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PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF THE ROOT BARK EXTRACTS OF *Strychnos spinosa* LAM.

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ABSTRACT
The phytochemical screening and antimicrobial properties of petroleum ether, ethylacetate and methanol root bark extracts of Strychnos spinosa. The extracts were tested against Staphylococcus aureus, Salmonella typhii, Klebsiella pneumonia and Candidas albicans by Agar well diffusion and micro-dilution methods. The phytochemical screening revealed the presence of alkaloids, saponins, anthraquinones, glycosides, steroids and terpenoids. The methanol extracts was found to be the most potent against Staphylococcus aureus and Klebsiella pneumonia with MIC values of 1.56mg/ml. The MBC revealed that the extracts were more of bacteriostatic than bactericidal.
Keywords; Phytochemical, Antimicrobial, Strychnos spinosa, Root bark.

INTRODUCTIONS

The plant kingdom represents an enormous reservoir of drugs principles that are distributed widely, particularly among angiosperms (Brantner *et al*., 1994). The phytochemicals varies widely in their potency and distribution within a plant. Some of these active compounds include alkaloids, glycoside, flavonoids and tannins. Therefore, the phytochemical research of plants is considered an effective approach in the discovery of new anti-infective agents from higher plants (Duraipandiyan *et al*., 2006).

The genus Strychnos belongs to the family Loganiaceae and consist of about 75 accepted species found throughout the tropics and subtropical Africa. *Strychnos spinosa* commonly known as monkey orange, kokiya (hausa), Angboroko (ibo) or Atako (Yoruba) is a small to medium sized spiny deciduous tree with their leaves turning yellow in autumn. The canopy is flattish and irregular, the tree is heavily branched, it produces small greenish white flower in dense heads at the ends of branches. The fruits tend to appear only after good rains, the smooth, hard fruit are large and green ripening to yellow. They take a long time to ripen, inside are tightly packed seed surrounded by a fleshy, edible covering (Schmidt, 2002). The plant is utilized in Africa traditional medicine for the treatment ailments such as dropsy, earache, snakebite, fever, and elephantiasis (De *et al*., 1988). It is also used as a tick control remedy, (Stevenson, 2010). The antimicrobial The respective extracts were filtered through a whatman filter paper no 42, The filtrates were activity of the leaf and stem bark extracts of *Strychnos spinosa* against *Candida albican* and *Aspergillus niger* among other microorganisms was reported (Nwozo *et al*, 2010), antiplasmodial (Fredrich *et al*., 2002), This paper was aimed at reporting the phytochemicals and antimicrobial activity of the root bark of *Stychnos spinoso* against some selected microorganisms.

MATERIALS AND METHODS

Plant collections, identifications and pre-treatment

The root bark of *Stychnos spinoso* was collected with the aid of a sterilized axe at Rigasa in Igabi Local government area of Kaduna state, Nigeria on 14th march, 2016. The plant material was identified and authenticated by a Taxonomist, of the Herbarium section of National Research Institute of chemical technology, Zaria (NARICT) with a voucher specimen number 2316. The root bark were washed and air dried under the shade for 3 weeks before pounding into powdered and kept in an air tight polythene bag until needed for extraction process.

Extraction of the plant materials

The coarse powdered plant material (500g) was sequentially extracted with solvent of increasing polarities including petroleum ether (60-80^{o}C) 1000ml, ethylacetate (500ml), and methanol (500ml) by percolation for 2 weeks.

evaporated to dryness using a vacuum rotary evaporator maintained at 40°C. The extracts were kept in a refrigerator at 4°C until needed.

Phytochemical Screening

The extracts of the root bark of S*trychnos spinosa* was screened for secondary metabolites namely alkaloids, steroids, tannins, antraquinones, saponins, flavonoids, reducing sugar and glycosides using standard methods (Trease and Evans, 1989, Olurinola, 1996).

Antimicrobial Assay

Preparation of test organisms

The stock culture of *Staphylococcus aureus, Salmonella typhii, Klebsiella pneumonia*and *Candida albicans*were obtained from the Department of Microbiology, Ahmadu Bello University, Zaria.

The culture media used for this analysis include Mueller Hinton Agar (MHA), Mueller Hinton Agar (MHB), Potato Dextrose Agar (PDA) and Nutrient Agar (NA). All these media were used for sensitivity test, determination of minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) and were prepared according to the manufacturer's instructions before sterilizing in autoclave at 121°C for 15 minutes.

Ciprofloxan (10mg/ml) and fluconazole (10mg/ml) were prepared and serve as anti-biotic and antifungal controls respectively.

Antimicrobial assay (sensitivity test)

The antimicrobial assay was carried out on the petroleum ether, ethyl acetate and methanol extracts using the Agar well diffusion method according to the method described by Mallikharjang *et al,*(2010) but with certain modifications.

