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ANTIMICROBIAL ACTIVITY OF *PILIOSTIGMA RETICULATUM* ON SOME PATHOGENIC BACTERIA

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ABSTRACT

The leaves and root of *Poliostigma reticulatum* were tested for their antimicrobial activity against some selected pathogens viz. *Salmonella typhii*, *E. coli*, *Staphylococcus aureus* and *Shigella spp* at a concentration of 60 mg/ml using agar diffusion method. Results show that extract inhibited the growth of these organisms with the diameter of zone of inhibitions ranging between 2.0 to 14.0mm. Result show that leaf extract has greater activity than the root extract of the plant. The minimum inhibitory concentration (MIC) of the extracts range from 15.0 to 60.0 mg/ml. The results suggest that *piliostigma reticulatum* could be used in treatment of disease caused by these pathogens.

KEYWORDS: *Piliostigma reticulatum, Salmonella, Shigella spp* and *Staphylococcus aureus, etc.*

INTRODUCTION

Plants have been one of man's major source of complex chemicals which interfere with biological processes until the 19th century when synthetic organic compound came up.[1] These complex phytochemicals have been found to possess therapeutic properties. The plants with one or more of its organs containing substances that can be used for therapeutic purpose are called medicinal plants.

Traditional medicine is a blending together of dynamic medical knowledge solidly founded on ancestral experience.[2] Plants based drugs have been used World Wide in traditional

medicine for the treatment of various diseases.[3] About three - quarter (3/4) of the world population still rely on medicinal plants for their primary health care.[4]

In Africa and other developing countries of the world, a large number of people die daily of preventable or curable diseases because of the absence of simple health care, the situation is further aggravated by the perpetual state of poverty and the continued rising trend of population.

Modern pharmaceutical cannot solve these problems effectively because some drugs have unwanted side effects and if the drug is available for a long time for therapeutic use it can even cause emergence of adulteration, drug resistance among microorganism which reduces their effectiveness.

Various parts of the *Piliostigma reticulatum* (camel foot leaves) have been used in African traditional medicine for the treatment of a wide range of disease conditions such as leprosy, ulcers, dysentery, cancer, malaria, headache and other bacterial disease. It is also based on preliminary research reported by [5,6,7] that has antibacterial and anti helminthic activity. Therefore in this research we have evaluated the antimicrobial activity of *Piliostigma reticulatum* on some selected gram positive and gram negative bacteria to see whether or not preparations from *Piliostigma reticulatum* are suitable for the treatment of bacterial diseases.

MATERIALS AND METHODS

Study Area

This study has been conducted at microbiology research laboratory of the Department of microbiology and biochemistry laboratory of the Department of biochemistry, Usmanu Danfodiyo University Sokoto.

Collection of Plant Material

Fresh roots and leaves of *Piliostigma reticulatum* were collected from Madaddabai village of Batsari Local Government, Katsina state, and identified and confirmed by a senior plant taxonomist of Biological Sciences Department, Usmanu Danfidiyo University, Sokoto.

Processing of Plant Materials

The identified root and leaves of *Piliostigma reticulatum* were washed and air-dried. The dried samples were milled into fine powder by pounding manually with a clean and sterile mortar.

The powder was collected into sterile cellophane bags and labeled. The samples were kept in a cool dry place till further use.

Extraction of Plant Materials

Methanol Extract

Forty grams (40g) powder of each root and leaf was weighted. 400ml of methanol was used as a solvent using shoxlet extractor model. The process was run for 6 hours after which the sample was evaporated to dryness using steam evaporator. The dried extract were weighed and kept in a well labeled sterile sample bottles. Methanol was used because it is a polar solvent.

Aqueous Extract

Forty grams (40g) powder of each root and leaf was weighted. 400ml of distilled water on was added to 40g sample for the aqueous extract using shoxlet extractors model. The process was run for 6 hours after which the sample was evaporated to dryness using steam evaporated. The dried extract were weighed and kept in well sterile sample bottles. Distilled water use because it is a universal solvent.

