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Phytochemical Screening and Antimicrobial Activities of Leaf Extracts of *Swietenia macrophylla*

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ABSTRACT
The phytochemical screening of *Swietenia macrophylla* was undertaken through controlled experiments. The results showed that flavonoids, alkaloids, steroids, terpenes, tannins, glycosides and saponins are present in all the leaf extracts. The result of the antimicrobial activity obtained from the extracts of the leaf of *S. macrophylla* revealed that all the crude extracts of the leaf inhibited or exhibited antibacterial activity against *Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Aspergillus niger* and *Penicillium chrysogenum.* All the extracts demonstrated antimicrobial activity against both the test bacteria and the fungi with the ethyl acetate extracts demonstrating the highest activity for *Salmonella typhi* test. The plant *S. macrophylla* is commonly used traditionally for the treatment of diarrhoea, wound, headache, malaria, dysentery and fevers. The overall results confirm the significance of the use of the plant in traditional medicinal treatment of diarrhea, wound, headache, malaria, dysentery and fevers, in line with reported claims.

Keywords:

INTRODUCTION
Medicinal plants contain organic compounds which could produce definite physiological action on the human body. These bioactive substances could include tannins, alkaloids, terpenoids, steroids and flavonoids etc. (Edeoga *et al.,* 2005). They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas (Vasu *et al*., 2009). In Nigeria many spices are used as food and medicine. Medicinal plants play a significant role in the health of humanity (Sofowora, 1993). The medicinal value of these plants lies in their phytochemical content. Phytochemicals are chemical constituents of plants and exert physiological action on man and animals (Subhashini *et al.,* 2010). The active principles isolated, have provided leads in the development of several lifesaving drugs, which are in use today. Different civilizations have developed their own indigenous system of medicines, across the various ages (Gupta *et al.,* 2010).

Swietnia macrophylla is one of the plants which have been used in traditional medicine for many years. The bark is astringent, bitter and febrifuge. An infusion of the plants leaves is used to treat diarrhea and fevers. To the best of our knowledge little or no work has been done on the plant *S. macrophylla* in Taraba, Nigeria. This work is designed to enrich the available scientific data on the phytochemistry and antimicrobial activities of *S macrophylla* leaves. This paper reports the phytochemistry and antimicrobial activities of *S. macrophalla* leaves on some bacterial and fungal isolates.

MATERIALS AND METHODS
Sample Collection
The leaves of *S. macrophylla* were collected from its natural habitat in Wukari local government area of Taraba State, Nigeria. The samples were identified and authenticated by plant scientists at the Department of Biological Sciences Federal University Wukari. The sample was air dried for two weeks before being milled into fine powder using milling machine

Extraction
All the solvents and reagents used for the extraction were of analytical grade (AR). The method of cold maceration was used in the extraction by serial exhaustive extraction method as described by Pavia, 1976. This involves successive extraction with solvents of increasing polarity from a non-polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compounds could be extracted. The leaf extracts were prepared by soaking 100g of each of the sample in 250ml hexane for four days with frequent agitation until soluble matter was

dissolved. The resulting mixture was filtered using filter paper and the filtrate was concentrated by evaporation using rotary evaporator. This was then kept in a vacuum oven overnight at room temperature to remove any residual solvent before the sample was weighed. The procedure was repeated on the residue using the following solvents; chloroform, ethyl acetate, acetone and ethanol sequentially in order of polarity. The extracts were kept in the refrigerator until required for testing.

