



ASSESSMENT OF ANTHELMINTIC ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *PSIDIUM GUAJAVA* LEAVES: AN *IN-VITRO* DESIGN

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ABSTRACT

Back ground: In the recent years, the use of herbal products has been increasing in developing countries. *Psidium guajava* Linn. (Guava), family: Myrtaceae is an important dietary plant used traditionally for medicinal purpose around the world. Objectives: The main objective of this research is to investigate the phytochemicals present in leaves and screen the anthelmintic activity of *Psidium guajava* leaves against Indian adult earthworms *Pheretima posthuma*. Method: The phytochemical screening was done by following standard protocols. The Anthelmintic assay was carried as per the method followed by Ajaiyeoba EO et. al, with minor modifications. Three concentrations (25, 50 and 100 mg/ml) of extract were studied, which involved for the determination of time of paralysis and time of death of the test worms. Results: It was found that ethanol extract of *Psidium guajava* leaves exhibited significant anthelmintic activity at high conc. (100 mg/ml). Albendazole in same concentration as that of extract was used as standard reference and saline water as control. Conclusion: Findings of the present investigations confirms that, the ethno-medicinal claim of anthelmintic activity of this plant is genuine. Therefore, it can be used as a source of natural anthelmintic and used in drug formulations for treatment of diseases.

Key words: *Psidium guajava*, Albendazole, tannins, anthelmintic activity, phytochemicals.

INTRODUCTION

Worldwide, the use of traditional medicines (TMs) has a long history and encompasses an easily accessible and affordable source of treatment. In India, one of the earliest uses of TM is cited in Rig Veda, a compilation of Hindu holy verses (1600–3500 BC). Traditional use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as ‘traditional herbal medicines’. In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs¹. Although modern medicine may exist side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons. Such products have become more widely available commercially, especially in developed countries. In this modern setting, ingredients are sometimes marketed for uses that were never contemplated in the traditional healing systems from which they emerged².

Guava (*Psidium guajava* Linn.) belonging to family Myrtaceae is a traditionally used plant because of its food and nutrition value. Guava is widely grown in tropical and many areas like India, Bangladesh, Florida, and West Indies. Different parts of the *Psidium guajava* are reported to be used in folk medicine. Various parts of the plant like root, bark, leaves and fruits are found to possess many pharmacological properties as it is used in the treatment of various disorders^{3,4}.

Psidium guajava, the common guava, yellow guava, or lemon guava is an evergreen shrub or small tree, native to the Caribbean, Central America and South America. It is easily

pollinated by insects; in culture, mainly by the common honey bee. Owing to its hardy nature, guava is grown successfully in tropical and subtropical regions up to 1,500 m (4,900 ft) above mean sea-level. Best quality guavas are obtained where low night temperatures, 10 °C, prevail during winter. It tolerates high temperatures and drought conditions in North India during summers but it is susceptible to severe frost as it can kill the young plants. An annual rainfall of about 100 cm (39 in) is sufficient during the rainy season (July–September). The rains during harvesting period, however, deteriorate the quality of fruits. Guava is cultivated on varied types of soils, from heavy clay to very light sandy soils. Nevertheless, very good quality guavas are produced in river-basins. It tolerates a soil pH of 4.5–8.2. Maximum concentration of its feeding roots is available up to 25 cm (9.8 in) soil depth. Thus the top soil should be quite rich to provide enough nutrients for accelerating new growth which bears fruits^{5,6}.

Psidium guajava tree is a small shrub-like tree up to 10 m in height. It is a shady tree with white and scented flowers. It is cultivated for its fruit and medicinal uses. *Psidium guajava* fruit (Guava) is an ethnomedicine. It has special importance in the traditional system of medicine⁷. In Indian Ayurveda, it is considered as an important herbal medicine for dysentery and diarrhea. In Traditional Chinese Medicine system, it is used to treat many diseases. It has been used since ages to improve the health of humans. *Psidium guajava* plant is full of medicinal values. Many parts of this plant possess the medicinal properties. Fruits, seeds, and leaves are the edible parts of this plant. Many delicacies are produced from the fruit and can be eaten raw too. Leaves are also used in cooking and seeds are used for oil extraction^{8,9}.

