https://chemclassjournal.com/
ChemClass Journal Vol. 9 Issue 4 (2025); 243 - 253
e-ISSN:3092-8214 p-ISSN:3092-8206
doi.org/10.33003/chemclass-2025-0904/17

Antibacterial Activity, and Isolation of Compound from *Piliostigma Thonnongii*

Zaruku Abdulkadir[1*], Abubakar Sani[1], Yusuf Hassan[1], Maryam Saddik[1], Ibrahim Salisu[1], Kamaluddeen Kabir[2]

[1]*Chemistry Department, Umaru Musa Yaradua University Katsina, Katsina State.*
[2]*Microbiology Department, Umaru Musa Yaradua University Katsina, Katsina State.*

***Corresponding author:** Email: zarukumdw@gmail.com (+234 9022605232)

Abstract

The present study focused on the antimicrobial activity of crude fractions from the extract of *Piliostigma thonningii* leaves. The pulverized plant leaves were soaked in methanol and a solvent-based fractionation was carried out using *n*-hexane, dichloromethane and ethyl acetate. The crude fractions obtained were screened for antimicrobial activity against *Escherichia coli*, *Salmonella typhi, Staphylococcus aureus* and *Bacillus subtilis*. Among all the solvents, the *n*-hexane fraction of *Piliostigma thonningii* (PT02) showed highest antimicrobial activity against the tested organisms. At 250 mg/mL, the *n*-hexane fraction showed inhibition of 13.5±0.5, 10.5±0.5, 12±0 and 12±1 mm against *E. coli*, *S. typhi, S. aureus* and *B. subtilis* respectively. The lower Minimum Inhibitory Concentration (MIC) value of 15.6 mg/mL was obtained against *S. aureus*. Therefore, the study justifies the use of leaves extract of the *Piliostigma thonningii* as an antimicrobial agent.

Keywords: *Piliostigma thonningii,* crude fraction, isolation, antimicrobial activity.

Introduction

After the discovery of antibiotics, it was thought that infectious diseases would no longer exist. But due to irrational use of antibiotics, a number of bacterial strains with multi-drug resistance have emerged and due to infectious diseases millions of people die every year [1]. It is a bitter fact that infectious diseases are the leading cause of the premature deaths which result in about fifty thousand deaths annually around the globe [1]. The unnoticeable use of antimicrobials in developing and developed countries led to the creation of microbial resistance problems [2]. The scarcity of alternative antimicrobial agents in the drug discovery pipeline has caused the emergence of many types of infections [3]. The World Health Organization recognizes medicinal plants as one of the sources of new drug varieties [4]. This is because plants are naturally endowed with phytochemicals capable of curing a number of diseases.

The West African plant, *Piliostigma thonningii* called 'Kalgo' in Hausa belongs to the subfamily

© CSN Zaria Chapter

Caesalpinioideae in the legume family, Leguminosae/Fabaceae [5]. It grows in open woodland and savannah regions. It is widely distributed in Africa and Asia [6]. *Piliostigma thoninngii* is a deciduous and ever green shrub or tree with a rounded crown, it can grow 3-15 meters tall. The short bole is often crooked [7]. The pulp surrounding the seed has a sweet flavor, the flat woody pods are 15-20 cm long, they persist on the tree but eventually decay on the ground to free the pea sized seeds [7].

The plant is used for various medicinal purposes. The decoction of the leaves and bark is used for the treatment of ulcers, wounds, heart pain, arthritis, malaria, pyrexia, leprosy, sore throat, diarrhea, toothache, gingivitis, cough, and bronchitis. Its roots and twigs are used in the treatment of dysentery, fever, wound infections, cough and skin diseases. The crude extract of *Piliostigma thonningii* was reported to possess antilipidemic, antibacterial, antihelminthic, and antinflammatory activities [5]. The results obtained in a study by [20] suggest that *Piliostigma thonningii* aqueous leaf extract likely contains antilipidaemic and anticholesterolaemic substance(s), which may be useful in the prophylactic and curative management of lipid peroxidation, high blood pressure, and cardiovascular disorders. The various parts of *Piliostigma thonningii* (root, bark, seed, and fruit) are used to treat wounds, ulcers, gastric and heart pain, gingivitis, and as an antipyretic [8] [9]. In this work, the antimicrobial activity of the crude fractions of *P. thonningii* leaves was studied.

