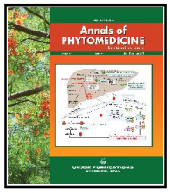
Annals of Phytomedicine 12(2): 765-768, 2023

DOI: http://dx.doi.org/10.54085/ap.2023.12.2.89

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Phytochemical analysis and *in vitro* anthelmintic activity of methanolic extract of *Samanea saman* (Jacq.) Merr. leaves

B. Srilehka, K. Raveendra Babu◆, S.K. Naseer Jani, G. Navya Sri, Jahangir Alom and M. Chinnaeswaraiah
Department of Pharmacy Practice, Anurag Pharmacy College, Kodad-50806, Telangana, India

Article Info

Article history
Received 11 July 2023
Revised 29 August 2023
Accepted 30 August 2023
Published Online 30 December 2023

Keywords
Samanea saman (Jacq.) Merr.,
Paralysis
Earthworm
Anthelmintic activity
Piperazine citrate

Abstract

Soil transmitted helminth (STH) infections are among the most common infections worldwide with an estimated 1.5 billion infected people or 24% of the world's population. The high cost of usual anthelmintic drugs and the development of anthelmintic resistance led to evaluation of remedial plants as an alternative source of anthelmintics. *Samanea saman* (Jacq.) Merr. is a popularly grown tropical avenue tree in Asia and Northern America, a member of the Fabaceae. It has antiulcer, antibacterial, antimicrobial, anthelmintic properties. Current study, the methanol extract of leaves of *S. saman* shows anthelmintic activity and shows a positive correlation to the alcohol, and flavonoids contents. The present research aimed to assess the anthelmintic activity of methanolic extract of *S.saman* leaves by *Pheretima posthuma* as test worms. *Piperazine citrate* (15 mg/ml) used as standard. Paralysis and death times were examined, the activity was correlated with piperazine citrate. At higher doses (500 mg/ml), the shortest paralysis time was noted. The earthworm utilized in the study was significantly affected by the methanolic extract of *S. saman* leaves, correlated by earthworm's paralysis and death.

1. Introduction

Samanea saman (Jacq.) Merr., member of the fabaceae family, is used in herbal medicine as an analgesic, anti-inflammatory, diuretic, febrifuge, anthelmintic, and antifungal, as well as for digestive issues. Word "helminth" is originated from Greek word "helminthes," means "worm, refers to a large category of parasitic worms that live inside the body (Patel, *et al.*, 2010). Over two billion people have parasitic worm diseases, according to the World Health Organization. By 2025, it is predicted that 57% of people living in developing nations would be affected (Mulla *et al.*, 2010). Helminth infections are a major contributor to these, particularly in small ruminant production, which results in significant economic losses, including loss of production due to death, weight loss, and decreased milk and meat production (Ketzis *et al.*, 2003; Githiori *et al.*, 2003). Antihelmintics are medicines that either eliminate or kill helminths that are present in gastrointestinal tract, despite the fact that certain species migrate inside tissues or reside there. They injure the host by depriving him of food, resulting in blood loss, organ damage, intestinal, secreting toxic substances, and causing injury to organs. Despite being a major cause of morbidity, helminthiasis seldom results in death (Bundy, 2004). In endemic locations, internal parasitic illnesses hit the population with relentless morbidity. The abdomen, small intestine and large intestine are all infected by these parasites. The aim of research was to determine the anthelmintic activity of methanolic extract of leaves of *S. saman* by *P. posthuma* as test worms.

Corresponding author: Dr. K Raveendra Babu,
Associate Professor, Department of Pharmacy Practice, Anurag Pharmacy College, Kodad-508206, Telangana, India
E-mail: ravipharma36@gmail.com
Tel.: +91-9959212028

