ANTI- BACTERIAL EFFECT OF *CHRYSOPHYLLUM ALBIDUM* PHYTO EXTRACT

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ABSTRACT

Aims: This study was carried out to investigate the antimicrobial activity of Chrysophyllum *albidum leaves extract on selected Gastro-instestinal bacteria* such as *Salmonella typhimurium, Shigella dysentariae, Vibrio cholera, Escherichia coli and Clostridium perfringens.*

Methodology: The leaves were extracted using ethanol, methanol and distilled water; the concentration of the extracts employed were 100mg/ml, 200mg/ml, 400mg/ml and 500mg/ml respectively; however the leaf extracts of *Chrysophyllum albidum* were screened for anti-microbial activity using the *in vitro* cup-plate method of agar diffusion technique with concentration of 10^{-5}cells/ml of the selected bacteria. Simultaneously, 30μg tetracycline and 30 μg metronidazole were used as positive control.

Result: The result showed that the most active among them is Tetracycline; followed by ethanolic extract, aqueous extract, methanolic extract and metronidazole extract respectively on the tested bacteria.

Conclusion: This research justifies the traditional use of the leaves of C*hrysophyllum albidum* for the therapeutic purposes; hence can be commercialized by pharmaceutical outfit; if not for anything but its availability and readily for human consumption.

Keywords: Anti-bacterial, *Chrysophyllum albidum,* Extract, Metronidazole, and Tetracycline

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1. INTRODUCTION

Antibiotics are one of the most important weapons in fighting bacterial infections and have greatly benefited the health related quality of human life; since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less effective against certain illness; not only because many of them produce toxic reactions; but also due to the emergence of drug-resistant bacteria. It is essential to investigate latest drugs with lesser resistance. In general; bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents. The emergence of multiple drug resistance bacteria (MDR) has become a major cause of failure of the treatment of infectious diseases (1). As a result, society is facing one of the most serious public health dilemmas over the emergence of infectious bacteria displaying resistance to many antibiotics as stated by (2). The continuous spread of multidrug resistance pathogens has become a serious threat to public health and a major concern for the infection control by the practitioners worldwide. In addition to; increasing cost of drug regimes; this scenario has paved way for the re-emergence of high frequency of opportunity and chronic infection cases in developing countries. The slow pace of the newer antibiotics development couple with the availability of fewer antimicrobial actions centered on the inhibition of the ergosterol synthesis; has provided the need to explore nature in search of the phytotherapeutic agent; work with novel targets and mode of actions.

Chrysophyllum albidum belongs to the Sapotaceae family and commonly found in Nigeria. It is common throughout the tropical central, East and West Africa regions for its sweet, edible and various ethno-medical uses. The plant is known as *Agbalumo* in Yoruba language in Nigeria. *Chrysophyllum albidum* fruits (known as African star apple) are widely eaten in Western and Southern Nigeria. The fruit is seasonal (December-March); when ripe, it is ovoid to sub-globose, pointed at the apex and up to 6cm long and 5cm in diameter. *Chrysophyllum albidum* leaves are used by the traditional medicine practitioners in Nigeria in the management and treatment of several disorders which include skin eruptions, diarrhoea and stomach-ache which are as a result of infection and inflammatory reactions (3). (4) Confirmed the antimicrobial effects of the seed oils from *Chrysophyllum albidum*. (5); validated the antibacterial activity of the *Chrysophyllum albidum* aqueous and methanolic leaves extracts. The methanolic extracts had stronger inhibitory effects on test microorganisms. *Chrysophyllum albidum* cotyledons are useful for the treatment of vaginal and dermatological infections (6). According to the (6); cotyledons of the *Chrysophyllum albidum* were also active against *Candida albicans and C. pseudorotropicalis.* The presence of the tannins in the seed cotyledon leaves and stem slash have also been reported by many researchers and these plant parts have anti-inflammatory effect which

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help control all indications of gastritis, oesophagitis, enteritis and irritating bowel disorders (7,8). Both the stem slash and seed cotyledon possess very high levels of alkaloids and flavonoids, and the latter show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity (9). These alkaloids may be toxic chemical element in the seed cotyledon; used as a remedy for fever; while the stem slash is used as emollients and for the treatment of skin eruptions, diarrhoea and stomach ache; which are as a result of infections and inflammatory reactions (10). The efficacy of the seed against vaginal infections and dermatological infections was confirmed by (11) and also its activity against *Candida albicans and C. pseudotropicalis*; this further explains the therapeutic and medicinal properties of the *Chrysophyllum albidium* and supported the use of this plant as an external application for the skin eruptions diseases. It has been observed that tannins are responsible for the anti-diarrhoeal activity (12). Evaluation of the potentials of *Chrysophyllum albidium* in wound care showed that the cotyledon extract exhibited haemostatic, antimicrobial and wound healing activities (13).

