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PHYTOCHEMICALS AND ANTIMICROBIAL ACTIVITIES OF ETHYL ACETATE AND METHANOL LEAF EXTRACTS OF *STERCULIA SETIGERA*

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Abstract

The leaf of *Sterculia setigera* obtained from Taraba State, Nigeria, were extracted by maceration with ethyl acetate and methanol solvents in increasing polarity, these were filtered, concentrated and subjected to phytochemical and antimicrobial screening. The results obtained from the phytochemical screening of the crude extracts showed the presence of alkaloids, flavonoids, glycosides, steroids, saponins, terpenoids, tannins, phenol and phlobatannins. The antimicrobial screening of the crude extracts showed that Methicilin resistant *Staphlococcus aureus* (MRSA), Vancomycin resistant *Enterococci* (VRE), *Escherichia coli*, *Campylobacter jejuni*, *Salmonella typhi* and *Candida krusei* were susceptible while *Staphlococcus aureus*, *Helicobacter pylori*, *Candida albicans* and *Candida tropicalis* resistant to the plant's extracts. The ethyl acetate extract was observed to be most potent on the test organisms having the highest mean zone of inhibition at concentration of 25 mm for *Campylobacter jejuni*, and the least at 22 mm for *Salmonella typhi*, while methanol extract showed the most potency on Methicilin resistant *Staphlococcus aureus* (MRSA) with mean zone of inhibition at 22 mm concentration and the least at 19 mm for *Salmonella typhi*. The bioactive compound detected in the leaf extracts of *Sterculia setigera* are known to be bactericidal, pesticidal or fungicidal in nature thus conferring antimicrobial property to the plant. This validates the claims by the indigenes in it use for medicinal purposes.

Keywords: *Sterculia setigera*, Maceration, Crude extracts, phytochemical screening

1. Introduction

Plants are known to have potent biochemical compounds (secondary metabolites) from which a wondrous assortment of pharmaceutical, agricultural and industrial chemicals are obtained. Plant based natural constituents can be derived from any part of plant like, bark, pod, leaves, flowers, roots, fruits and seeds etc (Eloff, 1998). These biochemical compounds which are natural constituents include alkaloids, flavonoids, glycosides, steroids, terpenoids, tannins, phenol and phlobatannins have been reported to be responsible for the curative properties of medicinal plants (Kendeson *et al*., 2019). They are produced in the correct chiral form to serve such functions as chemical defenses against insects, microorganisms, and other predators, or as pollinator attractants, and biosynthesized in specialized cell types, at only some of the life stages of the plant, and

usually accumulate in much lower quantities (Balandrin *et al*., 1985).

Medicinal plants act as potential source of therapeutic aids which has attained a significant role in health system all over the world for both humans and animals not only in diseased condition but also as potential material for maintaining proper health (Ajayi and Ojelere 2013).

Sterculia setigera plant belongs to the family of *Sterculiaceae* and is commonly called Karaya gum tree (Maigari and Hamza, 2017) and locally known by different indigenous cultural communities in Nigeria as "Kukuki" (Hausa); "Ose-awere" or "eso funfun" (Yoruba), "Kume-ndur" (Tiv) (Igoli *et al*., 2006; Tor-Anyiin *et al*., 2003). The plant *S. setigera* has been in use as a medicinal plant for the treatment of leprosy in Taraba State, this research was carried out to enrich the available scientific proof on the phytochemical and antimicrobial activities.

2. Materials and Methods

Sample collection and preparation

Fresh sample of the plant material (*S. setigera* leaf) was collected and identified by a Botanist from the Federal University Wukari, Taraba State, Nigeria. The freshly collected leaves were air dried at room temperature for three (3) weeks, after which; they were pounded with the laboratory mortar and pestle and were stored in a clean container for further analysis.

The method of cold maceration was used for the extraction by soaking 200 g of the sample in 400 ml of hexane for four days with frequent agitation. The resulting mixture was filtered using filter paper and the filtrates concentrated by evaporation using rotary evaporator and weighed. This procedure was repeated on the residue using the following solvents; ethyl acetate, acetone and methanol. The extracts were kept in the refrigerator until required for test.

3. Phytochemical Screening

Phytochemical examinations were carried out for all the extracts using standard procedures as described by Ushie *et al*., (2019; 2020; 2022), and Kendeson *et al*., (2019) to identify the presence of the classes of Secondary metabolites (alkaloids, anthraquinones, flavonoids, tannins, saponins, glycosides, terpenes, steroids, phenol, etc).

4. Antimicrobial Activities

The crude extracts were tested for antimicrobial activities using methods described by Ushie *et al.*, (2021) with modification. 0.1 g of the crude extracts was weighed and dissolved in 10 ml of DMSO to obtain a concentration of 10 mg/mL. This was the initial concentration of the extract used to determine its antimicrobial activities. The test organisms were collected from the department of Medical Microbiology Ahmadu Bello University teaching hospital, Zaria, Kaduna State, Nigeria. These microorganisms are Methicilin resistant *Staphlococcus aureus* (MRSA), Vancomycin resistant *Enterococci* (VRE), *Escherichia coli*, *Campylobacter jejuni*, *Salmonella typhi*, *Staphlococcus aureus*, *Helicobacter pylori*, *Candida krusei*, *Candida albicans*, and *Candida tropicalis*, they were sub-cultured and used for the assay.

