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Photochemical and Antimicrobial Activities of Stem-bark of *Stereospermum Kunthianum* Plant

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Abstract

The ethanolic stem-bark extract of Stereospermum kunthianum plant bark was subjected to preliminary phytochemicals screening and antimicrobial tests. The extract revealed the presence of flavonoid, terpenes, steroids, tannins, terpenoids and saponins. The antimicrobial activity of the plant extract was assayed by the agar plate disc diffusion and nutrient broth dilution techniques. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Test micro-organisms were Pseudomonas aeruginosa, staphylococcus aureus and Klebsiella spp, all the organisms were laboratory isolates. The extract inhibited the growth of all the test organisms' at different concentration especially against Pseudomonas aeruginosa which have mean inhibition zone of 26 mm when 1 ml crude extract was used, while streptococcus aureus and Klebsiella have mean inhibition zone of 21 mm each when 1 ml crude extract was used. The result showed the MIC of 0.125 mg/ml for streptococcus spp and pseudomonas and 0.0625 mg/ml for klebsiella spp. The MBC against staphylococcus spp was 0.125 mg/ml and that of klebsiella spp was found to be 0.625 mg/ml. The extracts showed varied inhibitory activity against the organisms studied. The spectra of activities displayed by the extract can be attributed to the presence of these phytochemicals which signifies the potentials of Stereospermum kunthianum stem-bark as a source of therapeutic agents.

Keywords: Stem-bark; photochemical; Stereospermum kunthianum; Antimicrobial; Micro organisms

INTRODUCTION

Plants constitute a rich untapped pool of natural resources. Man has depended on plants as a source of food, shelter, Medicine, clothing etc. Medicinal plants are an important natural wealth of a country, serving as therapeutic agents and important raw materials for the manufacture of modern medicine (Tor-Anyiin et al., 2013). The use of plants for medicinal purposes is an age old tradition in Africa (Aliyu et al., 2009). Today more than 70% of the people in Africa refer to traditional healers concerning health issues. The World Health Organization (WHO) encourages the inclusion of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries because of the great potential they hold in combating various diseases (Aliyu. et al., 2009).

Plant extracts represent a continuous effort to find new compounds with the potentials to act against multi-resistant bacteria. Most of the plants found and are used by traditional healers in developing countries have been submitted to pharmacological or biological test, and a substantial number of new anti-biotic introduced on the market are obtained from natural or semi-synthetic resources (Mothana and Lindequist, 2005). Thus, it is anticipated that the phytochemicals with adequate anti-bacterial efficacy will be used for the treatment of bacterial infection.

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Phytochemicals are known to vary with variation in climate, weather, soil conditions as well as time of collection. Many researchers have established the side effect of overuse and misused of antibiotics which can harm vital organs like liver and kidney as well as their impact on the immune system (Ibtisam et al., 2011). The known success of traditional medicine has guided the search for new chemotherapeutic alternatives to eliminate the infections caused by drug-resistant microbes and to reduce the harm caused by antibiotics (Bocanega et al., 2009. Giamarellou, H., 2006).

Stereospermum kunthianum is a deciduous shrub or tree found in the dry areas of deciduous forest, woodland, bush, rocky outcrops, termite mould and margin of ever green forests. The plant is known locally as pink jacaranda (English), Sansami (Hausa), Ayada (Yoruba), Alakiriti (Ibo), and is widely used by rural dwellers where the plant is commonly found in Biu , Borno state Nigeria for water treatment and for the treatment of various human diseases. The decoction or infusion of the stem-bark of *Stereospermum kunthianum* which is the main focus here, is used to cure bronchitis, pneumonia, cough, and dysentery .(Onige et al.,2002). The twigs are chewed to clean teeth and to treat toothache. (Kothai and Seshathri.,2012).

The primary focus of these investigation is to established the efficacy of *Stereospermum kunthianum* plant stem-bark commonly used by Biu community in Borno state of Nigeria in water treatment and is been employed in ethno medicine. This study therefore looks into the importance of *Stereospermum kunthianum* stem-bark along the lines of the plants behaviour towards three pathogens, *Pseudomonas aeroginosa, Staphylococcus aureus and klebsiella Spp.*

Phytochemicals screening of the plant stem-bark has been carried out with the hope of relating its anti-microbial behaviour to its phytochemistry.