Due to wide use in ayurvedic medicine in Africa, we design to study the antibacterial potential of *Chrysophyllum albidum leaves extract*.

2. MATERIAL AND METHODS

2.1 Collection of Material

Fresh leaves of *Chrysophyllum albidum* was plucked from its plants growing on the Power Line way; Magboro, Ogun State and identified in hebarium of Department of Botany of the University of Lagos. The drugs used as control for this study were tetracycline and metronidazole and bought from a registered pharmacy at Ikorodu. The test organisms were obtained from Federal Institute of Industrial Research, Oshodi (FIIRO) Lagos, Nigeria.

2.2 Preparation of Extract

The leaves of *Chrysophyllum albidum* were thoroughly washed and rinsed with distilled water. The leaves were air dried for 14 (fourteen) days at room temperature and grounded into fine powder using grinding machine. 30g of the finely ground sample was weighed into three different 500ml beakers of the extracting solvents e.g distilled water, methanol and ethanol respectively and kept in a dark cupboard for five days. The samples were aseptically filtered using Whatman no 4 filter paper. The resultant extracts were each concentrated

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using rotary evaporator model (Buchi Rotarvapour R-114) which ensures evaporation of bulky solutions to small volume concentrates without bumping at temperature 40^0C. The resultant extracts were sterilized using Millipore filter (0.45µm) and then used for the antibacterial activity.

2.3 Phytochemical Screening

The phytochemical analysis was carried out using the method described by (14). The plant extracts were screened for the presence of tannins, saponins, flavonoids, steroids, glycosides, terpenoids, alkaloids and phenolic compounds.

Test for tannins: About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for saponins: 1g of the each sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 min. the mixture was filtered and 2.5ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stopped and shaken vigorously for about 30second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins. Appearance of an oil stain or a grease spot on the filter paper when observed under direct sunlight indicated the presence of fixed oils.

Test for Flavonoids: Two milliliter filtrate was added to conc. HCl and magnesium ribbon. Pink-tomato red colour indicated the presence of flavonoids.

Test for Steroids: 0.5ml of the each extract was dissolved in 3ml of chloroform and was filtered. To the filtrate, concentrated sulphuric acid was added by the sides of the test tube, which formed a lower layer. A reddish brown was formed.

Test for glycosides: 2ml of extract with the addition of hydrochloric acid solution (HCl) was neutralized with sodium hydroxide (NaOH) solution, then few drops of ferric chloride solution ($FeCl_3$) was added as well with 1ml of concentrated H_2SO_4 sulphuric acid underlaid. A reddish brown ring at the interface was observed, indicating the presence of cardiac glycosides in all the extracts.

Test for terpenoids (Salkowskitest): 5ml of each extract was mixed in 2ml of chloroform, and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids.

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Test for alkaloids: 1cm 3 of 1 % HCl was added to 3cm^3 of each extract in a test tube. Each extract treated with a few drop of Meyer's reagent. A creamy white precipitate was observed indicating the presence of alkaloids.

Test for phenolic compounds: 2ml of extract was added to 5.0ml of 95% ethanol; they were boiled in water bath for five minutes and filtered hot. 5.0ml of distilled water was added and the ethanol was evaporated at a reduced pressure in the water bath. The resultant concentrate with the addition of five drops of 1% of Ferric Chloride and 1% Potassium Ferric cyanide solution were added. A violet, wine, red, purple colour was developed, indicating a positive test for phenolic compounds.

2.4 Sensitivity Test

The antimicrobial tests of the plant extract were carried out on the five selected Gastro-intestinal; namely; *Salmonella typhimurium, Shigella dysentariae, Clostridium perfringens, Vibrio cholera* and *Escherichia coli*. Mueller Hinton agar was prepared for the test according to the manufacturer prescription. Test organisms were cultured and incubated overnight; after which a suspension of each test organism was made to give a concentration of about 10^{-5}cells/ml.

