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Full Length Research Paper

Preliminary phytochemical and antimicrobial screening of the leaf extract *Pilostigma reticulatum* (dc) Hochst

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Ethanolic and aqueous extracts of *Pilostigma reticulatum (dc)* hochst were screened for their antimicrobial activity against *Staphylococcus aureus, Streptococcus pyogen, Escherichia coli, Salmonella thypi* and *Shigella dysentery*. The results indicated that the extracts inhibited the growth of one or more test pathogens. The ethanolic extract showed a broad spectrum of antimicrobial activity. Phytochemical investigation revealed the presence of tannins, alkaloids, glycosides, flavonoids, carbohydrates and terpenes. The minimum inhibitory concentration (MIC) ranges from $8.0x10^2$ to $1x10^4$ µg/ml.

Key words: Medicinal plant, antimicrobial activity, phytochemical screening, *Pilostigma reticulatum*, pathogens.

INTRODUCTION

For the past two decades, there has been an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agents (Borjar and Farrokhi, 2004). Medicinal plants represent rich sources from which antimicrobial agents may be obtained. Plants are used medicinally in different countries and are sources of many potent and powerful drugs (Srivastva et al., 1996).

Pilostigma reticulatum (dc.) Hochst (family Caesalpineacea) is an African medicinal plant, widely used in the treatment of diseases and inflammatory condition (Burkill, 1995). The active principles of many drugs found in plants are secondary metabolites (Ghani, 1990; Doelis, 1993). Therefore, basic phytochemical investigation of its extracts for major phytoconstituents is also vital. In the present study, the water and ethanolic extracts of *P. reticulatum* were screened for phytochemical constituents and antimicrobial activity against *Staphylococcus aureus, Streptococcus pyogen, Escherichia coli, Salmonella typhi* and *Shigella dysentery*.

MATERIALS AND METHODS

Plants used for this study were collected from Maiduguri metropolis, Borno State, Nigeria. The plant materials were identified by Professor S. S. Sanusi of the Biological Science Department, University of Maiduguri and a Voucher specimen No. 46BA was deposited in the research laboratory of chemistry Department, University of Maiduguri.

Preparation of plant extracts

The plant material was dried at room temperature and then powdered using a grinder. The powdered sample (100 g) was subjected to soxhlet extraction using 300 ml of each of the solvents (water and ethanol). The resulting extracts were concentrated on a hot water bath and kept for further investigation.

Phytochemical screening

Phytochemical screening for major constituents was undertaken using standard qualitative methods. The extracts were screened for the presence of glycosides, alkaloids, tannins, flavonoids, saponins, anthraquinones and terpenes.

Test organisms

Standard strains of *S.* aureus*, S. pyogen*, *E. coli, S. typhi* and *S. dysentery* were obtained from the department of medical microbiology, university of Maiduguri teaching hospital, Maiduguri, Nigeria.

Antimicrobial screening test

The paper disc diffusion method was used to determine the

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Table 1. Phytochemical screening of *Pilostigma reticulatum* (dc) Hochst water and ethanol extracts.

	Water extract	Ethanol extract
Tannins	+	++
Carbohydrate	+	+
Alkaloids	+	+
Glycoside	+	+
Flavonoid	++	+++
Terpenes	+	+
Saponins	-	-
Anthraquinones	-	-

+++=High concentration; ++ = moderate concentration; + = low concentration; - = absent.

Table 2. Inhibition zone of *Pilostigma reticulatum* (dc) Hochst water extract against the tested micro organisms.

	Zones of inhibition (mm)				
Extract/drug (mg/ml)	***Staphylococcus aureus***	***Staphylococcus pyogen***	***E. coli***	***Shigella dysentery***	***Salmmetta typhi***
200	6	4	12	26	22
300	10	7	15	26	24
400	12	10	15	28	26
500	14	13	18	30	30
250 GTC	20	22	25	25	32

GTC = Gentamicin.

antimicrobial activity of the extract from *P. reticulatum* (dc) Hochst using standard procedures (Erickson et al., 1960; Bauer et al., 1996). Solutions of the extract of varying concentrations, ranging from 200 to 500 mg/ml were prepared. Nutrient agar was prepared, sterilized and used as the growth medium for the microorganisms. 20 ml of sterilized medium was poured into each sterilized petri-dish, covered and allowed to solidify. The Mueller-Hinton sensitivity agar plate was then seeded with the test microorganisms by the spread plate technique, and was left for about 30 min to dry. The sterilized paper discs were soaked in the prepared solution of the extracts with varying concentration and were dried at 50°C. The dried paper discs were then planted on the nutrient Agar seeded with the test microorganisms. The plates were incubated at 37°C for 24 h and then inspected for zones of inhibition of growth. The zones of inhibition were measured and recorded in millimeters. A control experiment was also set up using pure DMSO for each tested organisms.