Materials and Methods

Sample Collection and Identification of Plant Material

Fresh leaves of *P. thonningii* were collected from Mai'adua town, Mai'adua Local Government Area, Katsina State, Nigeria. The plant was taxonomically identified and authenticated by a Botanist from the Biology Department, Umaru Musa Yar'adua University Katsina, Nigeria. A voucher specimen (Accession number UMYUH743) containing the identification characteristics was kept at the herbarium. The leaves were cleaned with tap water, dried at room temperature and pulverized. An electric blender was used to grind the leaves into fine powder and store them in a clean, airtight container prior to extraction.

Preparation of Crude Extract

The crude extract was prepared according to the method used by [10], [11] with some modifications. Powdered plant material (300 g) was macerated at room temperature in 2 L of 98 % methanol (ratio of 1:7 w/v) with occasional shaking for 72 hrs. The suspension was filtered using Buchner funnel and Whatman No.1 filter paper. Later, the extract was concentrated using rotary evaporator under reduced pressure at 40 ^{0}C to obtain methanolic crude extract which was weighed after drying and stored in a clean air tight

container. The crude Methanolic extract of the plant material obtained was subsequently fractionated using N-hexane, Dichloromethane and Ethyl acetate using the Kupchan scheme of fractionation. The N-hexane, dichloromethane, and ethyl acetate fractions were added into pre-weighed vials and labelled as PT02, PT03 and PT04 respectively. Their colors, textures, and yields were also recorded.

Antimicrobial Susceptibility Test

Antimicrobial activity of *P. thonningii* fractions (PT02, PT03 and PT04) was determined by the disc diffusion method as contained in the European Committee on Antimicrobial Susceptibility Testing and Clinical and Laboratory Standards Institute manual [12], [13], [14]. Four (4) different concentrations of the fractions (250 mg/mL, 125 mg/mL, 62.5 mg/mL and 31.2 mg/mL) were prepared using serial dilution method. Thus, prepared sterile culture media were poured into sterile plates (approximately 25 mL in each plate so that uniformity was maintained) and then kept at room temperature to solidify. A colony of 24 h culture of each isolate adjusted to 0.5 McFarland standard was spread evenly on the surfaces of the culture media plates using sterile swab sticks dipped into the isolate's suspension. After the cotton swab was streaked throughout the agar plates, the plates were kept aside for 5 minutes and fractions impregnated discs (6 mm) were aseptically placed on the agar surface with the aid of sterile forceps and ensured complete contact between the disc and the agar surface. Discs (6 mm) impregnated with ciprofloxacin (10 µg/mL) and 200 µg/ mL of 20% DMSO were used as positive and negative controls on separate media plates inoculated with the test organisms. The plates were incubated for 24 to 48 h at 37 ºC respectively. The susceptibility test was determined by measuring the diameter of zones of growth inhibition (mm) surrounding the discs and the readings were taken in 2 different fixed directions [12], [13], [14].

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined using serial dilution method. Varying amounts of the fractions in the following concentration order; 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml were prepared. The least concentration of the fractions that does not permit any visible growth of the inoculated test organisms in broth culture was taken as the minimum inhibitory concentration in each case. Control experiments of the antibiotic (ciprofloxacin) were also performed. [13], [14].

Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) was determined by sub-culturing from the tubes with concentrations equals to the MIC and above on fresh media plates. The lowest concentrations of the fractions associated with no visible bacterial growths were recorded [12], [13], [14].

Statistical Analysis

All experiments were performed in triplicate to safeguard the effectiveness of the method used. The zone of inhibition data is expressed as mean±standard error of the mean (SEM). The statistical difference of the mean zone of inhibition of the fractions for each individual isolate was carried out by employing One-Way Analysis of Variance (ANOVA).