The simple leaves are oppositely arranged along the stems and are borne on short stalks (petioles) 4-10 mm long. The leaf blades (7-15 cm long and 3-7 cm wide) are somewhat oval in shape (ovate-elliptic or oblong-elliptic) with rounded or pointed tips (obtuse or acute apex) and rounded (obtuse) bases. They have hairy (pubescent) undersides (especially when young), entire margins, and are generally dull green in colour. Each leaf has a prominent central vein (midrib) and 10-20 pairs of side veins (lateral veins) that are also relatively obvious^{10,11}.

***Psidium guajava* plant taxonomy^{12,13}**

Kingdom	: Plantae
Subkingdom	: Viridiplantae
Super Division	: Embryophyta
Division	: Tracheophyta (Tracheophytes or Vascular Plants)
Sub Division	: Spermatophytina
Class	: Magnoliopsida
Super Order	: Rosanae
Order	: Myrtales
Family	: Myrtaceae
Genus	: <i>Psidium</i>
Species	: <i>Psidium guajava</i> L.

Parasitic infections remain a major constraint to livestock production globally. One practical way of developing cheaper and effective anthelmintics is to study indigenous herbal remedies¹⁴. Evaluation of the activities of medicinal plants claimed for anthelmintic property is getting attention these days. There have been many reports, mainly from Africa, indicating the effectiveness of plant products against helminth infections in animals¹⁵⁻¹⁷.

The main objective of this research is to evaluate the phytochemical composition and screen the anthelmintic activity of *Psidium guajava* leaves against *Pheretima posthuma*.

MATERIALS AND METHODS

Collection of the plant

The fresh leaves of *Psidium guajava* were collected in the month of January from Vrukshashala, Medicinal Garden, School of Pharmacy, Anurag Group of Institutions, Venkatapur, Ghatkesar, Telangana, India. The plant material was washed with distil water and shade dried, pulverized in mechanical grinder and stored in an airtight container till further successive extractions¹⁸.

Drugs and chemicals

Albendazole suspension (Zentel, GSK Pharmaceuticals Ltd. Bangalore) and ethanol were used during the experimental protocol. All the chemicals used are laboratory and analytical grade.

Preparation of plant extract

Soxhlet extraction^{19,20}

The 100 gm of coarse powder was extracted with extra pure ethanol successively in a Soxhlet extractor repeatedly for 48 hours. The extract was dried by solvent evaporation in a thermostat water bath at 50-60 °C temperature. The extract was stored at 4 °C until used. The prepared extract is used for the anti-helminthic activity.

Selection of experimental model^{21,22}

Indian adult earthworms (*Pheretima posthuma*) were used to carry out the experiment. The earthworms were collected from the moist soil. Worms were washed with normal saline to remove all faecal matter. The earthworms of 7-9 cm in length and 0.3-0.4 cm in width were used for all the experimental protocol. Ready availability, anatomical and physiological resemblance of *Pheretima posthuma* made it to be used initially for *in-vitro* evaluation of anthelmintic activity.

Preliminary phytochemical screening

Phytochemical screening was carried out to assess the qualitative chemical composition of crude ethanolic extract of *Psidium guajava*. Standard screening tests using conventional protocol, procedure, and reagents were conducted using standard procedures to identify the constituents²³⁻²⁶.

Test for alkaloids

i) Dragendorff's test: In a test tube containing 1ml of extract, few drops of Dragendorff's reagent was added and the colour developed was noticed. Appearance of orange colour indicates the presence of alkaloids.

ii) Wagner's test: To the extract, 2 ml of Wagner's reagent was added; the formation of a reddish brown precipitate indicates the presence of alkaloids.

iii) Mayer's test: To the extract, 2 ml of Mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

iv) Hager's test: To the extract, 2 ml of Hager's reagent was added; the formation of yellow precipitate confirmed the presence of alkaloids.

Test for terpenoids

i) Salkowski test: To 1 ml of extract, tin (one bit) and thionyl chloride were added. Appearance of pink colour indicates the presence of terpenoids.

ii) Hirshon reaction: When the substance was heated with trichloroacetic acid, red to purple colour was observed.

Test for steroids

i) Liebermann burchard test: To 1ml of extract, 1ml of glacial acetic acid and 1ml of acetic anhydride and two drops of concentrated sulphuric acid were added. The solution become red, then blue and finally bluish green indicates the presence of steroids.