Phytochemical Screening

Phytochemical tests were carried out on all the extracts using standard procedures to identify the constituents. Qualitative analysis of the crude extracts were carried out as described by literature reports (Tiwari, *et al.*, 2011, Sagayaraj *et al.*, 2015, Edeoga *et al.*, 2005, Ushie *et al*., 2013 , Ushie *et al*., 2013a, Ushie *et al*, 2013b) to identify the presence of the classes of secondary metabolites (alkaloids, anthraquinones, flavonoids, tannins, saponins, glycosides, cardiac glycosides, terpenes, steroids, phenol, etc)

Detection of Alkaloids: Alkaloids were detected by both the Mayer's and Wagner's tests. Extracts were dissolved individually in 1% hydrochloric acid solution and filtered. The Mayer's test was done by treating filtrates with Mayer's reagent (Potassium iodide 5.0 g). Formation of a yellow coloured precipitate indicates the presence of alkaloids. In the Wagner's test filtrates were treated with Wagner's reagent (Potassium iodide 3.0 g). Formation of brown/reddish precipitate indicates the presence of alkaloids. (Tiwari, *et al*., 2011)

Detection of Glycosides: Extracts were hydrolysed with dilute 1% hydrochloric acid solution, and then subjected to test for glycosides using the Modified Borntrager's test. Extracts were treated with ferric chloride solution and immersed in boiling water for 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides (Tiwari, *et al.*, 2011).

Detection of Saponins: This was done by the Froth Test and Foam test. In the Froth test extracts were diluted with distilled water to a 20ml volume. This was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins. In the Foam test, exactly 0.5 g of the extract was shaken with 2 ml of water. If the foam that was produced persists for ten minutes, this indicates the presence of saponins.

Detection of Flavonoids: This was done by the Alkaline reagent and Lead acetate tests (Tiwari, *et al.*, 2011). In the Alkaline reagent test extracts were treated with few drops of 2M sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of 1% hydrochloric acid solution, indicates the presence of flavonoids. In the Lead acetate test extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of Tannins: A small quantity of the extract was mixed with distilled water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A blue black or brownish green indicate the presence of tannins.

Detection of Anthraquinone: 0.5 g of the extract was boiled with 2ml HCl for five minutes in a water bath. The resultant solution was filtered and allowed to cool at room temperature. Equal volume of chloroform was added to the filtrate. Few drops of 10% NH_3 solution was added to the mixture and heated. Formation of rose-pink colour indicates the presence of anthraquinone.

Detection of Terpenoids: Exactly 0.2 g of the extract was mixed with 2ml chloroform, and 3ml concentrated H_2SO_4. A reddish brown interface was formed which indicated the presence of terpenoids.

Detection of Phenol: To 1ml of the leaf extract 2ml of distilled water was added followed by a two drops of 10% ferric chloride. Formation of blue or black colour indicates the presence of phenols.

Test for Phlobatannins: A portion of each extract was boiled with 1% aqueous HCl. The solutions were observed for a red deposit of precipitate taken as evidence for the presence of phlobatannins.

Test for steroids: 5 drops of concentrated H_2SO_4 was added to 1 mL of each extract in a test tube. The solutions were observed for a red colouration indicating the presence of steroids in the extracts.

Bioassay

The bioassay was carried out by methods described by Ochi *et al.*, 2015. Using such procedures the crude extracts were tested for antibacterial and antifungal activities. The test organisms were collected from Abubakar Tafawa Balewa University Teaching Hospital, Bauchi State, Nigeria. *S. macrophylla* leaf extracts were tested on five clinical isolates comprising three bacterial isolates of *Salmonella tyhpi, Staphylococcus aureus, Escherichia coli,* and two fungi *Aspergillus niger* and *Penicillium* species.

Preparation of varying concentrations of the extracts

Various concentrations of the extracts were prepared ranging from 50 to 200 mg/mL. The concentrations were obtained by measuring 1 mg of the extract and dissolved in 10 mL dimethyl sulphur oxide (DMSO), a solvent that dissolved the

extract (100 mg/mL). A serial dilution of the dissolved extract (200 mg/mL) was carried out into three different bottles containing DMSO to obtain concentrations of 100 and 50 mg/mL respectively.