Thin-Layer Chromatography (TLC)

The PT02 was used to isolate the bioactive compound(s) since the fraction has the highest activity. The fraction was spotted on analytical aluminium TLC plate and the following solvent systems and ratios were used as mobile phase to determine the eluent with optimum performance; n-hexane: EtOAc in graded mixture i.e. 9.5:0.5, 9:1, 8.5:1.5, 8:2, 7.5:2.5, 7:3, 6.5:3.5, 6:4, 5.5:4.5 and 5:5. After each separation, the TLC plates were visualized under UV lamp and also the plates were stained with potassium permanganate solution for visualization as well. The solvent system that provided the best resolution/separation was used for column fractionation.

Column Chromatography (CC)

Column separation of the PT02 was carried out with a glass column of internal diameter 80 mm and length 100 cm. A column grade silica gel G (70 g) of 60-120 mesh size was wet-packed using n-hexane: EtOAc (60:40) as the solvent system and loaded onto the column. After the silica had set, 3.5 g amount of the crude extract (PT02) was first dissolved in 10 ml of the solvent system and the slurry was loaded onto the wet packed column and continuously eluted with the mobile phase (n-hexane: EtOAc 60:40). Total of 30 fractions were collected while the eluents were monitored by observing the distance traveled by the sample down the column and were analyzed on TLC plates. Bands of the same sample spots formed on TLC plates were also monitored. Different sample spots formed on the TLC plates were visualized using a UV lamp, $KMnO_4$ solution and Iodine vapor. Fractions labelled A1-A9 showed similar TLC mobility and band formation; they were pooled together and evaporated at room temperature.

Micro column

Fractions labelled A1-A9 (120 mg) were re-chromatographed using a micro glass column of internal diameter 50 mm and length 30 cm over a silica gel eluting with n-hex: EtOAc (80: 20) to yield compound A3 (24 mg) ascertained on TLC (n-hexane: EtOAc; 8:2), the solvent evaporated under a steady air current at room temperature which was then transferred into a sterile bottle prior to analyses.

Fourier Transform Infrared Spectroscopy (FTIR)

The FT-IR spectrum of the compound was recorded on an Agilent Technology's 630 FTIR spectrophotometer for studying the presence of functional groups. The sample was analyzed using the machine's attenuated total reflectance (ATR) technique and the sample was scanned in the range of 4000-650 cm^{-1} wavenumber.

Nuclear Magnetic Resonance Spectroscopy (NMR)

The ^{1}H and ^{13}C analyses were conducted using deuterated chloroform ($CDCl_3$) as solvent on a Bruker NMR DBX-400 MHz spectrometer. The chemical shifts expressed in ppm were recorded.

Results

Table 1: Yield of Crude Extract and Fractions of *P. thonningii*

S/N	Fraction	Weight (g)	Yield (%)	Color	Texture
1	PT01	59.05	19.68	Deep green	Oily and sticky
3	PT02	10.15	17.19	Green	Sticky
4	PT03	8.57	14.51	Greenish-brown	Sticky
5	PT04	12.26	20.76	Green	Sticky

PT01= Crude Methanol fraction, PT02= *P. Thonningii* N-Hexane fraction, PT03= *P. Thonningii* dichloromethane fraction, PT04= *P. Thonningii* ethylacetate fraction

Table 2: Susceptibility Pattern of Fractions from *P. thonningii* against Clinical Isolates

