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ANTIBACTERIAL STUDIES OF THE LEAVES EXTRACTS OF *SIDA CORYMBOSA* (*MALVACEAE*)

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ABSTRACT
Wound healing property of *Sida corymbosa* plant leaves was investigated. Standard procedures were then used to investigate the phytochemical constituents and the antibacterial activity of the extracts. Alkaloid, saponins, flavonoids, terpenoids, tannins and steroids were detected in the plant extracts. All the phytochemicals detected in the plant extracts were present in the methanol extract with the exception of steroids. Antibacterial activity showed zone of inhibitions that ranged between 10 mm and 21 mm. The methanol extract especially inhibited the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* at MIC of 25 mg/ml. The MBC for the methanolic extract on *Staphylococcus aureus* was 100 mg/ml whereas the MBC for *Pseudomonas aeruginosa* and *Streptococcus pyogenes* was 50 mg/ml. It can be concluded from this research work that Sida corymbosa leaves is a good source of antibacterial agents that possess wound healing property in line with its ethno-medicinal use.

Keywords: *Sida corymbosa leaves, phytochemical, antibacterial, MIC, MBC.*

INTRODUCTION

The emergence of resistant bacteria, especially those causing infections on wounds, has become a health care problem that has caused serious concern to medical practice (Arias and Murray, 2008). Antibacterial drugs have been in used since several years ago to handle these infections. However, in the recent decade, bacterial resistance to these drugs is being reported (Steven *et. al*., 2015). Drug resistant bacteria render many synthetic antibiotics ineffective or useless (Venkatesan *et al.*, 2009). Multi-drug resistance therefore has become a major concern to the treatment of bacterial related diseases. The quest to solve this problem has necessitated a search for new antibacterial substance from other sources including screening of medicinal plants for anti-bacterial activities and bioactive phytochemicals that could serve as templates for new compounds of therapeutic applications (Venkatesan *et al.*, 2009). The problem posed by new strain drug resistant bacteria and the quest to cope toxicity of antibiotics have led to ever increasing need for natural product researches in order to come up with chemical entities of medicinal value from plant sources. (Voravuthikunchai *et al.*, 2003; Motalleb *et al*, 2011; Sathish *et al*., 2013). Hence, Selection of plant of ethno-medicinal reputation is thus a crucial factor for the ultimate success of the identification of bioactive plant constituents of therapeutic importance (Hostettmann *et al*., 2000). In view of this, *Sida corymbosa* belonging to plant family *Malvaceae* was selected for this study. The plant has been used, by locals in northern Nigeria, for the treatment of ulcers and wounds. It is a shrubby semi-woody perennial plant of about 2.25 m high seen in roadsides, tracks and waste places (Ekpendu 2013; Lucy *et al*., 2013).

To the best of our knowledge there is no report on phytochemical studies of *Sida corymbosa* collected from Tsanyawa. This is justifiable with the fact that chemical composition of plants (and also its therapeutic value) vary with locations (Tijjani *et al*., 2016).Therefore, the objective of this study is to validate the folklore use of *Sida corymbosa* (collected for the first time from Tsanyawa Local Government Area of Kano State, Nigeria) on wound healing. Though similar studies was conducted on this plant (Lucy *et.al*, 2014) but that did not involve phytochemical screening on the plant's

extract and use of specific microorganisms that caused wound infections as reported in this study.

MATERIALS AND METHODS

Sample Collection and Preparation

The leaves of *Sida corymbosa* were collected from Tsanyawa Local Government Area of Kano State, Nigeria in June, 2017. The plant was identified and authenticated in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria and a voucher specimen number 7069 was deposited in the herbarium of the same Department. The leaves of the plant were washed and dried in a shed for 7 days, ground into powder with the help of mechanical grinder. It was kept in a cool dried place prior to phytochemical screening and antibacterial test.

