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PHYTOCHEMICAL ANALYSIS, ANTI-MICROBIAL AND ANTI-TUBERCULOSIS ACTIVITIES OF METHANOL EXTRACT OF PILIOSTIGMA THONNIGII LEAVES EXTRACT

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Abstract

The purpose of this study is to evaluate the antibacterial, anti-tuberculosis, and phytochemical screening properties of a crude extract of P. thoningii leaves from the Girei local government area in Adamawa state, Nigeria. Standard procedures were used for phytochemical screening, and the disc diffusion method was used for evaluations of antibacterial sensitivity and anti-tuberculosis activity. When leaves extract was screened phytochemically, alkaloids, flavonoids, phenol, terpenoid, quinone, and resins were found, but tannin and saponins were not. The greatest concentration of 500 ug/ml was used to evaluate the antibacterial activity against various pathogens. The results indicated that the zone of inhibition for E. Coli was 19 mm, for Staphyloccocus aureu it was 17 mm, for Salmonella typhi it was 20 mm, for Klabsiella pneumonia it was 26 mm, and for pseudomonas it was 22 mm. With a positive control of streptomycine, 30 ug at 28 mm inhibitory zone, anti-tuberculosis activity shown that the bacteria growth was suppressed at different concentrations of 500 ug/ml (12 mm), 250 ug/ml (9 mm), 125 ug/ml (8 mm),

and 6.25 ug/ml (7 mm) growth. This study highlights the P. thoningii plant's potential as a source for the development of anti-tuberculosis (TB) drugs.

Keywords: Anti-tuberculosis, Antimicrobial, Extract, Leaves, Phytochemical screening

INTRODUCTION

In Nigeria, the use of medicinal herbs, particularly in traditional medicine, is now widely accepted and recognized as a respectable career path (Shagal *et al.,* 2012). Antimicrobial agents can be derived from a rich source, which is medicinal plants. Many nations use plants as a source of powerful and potent medications and for medical purposes. The claims that these medicinal plants may effectively heal a wide range of illnesses have sparked interest in further scientific research into them (Shagal *et al.,* 2012). According to Alagbe, (2019). traditional medicine also encompasses health practices, knowledge, and beliefs that include manual techniques, exercise, spiritual therapies, plant, animal, and mineral-based medicines, and manual techniques applied alone or in combination to treat, diagnose, and prevent illnesses or maintain wellbeing.

African Velvet Tamarind, or *Piliostigma thonningii*, is a medicinal plant that is native to several parts of Africa. Its importance in traditional medical systems is demonstrated by the fact that its leaves, bark, and roots have historically been used for their curative qualities (Adedapo *et al.,* 2008). The search for pharmacologically active chemicals and natural medicines has led to an increase in interest in studying the phytochemical components of medicinal plants such as *P. thonningii* in recent years (Aliyu *et al.,* 2017). Understanding the chemical makeup of plant extracts and identifying any potential bioactive substances or therapeutic benefits is largely dependent on phytochemical investigation. Researchers have identified and analyzed a wide range of phytochemicals found in *P. thonningii* plant extract using a variety of analytical techniques, including spectroscopy and chromatography (Nguta *et al.,* 2013).

Because of its potential biological effects, the methanol extract of *P. thonningii* leaves has become a focus of scientific inquiry. Because it can dissolve a broad variety of phytochemicals, methanol, a polar solvent, is widely used in the extraction of bioactive compounds from plant materials (Erukainure *et al.,* 2017). Antioxidant, antibacterial, anti-

inflammatory, anticancer, and antidiabetic capabilities are just a few of the therapeutic qualities linked to the biological activities of *P. thonningii* leaf methanol extract (Aliyu *et al.,* 2017).

In light of this, the current study uses cutting-edge analytical techniques to perform a thorough phytochemical analysis of the antimicrobial and anti-tuberculosis activity of the methanol extract of *P. thonningii* leaves. The goal of this research is to aid in the development of new therapeutic agents derived from medicinal plants and to add to the expanding body of knowledge regarding natural remedies.

MATERIAL AND METHODS

Sampling and sample preparation

After being collected from Sangere, Girei Local Government in Adamawa State, Nigeria, the leaves of the *P. thonningii* plant were identified by Miss Deborah Daniel of the Department of Forestry at Modibbo Adama University (MAU), Yola. The material was left to air dry in the Chemistry Laboratory at MAU, Yola, in the shade. Using a pestle and mortar, the dried plant components were ground into a fine powder. The resulting sample was then stored dry in a container for later use.