5. Results and Discussions

5.1 Preliminary Phytochemical screening of extracts from leaves of *S. setigera*

The crude ethyl acetate and methanol extracts of *S. setigera* leaves were screened for the presence of some phytochemicals such as alkaloids, flavonoids anthroquanoids, steroids, glycosides, phtobatannis, tannins, terpenoids, saponins and phenols.

Table 1: Preliminary Phytochemical Screening of *S. setigera* Leaf Extracts

Phytochemical	Reagents	Crude Extracts	
		Ethyl acetate	Methanol
Alkaloids	(a) Mayer	-	+
	(b) Wagner	+	+
Flavonoids	NaOH + dil. HCl	-	+
Anthraquinones	Extract + HCl (10%) in boiling water + chloroform + ammona (10%) solution	-	-
Steroids	Extract + conc, H_2SO_4	+	+
Glycosides	Extract + dil. HCl + ferric chloride solution in boiling water + benzene + ammonia solution.	+	-
Phtobatannis	Extract + (1%) HCl in boiling water	+	+
Tannins	Extract + distilled water in boing water + ferric chloride	+	+
Terpenoids	Extract + chloroform + conc. H_2SO_4	+	+
Saponins	(a) Froth	-	+
	(b) foam	-	+
Phenols	Extract + distilled water + (1%) ferric Chloride	+	+

Key: + indicates presence of the constituents
- indicates absence of the constituents.

Table 3: Antimicrobial Activities of *S. Setigera* Leaf Crude Extracts

Test organisms	Crude Extracts	
	Ethyl acetate	**Methanol**
Methicilin resistant *S. aureus (MRSA)*	S	S
Vancomycin resistant *Enterococci (VRE)*	S	S
S. aureus	R	R
E. coli	S	S
H. pylori	R	R
C. jejuni	S	S
S. typhi	S	S
C. albicans	R	R
C. krusei	S	S
C. tropicalis	R	R

Key: R implies Resistance and **S** implies Susceptible

Table 4: Mean Zone of Inhibition of the Extract against the Test Micro-organism

Test organisms	Zone of Inhibition/ Extracts	
	Ethyl acetate	**Methanol**
Methicilin resistant *S. aureus (MRSA)*	22	20
Vancomycin resistant *Enterococci (VRE)*	24	22
S. aureus	0	0

E. coli	22	20
H. pylori	0	0
C. jejuni	25	22
S. typhi	22	19
C. albicans	0	0
C. krusei	23	20
C. tropicalis	0	0

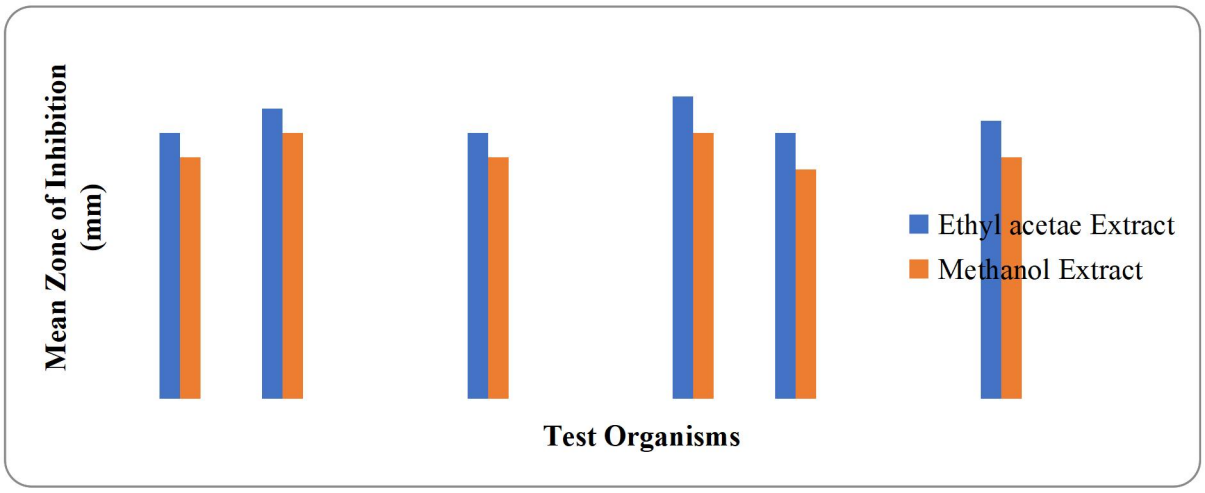

Fig 1: Graph representation of the Mean Zone Inhibition against the micro-organisms

