

Antibacterial activity of the leaf extract of *Piliostigma thonningii* against *Salmonella typhi* and *Shigella dysenteriae*

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ABSTRACT

The cold maceration, qualitative phytochemical analysis and agar well diffusion methods were applied to investigate the antibacterial activity of *Piliostigma thonningii* using hexane and water as extracting solvents. The phytochemical analysis reveals the presence of tannins, terpenines, flavonoids, alkaloids, steroids and phenol while glycosides were absent. Tannins, terpenes, alkaloids and phenol were present in both solvent extract while flavonoids, glycosides and steroids were absent in the hexane extract. Sensitivity result shows that the aqueous and hexane extracts had activity against the test organisms with mean zones of inhibition in millimeter of 17.33 ± 0.57 and 15.33 ± 1.15 at 30 mg/ml against *Salmonella typhi*, while the mean zone of inhibition against *Shigella dysenteriae* were 14.33 ± 0.57 and 11.00 ± 1.0 at 30 mg/ml for aqueous and hexane extracts, respectively. The least inhibition zones, 9.66 ± 1.15 and 11.00 ± 1.0 at 20 and 30 mg/ml respectively of the hexane extract were recorded against *S. dysenteriae*. The leaf extract exhibited the lowest MIC (1.8 mg/ml) against *S. dysenteriae* as compared to 15 mg/ml against *Salmonella typhi*. Summarily, the activity of the plant extract might be concentration dependent and therefore, based on the result of this research work a higher concentration and purification of the crude extract is recommended to achieve a considerable antimicrobial activity.

Keywords: *Piliostigma thonningii*, terpenines, cold maceration, *Salmonella typhi*, hexane.

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INTRODUCTION

The use of plant compounds for pharmaceutical purposes has gradually increased worldwide. Unlike pharmaceutical drugs which are mainly synthetic, herbs are easily assimilated in the body, they are easily eliminated and do not usually accumulate (Daniyan et al., 2012). The persistent increase in antibiotic resistant strain of microbes has brought about the production of more effective synthetic antibiotics by pharmaceutical companies (Abinu et al., 2004). Due to economic crisis, high cost, side effects and inefficient availability of these synthetic drugs particularly in developing countries (Johann et al., 2007). According to World Health Organization (WHO), medicinal plants could be the best source of drugs variety (Cohen, 1992). Herbal medicines

are the foundation for modern therapeutic agents (Daniel et al., 2012). They contain parts of plants or other plant material as active ingredients (WHO, 2008). The plants materials include seeds, berries, roots, leaves and bark (Ehrlich, 2010). It is also recognized by the WHO that herbal medicines are the most popular form of traditional medicine, and are highly lucrative in the international market. Also there is limited scientific evidence from studies done to evaluate the safety and effectiveness of traditional medicine products and practices (WHO, 2008). *Piliostigma thonningii* is a leguminous plant belonging to the family Caesalpiniaceae. The tree is perennial in nature and its petals are white to pinkish coloured, produced between November and April. The plant grows



Figure 1. Picture of leaf of *Piliostigma thonningii*.

to a height of 8 m with branches. It has a large two-lobed simple leaves without thorns or spines (Burkill, 1995) (Figure 1). Its leathery green leaves measure up to 15 × 17 cm, bi-lobed one eighth to one third the way down with a small bristle in the notch, glossy above and heavily veined and somewhat rusty-hairy below without thorns or spines (Daniyan et al., 2010). It is a multipurpose plant of vast economic importance (Daniyan et al., 2012) and it possesses edible and chewable leaves which is believed to relieve thirst. The cold maceration (using hexane and water as extracting solvents), qualitative phytochemical analysis and agar well diffusion methods were employed to investigate the claims of local herbal medicine marketers and consumers of the use of *P. thonningii* (cattle leg) for the treatment of typhoid fever, diarrhea, dysentery and intestinal upset.

MATERIALS AND METHODS

Sample collection and processing

Fresh leaves of *Piliostigma thonningii* were collected from the Federal Housing Estate, Girei Local Government Area, Adamawa State, Nigeria. The plant was identified in Plant Science Department, Modibbo Adama University of Technology Yola, Adamawa State by Professor Dimas Kumbrawa. The fresh leaves were washed and dried under shade until a constant weight is obtained. The dried leaves were crushed to a semi-powdery texture with the use of mortar and pestle (this is to aid the penetration of solvents and enhance the yield) while the test organisms were obtained from stock cultures in the Microbiology laboratory.

