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RESEARCH ARTICLE

Secondary Metabolites and Antimicrobial Profile of the Crude Stem and Rhizome Extracts of Lasimorpha Senegalensis (SCHOTT) Araceae

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Abstract

Medicinal plants have been used to treat a variety of disorders in African traditional medicine as well as other forms of treatment from around the world. Approximately 80% of the world's population, primarily in Africa and other underdeveloped countries, still relies mainly on traditional or herbal medicine for disease treatment. The study's goal was to evaluate the stem and rhizomes of Lasimorpha senegalensis for phytochemical and antibacterial properties utilizing clinical isolates. The stems and rhizomes were obtained in the wild in Korokorosei Community, Southern Ijaw LGA, Bayelsa state, and identified at the NDU Department of Pharmacognosy and Herbal Medicine. Following preliminary phytochemical screening of the crude stem and rhizome, methanol, water, and dichloromethane fractions were extracted from both stem and rhizomes and subjected to TLC and antimicrobial evaluation against some clinical isolates obtained from Niger Delta University Teaching Hospital, Okolobiri, Yenagoa, Bayelsa State, Nigeria. The presence of flavonoids, tannins, glycosides, and modest antibacterial activity on the stem was discovered. The methanolic and dichloromethane fractions of the stem showed mild activity against Pseudomonas aeruginosa, compared with the standard drug used, with the highest zone of inhibition at 8 mm and lowest at 4 mm, at 500 mg/ml and 62.5 mg/ml respectively, while the aqueous fraction showed no activity against all the test organism.

Keywords: Medicinal Plant, Lasimorpha Senegalensis, Antimicrobial, Phytochemicals, Korokorosei.

1. Introduction

Natural products are becoming more important in drug manufacture, not only when bioactive substances are employed directly as therapeutic medications, but also when used as a raw material for drug synthesis or as a model for the search for biologically active compounds (Di Nardo & Gilardi, 2020; Veeresham, 2012). Previous research has revealed that only approximately 10% of the 250,000 plant species known globally have been used for medical purposes. (Calixto, 2019; Thomford et al., 2018). As a result, the use of herbal pharmaceuticals as an alternative to conventional drugs and antibiotics is being studied all the time. For many years, a variety of plants have been employed in traditional medicine. Some appear to work, yet there may not be enough scientific evidence to back them up. Such plants should be classified as medicinal plants (Ekor, 2014; Yuan et al., 2016). A thorough compilation of medicinal plants that can be employed in illness prevention requires the collection of original data from traditional custodians of such knowledge. This necessitates prudence in the use of medicinal plants, which are becoming more popular

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because of their ease of availability, affordability, accessibility, and promising efficacy in comparison to the often-high cost and harmful effects of traditional synthetic pharmacological agents (Sofowora et al., 2013). All medicines, whether synthetic or natural, must meet the basic criterion of being both safe and effective. Herbal medications are plants or plant moist components that have been turned into phytopharmaceuticals through easy harvesting, drying, and storing techniques. As a result, they are adaptable (M.O. Nafiu, 2017). Differences in growth, geographical location, and harvesting time all contribute to this variability. A lack of scientific data, ineffective urgent medical care, insufficient standardization, and a lack of quality criteria all have an impact on the usage of medicinal herbs (Nilakshi Pradhan et al., 2015).

Lasimorpha senegalensis (Schott) is an Araecea species found in swampy environments where it generates vast populations due to the rapid development of underground suckers. There are no genetic resource collections for this plant (van der Burg, 2004). The plant has big leaves, soft-thorned striated stems (Figure 1B), tuber-like rhizomes (Figure 2), and maize-like fruit compartments. The species is not threatened by genetic degradation. L. senegalensis appears to have limited potential as a vegetable. It has previously been reported to have antimicrobial effects (Bunu et al., 2022). Its potential for usage as an indoor pot plant in temperate climates or as a garden pond decorative in warmer climates is promising. Although no particular mention of this species has been found, it is a member of a family in which the majority of the individuals contain calcium oxalate crystals. This substance is dangerous when fresh, and when eaten, it feels like hundreds of small needles are digging into the lips, tongue, and throat (van der Burg, 2004). However, calcium oxalate is easily broken down by properly boiling or drying the plant, and the plant is safe to eat in either of these forms. In the wild, L. senegalensis grows freely with little or no nursing (Figure 1) (Arnold, 2014). The plant is found commonly in Nigeria, and other parts of Africa, mainly seen on Edges of swamp forests, along slow-flowing streams, in ditches and ponds, often very abundant (Bunu et al., 2022).