The nutrient agar powder (9,5g) was suspended in 250 ml of cold distilled water, the mixture was stirred and boiled to dissolve. These standardized inoculate of both the bacterial and fungal isolates were streaked on sterilized Mueller Hinton and potato dextrose agar plate respectively with the aid of a sterilize swab sticks for wells. 0.2 ml each of different concentrations of the extracts as 50, 25, 12,5 and 6.25mg/ml were added respectively to each of the wells.

The inoculated plates with the extract were allowed to stayed for 30 minutes, these allowed the extracts to diffuses on the agar before incubating the plates of Mueller Hinton agar at 37°C for 24 hours while the plates of potato dextrose agar at room temperature for 4 days. At the end of the incubation period, the plates were observed for any evidence of inhibition which will appear as a clear zone that was completely devoid of growth around the wells, the diameter of the zones were measured with a transparent ruler calibrated in millimeter.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the extract was determined by using the macro broth dilution techniques as described by Triggs and Hill(1996) with certain modification, the lowest concentration of the extract showing inhibition for each of the organism, The extract used in the sensitivity test was serially diluted to a different concentrations of 50, 25, 12,5 and 6.25mg/ml in a test tube containing Mueller Hinton broth as the diluents. The organisms were inoculated into each of the test tube containing the broth and the extracts. The inoculated test tubes were corked and incubated at 37°C for 24 hours.

At the end of the incubation period, the tubes were examined for the presence or absence of growth using turbidity as criteria, the lowest concentration in the series without visible sign of growth (turbidity) was considered to be the minimum inhibitory concentration.

Determination of minimum Bactericidal concentration (MBC)

The result from the minimum inhibitory concentration (MIC) was used to determine the minimum bactericidal concentration (MBC) of the extracts. A sterilized wire loop was dipped into the test tube(s) that did not show turbidity (clear) in the MIC test, the loop full was taken and streaked on a sterile nutrient agar plates. The plates were incubated at 37°C for 24 hours. At the end of the incubation period, the plates were examined for the presence or absence of growth. The MBC was recorded as the least concentration at which no growth was observed. This is to determine whether the antimicrobial effect of the extract is bacteristatic or bactericidal.

Statistical Analysis

The test for significant difference at $P \leq 0.05$ was used to analysed the results with MINITAB version 14 statistical software.

RESULTS AND DISCUSSION

The dried root bark (500g) of *Strychnos spinosa* was extracted with petroleum ether, ethyl acetate and methanol to yield three crude extracts as a brown oil 5.2g (1.04%), a dark brown gummy solid 7.5g (1.5%) and a reddish brown solid 8.2g (1.64%) respectively. (Table 1) The highest yield was obtained with methanol indicating that there are more polar constituents in the root bark of the plant. The phytochemical screening results (Table 2) indicated the presences of alkaloids, glycosides, terpenoids/steroids, anthraquinones and saponins in all the tested extracts

Table 1; Physicalcharacteristics of the extracts

Pet Ether	**Ethyl acetate**	**Methanol**	
Mass of powder(g)	500	500	500
Mass of Extract recovered (g)	5.2	7.5	8.2
% recovery	1.04	1.50	1.54
Color of Extracts	Brown	Dark- Brown	Reddish- Brown
Texture of Extracts	Oil	Gummy	Solid

TABLE 2; Phytochemical constituents of Root bark of *Strychnos spinosa.*

COMPOUNDS	**ROOT BARK EXTRACTS**		
Pet Ether	**Ethyl acetate**	**Methanol**	
Alkaloids	+	+	+
Glycosides	--	+	+
Steroids & Terpenoids	+	+	+
Tannins	--	--	+
Anthraquinones	+	+	+
Phlobatannins	--	--	--
Saponins	+	+	+

KEYS+ = present --- = Absent

The phytochemical screening of the powdered root bark showed that *Strychnos spinosa* contains alkaloids, glycosides, steroids, terpenoids, anthraquinone and saponins (Table 2). This is consistent with the works of Ugoh *et al*(2013), where the leaves and stem bark of *Strychnos spinosa* revealed the presences of alkaloids, steroida, terpenoids, tannins, reducing sugar and saponins. Tannins and Phlobatanninswere absent in the Petroleum ether and ethyl acetate extracts which is in contrast with the finding of Kubmarawa *et al*(2017), and this contrast might be attributed to difference in geographical location and environmental condition where the plant sample were collected. The presence of alkaloids and saponins are known to exhibit medicinal and physiological activity (Rao and Newmark, 1998). The presence of glycosides are associated with the treatment of heart diseases, such as congestive heart failure and cardiac arrthythmia (Okwu, 2001) while steroids are important in pharmacy due to their relationship with some compounds as sex hormones (Okwu, 2001).

