Screening Of Methanolic And Aqueous Extracts Of Lasimorpha senegalensis For Antibacterial Activity

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ABSTRACT

Traditional medicine, though an old practice in disease prophylaxis and therapy, is still widely employed globally to treat various human ailments. In this study conducted at the Department of Microbiology, University of Nigeria between October 2018 and January 2019, methanolic and aqueous extracts of an aquatic plant *Lasimorpha senegalensis* were evaluated for antibacterial activities against human pathogens; *Escherichia coli* and *Staphylococcus aureus*. Agar well diffusion method was used to determine the potency of *L. senegalensis* against the test organisms at different concentrations. Also, minimum inhibitory concentration (MIC) was determined by tetrazolium chloride microtiter dilution assay. Results showed that; inhibition zone diameters ranging from 0-14mm for both test organisms using the plant extracts was less than that of the control (septrin and chloramphenicol) ranging from 0-26mm. MIC ranged from 62.5mg/ml to 500mg/ml, lowest MIC was obtained with methanolic stem extract. Preliminary phytochemical screening revealed the presence of flavonoids responsible for the antibacterial activity. Therefore, *L. senegalensis* should be considered medicinally important as they contain biologically active compounds with curative potentials against infectious diseases.

Keywords; Antibacterial; Escherichia coli; Lasimorpha senegalensis; Phytochemicals; Staphylococcus aureus.

1. Introduction

Worldwide, infectious diseases are the leading cause of death worsened by resistance of infectious agents to antibiotics [1]. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens.

In Sub-Saharan Africa, due to poor sanitation systems, it is often a common practice to dump environmental pollutant such as pharmaceutical wastes, agricultural discharges and other industrial wastes into aquatic systems. This results in the development of antimicrobial resistance by aquatic microflora [2]. Antimicrobial resistance in bacteria has been attributed primarily to the misuse and overuse of antibiotics as well as the possession of drug resistant plasmids; nevertheless, resistant strains also emerge through acquisition of mobile genetic elements such as plasmids, transposons, through horizontal gene transfer [3, 4]. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs which are utilized as therapeutic agents, this problem is growing and the outlook for use of antibiotics in the future is still uncertain which necessitates the need to take actions to combat this problem. The ultimate goal is to offer appropriate and efficient antimicrobial agents to patients. Plants are being used for certain purposes ranging from food sources to medicinal agents. *Lasimorpha senegalensis* is a herbaceous, perennial plant usually seen in swampy areas, producing a clump of leaves from a short, thick rhizome that is vigorously stoloniferous. The leaves each comprise an erect, spiny petiole up to 100cm long topped with an arrow-shaped leaf blade up to 50cm long and 30cm wide [5].

Fig. 1: Photograph of *L. senegalensis* growing in its natural habitat [Source: www.westafricanplants.senckenberg.de]

It is found commonly in Senegal, Sierra Leone, Chad, Nigeria, Central African Republic, DR Congo, Gabon and Angola [5]. In Gabon, the young leaves are eaten as vegetables while the rhizomes are used to treat ulcers. Also in Sierra Leone, the young leaves are eaten as famine food and as an ingredient of palaver sauce. In Congo the leaves are taken to cure cough and in larger doses to treat nervousness and agitation, it is also given to women during childbirth to accelerate delivery. In Côte d'Ivoire the leaf sap has been taken orally against hiccups. In southern Nigeria the fruits are an ingredient of remedies for gonorrhoea and dysentery [6]. Though consumed as vegetables, *Lasimorpha senegalensis* contains calcium oxalate crystals which are toxic if consumed raw. However, calcium oxalate is easily broken down either by thoroughly cooking the plant or by fully drying it. In addition, special caution should be taken when including this plant in the diet of people suffering from rheumatism, arthritis, gout, kidney stones and hyperacidity as it could induce adverse side effects [5]. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world and according to the World Health Organization (WHO), plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population [7]. Global burden of infectious diseases caused by bacterial agents is a serious threat to public health. The test organisms selected for this study include *Staphylococcus aureus* and *Escherichia coli*. *Escherichia coli* is a motile, non-spore forming facultative anaerobe that colonizes the human gut, based on its unique virulence factors, six pathogenic groups have been identified; enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC) , enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and perhaps others that are not yet well characterized [8]. On the other hand, *Staphylococcus aureus* is a major human bacterial pathogen that has been implicated in a wide range of clinical manifestations [9]

