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PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITIES OF *STEREOSPERMUM KUNTHIANUM* STEM-BARK USED IN WATER TREATMENT/PURIFICATION

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Abstract: The ethanolic stem-bark extract of Stereospermum kunthianum plant bark was subjected to preliminary phytochemical screening and antimicrobial testing. The extract revealed flavonoids, terpenes, steroids, tannins, terpenoids, and saponins. The antimicrobial activity of the plant extract was assayed using agar plate disc diffusion and nutrient broth dilution techniques. The MIC and minimum bactericidal concentration (MBC) were determined. The test microorganisms were Pseudomonas aeruginosa, Staphylococcus aureus, and Klebsiella spp. All organisms were laboratory isolates. The extract inhibited the growth of all the test organisms at different concentrations, especially against Pseudomonas aeruginosa, which had a mean inhibition zone of 26 mm when 1 ml of crude extract was used, whereas S. aureus and K. aureus had a mean inhibition zone of 21 mm each when 1 ml of crude extract was used. The MIC was 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 against and that of klebsiella spp. was 0.625 mg/ml. The extracts exhibited varying inhibitory activities against the studied organisms. The spectra of activities displayed by the extract can be attributed to the presence of these phytochemicals, indicating the potential of S. kunthianum stem-bark as a source of therapeutic agents.

Keywords: Stem-bark, phytochemicals, stereospermum, kunthianum, antimicrobial, microorganisms

INTRODUCTION

Plants constitute a rich, untapped pool of natural resources. Man has depended on plants as a source of food, shelter, medicine, and clothing. 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 old tradition in Africa (Aliyu et al., 2009). Today, more than 70% of the people in Africa refer to traditional healers regarding health issues. The World Health Organization (WHO)

encourages the inclusion of herbal medicines with proven safety and efficacy in the healthcare programs of developing countries because of their great potential in combating various diseases (Aliyu et al., 2009).

Plant extracts represent a continuous effort to find new compounds with the potential to act against MRSA. Most of the plants found and used by traditional healers in developing countries have been subjected to pharmacological or biological tests, and a substantial number of new antibiotics are obtained from natural or semi-synthetic resources (Mothana and Lindequist, 2005). Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for treating bacterial infections.

Phytochemicals vary with climate, weather, soil conditions, and time of collection. Many researchers have established the side effects of overuse and misuse of antibiotics, which can harm vital organs, such as the 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).

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

The primary focus of this investigation is to establish the efficacy of the stem-bark of Stereospermum kunthianum plant, which is commonly used by the Biu community in Borno state of Nigeria in water treatment and has been employed in ethnomedicine. Therefore, this study looks into the importance of *Stereospermum kunthianum* stem-bark along the lines of the plants behaviour toward three pathogens, *Pseudomonas aeroginosa, Staphylococcus aureus and Klebsiella spp.*

Phytochemical screening of the plant stem-bark has been conducted with the hope of relating its antimicrobial behavior 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 dried dead tissues by careful scraping with a spatula. It was chopped to pieces, air-dried for 2 weeks, and ground using a pestle and mortar. The pulverized sample was stored in a paper bag for further analysis.

Extraction and phytochemical screening

Air dried pulverized stem-bark powder (approximately 200 g) was 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 dried in vacuum desiccators over anhydrous copper sulphate to give a dry solid of the extract (7.0 g). This was used for phytochemical screening.

Phytochemical screening of the extract was performed to identify the constituents using standard phytochemical methods, as described by Sofowora (1993). Areas and Evans (1996).

Evaluation of antimicrobial activity

Nutrient agar was prepared by weighing 28 g of nutrient agar powder. This was prepared aseptically by dissolving 28 g of the nutrient agar powder in 1 L of distilled water (i.e., 1000 mL). The preparation was wrapped using autoclave tape and autoclave paper. The preparation was gently loaded in an autoclave machine and autoclave stream under pressure technique, i.e., 121° for 15 pound pressure for 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 15 ml of each plate was poured to 15 mls each so as to achieve the degree of depth. The media was allowed to set or gel to obtain a solid phase for clear inoculation. The preparation was stored in a refrigerator to avoid contamination.

Inoculation spray plate method

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

Ditch plate techniques

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

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

(i) Streptococcus aureus

(ii) Klebsiella spp.

(iii) P. aeruginosa

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

A hole was obtained at the three adjacent angles using a sterile coke borer to obtain the mean result. A hole of 2 mm in diameter was bored in all the plates. Gradient sterile needles of 1 ml, 0.5 ml, 0.25 ml, and 0.125 ml of dissolved extract were introduced.

