Journal of Medicinal Herbs

journal homepage:**www.jhd.iaushk.ac.ir**

Pharmacognostic standardization and hepatoprotective effect of the aqueous leaf extract of *Lecanoidiscus cupanoides* Planch (ex. Benth) in CCL_4 induced liver damage in Swiss Albino mice

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ARTICLE INFO

Type: *Original Research*
Topic: *Medicinal Plants*
Received *June 10th 2023*
Accepted *September 21th 2023*

Key words:
- ✓ *Lecanoidiscus cupanoides*
- ✓ *standardization*
- ✓ *hepatoprotective*
- ✓ *aqueous extract*

ABSTRACT

Background & Aim: *Lecaniodiscus cupanioides* Planch (ex Benth) is a small, resilient shrub and a tropical plant effective in treating burns, fevers, liver disease, inflammatory conditions, among others. This study was carried out to establish the quality standards according to WHO recommendations and also investigate the hepatoprotective potential of the aqueous leaf extract of *Lecanoidiscus cupanoides*.

Experimental: Swiss albino mice were used in this study to test the hepatoprotective effect against carbon tetrachloride-induced hepatotoxicity and to determine the pharmacognostic characteristics of the leaf of the aforementioned plant.

Results: According to the physicochemical analysis, the moisture content was 10.5± 0.13, the total ash was 5.00 ± 0.25, the acid insoluble ash was 0.75 ± 0.03, the alcohol soluble extractive was 17.25 ± 0.76, and the water extractive value was 14.25 ± 0.50. The macroscopic examination of the fresh leaves indicated that when dried, the leaves were meaty and leathery. Microscopically, prism-shaped calcium oxalate crystals, starch grains, lignified tissues, wavy epidermal cells, trichomes, xylem, parenchyma cells, anomocytic stomata, and palisade tissue were found. The presence of oxalate crystals, starch grains, lignified tissues, tannins, cellulose, protein, and oil was revealed in chemomicroscopic analysis. Pretreatment with aqueous extract of *Lecanoidiscus cupanoides* at 200 and 400 mg/kg significantly ($P<0.05$) protected against CCL_4 induced liver toxicity just like the reference standard (silymarin).

Recommended applications/industries: This study validates the ethnomedicinal claim of *Lecanoidiscus cupanoides* against liver disorder. We recommend that well standardized aqueous formulation would be beneficial for the populace as preventive remedy against liver disease.

1. Introduction

Hepatotoxicity, or liver injury, is caused by hepatotoxins, which can come from chemicals, dietary supplements, pharmaceutical medications, medicinal plants among others (Hussain *et al.,* 2017). Although, synthetic or conventional hepatoprotective drugs have been limited due to some side effects (Deepa *et al.,*

2014), natural products, especially medicinal plants, that are accessible, affordable, relatively safe are now used as alternatives (Ahlem *et al.,* 2015).

Various medicinal plants are used to treat illness as alternative to conventional medicine. Some therapeutic herbs have been reported to protect the liver from injury, while others cause liver injury (Thompson *et al.,* 2017).

Lecaniodiscus cupanioides is a tropical plant widely distributed in Africa and Asia. It belongs to the Sapindaceae family and it is identified by various names in Nigeria, such as Ukpo (Igbo), Utantan (Edo), Kafi-nama-zaki (Hausa), and Akika (Yoruba). The plant is ethnomedically reputed to be useful in the treatment of wounds and sores, abdominal swelling caused by liver abscess, fevers, measles, burns, among others (Yemitan and Adeyemi, 2005).

The extracts and phytochemicals of *L. cupanioides* have been investigated to possess antibacterial, anticancer, aphrodisiac, antifungal, cytotoxic, antidiabetic, antiprotozoal, antioxidant, antidiarrhoeal, analgesic and ameliorative properties (Taofeek *et al.,* 2014). As an avenue to validate the ethnomedicinal claim of *Lecanoidiscus cupanoides* against liver disease, we investigated the Pharmacognostic standardization and hepatoprotective effect of its aqueous leaf extract in CCL_4 induced liver damage model using swiss albino mice.

