

Phytochemical Investigation of the Leaves Extract of *Lecaniodiscus cupanioides* in South West, Ogun State, Nigeria

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Abstract: Medicinal plants are consumed globally for the treatment of various illnesses and they also serve as important raw materials in pharmaceutical industries. Medicinal plants are also of great importance to the health of individuals and communities. The Biological activities of this plant lies in some chemical substances that produces biological and physiological action in the body. In this study, phytochemical investigation and anti-microbial screening were carried out in the leaves of *Lecaniodiscus cupanioides* extract. Extraction of the plant leaves was preceded using maceration procedure. The presence of some bioactive compounds of plant leaves were also investigated. The data obtained from the phytochemical investigation revealed the presence of flavonoids, tannins, saponins, steroids, phlobatannins, terpenoids and cardiac glycosides in the leaves extract. Similarly, the quantitative estimation of the chemical constituents carried out on the crude extract of the leaves of *Lecaniodiscus cupanioides* indicates the presence of alkaloids (10%), flavonoids (3%) and saponins (1%). In the same vein, antimicrobial screening on the crude methanolic extract of the leaves of *Lecaniodiscus cupanioides* revealed the presence of a very strong activity on both grame-positive and grame-negative, and fungi organism. The presence of medicinal constituents or bioactive components in the leaves of *lecaniodiscus cupanioides* extract indicates that the medicinal plant could be of great source of useful drugs in the treatment of various diseases.

Keywords: *Lecaniodiscus cupanioides*, Antimicrobial, Tioconazole, *Esherichia coli*, Bioactive

1. Introduction

Plants have been used to treat or prevent illnesses since before recorded history. Plants and plants-based medicines are the basis of many of the present pharmaceuticals we use today for our various illnesses or ailments. The discovery of medicinal plants has usually depended on the experience of the populace based on long persistent and dangerous self-experiment. There is tremendous progress over the centuries towards a better understanding of a plant derived medicine has depended on two major factors that have gone hand in hand. One of which has been the development of increasingly strict criteria of proof that a medicine really does what it is

claimed to do and the other has been the current improvement of identification by chemical analysis of the active compounds in the plant [11].

According to World Health Organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs [13]. The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on the human body [13]. The most important of these bio-active compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds [5]. Also, the phytochemical research based on ethno-pharmacological information is generally considered as an effective approach in the discovery of new anti-

infective agents from higher plants. The knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials [1, 9].

Lecaniodiscus cupanioides (Sapindaceae) is a tropical plant widely distributed in Africa and Asia. The plant is identified by various names in Nigeria, such as ukpo (in Igbo), utantan (in Edo), Kafi-nama-Zaki (in Hausa) and Akika (in Yoruba). It is a small tree 6-12m high branching low down with a widely spreading crown. The leaves of the plant resemble these members of the genus blighia but with different fruits. The plant has a variety of traditional and ethno-medical uses [1].

Recently, many researchers have investigated various sources of antioxidant compounds such as flavonoids, saponins, tannins, and terpenoids rich in vegetables. Medicinal plant extracts has showed some potent compounds that are responsible for biological activity. Both traditional and modern medicines are obtained mainly from plant precursors. The potentials of antioxidants compounds are found in medicinal plants [20-22].

Lecaniodiscus cupanioides has played a wide role in natural medicines, not only in Nigeria but world-wide recognition as being effective in various treatments of diseases [12].

The aim of this study is to investigate the presence of chemical compounds such as alkaloids, terpenoids, steroids, tannins, flavonoids, saponins, phlobatannins, cardiac glycosides and anti-microbial activity of the methanolic extract of the leaves of *Lecaniodiscus cupanioides*.

2. Materials and Methods

2.1. Collection of Samples

Fresh leaves of *Lecaniodiscus cupanioides* selected for this research work were collected from different areas within Idowa community, Ijebu-Ode and Ijagba, Sagamu, South West, Ogun State, Nigeria. The plants were identified by a Botanist at Forestry Research Institute of Nigeria (FRIN) Ibadan, Oyo State, Nigeria.

The fresh plant leaves were washed with distilled water to remove dust particles and air-dried for 14 days after which, was grounded into powder using grinding mill. The powdered samples were stored in airtight bottles for further use. All the chemicals used in this study were of analytical grade.

