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Phytochemical Screening and Antibacterial Activity of Leaves of *Sterculia Setigera* Extracts

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To Assessment of phytochemicals and antibacterial activity of different solvent extracts of the leaf of *Sterculia setigera* were carried out using standard methods. Fifty grams of the dried leaf was used for each solvent extraction and 7.40 g (14.8%), 5.50 g (11.0%), 4.10 g (8.2%) of ethanol, methanol and aqueous extract was obtained respectively. Phytochemical screening showed the presence of alkaloids, flavanoids, saponins and tannins. Using the Agar well diffusion method, the different extract showed visible inhibitory effect when compared to the positive control. The methanol extract was observed to be most potent on the test organisms having the highest mean zone of inhibition at concentration of 500 mg/ml 20.5 mm for *Pseudomonas aeruginosa*, and 7.0 mm at 62.5 mg/ml for *Staphylococcus aureus* while the ethanolic extract showed the least potency on *S. aureus* with mean zone of inhibition at 500 mg/ml concentration to be 8.5 mm. The minimum inhibitory concentration (MIC) for the ethanol extract on *S. aureus* was 500 mg/ml while aqueous and methanolic extracts showed MIC at 125 mg/ml for both *E.coli* and *S. aureus* respectively. The minimum bactericidal concentration (MBC) observed to be 250 mg/ml on *S. aureus* and *E. coli* for both methanolic and aqueous extracts. These results suggest that *Sterculia setigera* used in this study has potential that could be harnessed for the production of drugs against bacterial infections.

Keywords: Antibacterial, phytochemical, Sterculia setigera, solvent extracts, inhibition

INTRODUCTION

Plants are potent biochemical and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plant based natural constituents can be derived from any part of plant like, bark, pod, leaves, flowers, roots, fruits and seeds etc (Eloff, 1998). The systematic screening of plant species with the purpose of discovering new bioactive compound is a routine activity in many laboratories. Scientific analysis of plant components follows a logical pathway. Plants are collected either randomly or by following leads supplied by local healer in geographical areas where the plants are found (Parekh *et al*., 2006). Fresh or dried plant materials can be used as a source for the extraction of secondary plant components. Plants are used in dry form (or as an aqueous extract) by traditional healers and due to differences in water content within different plant tissue, plant are usually air dried to a constant weight before extraction (Collan, 2006). In most of the reported works underground parts (roots, tuber, rhizome, bulb etc) of a plant were used extensively compared with other above ground parts in search of bioactive compounds possessing antimicrobial properties (Ncube *et al*., 2008; Das *et al*., 2010). Despite of tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health (Lapornik *et al*., 2010). Nature has provided an important source of remedies to cure all the ailments of mankind. Africa is rich in a wide range of flora that is exploited as herbal remedy (Zaruwa *et al*., 2016). For some years now, all the medicines used were from natural source, especially from plant (Sofowora, 2011) which has made plant-derived substance to become of great interest owing to their versatile application like; modern medicines, a gift of nature been used against various infection and diseases in the world; nutraceuticals, food supplement, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al*., 2008). *Sterculia setigeria* is a forest woody tree belonging to the family of *Sterculiaceae* with more than one thousand species (Felgger, 2009). The species grow under different ecological and soil conditions (Mueller and Tobin, 2009). The use of *Sterculia setigera* traditionally in the treatment

of malaria, jaundice, measles, syphilis, wound, asthma, tuberculosis and leprosy have been reported by several researchers over the years (Maigari and Hamza, 2017; Atakpama *et al*., 2012; Tor-Anyiin *et al*., 2011; Thangodurai, 2005; Igoli *et al*., 2005; Almagboul *et al*., 1985). About 80% of the western pharmaceuticals have their origin in plants (Cuellar *et al.,* 2010). Therefore, this research is designed to evaluate the phytochemical constituents and the antibacterial activity of the leaf of *Sterculia setigera* against some organisms.