The leaf extracts of *Chrysophyllum albidum* were screened for the anti-microbial activity using the *in vitro* cup-plate method of agar diffusion technique (15). Aliquot of 1ml of the test organism suspensions was inoculated using micropipette with sterile tips; dropped onto the agar surfaces respectively. The bacterial suspension was spread aseptically on the agar surface; with the aid of hockey stick. The plates were allowed to absorb the organism suspensions at room temperature. A sterile cork borer of diameter 5mm was punched on the agar surface to make four wells; for ethanolic, methanolic and aqueous plates and filled with 100mg/ml, 200mg/ml, 400mg/ml and 500mg/ml of the plant extracts each respectively. Simultaneously, tetracycline (30 μg) and metronidazole (30 μg) were used as positive control. Control wells containing the same volume (100 µl) of distilled water, methanol and Ethanol were made. The plates were incubated at 35^{o}C overnight. The antibiogram plates were observed for zones of inhibition. The bacterial strains resistant to antimicrobial agent grew up to the edges of the well as against the sensitive strain which were inhibited at a distance from the well. The zones of inhibition around each well were measured using a transparent metric ruler in millimetres (mm) and the average diameter was taken.

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3. RESULTS

The ethanolic, methanolic and aqueous extracts are dark green, light green and brown respectively. The plant extracts were screened for the presence of Tannins, Saponins, Flavonoids, steroids, Glycosides, Terpenoids, Alkaloids and Phenolic compounds.

3.1 PHYTOCHEMICAL CONSTITUENTS OF *CHRYSOPHYLLUM ALBIDUM* LEAVES

Active ingrédients	Inférence
Tannins	+ + +
Saponins	+ +
Flavonoids	+ + +
Steroids	+ +
Glycosides	+ + +
Terpenoids	+
Alkaloids	+ +
Phenolic compounds	+ +

KEYWORDS

\+ Means present

_ Means absent

3.2 *Sensitivity of Chrysophyllum albidum on Salmonella typhimurium, Shegella dysentariae Clostridium perfringens, Vibrio cholerae and Escherichia coli*

3.2.1 Diameter of zone of inhibition (mm) of the Ethanolic extracts on the organisms

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	Zone of inhibition in (mm)				
Test Organism	**100mg**	**200mg**	**400mg**	**500mg**	**MIC**
Salmonella typhimurium	31mm	36mm	31mm	39mm	100mg
Shigella dysentariae	34mm	31mm	28.5mm	28mm	500mg
Clostridium perfringens	26mm	28.5mm	26mm	30mm	100mg
Vibrio cholera	23.5mm	26mm	24mm	26.5mm	100mg
Escherichia coli	25mm	27mm	30mm	27mm	100mg

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	Zone of inhibition in (mm)				
Test Organism	**100mg**	**200mg**	**400mg**	**500mg**	**MIC**
Salmonella typhimurium	27.5mm	33mm	33mm	35.5mm	100mg
Shigella dysentariae	21mm	26mm	28mm	22mm	100mg
Clostridium perfringens	23mm	28mm	25mm	25mm	100mg
Vibrio cholera	23mm	25.5mm	25.5mm	31mm	100mg
Escherichia coli	20.5mm	22mm	27mm	29mm	100mg

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3.2.3 Diameter of zone inhibition in (mm) of aqueous extract

	Zone of inhibition in (mm)				
Test Organism	**100mg**	**200mg**	**400mg**	**500mg**	**MIC**
Salmonella typhimurium	28.5mm	31.5mm	37mm	29mm	100mg
Shigella dysentariae	R	R	18mm	23.5mm	400mg
Clostridium perfringens	26mm	28.5mm	26mm	29mm	100mg
Vibrio cholera	21mm	26.5mm	24mm	33mm	100mg
Escherichia coli	17mm	22.5mm	25mm	27mm	100mg

Positive controls inhibited all selected organisms; but only water among the negative controls did not inhibit any of the organisms at all

KEY WORDS

µl = microlitre

R = Resistant

mm = milimeter

4.0 DISCUSSION

The ethanolic extract was dark green; the methanolic extract was light green while the aqueous extract was brown. The most abundant ingredients were tannins, flavonoids and glycosides. Pytochemicals analysis of the extracts indicated the presence of typical plant constituents such as alkaloids, saponins, tannins and phenolic compounds; however, the phenolic compounds in *Chrysophyllum albidium* may be responsible for the therapeutic,antiseptic, antifungal or bacterial properties of the plant.(16)

Antimicrobial activity of chrysophyllum *albidium* leaf extract were tested against four selected gram-negative bacteria such as *Salmonella typhimurium*, *Shigella dysentariae*, Vibrio cholera, and *Esherichia coli,* then one gram-positive bacteria which is *Clostridium*