Antimicrobial Susceptibility test of the crude extract

This was done using Agar Well Diffusion technique .The organisms used were standardized using Mc- Farland turbidity standard scale l, to obtain a bacterial cell density of 10^6 colony forming unit per millilitre (cfu/mL). The standardized inoculum was uniformly streaked (swabbed) into freshly prepared Mueller Hinton agar and potato dextrose agar plates respectively for the bacterial and fungal growth. Five wells were punched on the inoculated plates with a cork borer (8 mm in diameter). The wells were properly labeled according to different number of the concentrations prepared. The wells were then filled up with the extracts about 0.2 mL per well. The plates were allowed to stay on the bench for 1 hour for the extract to diffuse into the agar. The Mueller Hinton agar plates for bacteria were incubated at 37^{o}C for 24 hours while the potato dextrose agar plates for fungi were incubated at room temperature (drawer) for three days. At the end of the incubation period, all plates were observed for any evidence of inhibition, which appear as clear zones completely devoid of growth around the wells (zone of inhibition). The diameters of the zones were measured with a transparent ruler calibrated in millimeters (mm).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the extract was determined using tube dilution method. Serial dilution of the extract was carried out in test tubes using Mueller Hinton Broth (MHB) and Potato Dextrose Broth (PDB) as diluents. The lowest concentration showing inhibition (clear zone) for each organism when the extract was tested during sensitivity test was serially diluted in test tubes containing MHB and PDB. Each tube containing the broth and the extract was inoculated with the standardized organisms. A tube containing sterile broth (MHB and PDB) without any organism was used as a control. All tubes were then incubated at 37^{o}C for 24 hours. After the incubation period, the tubes were examined for the presence or absence of growth using turbidity as a criterion. The lowest concentration (dilution) in the series without visible signs of growth was considered to be the MIC.

Determination of Minimum Bactericidal Concentration (MBC)

The results from the Minimum Inhibitory Concentration (MIC) were used to determine the MBC. A sterile wire loop was dipped into the tubes that did not show turbidity in the MIC test. This was then streaked unto a freshly prepared sterile nutrient agar plates. The plates were incubated at 37oC for 24 hours. After the incubation period the plates were then examined for the presence or absence of growth. This was done to determine if the antimicrobial effect of the extract was bactericidal.

RESULTS AND DISCUSSION

The results of the preliminary phytochemical screening revealed the absence of anthraquinones in hexane, chloroform, and acetone extracts. However, these substances were found to be present in ethyl acetate and methanol extracts. Phenol was found to be present in all extracts except chloroform and acetone extracts. Tannins were present in all extracts except chloroform extract. Phlobatannins were absent in all the extracts, Flavonoids, alkaloids, terpenes, steroids, saponin and glycoside is present in all the extracts as shown in Table 1.

S. macrophylla can be used as anti-inflammatory, antispasmodic, anti-analgesic agent because of their diuretic properties which can be attributed to the presence flavonoids, alkaloids, steroids, glycosides and saponins in their compositions (Savithramma 2011). Saponin causes complexation with cholesterol to form pores in cell membrane bilayers, e.g., in red cell (erythrocyte) membranes, where complexation leads to red cell lysis (hemolysis) on intravenous injection (Francis *et al.;* 2002). *S. macrophylla* is important in pharmacy because it contains steroidal compounds which are of importance and interest in pharmacy due to their relationship with sex hormones (Okwu 2001). These are known to effect the development and control of the reproductive tract in humans and molt insects. Other function inducing sexual reproduction in aquatic fungi. Saxena 2013 pointed out that recently the tannins have attracted scientific interest, especially due to the increased incidence of deadly illnesses such as acquired immune deficiency syndrome (AIDS) and various cancers (Blytt *et al*., 1988). Alkaloids were detected in all the extract. Hence, *S. macrophylla* has the potential to be used as an analgesic or anaesthetic since it contains alkaloids. The presence of terpenoids that have carboxylic acid groups could also be responsible for the activity of the organic extracts (Njoku and Obi 2009). *S. macrophylla* can potentially be used in the treatment of certain illnesses because it contains glycosides.

Table 1: Preliminary Phytochemical Screening of extracts of *Swietenia macrophylla* leaf.