Figure 1. *Lasimorpha senegalensis: Natural habitat (A), Stem (B)*

Lasimorpha senegalensis leaves are used to wrap cassava flour dumplings, the plant's young leaves are eaten as a vegetable, and the rhizomes are used to treat ulcers in Gabon, while the young leaf is eaten as a famine food and an ingredient in palaver sauce in Sierra Leone (Fern, 2023). In Congo, the leaves are given to women during labor to help them deliver faster. A decoction of the leaves is used as an analgesic and sedative, as a cough remedy, and in higher dosages to treat restlessness and agitation. In addition, in Côte d'Ivoire, the leaf sap has been used orally to treat hiccups (Lamxay et al., 2011). In the southern portion of Nigeria, the fruits are employed in gonorrhea and dysentery cures, while salt is produced from the ashes of burned plants in Sierra Leone and Gabon (Anumudu et al., 2019).

Figure 2. *RhizomeS of Lasimorpha senegalensis*

Plants are a potential source of antibacterial agents in several areas. Plant-derived medication is used by 60 to 90% of the population in underdeveloped countries (Wendakoon, 2012). Historically, crude plant extracts have been utilized as herbal medicine to treat human infectious disorders. Plants are high in phytochemicals such as tannins, terpenoids, alkaloids, and flavonoids, which have antibacterial activities in vitro. Although the mechanism of action and efficacy of these herbal extracts have yet to be experimentally verified in most cases, these preparations mediate major host responses (Amara et al., 2008; Bhalodia & Shukla, 2011). Infectious diseases produced by bacteria are a major public health concern worldwide. Controlling plagues such as bacterial and fungal infections, malaria, cancer, and many others is becoming more difficult due to the advent of disease resistance to many conventional medications. This resistance is increasing at an alarming rate, outpacing the discovery of novel methods of addressing these diseases (Baker et al., 2022; Institute of Medicine, 2003). When confronted with these public health challenges, traditional medicine may provide a treatment approach customized to the populations' financial resources and socio-cultural context. Because of their relative safety and efficacy, the recent rise in antibiotic resistance and associated toxicity issues is sparking renewed interest in the antimicrobial role of plants against resistant bacteria. As a result, natural remedies are becoming a realistic choice in primary care systems, as well as a promising avenue for the development of traditionally improved pharmaceuticals (Ventola, 2010; Yuan et al., 2016). The present study was undertaken to investigate Lasimorpha senegalensis stems and rhizomes for their potential activity against human bacterial pathogens.

2. Method

2.1 Plant Collection and Extraction

Lasimorpha senegalensis (Schott) Aracae stem and rhizome were obtained in the Korokorosei community of Southern Ijaw Local Government Area, Bayelsa state, Nigeria. The was identified and authenticated at Niger Delta University's Department of Pharmacognosy and Herbal Medicine. The stem and rhizome were collected, air-dried, and pulverized to a coarse powder using a mechanical blender. About 921g of the coarse stem powder was transferred into a reagent bottle and about 3.8L of Methanol, and 903g of the coarse rhizome powder were transferred into another reagent bottle and about 3.8L of Methanol were used to extract two weeks using the cold maceration technique. The extract was filtered, and the filtrate was concentrated in a 44°C water bath. And the concentrated extracts were allowed to cool before being carefully stored.

2.2 Phytochemical Screening

The presence of different phytochemicals including saponins, steroids (Cardiac glycoside), flavonoids, Ketones (secondary metabolites), and tannins, were screened from the plant, and observations were recorded (Viteri et al., 2022).

2.3 Antimicrobial Evaluation

An equivalent amount of 0.5g extract was diluted in 0.5 ml of 0.5% DMSO and thoroughly shaken. The extract was then concentrated to 500 mg/ml (5 ml) by adding 4.5 ml of distilled water to the solution. Half of the initial concentration's volume (2.5 ml) was transferred to a fresh test tube and diluted with 2.5 ml of distilled water, lowering the initial concentration

by half (250 mg/ml). Following the same technique, more serial dilutions were created, yielding five distinct concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml, and 62.5 mg/ml). This was repeated for all the fractions obtained (aqueous, methanol, and DCM), respectively. The antimicrobial screening was carried out against the following Organisms Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Proteus vulgaris. All of these organisms were taken from the laboratory stock that was kept in the refrigerator. The test organisms were cultivated from a molten agar plate and incubated for 24 hours at 37°C. The turbidity of the various bacteria cultures or isolates was adjusted and compared to McFarland's standard using a sterile container containing 5ml of sterile water (Giuliano et al., 2019). The weight of the agar-agar was estimated based on the amount of agar required in milliliters (40ml), then transferred to a beaker containing distilled water and autoclaved at 121°C for 15 minutes. The agar was then put into the Petri dishes, let to cool and set, and then dried in a hot air oven. To avoid contamination, these activities were carried out in aseptic conditions.

