Published in the Slovak Republic
European Journal of Molecular Biotechnology
Has been issued since 2013.
ISSN: 2310-6255
E-ISSN: 2409-1332
2017, 5(2): 74-79

DOI: 10.13187/ejmb.2017.2.74
www.ejournal8.com

Phytochemical Analysis and Antibacterial Assay of Stem Bark of Anogeissus Leiogarpus

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Abstract

Anogeissus leiocarpus is extensively used in *Nupe* traditional and folklore medicine to cure various human ailments. The preliminary phytochemical screening of the stem bark revealed the presence of flavonoids, soponins, steroids, terpeniods, cardiac glycosides and anthraquinones. In vitro antibacterial studies of the methanol and water extracts of the plant part were carried out on medically important bacterial strains including, Lacbacillus spp, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus spp, and Salmonella typhii using agar plug techniques. The results compared well with standard antibiotics (streptomycin) with susceptibly increasing with concentration. The extracts of the plant showed antibacterial activity, justifying their continued used in treatment of bacterial infections.

Keywords: *Anogeissus leiocarpus,* Lacbacillus spp, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus spp, Salmonella typhii.

1. Introduction

Anogeissus leiocarpus belongs to the family combretaceae (common name: Axle wood tree) is popular known as *marke* in Hausa northern Nigeria. Plant-drive compounds are a major area of interest to source for safer and more effected antibacterial agents (Longanga et al., 2000; Blench, Dendo, 2007; Musa et al., 2011).

A *leiocarpus* is used medically for the treatment of ascaricide, gonorrhea, general body pains, blood clots, asthma, coughing and tuberculosis (Nalule, Mbaria, Kimenju, 2013; Diab, Guru, Bhushan, Saxena, 2015). Information obtained from the Yurubas and south and eastern people of Nigeria illustrates that the plant is also used as an antimicrobial agent against bacterial infections (Blench, Dendo, 2007; Musa et al., 2011).

The leaves of the plant are used in Nigeria for the treatment of skin diseases and the itch of psoriasis. The powdered bark is applied to wounds, sores, boils, cysts and diabetic ulcers with good results. The powdered bark has also been mixed with 'green clay' and applied as an unusual face mask for serious blackheads (Longanga et al., 2000; Patil, Gaikwad, 2011; Olufunmilayo, 2017).

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A *leiocarpus* is traditionally acclaimed to be effective in treating infections wounds in man and Animals (Akinpelu, 1999; Barku et al., 2013).

A *leiocarpus* in folk medicine is used for the treatment of skin infections, wounds. mouth infections, parasitic infections such as malaria, as well as jaundice (Nalule et al., 2013; Rajeshwar et al., 2016). Plant medicine was commonly used for traditional treatment of some infections disease to show significant antimicrobial activity (Ibisi et al., 2017; Eltayeb et al., 2018). Higher plant procedure hundreds to thousands of diver's chemical compound with different biological activities. Antibacterial active principles isolated from higher plants used in traditional medicine were developed in to drugs. A substantial number of drugs currently being used are discovered as a result of chemical studies directed of the isolation of the active substance from plants used in rational medicine (Eltayeb et al., 2016). This plant based system continue to play an essential role in health care, and it has been estimated by the world health organization that approximately 80% of the world's inhabitants rely mainly on traditional medicine for their primary health care (Diab et al., 2015).

Nigeria flora has over seven thousand, three hundred and forty and forty nine species of higher plants that had make serious impact on health and wealth of Nigerians and could be an enormous sources of foreign exchange for country (Musa et al., 2011).

Materials and methods

Preparation of reagents

Wagner's reagent

About 3.0g of potassium iodide was weighed and dissolved in about 40cm^{3}of distilled water. To the resulting solution of potassium iodide, 2.0g of iodide crystal was added and properly stirred to homogenize into solution. This was transferred into 100cm^3 volumetric flask and filled up to the mark with distilled water.