2. Materials and Methods

2.1. Animals

Male swiss albino mice (25 – 30 g) were obtained from the animal house of the Department of Pharmacology, Enugu State University of Science and Technology, Enugu State, Nigeria. Animals were allowed to acclimatize for one week prior to the commencement of the study. Food and water were provided *ad libitum*. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals (National Institute of health (NIH) (2011) Pub No: 85-23).

2.1.1. Ethical approval

The ethical review committee of the college of pharmaceutical sciences at Chukwuemeka Odumegwu ojukwu University gave the animal study ethical clearance and issued an ethical approval number, PHACOOU/AREC/2023/019.

2.2. Plant collection

The healthy leaves of *Lecanoidiscus cupanoides* Planch were collected in July, 2022 from Orba, Nsukka, Enugu state, Nigeria. Proper identification and authentication were done by a taxonomist, Mr. Felix Nwafor in Department of Pharmacognosy and Environmental Science, University of Nigeria, Nsukka, with voucher reference number “UNH/04/0330C”. The fresh leaves weighing about 3 kg were cleaned and air/shade dried at room temperature. About 2 kg of the dry sample was pulverized.

2.3. Macroscopy

The leaves were examined with unaided senses (naked eyes, nose, and hands). The color was examined using an untreated sample under diffuse daylight. The macroscopic characters of the leaves which include types of margin, venation, base apex, mid-rib, size, etc. were observed and noted. The organoleptic properties such as colour, texture, odour, and taste of the plant material were also observed and noted (Evans, 2009).

2.4. Pharmacognostic standardization

2.4.1. Microscopic examination of powdered leaves

2.4.1.1. Fresh leaf microscopy

Foliar epidermis of the adaxial (upper surface) and abaxial (lower surface) surfaces of the leaves were prepared by clearing method. The leaf samples were cleared by soaking in commercial bleach “Hypo” containing 3.5% sodium hypochlorite for 18 hrs. Then, the epidermal strips of the leaf samples were scrapped gently with the aid of a pair of forceps and placed on a clean slide, and then stained with Safranin solution and covered with a cover slip. The slides were viewed under a light Olympus Tokyo (Japan No. 271961) microscope at x40, x100 and x400 magnifications and photomicrographs were taken with a Motican Camera 2.0. All parameters were observed on both the adaxial and abaxial surfaces of the leaves (Nwafor *et al.,* 2019).

2.4.1.2. Quantitative microscopy

This was determined following standard methods (Evans, 2002). A constant range of values for palisade

ratio, stomatal number, stomata index, vein-islet number and veinlet termination number were obtained.

2.4.1.3. Qualitative microscopy

The powdered leaf (1 g) was placed on a slide, two drops of chloral hydrate solution was added to moisten the powder and also act as a clearing agent. The slide was passed across the flame of a burnsen burner repeatedly until bubbles occurred. It was allowed to cool and the slide was covered with glycerin followed with cover slip and was viewed under the microscope. The microscopic characters were observed such as the palisade cells and lignified vascular tissues were noted

2.4.1.4. Microscopic examination of transverse section (TS) of the leaf

Transverse section (TS) of the leaf was made using a Reichert sledge microtome following the procedures of Johansen (1950) and Nwosu (2006). The sections were microtomed at 10-15 unimicrons and were picked with the aid of a camel hair brush from the tip of the microtome knife into separate Petri dishes containing 70% absolute alcohol and labeled appropriately. Safranine and Fast green served as biological stains in differentiating lignified tissues. Quality control standardization is very useful in the assessment of integrity of crude drugs from natural origin which includes proper identification, purity and quality of medicinal plant (Ezeonyi *et al.,* 2022).

2.4.1.5. Chemomicroscopy

The leaves were dried under shade and pulverized with local mortar and pestle. Chemo-microscopy checked on the powders to determine the presence of starch, calcium oxalate crystals and lignified vessels. The pulverized powdered leaves (1 g) were dropped on a glass slide. One drop of chloral hydrate was dropped and passed over a Bunsen burner repeatedly until bubbles formed. This signified the successful clearing of the tissues. The cleared sample was then used for the chemo-microscopy (Venkateswarlu and Ganapathy, 2018).

