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# PHYTOCHEMICAL ANALYSIS, ISOLATION AND BIOLOGICAL STUDIES OF *PILIOSTIGMA THONNIGII* STEM BARK EXTRACT

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## ABSTRACT

The current study ascertained the stem bark methanol extract of *Piliostigma thoningii (P. thoningii)* plant collected from Girei Local Government Area of Adamawa State, Nigeria, for phytochemical screening, Fourier transform infrared (FTIR) analysis, antibacterial and anti-tuberculosis activities. Phytochemical screening was carried out using standard methods, antibacterial sensitivity and anti-tuberculosis activity tests were carried out using disc diffusion method. To obtain pure fractions, column chromatography was used and characterization was done by FTIR spectroscopy. Phytochemical analysis revealed the presence of alkaloids, phenols, terpenoids, and quinones while resins were absent. Antibacterial activities were tested against different pathogens with 500 ug/ml as the highest concentration and showed that *E. Coli* had zone of inhibition of 19 mm, *Staphyloccocus aureu,* 6 mm; *Salmonella typhi,* 11 mm; *Klabsiella pneumonia,* 9 mm and *pseudomonas* 6 mm. Anti-tuberculosis activity showed that the bacteria growth was inhibited at different concentrations of 500 ug/ml (19 mm), 250 ug/ml (16 mm), 125 ug/ml (11 mm), 6.25 ug/ml (8 mm) with a positive control of Streptomycine, 30 ug at 28 mm inhibitory zone. Five fractions were obtained using column chromatography. The most sensitive fractions among the five obtained were identified by testing the antimicrobial activity of the fractions. The highest zone of inhibition was 27 mm found in fraction ST001. The functional groups contained in the separated portion of the crude methanol extract were identified. The most active fraction ST001's FT-IR analysis showed the presence of methyl, alkene, carbonyl, and hydroxyl groups. Peak values were observed at 3379.9 $cm^{-1}$, 2930.4 $cm^{-1}$, 2130.1 $cm^{-1}$, 1707.8 $cm^{-1,}$ and 1456.0 $cm^{-1}$, in that order. The stem bark of the *Piliostigma thonnigii* plant has been found to be a possible source of drug development for anti-tuberculosis (TB).

**Keywords:** Anti-tuberculosis, FTIR analysis, Methanol extract, *Piliostigma thonnigii*

## INTRODUCTION

Antimicrobial agents can be derived from medicinal plants. Many nations use plants as a source of strong 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 [1]**.** Due to growing drug resistance and increased costs associated with drug development, pharmaceutical corporations and medical researchers in affluent nations are increasingly looking to traditional medicine for answers to the most urgent health issues facing the globe [2]. Traditional medicine (TM) is used by a large population of the population either due to the system being accepted from cultural and spiritual perspectives or to the high cost of western pharmaceuticals [3]. There are many forms of TM systems that exist within cultures and in different regions [4].

Appropriate chemical compounds present in fruits, vegetables, and plant extracts are now widely acknowledged to offer protection against serious human illnesses. Some of which, like cancer and cardiovascular illnesses, are particularly dangerous. The potential of components including alkaloids, tannins, saponins, flavonoids, cardiac glycosides, terpenoids, phlobatannins and steroids is receiving increased attention due to the fact that numerous researches appear to support those beneficial properties [5].

Nigerians recognize and accept the use of medicinal plants, particularly in traditional medicine, as a legitimate field of work [6]. The health of both individuals and society greatly depends on medicinal plants. Certain chemical compounds in these plants have a specific physiological effect on humans, which account for their medical significance. Alkaloids, tannins, flavonoids, and phenolic compounds are the most significant of these bioactive components found in plants. Numerous of these naturally-occurring medicinal plants are used as culinary spices. Additionally, they are occasionally added to foods intended for nursing and pregnant women for therapeutic purposes [7].

` As alternative medicine, plants are the original source of remedies. However, with the rise in antimicrobial drug resistance and the frequent occurrence of unwanted side effects from certain antibiotics, the search for medicinal plants has gained significance in recent years. A commensurate increase for natural antimicrobial therapies has coincided with the introduction of increasingly resistant drugs [8]. A vast array of illnesses and ailments have been treated in several African nations using extracts from fruits, leaves, stems, bark, and root bark, which are typically given as infusions, decoctions, tinctures, syrups, and lotions [9]. The applications for treatment, prevention, and cure go back before conventional medicine was developed, particularly in Africa. Many plant species are known to have hypoglycemic effects worldwide. Despite this, many people, particularly in tropical regions, still rely on local medicinal plants to treat and manage a

variety of illnesses. The majority of these plants are purchased from local traditional healers who are unaware of the effects and dosages of the plant's chemical constituents. Therefore, in order to understand the biological activity and composition of these plants' chemical and bioactive ingredients as well as their use in primary healthcare, a scientific approach must be taken [10].