Potassium hydroxide

About 8.0g of potassium iodide was weighed and dissolved in about 600cm^{3}of dissolved water them transferred in to 100cm^{3}volumetric flask. This was filled to the mark with distilled water.

Lead acetate

10g of lead acetate was accurately weighted and dissolved in about 40cm^{3}of distilled water, this then transferred in to 100cm^{3}volumetric flask and filled to the mark with distilled water.

Sample collection and preparation

Fresh and healthy plant part of ***A. Leicarpus*** were collected from Rafin-Tambari, behind Abubakar Tatari Ali Polytechnic Bauchi, Bauchi State, Nigeria and authenticated by an agronomist in the same facility. The plant was washed, cut into small pieces and completely dried at room temperature (27^o) for two weeks. The dried plant materials were ground in to powder and stored in air tight glass bottles at room temperature prior to experiment.

Extraction

100g of the pulverize stem bark of dried plant part was macerated in 100ml of 70% methanol for 72hours with string. The extract was filtered through whatman No. 1 filter paper to remove all unextractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. The extracts were filtered through a whatman No 1. Filter paper and the filtrate concentrated to dryness using evaporator under reduced pressure.

Phytochemical analysis

Phytochemical analysis of the extract was conducted on the stem bark of A *leiocarpus* using standard methods. The presence of sugars, proteins, alkaloids, flavonoids, saponins, tannins cardiac glycosides, terpenoids and lipids were tested for.

Test for flavonoids

The presence of flavonoids was estimated using standard methods.

Test for tannins

2ml extract was added to 1% lead acetate a yellowish precipitate indicate the presence of tannins.

Test for Saponins

5ml extract was mixed with 20ml of distilled water then agitate in graduated cylinder for 15 minutes. Formation of foams indicates the saponins.

Test for cardiac Glycosides

Killer-killani test: plant extract treated with 2ml glacial acetic acid containing a drop of fetch. A brown colour ring indicated the presence of positive test.

Test for steroids

2ml ofscetic anhydride was added to 0.5mg of the extract of 2ml of $H_2 50_4$ The colour changed from boiled to blue or green indicating the presence of steroids.

Test for anthraquinones

0.5g extract was shaken with benzene (2.0cm^3) and filtered where necessary. 10% ammonia solution (4c m^3) was then added to the filtrate. The resulting mixture was shaken and the presence of a pink colour in the ammonia solution phase [lower layer] indicates the presence of Anthraquinones.

Bio – Assay

The extract was tested for antimicrobial activity using standardized agar dics diffusion methods. The bacteria were inoculated on the fresh media of nutrient agar slants incubated at 37^{o}C for 24 hours and were referred to as seeded broth. 0.3ml potion of the new culture was especially transferred in to Petri dishes containing 1m base medium, gently agitated and poured as over lay on assay plate containing 15m base medium.

The preparation was left to dry under hood, Different concentrations' of the extract were introduced to spots and streptomycin (200, 300, 400 mg/ml) was used as standard and a control experiment was set up by using drop of sterile water in place of different of concentrations extract.

The plates containing the bacteria, various concentrations of extract and the antibiotic used as standard as well as the control plates were allowed to stand for an hour at room temperature to allow the growth of the organisms to commence.

The plates were observed for zones of inhibition, which was measured in (mm), after 24 hours incubation at 3% in triplicate determination.

Extraction preparation for bio-assay

Antimicrobial activity of the aqueous and organic extract of the plant sample was evaluated by paper disc diffusion method of determination of antimicrobial activity, bacterial culture were subjected to 0.5ml for turbidity standard and inoculated on to nutrient agar plate (15cm diameter).

Preparation of media

28g of nutrient agar powder was suspended in 1 liter of distilled water, and boiled to dissolve completely and dispense as required. Sixty four (64g) of sabouraud dextrose agar (SNA) was suspended in 1 liter of distilled water and swilled continuously for even distribution, this was then sterilized in an autoclave at 12^{o}C for 15 minutes and allowed to cool.

