Phytochemical Screening and Antimicrobial Activities of *Piliostigma Thonningii* Ethanol Stem Bark Extract

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Abstract

The research was carried out to determine the phytochemical screening and antimicrobial activities of ethanol stem bark extract of P. thonningii plant. Preliminary phytochemical screening revealed the presence of alkaloids, triterpenes, saponins, phenols and quinones while anthraquinones steroids tannins, flavonoids and were absent. The antibacterial activity was determined using the Kirby Baur Disc Diffusion Method. the plant extract at 15µg, 20µg, 25µg and 30µg concentrations and Augmentin 30µg as control in 2ml saline was subjected to five different organism isolates which are Klebsiella. spp, P. mirabilis, E. coli, S. aureus and S. pyogenes. The results reveals that Augmentin 30µg/2ml and P. thonningii ethanol stem bark extract at 15µg/2ml, 20µg/2ml, 25µg/2ml and 30µg/2ml were ineffective against P. mirabilis, E. coli and S. pyogenes. The extract was ineffective against Klebsiella. spp at all the concentrations while Augumentin 30µg/2ml shows 5mm zone of inhibition. The zones of inhibition for the plant ethanol stem bark extract against S. aureus at 15µg/2ml, 20µg/2ml, 25µg/2ml and 30µg/2ml are 12mm, 14mm, 18mm, and 20mm respectively, while for Augmentin 30µg/2ml is 8mm. The highest zone of inhibition was found in the plant extract to be 20mm at the highest concentration of 30µg/2ml. The extract phytochemical constituent presence, and the ability to inhibit the growth of the test bacteria is an indication of its antimicrobial potency employed in the traditional treatment of microbial infections and degenerative diseases.

Keywords

Medicinal Plants Phytochemical, Antimicrobial, Anthraquinones

1. Introduction

For a long time, people have used plants to cure common illnesses and some of these old medicines are still used today as part of regular treatment for different diseases. Medicinal plants organs contain substances that can be useful for therapeutic purposes or which are precursors for the synthesis of useful drugs Natural products obtained from plant sources remain a major source of preventive and curative medicine They are the foundation for modern therapeutic agents, they contain parts or other plant material as active ingredients. The plant material includes seeds, berries, roots, leaves, and barks [1].

Medicinal plants are mostly used for the wide range of constituents present, which have been used to treat chronic as well as infectious diseases. They are rich sources of obtaining variety of drugs, a major part of traditional therapeutic involves the use of plant extracts or their active constituents.

In Nigeria the plant is found growing freely as wild uncultivated tree in many locations such as Ogun, Zaria, Bauchi, Kwara, Lagos and some parts of Kogi, Benue and Nassarawa States. *Piliostigma thonningii* is also known as Camel's foot or Monkey bread. In different Nigerian languages its names include *Abefe* in Yoruba, *Kargo or kalgo* in Hausa, *Okpoatu* in Igbo, *Nyihar* in Tiv and *Ejei - jei* in Igala [2].

2. Pharmacological Activity

African traditional medicine has used different parts of P. *Thonningii* to treat many different health problems and diseases. It is used for treating malaria, leprosy, ulcers, fever, wounds, arthritis, dizziness, coughs, dementia, and memory improvement. It is also used for stomach and heart pain, gum disease, and as a fever reducer. Traditional healers in Doila use it for children to treat headaches, hemorrhoids, and back pain, among other things. *Piliostigma thonningii* is used to manage fever, cough, wounds, and various types of ulcers. The bark is chewed to help with coughs, and the pods are used to dress wounds [3]. The leaves and bark are used in teas or chewed to relieve chest pain, stomach issues, diarrhea, and dysentery. The roots and twigs of P. *Thonningii* are used locally to treat dysentery, fever, respiratory problems, snake bites, hookworm, and skin infections. The leaves are also used for treating malaria fever. In Uganda, P. *Thonningii* is commonly used to stop diarrhea, dysentery, and stomach problems. It is also used in tea form to treat malaria and leprosy. The bark is also used as a pain reliever for sore throat, toothache, stomachache, and earache.

The leaves are edible and are chewed by the Masai people in East Africa to relieve thirst [4]. A tea made from the leaves acts as a laxative and is given to children. The tea is also given to newborn babies as a tonic and is used to massage mothers' bellies. The leaves, after soaking in hot water, are applied directly to wounds. In the southwest part of Africa, a tea made from the leaves is used to treat surgical wounds.

3. Sample Collection

Fresh *Piliostigma thonningii* stem bark were collected from Jakusko Local Government Area of Yobe State Nigeria.

3.1 Processing of the Plant Materials

The Fresh stem barks of the plant *Piliostigma thonningii* was washed, cut into small portions, and dried at room temperature for one month. The dried plant stem barks were grounded using pestle and mortar, and the powdered was kept at room temperature away from direct sunlight [5].