*P. thonningii* is a woody plant in the legumecaea family (Fabaceae), with a rounded crown and a short, crooked bole. It can grow to a height of 4 to 15 meters. The branches have hair. Rough and longitudinally fissured, the bark eventually turns a creamy brown color. It has leaves of size up to 15 x 17 cm, leathery, bi-lobed from one-eighth to one-third of the way down, shiny above, with highly veined and somewhat rusty hair below. It is pendulous, unisexual, with five white to pink petals; the male and female are typically on different trees; the ovary is capped by a large, flattened globose stigma; the pods are indehiscent, up to 26 x 7 cm, and have rusty brown hair that fall off as the pods mature and get somewhat consorted. The pods remain on the tree for a while before falling to the ground and breaking down into pea-sized seeds. These seeds are surrounded by a delicious pulp. This specie's deep roots enable plants to withstand severe gusts and obtain water during dry spells. The term *piliostigma* refers to stigma that resembles a cap. *P. thonningii* is frequently found at medium to low elevations in open woodland and wooded grasslands in subhumid Africa [11]. In Eastern Nigeria, the leaf extracts have been used to treat malaria, while the roots and twigs have been used locally to treat fever, respiratory conditions, snake bites, hookworm, and skin infections [12]. This study plant species have been examined for their phytochemical, antimicrobial, anti-tuberculosis, and spectroscopic analytical properties. Complex combinations of physiologically active chemicals, some of which may have genotoxic and antigenotoxic effects, are found in crude extracts [13]. Therefore, phytochemical analysis is required to determine any possible health risks associated with using plant extracts for medical purposes [14]. A crucial first step in identifying the active ingredients that are frequently investigated in the discovery and development of new medications is the preliminary screening of medicinal plants for their phytoconstituents [15]. Wafaa et al [16] reported the presence of tannins, saponins, alkaloids and phenols in the methanol extract of *P. thonningii*. The presence of tannins, phenols, saponins, flavonoids and alkaloids in stem bark of *P. thoningii* has also been earlier reported by Yadav et al [17]. The differences in physiological activities and also factors such as season, environmental conditions and differences in reproductive period may affect the availability and concentrations of these phytochemical constituents [18].

A study carried out by Musa [19] on the phytochemicals of the stem bark extract of *Piliostigma thonnigii* revealed the presence of alkaloids (5.11%), flavonoids (3.46%), terpenoids (0.49%), hydrolysable tannins (1.92%), condensed tannins (0.19%), phenols (4.00%), saponins (0.22%), steroids (1.18%), phytates (0.51%) and cyanates (0.15%).

The novelty of the present study is the isolation and anti-tuberculosis activity of the stem bark extract of the plant in which little or no work have been done on it. Therefore, the aim of this study is to evaluate *P. thoningii* stem bark extract for phytochemical, antibacterial and anti-tuberculosis properties.

## MATERIAL AND METHODS

### Sampling and sample preparation

The stem barks of the *P. thonningii* plant were collected from Sangere, Girei Local Government of Adamawa State, Nigeria, and identified by Mr. John Danladi of the Department of Forestry at Modibbo Adama University (MAU), Yola. The sample was allowed to air dry under shade in Chemistry Laboratory at MAU, Yola. The dried plant materials were pulverized using a pestle and mortar and the pulverized sample was kept dry in a container for further use.

### Sample Extraction

A soxhlet extraction device was fitted with a timble that held 50 g of powdered *P. thonningii* stem bark of the sample. One hour of extraction at 60 $^{o}$C was allowed for the soxhlet containing around 300 ml of methanol. After being reduced to a tenth of its initial volume in a water bath heated to 60 $^{o}$C, the extract was dry using rotary extractor [6].

### Phytochemical Screening

Phytochemical screening for major constituents was done using standard qualitative methods [1,5, 10 and 14]. The extract was screened for alkaloids, tannins, flavonoids, steroid, saponins, terpeniods, phenolic compounds and resins.

### Qualitative phytochemical analysis

### Test for alkaloids

About 3 ml of the extract and precisely 1 ml of 1% HCl were combined in a test tube. The mixture was heated, cooled, and filtered after 20 minutes. One milliliter of the filtrate was mixed with around two drops of Mayer's reagent. Alkaloids are present when the substance is creamy [1].

