



Science

ELEMENTAL AND MEDICINAL EVALUATION OF THE LEAF EXTRACT OF PILOSTIGMA RETICULATUM (DC) HOCHST

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Abstract

Elemental analysis, phytochemical screening and antimicrobial activities of aqueous and ethanolic leaf extract of *Pilostigma reticulatum (dc) Hochst* were studied using paper disc diffusion method against *Streptococcus pyogen*, *Escherichia coli* and *Salmonella thypi*. Elemental analysis of the plant sample revealed the presence of Ca ($1.51 \pm 0.01 \mu\text{g/g}$), Mg ($0.43 \pm 0.02 \mu\text{g/g}$), P ($0.29 \pm 0.01 \mu\text{g/g}$), Mn ($3.01 \pm 0.01 \mu\text{g/g}$), Fe ($1.04 \pm 0.01 \mu\text{g/g}$), Zn (1.05 ± 0.02) and Cu was below detectable limit(BDL). The results of the antimicrobial studies indicated that the extracts inhibited the growth of one or more tested pathogens. The ethanolic extract showed a broad spectrum of antimicrobial activity. Phytochemical investigation revealed the presence of tannins, alkaloids, glycosides, flavonoids, carbohydrates and terpenes. Anthraquinone and saponin were not present. Inhibition zone by the extract ranges from 4.0mm to 30mm. The minimum inhibitory concentration (MIC) ranges from $8.0 \times 10^2 \mu\text{g/ml}$ to $1 \times 10^4 \mu\text{g/ml}$. *Pilostigma reticulatum* leaf may be able to produce antimicrobial agents in drug delivery.

Keywords: Medicinal Plant; Antimicrobial Activity; Phytochemical Screening; *Pilostigma Reticulatum*.

Cite This Article: O. Adeyanju, S. V. Francis, R. R. Ede, P.S. Jang, and J.K. Bulus. (2019). "ELEMENTAL AND MEDICINAL EVALUATION OF THE LEAF EXTRACT OF PILOSTIGMA RETICULATUM (DC) HOCHST." *International Journal of Research - Granthaalayah*, 7(7), 391-397. <https://doi.org/10.29121/granthaalayah.v7.i7.2019.792>.

1. Introduction

Despite the great advances witnessed in modern medicine in recent decade, plants still make an important contribution to health care (Poojary et al., 2016). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on human body (Uahie et al., 2014) Medicinal plants are useful for healing as well as for curing human diseases because of the presence of phytochemical constituents in the plants. The most of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. (Anostro et al., 2000). Medicinal plants represent rich sources from which antimicrobial agents may be obtained. *Pitostigma reticulatum (dc) Hochst* (caesalpineacea) is an African medicinal plant, widely used in

the treatment of diseases and inflammatory condition. (Burkill, 1995). The local names are semellier in French, Kargo in Hausa, Ewe Abafin in Yoruba and Okpo atu in Igbo. (Awe and Omojasola, 2009). The active principles of many drugs found in plants are secondary metabolites as stated earlier, therefore basic phytochemical constituents is also vital. In the present study, Elemental analysis of the leaf was determined. Also Ethanolic and aqueous leaf extracts of *P. reticulatum* were screened for their phytochemical constituents and antimicrobial activities against streptococcus pyogen, Escherichia coli and Salmonella typhi respectively. The results of this present research is likely to highlight the elemental concentration and medicinal importance of the leaf of *P. reticulatum*.

2. Materials and Methods

Plant used for this study was 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, Nigeria.

2.1. Elemental Determination

Five grams (5g) of oven dried samples was weighed into a crucible. The crucible was then placed in a hot furnace and ashed at 600 °C for 3 h. The furnace was cooled to about 120 °C. the crucible was then removed and placed in a desiccator for 1 h to cool before weighing. The process was repeated until a constant weight was obtained. The ashed sample (0.5 g) was weighed and transferred into the digestion tube. 5ml each of distilled water, concentrated trioxonitrate (V) acid (HN03) and perchloric acid (HCIO4) were added and the content mixed. The tubes were placed into the digestion block inside a fume cupboard and the temperature

2.2. Preparation of Plants 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 300ml of each of the solvents (water and ethanol). The resulting extracts were concentrated on a hot water bath and kept for further investigation.

2.3. 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.

2.4. Test Organisms

Standard strain of *S.pyogen*, *E. Coli* and *Salmonella typhi* were obtained from the department of medical microbiology, university of Maiduguri teaching hospital, Maiduguri, Nigeria.

2.5. Antimicrobial Screening Test

The paper disc diffusion method was used to determine the 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. 20ml 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 minutes 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.

2.6. Determination of Minimum Inhibitory Concentration (MIC)

MIC of the ethanolic and aqueous extract of *P. reticulatum* (MIC) 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 microorganisms were prepared from 12h broth cultures. Stock solutions of extracts (200 mg/ml) were diluted with nutrient broth cultures. Stock solutions of extracts (200mg/ml) were diluted with nutrient broth in serial tenfold dilutions using nutrient broth to make dilution ranging from 200mg/ml ($2 \times 10^5 \mu\text{g/ml}$) to 0.2mg/ml ($2 \times 10^2 \mu\text{g/ml}$) 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 minimum inhibitory concentration (MIC).

