with ethanol leaf extract of *F. cienkowskii* as shown in figures 1-6. Histological examination of the liver sections of control animals revealed the presence of normal hepatocytes with central vein and sinusoidal dilation, as demonstrated by Jeyadevi *et al*.,[36] which states that the histopathological examination of *Ipomoea staphylina* Linn leaves, the liver sections of control animals revealed the presence of normal hepatocytes with well-preserved cytoplasm, a prominent nucleus, and distinct sinusoidal spaces. Also, the histopathological observations in paracetamol-treated rats showed severe degeneration of interstitial tissues and hepatocytes. This is due to the formation of highly reactive radicals because of the oxidative threat caused by paracetamol. All these changes were very much

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reduced histopathologically in rats treated with ethanol leaf extract of *F. cienkowskii* as the doses increased. When compared with liver tissue of PCM injured rats treated with chloroform leaf extract of *I. frutescens* (Family: Apocynaceae) at 250 mg/kg once daily for 7 days showing a mild degree of necrosis with normal cells, it can be inferred that the leaf extract of *F. cienkowskii* was more effective in reversing induced liver damage than leaf extract of *I. frutescens*).[41]

Effects of extract and its fraction of F. cienkowskii on antioxidant enzymes and lipid peroxidation in PCM induced liver damage

The assay of SOD, CAT and MDA levels in the liver gave the mean values shown in Table 4. However, treatment with the ethanol crude extract and fractions (ethyl acetate, n-Hexane, butanol and aqueous) of the leaves of *F. cienkowskii* improved the levels of these enzymes significantly in a dose-dependent manner (100 and 300 mg/kg) and ethyl acetate fraction at 200-400 mg/kg led to the significant reduction ($P < 0.05$) in the antioxidant enzymes (SOD, CAT and MDA). The DPPH antioxidant activity of the crude extract and fractions is represented in Table 5.

In this study, *in vitro* antioxidant study via the free radical scavenging using DPPH assay and the *in vivo* antioxidant enzyme concentrations of SOD, CAT, and MDA, has been carried out to explore the antioxidant activity of *F. cienkowskii*. These are the most commonly used assays by researchers to evaluate the antioxidant capacity of samples from plants.

The DPPH values indicated that the ethanol crude extract and fractions of the leaves of *F. cienkowskii* at different doses possess significant radical quenching properties in Table 5. However, among all the assays it demonstrated the highest scavenging activity towards DPPH free radicals, 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% respectively. Therefore, the antioxidant activity of the ethanol crude extract of *F. cienkowskii* may be attributed to the presence of the identified phytochemicals. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers.[49] Similarly, terpenoids, like vitamins, act as regulators of metabolism and play a protective role as antioxidants.[50] Antioxidants prevent oxidative stress, caused by free radicals by removing free radicals intermediates and inhibit other oxidation reactions.[51]

An antioxidant enzyme such as SOD converts superoxide anion into H_2O_2 and O_2, whereas CAT reduces H_2O_2 to H_2O, resulting in the quenching of free radicals. SOD and CAT play an important role in the elimination of ROS derived from the redox process in liver tissues and are considered to be the most important enzymes.[52]The assay of SOD, CAT and MDA levels gave the mean values shown in table 4. However, treatment with the ethanol crude extract and fractions (ethyl acetate, n-hexane, butanol, and water) of the leaves of *F. cienkowskii* improved the levels of the antioxidant enzymes significantly in a dose-dependent manner. The ethanol crude extract (100 and 300 mg/kg), ethyl acetate fraction at 200-400 mg/kg and the positive control (ascorbic acid 100mg/kg), when compared to the negative control (10 mL/kg Distilled water) leads to a significant reduction ($P < 0.05$) in the liver enzymes (SOD, CAT, and MDA).