MATERIALS AND METHODS

A sample of the stem-bark of *Stereospermum kunthianum* plant was collected in paper bags from Creek, Waka-Biu in Biu local government area of Borno state, Nigeria and identified by Prof.S.S Sanusi, Department of Biological sciences, University of Maiduguri, Nigeria. The stem-bark was freed from dead dried tissues by carefully scraping with spatula. It was chopped to pieces, air dried for two weeks and grinded using pestle and mortar. The pulverized sample was stored in paper bag for further analysis.

Extraction and Phytochemical Screening

Air dried pulverised stem-bark powder about (200 g) were soxhlet extracted with ethanol until the draining solvent was clear. The solvent was removed under reduced pressure of 40^{o}c to get the crude extract. The crude extract was further dried in vacuum desiccators over anhydrous copper sulphate to give a dry solid of the extract (7.0 g). This was used for phytochemicals screening.

Phytochemicals screening of the extract was carried out to identify the constituents using standard phytochemical methods as described by Sofowora, (1993). Trease and Evans (1996).

Evaluation of the antimicrobial activity

Nutrient agar was prepared by weighing 28 g of the nutrient agar powder. This was aseptically prepared by dissolving 28 g of the nutrient agar powder in 1 litre i.e. 1000 mls of distilled water. The preparation was wrapped using auto clave tape and auto clave paper. The preparation was gently loaded in an auto clave machine and auto clave stream under pressure technique i.e., 121 degree for 15 pound pressure for timing of 15 minutes. After achieving sterilization, the machine was allowed to cool and off loaded at a temperature of 40-50 degree, the nutrient agar plate was poured, and each of the plate was

poured to 15 mls each so as to achieve the degree of depth. The media was allowed to set or jell so as to obtain a solid phase for clear inoculations. The preparation was stored into refrigerator to avoid contamination.

Inoculation Spray Plate Method

Spray plate method in the system of inoculation that tends to enrolled bacteria unto a growth surface was employed. In spray plate method the isolates was obtained using a sterile wire loop and was emulsified in a peptone water after dispersing, the system was poured on a nutrient agar plates floated to spray on the entire surface then the excess preparation was discarded off, by such preparation, the bacteria's was introduced on the surface to grow.

Ditch Plates Techniques

This is an improvised technique to anti bio-gram. Ditch plates was obtained by creating a hole of a known diameter using a coke borer, the coke borer was a sterilized red hot flame and allowed to cool, a hole was bored at the center of the nutrient agar plate. The hole was filled to a known volume in other to see a zone of inhabitation by the extract.

Three (3) gram negative bacteria were emulsified in peptone water

(i) Streptococcus aureus

(ii) Klebsiella spp

(iii) Pseudomonas aeruginosa

These isolates were sprayed on a jel nutrient agar plates as explained above. The excess were drained off. Each of these isolates was poured onto their respective surface.

Using a sterile coke borer, a hole was obtained at the three adjacent angles to get mean result. In all the plates, a hole of 2 mm in diameter was bored. Using graduated sterile needles of 1 ml, 0.5 ml, 0.25 ml, and 0.125 ml of dissolved extract was introduced.

The preparation was safely incubated aerobically at 37 degree for 24 hrs in an incubator. The mixture of the stem-bark extract of *Stereospermum kunthianum* plant was dissolved by the volume RV/O. The decrease in concentration was determined to know the activity as volume and strength is reduced.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) was define as the lowest concentration where no visible turbidity was observed in the test tube. The concentration was determined as earlier described by Vollekova et al., 2001, with some modification by Usman et al., 2005. The MIC was determined for the micro-organism that showed reasonable sensitivity to the test extract. In this test, the micro organisms were prepared using the broth dilution technique stated above. After 24 hours, incubation at 37^{o}C, the tubes were observed for turbidity. The lowest concentration where no turbidity is observed was determined and noted (Usman et al., 2005)

Minimum Bactericidal Concentration (MBC)

The minimal bactericidal concentration (MBC) was determined from broth dilution test resulting from the MIC tubes as described previously (Vollekova et al., 2001; Usman et al., 2007) by inoculating the content of each test tube on a nutrient agar plate. The plates were then incubated at 37^{o}C for 24 hours. The lowest concentration of the extract that showed no growth was noted and recorded as the minimum bactericidal concentration.