Extraction

The cold maceration extraction method was used. The plant materials were soaked for 72 h (3 days) with intermittent shaking (Ewansiha et al., 2012). After soaking it was filtered using muslin cloth. The filtrate was then centrifuged in a test tube at 3000 rpm for 3 min and the supernatant poured out. The residue was poured in a

conical flask and evaporated to almost dryness (this is to prevent the extract from sticking to the walls of the flask and to enable complete recovery of all extract) and then it was freeze-dried to complete dryness. After the samples have evaporated the weight of the extract was determined.

Phytochemical screening

Phytochemical screening was done according to the method of Odebiyi and Sofowora (1999). The components screened for are tannins, terpenines, flavonoids, alkaloids, steroids, phenol and glycosides.

Preparation of extracts

One hundred milligram (100 mg) and 150 mg each of the hexane and aqueous extracts were weighed separately and dissolved into 5 ml of 10% dimethyl sulfoxide (DMSO) to give 20 and 30 mg/ml respectively which was used for the antibacterial sensitivity test.

Antibacterial sensitivity test

Standardization of inoculum

The sensitivity test was determined by the use of the well diffusion method according to National Committee for Clinical Laboratory standards (NCCLS). Three to five (3 to 5) identical colonies from each agar plate were subcultured onto a test tube containing 5ml of nutrient broth. The turbidity of each of the microbial suspension was adjusted to reach an optical comparison of 0.5 McFarland turbidity standards, resulting in a suspension containing approximately 0.5×10^6 CFU/ml.

Susceptibility test

One millilitre (1 ml) of each standardized microbial test suspension was used to inoculate the entire surface of Muller-Hinton Agar using the spread plate method. After the agar plate had dried for about 5 to 10 min, 6 mm diameter well was bored in each agar plate using a sterilized 6 mm diameter cork borer and the base was sealed with

molten Muller-Hinton Agar to prevent unnecessary spread of the extract. Each extract was checked for antimicrobial activity by introducing 1 ml of different concentrations of the extracts (20 and 30 mg) into triplicate wells. The plate was allowed to stand at room temperature for 15 min, to allow for the diffusion of the extract into the agar. The agar plates were incubated at 37°C for 24 h. Subsequently, the plates were examined for inhibition which is indicated by a cleared zone around the wells. The inhibition zone diameter (IZD) was measured to the nearest millimeter. Antibiotic disc of Ciproflaxin (10 µg/disc), metronidazole (30 µg/disc), and distilled water was used as positive and negative control respectively.

MIC and MBC assay

The micro tube dilution method was used to determine the minimum inhibitory concentration (MIC). Serial dilutions of the crude extracts were prepared to give a geometric decrease in concentration (ranging from 30, 15, 7.5, 3.75 and 1.8 mg/ml). This was achieved by diluting 150 mg of the crude extract in 5 ml of 10% dimethylsulfoxide (DMSO) in a test tube labelled A. From test tube A, 1 ml was transferred to a second test tube labelled B and 1 ml of 10% dimethylsulfoxide was added to test tube B (this will give 15 mg/ml w/v). This procedure continued until a concentration of 1.8 mg/ml in test tube E is obtained. One milliliter (1 ml) of the serially diluted leaf extract (30, 15, 7.5, 3.75 and 1.875 mg/ml) was dispensed into sterile test tubes and each of the test tubes were inoculated with a loop full of the standardized test organisms. Also, sterile nutrient broth was dispensed in test tubes without inoculation, which served as the control. The concentration of the least clear tube after incubation that shows no visible turbidity when compared with the control was recorded as the minimum inhibitory concentration (MIC) of the extract in mg/ml. The minimum bactericidal concentration (MBC) was determined by subculturing the tubes that shows no visible turbidity beginning with the MIC on to a freshly prepared Muller-Hinton agar plates and incubated for 18 to 24 h at 37°C. The concentration of the tube dilution that yielded no single microbial colony on the agar plates was recorded as the MBC of the extract in mg/ml.