The % inhibition of the ethanol crude extract and fractions of the leaves of *F. cienkowskii* at different doses showed that the ethanol crude extract at 300 mg/kg has the highest percentage inhibition of the antioxidant enzymes SOD (22.2%), CAT (39.3%) and MDA (9.6%), while for the different fractions, the ethyl acetate fraction of 400 mg/kg has the highest percentage inhibition of the antioxidant enzymes SOD (88.83%), CAT (71.43%) and MDA (68.97%) levels. Therefore, the extract can inhibit oxidative stress up to the same level as ascorbic acid (the reference standard). These observations are indicative of the antioxidant activity of the crude extract. Superoxidase dismutase protects cellular homeostasis by scavenging reactive oxygen species.[53, 54] Gas chromatography-mass spectrometry (GC-MS) is a method that identifies different substances within a test sample by combining the features of gas-liquid chromatography and mass spectrometry. In this study, the mass spectra of the identified compounds in the ethanol crude extract at different retention time showed the presence of Ursodeoxycholic acid, Ethyl iso-allocholate, Dodecanoic acid derivative, and Tetradecanoic acid derivative. The n-Hexane fraction at different retention time showed the presence of 1-Heptatriacotanol, Ethyl iso-allocholate, Hexadecanoic acid derivative, Tetradecanoic acid derivative, 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde,Chlordiazepoxide, and Pregn-4-ene-3,20-dione, 17,21-dihydroxy-, bis(O-methyloxime). The ethyl acetate fraction at different retention time showed the presence of Tetradecanoic acid, Ursodeoxycholic acid, Dodecanoic acid and 4H-Cyclopropa[5',6']benz[1',2':7,8]azuleno[5,6-b]oxiren-4-one,8-(acetyloxy)1,1a,1b,1c,2a,3,3a,6a,6b,7,8,8a-dodecahydro-3a,6b,8a-trihydroxy-2a(hydroxyl methyl)-1,1,5,7-tetramethyl-,(1aα, 1b*β*,1c*β*,2a*β*,3a*β*,6aα,6bα,7α,8*β*,8aα). The butanol fraction at different retention time showed the presence of Tetradecanoic acid derivative, Dodecanoic acid derivative, 4H-Cyclopropa[5',6']benz[1',2':7,8]azuleno[5,6-b]oxiren-4-one,8-(acetyloxy)-1,1a,1b,1c,2a,3,3a,6a,6b,7,8,8a-dodecahydro-3a,6b,8a-trihydroxy-2a-(hydroxyl-methyl)-1,1,-5,7-tetrame thyl-,(1aα,1b*β*,1c*β*,2a*β*,3a*β*,6aα,6bα,7α,8*β*,8aα),Gibberellic acid, and Butanoic acid derivative,1a,2,5,5a,6,9,10,10a-octahydro-5a-hydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-6,11-dioxo-1H-2,8a-methanocyclopenta[a]cyclopropa [e]cyclodecen-5-yl-ester,[1aR-(1aα,2α,5*β*, 5a*β*,8aα,9α,10aα)]. Lastly, the water fraction at different retention time showed the presence of Tetradecanoic acid derivative, Dodecanoic acid derivative, and Hexadecanoic acid derivative. The Gas chromatography-mass spectrometry (GC-MS) chromatogram of the ethanol crude extract and fractions identified twelve compounds as presented in Tables 6-10, which are discussed below.

Ursodeoxycholic acid is a steroid and has therapeutic potentials such as for the treatment of cholestatic liver injury, oxidative stress, gallstone formation, primary biliary cholangitis, cystic fibrosis.[40, 55]

Ethyl iso-allocholate is an ester and its therapeutic potential includes; antioxidant, hepatoprotective, neuroprotective, hypertensive, anti-inflammatory, anticancer, and antimicrobial properties.[56]

Dodecanoic acid derivative is a fatty acid and it is also called lauric acid. The therapeutic potential includes antioxidant,[57] hepatoprotective,[58] cardiovascular,[59] and for the treatment of atherosclerosis. The moiety attached to the derivative will determine, whether the moiety substitutes will enhance or reduce the activities of their parent compounds. Tetradecanoic acid derivation is a fatty acid and it is also called myristic acid. Its therapeutic activity includes antioxidant,[57] hepatoprotective,[58] cardiovascular[59]and for the treatment of Atherosclerosis. The moiety attached to the derivative will determine, whether the moiety substitutes will enhance or reduce the activities of their parent compounds.

Heptatriacotanol therapeutic uses include hepatoprotective,[60, 61] antioxidant,[60] acaricidal,[62] and antimicrobial properties.[63]

Hexadecanoic acid derivative is a fatty acid and its pharmacological use includes anti-inflammatory, antioxidant, antiandrogenic, nematicide, for the treatment of hypocholesterolemic. They are also used for the production of pesticides, lubricants and flavours.[63- 65]The moiety attached to the derivative will determine, whether the moiety substitutes will enhance or reduce the activities of their parent compounds. 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1carboxaldehy- de is an aldehyde, and its pharmacological use includes antibacterial,[66] hepatoprotective, antiarthritic, nematicide properties and for the treatment of hypocholesterol.[67] Chlordiazepoxide therapeutic uses includes antioxidant, analgesic, antihyperglycemia, neuro-pharmacological (anxiety) and antidiarrheal properties.[68] Pregn-4-ene-3,20-dione, 17,21-dihydroxy-, bis(O-methyloxime) therapeutic uses includes antioxidant, anticancer, antigenotoxic activity, antimicrobial Activity, anti–inflammatory, anthelmintic, nematicide, antihistaminic, antieczemic, antitumor , decreased risk of breast cancer, antidiarrheal, antifungal activity as described by Al-Gari *et al*.,[69] and Al-Rubaye *et al*.[70] 4H-Cyclopropa[5',6']benz[1',2':7,8]azuleno[5,6-b]oxiren-4-one,8-(acetyloxy)1,1a,1b,1c,2a,3,3a,6a,6b,7,8,8a dodecahydro-3a,6b,8a-trihydroxy-2a(hydroxyl-methyl)-1,1,5,7-tetramethyl-, (1aα,1b*β*,1c*β*,2a*β*,3a*β*,6aα,6bα,7α,8*β*,8aα)therapeutic uses includes antibacterial,[66]hepato-protective, hypocholesterolemic, antiarthritic, nematicide properties.[67]

