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Preliminary Phytochemical and Antimicrobial Activity Screening of Crude Extracts of Bird lime (*Tapinanthus globiferus*)

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ABSTRACT: Several species of the genus *Tapinanthus* have folkloric use by the Hausa and Fulani tribes of the Northern Nigeria as a remedy for numerous human and animal ailments including stomach ache, diarrhea, dysentery, wounds and swellings. *The* preliminary phytochemical screening of the stem bark of *Tapinanthus globiferus,* was carried out using standard method. The result of the phytochemical screening of crude ethylacetate, n-hexane extract revealed the presence of carbohydrate, flavonoids steroids and terpenes. The antimicrobial screening against *Candida krusei*, methicillin resistant *Staphylococcus aureus, Campylobacter jejuni, Streptococcus pyogenes, vancomycin resistant enterococci, Helicobacter pylori, Escherichia coli, Shigella dysenteriae, Staphylococcus aureus* and *Candida tropicalis* was done using agar well diffusion method. The zone of inhibition of growth of microorganisms ranged from 22-28mm for the ethylacetate extract, and 18-21mm for the n-hexane extract and the minimum inhibitory concentration of the extracts was found to be between 0.63mg/ml and 20mg/ml while the minimum bactericidal/fungicidal concentration were found to be between 0.63mg/ml and 20mg/ml. This research has establish a baseline information on the efficacy of the crude extracts of *Tapinanthus globiferus.*

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Mistletoes are often described as hemiparasites because they are partial parasites on various host, most of which are of economic value. They are partially parasitic in the sense, that though they are attached to the host plant from which they obtain nutrients and water, they have green leaves which carry out photosynthesis and thus manufacture food for the plant. Mistletoes of the *Loranthaceae* and *Viscaceae*, are widely used in various cultures in almost every continent to treat various ailments including hypertension, cancer, and diabetes, or used as a diuretic agent (Burkil, 2000; Sher and Alyemani, 2011; Jadhav *et al.,* 2010*). Tapinanthus globiferus* is a mistletoe of the family *Loranthaceae*. It grows parasitically on trees and shrubs, nearly all the Loranthaceae family grow in the tropics. It is a woody, spreading shrub with blackish, smooth stems made rough by the presence of lenticels, the leaves are opposite but sometimes alternate. Literature search on the plant reveals the previous studies conducted which includes antibacterial effect (Ndukwe *et al.,* 2001) antioxidant effect (Cook *et al.,* 1998) and antihypertensive studies via reduction of LDL and triglycerides. It was also reported that the leaf decoction from this plant group is used for treating epilepsy and helps to calm electrical activities of the brain (Dazeel, 1938; Irvine, 1961). The objective of this paper is to report the preliminary phytochemical and antimicrobial activity screening of crude extracts of bird lime (*Tapinanthus globiferus*)

MATERIALS AND METHODS

Plant collection: Fresh stem samples of *Tapinanthus globiferus* growing on *Terminalia catappa* were collected from Samara in Zaria, Nigeria, in the month of July-August 2017 and sundried, the sundried stem was pulverized using mortar and pestle.

Extraction: 500g of the pulverized stem was measured into a clean Winchester bottle and 1.5 liters of hexane was introduced and shaken intermittently and then filtered after 48 hours, the same was repeated for ethylacetate. Extracts were concentrated to one third of the original volume in vacuo using a rotary evaporator at about 42^0C, this yielded 1.2g (0.24%) of crude n- hexane extract, 1.2g (0.24%) of crude ethylacetate extract and 2.5g (0.5%) of methanol extract. These were then subjected to bioassay studies.

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Phytochemical screening: this was carried out on the pulverized plant sample to test for the presence of secondary metabolites using standard techniques (Brain *et al.,* 1975) for carbohydrates, alkaloids, tannins, saponins, flavonoids, steroids and triterpenes.

Test organisms: *Candida krusei,* Methicillin Resistant *Staphylococcus aureus, Campylobacter jejuni, Streptococcus pyogenes,* Vancomycin Resistant *enterococci, Helicobacter pylori, Escherichia coli, Shigella dysenteriae, Staphylococcus aureus* and *Candida tropicalis* were used as the test organisms.