**Test for Saponins**

Precisely five drops of olive oil were added to three milliliters of extract in a test tube and the mixture was violently agitated. The presence of saponin is indicated by the lack of foaming and a stable emulsion [14].

**Test for tannins**

In a test tube with 20 ml of water, precisely 0.5 g of the dry powder sample was boiled before being filtered. A few drops of 0.1% ferric chloride were added and the coloration was checked for brownish green or blue-black [5].

**Test for phenolic compound**

To one gram of dried plant material, exactly 10 ml of ethanol was added, then ultra-sonication at 30 ºC was carried out for 15 minutes. Two milliliters of the filtrate were added to five milliliters of distilled water after the combination had been filtered. For treatment, a few drops of 5% $FeCl_3$ were added to the filtrate. The dark green hue indicated phenolic compounds [10].

**Test for flavonoids**

A quantity of 1 ml of 10% NaOH was added to 3 ml of the extract. The absence of flavonoids shows yellow coloration [1].

**Test for Quinones**

Ten milliliters of ethanol were precisely mixed with one gram of the dried material, and the combination was ultrasonically sonicated for fifteen minutes at thirty degrees Celsius. The mixture underwent filtration. $H_2SO_4$ and filtrate were combined in one milliliter. Quinones could be identified by their red hue [1].

**Test for terpenoids**

About Five (5) ml of the extract was mixed in 2 ml of chloroforms and 3 ml concentrated $H_2SO_4$ was carefully added to form a layer. A reddish brown coloration of the interface formed shows the presence of terpenids [5].

**Test for Resins**

About 1 g of the dried sample and exactly 10 ml of distilled water were combined, and the mixture was ultrasonically sonicated for 15 minutes at 30 ºC. Filtration was done on the mixture. The turbidity's occurrence indicated the existence of resins [1].

**Antimicrobial Study**

**Collection of test organisms**

The following microorganisms were obtained from the microbiology laboratory of the specialized hospital, Jimeta in Yola: *Salmonella tyhi*, *Escherichia coli*, *Streptococcus pyrogene*, *Staphlocuccus aureaus, and Klebsiella pneumonia*. The antibiotic sensitivity tests were performed and the test organism's identification were carried out at the same hospital.

**Preparation of the nutrient agar**

This was done in accordance with Shagal et al [6] method. In a conical flask, 28 g of nutrient agar powder was dissolved in 1000 ml of distilled water. The mixture was then autoclaved for 15 minutes at 121 $^{o}$C, cooled to 47 $^{o}$C, and then dispensed into plates for use in the organism's culture and sensitivity test. similar to ethanol and ethanol-water mixtures.

**Antimicrobial sensitivity test**

Before every antimicrobial test, the stock was kept on nutrient agar plates and cultivated for incubation at 37 °C. The discs were sterilized in an oven at 121 °C for 15 minutes after being prepared with Whatman filter paper and stored in vitals bottles. Using a sterilizer for the caps in each instance, prepared discs containing the different extracts were carefully placed on the inoculation plates [6]. Then, the plates were inverted and inoculated for 24 hours at 37 °C in an incubator. The test organisms' growth and inhibition—both sensitive and non-sensitive—were taken into account while determining the results.

**Anti-mycobacterial Activity**

The crude extract of *P. thoningii* was tested for antimycobacterial activity against a strain of Mycobacterium TBMinimum inhibitory concentrations (MICs) were ascertained using the microtitre plate method using the middle brook 7H9 method, whereas susceptibility tests were conducted using the disc diffusion method on solid middle brook 7H9. The microtitre plate method was utilized to estimate the minimum inhibitory concentrations (MICs), whereas the disc diffusion method was employed for the susceptibility testing on solid middle brook 7H9.

**Isolation of active fractions**

A gradient of solvent system consisting of hexane-ethyl acetate methanol 5% stepwise increase was used to elute 50 g of the crude extract after it has been loaded dry by adsorption on silica gel

normal phase thin layer chromatography (NP TLC) and fractionated by vacuum chromatography. Similar fractions were combined to form a main fraction after the fractions were extracted and concentrated from the TLC profile of the fractions [6]. FT-IR spectroscopy (Perkin Elmer 1310 model) was used to further investigate the pure chemical that had been produced as a crystal.

## RESULTS AND DICUSSIONS

Table 1 displays the outcomes of the phytochemical screening results for *P. thonningii* stem bark methanolic crude extracts.

