

## NATURAL DRUGS

PHYTOCHEMICAL AND COMPARATIVE ANTIBACTERIAL STUDIES  
ON THE CRUDE ETHANOL AND AQUEOUS EXTRACTS OF THE LEAVES  
OF *LECANIODISCUS CUPANOIDES* PLANCH (SAPINDACEAE)ISRAEL E. OBOH<sup>1</sup>\*, OSAHON OBASUYI<sup>2</sup> AND JOHN O. AKERELE<sup>2</sup><sup>1</sup> Department of Pharmacognosy, <sup>2</sup> Department of Pharmaceutical Microbiology,  
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**Abstract:** Phytochemical investigation was carried out on the leaves of *Lecaniodiscus cupanoides* Planch (Sapindaceae). The diameter of the zones of inhibition of the 90% ethanol and aqueous extracts of the leaves were compared in order to determine the relative activity of the extracts against the tested microorganisms and also to verify its claimed ethnomedicinal use in the treatment of microbial infections. Phytochemical tests were carried out employing standard procedures. The antimicrobial activity of the extract was tested against standard strains and clinical isolates of some aerobic bacteria using the agar well diffusion method. Commercial antibiotics were used as positive reference standards to determine the sensitivity of the strains. The minimum inhibitory concentration (MIC) values were also determined using the agar well diffusion method. Preliminary phytochemical studies revealed the presence of flavonoids, tannins, saponins and cardiac glycosides as the chemical classes of compounds present in the crude extract. The extracts showed inhibitory activity against clinical isolates of *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* *Staphylococcus aureus* and a standard strain of *Staphylococcus aureus* (NCTC 10788). The ethanol extract was more active than the aqueous extract against all the microorganisms tested, except against the clinical isolates of *Staphylococcus aureus*. The MIC values ranged from 2.5 to 6.25 mg/mL for all the organisms tested. The results showed that the ethanol extract was more potent than the aqueous extract. The broad spectrum of activity displayed by the extracts would appear to provide the scientific basis for the use of the leaves of *Lecaniodiscus cupanoides* for dressing of boils, burns and cuts in ethnomedicine.

**Keywords:** *Lecaniodiscus cupanoides*, ethanol and aqueous extracts, phytochemical tests, antibacterial activity

*Lecaniodiscus cupanoides* Planch (Sapindaceae) is a small tree 6-12 m high branching low down with a widely spreading crown. The leaves of the plant resemble these of members of the genus *Blighia* but with different fruits. It grows mostly in Savanna areas. Local names include “*akika*” (Yoruba), *kafi-nama-zaki* (Hausa), *okpu* (Igbo), and *utantan* (Edo) (1). The plant has a variety of traditional uses. An infusion of the bark is used for fever in Nigeria and as a purgative in Uganda. The leaves steeped in milk are applied to burns in the Ivory Coast. The crushed leaves are used in Nigeria in dressing sores (2). The antifungal activity of the triterpenoid saponins from *Lecaniodiscus cupanoides* has been reported (3).

This study screened the leaves for phytochemical constituents, and compared the antibacterial activity of the ethanol and aqueous extracts in order

to verify some of the claimed uses of the plant material in the treatment of infections.

## EXPERIMENTAL

## Materials and methods

## Plant material

The leaves of *Lecaniodiscus cupanoides* Planch (Sapindaceae) were collected in June 2007, at Temboga village, Ikpoba hill, Benin City, Edo State, Nigeria. The plant was authenticated at Forestry Research Institute of Nigeria, Ibadan, where a herbarium voucher specimen (FHI No.107748) was deposited.

The plant material was air dried on the laboratory bench for 5 days and then ground to powder using an electric mill. 350 g of the powdered leaves were macerated with 2.2 litres of 95% ethanol for 72

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h. The process was repeated using 300 g of powdered leaves and 2 litres of distilled water and allowing it to stand for 24 h. Both extracts were filtered and concentrated by evaporation in a vacuum rotavapor at 40°C to yield 21.4 g of ethanol extract and 13.7 g of water extract.

#### Preliminary phytochemical screening

Phytochemical tests were carried on portions of the powdered drug differently extracted for the specific classes of plant constituents (4-6). The tests were carried out employing standard phytochemical procedures to establish the presence or otherwise of secondary metabolites such as alkaloids, tannins, saponin glycosides, flavonoids and steroids in the crude drug. For the presence of alkaloids, the extracts were tested with the alkaloidal reagents (Dragendorff's, Hager's, Mayer's and Wagner's). Tests for glycosides involving: Fehling's reagent for reducing sugars, Keller-Kiliani test for deoxysugar(s) in cardiac glycosides, sodium picrate paper test for cyanogenetic glycosides, Borntrager's test for anthracene derivatives, frothing test and blood hemolysis test for saponins and NaOH and conc. HCl tests for flavonoids were carried out. Lieberman-Burchard's test was applied for detecting steroidal/triterpenoidal nucleus, while the presence of tannins was tested with dilute FeCl<sub>3</sub> solution.