Inoculation and application of extract

For the determination of antimycotic activity all the fungal isolations and Candida albicans were first adjusted to the concentration 0.9% normal saline and the spore of the other filamentous dextrose agar plate. Bacterial culture and the Candida albicans, were then incubated at room temperature (30-32^{o}C) for 48 hours. Paper disc impregnated with 20 ul of a solution of 10 mg/ml of ciprofloxacin and cotrimoxazole (for bacteria) and nystatin and amphotericin B for fungi as standards.

Minimum Inhibition Concentration (MIC)

The minimum inhibitory concentration (MIC) of the plant extract that showed inhibition in the antimicrobial screening was determined. The MIC was carried out by preparing the dried plant extract in different concentrations 10^{-2} mg/ml, 10^{3} mg/ml, and 10^{-6} mg/ml, respectively.

The different concentrations of the diluted extracts were filled inside the wells on the inoculated nutrient agar plats and allowed to stand for I hour for proper diffusion of the extract and them inoculated after which the lowest concentration that showed inhibition was checked for.

Result and discussion

Results

Table 1. phytochemical screening result of *A. leicarpus*.

S/N	Test of extract	Methanol extract	Water extract
1.	Flavonoids	+	+
2.	Tannins	+	+
3.	Sponins	+	+
4.	Steroids	+	+
5.	Terpenoids	+	+
6.	CardialGlyvosides	+	+
7.	Antthaquinones	-	-

