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Phytochemistry and antimicrobial property of fruits of *Chrysophyllum albidum* against selected clinical isolates

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Abstract. The antimicrobial activity of methanolic and aqueous extracts of *Chrysophyllum albidum* fruits was investigated against clinical isolates(*Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia and Candida albicans*). Qualitative phytochemistry of the plant indicated that the plant contained Flavonoids, Steroids, Alkaloids, Tannin, Anthraquinone and Cardiac glycosides while Saponins were reported absent. The maximum activity of the aqueous extracts in the test isolates was observed on *Staphylococcus aureus*, which showed clear zones with diameters of 24.0mm, 20.0mm and 16.5mm at concentrations of 100mg/ml, 50mg/ml and 25mg/ml respectively while it had low activity on *Klebsiella pneumonia,* with clear zones of inhibition of 15.0mm, 12.0mm and 10.5mm at same concentrations. On the other hand, Methanolic extracts activity on *Staphylococcus aureus* produced clear zones of 21.0mm, 17.5mm and 12.0mm at concentrations of 200mg/ml, 100mg/ml and 50mg/ml respectively as its best activity while the it had least observed activity on *Klebsiella pneumonia* with clear zones of 14.0mm, 11.5mm and 10.5mm at same concentrations. The aqueous extracts had greater activity than the methanolic extracts at same concentrations. Therefore, the fruit of the plant can be a good source of remedy in phytomedicine.

Introduction

Healing with medicinal plants is as old as mankind itself [1]. The connection between man and his search for drugs in nature dates from the far past, of which there is ample evidence from various sources: written documents, preserved monuments and even original plant medicines. Awareness of medicinal plants usage is a result of the many years of struggles against illnesses due to which man learned to pursue drugs in barks, seeds, roots, fruit bodies, leaves and other parts of plants [2]. Medicinal plants are known to owe their curative potentials to certain biological active substances which exist in parts of plants. The chemicals which are referred to as active principles or phytochemical substances include terpenes, flavonoid, bioflavonoid, benzophonones, xanthenes, as well as some metabolites such as tannins, saponins, cyanates, oxalate and anthrax-quinones [3,4]. Plants have historically provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being [3]. Their role is two-fold in the development of a medicine, a natural blueprint for the development of new drugs. Their second role is to be used as a phytomedicine for the treatment of disease. The development of phytomedicines follows the ethnomedicinal approach. The first generation of plant drugs were simple botanicals employed in more or less their crude form. Several effective medicines used in their natural state such as cinchona, opium, belladonna and aloe were selected as therapeutic agents based on empirical evidence of their clinical application by traditional societies from different parts of the world [3]. Following the industrial revolution, a second generation of plant based drugs emerged based on scientific processing of the plant extracts to isolate their active constituents. The second generation phytopharmaceutical agents were pure molecules and some of the compounds were even more pharmacologically active than their synthe tic counterparts. Notable examples were quinine from *Cinchona*, reserpine from *Ranolfia*, and more recently taxol from *Taxus* species [3].

These compounds differed from the synthetic therapeutic agents only in their origin. They followed the same method of development and evaluation as other pharmaceutical agents. The sequence for development of pharmaceuticals usually begins with the identification of active lead molecules, detailed biological assays and formulation of dosage forms in that order, and followed by several phases of clinical studies designed to establish safety, efficacy and pharmacokinetic profile of the new drug. Possible interaction with food and other medications may be discerned from the clinical trials. In the development of third generation phytotherapeutic agent, a top-bottom approach is usually adopted. This consists of first conducting a clinical evaluation of the treatment modalities and therapy as administered by traditional doctors or as used by the community as folk medicine. This research therefore investigate and evaluate the antimicrobial properties or efficacy of the *Chrysophyllum albidum* fruit against four pathogenic microbial isolates.

Materials and Method

Sample collection

Fresh and ripe *Chrysophyllum albidum* fruit samples were bought at Eke-Onunwa market in Owerri, Imo state and transported to the laboratory in a bag.

Preparation of sample

The fruit samples were thoroughly washed with clean water. The seeds were removed and the pulp and peel of the fruit were cut into tiny pieces. The pieces of the sample were then dried and grinded to powder and stored in sterile bottles.

Extraction process

The extraction process was done by soaking 50g of the dried powdered sample in 100ml of the solvent (distilled water and methanol) for 5 days. The extracts were filtered using Whatman filter paper and allowed to evaporate to dryness. Different concentrations of the extracts were then prepared (25, 50 and 100mg/l for aqueous extracts; 50, 100 and 200mg/l for methanolic extracts).