Extraction

Cold maceration method was used to extract the plant material. The powdered sample (1k g) was soaked into 3 dm^3 of n-hexane and allowed to stand for one week with shaking at regular intervals. The extract obtained was filtered and concentrated *in vacuo* using rota vapor at 40^{o}C after which the extract was air dried. The residue (marc) was similarly macerated with chloroform (2 dm^3), ethanol (2 dm^3) and methanol (2 dm^3) in this order, for one week per each residue. At the end of the one week, the extracts were also filtered and concentrated using rotatory evaporator at 40°C to obtain the crude leaf extracts which were weighed and recorded.

Phytochemical Screening

The methods described by Sofowora (1993), Trease and Evans (2002) and Kwaji *et al* (2013) were used for qualitative phytochemical screening of extracts of *Sida corymbosa* leaves. Tannins, saponins, alkaloids, flavonoids, terpenoids and steroids were the secondary metabolites screened.

Test for Alkaloids

The extract (0.5g) was stirred with 5 ml of 1% Hydrochloric acid on a water bath for thirty minutes and filtered. The filtrate (1ml) was transferred into a test tube. This was followed by addition of few drops of Dragendorff's Reagent. An orange-red precipitate is a positive test.

Test for Flavonoids

The extract (0.2 g) was diluted with few drops of dilute sodium hydroxide. The appearance of a yellow solution which disappeared on addition of few drops of dilute hydrochloric acid indicates the presence of flavonoids.

Test for Saponins

The crude extract (0.5 g) was shaken vigorously for 2 minutes with 10 ml of distilled water. Frothing that persisted for 15 minutes indicates the presence of saponins.

Test for Tannins

A small quantity (0.25 g) of the extract was dissolved in 10 ml distilled water and heated on a water bath. Freshly prepared ferric chloride solution was subsequently added. A blue-black, green or blue-green precipitate is a positive test for the presence of tannins.

Test for Steroids (Salkowski Test)

A little quantity of the extract was dissolved in chloroform (1 ml) and concentrated sulphuric acid (1 ml) was added down the test tube to fom two phases. Formation of red coloration was taken as an indication for the presence of sterols.

Test for Terpenoids (Liebermann-Burchard Test)

Anhydrous acetic acid (1 ml) was added to chloroform (1 ml) and cooled to 0 °C; one drop of concentrated sulphuric acid was added to the cooled mixture. This was added to the extract. Formation of brown colour was observed with time.

Antibacterial Activity

The agar well diffusion and pour plate methods were used in determining the antibacterial activity as described by El-Mahmood (2009) with modifications. Briefly, 1ml of standardized bacteria culture (1×108 cfu/ml) was placed in a sterile petri-dish and 25 ml of Mueller Hilton Agar, MHA (Titan, India) was added and allowed to stand undisturbed so as to solidify. Five equidistant holes, 6mm in diameter each, were made in the nutrient agar with sterile cork borer. Three of the holes were filled with 100 µl of 400 mg/ml of extract; 1 hole was filled with 100 µl of 0.25 mg/ml of Chloramphenicol standard (positive control) and the last hole with 100 µl of sterile distilled water as negative control. The petri-dishes were incubated at 37 °C for 24 h and then observed visually for zones of inhibition. The diameter of zones of inhibition were measured with a transparent ruler and the mean values were recorded (Table 3). Tests were carried out in triplicates.