2.5 Physicochemical Evaluation

Analysis of physicochemical constants of the leaf powdered drug was determined to evaluate the quality and purity of the drug (Mukherjee, 2002; WHO, 2011). Parameters such as total ash, water-soluble ash and acid-insoluble ash values were calculated as per WHO guidelines. Alcohol and water-soluble extractive values were determined to find out the amount of water and alcohol soluble components.

2.6 Total Phenolic content determination

The total phenolic content of the extract were determined using folin ciocalteu's method described by Kim *et al*. (2003). One milliliter of the extracts (100 µg/mL) was mixed with 0.2 mL of Folin-Ciocalteu's phenol reagent. After 5 min, 1 mLl of 7.6% Na_2CO_3 solution was added to the mixture followed by the addition of 2 mL of distilled water. The mixture (in duplicate) was incubated at 40°C for 30 min, after which the absorbance were read at 760 nm using UV-VIS spectrophotometer against blank (containing every other component of the mixture except sample). The total phenolic content was estimated from the calibrated curve which was made by preparing gallic acid solution and expressed as milligrams of gallic acid equivalent (GAE) per gram of the extracts.

2.7. In vitro antioxidant assay

2.7.1. Free radical (DPPH scavenging test)

This assay was carried out as described by Erhihie *et al.* (2020). DPPH (1, 1 -diphenyl -2- picrylhydrazil) solution (0.6 mM) was freshly prepared using methanol. The reaction mixtures which contain 0.25 mL of various concentrations (31.25, 62.5, 125, 250, 500 and 1000 ug/mL) of samples. For ascorbic acid, 1.96, 3.91, 7.82, 15.63, 31.25, 62.5, 125, 250, 500 and 1000ug/mL were used. Samples/ascorbic acid were mixed with 0.25 of 0.6 mmol DPPH and 2 mL of ethanol were incubated in the dark for thirty minutes at room temperature. Thereafter,the absorbance of the mixture was measured spectrophotometrically at 517 nm. A tube containing 0.25 mL of DPPH solution and 2.25 ml of ethanol served as a control. Assays were carried out in triplicates. Free radical scavenging activities of the samples were obtained using the formula below:

DPPH scavenging activity=100 × (AC-AS)/AC).

Where, AC= Absorbance of control and AS= Absorbance of sample. A graph of percentage inhibition against concentration was plotted and the concentration at 50% inhibition (IC_{50}) was extrapolated using an equation.

2.7.2. Ferric Reducing Antioxidant Power (FRAP) assay

FRAP assay was carried out following the method described by Habibur *et al.* (2013). Two hundred and fifty microlitre (0.25 mL) of various concentrations (31.25, 62.5, 125, 250, 500 and 1000 ug/mL) of samples and ascorbic acid. For ascorbic acid, (1.96, 3.91, 7.82, 15.63, 31.25, 62.5, 125, 250,500 and 1000 ug/mL) were mixed with 0.625 ml of phosphate buffer and 0.625 mL of 1% potassium ferricyanide (K3FeCN6). The mixtures were heated at 50°c for twenty minutes. Then 0.625 mL of 10% trichloroacetic acid (TCA) was added and the mixtures were centrifuged at 3000 rpm for five minutes. From the upper layer, 0.625 mL was pipetted and mixed with 0.625 ml of distilled water and 0.125 mL of 0.1% (w/v) ferric chloride solution ($FeCl_3$) solution. Absorbances of the mixture were measured at 700 nm against air using a Spectrophotometer. Tests were performed in Triplicates. Percentage inhibition was calculated using the formula below:

% inhibition= (Absorbance of sample - Absorbance of blank) x 100

For ascorbic acid, a graph of percentage inhibition against concentration was plotted and the effective concentration (EC_{50}) was extrapolated using an equation.

2.8. Hepatotoxicity study: CCl_4 induced oxidative stress

2.8.1. Experimental design

Twenty-five swiss albino mice were divided into 5 groups of five animals each and treated as follows for 10 days. Groups 1 and 2 were treated with 200 and 400 mg/kg of the extract, respectively, based on its previously established LD_{50} values (Oloyede *et al.,* 2020). Groups 3 served as the normal control (uninduced). Group 4 served as reference/standard (100 mg/kg of Silymarin) while 5th group was served as CCL_4 control. Six hours after the last day of the treatment, oxidative stress was induced with carbon tetrachloride (CCL_4, 2 mL/kg) intraperitoneally in groups 1, 2, 4, 5 except group 3 (naive group).