Antimicrobial activity of the three extracts showed a broad spectrum activity, the methanol extracts is the most active as it shows activity against all the pathogens. (Table 3 & 4) while the petroleum ether extract was the least active. From Table 3 it can be seen that the petroleum ether extract exhibited a weak activity of 12mm against *S.typhii* and 18mm against *S.aureus*, at concentration of 6.25mg/ml, while the ethyl acetate extracts was found sensitivity in the range of 12mm to 25mm against *S.aureus*. The highest activity of the extracts is in the methanol extracts which is active against all the pathogens at a low concentration of 6.25mg/ml except *C.albcans*. The antimicrobial activities observed are in agreement with the finding of Verpoorte *et al,*(1983). The root bark extracts of *S.spinosa* shows a remarkable antimicrobial action on *K. pneumonia* in contrast to *C.albicans* which show resistance at different concentrations, these could be due to the fact that most fungi are resistance to phytochemicals (Kubmarawa *et al*., 2017) However the extracts show a remarkable activity against *Staphylococcus aureus. Salmonella typhii* and *Klebsiella pneumonia.*

TABLE 3 Inhibitory activity of root bark extracts of *Strychnos spinosa* on the test organisms

	Diameter of zone of inhibition(mm) at varying concentration of the extract (mg/ml)									
Test Organism	**Pet Ether**			**Ethyl acetate**			**Methanol**			**Control (Ciprflaxine/fluco zole)**
	5025	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**	
S. aureus.	18±0.7	15±0.5	13±0.3	25±0.7	20±0.7	16±0.5		34±0.3	28±0.7	19±0.7		35±0.3
S.typhii.	12±0.7			13±0.7				14±0.3				38±0.5
K.pneumonia	15±0.3	12±0.7	--	18±0.7	14±0.3	12±0.7		20±0.7	14±0.3	13±0.7		40±0.5
	---	--	--	--				12±0.7				35±0.3
C. albicans	--			16±0.5	--	---	---	24±0.5	22±0.5	19±0.7		
	-	--	--	15±07	---	---	---	14±0.7				
		---						18±0.7	--	--	--	

KEYS---- = No inhibition (no activity)

The secondary metabolite of various chemical types present in the plant species are known to possess antimicrobial activity. The phytochemicals are known to affect the organism by hindering some key enzymes of their metabolic pathways. This is done by forming hydrogen bond with the carbonyl group of the enzyme (Waterman *et al*, 1994). While saponins exert their antimicrobial action by complexing with the small amount of the sterols present in the bacteria cell membranes and thus interfering efficiency of the semipermeability of the bacteria cell membrane (Mitscher, 1975).

TABLE 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts against the test organisms

Test organism	Concentrations of the extracts					
	MIC			MBC		
	PE	EA	ME	PE	EA	ME
Staphylococcus aureus.	6.256.25		1.56	12.5	12.5	3.125
salmonella typhii.	50.0	12.5	12.5	--	25	25
klebsiella pneumonia.	---	2.50	1.56	---	50.0	3.125
candidas albicans	50.0	50.0	50.0	--	---	---

KEYS.---- = No effect**,**PE = Petroleum Ether extracts, EA = Ethyl acetate extracts, ME = Methanol extracts, MIC = Minimum inhibitory concentrations.MBC = Minimum Bactericidal concentrations.

The minimum inhibitory concentration (MIC) results corroborate with the agar dilution assay, it showed that the methanol extracts of the plants was the most potent against *S. aureus,* and *K pneumonia.*with MIC values of 1.56mg/ml (Table 4).The minimum bacteriocidal concentration (MBC) assay shows that most of the extracts were more of bacteriostatic than bactericidal at the tested concentration as it is reported in the work of Bowman *et al,* (1988). Bacteriostatic normally stop the bacteria from reproducing while not necessary killing them, the values of MBC are much higher than that of MIC, therefore the plant extract are more of bacteriostatic than bactericidal (Tripathi, 2013)
There is no significant different ($p \leq 0.05$) in the activities of most the plant extracts on the test organisms.

CONCLUSION

From the results obtained, it can be concluded that the root bark of *Strychnos spinosa* contains alkaloids, glycosides, antraquinones, saponins, Steroids and Terpenoids. It has also been confirmed that the root bark possesses a broad spectrum antimicrobial activity against S*taphylococcus aureus, Salmonella typhii Klebsiella pneumonia* and*Candida albicans* with varying degrees of sensitivity and MIC values. These antimicrobials activity may be attributed to various action of phytochemical present in it either by their individual or by synergistic action. This emphasizes the usefulness of the root bark of the plant in the treatment of certain bacterial diseases in the traditional medicines.

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