Test for coumarins

i) To 1 ml of extract, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow colour.

Test for tannins

i) To few mg of extract, ferric chloride was added, formation of a dark blue or greenish black colour showed the presence of tannins.

ii) The extract was mixed with basic lead acetate solution; formation of white precipitate indicated the presence of tannins.

Test for saponins

i) To 1 ml of the extract, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of saponins.

Test for flavones

i) **Shinoda test:** To the extract, a few magnesium turnings and 2 drops of concentrated hydrochloric acid were added, formation of red colour showed the presence of flavones.

ii) To the extract, 10% sodium hydroxide or ammonia was added; dark yellow colour shows the presence of flavones.

Test for quinones

i) To 1 ml of the extract 1 ml of concentrated sulphuric acid was added. Formation of red colour shows the presence of quinones.

Test for flavanones

i) To the extract, 10% sodium hydroxide was added and the colour changes from yellow to orange, which indicates the presence of flavanones.

ii) To the extract, conc. sulphuric acid was added, and the colour changes from orange to crimson red, which indicates the presence of flavanones.

Test for anthocyanins

i) To the extract, 10% sodium hydroxide was added, and the blue colour shows the presence of anthocyanins.

ii) To the extract, conc. sulphuric acid was added, and the yellowish orange colour confirms the presence of anthocyanins.

Test for anthraquinones

i) **Borntrager's test:** The extract was macerated with ether and after filtration; aqueous ammonia or caustic soda was added. Pink red or violet colour in the aqueous layer after shaking indicates the presence of anthraquinones.

Test for phenols

i) **Ferric chloride test:** To the extract, few drops of 10 % aqueous ferric chloride were added. Appearance of blue or green colour indicates the presence of phenols.

Test for proteins

i) **Biuret test:** To the extract, 1 ml of 40% sodium hydroxide solution and two drops of one percent copper sulphate solution were added. Formation of violet colour indicates the presence of proteins.

ii) **Xanthoprotein test:** To the extract, 1 ml of concentrated nitric acid was added. A white precipitate was formed; it is then boiled and cooled. Then 20% sodium hydroxide or ammonia was added. Orange colour indicates the presence of aromatic amino acids.

iii) **Tannic Acid test:** To the extract, 10% tannic acid was added. Formation of white precipitate indicates the presence of proteins.

Test for carbohydrates

i) **Molisch's test:** To the extract, 1 ml of alpha-naphthol solution, and concentrated sulphuric acid through the sides of test tube were added. Purple or reddish violet colour at the junction of the two liquids revealed the presence of carbohydrates.

ii) **Fehling's test:** To the extract, equal quantities of fehling's solution A and B were added and on heating, formation of a brick red precipitate indicates the presence of carbohydrates.

iii) **Benedict's test:** To 5 ml of Benedict's reagent, extract was added and boiled for two minutes and cooled. Formation of red precipitate showed the presence of carbohydrates.

Test for amino acids

i) **Ninhydrin test:** Two drops of ninhydrin solution were added to the extract, a characteristic purple colour indicates the presence of amino acids.

Test for fixed oils and fats

i) **Spot test:** A small quantity of extract was pressed between two filter papers. Oil stains on the paper indicates the presence of fixed oils and fats.

Experimental Design²⁷⁻³⁰

Ethanol extract from the leaves of *Psidium guajava* were investigated for anthelmintic activity against *Pheretima posthuma*. Various concentrations (25, 50 and 100 mg/ml) of extract was tested by bioassay, which involved determination of time of paralysis and time of death of the worms. Albendazole was used as standard reference and saline water as control. The assay was performed on adult Indian earthworms, *Pheretima posthuma* due to its anatomical and physiological resemblance with that of intestinal round worm parasite of human beings. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro*.

The anthelmintic activity was carried out as described by using standard protocols, with minor modifications²⁷. The Indian earthworm (*Pheretima posthuma*) of nearly equal size, six in each group was taken for the experiment. Each type of dried extract was suspended in 1% w/v Carboxy Methyl Cellulose, prepared in normal saline water in three different conc. (25, 50, 100 mg/ml). Albendazole suspension of same conc. was taken as standard and normal saline water with 1% CMC was taken as a control. Worms were placed in petridish containing 15 ml of sample (drug) solution. Time for paralysis was noted either when any movement could not be observed except when the worms were shaken vigorously or when dipped in warm water (50 °C). Death was included when the worms lost their motility followed by white secretions and fading away of their body colour.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Our observation revealed that in the preliminary phytochemical screening was found that the ethanolic dried leaves extract contain tannins, steroids, flavonoids, saponins, terpenoids, glycosides etc. The preliminary phytochemical screening results were illustrated in Table 1.