Keys:-
+ = Present
- = Absent

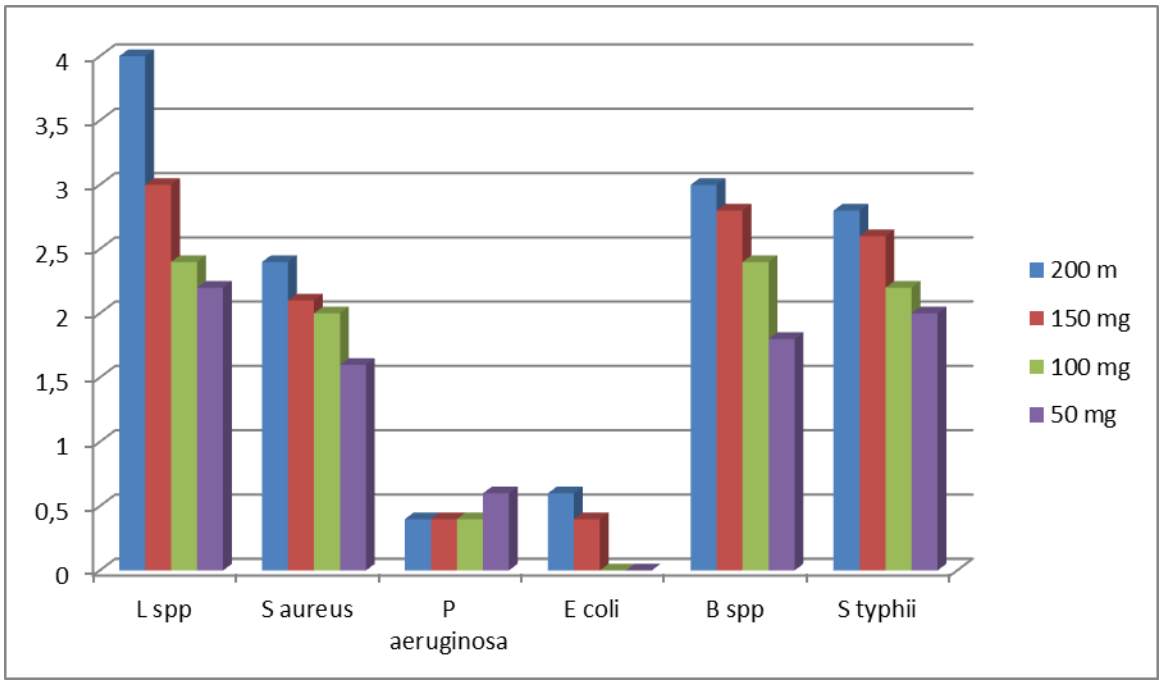

Fig. 1. Zone of inhibition [mm] of extract plant part against the test organism

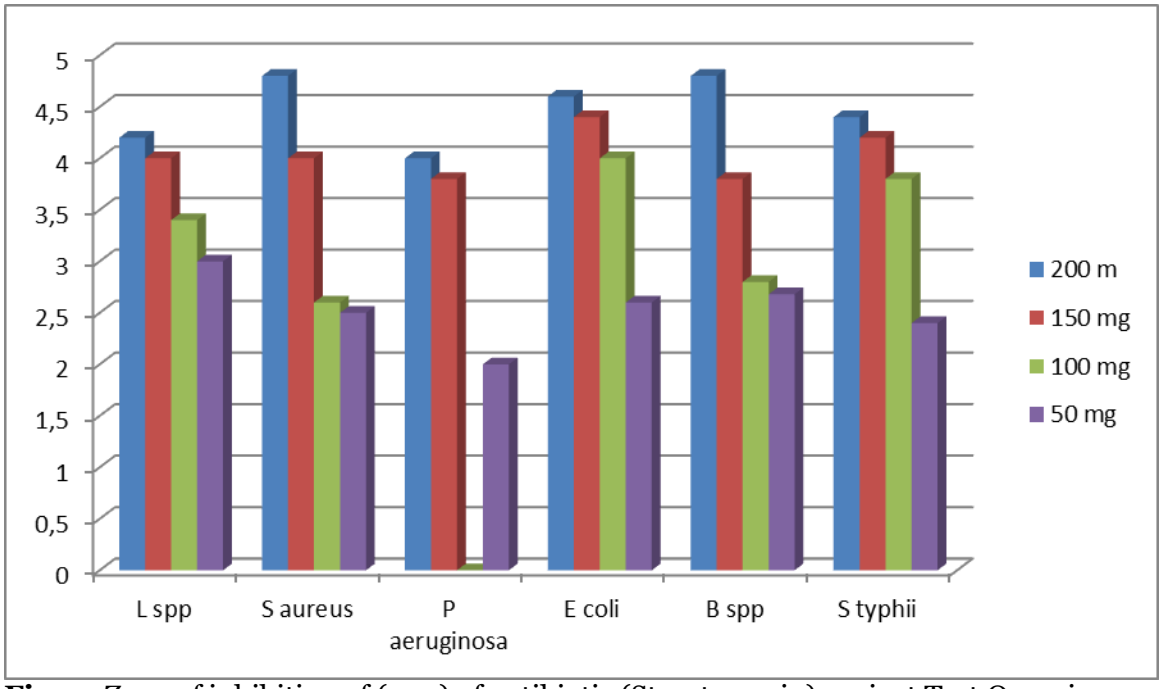

Fig. 2. Zone of inhibition of (mm) of antibiotic (Streptomycin) against Test Organism.

Discussion

The preliminary phytochemical analysis of the extracts revealed the presence of flavonoids. tannis, saponins, cardiac glycosides, terpenoids and anthraquinones as presented in the table 1 and fig. 1 and 2.

Susceptibility test of the methanolic extract of the tested plant part was positive on all tested organisms; comparable observations were made in similar work. It has been found that among all the tested organisms, the bacterial strain, lactobacilillus spp was found to be more susceptible to the plant extract by showing inhabitation as presented in table 2 and fig. 1 and 2.

The present study has shown that susceptibility increase with concentration as seen in both the extract and the standard. The extract compares favorably with the standard. The gradual increase in effect of extracts on test organisms with alteration of concentration has been reported by others (Ghamba et al., 2014; Bekele, 2015; Eltayeb et al., 2018).

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