Qualitative phytochemical Analysis of the Plant material.

The qualitative methods already established to test for classes of compounds in plant extracts by Ciulei [5] and Chitravadivu et al. [6] were used. The substances that were tested for included: Tanins, Saponins, and Flavonoids, Cardiac glycosides, Alkaloids, Anthraquinone and Steroids which are reported to have biological activities.

a) Test for Anthraquinones

The determination of Anthraquinones was carried out by the titration method of AOAC [7]. To 1ml of solution, 5ml of 10% HCL was added and allowed to stay for 5 minutes. The solution was filtered. The filtrate was decanted into a test tube and shaken with 5ml of benzene. The upper benzene layer was pipette off and transferred into test tube containing 5ml of 10% ammonium hydroxide. Production of pink, red or violet colouration in the lower ammonia

b) Test for Glycosides

The determination of Glycosides was carried out by the titration method of AOAC [7]. To 1ml of the test solution, 2 drops of Conc. Sulphuric acid was added and placed in water bath for about 15 minutes. 20% KOH will be added to make the solution alkaline. To this solution, few drops of $FeCl_2$ were added. The formation brick red precipitate indicates the presence of glycosides.

c) Test for alkaloids

One milligram of dried extract was dissolved in 6 props of 2% hydrochloric acid. The solution was divided into 3 aliquots; to the first portion which acted as a reference, 2 ml of distilled water was added. To the second test tube, 2 drops of Dragendorff's reagent whose Basic Bismuth nitrate were

added. A precipitate indicated presence of alkaloids. To the third portion, 2 drops of Mayer's reagent was added and a yellowish white precipitate indicated the presence of alkaloids.

d) Test for flavonoids (shinoda test)

A little amount of magnesium powder and a few drops of concentrated HCL were added to 3 ml of methanolic extract. A red or intense red coloration indicates the presence of flavonones.

e) Test for tannins

One milligram of plant extracts was dissolved in 1.5 ml of water; 3 drop of dilute ferric chloride from were added. A blackish blue colour indicated the presence of Gallic tannins and green blackish colour indicated catechol tannins.

f) Test for saponins

Three drops of dimethylsulfoxide were added to 1 mg of plant extract, 5 ml of distilled water added and shaken. Presence of foam which persisted for more than 15 min indicated the presence of saponins.

g) Test for steroids and triterpenoids

One milligram of dried extracts was dissolved in 0.5 ml of acetic anhydride; 0.5 ml of chloroform was added. The solution was pipette into a dry test tube and 1 ml of concentrated sulphuric acid added at the bottom of the tube. A brown-red ring at the interface between the two liquids and a green supernatant indicated the presence of steroids and triterpenoids.

Isolation and identification of test organisms

Isolation of test organisms

The test organisms used in the study were isolated from different clinical specimen at Ferdicon medical laboratory in Owerri, Imo state. The bacterial isolate, *Escherichia coli* was isolated from a faecal specimen, *Staphylococcus aureus* from sputum, *Klebsiella pneumonia* from a urine specimen and the fungal isolate, *Candida albicans* from a vaginal swab. All the test organisms were subcultured to get pure cultures and tested using appropriate biochemical tests. The results obtained was matched with standard identification manuals [8].

Biochemical tests

Biochemical tests were used in the identification and confirmation of the bacterial isolates in line with standard operational procedures outlined by Cheesbrough [9].

a) Indole test

This test was used to identify *Escherichia coli* as it can break down the amino acid tryptophan with the release of indole. The test organism was inoculated into a bijou bottle containing 3ml of sterile tryptone water and incubated at 35°c for up to 48 hours. 0.5ml of Kovac's reagent was added and shaken gently. A red colour was observed on the surface layer.

b) Catalase and Coagulase test

This test was used to identify *Staphylococcus aureus* as a catalase and coagulase enzymes producer. To test for catalase 3ml of hydrogen peroxide solution was poured into a test tube. The test organism was inoculated into the solution and active bubbling was observed. The ability of the isolate to coagulate horse serum was recorded as coagulase positive.

c) Citrate utilization test

This test was used to identify *Klebsiella pneumonia* as it uses citrate as its source of carbon. Slant Simmon's citrate agar was prepared in bijou bottles, and a saline suspension of the test organism was streaked on the slant and also stabbed to the butt. This was incubated at 35°c for 48 hours and a bright blue colour was observed.