MATERIALS AND METHODS

Sample collection and preparation

Fresh samples of the plant material (leaves of *Sterculia setigera*) were collected at biological garden of the Department of Biological Science, University of Abuja, F.C.T., Nigeria. The plant was identified at the herbarium of the Department of Biological Sciences, University of Abuja. The freshly collected leaves of *Sterculia setigera* were spread to air dry at room temperature for two weeks. After drying, the leaves were pounded with the laboratory mortar and pestle and were stored in a clean container for further analysis.

Extraction of the leaf extracts

Three different solvent (methanol, ethanol and distilled water) were used to extract the active components from the plant sample. About 50 g of the powdered leaf sample was weighed into three separate 500 ml conical flasks and 250 ml of the different solvents was added to each respectively. The flasks were corked and left for 48 h at room temperature with occasional shaking. The mixture was filtered using Whatman filter paper No.1 and the filtrate was evaporated using the water bath. The extracts were then stored in the refrigerator at 4°C until further use.

Preparation of stock solution of plant extracts and the control antibiotic (Ampicilin)

The stock solution of the leaf extracts (aqueous, ethanolic and methanolic) were prepared according to the methods described by Arekemase *et al*. (2011). The extracts were prepared by weighing different concentrations (500, 250, 125 and 62.5 mg/ml) into sterile sample bottles using a weighing balance. The various extracts concentrates were then dissolved separately in sterile bottle using 1 ml of sterile water for the aqueous extract and 1 ml of Dimethyl sulfoxide (DMSO) each for the ethanolic and methanolic extracts. The sterile extracts were then stored aseptically in the refrigerator until ready for use (Fatope *et al.,* 2005). For the control, a known standard antibiotic ampicilin was used which was also prepared into different concentration (62.5, 125, 250 and 500 mg/ml) using a weighing balance like the extracts. It was then dissolved in 1 ml of sterile water in different sterile universal containers that has been properly labeled to obtain stock culture of it (Idigo, 2006).

Determination of phytochemical constituents of extract

Phytochemical screening of the plant extracts was carried out to determine the presence of chemical constituents such as glycoside, alkaloids, saponins, tannins. This was carried out using appropriate phytochemical reagents for the various tests as described by Akinyemi *et al*. (2005); Edeoga *et* al. 2005; Junaid *et al*. (2006) and Prashant *et al*. (2011).

Preparation of inoculums

The test organisms used for the screening of the antibacterial activity were *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Stock cultures used were obtained from University of Abuja Teaching Hospital. The test organisms were prepared by transferring a loopful of organism from stock culture into nutrient agar plates and incubated at 37 °C for 24 h. This was done in order to obtain fresh colonies of the organisms. Innoculum for each organism was prepared in a test tube using Macfarland's standard (0.5). A loopful of each of organism was picked and added to different test tubes containing distilled water. Using the inoculating loop, it was stirred and the turbidity compared with that of the 0.5 Macfarland's standard. This was done for each organism until the turbidity was the same with that of 0.5 Macfarland's Standard (Kunle and Eghareuba*,* 2009).

Antibacterial assay

The antibacterial assay was performed using the agar-well diffusion method as described by Okoli and Iroegbu, (2004). This method was employed to determine the growth inhibition of the test organisms by the plant extracts. About 25 ml each of the prepared Muller Hinton Agar was poured into sterile Petri dishes that were already properly labeled for easy identification and left to solidify and dry. Using sterile pipettes, the agar was aseptically inoculated uniformly by flooding with 1ml suspension of the test organisms which had been prepared to 0.5 Marcfaland's standard. The plates were then rocked carefully for the inoculums to spread round the Agar and allowed to dry. Different Agar wells were then made on the plates with aid of sterile cork borer (6 mm) in diameter. Five wells were made on the agar sufficiently spaced away from the edge of the plate and 25 mm from well to well to prevent overlapping of zones