Eighteen hours later, blood sample was collected from animals through retro-orbital puncture and allowed to clot for 30 minutes and then centrifuged at 2500 rpm for 15 min and the serum was used for the estimation of serum liver marker enzymes - ALP, AST, ALT- and lipid peroxidation (Yesufu *et al.,* 2010).

2.8.2. Determination of liver biomarkers and lipid peroxidation (LPO) in serum

Serum alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Alkaline phosphatase were estimated by the method described by Colville (2002) using commercial test kit (Span Diagnostics Ltd., India). The level of thiobarbituric acid reactive substance (TBARS) and malondialdehyde (MDA) production was measured in serum by the modified method as described by Draper and Hadley (1990).

2.9. Statistical Analysis

The data obtained were expressed as mean ± SEM. Mean statistical analysis were done using SPSS version 20. All data were analyzed by Kruskal-Wallis ANOVA test. The differences between treatments were compared with multiple comparisons of mean ranks for all groups. In all cases, a probability error of less than 0.05 was selected as the criterion for statistical significance.

3. Results and discussion

3.1. Pharmacognostic characteristics

3.1.1. Macroscopic character

Modern research tools for analyzing plant treatments are now available, but the microscopic method remains one of the simplest and least expensive ways to begin discovering the correct identity of the parent materials. The macroscopic features were useful in the rapid identification of plant material and also served as an essential standardization specifications. Macroscopic examination of *Lecanoidiscus cupanoides* leaves revealed that the leaf is alternate, acute base and cupsidate apex type. It also has crenate margins, ovate/lanceolate shape of the lamina, pinnately parallel venation. The fruits are ovoid drupe and an orange colour when ripe (Figure 1).

Fig 1. Image of *Lecaniodiscus cupanioides* growing in its habitat.

3.1.2. Microscopic character

Microscopical examination of the leaf will aid in the identification of the crude drug. The quantitative estimation of several pharmacognostic characteristics is useful for creating standards for crude drugs. The assessment of stomatal number, stomatal index value, vein islet, and vein termination value is important in the standardization of phytomedicine.

The transverse section investigation revealed that the leaf had a single-layered epidermis. This is followed by a series of column-shaped palisade mesophyll cells and spongy mesophylls. The cup-shaped vascular bundle (consisting of phloem, cambium, and xylem) and central pith were evident. There are trichomes present (Figure 2).

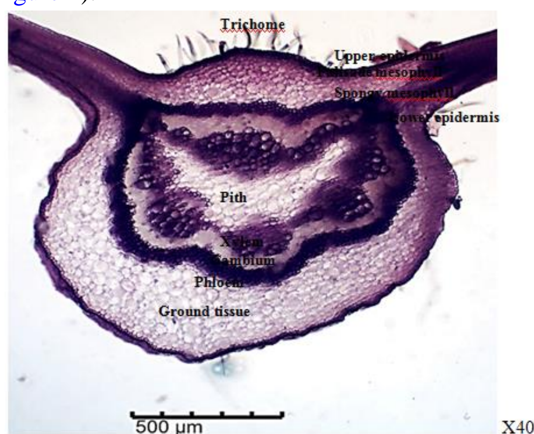

Figure 2. Transverse section of leaf of *Lecanoidiscus cupanoides*

The leaf is hypostomatic (stomata occur only on the lower surface) with anomocytic (various subsidiary cells surrounding it) type of stomata. Epidermal cells are irregularly shaped with wavy anticlinal cell walls on both the upper and lower surfaces. Adaxial surface of the leaf of *Lecanoidiscus cupanoides* showing wavy epidermal cells (red arrow) and glandular trichome (yellow arrow) (Figure 3). Abaxial surface of the leaf of *Lecanoidiscus cupanoides* showing anomocytic type of stomata (yellow arrow) and wavy epidermal cells (red arrow). Glandular and covering unicellular trichomes (black arrow) present (Figure 4). The microscopy of the leaf fragment depicted a venation pattern: vein islet (blue arrow) and veinlet termination (red arrow) as seen in Figure 5. Microscopy of the leaf fragment showing lignified vessel (white arrow) and fibre (yellow arrow) elements. Prism-shaped calcium oxalate crystals (blue arrow) are also present in the tissues (Figure 6). Powder microscopy of the leaf revealed presence of palisade cells and lignified vascular tissues (Figure 7).