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perfringens. They were compared with tetracycline and metronidazole. The result showed that the maximum inhibitory zone for ethanol extract on *Salmonella typhimurium* is 39mm at 500mg and the minimum inhibitory zone was 23.5mm at 100mg; for *Shigella dysentariae* the highest maximum inhibitory zone is 34mm at 100mg and the minimum inhibitory zone is 28mm at 500mg. The maximum inhibitory zone for the *Clostridium perfringens* is 30mm at 500mg and the minimum inhibitory zone is 26mm at both 100mg and 400mg; for the *Vibrio cholera;* the maximum inhibitory zone is 26.5mm at 500mg while the minimum inhibitory zone is 23.5mm at 100mg, the maximum inhibitory zone for the *Escherichia coli* is 30mm at 400mg while the minimum inhibitory zone is 25mm at 100mg.

The methanolic extract showed the following zones of inhibition; for the *Salmonella typhimurium* the maximum inhibitory zone is 35.5mm at 500mg while the minimum inhibitory zone is 33mm at both 200mg and 400mg; for *Shigella dysentariae* the maximum inhibitory zone is 28mm at 400mg while the minimum inhibition inhibitory zone is 21mm at 100mg; for the *Clostridium perfringens,* the maximum inhibition concentration is 28mm at 200mg while the minimum inhibitory zone is 23mm at 100mg. The maximum inhibitory zone of *Vibrio cholera* is 31mm at 500mg while the minimum inhibitory zone is 23mm at 100mg and for *Escherichia coli* the maximum inhibitory zone is 29mm at 500mg while the minimum inhibitory zone is 20.5mm at 100mg. (5); validated the antibacterial activity of *Chrysophyllum albidum* leaves aqueous and methanolic extracts; while the methanolic extracts had stronger inhibitory effects on test microorganisms.

The aqueous extract showed the following zones of inhibitions; for *Salmonella typhimurium* the maximum inhibitory zone is 37mm at 500mg while the minimum inhibitory zone of 28.5mm at 100mg; for the *Shigella dysentariae* there was no zone of inhibition at both 100mg and 200mg but the minimum inhibitory zone is 18mm at 400mg, for *Clostridium perfringens* the maximum inhibitory zone is 29mm at 500mg while the minimum inhibitory zone is 26mm at both 100mg at 400mg; for the *Vibrio cholerae* the maximum inhibitory zone is 33mm at 500mg while the minimum inhibitory zone is 21mm at 100mg; however, for *Escherichia coli* the maximum inhibitory zone is 27mm at 500mg while the minimum inhibitory zone is 17mm at 100mg. (5); validated the antibacterial activity of *Chrysophyllum albidum* leaves aqueous extract.

The antibiotics used showed that the following zones of inhibition; for tetracycline, the maximum inhibitory zone is 39.5mm on *Shigella dysentariae* at 30mg; while the minimum inhibitory zone was 33mm on *Escherichia coli* at 30mg; for the Metronidazole; the maximum inhibitory zone is 34.5mm on *Escherichia coli* at 30mg while the minimum inhibitory zone was 28mm on *Vibrio cholerae* at 30mg. The antimicrobial activity of *Chrysophyllum albidium* extract showed potent inhibition on some microorganisms. *Chrysophyllum*

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albidium root extracts successfully inhibited *P. aeruginosa*, *E. coli*, *S. aureus, C. tetani, B. subtilis, and C. albicans*. The stem slash also showed potent inhibition on these microorganisms,(Okoli and Okere,2010)(16).

The result showed that Tetracycline was more effective amongst all; followed by ethanolic, aqueous, methanolic extract and Metronidazole. However; the discriminate and proper use of some herbal products is safe and may provide some therapeutic benefits, but the indiscrimate or excessive use of herbs can be unsafe and even dangerous (17)

5.1 Conclusion

The result of this work justifies the traditional use of the leaves of *chrysophyllum albidum* for therapeutic purposes. The findings could also be of commercial interest to both pharmaceutical companies and research institute in the production of new drugs. The plant extract has active ingredients which are able to inhabit the growth of microbes that are capable of causing gastro-intestinal diseases. However; ethanolic extract was very active amongst the other extracts used, hence it is highly recommended that ethanol should be used for extraction of this plant; whenever is to be used to cure gastro-intestinal ailment caused by these selected organisms used in this work.

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