Antimicrobial screening

The antimicrobial properties of the extracts were evaluated using the Kirby-Bauer disc diffusion test. A microbial cell suspension was inoculated into a nutrient agar medium and poured into sterile petri dishes. Sterile paper discs 6mm in diameter were impregnated with 20µl of each extract concentration (25, 50 and 100mg/l for aqueous extracts; 50, 100 and 200mg/l for methanolic extracts), which were prepared using the same solvents employed to dissolve the plant extracts. The impregnated discs were then placed on the inoculated agar surface. Standard Ceftriaxone and Ofloxacine discs were used as positive control while discs loaded with 20µl of solvents were used as negative control. The plates were pre-incubated for 2 hours in a refrigerator and subsequently incubated overnight at 37°C for 24 hours. The resulting zones of inhibition were measured and recorded.

Results

Table 1 shows the qualitative phytochemical analysis of the fruits of *Chrysophyllum albidum*. From the results, Flavonoids, Steroids, Alkaloids, Tannin, Anthraquinone and Cardiac glycosides were found to be present in the plant's fruit while Saponin was found absent.

Table 1: Qualitative phytochemical analysis of the fruit of *Chrysophyllum albidum*.

Phytochemical	Amount
Flavonoids	+
Saponin	-
Steroids	+
Tannin	+
Alkaloids	+
Anthraquinone	+
Cardiac glycoside	+

Key: (+) Present, (-) Absent

The result of the antimicrobial screening test showed clear zones of inhibition around the impregnated discs of each of the three concentrations of both the aqueous and methanolic extracts of the *Chrysophyllum albidum* fruit which were inoculated on all four clinical isolates. The mean zones of inhibition for the aqueous and methanolic extracts are shown in Tables 2 and 3. The presence of the zones of inhibition is a clear evidence of the antimicrobial properties of the *C. albidum* fruit. The Minimum Inhibitory Concentration (MIC) of both aqueous and methanolic extracts of the C. albidum fruit was also determined from the results of the antimicrobial test. This is shown in Table 4. The results of the test were also analysed statistically using the Analysis of Variance (ANOVA) to check the significance of the variations of the result. Comparison of the observed variance ratio F_s and the conservative critical value as well as the next tabled F with less degree of freedom is convincing enough to reject the null hypothesis for both the aqueous and methanolic extracts. Thus, both results are significant.

Means zones of inhibition

The tables show the mean zones of inhibition (mm) of each of the three concentrations of the discs impregnated with plant extracts on the test organisms.

Table 2: Mean Zones of Inhibition (mm) of aqueous extracts of *Chrysophyllum albidum* fruit on some selected organisms.

Organisms	**Zones of Inhibition(mm)**		
	25**(mg/ml)**	50**(mg/ml)**	100**(mg/ml)**
Staphylococcus aureus	16.5	20.0	24.0
Klebsiella pneumonia	10.5	12.0	15.0
Candida albicans	11.0	13.0	15.5
Escherichia coli	12.0	13.5	14.5

Table 3: Mean Zones of Inhibition (mm) of methanolic extracts of *Chrysophyllum albidum* fruit on some selected organisms.

Organisms	**Mean zones of inhibition(mm)**		
	50**(mg/ml)**	100**(mg/ml)**	200**(mg/ml)**
Staphylococcus aureus	12.0	17.5	21.0
Klebsiella pneumonia	10.5	11.5	14.0
Candida albicans	11.5	14.0	16.5
Escherichia coli	11.0	13.0	15.0

Minimum Inhibitory Concentrations (MIC)

The table shows the Minimum Inhibitory Concentrations (MIC) of the test organisms which is the lowest concentration of the plant extracts which can inhibit the growth of the organisms as determined from the zones of inhibition.

Table 4: Minimum Inhibitory Concentrations (MIC) of the aqueous and methanolic extracts of *Chrysophyllum albidum* fruit on selected organisms.

Organisms	**Minimum Inhibitory Concentration (mg/l)**	
	Aqueous	Methanolic
Staphylococcus aureus	25	50
Klebsiella pneumonia	25	50
Candida albicans	25	50
Escherichia coli	25	50

Discussions

Phytochemicals are believed to confer antimicrobial property to plants. In this work, the analysis of the phytochemicals of the fruits of *Chrysophyllum albidum*, revealed the presence of Flavonoids, Steroids, Alkaloids, Tannin, Anthraquinone and Cardiac glycosides while Saponin was found absent. The results obtained in this work was in agreement to the work of Duyilemi and Lawal [10]. They also reported that the plant contains saponons Tanins and Anthraquinine. Analysis of a part of the fruit by Orijajogun et al [11] also agreed with the components seen in this research. Plants that contains alkaloids have been discussed extensively by researchers to be antibacterial [12]. Also, Plants containing polyphenols are associated with their antioxidant activities and good potentials as anti-inflammatory, anti-diarrheal and anti-hemorrhoidal compound [11, 13]. Therefore, the presence

of cardiac glycoside in C. *albidum* makes it to be likelihood in application in congestive heart failure as reported by Aboaba [14].