The popular names in english include saman, rain tree and monkey pod**.** Scientific classification of Taxonomy Kingdom: Plantae, Order: Fabales, Family: Fabaceae, Genus: *Samanea saman* (Jacq.) Merr.. It exhibits the synonym names of *Samanea saman* such as *Albizia saman, Enterolobium saman, Inga saman, Pithecellobium saman and Mimosa saman*. *S. saman* on the other hand; leaves are arranged next to twigs and have a noticeable enlargement at the base of the petiole. The leaf measures 2-4 cm when stretched and 1-2 cm when expanded. The largest apical flyers and foliage are grouped into 2-6 sets of pinnae, with each pinna containing 6-16 precious stone-shaped promotions and having stipules that resemble yarn in the leaves. The leaf appears to have sharp edges and is twice as intricately pinnate. The leaf has a spherical organ and a silky state. For a brief time, the leaf appears dry and dreary because of its lustrous green top and its meagerly bristly base. *S. saman* trees lose their leaves in the summer because they are semi deciduous is because of this that it remains leafless for a while before defoliating as soon as there is enough moisture. *S. saman* (rain tree), in moist conditions, appears to be evergreen as a result. Piperazine citrate is the reference standard drug utilized in the study. As a result of the muscle being hyperpolarized, chloride channels are opened, which relaxes the muscle and reduces the ability of acetylcholine to contract the muscle, resulting in flaccid paralysis.

2. Materials and Methods

2.1 Drugs and chemicals

Each chemical employed in the investigation is of analytical quality such as piperazine citrate (SD Fine Chemicals Ltd., Mumbai) and Methanol (Merck, India).

2.2 Plant material

Plant fresh leaves are received from the village of Ananthagiri of District Suryapet from Telangana. The leaves were cleaned to remove unwanted material. Botanists verified the authenticity of the plant material, Dr. K. Srinivasa Reddy, Assistant Professor, Department of Botany, Govt. Degree College for Woman, Nalgonda, Telangana. A voucher specimen was deposited as Herbarium, in Department for further use (Pcog-2023/3).

2.3 Preparation and preliminary phytochemical screening of the extracts

The shade-dried leaves were then chopped up into large pieces and allowed to macerate in water with chloroform and methanol for 72 h before being extracted using a Soxhlet. To stop evaporation, aluminum foil was used to cover this entire setup (Kuntal Das *et al*., 2021). After extraction, the extract is cleaned using muslin that has been folded twelve times before being concentrated in a rotary evaporator at a temperature of 45°C. Scratching is used to remove dried extract, and the percentage of yield, colour, and uniformity has been taken into consideration. The final product was employed for anthelmintic activity screening, quantitative phytochemical tests, and phytochemical screening (Sudhakar *et al*., 2022).

2.4 Phytochemical screening

Preliminary analysis of extract done to identify the different phytoconstituents by employing routine protocols (Chaudhary *et al.,* 2010).

2.5 Test organism

Indian earthworms (Annelida: *Pheretima posthuma*) had been collected from nearby soil patches, the mean size found to be approximately 6-8 cm. They live between three and ten years (Kutschera *et al.,* 2010). The clinging particles were removed by washing them with tap water; it is a suitable model for testing anthelmintic drugs owing to its accessibility and physiological and anatomical similarity to intestinal roundworms (Vigar, 1984). Before the initiation of experiment, the earthworms were washed with normal saline (Sudhakar *et al.,* 2023).

2.6 Preparation of piperazine citrate

Piperazine citrate (15 mg/ml) was produced using 0.2% v/v tween 20 as a suspending agent.

2.7 Anthelminthic activity

The assay was performed on adult Indian earthworm *P. posthuma* (Ajayieoba *et al.,* 2001) with a few adjustments. 20 ml of a formulation containing three distinct strengths of crude methanol extract (100, 250, and 500 mg/ml in normal saline), each concentration in its own petri dish. All three extracts and the standard solution were newly constituted just before execution of the experiment. Earth worm was freed in each petri dish and examined for paralysis, followed by death time. When there was no visible movement other than when the parasite was vigorously agitated, an average time for paralysis (in min) was noted, time for death of worms (in min) was noted following worms' loss of motility, subsequently fading away of the body color, by observing keenly with the naked eye as well as with the aid of a hand lens magnifier. *Piperazine citrate* (15 mg/ ml) was used as standard reference. This outcome was tested three times. The result of anthelminthic activity is tabulated. Group I served as the control group, Group II received the standard medicine (piperazine citrate), and Groups III, IV, and V received various amounts of methanolic plant extracts for the anthelmintic activity. All the results were expressed as mean ± S.D. of six animals in each group and are tabulated and graphically represented in Table 2 and Figure 1.

3. Results

Preliminary phytochemical screening has revealed the presence of carbohydrates, alkaloids, glycosides, steroids, terpenoids, tannins, and phenolic substances in methonolic leaf extracts was illustrated in Table 1. The above constituents provide evidence that some of these phytochemical components may produce antihelminthic action.