3. Results

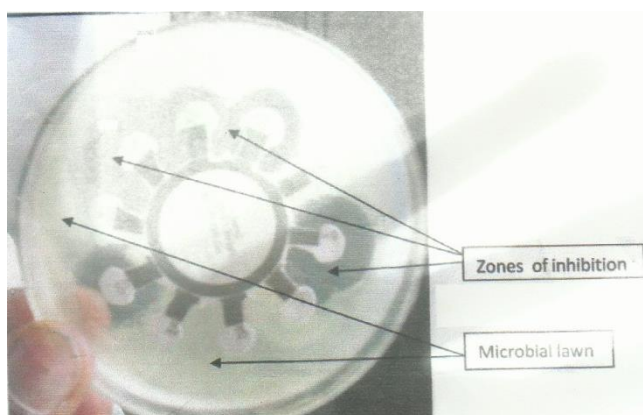


Figure 1: Inhibition zones and microbial Lawn



Figure 2: MIC Determination

The results of the elemental analysis, phytochemical screening, antimicrobial tests and minimum inhibitory concentrations for the water and ethanolic extracts are presented in Tables 1 to 6.

3.1. Elemental Analysis

The elemental analysis results in table 1 shows that calcium, (Ca), Magnesium (Mg), Iron (Fe), Zinc (Zn), Manganese (Mn) and Phosphorous (P) were present at moderate concentrations.

Table 1: Elemental analysis of the Leaf of *P.reticulatum*

Elements	Ca	Mg	Mn	Fe	Cu	Zn	P
Concentration (mg/g)	1.5±0.01	0.43±0.02	3.01±0.01	1.04±0.01	BDL	1.05±0.02	0.29±0.01

BDL=Below detectable limit; Results are means of triplicate determination ± standard deviation.

Table 2: Phytochemical screening of *Pilostigma reticulatum* (dc) hochst water and ethanolic leaf extracts.

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

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

Table 3: Inhibition zone of *Pilostigma reticulatum* (dc) hochst water extract against the tested micro organisms.

Zone of Inhibition (mm)			
Extract/drug (mg/ml)	<i>Streptococcus pyogen</i>	<i>E. coli</i>	<i>Salmonella typhi</i>
200	4±0.01	12±0.02	00±0.02
300	7±0.01	15±0.01	24±0.02
400	10±0.04	15±0.01	26±0.01
500	13±0.01	18±0.02	30±0.04
250 GTC	22±0.02	25±0.03	32±0.04

GTC = Gentamicin, Results are means of triplicate determination ± standard deviation.

Table 4: Inhibition zone of *Pilostigma reticulatum* (dc) ethanol extract against the tested microorganisms.

Extract/drug (mg/ml)	<i>Streptococcus pyogen</i>	<i>E. coli</i>	<i>Salmonella typhi</i>
200	4±0.00	17±0.03	17±0.02
300	4±0.04	17±0.01	15±0.01
400	8±0.00	19±0.01	19±0.04
500	10±0.02	21±0.02	20±0.01
250 GTC	25±0.01	25±0.01	23±0.02

GTC = Gentamicin, Results are means of triplicate determination ± standard deviation.

Table 5: Minimum inhibitory concentration (MIC) of *Pilostigma reticulatum* (dc) (water extract) against the tested microorganisms

Extract/drug (mg/ml)	Concentration $\mu\text{g/ml}$				
	8×10^2	2×10^3	3×10^3	6×10^3	1×10^4
<i>Streptococcus pyogen</i>	-	-	-	-	O+
<i>Escherichia coli</i>	-	-	-	O+	+
<i>Salmonella typhi</i>	-	O+	+	+	+

+ = inhibition, O+ = minimum inhibition, - = no inhibition

Table 6: Minimum inhibitory concentration (MIC) of *Pilostigma reticulatum* (dc) (ethanol extract) against the tested microorganism

Extract/drug (mg/ml)	Concentration $\mu\text{g/ml}$				
	8×10^2	2×10^3	3×10^3	6×10^3	1×10^4
<i>Streptococcus pyogen</i>	-	-	-	-	O+
<i>Escherichia coli</i>	-	O+	+	+	+
<i>Salmonella typhi</i>	-	-	+	O+	+

+ = inhibition, O+ = minimum inhibition, - = no inhibition

4. Discussion

The elemental analysis results (table 1) shows that the presence of calcium, magnesium, Iron, zinc, manganese and phosphorus at different concentration. The concentration of the essential elements appear to be lower and within the safety limit according to W.H.O. (1996). The low concentration of Iron (Fe), Zinc (Zn) and absence of Copper (Cu) is an indication of little or no toxicity of plants as heavy metals are known to cause cancer, liver and kidney problems. (Ogugbuaja, et., al., 1997).

The phytochemical screening (Table 2) revealed presence of Tannins, alkaloids, glycoside, flavonoid terpenes. The chemical constituents present in extract have many therapeutic values. Tannins are metabolites well known for their antimicrobial properties (Tsechesche, 1971). Flavonoids have both antifungal and antibacterial activities. They possess anti-inflammatory activity (Ogundaini, 2005) 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 leaf extract of *P. reticulatum* (dc) hochst. According to Trease and Evans (1978), the anti-bacterial activity and inhibitory effect of plant extracts may be due to the presence of secondary metabolites.

In Table 6, the ethanol extract of *P. reticulatum* (dc) hochst was active against the entire microorganisms. *S. Pyogen*, *E. coli* and *S. typhi*. It has MIC value of $2 \times 10^3 \mu\text{g/ml}$ against *E. coli* and $6 \times 10^3 \mu\text{g/ml}$ against *S. typhi* and $1 \times 10^4 \mu\text{g/ml}$ *S. pyogen*. These findings are consistent with Etuk et. al. (2009; who reported that the bark extract of *P. reticulatum* had antidiarrhoe activity in vivo. Previous reports have demonstrated the antidiarrhoe activity of tannins (Murkherjee et. al., 1995), flavonoids (Galvez et al., 1993; and saponins (Otshudi et al., 2000).

5. Conclusion

The result of the experiment showed that the *P. reticulatum* leaves 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 herbal medicine.

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