Minimum Inhibitory Concentration

Broth dilution technique was used in determining the minimum inhibitory concentration (Adesokan *et al*., 2007; Oyeleke *et al*., 2008). Nine test tubes labeled 1-9 were filled with 2 ml of sterile distilled water. The extract (2 ml) was added, at a concentration of 0.8 mg/ml, to test tube 1 and shaken properly to obtain a concentration of 400 mg/ml. Subsequently, two fold serial dilutions were carried out on test tubes 2-8 by transferring 2 ml from test tube 1 to test tube 2 and mixing it. This procedure was repeated until the 8^{th} test tube was reached where 2 ml of solution was drawn out and discarded. The 9^{th} test tube contains only sterile distilled water. Test tubes 1-8 had the following concentration (mg/ml); 400, 200,100, 50, 25, 12.5, 6.5, 3.13.1ml of standardized bacteria sample. The nutrient broth (1ml) was added to each of the 9 test tubes, mixed and then incubated for 24 h. The tubes were inspected visually to determine the growth of microorganisms by the presence of turbidity and the tubes in which the extract is present in minimum concentration sufficient to inhibit the microbial growth which remains clear was noted as MIC of the extract. In experimental terms MIC is the concentration of the extract present in the last clear tube that is the tube having the lowest antibacterial concentration in which growth is not observed (Nazaruk and Jakoniuk, 2005).

Minimum bactericidal concentration

The MBC is defined as lowest concentration where no bacterial growth is seen (indicated by the absence of turbidity). This was determined using broth dilutions from the MIC tubes by sub-culturing to antibacterial free agar as described by Reuben *et al* (2008). In this method, the contents of the test tubes from MIC experiments were streaked with sterile wire loop onto agar plate free of antibacterial agent and incubated at 27°C for 24 h. The lowest concentration which produced no bacterial growth was recorded as the MBC.

RESULTS AND DISCUSSION

Table 1. **Percentage yield of extracts (w/w) per 1K g of dried plant sample**

EXTRACT NAME	EXTRACT MASS (g)	% YIELD
Methanol Extract	31	31
Ethanol Extract	43	43
Chloroform Extract	25	25
n- Hexane Extract	18	18

The result of percentage extraction yield is presented in Table 1. The result shows that the percentage yield ranges from 18 to 43. Ethanol has the highest yield while n-hexane has the lowest percentage yield. Percentage yield may be used as a measure of the capacity of a solvent to extract chemical constituents from plant.

Table 2. Results of the Phytochemical Screening of extracts of *Sida corymbosa*

Phytochemical	Hexane Extract	Ethanol Extract	Methanol Extract	Chloroform Extract
Alkaloids	+	+	+	−
Flavonoids	–	+	+	+
Terpenoids	+	–	+	–
Tannins	–	+	+	+
Saponins	–	+	+	+
Steroids	–	–	–	+

Key: + = Present and − = Absent

Table 2 shows the results for the phytochemical screening of *S. corymbosa.* Alkaloids, flavonoids, terpenoids, tannins, saponins and the steroids were detected. All the above phytochemicals were found in methanol extract except steroids. Alkaloids and terpenoids were not found in chloroform extract. This result agrees with the fact that the phytochemical constituents extracted from plant source depend on the solvent used. The phytochemicals detected in *S. corymbosa* were found to have medicinal and physiological properties (Reuben *et al.*, 2008).

Table 3. Antibacterial Activity of Methanol and Chloroform extracts at 400mg/ml

Bacteria	Zone of inhibition[a] (mm)		
	Methanolic Extract	Chloroform Extract	Chloramphenicol
Staphylococcus aureus	21	12	35
Escherichia. Coli	0	0	32
Pseudomonas. Aeruginosa	19	15	22
Streptococcus pyogenes	17	10	29

[a]Values are means of triplicate determinations

The result of the antibacterial activity for methanol and chloroform extracts of *S. corymbosa* presented in table 3 shows that the zone of inhibitions ranges between 10 and 21 mm for the two extracts. Specifically, zone of inhibitions for methanol extract ranged from 17 to 21 mm while those of chloroform extract ranged from 10 to 16 mm. The result revealed that methanol extract has the highest inhibition zones. The two extracts inhibited the growth of *S. aureus, P. aeruginosa, and S. pyogenes* but not *E. coli,* a multi-resistant bacterium. According to the work published by Johnson and case in 1995, zone of inhibition equal to or greater than 16 mm is associated with microbial susceptibility. Therefore, this research has proved that the methanol extract of *S. corymbosa* possesses antibacterial activity