Fractions	Conc. (mg/mL)	Organisms			
		E. coli	***S. aureus***	***S. typhi***	***B. subtilis***
		Mean Zone of Inhibition (mm) ± SD			
	250	13.5±0.5	12.0±0.0	10.5±0.5	12.0±0.0
	125	10.0±0.0	10.0±0.0	10.0±0.0	09.0±0.0
PT02	62.5	09.0±0.0	09.0±0.0	09.0±0.0	08.0±0.0
	31.2	08.0±0.0	09.0±.00	09.0±0.0	06.0±0.0
	250	11.5±0.5	09.0±0.0	08.0±0.0	11.0±0.0
	125	10.0±0.0	07.0±0.0	07.0±0.0	10.0±0.0
PT03	62.5	08.0±0.0	06.0±0.0	06.0±0.0	06.0±0.0
	31.2	07.0±0.0	06.0±0.0	06.0±0.0	06.0±0.0
	250	10.0±0.0	09.5±0.0	09.0±0.0	10.0±0.0
	125	09.0±0.0	08.0±0.0	08.0±0.0	08.5±0.0
PT04	62.5	08.0±0.0	08.0±0.0	07.0±0.0	08.0±0.0
	31.2	08.0±0.0	07.0±0.0	06.0±0.0	06.0±0.0
+ve control		46.0±1.5	30.0±0.0	30.0±0.0	59.0±0.0
-ve control		06.00	06.00	06.00	06.00

PT02= *P. Thonningii* N-Hexane fraction, PT03= *P. Thonningii* dichloromethane fraction, PT04= *P. Thonningii* ethylacetate fraction, -ve control= DMSO and +ve control= ciprofloxacin.

Table 3: Minimum Inhibitory and Minimum Bactericidal Concentration of *P. thonningiii*

		Test Organisms/Concentration (mg/ml)							
		E. coli		*S. typhi*		*B. subtilis*		*S. Aureus*	
S/N	Fraction	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	PT02	62.5	62.5	62.5	125	125	R	15.6	15.6
2	PT03	125	125	125	R	62.5	125	125	R
3	PT04	125	R	125	R	62.5	125	62.5	125
4	Control	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6

PT02= *P. Thonningii* n-hexane fraction, PT03= *P. Thonningii* dichloromethane fraction, PT04= *P. Thonningii* ethylacetate fraction, R= Resistant and control= ciprofloxacin.

Table 4: ^{1}H, ^{1}3C and NMR Spectroscopic Data of Compound A3

δH (ppm)	C-number	δC (ppm)	Type of C-atom
4.69	C1	109.26	CH_2
5.81	C2	150.50	CH
Not assigned	C3	146.94	C (Quaternary)
1.54	C4	31.08	CH_2
1.63	C5	29.78	CH_2
5.06	C6	124.47	CH
Not assigned	C7	122.19	C
1.26	C8	21.06	CH_3
0.86	C9	17.72	CH_3
1.98	C10	118.41	CH_2

Table 5: FTIR Spectroscopic Data of Compound A3

Wavenumber (cm^{-1})	**Functional Group**	**Assignment**
3354	Vinyl	=C-H Stretch
2922	Alkane	C-H Stretch
1640	Alkene	C=C Stretch

Discussion

Extraction and Fractionation

In this study, the maceration method of extraction and solvent partitioning (liquid-liquid extraction) using solvents of increasing polarities was adopted. The solvents used are *n*-hexane, Dichloromethane and Ethyl Acetate. Ethyl Acetate showed the highest fractionation yield (12.26g) in *P. thonningii* followed by Hexane fraction (10.15g), then Dichloromethane fraction (8.57g) using 59.05 g of the crude extract (Table 1).

Antimicrobial Activity

The antimicrobial potentials of the soluble fractions were tested against four potentially clinical
isolates viz; *Escherichia coli, Salmonella typhi, Staphylococcus aureus and Bacillus subtilis* at different concentrations of 250, 125, 62.5 and 31.2 mg/mL. The experiment was carried out to determine on which fraction the constituents of the leaves of *Piliostigma thonningii* that may be responsible for its antimicrobial activity is concentrated.

All the fractions evaluated exhibited varying degree of inhibitory effect against the standard
strain of human and animal pathogenic bacteria (Gram-positive as well as Gram-negative) as evidenced in the zones of inhibition (Table 2). But, there were differences in the spectrum of activity for the fractions, indicating that they contained different bioactive compounds at varying proportions. PT02 showed better activity against all the tested clinical bacterial isolates with MIC range of 15.6–125 mg/ml (Table 3).

A lower MIC value suggested that the fraction was highly efficient as an antibacterial agent in a little amount that might be below the extracts' toxicity level without endangering the host.