Acetone Extract

Forty grams (40g) powder of each root and leaf was weight and mixed with 400ml of acetone that was used as solvent using shoxlet extractor model. The process was run for 6 hours after which the sample was evaporated to dryness using steak, evaporator. The dried extract were weighed and kept in well labeled sterile sample bottles.

Screening of Test Organisms

Clinical samples of the organisms were collected from Usmanu Danfodiyo university teaching hospital (UDUTH) which include *Escherchia coli, streptococci pyogens, salmonella Typhi, Staphylococcus, Shigella,* the bacterial isolate were culture on nutrient agar and incubated at 37^0c for 24 hours. The microorganism were repeatedly sub cultured in order to obtain pure culture. Gram stain reactions and after biochemical were carried out for proper confirmation. They were inoculated into nutrient agar slants and stored at 4^0c until further use.

Standardization of Test Organisms

A loop full of test organism was inoculate in nutrient broth and incubated for 24hours. 0.2ml from the 24hourcs culture of the organism was dispensed into sterile nutrients broth and incubated for 3-5hours to standardize the culture to $10^{6cfu/cm}$.

Screening of Extract for Antimicrobial Activity

The agar well diffusion method was used. Sterile nutrient agar were prepared. In 1ml of test organism was added to 9ml of the nutrients agar, each plate was properly labeled. A sterile cork borer (4mm) was used to make wells in each plate for the extracts. The base of each well was filled with sterile molten nutrient agar to seal the bottom and left for some time to allow it to gel. 0.2ml of the extract was dispensed into each well. The plates were left to allow diffusion of the extracts before being placed in the incubator at 37^0c for 24 hours. The zones of inhibition produce after incubation was measured and recorded.

Determination Minimum Inhibitory

Concentration (Mic)

The minimum inhibitory concentration (MIC) was determined as the least concentration that showed an inhibitory effect on any of the test microorganism using the tube Method.[8] Tow – fold serial dilutions were made using nutrient broth one ml (1ml) of a solution of the test compound (plant extract of 0.3g/5ml) was added aseptically to one ml (1ml) of double strength medium (nutrient broth) and mixed by shaking. Using a fresh pipette are ml (1ml) of the mixture was transferred to test tube two (2) which contained one ml (1ml) single strength medium. This too, was mixed by shaking and from it one ml (1m) was taken into test tube three (3) aseptically and mixed by shaking. This procedure was repeated up to test tube six (6) and one ml (1ml) from the same test tube six (6) was discarded after shaking. The seven (7^{th}) test tube contained no test compound being the control. Finally, to each test tube was added zero point one ml (0.1ml) incolumns of the test organism aseptically. The test tubes were covered with aluminum foil immediately and incubate at 37^0 for 24 hours and then observe for turbidity.

The lowest concentration that inhibited growth of test organism was note as the MIC.

RESULTS

Antimicrobial Activity of The Crude Extracts of *Piliostigma reticulatum*

The result of the antimicrobial activity of the crude extract of leaf and root of *Piliostigma reticulatum* on test organisms showed that the aqueous, and methanol leaf extract inhibited

two (*staphylococcus aureus* and *Shigella spp* out of four tested bacteria. Extract inhibited (*Salmonella, shigella spp* and *Staphylococcus aureus*) out of four tested bacteria. It was observed that the aqueous leaf extract showed wide zones of inhibition than acetone leafextract, methanol leaf extract and aqueous root extracts.

The aqueous root extract inhibited only (*Staphylococcus aureus*) out of four tested bacteria.

Minimum Inhibitory Concentration of Crude Extract of Leaf and Root of *Piliostigma reticulatum*

The result obtained for the minimum inhibitory concentration (MIC) for both leaf and root of aqueous, acetone and methanonic extract of *Piliostigma reticulatum* are represented in table 3,4 and 5 respectively. The aqueous leaf extract had MIC of 15mg/ml for both *staph. aureus* and *Shigella spp* and aqueous root extract had MIC of 30mg/ml for *staph. aureus*.