#### Test organisms

The following microorganisms were used for the study: clinical isolates of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and a standard strain of *Staphylococcus aureus* (NCTC 10788). These microorganisms were obtained from the laboratory stock of the Department of Pharmaceutical Microbiology, University of Benin, Benin City, Nigeria.

#### Drugs and microbiological media

The antimicrobial agents used were: amoxicillin (SmithKline Beecham Pharmaceuticals, U.K), ciprofloxacin (Fidson Pharmaceuticals, Nigeria), nutrient broth and nutrient agar (Oxoid Ltd, Basingtone, Hampshire, England).

#### Preparation of plates for susceptibility tests

The agar-well diffusion method, suitably modified was adopted for the susceptibility studies (7-9). Inocula of the test organisms obtained from the source were prepared by growing each pure isolate in nutrient broth overnight at 37°C. The overnight broth culture was, subcultured in fresh nutrient broth and grown for 3 h, to obtain log phase culture. This

was matched with Mac Farland's turbidity standard to give approximately 10<sup>8</sup> cfu/mL. Aliquots of 0.2 mL were used to seed a molten nutrient agar medium, which was cooled to 45°C to obtain approximately 10<sup>6</sup> cfu/mL. This was poured onto the sterile Petri dishes, allowed to set and used for the investigations. The diameters of zones of inhibition were measured in millimeters with a ruler and recorded. This was repeated three times and average diameters were recorded.

The crude extracts were reconstituted with sterile distilled water and stock concentration of 400 mg/mL was made. The extract was tested at a concentration of 80 mg/mL. This was delivered into a well (8 mm in diameter) bored into the already seeded nutrient agar plates. Equal volume of distilled water was assayed as a control.

Ciprofloxacin (5 µg/mL) and amoxicillin (25 µg/mL) were used as standard antimicrobial agents and tested along with the extracts. The nutrient agar plates were incubated at 37°C for 24 h. The diameters of zones of inhibition were measured in millimeters with a ruler and recorded. This was repeated three times and average diameters were recorded.

#### Determination of minimum inhibitory concentrations (MICs)

The standard agar dilution protocol with doubling dilution was used. The extract was incorporated into nutrient agar at concentrations of 2.5 mg/mL to 20 mg/mL. A control without the extract was also set up. 10 µL each of the test organisms, previously diluted to give 10<sup>6</sup> cfu/mL, was used to inoculate the plates. These were incubated at 37°C for 24 h, and observed for growth. The minimum inhibitory concentrations (MICs) of the extract for each test microorganism were regarded as the lowest concentration that inhibited visible growth of the test organisms.

## RESULTS

The preliminary phytochemical studies revealed the presence of flavonoids, tannins, saponins and cardiac glycosides (Table 1). Minimum inhibitory concentrations (MICs) of the aqueous and ethanol extracts of the leaves of *Lecaniodiscus cupanoides* against test organisms are shown in Table 2.

The results of the antibacterial activity of the ethanol and aqueous extracts of the leaves of *Lecaniodiscus cupanoides* against a standard strain of *Staphylococcus aureus* (NCTC 10788) and clinical isolates of *Staphylococcus aureus*, *Bacillus sub-*

*tilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are shown in Figure 1.

The ethanol extract showed the highest zones of inhibition against *Bacillus subtilis* and *Staphylococcus aureus* (NCTC 10788), whereas the aqueous extract showed the highest zones of inhibition against *Escherichia coli* and *Staphylococcus aureus* (NCTC 10788) (Figure 1).

The zones of inhibition produced by both the aqueous and ethanol extracts against Gram positive organisms were in the order: Standard strain of *Staphylococcus* (NCTC 10788) > *Bacillus subtilis* > clinical isolates of *Staphylococcus aureus*, while the zones of inhibition of both extracts against Gram negative microorganisms were in the order: *Escherichia coli* > *Klebsiella pneumoniae* > *Pseudomonas aeruginosa*. However, the ethanol extract was more active against all the Gram positive and Gram negative microorganisms tested

except against the clinical isolates of *Staphylococcus aureus* (Figure 1).

The activity of both extracts against the bacterial strains used were, however, lower than that of ciprofloxacin except against *Escherichia coli*, for which the standard drug used (ciprofloxacin) did not show any activity. Amoxycillin showed the highest activity against the clinical isolates of *Staphylococcus aureus*. Amoxycillin also showed equal activity against the standard strain of *Staphylococcus* (NCTC 10788), *Bacillus subtilis* and *Pseudomonas aeruginosa*, whereas it showed no activity against *Escherichia coli* and *Klebsiella pneumoniae* (Figure 1).

The zones of inhibition produced by 80 mg/mL of both extracts against the standard strain of *Staphylococcus aureus* (NCTC 10788) was much higher (23 mm for the aqueous extract and 26 mm for the ethanol extract) when compared with that of the clinical isolates of *Staphylococcus aureus* (18 mm for the aqueous extract and 17 mm for the ethanol extract, respectively – Figure 1).