and labeled properly according to the five different crude extract concentrations at the bottom of the Petri dish and the same was done for the standard control plates for easy identification. The stock solution of the crude extracts (aqueous, ethanolic and methanolic) were introduced (2-3 drops) into each agar well using sterile Pasteur pipette, according to the labels including that of the standard antibiotics plates. The plates were then incubated without inverting them at 37°C for 24 h after which the sensitivity of the test organism to aqueous, ethanol and methanol extracts of the plant were determined by measuring the zone of inhibition. The measurement of the zone of inhibition was carried out using a transparent meter rule and read to the nearest millimeter (Okigbo, 2007). The diameter of the clear zone was taken as an index of the degree of sensitivity.

Determination of minimum inhibitory concentration (MIC)

MIC is defined as the lowest concentration where no visible turbidity is observed in the test. The MIC values of the extracts were determined by using the dilution method for the extract. Various concentration (62.5, 125, 250 and 500 mg/ml) of the plant extract were prepared. Thirty-six test tubes were set-up; 2 ml of nutrient broth was pipette into each sterile test tube that was readily labeled including the control tubes. Using sterile pasteur pipettes, 0.2 ml suspension of the test organisms were introduced into the test tube according to their labels. Also 2-3 drops of different stock solution were introduced using sterile Pasteur pipettes into the test tube containing both broth and the inoculums. The tubes were incubated at 37°C for 24 h after which the test tubes were observed for turbidity or clearness. The least concentration where growth was not observed was determined and noted as the minimum inhibitory concentration (MIC) value.

Determination of minimum bactericidal concentration (MBC)

The least concentration of plant extracts that has an antibacterial effect on the organism is considered as the minimum bactericidal concentration (MBC). This was determined from the broth dilution resulting from the MIC tube by sub culturing to the surface of freshly prepared nutrient agar plates using a sterile inoculating loop to streak on the plates as described by (Akinyemi *et* al. 2005). All the test tubes that showed no microbial growth after 24 hours of incubation were sub-cultured onto the surface of freshly prepared nutrient Agar plates and incubated at 37 °C for 24 hours, after which the plates were observed for growth. The plates with the lowest concentration of the extract showing no bacterial growth after incubation was noted and recorded as the MBC.

RESULTS

The result of phytochemical screening of the three different extracts of *Sterculia setigera* leaves showed methanol extract had the highest number of phytochemicals while the aqueous extract had the least phytochemicals. Some secondary metabolites were observed in the phytochemicals (Table 1). The antibacterial screening revealed that *P. aeruginosa* is resistant to both the aqueous and ethanolic extracts even at the highest concentration of 500 mg/ml; while *S. aureus* was observed to be susceptible to the three different leaf extracts of *Sterculia setigera* when compared to the standard antibiotics Ampicilin (Table 2). The result of the minimum inhibitory concentration of leaf extracts on the test organisms showed that only *S. aureus* has MIC at 500 mg/ml of the ethanol extract while *P. aeruginosa* and *S. aureus* showed the lowest MIC (125 mg/ml) of methanol extract (Table 3). The result of minimum bactericidal concentration (MBC) of leaf extracts of *Sterculia setigera* on the test organisms is shown in (Table 4).