3.2 Solvent Extraction of the Plant Materials

The powdered sample (435g) was macerated using Ethanol (1800ml) for 7 days with occasional agitation. The solvent extract was filtered using a Whatman filter paper No.1.

3.3 Qualitative Phytochemicals Screening

The Qualitative phytochemical screening was conducted using standard phytochemical analysis procedures.

3.4 Test for Alkaloids

The extracts ($2cm^3$) was taken into a test tube, $0.2cm^3$ of dilute HCl was added, followed by $1cm^3$ of Dragendroff's reagent. Orange yellow colour was observed indicating the presence of alkaloids.

3.5 Test for Flavonoids

The plant extracts, which were 0.5 grams, were heated along with 10 cubic centimeters of ethyl acetate using a steam bath for 3 minutes [6]. The mixture was then filtered, and 4 cubic centimeters of the filtered liquid were mixed with 1 cubic centimeter of dilute ammonia solution. A yellow color appeared, showing that flavonoids were present.

3.6 Test for Saponins

The extract ($1cm^3$) was taken in a test tube and added $20cm^3$ of Distilled water. It was shaken with hand for 15 minutes. Persistent foam at the top of the test tube was observed indicating the presence of saponins.

3.7 Test for Tannins

$0.5cm^3$ of the stock solution was dissolved in 5ml of chloroform and 1ml acetic anhydride was added. Finally, concentrated H_2SO_4 ($1cm^3$) was added carefully to the solution by the side of the test tube. A green color was observed indicating the presence of tannins [7].

3.8 Test for Steroids

The plant crude ethanol extract (1g) was taken in a test tube and dissolved with $10cm^3$ chloroform, $1cm^3$ of concentrated H_2SO_4 was added to the test tube by the sides. Formation of red colour at the upper layer and yellow with green fluorescence in the acid layer was observed indicating the presence of steroids [8].

3.9 Test for Anthraquinones

The crude ethanol extract, which was 0.5 grams, was placed into a dry test tube. Then, 5 cubic centimeters of chloroform were added, and the mixture was shaken for 5 minutes [9]. After that, it was filtered. The filtrate was then mixed with 5 cubic centimeters of a 10% ammonia solution and shaken again. A pink violet or red color appeared in the lower layer, showing that anthraquinones were present.

3.10 Test for Triterpenoids

The dry crude ethanol extract, which was 5mg in amount, was dissolved in $2cm^3$ of chloroform. Then, 1ml of acetic anhydride was added to the mixture [10]. After that, $1cm^3$ of concentrated sulfuric acid was added to the solution. A reddish violet color appeared, which shows that triterpenoids are present.

3.11 Test for Phenols (Ferric Chloride Test)

To 1ml of extract 2ml of distilled water were added followed by few drops of 10% ferric chloride ($FeC1_3$). Appearance of blue or green colour indicates presence of phenols.

3.12 Test for Quinones

To 1ml of extract, 1ml of concentrated Sulphuric acid (H_2SO_4) was added formation of red colour shows a positive result.

3.13 Antimicrobial Test

Pure bacterial cultures were obtained by sub culturing them into another culture media of 5% chocolate agar and CLED agar. The discs were dried and sterilized at 37°C for 30minutes using hot air oven [11]. Each of the Muller Hinton agar was inoculated with 4 to 5 colonies of *Klebsiella Spp*, *Staphylococcus aureus*, *Proteus mirabilis*, *Streptococcus pyrogenes* and *Escherichia coli*. The inoculated plates were incubated in thermostat incubator at 37°C for 18 to 24 hours.

3.14 Antimicrobial Susceptibility Testing

The plant extracts were diluted with 2ml normal saline in four different concentrations of 15ug, 20ug, 25ug and 30ug [12]. The concentrations diluted were dispersed to each of the prepared antimicrobial disc and 30ug of Augumentin was used as control.

3.15 Antimicrobial Susceptibility Testing Using the Bacterial Isolates

The prepared prepare cultured bacteria were tested using the following procedures.

Escherichia coli test:

Each of the Muller Hinton Agar was seeded with the 5 to 6 colonies of E. Coli using streaking method and the prepared antimicrobials disc (of the extract 15µg, 20µg, 25µg and 30µg, Augmentin 30µg) were placed gently using sterile forcep.

Staphylococcus aureus test:

Muller Hinton Agar was inoculated with 5 to 6 colonies of S. aureus using streaking method and the prepared antimicrobials disc (of the extract 15µg, 20µg, 25µg and 30µg, Augmentin 30µg) were placed gently using sterile forcep.

Proteus mirabilis test:

Each of the Muller Hinton Agar was inoculated with 4 to 5 colonies of P. mirabilis using streaking method and the prepared antimicrobials disc (of the extract 15µg, 20µg, 25µg and 30µg, Augmentin 30µg) were placed gently using sterile forcep [13].