Phytochemicals	Reagents	Extracts				
		HE	CE	EAE	AE	ME
Alkaloids	(a) Mayer	-	-	-	-	+
	(b) Wagner	+	+	+	+	+
Flavonoids	(a) Lead acetate			+		+
	(b) NaOH + Dil. HCl	+	+	-	+	-
Anthraquinone	Extract +HCL (10%) in boiling water +chloroform +ammonia (10%) solution.	-	-	+	-	+
Steroids	Extract + conc. H_2SO_4	+	+	+	+	+
Glycosides	Extract + dil. HCl +ferric chloride solution in boiling water + benzene +ammonia solution.	+	+	+	+	+
Phlobatannins	Extract + (1%) HCL in boiling water	-	-	-	-	-
Tannins	Extract + distilled water in boing water +ferric chloride.	+	-	+	+	+
Phenol	Extract + distilled water + (%) ferric chloride.	+	-	+	-	+
Terpenoids	Extract + chloroform + conc. H_2SO_4	+	+	+	+	+
Saponnins	(a) Froth	+	+	+	+	+
	(b) foam	+	+	+	+	+

Key: HE = Hexane extract, CE= Chloroform, EAE= Ethyl acetate extract, AE = Acetone extract, ME = Methanol extract, + = Detected, - = Not detected

The result of the antimicrobial activity as shown in Tables 2 and 3 obtained from the extract of the leave of *S. macrophylla* revealed the following; all the crude extracts of the leaf inhibited or exhibited antibacterial activity against *Salmonella paratyphi*, *Staphylococcus aureus*, and *Escherichia coli*. All the extracts demonstrated antimicrobial activity against both the test bacteria and fungi with the ethyl acetate extracts demonstrating the highest activity for *Salmonella typhi* test (20 mm zone diameter of inhibition), followed by the methanol extracts for *Staphylococcus aureus* and *Escherichia coli* (20 and 17 mm zone diameter of inhibition) while the antimicrobial test for fungi did not demonstrate any reasonable activity against *Aspergillus niger* but demonstrated little in *Penicillium* sp. for the extracts of hexane, chloroform and methanol respectively. The acetone extracts were active against three of the laboratory isolates; *Salmonella typhi* (14 mm zone diameter of inhibition), *S. aureus* (15 mm zone diameter of inhibition) and *Escherichia coli* (15 mm zone diameter of inhibition), the methanol extracts were active against *E. coli* (17 mm zone diameter of inhibition) *S. typhi* (18 mm zone diameter of inhibition), *S. aureus* (20 mm zone diameter of inhibition), the chloroform extracts were active against *E. coli* (14 mm zone diameter of inhibition) *S. typhi* (17 mm zone diameter of inhibition), *S. aureus* (18 mm zone diameter of inhibition), the ethyl acetate extracts were active against *E. coli* (12 mm zone diameter of inhibition) *S. typhi* (20 mm zone diameter of inhibition), *S. aureus* (19 mm zone diameter of inhibition), the Hexane extracts were active against *E. coli* (09 mm zone diameter of inhibition) *S. typhi* (16 mm zone diameter of inhibition), *S. aureus* (17 mm zone diameter of inhibition) and *Penicillium chrysogenum* were active at (09 and 08 mm zone diameter of inhibition) for hexane, chloroform and methanol extracts while *A. niger* did not inhibit reasonably at 200 mg/ml. Augmentin and Mycotin demonstrated the highest activities against both bacteria and fungi respectively.

The MIC and MBC of the extracts ranged from 50-200 mg/mL, with the acetone extracts demonstrating the lowest values (MIC 50 mg/mL: MBC 50 mg/mL each) against *E. coli*, *S. aureus* and *S. typhi* followed by the chloroform extracts against *E. coli* (MIC 50 mg/mL, MBC 50 mg/mL), ethyl acetate demonstrating the lowest values (MIC 100 and 50 mg/mL: MBC 100 and 50 mg/mL each) against *E. coli*. Hexane demonstrating the lowest values (MIC100 and 50 mg/mL: MBC 50 mg/mL each) against *E. coli*, with almost all extracts demonstrating the lowest values (MIC 200, 100 and 50 mg/mL: MBC 200, 100 and 50 mg/mL each) against *A. niger* and *Penicillium* sp. Most of the MIC values were lower than the MBC values indicating that the extracts could be bactericidal in action. Low MIC and MBC values are also an indication of high efficacy. The demonstration of activity against both gram-negative and gram-positive bacteria and fungi is an indication that the plant can be a source of bioactive substances that could be of broad spectrum of activity. The fact that the plant was active against laboratory isolates

is also an indication that it can be a source of very potent antimicrobial substances that can be used against drug resistant microorganisms prevalent in hospital environments.