This plant also have antimicrobial activity. From the result of this research, it was observed that all the concentrations of both the aqueous and methanolic extracts showed clear zones of inhibition against the four pathogenic bacterial and fungal isolates tested, which are *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia and Candida albicans* respectively. This means that all four organisms are susceptible to the extracts of *Chrysophyllum albidum* fruit.

This agreed with the findings of Adewusi [15], who reported that the latex or exudates from *C. albidum* plants has antimicrobial properties against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Streptococcus pyogenes* and *Candida albicans*. Okoli and Okere [16] also reported that *C. albidum* root extracts and stem slash successfully inhibited *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, C. tetani, Bacillus subtilis*, and *Candida albicans,* while the seed cotyledon inhibited *C. albicans*. It is obvious that *C. albidum* fruit has similar antimicrobial activity as other parts of the plant as they inhibit virtually the same microorganisms.

The maximum activity of the aqueous extracts in the test isolates was observed on *Staphylococcus aureus*, which showed clear zones with diameters of 24.0mm, 20.0mm and 16.5mm at concentrations of 100mg/ml, 50mg/ml and 25mg/ml respectively. The lowest zone of inhibition for the aqueous extracts was observed on *Klebsiella pneumonia,* with clear zones of inhibition of 15.0mm, 12.0mm and 10.5mm at same concentrations.

This results for the methanolic extracts also shown the same pattern. Once again, the highest zones of inhibition was observed on *Staphylococcus aureus* with clear zones of 21.omm, 17.5mm and 12.0mm at concentrations of 200mg/ml, 100mg/ml and 50mg/ml respectively. Also, the lowest zones of inhibition were seen on *Klebsiella pneumonia* with clear zones of 14.0mm, 11.5mm and 10.5mm at same concentrations. Thus, it can be inferred that *Staphylococcus aureus* is the most susceptible organism to both the aqueous and methanolic extracts of *Chrysophyllum albidum* fruit, while *Klebsiella pneumonia* is the least susceptible organism.

The type of extracts used in extraction also plays a role in the activity of *Chrysophyllum albidum* [17]. It is also pertinent to note that in this research, the aqueous extracts had greater activity than the methanolic extracts at same concentrations. For example, at 50mg/ml the zones of inhibition for the aqueous extracts on *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia and Candida albicans* were 20.0mm, 13.5mm, 13.0mm and 12.0mm respectively while their corresponding inhibition zones for the methanolic extracts at the same concentration were 17.5mm, 13.0m, 11.5mm and 14.5mm respectively. The Minimum Inhibitory Concentration (MIC) of both extracts was determined from the concentrations of the zones of inhibition, as 25mg/ml for the aqueous extracts and 50mg/ml for the methanolic extracts.

The results of the tests of both extracts were analysed with the analysis of variance (ANOVA). The probability of the result for aqueous extracts, assuming the null hypothesis was 0.245. Thus, the null hypothesis was rejected because the result is significant at ($P < 1$). Likewise, the result for the methanolic extracts, assuming the null hypothesis was 0.031. Also, the null hypothesis was rejected as the result is significant at ($P < 1$).

Conclusion

In conclusion, it was confirmed that the *Chrysophyllum albidum* fruit, specifically the pulp and peel also has antimicrobial properties against both bacterial and fungal isolates like other parts of the plant. This is because of its evident inhibition of the four pathogenic microbial isolates which were all susceptible to its aqueous and methanolic extracts. The antimicrobial activity could be because of the phytochemicals, bioactive compounds or principles in the fruit. This research therefore provides a scientific rationale for the traditional treatment of diseases with antimicrobial plants as well as the isolation and identification of the bioactive compounds.

Recommendation

In cognizance of the problems of antibiotic resistance by many pathogenic microorganisms, it is expedient that the use of antimicrobial or medicinal plants be encouraged in the traditional treatment of diseases in place of antibiotics which have lost their potency. It is also encouraged that more plants be studied for possible antimicrobial properties and efficacy against all kinds of organisms. Furthermore, efforts should be made to go one step further to isolate and identify the bioactive compounds or principles present in these plants and subsequently determine its spectra and potency (Rios and Recio, 2005). Further research is also required to utilize these bioactive compounds in the ethnomedicinal or ethnopharmacological production of phytopharmaceuticals. Hence, all pharmaceutical companies are encouraged to establish research and development units to achieve this purpose.

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