Phytochemical and Antimicrobial Properties of Leaves of Achornea Cordifolia

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Abstract: The phytochemistry of Achornea cordifolia leaf extract using different solvents was studied using standard methods. The effects of the leaf extract on some pathogenic bacteria and fungi were also examined. The Phytochemical screening of the leaves shows the presence of useful ethno-botanical bioactive substances such as tannin, saponin, flavonoid, cardiac glycoside and anthraquinone, while alkaloid, phlobatanin and terpene also tested for were absent. The butanol fraction of the extract gives the highest zone of inhibition (13.0 mm) against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa, which are in parity. The control, gentamycin injection gives 12.5 mm, 11.0 mm and 12.0 mm respective zones of inhibition against the Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. Also, the butanol fraction of the extract shows highest zone of inhibition of 17.0 mm against Candida albican, while ethanolic extract gives 13.0 mm zone of inhibition against Trichophyton violaceum.

Keywords: Achornea cordifolia, Phytochemical screening, Bioactive constituents, Antimicrobial.

Introduction

The Nigerian Vegetations are rich in Medical Plants These plants are usually employed in traditional medicines as remedies for treatment of various ailments. However, in traditional medicines, these plants are arbitrarily used with non-precise dose and for this reason; the therapeutic and toxic concentrations are unknown.

Medicinal plants are those plants which contain substances (usually secondary metabolites) in their organs, which make them to be useful as therapeutic agents or as precursors for the synthesis of useful drugs. Medical plants such as Magnifera indica (stem
and bark), *Solocia purifemus* (roots), *Carica papaya* (leaves) and *Morihala indica* (leaves) have inhibiting effect on the growth of pathogenic bacteria. The aqueous and alcoholic extracts of the plants mentioned also inhibit the growth of bacteria such as *Trichophyton mentagrophyte*, *Staphylococcus epidermidis*, *Bacillus spp.* and *Microsporum spp.* However, the medicinal plant used in this research is *Alcornea cordifolia*.

*Alcornea cordifolia* belongs to the family of *Euphorbiaceae* and it is erect or straggling perennial shrub to a small tree. It is a common West African tropical flora. The plant which has heart-shaped leaves with brown stem and green hanging fruits is widely distributed in West Africa, mostly Nigeria and Zaire republic.

The fruit of *Alcornea cordifolia* yield a black dye used in dying fabrics, pottery, calabash and leathers. The leaf infusion or decoction is used to treat colds, bronchial problems, stomachache, dysmenorrhea, fever and eye problems. The leaves are also used to feed ruminants as fodder. Extract of the leaf is used for the treatment of Gonorrhea and toothaches. The powdered and pulverized leaf is used for treatment of cuts, burns, bruises, ulcers and piles. The antibacterial activity of the leaf extract on *Staphylococcus aureus* and *Escherichia coli* was attributed to isopentenyl guanidine. The present work is an attempt to examine the bioactive components of the leaf extracts and also to examine the antimicrobial properties of different solvent fractions of the leaf extract on some pathogenic microorganisms.

**Experimental**

All the leaf samples were collected in the month of October 2007 from a bush in Iba Oku, Uyo Local Government Area, Akwa Ibom State, Southern Nigeria. The leaves were collected from mature *Alcornea cordifolia* plant and were taken to the pharmacognosy laboratory for analysis of bioactive components. The fresh leaves were pounded to powder using a clean mortar and pestle. The pounded samples were soaked in 98% ethanol for 72 hours at room temperature in a close container. The filtrate obtained was evaporated to dryness by heating it at 45°C in a steam bath for some hours. The dry extract was stored in the refrigerator at 5°C till required for analysis.

Phytochemical bioactive component identifications were carried out using the procedures recorded in Trease and Evans and Sofowora. Test for tannin: About 0.5 g of the leaf extract was stirred with 10 mL of distilled water and filtered. 5% ferric chloride reagent was added to the filtrate. A blue-black precipitate indicates the presence of tannin. 0.5 g of the leaf extract also stirred with bromine water decolourised bromine water for a positive test of the presence of tannin in the extract.

Test for saponin: 0.5 g of the plant extract was dissolved with 5 mL of distilled water and filtered. Persistent frothing observed when the filtrate was shaken vigorously indicates the presence of saponin. A Fehling’s test was carried out by adding the filtrate of the leaf to 5% sodium carbonate (Na₂CO₃) followed by Fehling’s solution and boiled. The formation of brown precipitate indicates the presence of saponin. Addition of 0.5 g of leaf extract with Fehling’s solution A and B and boiled resulted in the formation of precipitate which was taken as positive test for the presence of saponin.