Table 1: Preliminary phytochemical screening of methanolic extract of *S. saman* leaves

1	**Test for carbohydrates**	
	Molish test	+
	Fehling test	+
	Benedict test	+
	Barfoed test	+
2	**Test for proteins and amino acids**	
	Million test	–
	Biuret test	–
	Ninhydrin test	–
3	**Test for fixed oils and fats**	
	Saponification test	–
4	**Test for alkaloids**	
	Mayers test	+
	Wagners test	+
	Dragendroff test	+
	Hagers test	+
5	**Test for glycosides**	
	Kellar killani test	+
	Borntragers test	–
6	**Test for steroids and terpenoids**	
	Liebermann-burchard test	+
	Salkowski test	+
7	**Test for tannins and phenolic compounds**	
	Ferric chloride test	+
	Lead acetate test	+
	Bromine water	+
8	**Test for flavonoids**	
	Shinoda test	–
	Alkaline reagent test	–
9	**Test for saponin glycosides**	
	Foam test	–
	Haemolysis test	–

Table 2: ***In vitro*** **anthelmintic activity of methanolic extract of** ***S. saman*** **leaves**

Treatment groups	Group	Conc. (mg/ml)	Paralysis time (min) (Mean ± S.D.)	Death time (min) (Mean ± S.D.)
Control	I	—	—	—
Standard piperazine citrate	II	15	22.66 ± 6.18	74.33 ± 2.054
Methanolic leaf extract	III	100	88.00 ± 3.0	190.66 ± 2.51
	IV	250	43.33 ±3.21	145.00 ± 2.0
	V	500	33.33 ± 4.50	81.33 ± 3.05

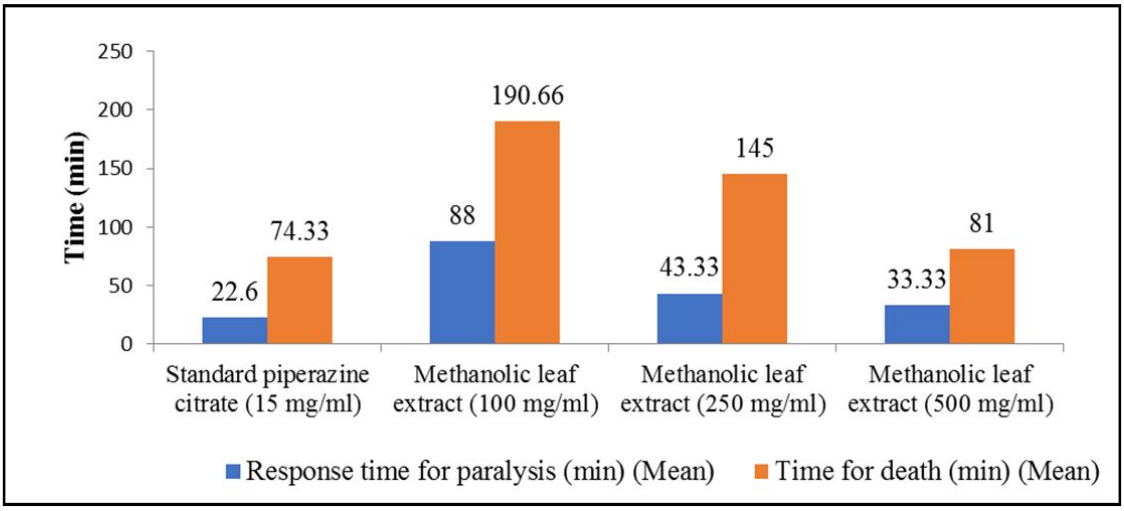


Figure 1: ***In vitro*** **anthelmintic activity of methanolic extract of** ***S. saman*** **leaves.**