Among the tested clinical bacteria, *S. aureus* was the most sensitive bacteria followed by *B. subtilis* and lastly *E. coli* and *S. typhi* with similar sensitivities. An increase in growth inhibition was noted with an increase in concentration of the fractions. The growth inhibition was in the order of *S. aureus*> *B. subtilis*> *E. coli*=*S. typhi* and PT02>PT04>PT03 for the fractions. Sensitivity of these selected bacterial strains to the fractions supports the traditional use of *P. thonningii* as a potential in curing injuries, inflammation and pain, gastrointestinal problems, fever and malaria, gynecological and pregnancy disorders, ethnoveterinary medicine, haemorrhoids, skin diseases, and infections [9]. The results obtained clearly indicated PT02 to be more efficient in growth inhibition against the tested clinical bacterial isolates in comparison with the control (Table 2). The result also agrees with previous reports on the antimicrobial activities of *P. thonningii* [15].

Negative control (20 % DMSO) did not show activity on the clinical isolates. This agrees with the study conducted by [16] where he demonstrated the use of DMSO

as a chemical solvent in a concentration not exceeding 80 % as non-inhibitor against growth.

Similarly, [17] investigated the antimicrobial activity of marine cyanobacteria where DMSO was used as a negative control and no interference with the growth of the tested organisms was detected at the concentrations used.

NMR Spectroscopic and FTIR Spectroscopic Analyses

The ^{1}H NMR signal at δ 1.26 ppm and δ 0.86 ppm corresponds to both methyl groups at the C8 and C9 position. The signal at δ 4.69 ppm represents the proton at C1. The ^{1}H NMR signals in the region at δ 1.98 ppm corresponds to the proton at C10 and whereas the signals at δ 5.81 ppm and δ 5.06 ppm are of the protons at C2 and C6 respectively. The presence of three double bonds in the structure is evidenced by six ^{13}C signals at δ 109.26, 150.50, 146.94, 124.47, 122.19, 118.41 among which δ 118.41 and 109.26 corresponds to the two terminal double bonds in the structure. The NMR signals of sample A3 was compared and found to be almost matching in the same range with the reported NMR signals of b-myrcene [18], [19]. The FTIR data (Table 5) of compound A3 displayed an absorption band as thus; weak peak at 3354 cm^{-1} represents the =C-H stretching of C_{10}, C_2 and C_1. The broad absorption peaks at 2922 cm^{-1} are assigned to the asymmetric stretching vibration of the aliphatic moieties. The strong absorption peak at 1461 cm^{-1} is assigned to C_{10}=C_3 and C_2=C_1 stretching frequency. Due to conjugation, this stretching appears at a lower wave number value. A small peak at 1640 cm^{-1} can be ascribed to the presence of isolated C_6=C_7 in the structure. The characteristic absorption peaks around 1461 and 1371 cm^{-1} are due to the $–CH_2$ and $–CH_3$ bending vibration of C_4-C_5 and C_8-C_9 centers respectively.

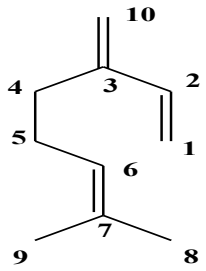


Figure 1: Proposed Structure of compound A3 (Mrycene)

Conclusion

P. thonningii are plants used by traditional healers and elderly people for the treatment of cholera, diarrhea, fever, piles and colds etc. The current work focused on the investigation of

phytochemicals, antibacterial activities of the leaves extract *P. thonningii* and isolation of pure compound in order to justify its use in traditional medicine. The study revealed the presence of phytochemicals considered as active medicinal chemical constituents in the fractions and good antibacterial activities against the clinical bacterial isolates especially the non-polar fraction of hexane (in comparison with the controls). Furthermore, the isolated compound from PT02 obtained from the fractionation of the crude extract was subjected to NMR and FTIR analyses. Based on the results, the compound was characterized as Mrycene, which is a well-known compound. In conclusion, the compound isolated, alongside other unidentified phytochemicals may be responsible for the observed biological activities. Thus, the pure compound isolated can serve as a lead compound in drug development.