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Table 1: Qualitative Phytochemical Analysis of *F. cienkowskii* leaf extract

Constituents	Ethanol crude	n-Hexane fraction	Ethyl acetate fraction	Butanol fraction	Aqueous fraction
Tannins	+	-	+	+	+
Flavonoids	+	-	+	+	+
Phenol	+	-	+	+	+
Reducing sugars	+	-	+	+	+
Glycosides	+	-	+	+	+
Terpenoids	+	+	+	+	+
Alkaloids	+	-	+	+	+
Hydrogen cyanide	-	-	-	-	-
Saponins	+	-	+	+	+
Steroids	+	+	+	+	+

Table 2: Quantitative phytochemical test of *F. cienkowskii* leaf extract (%)

Constituents (mg/g)	Ethanol crude	n-Hexane fraction	Ethyl acetate fraction	Butanol fraction	Aqueous fraction
Tannins	18.61	0.00	15.71	18.08	17.91
Flavonoids	16.70	0.05	14.51	20.67	18.58
Phenol	7.80	0.20	10.72	17.88	8.41
Reducing sugars	5.63	0.00	4.43	5.91	6.54
Glycosides	3.46	0.40	1.05	2.30	2.39
Terpenoids	7.60	4.36	6.79	7.07	7.10
Alkaloids	4.28	0.76	3.81	4.21	3.91
Hydrogen cyanide	0.00	0.00	0.00	0.00	0.00
Saponins	1.59	0.23	1.64	1.58	1.69
Steroids	2.13	1.57	1.04	1.15	1.95

Table 3: Hepatoprotective effects of extract and its fractions of *F. cienkowskii* in PCM induced liver damage

Treatment & doses	ALT (U/L)	AST (U/L)	ALP (U/L)	Bil (U/mol)
1800 mg/kg PCM	47.40 ± 0.40^{ns}	$65.85 \pm 7.82^{*}$	$53.74 \pm 1.31^{**}$	26.23 ± 1.06^{ns}
100 mg/kg Silymarin	$23.25 \pm 1.18^{*}$	$31.60 \pm 0.69^{**}$	$20.97 \pm 3.75^{**}$	$17.40 \pm 0.53^{**}$
100 mg/kg Extract	43.30 ± 1.62^{ns}	$48.65 \pm 2.63^{*}$	$30.68 \pm 4.60^{**}$	21.60 ± 0.64^{ns}
200 mg/kg Extract	$35.80 \pm 1.39^{**}$	$44.95 \pm 0.66^{**}$	$19.23 \pm 0.64^{**}$	$19.73 \pm 0.79^{**}$
400 mg/kg Extract	$24.55 \pm 0.89^{**}$	$38.40 \pm 1.21^{**}$	$17.58 \pm 0.32^{**}$	$18.03 \pm 1.24^{**}$
200 mg/kg n-Hexane	$74.1 \pm 7.28^{**}$	$138.75 \pm 8.86^{**}$	$113.85 \pm 2.74^{**}$	3.35 ± 0.20^{ns}
400 mg/kg n-Hexane	$149.7 \pm 12.70^{**}$	$145.85 \pm 8.34^{**}$	$119.95 \pm 1.36^{**}$	3.23 ± 0.13^{ns}
200 mg/kg Ethyl acetate	$90 \pm 1.62^{**}$	$85.05 \pm 5.40^{**}$	$95.1 \pm 6.58^{**}$	3.15 ± 0.10^{ns}
400 mg/kg Ethyl acetate	$76.3 \pm 7.97^{**}$	$71.7 \pm 5.31^{**}$	$91.05 \pm 5.98^{**}$	3.16 ± 0.03^{ns}
200 mg/kg Butanol fraction	$97.95 \pm 2.34^{**}$	$163.7 \pm 11.03^{**}$	$151.1 \pm 9.64^{**}$	3.28 ± 0.15^{ns}
400 mg/kg Butanol fraction	$80.8 \pm 5.14^{**}$	$128.5 \pm 4.82^{**}$	$136.75 \pm 6.90^{**}$	3.27 ± 0.42^{ns}
200 mg/kg H_2O fraction	$103.15 \pm 13.42^{**}$	$167.4 \pm 16.51^{**}$	$165.15 \pm 7.94^{**}$	3.27 ± 0.04^{ns}
400 mg/kg H_2O fraction	$96.15 \pm 16.08^{**}$	$138.65 \pm 3.09^{**}$	$162.25 \pm 2.05^{**}$	3.25 ± 0.49^{ns}