Antimicrobial activity screening: The antimicrobial activity tests of the extracts were carried out using some pathogens. The pure clinical bacterial and fungi isolate of vancomycin resistant *enterococci, Helicobacter pylori*, *Escherichia coli, Shigella dysenteriae, Staphylococcus aureus, Candida tropicalis,* of *Candida krusei,* methicillin resistant *Staphylococcus aureus, Campylobacter jejuni* and *Streptococcus pyogenes* were obtained from the department of medicinal microbiology, Ahmadu Bello University Teaching Hospital Zaria.

All the microbes were screened for purity and maintained in slants of nutrient agar for bacteria and slants of Sabouroud dextrose agar for fungi. In determining the antibacterial activities of the extracts, well diffusion method was used. The stock solutions of the plant extract was made by weighing 0.2g of each extracts and dissolving in 10ml of dimethylsuphoxide (DMSO) to obtain a concentration of 20mg/mL this was the initial concentration. This was also done for each of the extracts. Concentrations of 10mg/mL, 5mg/ml, 2.5 mg/ml, 1.25 mg/ml and 0.63 mg/ml were prepared, and the media prepared above was inoculated with 0.1mL standard inocula of the test organisms. The inocula were spread evenly over the surface of the medium by the use of a sterile swab.

The agar plates were seeded with 0.1ml of the standard inoculum of the test microbe. Inoculation was made at 37^0C for 24 hours. Standard cork borer of 6mm diameter was used to cut a well at the center of each inoculated medium and 0.1mL of the solution of the extracts of the concentration of 20mg/ml was introduced into the well on the inoculated medium, the plates were then incubated at 37^0C for 24 hours for the bacteria and at 25^0C for the fungi. They were observed after the periods of incubation as to note the zone of inhibition of growth. The zones were recorded in millimeters of their diametrical section as shown table 3. The minimum inhibitory concentration of the extracts was carried out on the test organisms using the broth dilution method. Muller-Hinton broth was prepared according to the manufacturers guidelines. Mac-farlands turbidity scale number 0.5 was prepared and 10ml was dispensed into sterile test tubes and the test microbes were then inoculated and incubated at 37^0C for 6 hours. Dilution of the test microorganisms in the in the normal saline was performed until the turbidity marched that of the Mac-farlands scale by visual comparison, at this point the microorganisms had a concentration of about 1.5×10^8cfu/ml. Two fold serial dilution of the extracts in the broth were performed to obtain the concentration of 20mg/ml,5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, and 0.63 mg/ml respectively, the initial concentration was obtained by dissolving 0.2g of the extracts in 10ml of the sterile broth. Having obtained the different concentration of the extracts in the sterile broth, 0.1ml of the standard inoculum of the test microorganism in the normal saline was then inoculated into the different concentration incubation was carried out at 37^oC for 24 hours after which each broth was observed for turbidity. The lowest concentration of the extract in the broth which showed no turbidity was recorded as the minimum inhibitory concentration recorded for the various microbes (Table 4.)

RESULTS AND DISCUSSION

Phytochemical screening: the preliminary phytochemical screening of the extracts of *Tapinanthus globiferus* showed the presence of carbohydrates, alkaloids and steroids in the hexane, ethylacetate and methanol extracts, flavonoids and triterpenes were present in both ethylacetate and methanol extracts, while cardiac glycosides and anthraquinones where present only in the methanol extracts. The results obtained in this screening are shown in table 1.

Table 1: Result of phytochemical screening

S/N	Phytochemical constituents/test	EA	HE	ME
1	Carbohydrates	+	+	+
2	Alkaloids	+	+	+
3	Tannins	-	-	+
4	saponins	-	-	-
5	Flavonoids	+	-	+
6	Steroids	+	+	+
7	Triterpenes	+	-	+
8	Cardiac glycosides	-	-	+
9	Anthraquinones	-	-	+

Key: + = present, - = absent HE =Hexane extract, EA = ethyl acetate extract, ME = methanol extract

Table 2: Results of the antimicrobial screening of the crude extracts

Test organisms	EA	HE	ME	CIP	SP	FLU
Methicillin-resistant *Staphylococcus aureus*	R	R	R	R	S	R
Vancomycin resistant *enterococci*	S	S	S	S	R	R
Staphylococcus aureus	S	S	S	S	S	R
Streptococcus pyogenes	R	R	R	S	R	R
Escherichia coli	S	S	S	S	S	R
Campylobacter jejuni	R	R	R	S	R	R
Helicobacter pylori	S	S	S	R	S	R
Shigella dysenteriae	S	S	R	S	S	R
Candida krusei	R	R	S	R	R	S
Candida tropicalis	S	S	S	R	R	S