Preliminary phytochemical analysis showed the presence of tannins, steroids, flavonoids, saponins, terpenoids, glycosides etc. like phytoconstituents in the extracts of *Psidium guajava*

leaves. Some of these phytoconstituents may be responsible to show a potent anthelmintic activity. The peak anthelmintic activity exhibited by the extract at highest concentration (100 mg/ml) which takes 09.19 ± 0.12 minute for paralysis and 32.11 ± 0.11 minute for death of the worms (illustrated in Table 2). Potency of the extract was inversely proportional to the time for paralysis (vermifuge) and death (vermicidal) of the worms.

Phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, tannins and steroids. Tannins chemically polyphenolic compounds, were shown to produce anthelmintic activities. Reported anthelmintic effect of tannins, can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death. Further studies are under process to identify the possible phytoconstituents responsible for anthelmintic activity. *Psidium guajava*, guava, documented to possess several medicinal properties, has been extensively researched for various pharmacological properties. The laboratory studies and clinical trials provide a strong scientific base supporting the various

ethnobotanical/ ethnopharmacological reports from across the world. In addition, as guava propagates easily and thrives in almost all the climatic conditions, it is widely available for medicinal use as well as commercial applications. A single plant with multiple benefits has the advantage especially where backyard plots are small and/or water limited. In addition, it is preferable to limit the variety of plants suggested for cultivation so as not to overwhelm the individuals maintaining the backyard nurseries.

The traditional use of the leaves of *Psidium guajava* as anthelmintic has been confirmed using the ethanolic extract and showed significant anthelmintic activity. Further it would be interesting to isolate the responsible phytoconstituents, which are responsible for the anthelmintic activity and the mechanism of action, which is being attempted in the laboratory. In addition, guava can also be useful for treatment of animals and explored for its commercial applications.

Table 1: Preliminary phytochemical screening of *Psidium guajava* leaves

Constituents	Ethanol extract
Terpenoids	+
Saponins	+
Steroids	+
Carbohydrates	+
Flavonoids	+
Alkaloids	-
Quinones	-
Tannins	+
Fixed oils and fats	-
Phenols	+
Glycosides	+
Amino acids	-
Anthraquinones	-

(+) Present, (-) Absent

Table 2: Anthelmintic activity of *Psidium guajava* leaves

Treatment	Conc. (mg/ml)	Paralysis time (min)	Death time (min)
Control (1% CMC in Normal Saline)	-	-	-
Albendazole (Standard)	25	2.45 ± 0.21	19.73 ± 0.26
	50	1.34 ± 0.15	12.76 ± 0.56
	100	0.39 ± 0.21	6.97 ± 0.48
Ethanol	25	21.8 ± 0.31	76.2 ± 0.04
	50	15.61 ± 0.23	64.68 ± 0.21
	100	9.19 ± 0.12	32.11 ± 0.11

[Values are expressed a mean \pm SEM (n=6)]



Fig. 1: *Psidium guajava* plant



Fig. 2: *Psidium guajava* leaves



Fig. 3: Soxhlet extraction



Fig. 4: Indian adult earthworms (*Pheretima posthuma*)

CONCLUSION

In the light of the present observations, the *Psidium guajava* leaf extract has showed significant anthelmintic activity at all the tested doses when compared to control as vermifuge and vermicide while highest activity exhibited by the higher conc. (100 mg/ml) which assures the ethno-medicinal claim. Hence, we can think about this herb as alternate source of anthelmintic drugs and also can generate new active lead for suitable anthelmintic drug.

The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant as anthelmintic. The plant may be further explored for its phytochemical profile to recognize the active constituent accountable for anthelmintic activity. However, further studies are needed to isolate, characterize and evaluate the actual bioactive components and their mechanism of actions. Also, studies on the toxicity, evaluation of the effect *in-vivo* condition and the establishment of the recommended doses for animals are to be recommended. In conclusion, popularization of guava can have multiple applications for rural communities.

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