2.4 Antimicrobial Susceptibility Testing

The standardized bacteria suspension was placed into Muller Hinton Agar plates, and the excess fluid was poured into a sodium hypochlorite-containing beaker. To achieve a uniform and confluent growth, the suspension was poured twice over the entire surface by repeating the operation, with the second time being taken care of to spin the plate through 60°. For 5-15 minutes, the inoculum on the agar plate was allowed to dry. A sterile cork borer was used to aseptically make four holes of 10 milliliter diameter, 15mm apart, in each agar plate. Two drops of molten agar were used to close each hole using a sterile pasture pipette. Concentrations of the test sample extract of 500 mg/ml, 250 mg/ml, 125 mg/ml, and 62.5 mg/ml were put into the various holes and let to stand for 1 hour for adequate pre-diffusion of the extracts to occur. Each fraction (aqueous, methanolic, and dichloromethane fractions) was tested against each of the test species (Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vulgaris) using this procedure separately and incubated at 37°c for 18-24hour and inhibition zone measured and recorded.

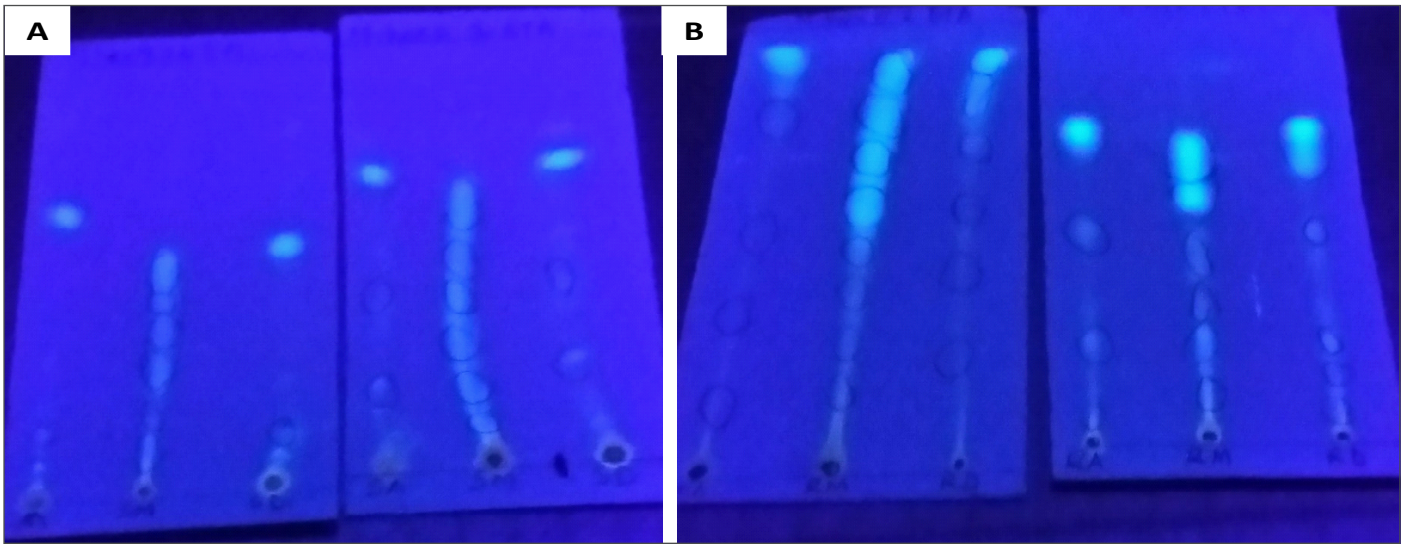
2.5 Thin Layer Chromatography

The varied fractions were spotted on the plate using a capillary tube, and the plate was placed in a hot air oven for a few minutes to evoke the polarity of the TLC plate. A saturated chamber with the solvent systems n-hexane and Ethyl acetate in the ratios 5:2 and 9:1 was created. The TLC plate was then placed inside the chamber. After a few minutes, the plate is removed from the chamber when the solvent has reached the mark. The plate was allowed to dry before being examined under an ultraviolet lamp and the spots observed were noted. It was doused with anisaldehyde and sulfuric acid and allowed to dry, revealing the dots. The retardation factor was calculated using the distance moved by the solute and the distance moved by the solvent front.