RESULTS AND DISCUSSION

The result of the phytochemical screening on *P. thonningii* showed that of the seven active ingredients screened for, six were present in the plant namely: tannins, terpenines, flavonoids, alkaloids, steroids and phenols (Table 1). Glycosides was absent in both solvent extract while flavonoids and steroids was only present in the aqueous extract. This might be attributed to the fact that secondary metabolites in plant are a group of compound classes occurring naturally and biosynthesized by differing biochemical pathways whose content and regulation is highly susceptible to environmental influences and to potential herbal predators. Such abiotic and biotic factors might be specifically induced by means of various mechanisms, which create variation in the accumulation or biogenesis of secondary metabolites (Daniel et al., 2012). The presence of these phytochemical compounds may be responsible for its antibacterial activity as confirmed by Robinson (2006); owing to the fact that these plants demonstrate considerable antibacterial activity as seen in

Table 1. Phytochemical constituents of the leaf extract of *Piliostigma thonningii*.

Phytochemical constituents	Leaf	
	ALE	HLE
Tannins	+	+
Terpenines	+	+
Flavonoids	+	-
Alkaloids	+	+
Glycosides	-	-
Phenol	+	+
Steroids	+	-

Key: ALE = Aqueous leaf extract, HLE = Hexane leaf extract.

the result of the sensitivity test (Table 2). Rios and Recio (2005) also described medicinal plants as one in which one or more of its organs contain substances that can be used for therapeutic purpose. According to Kolodziej and Kiderlen (2005), tannins and phenolic compounds have been found to inhibit bacterial and fungal growth and also capable of protecting certain plants against infection. Tannins have also been reported to have antidiarrheal, homeostatic and antihemorrhagal activity (Akiyama et al., 2001). The result of this study confirms partly the above claims. The antibacterial activity test as depicted by the inhibition zone diameter was seen to be concentration dependent, that is, the higher the concentration, the higher the inhibition zone diameter. Ciprofloxacin and metronidazole, the positive control had higher activity against the test as compared to the extract. This could also be attributed to the fact that the antibiotics are in their pure form while the extract is still in the crude form and needed to be purified to remove all inhibitory substances to its activity. The minimum inhibitory concentration (MIC) is the smallest concentration capable of inhibiting the growth of the organisms, while the minimum bactericidal concentration (MBC) is the concentration capable of killing the organism. Going by this definition, the MIC and MBC (Table 3) result gives a clue as to the required dose that can give desired result against the test organisms if implicated in any infection. According to Suffredini et al. (2006), the antibacterial activity of plant extract is considered significant if the MIC of the extract is less than or equal to 200 mg/ml. This is an indication that the leaf extract of *P. thonningii* exhibited significant antimicrobial activity against the tested organisms.

CONCLUSION

The presence of phytochemical compounds such as flavonoids, alkaloids, glycosides, terpenes, tannins, steroids and phenol provides a confirmation to the antibacterial potency for the use of this plant in traditional medicine to treat ailments. Based on these findings, the

Table 2. Mean zones of inhibition of aqueous and hexane extracts of *Piliostigma thonningii* (mm).

Test organism	ALE		HLE		CPX	MT	DW
	20 mg/ml	30 mg/ml	20 mg/ml	30 mg/ml	10 µg/ml	30 µg/ml	1 ml
<i>Salmonella typhi</i>	15.33 ± 1.53	17.33 ± 0.57	15.66 ± 0.57	15.33 ± 1.15	24.66 ± 1.53	*-	-
<i>S. dysenteriae</i>	13.0 ± 1.0	14.33 ± 0.57	09.66 ± 1.15	11.00 ± 1.0	*-	22.0 ± 1.73	-

Key: ALE = Aqueous leaf extract, HLE = Hexane leaf extract, CPX = Ciprofloxacin, (*) = Not applicable, (-) = No reaction, MT = Metronidazole and DW = Distilled water.

Table 3. MIC and MBC of *Piliostigma thonningii* leaf extract against *Salminella typhi* and *Shigella dysenteriae* (mg/ml).

Organism	MIC		MBC	
	ALE	HLE	ALE	HLE
<i>Salmonella typhi</i>	3.75	3.75	15	15
<i>Shigella dysenteriae</i>	1.8	1.8	7.5	15

Key: ALE = Aqueous leaf extract; HLE = Hexane leaf extract; MIC = Minimum inhibitory concentration; MBC = Minimum bactericidal concentration.

aqueous leaf extracts is most active against *S. dysenteriae* considering the result of the MIC. Also, the result of these findings confirms the claims by local user against enteric infection due to the test organisms and it is therefore recommended that purification of the crude extract be done to ascertain the particular compound that is involved in the antimicrobial activity and clinical test to ascertain its potency *in vivo*.

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