Test for phlobatannin: Three drops of 40% formaldehyde was added to 5 mL of filtrate, followed by 6 drops of hydrochloric acid and raised to boiling point and then cooled. Absence of a red precipitate was an indication for the absence of phlobatannin.
Test for terpene
0.5 g of Leaf extract was dissolved with 5 mL of chloroform and filtered. 10 drops of acetic anhydride was added to the filtrate followed by two drops of concentrated acid. Absence of pink colour at the interphase was an indication of the absence of terpene.

Test for flavonoid
Few pieces of magnesium metal were added to 5 mL of the extract and concentrated hydrochloric acid was carefully added. The formation of orange colour or crimson was taken as evidence of the presence of flavonoid.

Test for cardiac glycoside
0.5 g of the leaf extract was dissolved in 2 mL of chloroform. Concentrated sulphuric acid was carefully added to form a lower layer. A reddish-brown colouration at the interphase indicates the presence of a steroidal ring of cardiac glycoside. This test is called salkowski test.

Test for anthraquinone
2.5 g of Leaf extract was boiled with 5 mL of 10% sulphuric acid and filtered. The filtrate was shaken with 2.5 mL benzene. The benzene layer separated, and 10% NH₄OH added. A rose pink colouration in ammonia phase (lower phase) indicates the presence of anthraquinone.

Test for alkaloids
5 mL of 1% aqueous hydrochloric acid was added to 5 g of the leaf extract and warmed in a steam bath while stirring. It was filtered and the filtrate was used to test for alkaloid as follows:
(i) 1 mL of the filtrate was treated with a few drops of Dragendoff’s reagent. There was no formation of a reddish-brown turbid dispersion or precipitate indicating the absence of alkaloid.
(ii) 1 mL of the filtrate was also treated with a few drops of Mayer’s reagent and no creamy turbid dispersion was formed. This indicates the absence of alkaloid. Also, absence of white or yellow precipitate when 0.5 g of the filtrate was treated with a few drops of picric acid reagent indicates the absence of alkaloid.

In each of the tests, distilled water was used as control and the result of the photochemical screening of Alcornea cordifolia leaf is tabulated in Table 1.

Chromatographic technique was used to partition the ethanolic extract successively with n-hexane, chloroform, distilled water, ethyl acetate and butanol to yield n-hexane fraction, chloroform fraction, aqueous fraction, ethyl acetate fraction and butanol fraction respectively.

Antimicrobial tests were carried out using bacteria and fungi obtained from clinical samples, isolated from the medical laboratory section of the University of Uyo Health Centre. The microorganisms were Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albican and Trichophyton violaceum. The classical method by which these organisms were isolated was by plating out on a solid medium. A small quantity of material from infected patient was streaked out with a sterile loop into a defined area on the surface of a nutrient agar and Saboraud Dextrose agar medium respectively.

Filter papers were perforated in circular form at a diameter of 1 mm using perforating machine and introduced into aliquot of the extract and allowed to soak for some minutes before they were removed and placed on the inocula using sterile forceps. The cold broth culture of each of Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus were
streaked onto the surface of already prepared nutrient agar plates and *Candida albican* was streaked on the surface of already prepared Saboraud Dextrose agar plate. The excess of the broth found in different organisms were discarded and the surface of each plate dried by flaming. The prepared susceptibility dish papers were subjected into each of the ethanolic extract, *n*-hexane, chloroform, ethylacetate and butanol fractions and allowed to soak. A sterile forceps was used to place the sensitivity dish aseptically on the inoculum and was pressed down gently. The plates were incubated at 37 °C for 24 hours. The external diameters of visible zones of growth of inhibition were measured.

Ten milliliters or grams of Saboraud Dextrose Agar (SDA) was mixed with 0.1 mL of each of ethanolic leaf extract, *n*-hexane, chloroform, ethyl acetate and butanol fractions and allowed to solidify. This was done by pour-plating method and *Trichophyton violaceum* was planted in them using standard sterile loop to ensure the amount of organisms planted in each plate. They were incubated at 37 °C for an hour and their visible zones of growth parameter were measured. This was done by subtracting the growth parameter of each of the fractions by the control experiment.

As a control experiment 80 mg of gentamycin injection was diluted with 40 mL of distilled water to obtain 40 mg/mL and used as standard for antibacterial activity. 100 mg weighed out of 300 mg nystatin tablet was dissolved in 1 mL of distilled water to obtain concentration of 100 mg/mL and this was also used as standard for *Candida albican*, (fungus). *Trichophyton violaceum* was planted in pure Saboraud dextrose Agar (SDA) medium using standard sterile loop for measurement and this was used as standard for *Trichophyton violaceum*. In each of these experiments, the measured zones of inhibitions are shown in Table 2 & 3.