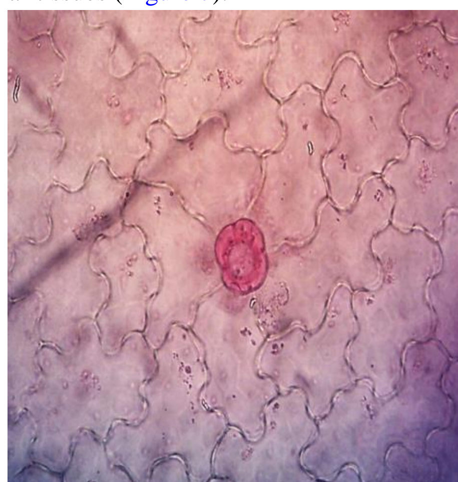

Figure 3. Adaxial surface of the leaf of *Lecanoidiscus cupanoides* showing wavy epidermal cells (red arrow) and glandular trichome (yellow arrow) x400.

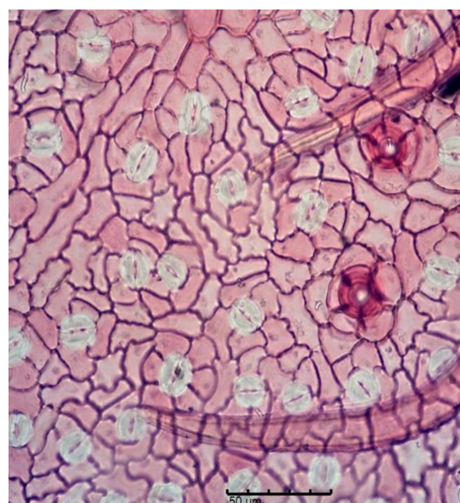

Figure 4. Abaxial surface of the leaf of *Lecanoidiscus cupanoides* showing anomocytic typeof stomata (yellow arrow) andwavy epidermal cells (red arrow). Glandular and covering unicellular trichomes (black arrow) present x400.

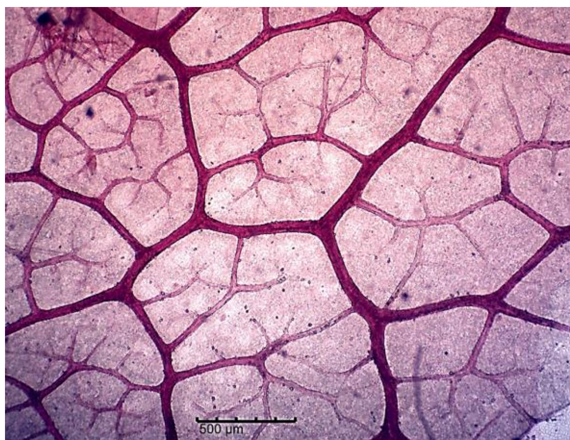

Figure 5. Photomicrograph of the leaf fragment showing the venation pattern: vein islet (blue arrow) and veinlet termination (red arrow) X400.

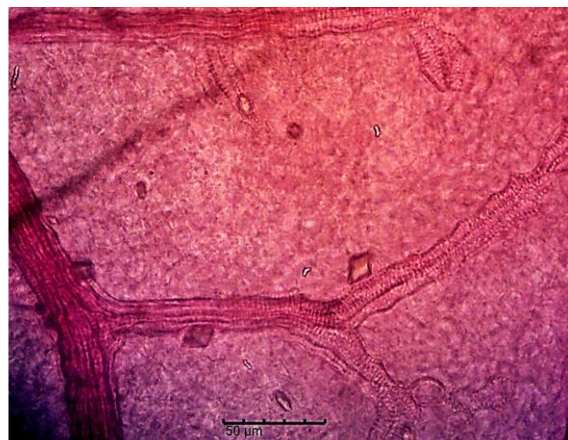

Figure 6. Microscopy of the leaf fragment showing lignified vessel (white arrow) and fibre (yellow arrow) elements. Prism-shaped calcium oxalate crystals (blue arrow) are also present in the tissuesX400.