Table 5: Minimum Inhibitory Concentration (MIC) of *S. setigera* Leaf Crude Extracts

Test organism	10.00 mg/ml	5.00 mg/ml	2.50 mg/ml	1.25 mg/ml	0.63 mg/ml	0.31 mg/ml	**(Ethyl acetate)**	10.00 mg/ml	5.00 mg/ml	2.50 mg/ml	1.25 mg/ml	0.63 mg/ml	0.31 mg/ml	**(Methanol)**
MRSA	-	-	0*	+	++	+++		-	0*	+	++	++	+++	
VRE	-	-	0*	+	++	+++		-	0*	+	++	++	+++	
S. aureus														
E. coli	-	-	0*	+	++	+++		-	0*	+	++	++	+++	
H. pylori														
C. jejuni	-	-	0*	+	++	+++		-	-	0*	+	++	+++	
S. typhi	-	-	0*	+	++	+++								
C. albicans														
C. krusei	-	-	0*	+	++	+++		-	0*	+	++	++	+++	
C. tropicalis														

Key: - = No turbidity (No growth); **0*** = MIC; + = Turbidity (light growth);
++ = Moderate turbidity; +++ = High Turbidity.

ble 6: Minimum Bactericidal Concentration (MBC) / Minimum Fungicidal Concentration (MFC) of *S. setigera* Leaf Crude Extracts

Test organism	10.00 mg/ml	5.00 mg/ml	2.50 mg/ml	1.25 mg/ml	0.63 mg/ml	0.31 mg/ml	(Ethyl acetate) 10.00 mg/ml	5.00 mg/ml	2.50 mg/ml	1.25 mg/ml	0.63 mg/ml
MRSA	0*	+	++	++	++	++	0*	+	++	++	++	++
VRE	-	0*	+	++	++	+++	0*	+	++	++	++	++
S. aureus											
E. coli	0*	+	++	++	++	++	0*	+	++	++	++	++
H. pylori											
C. jejuni	-	0*	+	++	++	+++	0*	+	++	++	++	++
S. typhi	0*	+	++	++	++	++					
C. albicans											
C. krusei	-	0*	+	++	++	+++	0*	+	++	++	++	++
C. tropicalis											

Key: - = No colony growth**; 0*** = MBC; + = Scanty colonies growth;
++ = Moderate colonies growth; +++ = Heavy colonies growth.

Discussion

The phytochemical screening of the crude extracts of *S. setigera* leaf showed that the leaves were rich in phytocompounds as shown in Table 1. The ethyl acetate and methanol crude extracts revealed the presence of the presence of alkaloids, flavonoids, glycosides, steroids, terpenoids, tannins, saponins, phenol and phlobatannins. Flavonoids, terpenoids and saponins were not detected in the ethyl acetae extract, likewise, glycosides were not detected in methanol extract; on the other hand, anthraquinones were completely absent in both extracts. This may be an impact from the type of solvents and the polarities of these solvents on the phytochemicals, as polar solvents usually have salvation of polyphenols than non-polar or lesser polar solvents (Thouri *et al*., 2017).

These bioactive compounds (phytochemicals) are used in the production of drugs, flavours, fragrances, insecticides, pesticides and dyes because of their great economic value (Pagare *et al*., 2015), and have been reported to work in synergy exhibiting potential health benefits which include antimicrobial, antioxidant (Pradeepa *et al*., 2016; Kendeson *et al*., 2021), anti-diarrheal and antihypertensive (Ghosal *et al*., 1996), anti-inflammatory (Onwuliri, 2004; Tawheed and Monika, 2014), anti-analgesic (Ahmed and Mohammad, 2014), anti-diabetic and antibiotics (Opara *et al*., 2019) activities , etc in humans as well as animals.

The results of the antimicrobial activity in Table 3 - 6 and Fig 1 revealed that both extracts of the leaf inhibited antimicrobial activities against Methicilin resistant *S.aureus (MRSA),* Vancomycin resistant *Enterococci (VRE)*, *E. coli, S. typhi, C. jejuni,* and *C. krusei* while *S. aureus, H. pylori, C. albicans,* and *C.tropicalis* were resistant to the plant's extracts. The resistance exhibited by these micro-organisms may be due to the low concentration (10 mg/mL) of these extracts used or mutation from the microorganisms. The antimicrobial activity as indicated by the

crude extracts against the selected microorganisms' shows that the plant possesses the potency to kill certain microorganism and can be used as a prospective antimicrobial agent, hence, its use by the locals for the treatment of the various diseases mentioned.

Conclusion

The ethyl acetate and methanol crude extracts of *S. setigera* leaf revealed the presence of alkaloids, flavonoids, glycosides, steroids, terpenoids, tannins, saponins, phenol and phlobatannins. These secondary metabolites have been reported to work in synergy exhibiting potential health benefits which include antimicrobial activity which is evident from the results of the antimicrobial assay, thus, supporting the traditional use of the plant's leaf for medicinal purposes by the locals.

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