**Results and Discussion**

The result of the phytochemical screening of the ethanolic extract of *Alchornea cordifolia* leaf reveals the presence of remarkable concentrations of tannin, saponin, flavonoid, anthraguinone and glycoside while alkaloid, terpene and phlobatannin are absent (Table 1). Table 2 shows the result of different solvent fractions of *Alchornea cordifolia* extract. The result shows that *Candida albican* exhibits the highest zone of inhibition of 17.0 mm with butanol fraction of the extract. This is followed by *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* with zones of inhibition of 13.0 mm each. *Trichophyton violaceum* shows the least zone of inhibition with butanol fraction of the extract, but was higher with ethanolic fraction of the extract. *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albican* were all resistant to *n*-hexane and chloroform fractions but sensitive to ethyl acetate fraction. The test microorganisms show various sensitivity indices for different leaf extracts.

The result of sensitivity of the test organisms to pure solvents is shown in Table 3. Gentamycin injection at concentration of 40 mg/mL used as standard for the bacterial isolate exhibited the highest inhibitory activity against *Staphylococcus aureus* with an inhibition zone of 12.5 mm; followed by *Pseudomonas aeruginosa* (12.0 mm) and *Escherichia coli* with 11.0 mm zone of inhibition. Nystatin tablet at the concentration of 100 mg/mL used as standard for *Candida albican* (fungus) exhibited 12.0 mm zone of inhibition. All the test organisms were not sensitive to sterile distilled water, but they show low zones of inhibition against pure solvents. As shown in Figures 1-5 obtained from Tables 2 and 3.
Figure 1. Inhibitory activity against *Escherichia coli*.

Figure 2. Inhibitory activity against *Pseudomonas aeruginosa*.

Figure 3. Inhibitory activity against *Staphylococcus aureus*.
The presence of tannin, flavonoid, saponin, anthraquinone and cardiac glycoside in the leaf of *Alcornea cordifolia* as observed in the study is the proper evidence to regard the plant as medicinal plant. Tannin is heterogeneous group of complex polyhydroxybenzoic acid derivatives, which are in plant and occur as solution in cell sap often in distinct vacuoles. Tannin has inhibitory effect on many enzymes due to protein precipitation. It also has stringent properties and hence may be applied to wounds as protective coating. It is possible therefore that this reason makes *Alcornea cordifolia* leaf to be used locally for treatment of wounds, sores and skin diseases.

Saponin containing plants are important because of their detergent and haemolytic properties. Saponin when injected into the blood stream are highly toxic because of their reaction with enzymes, but when administered orally, becomes comparatively harmless. Their presence in *Alcornia cordifolia* may be confirmed as this plant is used as chewing sticks. The presence of flavonoid in the plant suggests that it can be used as anti-spasmodic, anti-fungal and anti-bacterial drug plant. These findings confirm the reason for the use of the
plant in the treatment of diarrhoea, spasmodic bronchitis and other microbial infections as reported by Etukudo\(^7\). The results obtained from the antimicrobial test (Tables 2 & 3) confirm that *Alchornea cordifolia* leaf extract has antimicrobial properties.

**Table 1. Phytochemical screening of *Alchornea cordifolia* leaf.**

| Test                      | Observation                  | Inference |
|---------------------------|------------------------------|-----------|
| **Tannin test**           |                              |           |
| (a) Ferric Chloride test  | Blue Black Colouration       | +++       |
|                           | formed                       |           |
| (b) Bromine Water test    | Tannin decolourized          | ++        |
|                           | bromine water                |           |
| **Phlobatannin test**     |                              |           |
| Formaldehyde test         | No ppt formed                | -         |
| **Flavonoid test**        |                              |           |
| Magnesium metal test      | Orange Colour Crimson        | +++       |
|                           | or Magenta formed            |           |
| **Saponin test**          |                              |           |
| (a) Frothing test         | Persistent frothing          | ++        |
| (b) Fehlings solution test| Brick red ppt formed         | ++        |
| (c) Extract + Fehlings    | brown ppt formed             | ++        |
| Solution + Na\(_2\)C\(_O_3\) |                        |           |
| **Anthraquinones**        |                              |           |
| Acid + 10% Sulphuric      | Rose Colouration             | ++        |
|                           | formed                       |           |
| **Terpene test**          |                              |           |
| Chloroform + acetic       |                             |           |
| Anhydride + Conc.         |                             |           |
| Sulphuric acid            | No Colour change             | -         |
| **Alkaloid test**         |                              |           |
| (a) Dragendorff’s reagents| No ppt. formed               | -         |
| (b) Mayer’s reagent test  | No Colour change             | -         |
| (c) Picnic acid reagent test| No ppt. formed              | -         |
| **Test for glycosides**   |                              |           |
| (a) Hydrolysis of glycoside| Brick red ppt. Occurred     | ++        |
| **Test For Cardiac Glycosides**|                        |           |
| (i) Salkawski test        | Formation of reddish-        | ++        |
|                           | brown colour at interphase   |           |
| (ii) Keller-kiliani test  | Violet ring appears          | ++        |
|                           | below ring at interphase     |           |
| (iii) Lieberman’s test    | No colour change             | -         |