The MIC for the methanolic leaf extract on the test bacteria were 15 and 30 mg/ml for *Staph. aureus* and *Shigella spp* respectively. The MIC for the acetone leaf extract on the test bacteria were 60 mg/ml for both *Staph. aureus* and *Shigella* spp and 15 mg/ml for *S. typhii*.

Table 1: Antibacterial activity of crude extract of *Piliostigma reticulatum*.

Scientific name	Part of plant use	Mode of extraction		Concentration mg/ml		
				S. typhii	*E.coli*	*Shigella sp*
Pi. Reticulatum	Leaf	Methanol acetone aquesous control	60mg/ml “ “ “	0mm 2mm 0mm 2mm	0mm 0mm 0mm 4mm	11mm 12mm 9mm 0mm
	Root	Methanol Acetone Aqueous Control	60mg/ml “ “ “	0mm 0mm 0mm 0mm	0mm 0mm 0mm 0mm	0mm 0mm 0mm 4mm

Table 2: Minimum inhibitory concentration of the aqueous leaf and root extract of *Piliostigma reticulatum*.

Test organism	Concentration of extract mg/ml						Leaf	Root
	60	30	15	7.5	3.37	1.875	MICmg/ml	MIC mg/ml
1 *Staph. Aureus*	-	-	+	+	+	+		30
2 *S. typhii*	-	-	-	+	+	+	15	
3 *Shigella spp*	-	-	-	+	+	+	15	

Table 3-Minimum inhibitory concentration of the methanolic leaf extract of *Piliostigma reticulatum*

Test organism	Concentration of extract mg/ml						Leaf
	60	30	15	7.5	3.75	1.875	MICmg/ml
Shigella spp	-	-	+	+	+	+	30
Staph. Aureus	-	-	-	+	+	+	15

Table 4- Minimum inhibitory concentration of leaf extract of *Piliostigma reticulatum* using acetone

Test organism	Concentration of extract mg/ml						Leaf
	60	30	15	7.5	3.75	1.875	MICmg/ml
Shigella spp	-	+	+	+	+	+	60
S. typhii	-	-	-	+	+	+	15
Staph aureus	-	+	+	+	+	+	60

Key

- = no growth

+ = growth

DISCUSSION

It is clear from result that the extracts had varying degree of antimicrobial activity against the tested organisms. The leaf aqueous extract had the highest activity with zone of inhibitions ranges between (9-14mm) as compared to that of the root (0-11mm). The methanol extracts of the leaf had zone of inhibition of (5-11mm) and no zone of inhibition in root was observed. The acetone extracts of leaf had zone of inhibition ranges between (2-12mm) and no inhibition in root. Inhibition was due to the presence of active phytochemical compounds alkaloids as reported by [7] that they had inhibitory effects on *E.coli* and *Bacillus subtilis.*

The minimum inhibition concentration (MIC) values of both aqueous (leaf and root), methanolic leaf, and acetone leaf extracts varied with the bacteria tested. The minimum inhibitory concentration has a range between (15-30mg/ml) which confirms the study by [6], who found that at 20mg/ml. the crude extracts of *piliostigma reticulatum* inhibits *S. aureus, E. coli,Bacillus, Substilis, S. dysentriea* and *Proteis vulgaris.*

The preliminary phytochemical screening of the leaves and root crude extract of *Piliostigma reticulatum* revealed the presence of alkanoids, carbohydrates, flavonoids, tannins, steroids. Glycosides were not present in the root and leaf (acetone an aqueous crude extract) but present in the root and leaf of methanol crude extract. Balsams were not present in the leaf (Methenolan acetone crude extracts) but present in the root methanol crude extract. Balsams

were not present in the root methanol crude extract but present in the root (acetone an aqueous crude extracts). The presence of these phytochemicals agrees with the work of [7] who also confirm their presence in the plant. The presence of these compounds or some of them like, flavonoids, glycosides may be responsible for its activity against pathogenic bacteria.

CONCLUSION

It can be said conclusively that *Piliostigma reticulatum* can be used to treat the infectious diseases caused by studied bacteria, but care should be taken in excess use of it, because effects of phytochemicals are species specific on organisms.

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