Determination of minimum inhibitory concentration (MIC)

MIC of the ethanolic and aqueous extract of *P. reticulatum (*dc) hochst which showed the highest antibacterial activity in the disc diffusion assay were determined based on broth dilution technique with a standard method (Krivoshan et al., 1989). The inocula of micro organisms were prepared from 12 h broth cultures. Stock solutions of extracts (200 mg/ml) were diluted with nutrient broth cultures. Stock solutions of extracts (200 mg/ml) were diluted with nutrient broth in serial tenfold dilutions using nutrient broth to make dilution ranging from 200 mg/ml ($2x10^5$ µg/ml) to 0.2 mg/ml ($2x10^2$ µg/ml) and inoculated with 0.2 ml of the test microorganisms. The inoculated tubes were then incubated at 37°C for 24 h and were inspected for non-turbidity. The last concentration of the extract which prevented visible growth was noted and recorded as the minimum inhibitory concentration (MIC).

RESULT

The results of the phytochemical screening, antimicrobial tests and minimum inhibitory concentrations for the water and ethanol extracts are presented in Tables 1 to 5.

DISCUSSION

The phytochemical screening (Table 1) revealed the presence of Tannins, alkaloids, glycoside, flavonoid and terpenes. The chemical constituents present in the extract have many therapeutic values. Tannins are plant metabolites well known for their antimicrobial properties (Tsechesche, 1971). Flavonoids have both antifungal and antibacterial activities. They possess anti-inflamatory activity (Ogundaini, 2005; Iwu, 1984). Flavonoids, terpenes and steroids are known to have antimicrobial and bactericidal properties against several pathogens (Usman et al., 2007; Hassan et al., 2004).

In the antimicrobial studies, the majority of the organisms were more sensitive to the ethanol extract of *P. reticulatum* (dc) hochst. According to Trease and Evans (1978), the anti-bacterial activity and inhibitory

Table 3. Inhibition zone of *Pilostigma reticulatum* ethanol extract against the tested microorganisms.

Extract/drug (mg/ml)	*Staphylococcus aureus*	*Staphylococcus pyogen*	*E. coli*	*Shigella dysentery*	*Salmonella typhi*
200	15	4	17	19	17
300	15	4	17	20	15
400	15	8	19	22	19
500	17	10	21	24	20
250 (GTC)	26	25	25	27	23

GTC = Gentamicin.

Table 4. Minimum inhibitory concentration (MIC) of *Pilostigma reticulatum* (water extract) against the tested microorganisms.

Test organism	Concentration µg/ml				
	$8x10^2$	$2x10^3$	$3x10^3$	$6x10^3$	$1x10^4$
Staphylococcus aureus	-	-	-	+	+
Streptococcus pyogen	-	-	-	-	+
Escherichia coli	-	-	-	+	+
Shigella dysentery	-	+	+	+	+
Salmonella typhi	-	+	+	+	+

+++ = High concentration; ++ Moderate concentration; + = Low concentration; - = Absent.

Table 5. Minimum inhibitory concentration (MIC) of *Pilostigma reticulatum* (ethanol extract) against the tested microorganisms.

Test organism	Concentration µg/ml				
	$8x10^2$	$2x10^3$	$3x10^3$	$6x10^3$	$1x10^4$
Staphylococcus aureus	-	+	+	+	+
Streptococcus pyogen	-	-	-	-	+
Escherichia coli	-	+	+	+	+
Shigella dysentery	-	+	+	+	+
Salmonella typhi	-	-	-	+	+

+++ = High concentration; ++ Moderate Concentration; + = Low concentration; - = Absent.

effect of plant extracts may be due to the presence of secondary metabolites.

In Table 5, the ethanol extract of *P. reticulatum* (dc) Hochst was active against the entire microorganisms, *S. aureus, S. pyogen, E. coli, S. dysentery* and *S. typhi*. It has MIC value of $2x10^3$ µg/ml against *S. aureus*, *E. coli* and *S. dysentery,* $6x10^3$ µg/ml against *S. typhi* and $1x10^4$ µg/ml against *S. pyogen*.

These findings are consistent with Etuk et al. (2009) who reported that the bark extract of *P. reticulatum* had antidiarrhae activity *in vivo*. Previous report have demonstrated the antidiarrheae activity of tannins (Murkherjee et al., 1995), flavonoids (Galvez et al., 1993) and saponins (Otshudi et al., 2000).

Conclusion

The result of the experiment showed that the leaf of *P. reticulatum* may have some valuable anti-microbial activities against gram positive and gram negative microorganisms. This property tends to support the traditional medicinal stage in the treatment of bacterial infections. The result of the study justified the use of the plant in the treatment of diseases of microbial origin in herbal medicine.

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