**Key**

| (+++) | (+) | (+) | (+) |
|-------|-----|-----|-----|
| Highly positive | Moderately positive | Sparingly positive (Trance) | Negative |
Table 2. Effect of the Achornia cordifolia leaf extracts of different solvent fractions on the zone of inhibition of test microorganisms.

| Organism used       | Zones of inhibition | Ethanol extract, mm | n-hexane fraction, mm | Ethyl acetate fraction, mm | Chloroform fraction, mm | Butanol Fraction, mm |
|---------------------|---------------------|---------------------|-----------------------|---------------------------|-------------------------|----------------------|
| *Escherichia coli*  | R                   | 10.0                | R                     | 12.0                      | 5.0                     | 13.0                 |
| *Pseudomonas aeruginosa* |                   | 12.0                | R                     | 8.0                       | R                       | 13.0                 |
| *Staphylococcus aureus* |                   | 10.0                | R                     | 8.0                       | R                       | 13.0                 |
| *Candida albican*    | R                   | 9.0                 | R                     | 10.0                      | R                       | 17.0                 |
| *Trichophyton violaceum* |                | 13.0                | 13.0                  | 03                        | 05                      | 03                   |

*R = Resistant*

Table 3. Effects of pure solvents, gentamycin injection and nystatin tablet alone on the test microorganism as control on they zones of inhibition.

| Concentration | Gentamycin Injection | Distilled H₂O | Ethanol, 89% | n-hexane | Ethyl acetate | Chloroform | Butanol |
|---------------|----------------------|---------------|--------------|----------|---------------|------------|---------|
| Organisms used | Zonal zone of inhibition |
| *Escherichia coli* | 11.0 mm | R | 1.0 mm | 1.0 mm | 1.0 mm | 1.5 mm | 2.0 mm |
| *P. aeruginosa* | 12.0 mm | R | 0.5 mm | 1.0 mm | 2.0 mm | 1.0 mm | 1.0 mm |
| *S. aereus* | 12.5 mm | Nystatin tablet | R | 2.0 mm | 1.0 mm | 1.0 mm | 2.0 mm |
| *Candida albican* | 12.0 mm | Pure SDA | R | 1.0 mm | 2.0 mm | R | R | 1.0 mm |
| *T. Violaceum* | 33.0 mm | R | 1.0 mm | 2.0 mm | R | 1.0 mm | 0.5 mm |

*R = Resistant*

The ethanolic extract shows it’s highest sensitively against *Trichophyton violaceum* followed by *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Candida albican*. Butanol fraction of the extract gives the highest inhibitory zone against *Candida albican* followed by *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* which are in parity, but shows no sensitivity to *Trichophyton violaceum*. This shows that butanol fraction can be used to treat infection caused by *Candida albican, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. Ethyl acetate fraction of the extract is effective against *Escherichia coli, Candida albican, Pseudomonas aeruginosa and Staphylococcus aureus*, but shows low sensitivity against *Trichophyton violaceum*. This proves that ethyl acetate fraction of the extract can be used to treat diseases caused by the above micro-organisms.

**Conclusion**

Gentamycin injection used as standard for antibacterial shows low zone of inhibition compared to butanol fraction of the extract despite its high standard of purification. Again
nystatin tablet shows low inhibition zone compared to butanol fraction of the extract. Hence, if *Alcornea cordifolia* leaf extract is purified, it can be more effective to most diseases caused by the test microorganisms like *Candida albican*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Trichophyton violaceum*. The presence of the bioactive components of *Alcornea cordifolia* plant shown in Table 1 suggests the presence of phenolic compounds which have antimicrobial properties. This implies that *Alcornea cordifolia* extract is a useful antimicrobial plant against the test micro-organisms.

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