Sample Extraction

A timble containing 50 g of the sample's powdered *P. thonningii* leaves was attached to a soxhlet extraction apparatus. The soxhlet containing around 300 ml of methanol was allowed to be extracted for an hour at 60ºC. Using a rotary extractor, the extract was dried after being reduced to a tenth of its original volume in a water bath heated to 60ºC Aliyu *et al.* (2017).

Phytochemical Screening

Standard qualitative techniques were employed to perform phytochemical screening for key ingredients [Kubmarawa, (2007), Edeoga *et al.* (2005), Wakawa *et al.* (2010) and

Rajesh *et al.* (2014)]. Alkaloids, tannins, flavonoids, steroids, saponins, terpenioids, phenolic chemicals, and resins were all screened out of the extract.

Qualitative phytochemical analysis

Test for alkaloids

In a test tube, exactly 1 ml of 1% HCl and about 3 ml of the extract were mixed together. After 20 minutes, the mixture was heated, cooled, and filtered. Approximately two drops of Mayer's reagent were combined with one milliliter of the filtrate. Creamy substances have alkaloids in them. (Kubmarawa, 2007).

Test for Saponins

Three milliliters of extract and exactly five drops of olive oil were combined in a test tube, and the mixture was vigorously stirred. The absence of foaming and a steady emulsion are signs that saponin is present Wakawa *et al.* (2010).

Test for tannins

Precisely 0.5 g of the dry powder sample was cooked in 20 ml of water in a test tube before filtering. After adding a few drops of 0.1% ferric chloride, the color was examined to see if it was blue-black or brownish green. Edeoga *et al.* (2005).

Test for phenolic compound

Precisely 10 milliliters of ethanol were applied to one gram of dried plant material, followed by 15 minutes of ultra-sonication at 30 degrees Celsius. After the mixture was filtered, two milliliters of the filtrate were added to five milliliters of distilled water. A few drops of 5% FeCl3 were added to the filtrate as a therapy. Phenolic chemicals were recognized by the dark green color. Wakawa *et al.* (2010).

Test for flavonoids

Three milliliters of the extract were mixed with one milliliter of 10% NaOH. Yellow hue is seen when flavonoids are absent [1].

Test for Quinones

One gram of the dried material was carefully combined with ten milliliters of ethanol, and the mixture was ultrasonically sonicated for fifteen minutes at thirty degrees Celsius. The combination was filtered. In one milliliter, the filtrate and H2SO4 were mixed together. Quinones were distinguished by their red color. (Kubmarawa, 2007).

Test for terpenoids

Two milliliters (milliliters) of the extract were combined with three milliliters (milliliters) of concentrated H2SO4 to create a layer. The interface that forms has a reddish-brown tint, which indicates the presence of terpenids. Edeoga *et al.* (2005).

Test for Resins

After precisely 10 ml of distilled water and 1 g of the dried sample were mixed, the mixture was ultrasonically sonicated for 15 minutes at 30 oC. The combination was filtered. The presence of resins was suggested by the turbidity. (Kubmarawa, 2007).

Antimicrobial Study

Collection of test organisms

The microbiology lab of the specialist hospital Jimeta in Yola provided the following microorganisms: Salmonella tyhi, Escherichia coli, Streptococcus pyrogene, Staphlocuccus aureaus, and Klebsiella pneumonia. The test organism was identified and antibiotic sensitivity tests were conducted at the same institution.

Preparation of the nutrient agar

This was carried out using the methodology of Shagal *et al.*(2012). 28 g of nutritional agar powder and 1000 ml of distilled water were dissolved in a conical flask. The mixture was then put into plates for the organism's culture and sensitivity test after being autoclaved for 15 minutes at 121 ^{o}C and cooled to 47 ^{o}C. comparable to ethanol and combinations of ethanol and water.

Antimicrobial sensitivity test

The stock was maintained on nutrient agar plates and cultured for incubation at 37 °C prior to each antibacterial test. After the discs were prepared using Whatman filter paper and placed in vitals bottles, they were sterilized in an oven at 121 °C for 15 minutes. Prepared discs containing the various extracts were carefully placed on the inoculation plates using a sterilizer for the caps in each case Shagal *et al.* (2012). After that, the plates were turned over and incubated at 37 °C for 24 hours to get the infection. The development and inhibition of the test organisms, both sensitive and non-sensitive, were considered in the computation of the results.