Table 2: Inhibition Zone Diameters of extracts of *Swietenia macrophylla* leaf

Organism	Conc. in mg/ml	IZD of HE	IZD of CE	IZD of EAE	IZD of AE	IZD of ME	Positive control (Augmentin)/ Mycotin	Negative Control (DMSO)
Salmonellat yphi	200	16	17	20	14	18	31	00
	100	14	14	16	12	14	30	00
	50	10	12	12	09	12	28	00
S. aureus	200	17	18	19	15	20	29	00
	100	15	16	13	11	16	28	00
	50	13	13	11	09	11	26	00
E. coli	200	09	14	12	15	17	30	00
	100	03	08	07	09	12	30	00
	50	01	05	03	06	08	29	00
A. niger	200	03	01	01	02	01	29	00
	100	02	01	01	01	01	29	00
	50	01	01	01	01	01	27	00
Penicillium spp	200	09	08	06	07	08	30	00
	100	06	06	03	05	06	28	00
	50	01	01	01	01	02	27	00

Key: HE = Hexane extract, CE= Chloroform, EAE= Ethyl acetate extract, AE = Acetone extract, ME = Methanol extract, IZD= Inhibition zone diameter

Table 3: Minimum Inhibitory Concentration (MIC) of extracts of *Swietenia macrophylla* leaf

Organism	MIC of HE (mg/ml)	MIC of CE (mg/ml)	MIC of EAE (mg/ml)	MIC of AE (mg/ml)	MIC of ME (mg/ml)
S. typhi	50	50	50	50	50
S. aureus	50	50	50	50	50
E. coli	50	50	50	50	50
Aspergillus. niger	100	100	100	100	100
Penicillum sp.	50	50	50	50	50

Table 4: Minimum Bactericidal Concentration (MBC) of extracts of *Swietenia macrophylla* leaf

Organism	MBC of Hexane (mg/ml)	MBC of Chloroform (mg/ml)	MBC of Ethylacetate (mg/ml)	MBC of Acetone (mg/ml)	MBC of Methanol (mg/ml)
S. typhi	50	50	50	50	50
S. aureus	50	50	50	50	50
E. coli	50	50	50	50	50
Aspergillus. niger	100	100	100	100	100
Penicillum sp.	50	50	50	50	50

CONCLUSION

The bioactive components extracted from the leaves of *Swietenia macrophylla* include; flavonoid, alkaloids, steroids, terpenes, glycosides, saponins and tannin were detected in all the extract except in chloroform extract, these component are naturally occurring in most plant materials, and are known to be bactericidal, pesticidal or fungicidal in nature thus conferring the anti-microbial property to plants

All the extracts demonstrated antimicrobial activity against both the test bacteria and fungi with the ethyl acetate extracts demonstrating the highest activity for *Salmonella typhi* test (20 mm zone diameter of inhibition), followed by the methanol extracts for *Staphylococcus aureus* and *Escherichia coli test* (20 and 17 mm zone diameter of inhibition) while the antimicrobial test for fungi did not demonstrate any reasonable activity against *Aspergillus niger* but demonstrated little in *Penicillium chrysogenum* for the extracts of Hexane, Chloroform and Methanol respectively. The minimum inhibitory activity (MIC) of the extracts of *Swietenia macrophylla* against tested microbes ranges from 50 to 200 mg/ml in almost all the extracts for the tested fungi. These could explain the rationale for the use the plant in the treatment of the various conditions in traditional medical practice.

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