3. Results

Table 1. *Phytochemical and Secondary metabolites screening of the stem and the fruit of L. senegalensis*

Phytochemicals	Test	Positive indicator	Result (Stem)	Result (Rhizome)
Tannins	0.5 g extract, 10 ml of distilled water, few drops of ferric chloride	A brownish-green coloration	++	+
Ketones	2 ml extract of aqueous extract, a few drops of resorcinol, 2 ml of conc. HCL.	A rose coloration	++	++
Cardiac glycoside	0.5 g extract in 5 ml chloroform, filter, 1 ml acetic acid, 1 ml conc. sulfuric acid.	Change in color from violet to green	+	+++
Saponins	0.5 g extract, 10 ml distilled water, shake vigorously	Frothing which persists in warming	+	-
Flavonoids	5 ml Ammonia (10%), 3 ml aqueous filtrate of the extract, concentrate, equal volume of conc. sulfuric acid	A yellow coloration	+++	+++



Legend: *SA/RM – Stem/Rhizome aqueous fraction, SM/RM – Stem/Rhizome methanol fraction, SD/RD – Stem/Rhizome dichloromethane fraction.*

Figure 3. *TLC spots on the plate for the different fractions of the stem (A) and rhizome (B) Under the UV light*

Table 2. *Showing the antimicrobial activity of the stem of L. senegalensis (Schott) Aracae*

Organisms	Aqueous fraction (mg/ml)				Methanol fraction (mg/ml)				Dichloromethane fraction (mg/ml)				-ve Control (H_2O)	+ve Control (Amoxil)
Conc (mg/ml)	500	250	125	62.5	500	250	125	62.5	500	250	125	62.5		
E. coli (mm)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa (mm)	0	0	0	0	8	5	5	4	5	5	4	4	0	19
Staphylococcus aureus (mm)	0	0	0	0	0	0	0	0	0	0	0	0	0	18
Proteus vulgaris (mm)	0	0	0	0	0	0	0	0	0	0	0	0	0	10

Legend: *mm = millimeter, +ve = positive, -ve = negative*

4. Discussion

From the results obtained, the stem has flavonoids, tannins, ketones, steroids, or cardiac glycosides, and saponins while the rhizomes showed negative on the saponins test but positive on every other phytochemical test, as shown in Table 1. The Thin Layer Chromatography showed that the stem aqueous fraction with the solvent system ratio of n-hexane 5:2 Ethyl acetate showed the presence of three (3) spots. Methanol fraction showed six (6) spots and the dichloromethane showed three (3) spots. While a solvent system with the ratio of n-hexane 9:1 Ethyl acetate, the aqueous fraction showed three (3) spots. Methanol fraction showed the presence of six (6) spots and the dichloromethane showed two (2) spots (Figure 3). TLC for the rhizome with solvent system n-hexane 9:1 Ethyl acetate. The aqueous fraction showed four (4) spots, the methanol fraction showed the presence of six (6) spots and the dichloromethane fraction showed the presence of five (5) spots. For the ratio of n-hexane 5:2 Ethyl acetate, the aqueous fraction showed five (5) spots, the methanol fraction showed the presence of eight (8) spots and dichloromethane showed the presence of six (6) spots (Figure 3).

From the antimicrobial susceptibility test, all extracts, fractions, and concentrations showed no activity against E. coli, Staphylococcus aureus, and Proteus vulgaris. The methanolic and dichloromethane fractions of the stem showed mild activity against Pseudomonas aeruginosa, compared with the standard drug used, with the highest zone of inhibition at 8mm and lowest at 4mm, at 500mg/ml and 62.5mg/ml respectively, while the aqueous fraction showed no activity against all the test organism (Table 2).

All the fractions of the rhizome showed no activity against the test organisms. This result is similar to the report by Anumudu et al. (2019), who conducted an antimicrobial screening of Lasimorpha senegalensis and revealed that the methanolic and aqueous extract showed mild antimicrobial activities against the growth of pathogens such as S. aureus and E. coli. Also, previous has it that, the leaves and fruits part of this plant showed activities against Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli, but activity against Proteus vulgaris (Bunu et al., 2022).

Each of these spots from the TLC results indicates distinct chemical entities, hence, the plant is poised with many chemical constituents, that might be medicinally useful, and possibly serve as potential lead compounds for the discovery, design, and development of useful antimicrobial agents and other medicinal molecules based on the plant reported ethnobotanical applications.

5. Conclusion

Secondary metabolites found in the plant stem include flavonoids, tannins, ketones, steroids (cardiac glycosides), and saponins, while the rhizomes tested positive for all of the aforementioned phytochemicals except saponin. The results suggest that the antibacterial activity is mild. The stem's methanolic extract displays antibacterial activity against Pseudomonas aeruginosa and Staphylococcus aeruginosa.

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