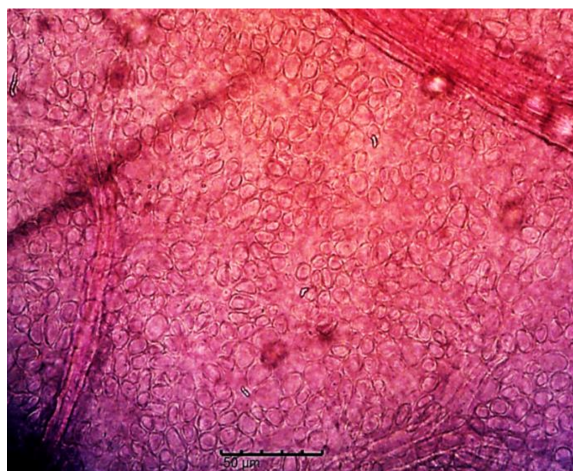

Figure 7. Powder microscopy of the leaf showing palisade cells and lignified vascular tissues.

3.1.3. Quantitative investigation

Table 1 revealed the different microscopical features of *Lecaniodiscus cupaniodes* leaf. It showed 30.25 ± 0.48 stomata number per field of view, stomata density of 177.94 ± 2.82 mm^{-2} while the stomata length and width are 18.51 ± 0.60 µm and 14.58 ± 0.21 µm, respectively. Another feature of the leaf is the stomatal index, which was found in the lower epidermis as 17.64 ± 0.19. The vein islet present in the leaf is 129.41 mm^{-2}and vein termination number found in the leaf is 164.71 mm^{-2}.

Table 1. Quantitative leaf microscopy of *Lecanoidiscus cupanoides.*

Characteristics	Description
Epidermal cell	Epidermal cells are irregularly shaped with wavy anticlinal cell walls on both the upper and lower surfaces.
Stomata type	The leaf is hypostomatic (stomata occur only on the lower surface) with anomocytic (various subsidiary cells surrounding it) type of stomata.
Trichome	Present – both glandular and covering unicellular trichomes
Stomata number	30.25 ± 0.48 per field of view
Stomata density	177.94 ± 2.82 mm^{-2}
Stomata length	18.51 ± 0.60 µm
Stomata width	14.58 ± 0.21 µm
Stomata size	270.16 ± 11.18 µm^{2}
Stomata index	17.64 ± 0.19
Vein islet number	129.41 mm^{-2}
Veinlet-termination number	164.71 mm^{-2}

3.2. Chemomicroscopical constituents

Table 2 revealed the presence of starch gain, lignified tissues, calcium oxalates, tannin, cellulose, gum/mucilage and oil. However, gum/mucilage and proteins were absent.

Table 2. Showing the presence and absence of different chemomicroscopical constituents.

Parameter	Reagent(s)	Result
Starch grains	Iodine solution	Present
Lignified tissues	Concentrated. HCl + Phloroglucinol	Present
Calcium oxalates	Iodine solution Concentrated Sulphuric acid	Present; Prism-shaped
Tannin	Ferric chloride	Present
Cellulose	Zinc chloride; Concentrated. Sulphuric acid	Present
Gum/Mucilage	Ruthenium red	Absent
Protein	Biuret reagent; Nihydrin	Absent
Oil	Sudan III reagent	Present

3.3. Physiochemical parameters

The moisture content in the leaf of *Lecaniodiscus cupaniodes* was found to be 10.5 ± 0.13. The ash values were as follows: 3.3 ± 0.29 total ash, 1.53 ± 0.08 water soluble ash and 0.75 ± 0.03 acid insoluble ash while the alcohol soluble extractive value and water soluble extractive value were 17.25 ± .76 and 14.25 ± 0.50, respectively (Table 3).