DISCUSSION

The phytochemical study revealed that the ethanolic extract of *S. setigera* have alkaloids and reducing sugar present in the leaf, while saponnins and resins were present in both aqueous and methanolic leaf extracts. Methanolic extract also have tannins, philobatannins, balsams and resins while flavonoids, alkaloid and reducing sugar were absent in the aqueous extract. The observed phtyochemicals in the leaf extracts corresponds to the reports of Hamidu, (2012). Tor-Anyiin *et al*. (2011) reported the presence of tannins and alkaloids in the methanol extract of the stem bark of *S. setigera.* The medicinal activity of the plant may be related to its chemical constituents for example tannins are plant secondary metabolites well known for their antimicrobial properties and can aid in wound healing and burns (Mohan *et al*., 2014). Also, about 80% of the western pharmaceuticals have their origin in plants (Cueller *et al*., 2010). Flavonoids have important dietary significant because being phenolic compound they are strongly antioxidant and this probably explains why Africans eat the young leaf of *Sterculia setigera* as vegetable. Since, they are known to be synthesized by plant and responsible for microbial inhibition in curing ailment and also therapeutic agent for relieving pain (Adebajo *et al*., 1983). Ouedraogo *et* al. (2013) reported the antifungal effect of the bioactive fractions of the leaf of *S. setigera*. Their results showed that *S. setigera* possessed a high amount of polyphenol content which may explain the ability of the plant in the treatment of infectious diseases. Some researchers have also reported that some saponnins

Table 1. *Phytochemical properties of* S. setigera *leaf extracts.*

Parameters	Aqueous extract	Ethanol extract	Methanol extract
Tannins	+	+	+
Saponins	+	+	+
Alkaloids	-	+	+
Flavonoids	-	-	+
Philobotannins	+	+	-
Balsams	+	-	+
Reducing sugars	-	+	+
Resins	+	-	+

Key: + = Present, - = Absent

Table 2. Antibacterial activity of the leaf extracts of S. *setigera* at different concentrations mean diameter zone of inhibition (mm) ($x \pm \sigma$)*

Organisms	Concentrations (mg/ml)	Ethanol Extract	Methanol Extract	Aqueous Extract	Amp. (control)
Pseudomonas aeruginosa	500	N Z I	20.5 ± 0.35	N Z I	19
	250	N Z I	17.0 ± 0.71	N Z I	17
	125	N Z I	9.5 ± 0.35	N Z I	16
	62.5	N Z I	N Z I	N Z I	14
Escherichia coli	500	N Z I	19.5 ± 0.35	15.0 ± 0.35	30
	250	N Z I	16.0 ± 0.71	13.0 ± 0.71	25
	125	N Z I	N Z I	8.5 ± 0.35	20
	62.5	N Z I	N Z I	N Z I	17
Staphylococcus aureus	500	8.5 ± 0.35	17.0 ± 0.71	17.0 ± 0.71	28
	250	6.0 ± 0.71	9.5 ± 0.35	10.5 ± 0.35	26
	125	N Z I	9.0 ± 0.71	9.0 ± 0.71	22
	62.5	N Z I	7.0 ± 0.71	5.0 ± 0.71	19

KEY: Amp = Ampicilin, N Z I = no zone of inhibition.

have anti-cancer and immune modulating properties (Kunle and Eghareuba, 2009). The result obtained from this study in (Table 2) showed dose dependent activities of *Sterculia setigera* leaf extracts (aqueous, ethanol and methanol) at the tested doses on the tested organism (*Escherichia coli, Pseudomonas aeruginosa and staphylococcus aureus*). The largest zone diameter of inhibition was obtained with the methanolic extract against *Pseudomonas* (20.5 mm) which compared favourably with the value (19.0 mm) of the standard antibiotic ampicilin and the lowest was obtained from both aqueous and ethanolic extracts against *E. coli* and *Pseudomonas aeruginosa* (0 mm). *Pseudomonas aeruginosa* and *E.coli* were highly sensitive to the methanolic extract. However, Tor-Anyiin *et* al. (2011) reported that the crude stem bark extracs showed low antibacterial activity against *Staphylococcus aureus, Proteus mirabilis and Klesiella pneumonia.* The result of antibacterial activity of crude extracts showed that the methanolic leaf extract was active against the three selected microorganism. It was also observed that the standard antibiotic (ampicilin) showed higher zone of inhibition compared to the leaf extracts. This may be because the standard antibiotic is in its pure form while the extracts are not yet purified. The results of this research strongly agree with several literature reports on the significant antimicrobial activities of *Sterculia setigera* against several pathogens such as *Salmonella typhi and B. subtilis* (Ibrahim *et al*., 2012; Cueller *et al*., 2010). This observation therefore revealed the use of *Sterculia setigera* by traditional medicine practitioners in the treatment of infectious disease. The MIC of ethanol, methanol and aqueous leaf extracts of *S. setigera* on the test organisms as shown in (Table 3). The ethanol leaf extract showed no MIC for *P. aeruginosa* and E.coli while for *S. aureus* was at the concentration of 500 mg/ml. Also, the least MIC of the methanol leaf extract for both *S. aureus* and *P. aeruginosa* was at 125 mg/ml and 250 mg/ml for *E. coli*. However, there was no MIC for the aqueous leaf extract on *P. aeruginosa*, while the least MIC for both *E. coli* and *S. aureus* was at 125mg/ml. According to Doughari *et al*. (2007), high MIC may be an indication of low efficacy or the organism has the potential for developing resistance to bioactive compounds