References

[1]. Dubey, D., Sahu, M. C., Rath, S., Paty, B. P., Debata, N. K., and Padhy, R. N. (2012). Antimicrobial activity of medicinal plants used by aborigines of Kalahandi, Orissa, India against multidrug resistant bacteria. *Asian pacific journal of Tropical biomedicine*, *2*(2), S846-S854.

[2]. Ahmad, I., and Beg, A. Z. (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *Journal of Ethnopharmacology*, *74*(2): 113-123.

[3]. Munita, J. M., & Arias, C. A. (2016). Mechanisms of antibiotic resistance. Virulence Mechanisms of Bacterial Pathogens, 481-511.

[4]. World Health Organization. (2021). WHO report on the global tobacco epidemic, 2021: addressing new and emerging products. World Health Organization.

[5]. Michael. A., Radhakrıshnan, S.. Olay inka, T.A., Oluwale, B.F., Abayomi, O., Fazila, Z.., Mobarmed, A.L and Samir, A.R. (2018). Phytochemical study of *Piliostigma thorningii*, a medicinal plant grown in Nigeria. *Medicinal Chemistry Resource*. 27(10): 2325-2330.

[6]. Ajayi, J.A., Madara, A. A., Salawu, 0. A. and Tijani, A.Y. (2010). Anti-malarial activity of ethanolic leaf extract of *Piliostigma thoningii* Schun. (Caesalpiniacea) in mice infected with Plasmodium berghei. *African Journal of Biotechnology*. 9(23): 3475-3480.

[7]. Ruffo, E.K, Birnie, A, and Tengnas, B, (2000). Regional land management unit Nairobi. Edible wild plant Tanzania. 4(2): 77-80.

[9]. Maroyi, A. (2019). Review of ethnomedicinal, phytochemical and pharmacological properties of *Lannea schweinfurthii* (Engl.) Engl. *Molecules*, *24*(4), 732.

[10]. Tiwari, P., Kumar, B., Kaur, M., Kaur, G., and Kaur, H. (2011). Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Sciencia*, *1*(1), 98-106.

[11]. Pandey, A., and Tripathi, S. (2014). Concept of standardization, extraction and pre-phytochemicalscreening strategies for herbal drug. *Journal of Pharmacognosy and Phytochemistry*, *2*(5).443-447.

[12]. European Committee on Antimicrobial Susceptibility Testing (2013). Reading guide. EUCAST disk diffusion method for antimicrobial susceptibility testing. v 3.0, 2013. Available at: http://www.eucast.org).

[13]. CLSI (2018). Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—12th ed. M02-A13. Clinical and Laboratory Standards Institute, Wayne, PA, USA.

[14]. CLSI (2021). Performance Standards for Antimicrobial Susceptibility Testing, M100, 31st ed. Clinical and Laboratory Standards Institute, Wayne, PA, USA.

[15]. Echu, F.A. 2014. Phytochemical and antimicrobial studies of the leaves extract of *Piliostigma thonningii*. Masters thesis. Ahmadu Bello University Zaria.

[16]. Brito, R., Silva, G., Farias, T., Ferreira, P., and Ferreira, S. (2017). Standardization of the safety level of the use of DMSO in viability assays in bacterial cells.

[17]. Macieira, R.V.S. (2013). Antibiotic properties of marine cyanobacteria: in search of new antibiotics.

[18]. Rucker, G., Mayer, R., and Manns, D. 1987. a- and b-Myrcene hydroperoxide from Artemisia annua. Journal of Natural Products. 50(2):287–289.

[19]. Sahu, P., and Bhowmick, A.K. 2019. Redox emulsion polymerization of terpenes: mapping the effect of the system, structure, and reactivity. Industrial and Engineering Chemical Research. 58(46):20946–20960.

[20]. Ighodaro, O.M. and Omole, J.O. (2012). Effects of Nigerian *Piliostigma thonningii* species Leaf Extract on lipid Profile in Wistar rats. International scholarly research network of pharmacy. 4 pages.