Values are expressed as mean ± SEM (n = 5); ** $p < 0.01$, *$p < 0.05$, $^{ns}p > 0.05$ vs. control, p.o: per oral

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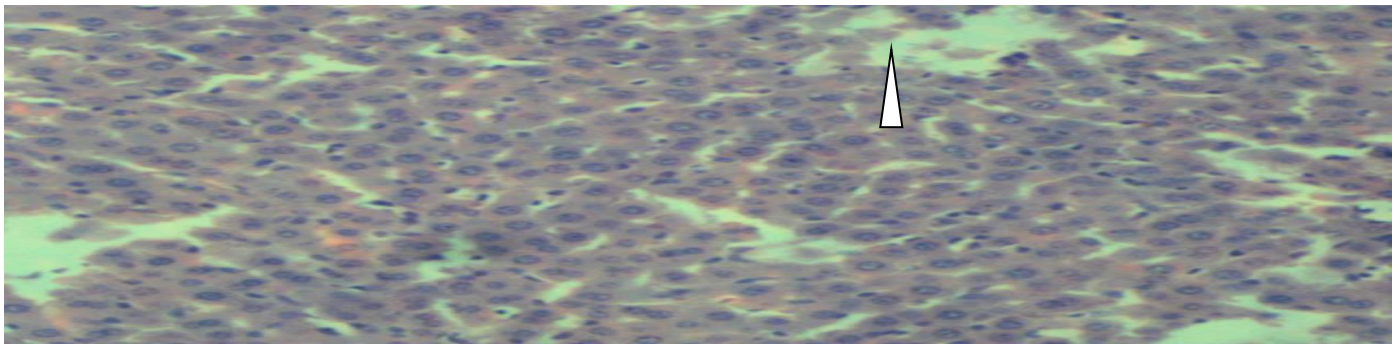

Figure 1: Photomicrograph of untreated liver tissue (normal control) showing normal hepatocytes with central vein and sinusoidal dilation (×100, H&E)

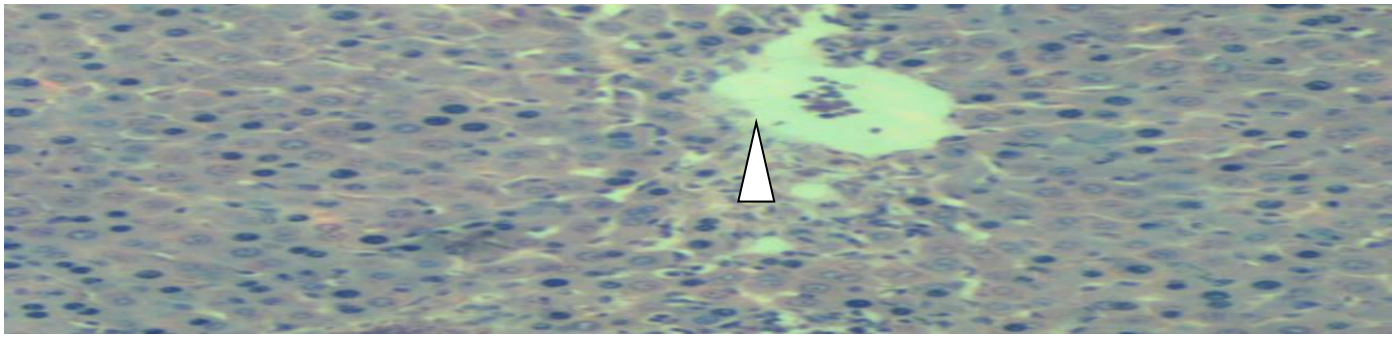

Figure 2: Photomicrograph of liver tissue treated with 1800 mg/kg PCM showing focal degeneration of interstitial tissue and hepatocytes (× 100(H&E)