RESULTS

The result of the phytochemical screening of stem-bark extract of *Stereospermum kunthianum* is presented in table 1 . Stem-bark extract of *Stereospermum kunthianum* and its partitioned portions were subjected to anti-microbial studies. The susceptibility pattern against the test organisms is shown in Table 2 - 4. Figure 1 is the spray plate technique and Figure 2 is the inoculation by spray plate technique. Figure 3 is the Ditch plate on blood agar while Figure 4 is the ditch plate on nutrient agar plate. Mean

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while the minimal inhibitory concentration (MIC) is presented in Table 5. While Table 6 below is the minimal bactericidal concentration (MBC) on the test organism.

Table 1;

Phytochemicals Constituent of the Stem-bark extract of Stereospermum kunthianum

Phytochemicals	Ethanol Extract
Saponin	+
Tanins	+
Alkaloids	–
Flavonoids	+
Glycosides	–
Steroids	+
Terpenes	+
Terpenoids	+

+ = Present, -- = Absent

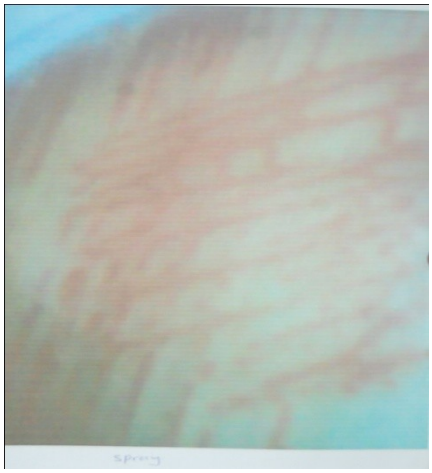

Figure 1; Spray Plate Technique

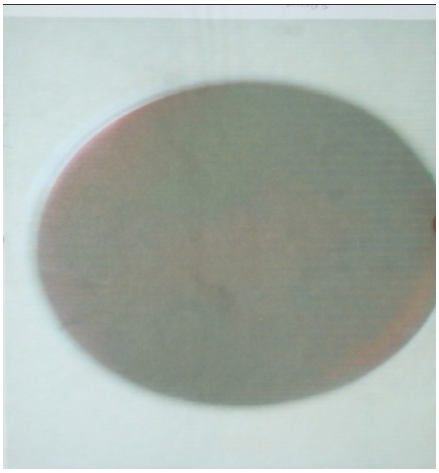

Figure 2; Inoculation by Spray Plate Technique

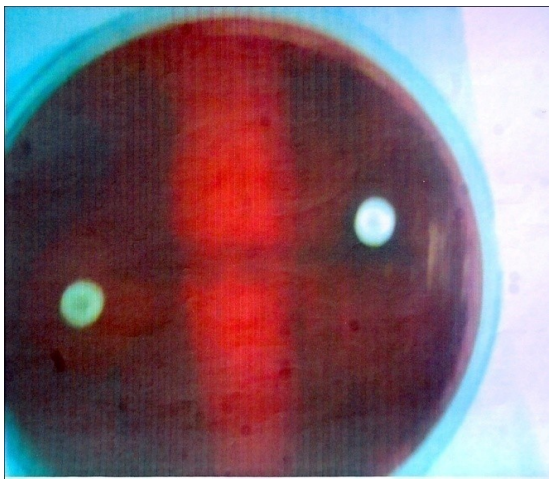

Figure 3; Ditch Plate on Blood Agar.

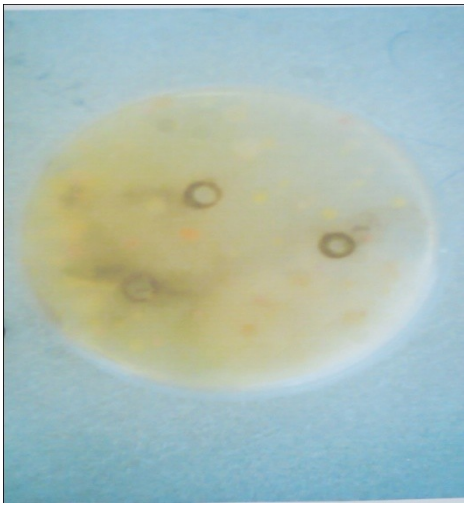

Figure 4; Ditch Plate on Nutrient Agar.