Due to the ever increasing incidence of antimicrobial incidence, medicinal plants have been sought as valuable alternatives for maintaining human health, especially in the last decade owing to the fact that they produce a wide variety of secondary metabolites with antimicrobial properties such as tannins, terpenoids, alkaloids and flavonoids [10, 11]. While antibiotic treatment is a preferred choice to treat bacterial infections, however, emergence of antimicrobial resistance and toxicity issues threatens the use of antibacterial agents [12]. This study was conducted to evaluate the antimicrobial properties of

both the methanol and aqueous extracts of *Lasimorpha senegalensis* against bacterial pathogens; *Staphylococcus aureus* and *Escherichia coli*

2. METHODOLOGY

2.1 Study Area

Awka is a town in Akwa South LGA in Anambra state, Nigeria. Akwa lies geographically on 6^{o} 12' 45" N and 7^{o} 4' 19" E with a total population of about 250, 900 and about 320km south of Abuja, the country's capital city.

2.2 Collection of Samples

Leaves and stems of Lasimorpha senegalensis where collected from a swamp in Awka Local government area in Anambra state, Nigeria, washed and taken to the Department of Plants Science and Biotechnology University of Nigeria Nsukka for identification and taxonomical authentication.

2.3 Preparation of Plant Extracts

The freshly collected leaves and stems of *Lasimorpha senegalensis* were washed and dried at room temperature for 8 days to ensure proper drying. The dried leaves and stems were finely ground separately and transferred into an airtight container for the extraction of bioactive compounds.

2.3.1 Methanolic Extraction for leaf and stem

For methanolic extraction, the leaf (34.20g) and stem (34.20g) powder of *L. senegalensis* was mixed separately with 250ml of methanol in 500ml conical flasks. The flasks were properly closed and left for 5 days at room temperature with constant agitation for proper mixing. After 5days, the extraction was filtered with a sieve followed by fiilteration with filter paper to remove the residues. The methanolic extract filtrate was evaporated using a rotary evaporator (KNF RC 900, USA) leaving the dried extract in a sterile bottle. The percentage yield of methanolic extracts was between 7–18%w/w.

2.3.2 Aqueous Extraction for leaf and stem

Finely ground leaf and stem powder (34.20g each) were soaked separately in distilled water (250ml) in 500ml conical flask. The flask was properly closed and left for 5 days at room temperature with constant agitation for proper mixing, after which the mixture was filtered and the filtrates subjected to water bath evaporation at 40^{o}C. After evaporation, the resulting distilled water extracts gave a percentage yield of 6-14%w/w.

2.3.3 Preparation of stock solution

Stock solution of both methanolic and distilled water extracts was prepared as follows; 0.5g of the extract in 0.5ml of 5% DMSO and 4.5ml of distilled water [13].

2.4 Collection of the test organisms

Pure cultures of two test organisms; *Staphylococcus aureus* and *Escherichia coli* were collected from the University of Nigeria Medical Centre and maintained in slants at 4^{o}C.

2.5 Preparation of MacFarland's Standard.

The MacFarland standard was prepared with 0.118 g of barium chloride crystals and 10ml of distilled water, to make a 10% barium chloride solution.0.1ml of concentrated $H_2SO_4$was added to 10ml of distilled water in a clean test-tube to make a 10% H_2SO_4 solution, then 9.9ml of 10% H_2SO_4 solution was mixed with 0.1ml of barium chloride solution to make a 1.0 MacFarland standard and stored in the refrigerator.

2.5.1 Standardization of the test organisms

Sterilized normal saline (1ml) was pipetted into a sterilized test tube and 1ml of MacFarland's standard reagent was transferred to another sterilized test-tube. Using a sterile wire loop, the normal saline was inoculated with the test organisms and the turbidity was compared with MacFarland's standard till both had same turbidity.

2.6 Antibacterial Sensitivity Screening

Agar well diffusion method was used. Sterile petri dishes containing solidified Muller Hinton agar were inoculated with 0.1ml of the standardized organisms. Using a sterile corkborer, five agar wells of about 5mm were made on the plates and filled with different dilutions of the leaf and stem extract and incubated at 37^0C for 24hours. After incubation, the zones of inhibition were measured, recorded and interpreted according to the Clinical Laboratory Standard Institute [14].