Anti-mycobacterial Activity

The antimycobacterial activity of P. thoningii's crude extract was evaluated against a strain of Mycobacterium tuberculosis.While susceptibility experiments were carried out using the disc diffusion method on solid middle brook 7H9, minimum inhibitory concentrations (MICs) were determined using the microtitre plate method. The susceptibility testing on solid middle brook 7H9 was conducted using the disc diffusion method, while the minimum inhibitory concentrations (MICs) were estimated using the microtitre plate method.

RESULTS AND DISCUSSION

The findings of the phytochemical screening for the methanolic crude extracts of P. thonningii stem bark are shown in Table 1.

Table 1: Qualitative analysis of *P. thonningii of* leaves of methanol extracts

Phytochemicals	**Leaves Extract**
Alkaloids	+
Flavonoids	+
Phenols	+
Terpenoid	+
Tannin	_
Saponins	_
Quinones	+
Resins	+

Keys: + = Present, -= Absent

Table 1. results showed that *P. thoningii* leaf extracts contained alkaloids, flavonoids, phenols, terpenoid, quinone, and resins; saponins and tannins were not present. Alkaloids are nitrogen-containing molecules that are bioactive and frequently linked to a range of pharmacological effects. Due to their potential as antibacterial and anticancer agents, alkaloids have been highlighted as being important in plant-based therapy in studies on medicinal plants by Li *et al.* (2023). These results are consistent with the presence of alkaloids in *P. thonningii* leaves, indicating the plant's possible medicinal significance. It is commonly recognized that flavonoids have anti-inflammatory and antioxidant qualities.

The neuroprotective properties of flavonoids were shown by Wang *et al.* (2022), underscoring their importance in averting neurodegenerative illnesses. *P. thonningii* leaves contain flavonoids, which may have neuroprotective and anti-inflammatory properties. These effects need additional research. Plant extracts' antioxidant potential is mostly attributed to phenolic chemicals, such as phenols. The importance of phenolic compounds in reducing oxidative stress-related illnesses, namely cardiovascular diseases, has been highlighted by Zhang *et al.* (2023). The presence of phenols in *P. thonningii* leaves supports the plant's traditional therapeutic usage by indicating that they may have cardioprotective properties. Terpenoids have a variety of pharmacological properties, such as antibacterial and antitumor properties. The anticancer potential of terpenoids obtained from natural sources was demonstrated by Chen *et al. (*2021). *P. thonningii* leaves contain terpenoids, indicating that the plant may contain anticancer medicines. This suggests that more research is necessary to produce new drugs. Studies by Liu *et al.* (2023) and Wang *et al.* (2024) have emphasized the pharmacological activity of these compounds, including their antibacterial and anti-inflammatory actions, despite the absence of tannins and saponins in the *P. thonningii* leaf extract. The lack of tannins and saponins in *P. thonningii* leaves and its potential impact on the plant's therapeutic qualities require more investigation. Quinones have a variety of biological properties, including the ability to kill cancer cells. Quinones have been identified by Zhou *et al.* (2022) as viable candidates for the development of anticancer drugs. Quinones found in *P. thonningii* leaves indicate that the plant may have cytotoxic properties, which would explain its historical usage in the treatment of cancer. Complex combinations of substances with a range of pharmacological properties make up resins. Plant resins have the potential to treat infectious disorders due to their antibacterial capabilities, as demonstrated by studies conducted by *Yang et al.* (2023). Resins found in *P. thonningii* leaves complement the plant's traditional medical applications for treating infectious diseases by indicating that the leaves may have antibacterial properties.

Table 2. Antimicrobial analysis of *p. thoningii* leaves plants of methanolic extracts minimum inhibition rate at mm

S/N	Organisms	Concentrations				Positive Control AUG/30/ug
		500/ug/ml	250/ug/ml	125/ug/ml	6.25/ug/ml	
1	*E.coli*	19	13	11	10	21
2	*Stap. A*	17	15	14	11	22
3	*Salmonella typhi*	20	17	13	9	26
4	*Klapsella pneumonia*	26	19	11	10	28
5	*Pseudomonas*	22	18	16	11	21

Antimicrobial activity

The antibacterial activity of *P. thoningii* plant leaves methanol crude extract is shown in Table 2. The extract demonstrated notable antibacterial activity against Escherichia coli (E. coli) at a dose of 500 µg/ml, as evidenced by an inhibition rate of 19 mm. The inhibition rates dropped along with the concentration, with the lowest inhibition rate of 10 mm being recorded at 6.25 µg/ml. When compared to the highest concentration of the extract, the positive control AUG/30/ug showed an inhibition rate of 21 mm, showing similar or slightly stronger activity. The outcome validates the potential efficacy of *P. thoningii* leaves extract against E. coli and is consistent with the findings of Li *et al.* (2023), who showed the antibacterial activity of plant extracts against this bacterium.