2.2. Extraction of *Lecaniodiscus cupanioides* Leaves

Extraction of the plant leaves was preceded using maceration method. 550 g of the powdered leaves of *Lecaniodiscus cupanioides* was weighed using analytical balance. The weighed plant sample was carefully poured into an aspirator bottle and soaked with one litre (1 L) of distilled methanol for seven (7) days. After 7 days, the mixture was decanted and the extract was recovered by

distillation [18].

2.3. Antimicrobial Method of *Lecaniodiscus cupanioides* Extract

Antimicrobial method of *Lecaniodiscus cupanioides* Extract was carried out using modified methods [3] as shown in Figure 1. one gram (1 g) of the plant extract was weighed and dissolved into the 5 mL of the solvent of extraction to give us 200 mg/mL. There were other 5 tubes that contain 2.5 mL of the same solvent, from the first tube that contains 200 mg/mL, 2.5mL was taken into the second tube to give us 100 mg/mL, also from the second tube, 2.5 mL was taken into the third tube to give us 50 mg/mL. This was done till the sixth tube which was 6.25 mg/mL. There was also the seventh tube serving as a negative control, which contains methanol only.

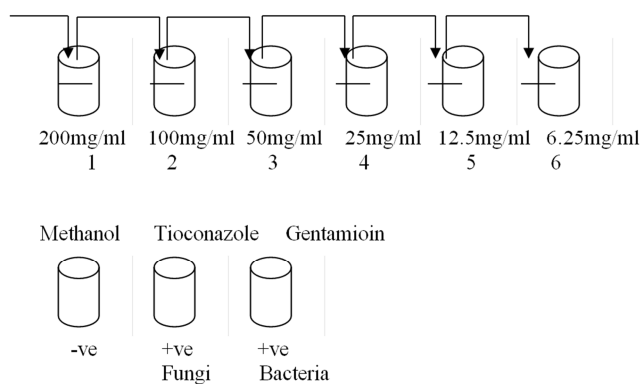


Figure 1. Antimicrobial method of *Lecaniodiscus cupanioides* extract

An overnight culture of each organism was prepared. 0.1 mL of each organism was taken into the 9.9 mL of sterile distilled water (SDW) to give us 10 mL at 1:100 (10^{-2}) dilution from 10^{-2} dilution 0.2 mL was taken into the sterile molten nutrients agar (NA) at 45°C. This was aseptically poured into the sterile plate and allowed to set on the bench for about 45 minutes. A sterile cork-borer was made to create wells/holes inside the set plate. Into the wells, different prepared concentrations of the sample were introduced. All the concentrations were introduced into the wells with negative and positive control. Positive control for bacterial is germicin at 10 mg/mL. These were allowed to stay on the bench for 2 h before incubation at 37°C for 18 - 24 h. This method is Agar distillation using pour plate method only for bacteria [19].

For fungi, molten sabourand dextrose agar (SDA) fungi, was poured aseptically in the sterile plates, allowed to cool and set for about 45 minutes. Then 0.2 mL of 1:100 dilution of the organism was spread on the surface using a sterile spreader. Then, a sterile cork borer was made to create wells/holes inside the set plate. Then, the procedure for bacteria was then followed from this stage. Meanwhile, positive control for fungi is Tioconazole 70%. All these plates were then incubated at 26-28°C for 48 h. This method is surface plate method only for fungi [9, 19].

2.4. Preliminary Phytochemical Screening of *Lecaniodiscus cupanioides* Extract

The plant extract were subjected to chemical test. The test was done to detect the presence of the active chemical constituents such as tannins, phlobatannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, and reducing sugar.

2.4.1. Test for Tannins

Five gram (0.5 g) of the plant extracts was boiled in 20 mL of distilled water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added. The presence of a brownish green or a blue-black colouration indicates the presence of tannin.

2.4.2. Test for Phlobatannins

The aqueous extract of the plant leaves were boiled with 1% aqueous hydrochloric acid. A deposition of a red precipitate indicated the presence of phlobatannins.

2.4.3. Test for Saponins

Two gram (2 g) of the plant extract was boiled in 20 mL of distilled water on a water bath and filtered. 10 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and was shaken vigorously, and then the formation of emulsion was observed [2], modified.

2.4.4. Test for Flavonoids

Few drops of 1% aluminum solution was added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids.