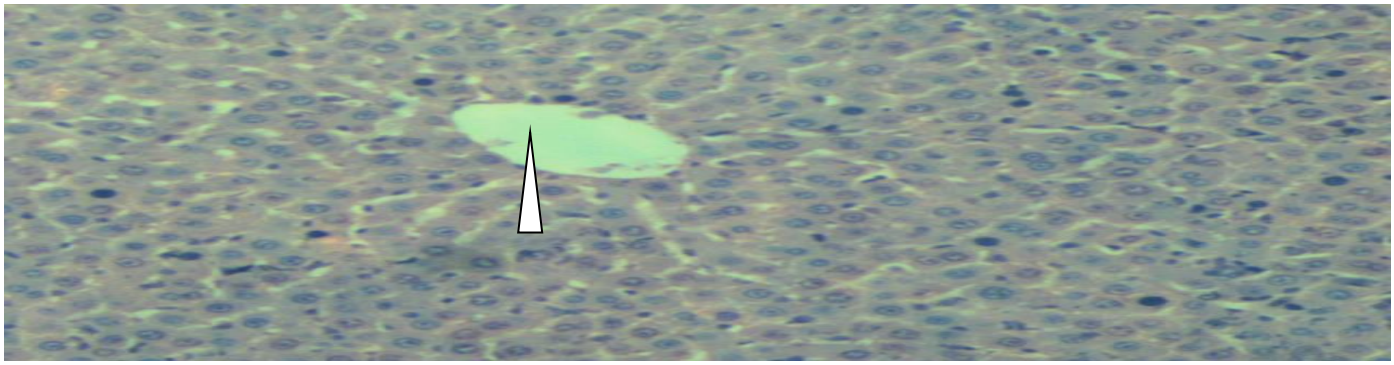

Figure 3: Photomicrograph of liver tissue treated with 100 mg/kg Silymarin showing normal hepatocytes and central vein (× 100, H&E)

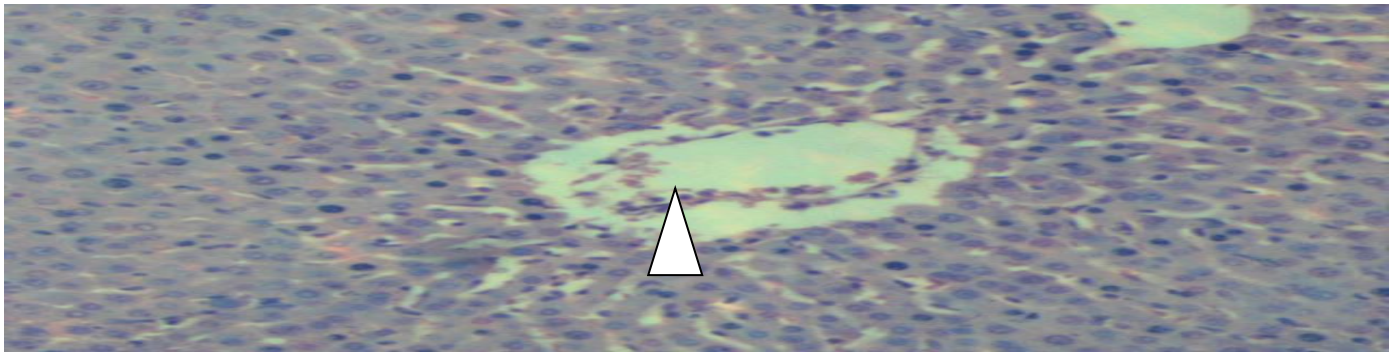

Figure 4: Photomicrograph of liver tissue treated with 100 mg/kg extract showing normal hepatocytes but with distorted endothelial lining of the central vein (× 100, H&E)

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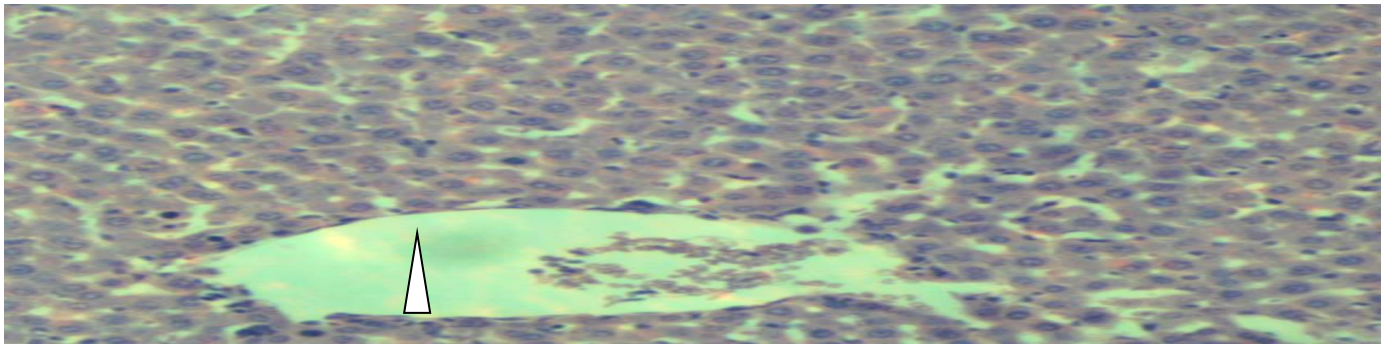