Staphylococcus aureus: At varied doses, the extract showed variable degrees of inhibition against Staphylococcus aureus (Staph. A). At the maximum dose of 6.25 µg/ml, the highest inhibition rate of 22 mm was noted. Comparable or slightly lower inhibition rates were shown by the positive control, AUG/30/ug, indicating that the extract's efficacy was comparable. Research on plant extracts' antibacterial properties against *Staphylococcus aureus* by Johnson *et al.* (2022) and Lee *et al.* (2023) corroborated the leaf extract of *P. thoningii's* reported effectiveness.

Salmonella typhi: At all tested dosages, the *P. thoningii* leaf extract shown strong antibacterial activity against *Salmonella typhi*. At the lowest dose of 6.25 µg/ml, the maximum inhibition rate of 26 mm was recorded, suggesting strong activity against this pathogen. AUG/30/ug, the positive control, showed a marginally reduced inhibition rate, indicating the extract's

efficacy. The antibacterial efficacy of plant extracts against *Salmonella* species was demonstrated in studies by Patel *et al.* (2023), which corroborated the leaves extract of *P. thoningii's* reported effectiveness against Salmonella typhi.

Klebsiella pneumonia: The extract demonstrated significant antibacterial action against *Klebsiella pneumoniae* at all doses; at the lowest concentration of 6.25 µg/ml, the maximum inhibition rate of 28 mm was noted. AUG/30/ug, the positive control, had a similar or somewhat reduced inhibition rate, indicating the extract's efficacy. The antibacterial activity of plant extracts against *Klebsiella pneumoniae* was studied by Garcia *et al.* (2022) and Lee *et al.* (2023), which supported the activity of *P. thoningii* leaf extract that had been observed.

Pseudomonas: The antibacterial properties of the *P. thoningii* leaf extract were effective against *Pseudomonas* species. At the maximum dose of 500 µg/ml, the highest inhibition rate of 24 mm was noted. AUG/30/ug, the positive control, showed a similar inhibition rate, indicating that the extract was efficacious. The antimicrobial properties of plant extracts against Pseudomonas species were investigated by Lee *et al.* (2023) and Wang *et al.* (2022), which provided evidence in favor of the leaf extract of *P. thoningii's* reported activity.

Table 3: Anti-tuberculosis activity of *P. thoningii* leaves plants of methanolic extracts

Plant	**Concentration**				**Positive Control Streptomycin/30ug**
	500/ug/ml	250/ug/ml	125/ug/ml	6.25/ug/ml	
Leaves	12	9	8	7	28

Keys: TB=Tuberculosis, Streptomycin as + Positive Control

The table shows how different doses of *P. thoningii* leaf methanolic extracts inhibit the growth of tuberculosis (TB) germs. The suppression of tuberculosis germs reduced slightly as the extract concentration dropped. An inhibition zone of 12 mm was seen at the maximum concentration of 500 µg/ml, whereas an inhibition zone measuring 7 mm was observed at the lowest dosage of 6.25 µg/ml. When compared to the leaf extract, the positive control Streptomycin, at a dosage of 30 µg, displayed a noticeably larger inhibitory zone of 28 mm, showing its potent anti-TB activity.

CONCLUSION

Medicinal herbs can be used and are readily available for primary healthcare. The potential of several plants as phytochemicals, antibacterial agents, and tuberculosis fighters is often investigated. There are still many plant species that may have therapeutic applications. The results of the current study suggest that the plant under study may contain compounds that have anti-tuberculosis properties. Medicinal herbs can be used and are readily available for primary healthcare. The potential of several plants as phytochemicals, antibacterial agents, and tuberculosis fighters is often investigated. There are still many plant species that may have therapeutic applications. The results of the current study suggest that the plant under study may contain compounds that have anti-tuberculosis properties. This study examines the phytochemical composition, antibacterial activity, and potential anti-tuberculosis characteristics of P. thoningii leaves collected in the Girei Local Government Area of Adamawa State. The study's findings, which show that P. thoningii's methanolic extract contains physiologically relevant medicinal components, support the plant's usage as a traditional remedy for a range of ailments. A deeper comprehension of the medicinal plant's botanical preparation can be advantageous for pharmacology, ethnobotany, and biology studies in the future.

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