

A Comparative Study of the Antibacterial Activity of *Piliostigma reticulatum* Bark Extract with Some Antibiotics

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Abstract

Aqueous and ethanolic extracts of *Piliostigma reticulatum*, a herb widely used for the treatment of diarrhea in southwest Nigeria, was challenged with clinical isolates of *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The ethanol extract exhibited the highest activity against the test organisms with zones of inhibition ranging from 16.0 – 20.0 mm, followed by the hot aqueous extract and the cold aqueous extracts, with zones of inhibition ranging between 8.0 – 10.0 mm and 4.0 – 7.0 mm respectively. The Minimum Inhibitory Concentration (MIC) ranged between 0.025 – 0.25% w/v and photochemical analysis showed that the plant possesses saponins, tanins, phenolics, phlobatinins and glycosides. A comparative analysis of the extract with some conventional antibiotics namely; Chloramphenicol, Ampicillin and Tetracycline showed that there was significant difference in the antibacterial activity of the bark extract and the antibiotic standard ($P < 0.05$).

Key words: Antimicrobial activity, Minimum Inhibitory Concentration, Antibiotics, Phytochemical and *Piliostigma reticulatum*.

Introduction

Herbal medicine has been shown to be effective and about 60% of rural populations depend on it for their primary health care (WHO, 1988; Akinyemi *et al.*, 2000). This could be attributed to affordability, accessibility, in the economic sense and socially, an uneven distribution of health personnel between rural and urban areas. Traditional medical practitioners in Nigeria use a variety of herbal preparations to treat different kinds of ailments such as typhoid and paratyphoid fevers, dysentery, malaria, diarrhea, etc. Various research works conducted in recent years have shown that pathogenic microorganisms are becoming increasingly resistant to existing antibiotics at alarming rates (Rotimi and Mosadimi, 1984; Spencer *et al.*, 1986; Olayemi and Oyagade, 1987; Monetiore *et al.*, 1993, Olukoya *et al.*, 1993). Such high wave of antibiotic resistance among pathogenic microorganisms creates problems in health care delivery in relation to microbial infections in man and animals.

Piliostigma reticulatum (DL.) Hochst. (common name; Yoruba: 'abafin', Hausa: 'kalgo', Igbo: okpo atu') belongs to the family Leguminosae - Caesalpiniaceae and is found in the savannah region of Nigeria. It is a tree, occurring up to 30ft in height with an evergreen, dense spreading crown (Keay, 1989).

In recent past, attention has been directed towards medicinal research to substantiate the claims of cure made by traditional healers and thus provide scientific basis for their efficacy. Several important drugs have been discovered in plants and are now synthesized chemically for commercial purposes. However, the search for new ones continues especially in the tropical plants. Akinsinde and Olukoya, (1995) demonstrated activity of *Terminalia avicennoides* against *Vibrio cholerae* and *Salmonella typhi*, Omojasola and Awe (2004) reported the antimicrobial activity of the leaf extract of *Anacardium occidentale* and *Gossypium hirsutum* against *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

In Nigeria, the research for new drugs is on course hence the need to investigate the pharmacological potentials of *Piliostigma reticulatum* used traditionally in the treatment of diarrhea.

Materials and Methods

Sample Collection

Sun dried bark of the plant was purchased from the trade-medical section of the Ilorin market. It was identified and authenticated at the Department of Plant Biology, University of Ilorin.

Extraction

Cold aqueous, hot aqueous and ethanolic extractions of the plant materials were prepared as described by Oyagade

et al. (1999). A preliminary test had shown that the aqueous extract showed greater activity at 80°C than 28°C. So the aqueous extraction was done at 80°C in water bath for 1½ hours. The extracts were decanted and filtered through Whatman No. 1 filter paper. The filtered extract was sterilized using 0.45µm pore size membrane filter and evaporated to dryness at 45°C. The residues obtained were reconstituted in 95% ethanol as stock concentration of 250mg/ml.

Test Organisms

Clinical isolates of *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were collected from the University of Ilorin Teaching Hospital, Ilorin, Nigeria. All inocula were standardized using the method described by Bauer *et al.* (1966).

Antibacterial assay

The well method of the agar dilution was used to determine the antibacterial activity of the plant extracts and the antibiotics. The bacterial cultures were inoculated on Mueller Hinton Agar (MHA) and were incubated at 37°C for 24 hours. The MIC for the plant extracts was determined using the method of Mann *et al.*, 1997 and Oyagade *et al.*, 1999).

Phytochemical screening of ethanolic extracts

The extract was subjected to phytochemical screening for the presence of alkaloids, tannins, phenolics, saponins, steroids, triterpenes, phlobatinin and cardiac glycosides using the method described by Odebiyi and Sofowora, (1978).