Klebsiella spp test:

Muller Hinton Agar culture plate was seeded with 4 to 5 colonies of Klebsiella spp using streaking method and the prepared antimicrobials disc (of the extract 15µg, 20µg, 25µg and 30µg, Augmentin 30µg) were placed gently using sterile forcep.

Streptococcus pyogenes test:

The culture plate Muller Hinton Agar was inoculated with 5 to 6 colonies of S. Pyogenes using streaking method and the prepared antimicrobials disc (of the extract 15µg, 20µg, 25µg and 30µg, Augmentin 30µg) were placed gently using sterile forcep.

3.16 Determination of Antimicrobial Analysis Results

All the inoculated plates were incubated in thermostat incubator at 37°C for 18 to 24 hours. The results were read by measuring the zones of inhibition of the plates using a ruler [14].

4. Results

4.1 Phytochemical Screening Ethanol Stem Bark Extracts

The phytochemical analysis carried out on the stem bark extracts of *P. thonningii* for the following secondary metabolites: alkaloids, flavonoids, saponins, tannins, steroids, anthraquinones, triterpenoids, phenols, and quinones. the results were shown in Table 1.

Table 1. phytochemical Analysis Results

Phytochemical	Inference
Alkaloids	+
Anthraquinones	-
Steroids	-
Triterpenes	+
Saponins	+
Tannins	-
Flavonoids	-
Phenols	+
Quinones	+

Key: + Present

- Absent

4.2 Antimicrobial Analysis

The plant extract was subjected to five different isolates of bacteria which includes *Klebsiella. spp, P. mirabilis, E. Coli, S. aureus and S. pyogenes* [15]. The concentration of the antimicrobial discs at 15µg/2ml, 20µg/2ml, 25µg/2ml and 30µg/ 2ml for ethanol extract from the stem bark of *Piliostigma thonningii* and control Augmentin 30µg/2ml was used as control for each of the isolate and the results shows the zone of inhibition (mm).

Table 2. Antimicrobial Activity Results

Test Organisms	**Zone of Inhibition (mm)**				
	P. thonningii Ethanol Extract				Control Augmentin 30 µg
	15 µg	20 µg	25 µg	30 µg	
Staphylococcus aureus	12	14	18	20	8
Proteus mirabilis	0	0	0	0	0
Escherichia coli	0	0	0	0	0
Klebsiella spp	0	0	0	0	5
Streptococcus Pyogenes	0	0	0	0	0

5. Discussion

5.1 Phytochemical Screening *P. Thonningii* Stem Bark Ethanol Extract

The preliminary phytochemical screening of *P. thonningii* stem bark extract (Table 1) showed that alkaloids, saponins, triterpenoids, phenols and quinones were present while flavonoids, tannins, steroids and anthraquinones were absent. The result on Phytochemical screening is in agreement with the literature, that various phytochemicals constituents can be found in various part of the plants and may differ based on the methods of extraction and solvents used [16].

5.2 Antimicrobial Activity

The results reveals that *P. thoningii* ethanol stem bark extract concentrations at 15µg/2ml, 20µg/2ml, 25µg/2ml and 30µg/2ml were ineffective against *P. mirabilis, E. Coli, Klebsiella. spp and S. pyogenes* while Augmentin 30µg/2ml is also ineffective against all the organisms with exception of *Klebsiella. spp*. The concentration of the antimicrobial discs used shows that zones of inhibition (mm) against *S. aureus* for *P. thonningi* ethanol stem bark extract concentrations at 15µg/2ml, 20µg/2ml, 25µg/2ml and 30µg/2ml is 12mm, 14mm, 8mm, and 20mm respectively, while for Augmentin 30µg/2ml is 8mm against *S. aureus* and 5mm against *Klebsiella. spp*. The highest zone of inhibition was found in the plant extract to be 20mm at the highest concentration of 30µg/2ml, the results are similar with the findings in the literature, that *P. thonningii* has antimicrobial activity against various organisms and the effectiveness is usually concentration and solvent dependent [17].

5.3 Recommendations

Based on the research finding indicates that the activity of the plant extract might be concentration, solvent, procedure of extraction dependent. Therefore, adequate research work on different concentration, solvent and extraction procedure and it is suggested to purify the crude extract in order to get better antimicrobial activity.

6. Conclusion

The results of this research work which confirmed the presence of numerous phytochemical inside the stem bark extract of the plant P. *thonningii*. The plant has chemicals like alkaloids, saponins, triterpenoids, quinones, and phenols, which show that it has been used in traditional medicine to treat many health problems. When tested, the extract from the stem bark stopped the growth of S. aureus bacteria at the tested concentrations. This means the ethanol extract from the stem bark has strong antimicrobial power, which could be used to treat infections and other diseases caused by microbes.

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