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Research Article

**IN VITRO ANTHELMINTIC ACTIVITY OF *PSIDIUM*
GUAJAVA L. EXTRACTS AGAINST *PHERETIMA*
*POSTHUMA***

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Abstract:

Background: Helminthic infections remain an important parasitic health problem, while resistance to established anthelmintic agents has encouraged investigation of plant-derived alternatives. Psidium guajava L. (Myrtaceae) is a medicinal plant reported in the source study to contain flavonoids, tannins, saponins, triterpenoids and other bioactive constituents. Objective: To evaluate the in vitro anthelmintic activity of petroleum ether and aqueous extracts of P. guajava against Pheretima posthuma using paralysis time (PT) and death time (DT) as outcome measures. Methods: Plant material was shade-dried, powdered and extracted using petroleum ether and water. Physicochemical parameters, preliminary phytochemical screening and an acute oral toxicity assessment were reported. Adult P. posthuma were exposed to extract concentrations of 25, 50, 75 and 100 mg/mL and compared with albendazole (10 mg/mL) and control treatment. Results: Petroleum ether extract produced PT/DT values of 90.3/100.0, 70.3/90.3, 65.8/85.8 and 56.1/70.3 min at 25, 50, 75 and 100 mg/mL, respectively. The aqueous extract produced PT/DT values of 81.3/90.1, 49.3/60.2, 44.8/55.8 and 26.1/30.9 min at the same concentrations. Albendazole produced PT/DT values of 20.1/26.4 min. Conclusion: Both P. guajava extracts demonstrated concentration-associated reductions in paralysis and death times in the reported assay, with the aqueous extract showing the shorter times at the tested concentrations. The findings support further investigation of P. guajava constituents and mechanisms responsible for the observed activity. Keywords: Psidium guajava; anthelmintic activity; Pheretima posthuma; aqueous extract; petroleum ether extract; albendazole; phytochemical screening.

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1. INTRODUCTION:

Medicinal plants have long been used as sources of therapeutic agents, and plant-derived preparations continue to be investigated for pharmacological activity. The source document emphasizes the medicinal importance of plants and the continuing search for naturally derived compounds with therapeutic potential.

Helminths are parasitic worms that may inhabit the intestinal tract or tissues. Anthelmintic resistance is described in the source study as the ability of parasites to survive treatment with an anthelmintic chemical that had previously been effective. The development of resistance is an evolutionary process influenced by selection pressure and management practices. The source document also notes the relevance of zoonotic parasites to human health.

Psidium guajava L. (guava), belonging to the family Myrtaceae, is a tropical medicinal plant. The reviewed material describes leaves containing flavonoids, particularly quercetin, together with tannins, triterpenoids, saponins and essential oils, and reports antioxidant, antimicrobial, anti-inflammatory, antidiabetic, antidiarrheal and anthelmintic-related pharmacological interest.

The study was therefore designed to investigate the anthelmintic effect of *P. guajava* extracts using *Pheretima posthuma* as an in vitro experimental model, with paralysis and death times used to assess activity.

2. Aim and Objectives

The stated aim was to scientifically evaluate the anthelmintic potential of *P. guajava* extracts against *P. posthuma* and compare their effects with a standard anthelmintic drug. The reported objectives included plant collection and authentication, preparation of petroleum ether and aqueous extracts, preliminary phytochemical analysis, collection of worms, acute toxicity assessment according to OECD Guideline 423, evaluation of anthelmintic activity and data collection.

3. MATERIALS AND METHODS:

3.1 Plant material and authentication

The source document reports collection of *P. guajava* from local fields in Gulbarga. The plant material was washed, shade-dried, powdered and passed through sieve No. 40. Authentication was reported to have been performed by Prof. Dr. (Mrs.) Rasika Kapale, Department of Botany, VG Women's Degree College, Gulbarga, with a voucher specimen retained in the herbarium



Leaves of Psidium guajava.



Project group with guide and Principal



Specimen of plant for authentication.

3.2 Physicochemical evaluation

The reported physicochemical parameters included total ash, acid-insoluble ash, water-soluble ash, loss on drying and solvent-soluble extractive values. The source describes standard ashing, loss-on-drying and extractive-value procedures.