2.7 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) for plant extract was evaluated according to method described by [15]. MHB (50ul) was dispensed into each column of a 50-well micro plate followed by addition of 100ul (500mg/ml) of the plant extracts into the first column of the microplates. Serial dilution was subsequently conducted and the columns were inoculated with 100ul of the bacterial suspension and incubated for 24hrs at 37°C. MIC was determined by adding 30ul (2mg/ml) of 0.02% p-iodonitrotetrazolium chloride (INT) and incubated at 37°C for 30 minutes.

2.8 Phytochemical Screening

2.8.1 Test for saponins: the leaves and stems extracts (2g) were was boiled in 20ml of distilled water in a water bath and filtered. Then, 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil, shaken vigorously and observed for the formation of emulsion [16].

2.8.2 Test for Tannins: the dried powdery extract (0.5g) was boiled in 20ml of water in a test tube and then filtered. A few drop of 0.1% ferric chloride was added and observed for brownish green or a blue black coloration [13].

2.8.3 Test for Flavonoid: 10% dilute ammonia solution (5ml) was added to a portion of the aqueous filtrate of the plant extract, followed by addition of concentrated H_2SO_4. A yellow coloration observed in the extract indicated the presence of flavonoid [16].

3. RESULTS

3.1 Antibacterial Sensitivity of the extracts

Result of the sensitivity screening for methanolic leaf/stem extracts and aqueous leaf/stem extracts is as shown (Table 1; Fig. 2). The zone of inhibition ranged from 4mm to 14mm for *E.coli* and 5mm to 14mm for *S. aureus.* Highest zones of inhibition for *E.coli* and *S. aureus* were recorded for methanolic stem extracts at 500mg/ml and 62.5mg/ml respectively.

Table 1: Antimicrobial sensitivity test of the extract

Extracts/Test organisms	500mg/ml	250mg/ml	125mg/ml	62.5mg/ml	31.25mg/ml	CCA
			Methanolic stem extract			
Escherichia coli	14mm	-	-	-	-	26mm
Staphylococcus aureus	-	-	-	14mm	-	23mm
			Methanolic leaf extract			
Escherichia coli	5mm	-	-	4mm	-	25mm
Staphylococcus aureus	-	-	10mm	-	-	23mm
			Aqueous stem extract			
Escherichia coli	12mm	-	-	7mm	5mm	-
Staphylococcus aureus	7mm	5mm	5mm	-	6mm	12mm
			Aqueous leaf extract			
Escherichia coli	7mm	-	-	-	-	-
Staphylococcus aureus	8mm	10mm	-	6mm	-	20mm

Legend;

CCA= Ampicillin (control for extracts against Gram positive *Staphylococcus aureus*)

CCA= Septrin (control for extracts against Gram negative *Escherichia coli*)

Fig. 2. Photographs showing inhibition of growth of the test organisms by *L. senegalensis*' leaf and stem extracts

3.3 Minimum Inhibition Concentration (MIC) Test

Methanolic stem extracts had the lowest MIC (62.5-125mg/ml) against both test organisms while the MIC for the aqueous extracts were significantly higher (250-500mg/ml) as shown in Fig. 3.