2.4.5. Test for Steroids

Two milliliters (2 mL) of acetic anhydride was added to 0.5 g methanolic extract of the plant with 2 mL Tetraoxosulphate (VI) acid, H_2SO_4 . The colour changed from violet to blue/green indicating the presence of steroids.

2.4.6. Test for Terpenoids

Five milliliters (5 mL) of the plant extract was mixed in 2 mL of chloroform and 3 mL of concentrated Tetraoxosulphate (VI) acid, H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the interface was formed indicating the presence of terpenoids [17].

2.4.7. Cardiac Glycosides

Five milliliters (5 mL) of the plant extract was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underlayered with 1 mL of concentrated sulphuric acid. A brown ring of the interface indicates deoxy sugar characteristics of cardenolides. A violet ring appeared below the brown ring, while in the acetic acid layer, a greenish ring form just gradually throughout the layer [17].

2.4.8. Test for Reducing Sugar

Volume of 0.5 mL of the plant extract was made a solution

with 1 mL of distilled water. 5 – 8 drops of Fehling's solution was added while hot. A brick red precipitate was observed indicating the presence of reducing sugar [10].

2.5. Quantitative Determination of Chemical Constituents

2.5.1. Alkaloid Determination Using Harborne, 1983 Method

Five gram (5 g) of the plant extract was weighed using analytical balance into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and precipitate was collected and washed with dilute ammonium hydroxide and was then filtered. The residue was the alkaloid, which was dried in the oven and weighed [6].

2.5.2. Saponin Determination Using Obadoni and Ochuko, 2001 Method

Twenty gram (20 g) of the plant extract was weighed into a conical flask and 100 mL of 20% aqueous ethanol was added. The extract was heated over a water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 mL of 20% ethanol. The combined extracts were reduced to 40 mL on a water bath at about 90°C. The concentrate was transferred into a 250 mL separating funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 mL of n-butanol was added. The combined n-butanol extract was washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated on water bath. After evaporation the extracts were dried in the oven to a constant weight, the saponin content was calculated as percentage [14].

2.5.3. Flavonoid Determination Using Bohm and Kocipai-Abyazan (1994) Method

Ten gram (10 g) of the leave extract was shaken repeatedly with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper. The filtrate was later transferred into a crucible and evaporated to dryness on a water bath and was weighed to a constant weight using analytical balance [15].

2.5.4. Tannins Determination Using Van-Burden and Robinson (1981) Method

Accurately, 0.5 g of the plant extract was weighed into a 50 mL plastic bottle, and 50 mL of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. Then, 5 mL of the filtrate was pipette out into a test tube and was mixed with 2 mL of 0.1M $FeCl_3$ in 0.1N HCl and 0.01M potassium ferrocyanide. Then, UV/visible spectrometer were used to measure the absorbance at 120 nm within 10 minutes [16].

2.5.5. Total Phenol Determination Using Spectrophotometer Method

About 5g of the plant extract was boiled with 50 mL of ether for the extraction of the phenolic component for 15 minutes. 5 mL of the extract was pipetted into a 50 mL flask, and then 10 mL of distilled water was added. 2 mL of ammonium hydroxide solution and 5 mL of concentrated amylacohol were also added. The mixture was made up to the mark and left to react for 30 minutes for colour development before it was then taken to UV/visible spectrophotometer for measurement, which was then measured at 505 nm [4, 8].

3. Results and Discussion

The result of antimicrobial screening of *Lecaniodiscus cupanioides* plant leaves showed highly active on both gram positive (+ve), gram negative (-ve) and also on fungi. This shows that the crude extract of the plant is a broad spectrum analysis. It was observed that at 200 mg/ml and 100 mg/ml of the solvent of extraction, the plant extract showed activity on both fungi and bacteria. At 50 mg/ml and 25 mg/ml, it shows more activity on fungi than bacteria. At 12.5 mg/ml, it was also active on fungi except organism like *Aspergillus niger* and also show little activity on bacteria. It reviewed no activity on both fungi and bacteria at 6.25 mg/ml. It was observed that it has little activity on spore forming organisms like *Salmonellae typhi*, *Escherichia coli* and *Pseudomonas aeruginosa* which are resistant in nature.