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

| Phytochemicals | Reults |
|---|---|
| Alkaloids | + |
| Flavonoids | + |
| Phenols | + |
| Terpenoid | + |
| Tannin | + |
| Saponins | + |
| Quinones | + |
| Resins | _ |

Keys: + = Present, -= Absent

The findings in Table 1 demonstrated that the stem bark extracts of *P. thoningii* contained alkaloids, flavonoids, phenols, tannins, terpenoid, saponins, and quinone.; Resins were absent. Flavonoids are important group of polyphenols known to inhibit the initiation, promotion and progression of tumors [20]. The flavonoids have been reported to exert multiple biological effects including antimicrobial, antidiarrheal, antioxidant, free radical scavenging abilities, anti-inflammatory, ant carcinogenic.

Alkaloids are the largest group of secondary chemical constituents and most efficient plant substances used therapeutically because of their well-documented antimicrobial, anthelmintic and antidiarrheal activities. The solutions of alkaloids are intensely bitter. These heterocyclic nitrogenous compounds function in the defense of plants against herbivores and pathogens, and are widely exploited as pharmaceuticals, stimulants, narcotics, and poisons due to their potent

biological activities. They play some metabolic roles and control development in living system. They are also involved in protective function in animals and are used as medicine especially the steroidal alkaloids. Pure isolated alkaloids and the synthetic derivatives are used as the basic medicinal agent because of their analgesic, and antispasmodic properties [21].

Tannins are phenolic compounds of high molecular weight (500-3000) also known for their antimicrobial, anthelmintic and antidiarrheal activities. They are used as antiseptic and this activity is due to presence of the phenolic group. The presence of tannins in *P. thoningii* in the stem bark is thought to be responsible for its inherent anti-tuberculosis property. The proposed mechanisms of action of tannins include: binding to adhesions, enzyme inhibition, substrate deprivation, complex with cell wall, membrane disruption and metal ion complexation. The presence of tannins in *P. thoningii* plant has been documented to support its strong use for treatment of candidiasis, healing of hemorrhoids, frost bite and varicose ulcers in herbal medicine. Phenolic acids act essentially by helping in the reduction of particular adherence of organisms to the cells lining the bladder, which ultimately lowers the incidence of urinary-tract infection [22].

Table 2. Antimicrobial activity of methanolic extract of stem bark of *P. thoningii* plant (Zone of inhibition in mm)

| S/N | Organisms | Concentrations | | | | Positive Control |
|---|---|---|---|---|---|---|
| | | 500 ug/ml | 250 ug/ml | 125 ug/ml | 6.25 ug/ml | AUG/30 ug |
| 1 | *E.coli* | 19 | 14 | 11 | 9 | 29 |
| 2 | *Stap. A* | 6 | 6 | 6 | 6 | 7 |
| 3 | *Salmonella typhi* | 11 | 9 | 8 | 6 | 16 |
| 4 | *Klabsella pneumoniae* | 9 | 8 | 6 | 6 | 19 |
| 5 | *Pseudomonas* | 6 | 6 | 6 | 6 | 20 |

**Antimicrobial activity**

Table 2 displays the stem bark methanolic crude extract of *P. thoningii* plant's antibacterial activity. For *E. Coli*, the stem bark extract's maximum and lowest zones of inhibition were 19 mm and 9 mm, respectively. For Staph. A and Pseudomonas, the zones of inhibition were 6 mm and 19 mm, respectively. Similar to this, *Klapsella pneumoniae* and *Salmonella typhi* had the highest

zones of inhibition of stem bark, measuring 9 mm and 11 mm, respectively. For *Klapsella pneumoniae* and *Salmonella typhii*, the stem bark's lowest zone of inhibition was 6 mm.

The antibacterial activity of *P. thoningii* plant stem bark methanolic preparations was displayed in Table 2. The outcomes demonstrated every pathogen examined. At varying doses, *Aureus*, *Salmonella typhi, Klapella pneumonia*, and *Pseudomonas* showed inhibitory action.

The results obtained in Table 2 showed strong antimicrobial activity, which may be due to the bioactive constituents, such as alkaloids, tannins, and flavonoids, present in the extracts [5]. The methanolic crude extract of *P. thoningii* plant (stem bark) showed antimicrobial activity against all the organisms. The control (AUG) gave the best antimicrobial activity against all the test organisms. The zone of inhibition (sensitivity) of the plant extract was considered from 7 mm and above, while 6 mm is resistance. The plant's methanol extract demonstrated a noteworthy rise in its antimicrobial activity towards the test organisms as the concentration increased. This resulted in the highest zone of inhibition, measuring 19 mm at 500 ug/ml, and the lowest zone of inhibition, measuring 9 mm at 6.25 ug/ml. Notably, the organisms remained susceptible to the stem bark at all concentrations.