Key: S = Sensitive, R = Resistance, HE =Hexane extract, EA = ethyl acetate extract, ME = Methanol extract

Table 3: Zone of inhibition of the test organism by the extracts (mm)

Test organism	EA	HE	ME	CIP	SP	FLU
Methicillin-resistant *staphylococcus aureus*	0	0	0	0	30	0
Vancomycin resistant enterococci	25	20	23	32	0	0
Staphylococcus aureus	28	21	25	35	32	0
Streptococcus pyogenes	0	0	0	32	0	0
Escherichia coli	23	18	21	38	35	0
Campylobacter jejuni	0	0	0	31	0	0
Helicobacter pylori	22	18	20	0	30	0
Shigella dysenteriae	27	20	0	38	37	0
Candida krusei	0	0	22	0	0	35
Candida tropicalis	24	20	24	0	0	32

Key; *HE =Hexane extract, EA = ethyl acetate extract, ME = Methanol extract; CIP = ciprofloxacin, SP = sparfloxacin, FLU = fluconazole*

Table 4: Determination of MIC MBC/MFC of the extracts on test organism

Test organisms;	MIC (mg/mL)			MBC/MFC (mg/mL)		
	HE	EA	ME	HE	EA	ME
Vancomycin resistant *enterococci*	5	2.5	12.5	20	10	25
Staphylococcus aureus	5	1.25	6.25	20	5	25
Streptococcus pyogenes	-	-	-	-	-	-
Escherichia coli	10	5	12.5	20	10	50
Campylobacter jejuni	-	-				-
Helicobacter pylori	10	5	12.5	20	20	50
Shigella dysenteriae	5	2.5	12.5	20	5	25
Candida krusei	-.	-	-	-	-	-
Candida tropicalis	5	5	12.5	20	10	50

Key; HE =Hexane extract, EA = ethyl acetate extract, ME = Methanol extract; MIC = minimum inhibitory concentration, MBC/MFC = minimum bactericidal concentration/minimum fungicidal concentration

The result of antimicrobial activity of the n-hexane, ethylacetate and methanol fractions showed inactivity to methicillin resistant staphylococcus aureus, streptococcus pyogenes and campylobacter jejuni and active on vancomycin resistant *Enterococci, Staphylococcus aureus, Escherichia coli, Helicobacter pyroli, Shigella dysenteriae and Candida tropicalis* with zones of inhibition on the microbes for the n-hexane extract to be 20, 21, 18, 18, 20, 20mm respectively. However the ethylacetate extract showed a higher zone of inhibition than that of n-hexane extract having the following values of 25, 28, 23, 22, 27 and 24mm. The highest was recorded on *Staphylococcus aureus* to be 28 and the lowest at 22mm on *Helicobacter pyroli*. The methanol fraction showed zones of inhibition at 23, 25, 21, 20, 22, 24mm having the highest zone of inhibition to be 25m staphylococcus aureus. Table 3. Showed the zone of inhibition of the three extracts against the test organisms. The ethylacetate extract shows the more toxicity on the test organisms than the hexane and methanol extract. The extracts are rather bacteriostatic to the test organisms.

The result of the minimum inhibitory concentration of the ethylacetate extract revealed that the extract could inhibit the growth of *Escherichia coli*, *Helicobacter pyroli* and *Candida tropicalis* at a concentration of 5mg/ml, it could also inhibit the growth of vancomycin resistant *Enterococci* and *Shigella dysenteriae* at a concentration of 2.5mg/ml and could also inhibit the growth of *Staphylococcus aureus* at a concentration of 1.25mg/ml. The result of the minimum inhibitory concentration of the hexane extract revealed that the extracts could inhibit the growths of vancomycin resistant *enterococci*. *Staphylococcus aureus, Shigella dysenteriae*, and *Candida tropicalis* at a concentration of 5mg/ml, it could also inhibit the growth of *Escherichia coli* and *Helicobacter pyroli* at a concentration of 10mg/ml.

Conclusion: From the overall results of the analysis, the zone of inhibition produced by the ethylacetate and n-hexane extract on these organisms compared fairly well with the zones of inhibition produced by Ciprofloxacin and Sparfloxacin and Fluconazole. Which are regular drugs used internationally for the treatment of disease associated with these microbes. These therefore establish the ethnomedicinal claims.

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