Table 3. Minimum inhibitory concentration of leaf extracts of S. *setiger*.

Test organisms	**Concentration (mg/ml)**	**Ethanol Extract**	**Methanol Extract**	**Aqueous Extract**
Pseudomonas aeruginosa				
	500	*+*	*-*	*-*
	250	*+*	*-*	*-*
	125	*+*	*-*	*-*
	62.5	*+*	*+*	*+*
Escherichia coli				
	500	*+*	*-*	*-*
	250	*+*	*-*	*-*
	125	*+*	*+*	*-*
	62.5	*+*	*+*	*+*
Staphylococcus aureus				
	500	*-*	*-*	*-*
	250	*+*	*-*	*-*
	125	*+*	*-*	*-*
	62.5	*+*	*+*	*+*

KEY: + = growth (turbid), - = no growth (clear/ less turbid).

Table 4. Minimum bactericidal concentration of leaf extracts of *S. setigera*

Test organisms	**Concentration (mg/ml)**	**Ethanol Extract**	**Methanol Extract**	**Aqueous Extract**
Pseudomonas aeruginosa				
	500	*+*	*-*	*+*
	250	*+*	*-*	*+*
	125	*+*	*+*	*+*
	62.5	*+*	*+*	*+*
Escherichia coli				
	500	*+*	*-*	*-*
	250	*+*	*+*	*+*
	125	*+*	*+*	*+*
	62.5	*+*	*+*	*+*
Staphylococcus aureus				
	500	*-*	*-*	*-*
	250	*+*	*-*	*-*
	125	*+*	*+*	*+*
	62.5	*+*	*+*	*+*

and low MIC is an indication of high efficacy. This corroborates the antibacterial results observed in this study. There was no MBC for the ethanol leave extract on *P. aeruginosa* and *E. coli* while, *S. aereus* was 500 mg/ml. For methanol, the MBC of *S. aureus* and *P. aeruginosa* were 250 mg/ml, *E. coli* was 500 mg/ml. For aqueous, the MBC of *E. coli* was 500 mg/ml, while for *S. aureus was* 250 mglml and *P. aeruginosa* had no MBC (Table 4).

Conclusion

The evaluation of phytochemical and antibacterial screening of *Sterculia setigera* concluded that aqueous, ethanolic and methanolic extract contain saponnins, tannins, philobotannin. Aqueous and methanol extract contain reducing sugar and alkaloid but only methanolic extract has flavonoid and these constituents could be responsible for its activities against *E.coli, S. aureus* and

Pseudomonas aeruginosa. The bioactive compounds explain to some extent its traditional usage for various ailments. This activity may be due to the combination of secondary metabolites present in the plant. It was evident from the result of this study that *E. coli* was more susceptible to the plant extracts compared to *P. aeruginosa* and *S. aureus.* The present study therefore offers a scientific basis for the traditional use of plant *Sterculia setigera* for the treatment of *Mycobacterium tuberculosis*, malaria, jaundice, measles, syphilis and leprosy.

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