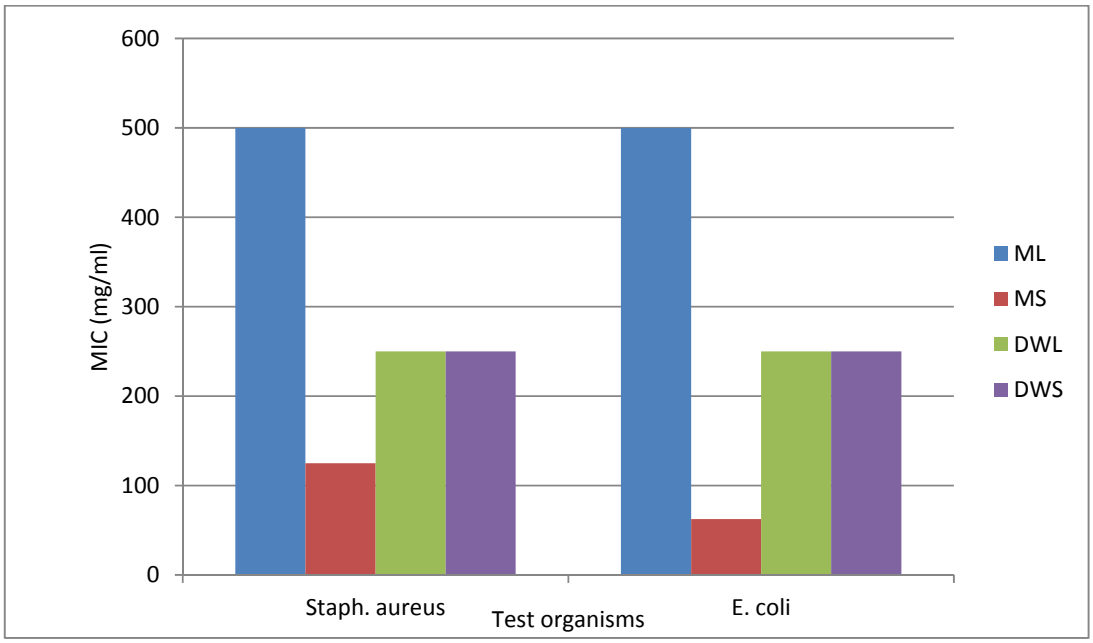

Fig. 3. MIC of *L. senegalensis*' leaf and stem extracts

Legend: ML- Methanol leaf extract; MS-Methanol stem extract

DWL- Aqueous leaf extract; DWS- Aqueous stem extract

3.4 Phytochemical Screening Test

Phytochemical screening showed that both stem and leaf extracts were highly abundant in a bioactive compound flavonoid but lacked other tested constituents such as saponins and tannins (Table 2).

Table 2: Qualitative analysis of phytochemical constituents of *Lasimorpha senegalensis*

Constituents	Leaf extract	Stem extract
Saponin	-	-
Tannin	-	-
Flavonoid	+++	+++

Legend:

(--)= Absent; (+++)= High in abundance.

4. DISCUSSION

In this study, the antimicrobial activity of an aquatic plant, *Lasimorpha senegalensis* was investigated. The biological activity of these plant extracts was tested against known human pathogens, *Escherichia coli* and *Staphylococcus aureus*. Findings from the study revealed that both the methanolic and aqueous stem and leaf extracts of the plant had antibacterial activity against the test organisms between 62.5mg/ml to 500mg/ml of the test concentration. This is in consonance with previous research [17] where antimicrobial activity against *K.pneumonia*, *S.aureus*, *S.typhi*, *Citrobacter* sp and *E coli* was recorded for the medicinal plant, *Sida rhombifolia*. Though the extracts (both leaf and stem) showed more potency against *Staphylococcus aureus* than *Escherichia coli*, the methanolic stem extracts were more effective at lower MIC (62.5-125mg/ml) with larger zones of inhibition (14mm±0.02). However, another study[18] reported a dissimilar finding where the aqueous extract (400 mg/ml) zone of inhibition for the growth of *S. aureus* was recorded as 19.75±1mm and 18.5±0.7mm for aqueousand methanol extracts accordingly. [18] attributed their findings to the loss of bioactive compounds responsible for antimicrobial activity via evaporation during methanol extraction. The phytochemical screening of the crude extract of *L. senegelensis* revealed high abundance of flavonoids in both stem and leaf extract. Similarly, [17] also reported the presence of phytochemicals including terpenoides, alkaloids, quinine, polyphenols, and flavonoids but absence of saponins and cardiac glycosides in *Sida rhombifolia*'s extracts. Hence, flavonoids may be responsible for antibacterial activities in *Lasimorpha senegalensis* crude extracts. In addition, the higher antibacterial activity recorded for the stem extracts indicates more quantitative composition of such flavonoids by the stem. Generally, the antimicrobial activities of plant crude extracts depend on its; phytochemical constituents, concentration, the type and population of microbial strains involved.

5. CONCLUSION

This study revealed that *Lasimorpha senegalensis* methanolic and aqueous extracts revealed have antimicrobial activities against the growth of pathogens such as *S. aureus* and *E. coli.* However, further studies are required to understand the mechanism of action of these extracts as antimicrobial agents.

COMPETING INTEREST

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Anumudu, O.H and Ibediala, J. C designed the study, wrote the protocols and the first draft of the manuscript. Akaniro, I.R., Ibediala, J. C and Ofonegbu, M. C managed the analyses of the study and literature searches. All authors read and approved the final manuscript

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