Anti-tuberculosis findings at varying concentrations are displayed in Table 3.

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

| Plant | Concentrations | | | | Positive Control Streptomycine/30ug |
|---|---|---|---|---|---|
| | 500/ug/ml | 250/ug/ml | 125/ug/ml | 6.25/ug/ml | |
| Stem bark | 19 | 16 | 11 | 8 | 28 |

Keys: TB= Tuberculosis, Streptomycin as + Positive Control

The stem bark methanolic crude extract of *P. thoningii* plants has been shown to have anti-tuberculosis (TB) properties. Specifically, the extract inhibits the development of bacteria within an inhibitory zone spanning 7 mm to 19 mm. Stronger activity was found to correspond with higher crude extract concentrations. The stem bark extract demonstrated that an activity of 19 mm was seen at 500 ug/ml of extract concentration.

In comparison to the methanol crude extract, streptomycin, the positive control in this investigation, exhibited greater activity (28 mm) against the test organisms. This could be because

the antibiotics, such as streptomycin, are in their pure form, whereas the crude extract is still in its crude state and needs to be processed in order to get rid of all the elements that prevent it from acting as intended.

**Column chromatography**

The following process was used to separate the bioactive components from the methanolic crude extract of stem bark: Hexane, ethyl acetate, and butanol were used to extract the plant's 32 g aqueous extract. Following the separation process, the butanol extract was separated via column chromatography employing a gradient elution approach as follows:

Three fractions (butanol: EtOAc = 10:90), six fractions (butanol: EtOAc = 20:80), and six fractions (100% hexane). There are three fractions (butanol: EOAC = 40:60), four fractions (butanol: EOAC = 50:50), and nine fractions (butanol: EOAC = 30:70). The following fractions are found in butanol: 11 (butanol: etOAc = 60:40), 2 (butanol: etOAc = 70:30), 5 (butanol: etOAc = 80:20), 9 (butanol: etOAc = 90:10), and 13 (butanol 100).

RF = (EtOAc: Butanol = 10:90) 0.57.

Table 4: Anti-microbial activity of the Stem bark fraction obtained from column chromatography

| Fractions | Concentrations | | | | Positive control |
|---|---|---|---|---|---|
| | 500/ug/ml | 250/ug/ml | 125/ug/ml | 6.25/ug/ml | Streptomycine/30ug |
| ST 001 | 27 | 24 | 19 | 14 | 28ug |
| ST 002 | 25 | 9 | 8 | 6 | 30ug |
| ST 003 | 19 | 9 | 8 | 7 | 26ug |
| ST 004 | 15 | 7 | 7 | 6 | 26ug |
| ST 005 | 11 | 6 | 5 | 5 | 23ug |

From Table 4, the maximum sensitivity or activity against antimicrobial activity is seen in fraction ST001 coded. For the assay, *klabsella pneumoniae* was the microbe.

Following the test, FTIR was performed using the isolate fraction ST001 code to identify the functional groups that were present in the isolated fraction.

**Fourier transform infrared spectroscopy analysis**

Table 5 displays the functional groups and FTIR peak values of *P. thoningii* stem bark plant methanolic extracts. The FTIR analysis of *P. thoningii* stem bark extracts yielded wave numbers (cm-1) for the major peaks that were obtained (Table 5). A distinctive peak was seen at 3379.9 cm-1, 2930.4 cm-1, 2130.1 cm -1, 1707.8 cm -1, and 1456.0 cm -1 in the *P. thoningii* stem bark extract plant. The presence of alkanes was linked to the band at 2930.4 cm-1 and 1456.0 cm-1, whereas the peak at 3379.9 cm-1 was related to alcohol's hydroxyl vibration. C-H stretching and C=C conjugated alkene stretching were identified as the causes of the peaks at 2130.1 cm-1 and 1707.8, respectively.

Based on the peak value in the infrared radiation area, the functional group of the active components was determined using the FTIR spectrum. Each functional group in the solvent, such as the hydroxyl, alkene, carbonyl, and methyl groups, had its own group. Therefore, the chemical components contained in the methanol crude extract of *P. thoningii* were identified by FTIR analysis. Furthermore, it has been demonstrated that FTIR spectroscopy is a sensitive and dependable technique for determining the bimolecular composition [6].