Table 3. Physiochemical evaluation of *Lecaniodiscus cupaniodes.*

Parameters	% Composition
Total ash	5.00 ± 0.25
Water soluble ash	1.53 ± 0.08
Acid insoluble ash	0.75 ± 0.03
Moisture content(Loss on drying)	10.50 ± 0.13
Alcohol soluble extractive value	17.25 ± 0.76
Water soluble extractive value	14.25 ± 0.50

(Values are expressed as Mean ± SEM, n=3).

The moisture content of crude drugs are critical and there are specified limits established by the British Pharmacopoeia (BP) to ensure high quality of such drugs. Most drugs may be stored safely if the moisture content is reduced by 6% or less (Table 3).

3.4. Preliminary phytochemical analysis

Phytochemical screenings of the aqueous extract of *L. cupanioides* revealed the presence of alkaloids, phenolics, saponins, tannins and Triterpenoids, cardiac glycoside, Carbohydrates, Proteins, steroids, and flavonoids (Table 4). Presence of these phytoconstituent substantiates the medicinal values and applications of *L. cupanioides* against various diseases.

Table 4. Preliminary phytochemical analysis.

Phytoconstituents	Methods	Aqueous Extract
Alkaloids	Wagner test	Present
	Hager's test	Present
	Drangendorff test	Present
Flavonoids	Lead acetate test	Present
	Zn.Hydrocholride test	Present
Tannins & Phenols	$Fecl_3$ test	Present
Saponins	Foamingtest	Present
	Emulsion test	Present
Steroids& Triterpenoids	Salkowski test	Present
Glycosides	Killer killani	Present
	Leiberman Buchard's test	Present
Carbohydrates	Molisch test	Present
Proteins	Biuret	Present

3.5. Total phenolic contents

From Table 5, aqueous extract produced the highest total phenolic content of 316.61 GAE/g when compared with ethyl acetate extract (90.50 GAE/g), dichloromethane, DCM (53.84 GAE/g), and methanol (145.22 GAE/g).

Table 5. Effect of solvent on total phenolic content.

Solvent	GAE/g extract
Ethyl acetate	90.50
DCM	53.84
Methanol	145.22
Water	316.61

3.6. In-vitro antioxidant result

From the antioxidant result, *L. cupanioides* has IC_{50} of 118.69 µg/mL in DPPH scavenging assay, and EC_{50} value of 329.78 µg/mL in FRAP assay (Table 6).

Table 6. In-vitro antioxidant result of *Lecaniodiscus cupaniodes*.

	DPPH scavenging activity:IC_{50}	FRAP: EC_{50}
L. cupanioides	118.69 µg/ mL	329.78 µg/mL
Ascorbic acid	5.58 µg/ mL	41.89 µg/mL

Though, these values are different when compared with ascorbic acid, the level of antioxidant activity of the aqueous extract is an indication that it has good antioxidant activity.

3.7. Effects of the extract on liver biomarkers and lipid peroxidation

The result of the effect of the extract on carbon tetrachloride (CCL_4) induced hepatotoxicity on liver function enzymes revealed over double fold

statistically significant (*P<0.05) increase in serum concentrations of ALT, AST, ALP and MDA when normal control group was compared with induced control group (Table 7) Pretreatment with the extract at 200 and 400 mg/kg of the extract and Sylimarin significantly (P<0.05) reversed the elevated levels of ALT, AST, ALP and MDA. When compared with Sylimarin, the extract did not produce statistically significant alteration in ALT, AST, ALP and MDA levels (Table 7).

Table 7. Effects of the extract on liver biomarkers and lipid peroxidation.