Figure 5: Photomicrograph of liver tissue treated with 200 mg/kg extract showing normal hepatocytes with moderately hypertrophied central vein (× 100, H&E)

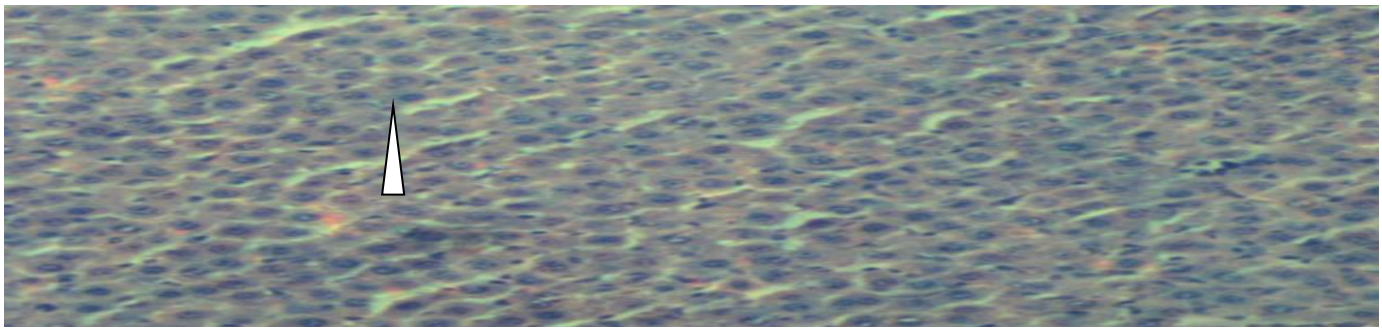

Figure 6: Photomicrograph of liver tissue treated with 400 mg/kg extract showing normal hepatocytes and sinusoids (× 100, H&E)

Table 4: Effect of crude extract and its fraction of *F. cienkowskii* on antioxidant enzymes and lipid peroxidation in PCM induced liver damage

Treatment & doses	SOD (U/mL protein)	CAT (U/mL protein)	MDA (U/mL protein)
10 mL/kg Distilled Water	$0.45 \pm 0.16^{**}$	$0.15 \pm 0.01^{*}$	1.79 ± 0.58^{ns}
100 mg/kg Ascorbic Acid	$1.87 \pm 0.06^{**}$	$0.2 \pm 0.01^{*}$	3.22 ± 0.67^{ns}
50 mg/kg Ethanol Extract	$1.83 \pm 0.04^{**}$	$0.41 \pm 0.04^{**}$	2.71 ± 0.22^{ns}
100 mg/kg Ethanol Extract	$1.45 \pm 0.02^{**}$	$0.33 \pm 0.04^{**}$	2.64 ± 0.73^{ns}
300 mg/kg Ethanol Extract	$1.67 \pm 0.16^{**}$	$0.74 \pm 0.47^{**}$	3.51 ± 0.6^{ns}
200 mg/kg n-Hexane fraction	$1.4 \pm 0.04^{**}$	0.31 ± 0.02^{ns}	$2.06 \pm 0.03^{**}$
400 mg/kg n-Hexane fraction	$1.03 \pm 0.04^{**}$	0.23 ± 0.0^{ns}	$2 \pm 0.01^{**}$
200 mg/kg EA fraction	$0.56 \pm 0.07^{**}$	$0.15 \pm 0.01^{**}$	$1.51 \pm 0.09^{**}$
400 mg/kg EA fraction	$0.2 \pm 0.02^{**}$	$0.1 \pm 0.04^{**}$	$1.21 \pm 0.08^{**}$
200 mg/kg Butanol fraction	$0.74 \pm 0.04^{**}$	$0.23 \pm 0.01^{**}$	$1.97 \pm 0.03^{**}$
400 mg/kg Butanol fraction	0.68 ± 0.03^{ns}	$0.2 \pm 0.01^{**}$	$1.91 \pm 0.02^{**}$
200 mg/kg H_2O	0.7 ± 0.01^{ns}	$0.21 \pm 0.01^{**}$	$1.85 \pm 0.01^{**}$
400 mg/kg H_2O	0.67 ± 0.02^{ns}	$0.17 \pm 0.01^{**}$	$1.81 \pm 0.03^{**}$