Results and Discussion

The antibacterial activity of the cold aqueous, hot aqueous and ethanolic extracts of *P. reticulatum* show that the ethanolic extract exhibited greater activity than both the hot and cold aqueous extracts (Table 1). The higher activity of the ethanolic extracts may not be unconnected with the extraction solvent because ethanol has been shown to be better solvent than water (Akinyemi *et al.*, 2000). This may also account for the reason why the herbal physicians prefer to use local alcoholic decoctions in extracting the herbs rather than aqueous extracts in treating their patients. The ethanolic extract was strongly positive for the presence of alkaloids, tannins and phenolics and positive for the presence of triterpenes and phlobatinins (Table 2). The presence of these phytochemicals is indicative of antibacterial activity (Odebiyi and Sofowora, 1978).

The potential of any drug is the active principle contained therein; many of these secondary metabolites have prominent effect on animal systems and microbial cells (Jung *et al.*, 1990; Triguna *et al.*, 1992 and Fatoba *et al.*, 2003). The presence of these metabolites therefore confers pharmacological potentials on this plant. Other workers have also reported bioinsecticidal and antitrypanocidal properties of *P. reticulatum* extract (Atawodi *et al.*, 2003, Abdullahi and Muhammed, 2004).

The cold water extract had least inhibitory effect on all the organisms used. The MIC ranged from 0.025 – 0.25 % w/v with *P. aeruginosa* having the highest value of 0.25% w/v (Figure .1). In the comparison of the antibacterial activity of *P. reticulatum* with some conventional antibiotics, the results indicate that the conventional antibiotics (Chloramphenicol, Ampicillin and Tetracycline) demonstrated greater activity than the plant extract at the same concentration (Fig. 2). The results also show that these differences are significant at 5% significance level. The fact that the plant extract is only a crude extract may account for some of these differences in activity. Higher levels of antibacterial activity are expected from a purified sample of *P. reticulatum* whose active principle has been isolated. The comparison of the activity of the plant extract with conventional antibiotics confirmed reports by other workers. Emeruwa (1982) reported that conventional antibiotics are more active than plant extracts. These results thus confirm the basis of traditional use of *P. reticulatum* in diarrhea treatment. Further study needs to be carried out on the isolation of the bioactive components of the tested plant and their effect *in vivo*.

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Table 1. Antimicrobial activity of the bark extract of *Piliostigma reticulatum*.

Test Organism	Cold aqueous extract	Hot aqueous extract	Ethanolic extract	Control	
				95% Ethanol	Distilled water
Diameter of Zone of inhibition (mm)					
<i>S. aureus</i>	4.00 ± 0.01	10.00 ± 0.02	16.00 ± 0.00	0.00	0.00
<i>Escherichia coli</i>	6.00 ± 0.00	8.00 ± 0.00	18.00 ± 0.01	0.00	0.00
<i>Ps. aeruginosa</i>	5.00 ± 0.02	9.00 ± 0.01	12.00 ± 0.02	0.00	0.00
<i>Shigella dysenteriae</i>	7.00 ± 0.01	10.00 ± 0.01	20.00 ± 0.02	0.00	0.00
<i>Salmonella typhimurium</i>	6.00 ± 0.00	8.00 ± 0.02	16.00 ± 0.00	0.00	0.00

Concentration used 250mg/ml
Each value is a mean of 2 replicates ± S.D
Values along the row are significantly different. (P<0.05)

Table 2. Phytochemical Screening of bark extract of *P. reticulatum*.

Chemical Constituents	<i>Piliostigma reticulatum</i>
Alkaloids	++
Tannins	++
Phenolics	++
Saponins	-