The broad spectrum of activity revealed by the antimicrobial screening result would appear to explain the scientific basis for the use of *Lecaniodiscus cupanioides* leaves for dressing of burns and cuts in various parts of West Africa. The phytochemical screening and quantitative estimation of the percentage crude extract yields of chemical constituents of the plants studied showed that the leaves of *Lecaniodiscus cupanioides* are rich in alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, phlobatannins and glycosides.

The plant reviewed medicinal activity as well as exhibiting physiological activity. From the quantitative estimation of the percentage crude extract yields of chemical constituents of the plant studied showed that the leaves of *Lecaniodiscus*

cupanioides contains percentage yields of alkaloids (1%). Alkaloids have neuroactive properties and interact with the receptors at the nerve endings. Many alkaloids have fragments buried within their overall structure which resemble the natural substances (the neurotransmitters) that bind to these receptors. Alkaloids have valuable pain-killing properties. They are used as an anti-malaria drug and in antibiotic and cancers [7]. *Lecaniodiscus cupanioides* is used for fever and dressing of wounds. It is also used for eye infections and in the treatment of leukaemia and cancer. Saponins were also present in appreciable amount (10%), which is an excellent remedy for tissue repair and tissue healing. The leaves of *Lecaniodiscus cupanioides* are commonly used for tissue repairing and also applied to wounds to assist fast healing. Saponins are also used in the treatment of cellulite. Cellulite is the term given to dimpled and humpy appearance of skin. It affects 90% of women after puberty. Cellulite usually appears around the arms, thighs and the posterior, and needs to be treated by consumption as well as external application of the leaves of *Lecaniodiscus cupanioides* containing saponins.

Also, the steroids are cardiac active and highly useful as a starting material for the synthesis of sex hormones. The leaves of *Lecaniodiscus cupanioides* contain percentage yields of flavonoids (3%) which possess wound healing activities; they promote hair growth and also used to reduce blood pressure. Since flavonoids are used in the treatment of hypertension, leaves of *Lecaniodiscus cupanioides* are recommended for hypertension patients. Steroids and phlobatannins that were found to be present in this plant contain steroidal compounds. Steroidal compounds are of importance and interest in pharmacy due to their relationship with such compound as sex hormones. This is the reason while leaves of *Lecaniodiscus cupanioides* are used as vegetables for expectant mothers or breast feeding mothers to ensure their hormonal balance.

The presence of terpenoids in the leaves of *Lecaniodiscus cupanioides* plant has been reported by researchers that this plant is widely used in herbal medicines. The presence of other chemical constituents like glycosides and reducing sugar also prove the effective use of the plant in the treatment of various diseases.

Table 1. Result of Antimicrobial Screening of *Lecaniodiscus cupanioides* plant

	mg/ml	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumoniae</i>	<i>Salmonella typhi</i>	<i>Candida albicans</i>	<i>Candida krusei</i>	<i>Aspergillus niger</i>	<i>Penicillium notatum</i>
1	200	24	14	20	14	18	12	20	20	20	24
2	100	22	12	18	12	14	10	18	16	18	18
3	50	18	10	14	10	10	-	16	14	14	16
4	25	12	-	12	-	-	-	14	12	12	14
5	12.5	10	-	10	-	-	-	12	10	-	12
6	6.25	-	-	-	-	-	-	-	-	-	+0
-ve		-	-	-	-	-	-	-	-	-	-
+ve		38	36	34	38	30	26	28	24	22	20

-ve = methanol

+ve (bacterial) = Gentamicin (10mg/ml)

+ve (fungi) = Tioconazole (70%)

Table 2. Result of Phytochemical Screening of *Lecaniodiscus Cupanioides*.

Test	Results
Tannins	+ve
Phlobatannins	+ve
Flavonoids	+ve
Saponins	+ve
Steroids	+ve
Terpenoids	+ve
Cardiac glycoside	+ve
Reducing sugar	-ve

+ve = present,

-ve = absent

4. Conclusion

This present study reviewed that leaves of *Lecaniodiscus cupanioides* plant extract could be seen as a potential source of useful drugs. This finding justifies the traditional use of the leaves of *Lecaniodiscus cupanioides* for prophylactic and therapeutic purposes. The importance of the findings could also be of commercial interest to both pharmaceutical companies, herbal practitioners and research institutes in the production of new drugs. Further studies are ongoing on this plant in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds. The antimicrobial activities of this plant for the treatment of diseases as claimed by traditional healers are also being investigated.

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