Values are expressed as mean ± SEM (n = 5); ** $p < 0.01$, *$p < 0.05$, $^{ns}p > 0.05$ vs. control, p.o: per oral. EA = Ethyl acetate

Gibb-3-ene-1,10-dicarboxylic acid, 2,4a,7-trihydroxy-1-methyl-8-methylene-, 1,4a-lactone, 10-methyl ester, (1α,2*β*,4aα,4b*β*,10*β*) is a terpene, and its pharmacological use includes antioxidant activity and hepatoprotective activity.[71]

Butanoic acid derivative, 1a,2,5,5a,6,9,10,10a-octahydro-5a-hydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-6,11-dioxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-5-yl-ester,[1aR-(1aα,2α,5*β*, 5a*β*,8aα,9α,10aα)] therapeutic uses includes antioxidant, antidiabetic, antidepressant and antibacterial properties.[65] The moiety attached to the derivative will determine, whether the moiety substitutes will enhance or reduce the activities of their parent compounds. Therefore, these constituents have reported therapeutic potentials including hepatoprotective and antioxidant activities and also the search and discovery of new hepatoprotective and antioxidant constituents.

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Table 5: DPPH Antioxidant Activity of the Extract and Fractions

Treatment & doses	500 µg	250 µg	125 µg	62.5 µg	31.25 µg
Crude Extract	0.33 ± 0.030**	0.4 ± 0.02 ns	0.46 ± 0.005**	0.54 ± 0.020***	0.56 ± 0.006 ***
n-Hexane Fraction	0.41 ± 0.006**	0.43 ± 0.00 ns	0.48 ± 0.005***	0.56 ± 0.012***	0.58 ± 0.002 ***
Ethyl acetate Fraction	0.29 ± 0.006**	0.35 ± 0.005**	0.42 ± 0.005**	0.46 ± 0.004**	0.51 ± 0.004**
Butanol Fraction	0.48 ± 0.004**	0.5 ± 0.001**	0.53 ± 0.002**	0.59 ± 0.002**	0.62 ± 0.003**
Water Fraction	0.59 ± 0.005ns	0.59 ± 0.002**	0.62 ± 0.002**	0.61 ± 0.003 **	0.62 ± 0.002**
Ascorbic Acid (Positive control)	0.03± 0.0003**	0.04 ± 0.0001ns	0.06 ± 0.0009 ns	0.1 ± 0.0005 ns	0.11 ± 0.001*

Values are expressed as mean ± SEM (n = 5); ** p < 0.01, *p < 0.05, ns p > 0.05 vs. control, p.o: per oral

Table 6: Metabolites from GC-MS Analysis of *F. cienkowskii* leaves ethanol extract (FC1)

S/N	Phytochemical Compounds	Retention time(Min)	Molecular Formular	Molecular weight (g/mol)
1	Ursodeoxycholic acid	16.953	$C_{24}H_{40}O_4$	392
2	Ethyl iso-allocholate [Ethyl-3,7,12-trihydroxycholan-24-oate]	16.593	$C_{26}H_{44}O_5$	436
3	Dodecanoic acid derivative [Lauric acid derivative]	14.387	$C_{32}H_{48}O_6$	528
4	Tetradecanoic acid derivative [Myristic acid derivative]	14.088	$C_{31}H_{50}O_6$	518

FC1: Ethanol crude extract of *F. cienkowskii* leaves

Table 7: Metabolites from GC-MS Analysis of *F. cienkowskii* leaves n-Hexane fraction (FC2)

S/N	Phytochemical Compounds	Retention time(Min)	Molecular Formular	Molecular weight (g/mol)
1	Heptatriacotanol **[**Heptatriacontanol]	16.953	$C_{37}H_{76}O$	536
2	Hexadecanoic acid derivative [Palmitic acid]	16.377	$C_{36}H_{58}O_6$	586
3	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde[2-[(1E,3E,5E)-4-Methyl-6-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,3,5-hexatrienyl]-1-cyclohexene-1-carbaldehyde]	14.316	$C_{23}H_{32}O$	324
4	Tetradecanoic acid derivative [Myristic acid]	14.088	$C_{31}H_{50}O_6$	518
5	Chlordiazepoxide [1. 3H-1,4-Benzodiazepin-2-amine,7-chloro-N-methyl-5-phenyl-, 4-oxide	13.477	$C_{16}H_{14}ClN_3O$	299
6	Pregn-4-ene-3,20-dione, 17,21-dihydroxy-, bis O-methyloxime[17,21-Dihydroxypregn-4-ene-3,20-dione bis(*O*-methyloxime)]	4.722	$C_{23}H_{36}N_2O_4$	404