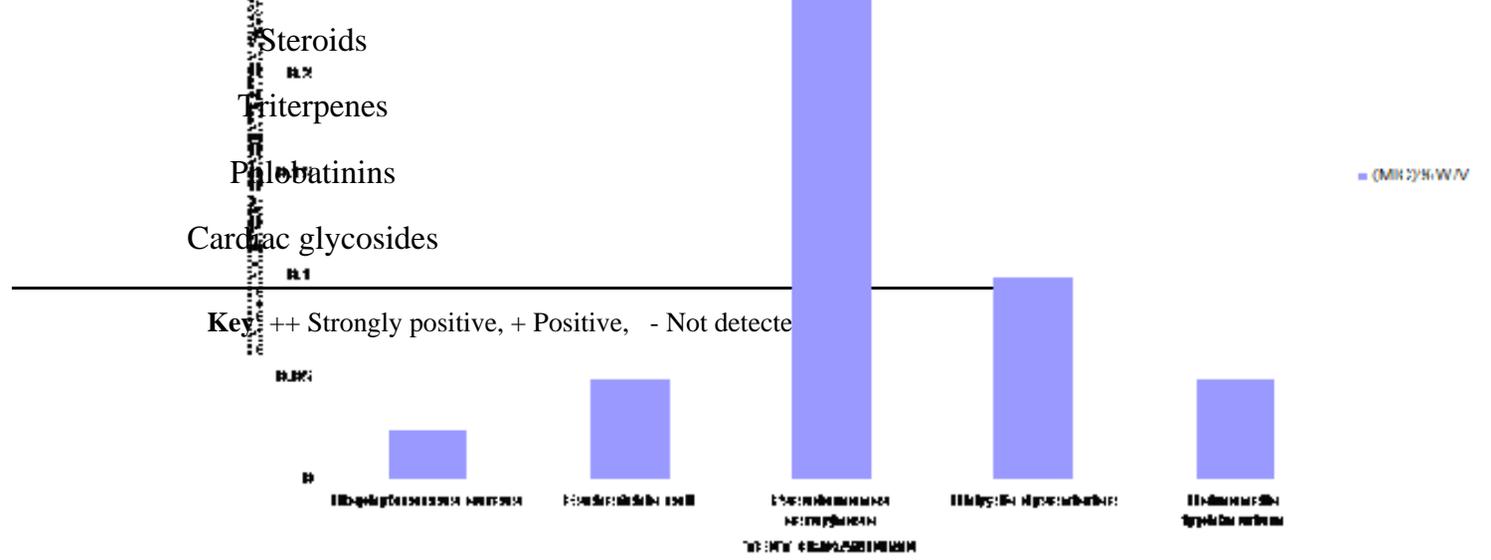
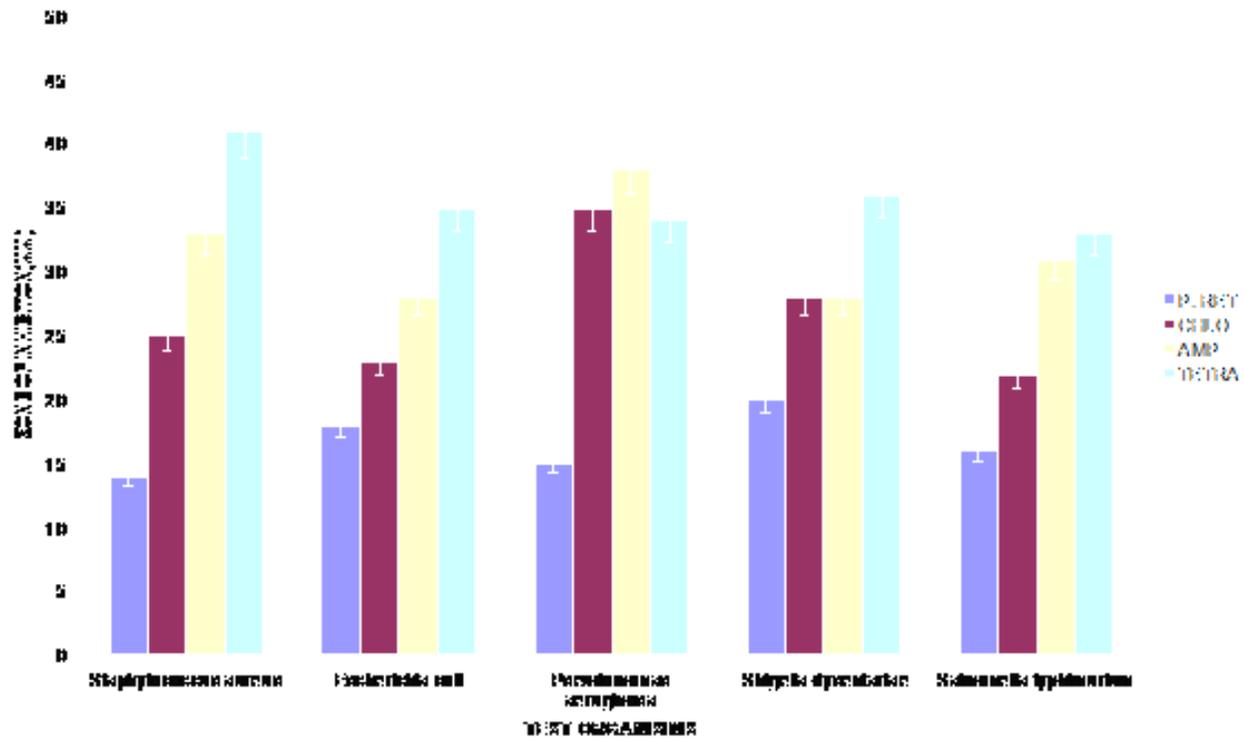


Figure 1. Minimum Inhibitory Concentration (MIC) of Ethanolic extract.



Key: P.RET - *P. reticulatum*, CHLO-Chloramphenicol, AMP- Ampicillin, TETRA- Tetracycline.

Fig .2 Antibacterial activity of ethanolic extract compared with the conventional antibiotics.