Original Research Article

ANTI-GASTRO-INTESTINAL BACTERIA OF CHRYSOPHYLLUM ALBIDUM LEAF EXTRACT

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ABSTRACT

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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 by the in vitro cup-plate method of agar diffusion technique with concentration of about 10^{-5}cells/ml of the selected bacteria. Simultaneously, 30mg/ml tetracycline and 30mg/ml 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 *chrysophyllum albidum* for the therapeutic purposes; hence can be commercialized by pharmaceutical outfit; if not for anything but its availability and non toxicity for human consumption.

Keywords: Anti- gastro-intestinal, bacteria, *Chrysophyllum albidum* Metronidazole, and Tetracycline

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.

Chrysophyllum albidum belongs to the sapoyaceae 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 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.

(1) Confirmed the antimicrobial effects of seed oils from Chrysophyllum albidum. Duyilemi and (2); validated the antibacterial activity of *Chryaophyllum albidum* water and methanolic leaves extracts. The methanolic extracts had stronger inhibitory effects on test microorganisms. *Chryaophyllum albidum* cotyledons are useful for the treatment of vaginal and dermatological infections (3). According to the Adesanya, (3); cotyledons of *Chryaophyllum albidum* were also active against *Candida albicans and C. pseudorotropicalis.*

In an effort to expand the spectrum of antibacterial agents from natural resources, *Chrysophyllum albidum* has been selected for study .The specific objectives of this study are as to determine the antimicrobial activity of *chrysophllum albidum* leaves on five selected Gastro-intestinal bacteria, to investigate the phytochemical constituents present in *Chrysophllum albidum* leaves, to determine the effectiveness of the ethanolic, methanolic and aqueous leaves extract of *Chrysophyllum albidum* on five selected Gastro-intestinal organisms; namely; *Salmonella typhimurium, Shigella dysentariae, Clostridium perfringens, Vibrio cholera* and *Escherichia coli* and compare them with tetracycline and metronidazole*; so as* to validate and justify the rationale behind the use of the leaves of *Chrysophyllum albidum* by local traditional health service provider for the treatment of stomach-ache and diarrhoea.

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; which were 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 days (fourteen days) at room temperature and grounded into fine powder using grinding machine. 30g of finely ground sample was weighed into three different 500ml beakers of 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 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 (0.45µ/m) and then use for the antibacterial activity.

2.3 Phytochemical Screening

The phytochemical analysis was carried out using the method described by (4). The plant extracts were screened for the presence of Tannins, Saponins, Flavonoids, steroids, Anthroquinones, Glycosides, phylobatannins, Terpeniids, Alkaloids and Phenolic compounds.

2.4 Sensitivity Test

The antimicrobial tests of the plant extract were carried out on the test isolates; using well diffusion method. Mueller Hinton agar was prepared for the test according to manufacturer prescription. Test organisms were first subculture and incubated for 24hrs; 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 anti-microbial activity by the in vitro cup-plate method of agar diffusion technique (5). 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 suspension 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 (30mg) and metronidazole (30mg) 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 for 24hours. The antibiogram plates were observed for zones of growth inhibition. The bacterial strains resistant to antimicrobial agent grew up to the edges of the well as against the sensitive strain which are 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.

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, Anthroquinones, Glycosides, phylobatannins, Terpenoids, Alkaloids and Phenolic compounds.

3.1 PHYTOCHEMICAL CONSTITUENTS OF CHRYSOPHYLLUM ALBIDUM LEAVES

Active ingrédients	Inférence
Tannins	+ + +
Saponins	+ +
Flavonoids	+ + +
Steroids	+ +
Anthroquinones	+ +
Glycosides	+ + +
Phlobatannins	+
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

	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

3.2.2 Diameter of zone of inhibition (mm) of methanolic extract

	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

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

3.2.4 Diameter of zone of inhibition in (mm) of standard antibiotics

	Zone of inhibition in (mm)				
Test Organism	Tetracyline	Metronidazole	Aqueous	Ethanol	Methanol(100 µl)
Salmonella typhimurium	35mm	29mm	R	R	R
Shigella dysentariae	39.5mm	34mm	R	12mm	R
Clostridium perfringens	38mm	31mm	R	10mm	6.5mm
Vibrio cholera	34mm	28mm	R	R	R
Escherichia coli	33mm	34.5mm	R	R	R

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.

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

inhibition concentration for *Escherichia coli* is 30mm at 400mg while the minimum inhibition concentration is 25mm at 100mg.

The methanolic extract showed the following zones of inhibition; for the *Salmonella typhimurium* the maximum inhibition concentration is 35.5mm at 500mg while the minimum inhibition concentration is 33mm at both 200mg and 400mg; for *Shigella dysentariae* the maximum inhibition concentration is 28mm at 400mg while the minimum inhibition concentration is 21mm at 100mg; for *Clostridium perfringens,* the maximum inhibition concentration is 28mm at 200mg while the minimum inhibition concentration is 23mm at 100mg. The maximum inhibition concentration of *Vibrio cholera* is 31mm at 500mg while the minimum inhibition concentration is 23mm at 100mg and for *Escherichia coli* the maximum inhibition concentration is 29mm at 500mg while the minimum inhibition concentration is 20.5mm at 100mg.

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

The antibiotics used showed that the following zones of inhibition; for tetracycline, the maximum inhibition is 39.5mm on *Shigella dysentariae* at 30mg; while the minimum inhibition concentration was 33mm on *Escherichia coli* at 30mg; for Metronidazole the maximum inhibition is 34.5mm on *Escherichia coli* at 30mg while the minimum inhibition concentration was 28mm on *Vibrio cholerae* at 30mg.

The result showed that Tetracycline was more effective amongst all; followed by ethanolic, aqueous, methanolic extract and Metronidazole.

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.

It was observed that tetracycline is the most effective of them all; followed by ethanolic extract, aqueous extract, methanolic extract and metronidazole on the tested organisms.

The phytochemical analysis showed that the most abundant ingredients were *Tannins, Flavonoids and Glycosides.*

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(6)

5.2 Recommendation

I hereby recommend the use of plant extract of *chrysophyllum albidum* for therapeutic purpose. 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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