The minimum inhibitory concentration of both extracts ranged from 2.5 mg/mL to 6.25 mg/mL. The MICs for both extracts were also similar (2.5 mg/mL) for the standard strain of *Staphylococcus aureus*, and the clinical isolates of *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* (Table 2).

The control did not produce any inhibitory activity against the test organisms.

## DISCUSSION AND CONCLUSION

The antimicrobial evaluation of the leaves of *Lecaniodiscus cupanioides* showed that the ethanol extract (solvent extract) was more active than the aqueous extract against all the microorganisms test-

Table 1. Phytochemical constituents of the leaves of *Lecaniodiscus cupanioides*

| Constituents tested for  | Observations* |
|--------------------------|---------------|
| Flavonoids               | +++           |
| Saponins                 | ++            |
| Tannins                  | +++           |
| Alkaloids                | -             |
| Anthraquinone glycosides | -             |
| Cardiac glycosides       | ++            |
| Cyanogenic glycosides    | -             |

\* +++ appreciable amount, ++ moderate amount, + trace amount, – completely absent

Table 2. Minimum inhibitory concentrations (MICs) of the aqueous and ethanol extracts of the leaves of *Lecaniodiscus cupanioides* against test microorganisms.

| Microorganism                             | MIC (mg/mL)*  |                 |
|---|---------------|-----------------|
|   | Water extract | Ethanol extract |
| <i>Staphylococcus aureus</i> (NCTC 10788) | 2.5           | 2.5             |
| <i>Staphylococcus aureus</i>              | 2.5           | 2.5             |
| <i>Bacillus subtilis</i>                  | 2.5           | 2.5             |
| <i>Escherichia coli</i>                   | 2.5           | 2.5             |
| <i>Pseudomonas aeruginosa</i>             | 6.25          | 5.0             |
| <i>Klebsiella pneumoniae</i>              | 5.0           | 2.5             |

\* The mean of 3 determinations

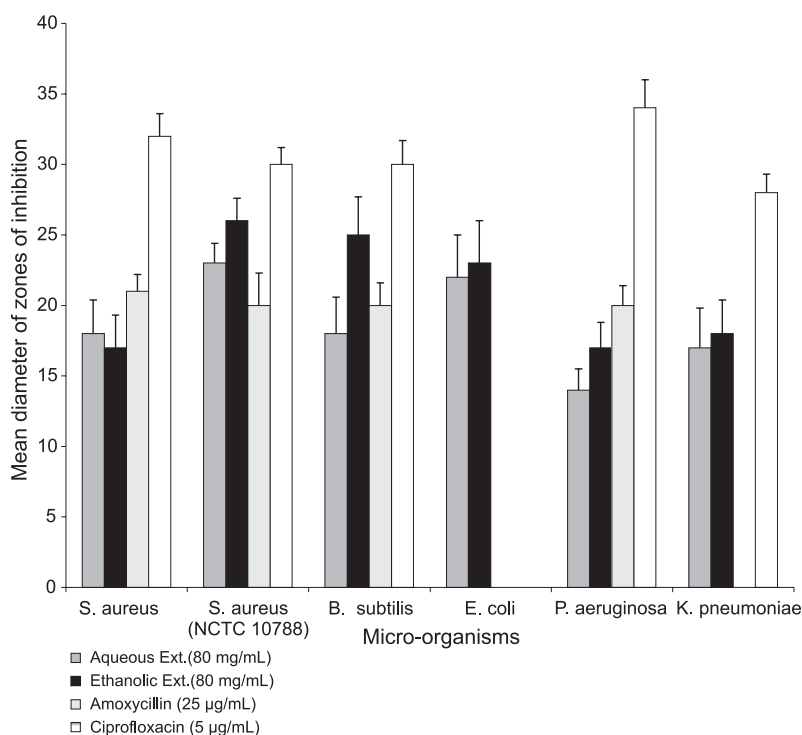


Figure 1. *In vitro* antimicrobial activity of 80 mg/mL of the aqueous and ethanol extracts of the leaves of *Lecaniodiscus cupanoides*. The mean diameter of zone of inhibition  $\pm$  S.D.

ed except against the clinical isolates of *Staphylococcus aureus*.

The results of this study conforms with earlier studies that showed that the antibacterial activity of some Iranian medicinal plants were more significant in the solvent extracts compared with aqueous extracts in all the plants, indicating that the active principle(s) responsible for antibacterial activity were more soluble in organic solvents (10). Similarly, the solvent extract of cloves flower was also found to exhibit a significantly higher inhibitory effect on the caries-inducing properties of *Streptococcus mutans* compared with the crude aqueous extracts (11).

In other studies the methanolic and petroleum spirit extracts of *Pelargonium* essential oils were more potent antibacterial agents than the steam distilled volatile samples (12).

The broad spectrum of activity displayed by the extracts would appear to explain the scientific basis for the use of the leaves of *Lecaniodiscus cupanoides* for dressing of boils, burns and cuts in various parts of West Africa.

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