FC2: n-Hexane fraction of *F. cienkowskii* leaves

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Table 8: Metabolites from GC-MS Analysis of *F. cienkowskii* leaves ethyl acetate fraction (FC3)

S/N	Phytochemical Compounds	Retention time (Min)	Molecular Formular	Molecular weight (g/mol)
1	Ursodeoxycholic acid	16.953	$C_{24}H_{40}O_4$	392
2	Dodecanoic acid derivative [Lauric acid]	14.387	$C_{32}H_{48}O_6$	528
3	Tetradecanoic acid derivative [Myristic acid]	14.088	$C_{31}H_{50}O_6$	518
4	4H-Cyclopropa[5',6']benz[1',2':7,8] azuleno[5,6-b]oxiren-4-one,8-(acetyloxy)-1,1a,1b,1c,2a,3,3a, 6a,6b, 7,8,8a-dodecahydro-3a,6b,8a-trihydroxy 2a-(hydroxymethyl)-1,1,5,7-tetramethyl-(1aα, 1b*β*,1c*β*,2a*β*,3a*β*,6aα,6bα,7α,8*β*,8aα)	13.278	$C_{22}H_{30}O_8$	422

FC3: Ethyl acetate fraction of *F. cienkowskii* leaves

Table 9: Metabolites from GC-MS Analysis of *F. cienkowskii* leaves butanol fraction (FC4)

S/N	Phytochemical Compounds	Retention time(Min)	Molecular Formular	Molecular weight (g/mol)
1	Butanoic acid derivative, 1a,2,5,5a,6,9,10,10a-octahydro-5a-hydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-6,11-dioxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-5-yl ester, [1aR-(1aα,2α,5*β*,5a*β*,8aα,9α,10aα)]	17.625	$C_{24}H_{32}O_6$	416
2	Gibb-3-ene-1,10-dicarboxylic acid, 2,4a,7-trihydroxy-1-methyl-8-methylene-,1,4a-lactone, 10-methyl ester, (1α,2*β*,4aα,4b*β*,10*β*) **(**Terpene**)** [1.Gibberellic acid, methyl ester 2.Gibberellin A3 methyl ester 3.GA3 Methyl ester 4.Methyl gibberellate]	15.927	$C_{20}H_{24}O_6$	360
3	Dodecanoic acid derivative [Lauric acid]	14.387	$C_{32}H_{48}O_6$	528
4	Tetradecanoic acid derivative [Myristic acid]	14.088	$C_{31}H_{50}O_6$	518
5	4H-Cyclopropa[5',6']benz[1',2':7,8]azuleno[5,6-b]oxiren-4-one,8-(acetyloxy)-1,1a,1b,1c,2a,3,3a,6a, 6b,7,8,8a-dodecahydro-3a,6b,8a-trihydroxy-2a-(hydroxymethyl)-1,1,5,7-tetramethyl(1aα,1b*β*,1c*β*,2a*β*, 3a*β*,6aα,6bα,7α,8*β*,8aα)-[1.3a,6b,8a-Trihydroxy-2a-(hydroxymethyl)-1,1,5,7-tetramethyl-4-oxo-1a,1b,1c,2a,3,3a,4,6a,6b,7,8, 8a-dodecahydro-1H-cyclopropa[5',6']benzo[1',2':7,8] azuleno[5,6-b]oxiren-8-yl acetate]	13.307	$C_{22}H_{30}O_8$	422

FC4: Butanol fraction of *F. cienkowskii* leaves

Table 10: Metabolites from GC-MS Analysis of *F. cienkowskii* leaves water fraction (FC5)

S/N	Phytochemical Compounds	Retention time(Min)	Molecular Formular	Molecular weight (g/mol)
1	Hexadecanoic acid derivative [Palmitic acid]	16.380	$C_{36}H_{58}O_6$	586
2	Dodecanoic acid derivative [Lauric acid]	14.387	$C_{32}H_{48}O_6$	528
3	Tetradecanoic acid derivative [Myristic acid]	14.088	$C_{31}H_{50}O_6$	518

FC5: Water fraction of *F. cienkowskii* leaves

Conclusion

The effects of the ethanol extract and fractions led to a significant reduction ($p < 0.05$) in the liver and antioxidant enzymes activities, and DPPH free radicals. The GC-MS of the extract, fractions and sub-fractions identified twelve compounds,which poses reported therapeutic potentials, including hepatoprotective and antioxidant activities. This supports the claims by traditional herbalists that the plant could be used in the management of liver diseases and oxidative stress-related diseases. The pharmacognostic standards of *F. cienkowskii* could act as a reference point or baseline data on this plant for possible inclusion in the Pharmacopoeia.

Conflict of Interests

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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