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Table 4. Minimum Inhibitory Concentration (MIC) of Methanol Extract against test bacteria

Bacteria	Concentration (mg/ml)								
	400	200	100	50	25	12.5	6.25	3.13	1.0
S. aureus	_	_	_	_	o•	+	+	++	++
E. coli									
P. aeruginosa	−	−	_	_	o•	+	+	++	++
S. pyogenes	−	−	_	_	o•	+	+	++	++

Key: − = No inhibition, **o•** = Minimum inhibition, **+** = Moderate inhibition, **++** = Heavy inhibition

Table 4 represents the results of the minimum inhibitory concentration of the methanol extract. MIC is determined to investigate the efficacy of antibacterial agents (Macias *et al*., 2008). The extract shows activities at the concentration of 12.5 mg/ml, 6.25 mg/ml, 3.13mg/ml and 1.0 mg/ml. However, the minimum inhibitory concentration is 25 mg/ml (Table 4). The inhibitions of the methanol extract against the pathogenic bacteria increases with decrease in extract concentration.

Table 5. Minimum Bactericidal Concentration (MBC) of Methanol Extract of *S. corymbosa* Against test bacteria

Bacteria	Concentration (mg/ml)								
	400	200	100	50	25	12.5	6.25	3.13	1.0
S. aureus	−	−	o•	+	+	+	+	++	++
E. coli									
P. aeruginosa	−	−	−	o•	+	+	+	++	++
S. pyogenes	−	−	−	o•	+	+	+	++	++

Key: − = No colony growth, **o•** = Minimum colony growth, **+** = Colony growth

Table 5 presents the results for minimum bacterial concentration. Minimum bacterial concentration is the lowest concentration required for an antimicrobial agent to kill a bacterium. From the table above, the MBC for *S. aureus* is 100 mg/ml while those of *P. aeruginosa and S. pyogenes* is 50 mg/ml. This implies that at a concentration of 100 mg/ml, methanol extract of *Sida corymbosa* killed *Staphylococcus aureus*. While *Pseudomonas aeruginosa and Streptococcus pyogenes* were killed at concentration of 50 mg/ml of the same extract (Table 5).

Increasing evidence from scientific studies suggests that bacteria play a role in wound chronicity. The bacteria key players implicated for wound infections and chronicity are *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes* and *Escherichia coli* (Hani and Adnan, 2009). Therefore, the activity exhibited by the plant leaves against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Streptococcus pyogenes* suggest that the plant has wound healing properties in line with its ethno-medicinal uses. This is not surprising because from the results of the phytochemical screening, some metabolites of immense pharmaceutical importance were detected (Table 2). For example saponins were detected, which have been reported to possess antimicrobial activity (Edewor *et al*., 2009). Flavonoids which have been reported to possess both antifungal and antibacterial activity were also present (Susana *et al.*, 2007; Leonidah *et al*., 2014).There are tannins that are reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and anti-parasitic effects (Abdul, 1990). Phytotherapeutically tannin-containing plants are used to treat nonspecific diarrhoea, inflammations of mouth and throat and slightly injured skins (Westendarp 2006). Steroids are also there, which are important drugs used as hypotensives, cardiac depressants, sedatives and anti-dysenteric agents (Abdul, 1990). Alkaloids that act as antimalarial, anti-amoebic agents, astringents were present (Abdul, 1990).

CONCLUSION

The antibacterial activity against the selected bacteria studied (*Staphylococcus aureus, Pseudomonas aeruginosa* and *Streptococcus pyogenes*) indicated the wound healing potentials of *Sida corymbosa* leaves. This activity is attributable to the presence of the phytochemicals present in the plant's leaves. Hence, the study scientifically provided evidence for the use of *Sida corymbosa* leaves to cure wounds in ethno-medicine. This plant's leaves could therefore be a very good source of antibacterial agent.

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