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DISCUSSION

Susceptibility Test by Ditch Plate Method

Table 2-4 below showed the susceptibility test against gram negative organisms. The ethanol extract exhibited considerable level of inhibition against all the test organisms. The results from all portions were found to be significantly higher. The higher the concentration of the plant extract, the bacterial inhibition concentration is less. The extract exhibited considerable level of inhibition against all the test organism with the highest activity on *pseudomonas aeroginosa* (26 mm) for 1 ml concentration of the plant extract and (21 mm) for 0.125 ml of the ethanol crude extract. While *streptococcus Spp* and *klebsiella Spp* have 21 mm each when 1 ml crude extract was used and 8 mm and 4 mm was obtained respectively when 0.125 ml crude plant extract was used

Table 2;
Streptococcus aureus

1.0 ml	MEAN	0.5 ml	MEAN	0.25 ml	MEAN	0.125 ml	MEAN
21		20		19		9	
22		20		18		8	
21		19		15		9	
	21 mm		19.6 mm		17 mm		8.6 mm

Table 3;
Pseudomonas aeruginosa

1.0 ml	MEAN	0.5 ml	MEAN	0.25 ml	MEAN	0.125 ml	MEAN
26		23		20		18	
25		22		21		19	
26		25		21		19	
	26 mm		22 mm		22 mm		21 mm

Table 4;
Klebsiella SPP

1.0 ml	MEAN	0.5 ml	MEAN	0.25 ml	MEAN	0.125 ml	MEAN
20		9		7		5	
20		8		7		4	
21		9		6		5	
	21 mm		8 mm		6 mm		4 mm

Minimum Inhibitory Concentration (MIC) of the Test Extract.

From the result of the MIC and MBC shown on table 5 and 6 below, it was noticed that the broadest activity of the extract against most of the gram negative organism was 0.625 mg/ml for *klebsiella Spp* and 1.25 mg/ml for *streptococcus spp and pseudomonas* as MIC, while the MBC of 1.25 mg/ml was for *streptococcus and pseudomonas* and 0.625 mg/ml was recorded for *klebsiella.* The extract exhibited some appreciable level of activity against the organisms.

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According to research by Ugbabe et al., 2010 ; Aliyu et al., 2009. who worked on the crude leaves extract of *Stereospermum kunthianum* against similar organisms, found similar broad activity recorded against must gram negative organisms . Studies compared to the results on table 4 which shows ethanol aqueous crude extract exhibiting highest activity of 0.625 mg/ml against the three gram negative bacteria assayed.

Table 5; Minimum Inhibition Concentration (MIC) on Test Organisms.

Streptococcus aureus			
2.5 mg/ml	1.25 mg/ml	0.625 mg/ml	0.3125 mg/ml
Clear	Clear	Turbid	Turbid
Pseudomonas aeruginosa			
1.25 mg/ml	0.625 mg/ml	0.3125 mg/ml	0.1565 mg/ml
Clear	Turbid	Turbid	Turbid
Klebsielly SPP			
1.25 mg/ml	0.625 mg/ml	0.3125 mg/ml	0.15625 mg/ml
Clear	Clear	Turbid	Turbid

Table 6; Minimum Bactericidal Concentration (MBC) of the Test Extract.

Streptococcus aureus	***Pseudomonas aeroginosa***	***Klebsiella SPP***
1.25 mg/ml	1.25 mg/ml	0.625 mg/ml

Conclusion

The broad antibacterial activities of this extracts could be as a result of the plant secondary metabolites present in the extract. The extract showed high inhibitory activities against all the test organisms. The results of these studies has provided more basis and credence for the use of this plant in treatment of ailments whose causative agents are some of the pathogenic microbes used in this study and thus suggest the possible usefulness of stem-bark of *Stereospermum kunthianum* in the treatment of bacterial in water. Therefore the use of this part of the plant by the traditional healers and communities where it is commonly found for the treatment/purification of water has been validated.

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