The preparation was safely incubated aerobically at 37°C for 24 h in an incubator. The stem-bark extract mixture 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)

MIC was defined as the lowest concentration where no visible turbidity was observed in the test tube. The concentration was determined as described by Vollekova et al. (2001), with some modification by Usman et al. (2005). The MIC was determined for microorganisms that showed reasonable sensitivity to the test extract. In this test, the microorganisms were prepared using the abovementioned broth dilution technique. After 24 h of incubation at 37^{o}C, the tubes were observed for turbidity. The lowest concentration where no turbidity was observed was determined and noted (Usman et al., 2005).

Minimum Bactericidal Concentration

The minimal bactericidal concentration (MBC) was determined from the 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. Plates were then incubated at 37ºC for 24 h. The lowest extract concentration that showed no growth was recorded as the minimum bactericidal concentration.

RESULTS

The phytochemical screening results of the stem-bark extract of *S. kunthianum are* presented in Table 1 . The stem-bark extract of *S. kunthianum and* its partitioned portions were subjected to antimicrobial studies. The susceptibility pattern against the test organisms is shown in Table 2-4. Figure 1 shows the spray plate technique, and Figure 2 shows the inoculation by the spray plate technique. Figure 3 shows the ditch plate on blood agar, whereas Figure 4 shows the ditch plate on nutrient agar plate. The mean while the MIC is presented in Table 5. Table 6 shows the minimal bactericidal concentration (MBC) on the test organism.

Table 1: Phytochemical constituents 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 using the spray plate technique

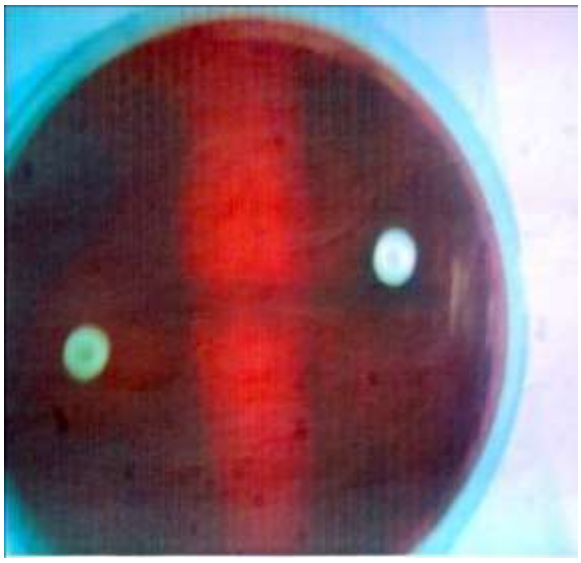

Figure 3: Ditch plate on blood agar.

Figure 4. Ditch plate on nutrient agar

DISCUSSION

Susceptibility Test by the Ditch Plate Method

Table 2-4 shows the results of the susceptibility test against gram-negative organisms. The ethanol extract exhibited a considerable level of inhibition against all test organisms. The results from all portions were significantly higher. The higher the plant extract concentration, the lower the bacterial inhibition concentration. The extract exhibited a considerable level of inhibition against all the test organisms, with the highest activity on *P. aeroginosa (*26 mm) for 1 ml of the plant extract and (21 mm) for 0.125 ml of the crude ethanol extract. Streptococcus *Spp* and *Klebsiella Spp had* 21 mm each when 1 ml crude extract was used and 8 mm and 4 mm were obtained when 0.125 ml crude plant extract was used, respectively.

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 results of the MIC and MBC shown in Tables 5 and 6, it was noticed that the broadest MIC of the extract against most of the gram-negative organisms was 0.625 mg/ml for Klebsiella spp. and 1.25 mg/ml for Streptococcus spp. and Pseudomonas, 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 activity against the organisms.

Ugbabe et al. (2010) and Aliyu et al. (2009), who worked on the crude leaf extract of *Stereospermum kunthianum* against similar organisms, found similar broad activity recorded against gram-negative organisms . Studies compared to the results in Table 4, which shows that the ethanol aqueous crude extract exhibited the highest activity of 0.625 mg/ml against the three gram-negative bacteria assayed.

Table 5; Minimum inhibitory 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	Turbid

Klebsielly SPP

1.25 mg/ml	0.625 mg/ml	0.3125 mg/ml	0.15625	0.15625 mg/ml
Clear	Clear	Turbid	Turbid	

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

SPP

1.25 mg/ml 1.25 mg/ml mg/ml

Conclusion

The broad antibacterial activities of this extract could be a result of the presence of plant secondary metabolites in the extract. The extract exhibited high inhibitory activity against all test organisms. The results of these studies have provided more basis and credence for the use of this plant in the treatment of ailments whose causative agents are some of the pathogenic microbes used in this study and thus suggest the possible usefulness of *S. kunthianum* stem-bark in the treatment of bacteria 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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