4. Discussion

An anthelmintic medication such as piperizine citrate is an example that kills worms by paralysing them and forcing their elimination in both animal and human faeces. Preliminary phytochemical screening has shown the existence of carbohydrates, alkaloids, glycosides, steroids, terpenoids, tannins and phenolic compounds in methanolic extract. The existence of these secondary metabolites may fabricate antihelminthic activity. Infections caused by parasites can affect people. Immature forms develop into well differentiated worms with tissue distribution features after entering humans through the skin or gastrointestinal system (GIT). Drugs known as anthelmintics work either locally to eliminate worms from the GIT or systemically to eliminate fully developed helminthes or their development forms that cause organ and tissue damage (Singh *et al*., 2002). Methanolic extract of *S. saman* plant of concentrations, 100, 250, and 500 mg/ml evidenced paralysis at 88.00, 43.33 and 33.33 min and death at 190.66, 145.00 and 81.33 min. Piperazine citrate manifested paralysis at 22.66 min and death 74.33 min at 15 mg /ml, respectively, shown in Table 2. From the above outcome, it is clear that methanolic extracts of *S. saman* plant have anthelmintic activity in terms dose dependent method when correlated with standard anthelmintic drug shown in Figure 1.

Earthworms can move by using their cilia. The mucilaginous outer layer of the earthworm is composed of intricate polysaccharides. This layer is slimy, allowing the earthworm to move about without restriction. The outer layer will be exposed, if the mucopolysaccharide membrane is damaged, which would limit its ability to move and may even result in paralysis (Sudhakar *et al.,* 2023). This strategy may cause injury to the mucopolysaccharide layer, which would kill the worm. The body gets stressed by this, resulting in paralysis. All anthelmintics kill worms by starving them to death or paralyzing them. Worms are unable to retain energy, thus they must eat almost continually to meet their metabolic needs. Every time, this process ceases, energy is lost. By depriving them of food for 24 h less, the majority of adult parasites can be destroyed. Paralyzed parasites will also perish as a result of their inability to maintain their position in the gut. During a preliminary phytochemical screening, these compounds were found: alkaloids, tannins, glycosides, phenols, and saponins. According to reports, tannins hinder energy generation by decoupling oxidative phosphorylation from other reactions. Tannin also has the capacity to connect with free proteins in the host animal's digestive system or with a glycoprotein on the cuticle of the worms, which results in death. According to sources, the plant's tannins can improve a person's ability to absorb protein (Suman *et al*., 2022). Lower rates of nematode worm infection were seen in the host animal as a result of increased protein absorption (Patel *et al*., 2010), whereas hydrogen bonding is responsible for tannin's direct impact on the nematode cuticle. This response stiffens the skin, paralyzing it and killing the nematodes (Vidyadhar *et al*., 2010). The inhibitory impact of sucrose transfer to the small intestine is provided by the alkaloids and steroids in the extracts, which can lower worm glucose support (Rana *et al*., 2021). Together with flavonoids' antioxidant properties, these effects can lessen the amount of nitrate produced for use in protein synthesis (Cruz, 2008). Worm paralysis and death may also result from saponin and tannin damage to the mucopolysaccharide

membrane. The surface muscles are shielded by this mucoid-shaped membrane, a mucilaginous builder. The membrane disruption will expose the outer layer and enable the worm's body to absorb the chemical content of the extract (Mulla *et al*., 2010; Singh *et al.,* 2002). These findings suggested that *S. saman* leaf extract might be utilized as a substitute anthelmintic.

5. Conclusion

A significant improvement in the treatment of helminthic diseases will result from the identification of an effective drug of plant origins. The use of these plants in conventional medicine demonstrates that they provide an accessible and reliable alternative to treat infectious diseases. The methonolic extract of *S. saman* leaves confirms the presence of phytochemicals, such as total carbohydrates, alkaloids, steroids, phenolic compounds and tannins, supports the biological activities of the plants and also explains their medicinal applications. The present study concludes that the methanolic extract of *S. saman* leaves exhibit significant anthelmintic activity against *P. posthuma* in dose dependent manner. Further investigations are needed for the isolation and identification of the active components and to elucidate its mechanisms of action.

Acknowledgements

The authors wish to thank the management and Professor M. Chinnaeswaraih, Principal of Anurag Pharmacy College, Kodad, Telangana, India for providing requisite equipment facilities for research, encouragement, and support.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **B. Srilekha, K. Raveendra Babu, S.K. Naseer Jani, G. Navya Sri, Jahangir Alom and M. Chinnaeswaraiah (2023). Phytochemical analysis and *in vitro* anthelmintic activity of methanolic extract of *Samanea saman* (Jacq.) Merr. leaves. Ann. Phytomed., 12(2):765-768. http://dx.doi.org/10.54085/ap.2023.12.2.89.**