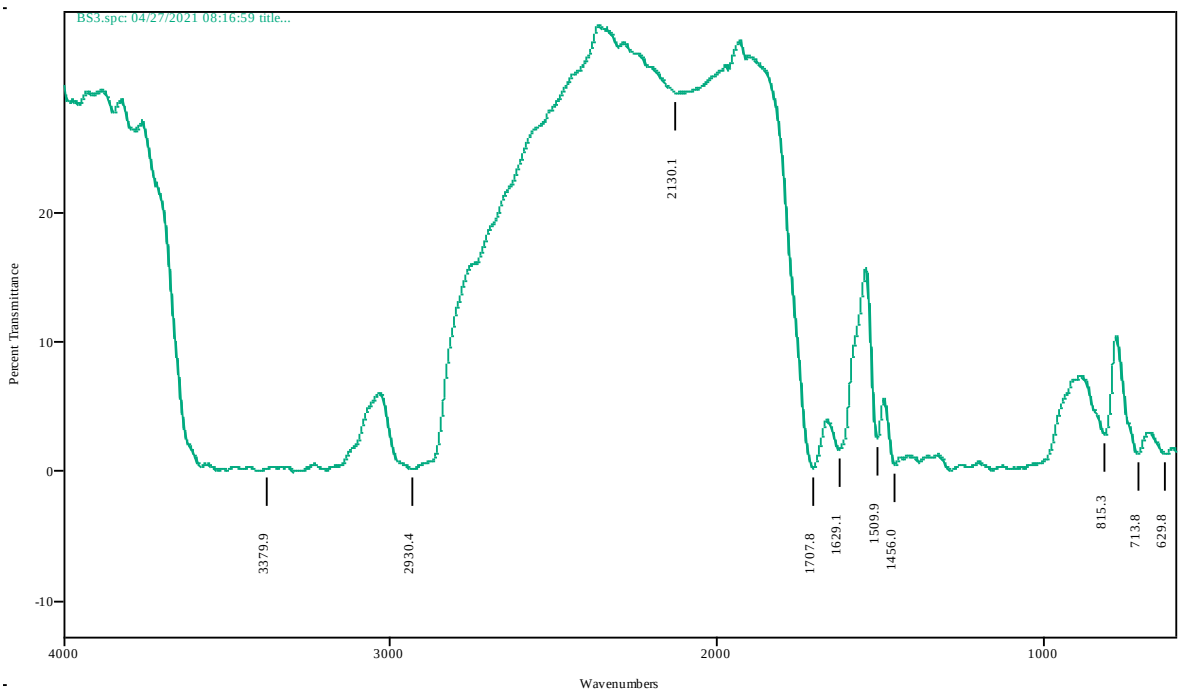


Figure 1: FTIR analysis of the isolate of P.*thoningii* stem bark

Table 5: Fourier Transform Infrared (FTIR) Spectral peak values and Functional groups of the isolate of P. *thoningii* stem bark

| Peak value ($cm^{-1}$) | Functional group | Type of vibration | Characteristic absorptions ($cm^{-1}$) | Intensity |
|---|---|---|---|---|
| 3379.9 | O-H | Stretch, (-H bonded) | 3200-3600 | Strong, broad |
| 2930.4 | C-H | Stretch | 2500-3300 | Strong, very broad |
| 2130.1 | –C≡C– | Stretch | 2100-2260 | Variables, not present in symmetrical alkynes |
| 1707.8 | C=O | Stretch | 1670-1820 | Strong |
| 1456.0 | -C-H | Bending | 1350-1480 | Variables |

## CONCLUSION

For primary healthcare, medicinal plants are useful and easily accessible. A lot of plants are routinely tested for their potential as antimicrobial agents, phytochemicals and tuberculosis fighters. There are still a lot of plant species that could be useful medically. The present study's findings indicate that the plant under investigation may possess chemicals with anti-tuberculosis efficacy. The present study identifies the phytochemical component, antibacterial activity, and possible anti-tuberculosis properties of *P. thoningii* stem bark that was gathered in Adamawa State's Girei Local Government Area. The results of this study support the use of *P. thoningii* as a traditional medicine for a variety of illnesses by demonstrating that its methanolic extract contains biologically significant medicinal components. Future research in the fields of pharmacology, ethnobotany and biology for drug development can benefit from an expanded understanding of the botanical preparation of the medicinal plant.

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