	ALT (U/L)	AST (U/L)	ALP (IU/L)	MDA (umol/ml)
Extract; 200 mg/kg	59.60 ± 4.07*	69.00 ± 2.43*	100.06 ± 3.54*	0.81 ± 0.01*
Extract; 100 mg/kg	44.40 ± 2.48*	50.80 ± 2.85*	75.60 ± 3.94*	0.68 ± 0.01*
Sylimarin; 100 mg/kg	43.80 ± 2.91*	52.60 ± 3.41*	83.20 ± 2.82*	0.71 ± 0.02*
Induced control	72.00 ± 2.43	88.60 ± 3.14	128.00 ± 4.97	1.20 ± 1.10
Normal control	30.20 ± 1.71*	41.00 ± 2.19*	73.60 ± 2.54*	0.52 ± 0.02*

Values are presented as mean ± Standard deviation. *P<0.05 compared to induced control. ALT (Alanine Transaminase), AST (Aspartate Transaminase), ALP (Alkaline Phosphatase), MDA (Malondialdehyde).

Assay of liver biomarkers and lipid peroxidation is of immense importance in the evaluation of liver toxicity. ALT, ALP, and AST are common liver enzymes utilized as biological indicators of liver damage (Hustead *et al.,* 2017). Aspartate aminotransferase (AST) is an essential enzyme in the metabolism of amino acids that catalyzes the reversible transfer of α-amino group from aspartate to glutamate. It is a pyridoxal phosphate dependent transaminase enzyme. While AST is found in the heart, kidney, red blood cells, liver, skeletal muscles, and brain, it is often used clinically as a marker for liver health (Wazis *et al.,* 2012). ALT specifically serves as an indicator of inflammation in the liver when compared to AST (Wazis *et al.,* 2012). ALP is frequently used to evaluate the plasma membrane integrity of the liver cells.

The result of the study revealed that CCL_4 induce liver damage as seen in over double-fold increase in serum concentrations of ALT, AST and increase in serum ALP when induced control group was compared with normal control (naïve group). These enzymes are concentrated mainly within the liver cells. Thus, increase in serum concentration of these enzymes is an indicator of liver damage.

Pretreatment with the extract at 200 and 400 mg/kg extract significantly (P<0.05) reversed increased ALT, AST and ALP levels, which suggests their protective effects against liver damaging effect of CCL_4. Also from previous studies, repeated administration of 250, 500, and 1000 mg/kg doses of the aqueous leaf extract of *L. cupanioides* over 21 days revealed no deleterious effect on body weight, hematological, liver, and kidney biomarkers, and histology (Ezeonyi *et al.,* 2024).

The effect of the extract on lipid peroxidation was monitored through serum concentration of malondialdehyde (MDA) – a product of lipid peroxidation. Increase in lipid peroxidation corresponds with increase in MDA. (Zou *et al.,* 2019). Comparison between the normal and induced control group showed that CCL_4 caused over double fold significant increase in lipid peroxidation just as was recorded in the liver function enzymes (ALT, AST and ALP). Treatment with the extract protected against lipid peroxidation as shown by significant (P<0.05) decrease in serum concentration of MDA when compared to the induced control group. The results also suggest that the extracts can be used as an enhancer due to its increased effects on the anti-oxidant parameters. This is strongly supported by the good antioxidant activity as well as high total phenolic content of the aqueous extract, which prompted its selection for the hepatoprotective study. A significant correlation has been established between agents with high phenolic content and good antioxidant activity (Erhirhie *et al.,* 2020).

Phenolic compounds from medicinal plants have played significant role in the treatment and prevention of oxidative stress and free radicals mediated tissue damage cannot (Li *et al.,* 2016). Serving as reducing agents, phenolic compounds have capacity to neutralize several forms of oxidizing free radicals due to their electron donating abilities and inhibiting lipid peroxidation in various biological systems, which was observed in this study (Erhirhie *et al.,* 2020).

4. Conclusion

The present study has established the macroscopic features of *Lecanoidiscus cupanoides* which is an

essential standardization specifications. Also, aqueous extract of *Lecanoidiscus cupanoides* at 200 and 400 mg/kg produced a protective effect against CCL_4 -induced liver toxicity, which validates the ethnomedicinal claim of *Lecanoidiscus cupanoides* in the treatment of liver disorders. Thus, well standardized aqueous extract of this *Lecanoidiscus cupanoides* would be of benefit for the populace as preventive remedy against liver disease.

5. Acknowledgments

The authors of this article would like to express their gratitude to the host of this work, Nnamdi Azikiwe University in Awka, Anambra State.

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