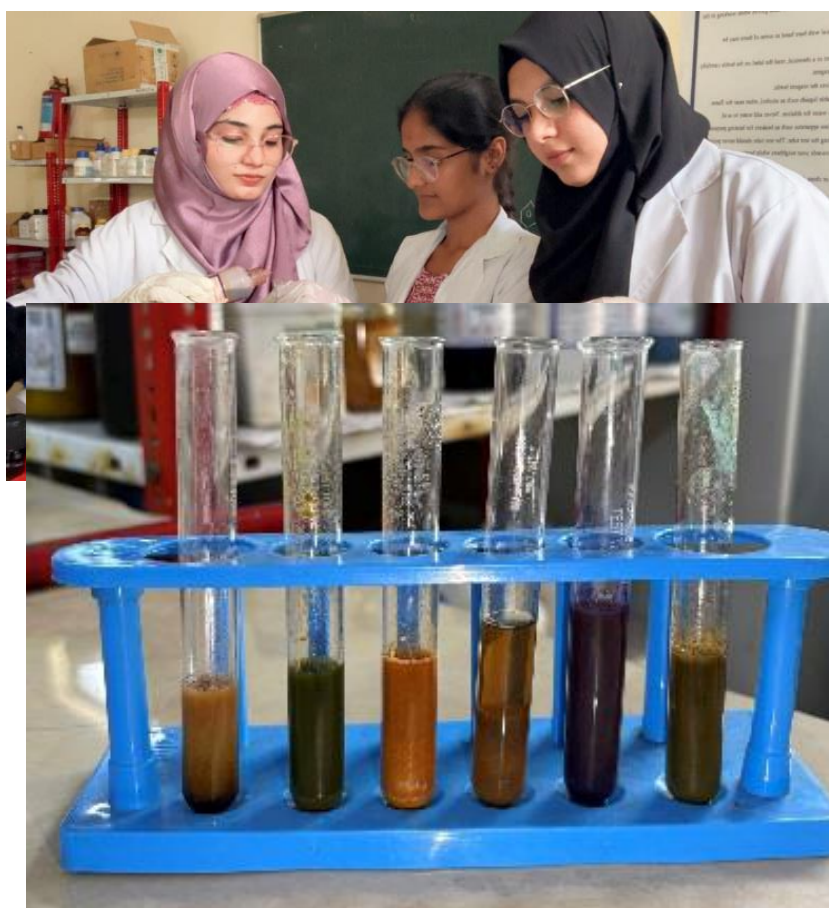
3.3 Preparation of extracts

For petroleum ether extraction, powdered plant material was subjected to Soxhlet extraction with petroleum ether. The filtrate was evaporated under reduced pressure and the dried extract was preserved at 4°C. For the aqueous extract, 1000 g of powdered

material was macerated with 3000 mL distilled water and 100 mL chloroform as preservative for seven days with daily shaking. The marc was separated and the extract concentrated at 50°C on a water bath.

3.4 Preliminary phytochemical screening

The source document reports qualitative testing for carbohydrates, proteins, steroids, amino acids, glycosides, flavonoids, alkaloids, tannins/phenolic compounds, saponins and vitamins using the chemical reactions described in the original project.



Phytochemical screening

3.5 Acute oral toxicity

An acute oral toxicity assessment is reported according to OECD Guideline 423 using female mice at 5, 50, 300 and 2000 mg/kg body weight. The source document reports no mortality or morbidity at 2000 mg/kg and records normal findings for several observed parameters.

3.6 Experimental worms

Healthy adult *Pheretima posthuma* of approximately 6–10 cm length were collected from moist soil/vermicompost areas, washed and acclimatized under laboratory conditions. Worms of approximately uniform size were selected.

3.7 In vitro anthelmintic assay

The source protocol used a control treatment, albendazole as the standard and petroleum ether and aqueous *P. guajava* extracts at graded concentrations. The reported assay observed time to complete paralysis (PT) and death (DT). Paralysis

was considered when the worm did not revive after stimulation in normal saline, while death was associated with loss of motility and fading of body colour, followed by confirmation as described in the source protocol.



Control group

Extract group

4. RESULTS:

4.1 Physicochemical characteristics

Parameter	Result (% w/w)
Total ash	8.14
Acid-insoluble ash	0.70
Water-soluble ash	4.50
Loss on drying	6.31
Petroleum ether-soluble extractive	0.16
Water-soluble extractive	0.36

4.2 Extract yield

Extract	Nature	Colour	Weight (g)	Yield (%)
Petroleum ether	Semi-solid	Yellowish green	22.5	2.25
Aqueous	Semi-solid	Dark brown	28.0	2.80

4.3 Phytochemical screening

Constituent	Petroleum ether extract	Aqueous extract
Alkaloids	—	—
Flavonoids	—	—
Saponins	—	++
Tannins	—	++
Phenolics	—	++
Glycosides	—	—
Carbohydrates	—	++
Amino acids	—	—

In the source table, '+' denotes presence, '-' absence and '++' high concentration. The discussion section of the source separately mentions alkaloids, phenols, flavonoids, steroids and tannins; this discrepancy should be resolved against the original laboratory records before journal submission.

4.4 Acute toxicity

At 5, 50, 300 and 2000 mg/kg, the source reports no mortality and records normal motor activity, pain response, righting reflex, gripping strength, pupils, urination, salivation, skin colour and lacrimation. Tremors, convulsions and writhing were reported as absent at all tested doses.

4.5 Anthelmintic activity

Treatment	Conc. (mg/mL)	Paralysis (PT) (min)	Death Time (DT) (min)	Reported observation
Normal saline control	—	—	—	Control
Albendazole	10	20.1 ± 0.7	26.4 ± 0.5	Standard
Petroleum ether extract	25	90.3 ± 1.4	100.0 ± 2.1	Extract
Petroleum ether extract	50	70.3 ± 1.5	90.3 ± 1.2	Extract
Petroleum ether extract	75	65.8 ± 1.7	85.8 ± 1.8	Extract
Petroleum ether extract	100	56.1 ± 1.0	70.3 ± 1.2	Extract
Aqueous extract	25	81.3 ± 1.9	90.1 ± 0.2	Extract
Aqueous extract	50	49.3 ± 1.0	60.2 ± 0.1	Extract
Aqueous extract	75	44.8 ± 1.1	55.8 ± 0.6	Extract
Aqueous extract	100	26.1 ± 1.0	30.9 ± 0.3	Extract

The reported data show shorter paralysis and death times with increasing extract concentration for both extracts. At 100 mg/mL, the aqueous extract produced PT and DT values of 26.1 ± 1.0 and 30.9 ± 0.3 min, respectively, compared with 56.1 ± 1.0 and 70.3 ± 1.2 min for the petroleum ether extract.

5. DISCUSSION:

The study reports measurable in vitro anthelmintic activity for both *P. guajava* extracts, as demonstrated by paralysis and death of *P. posthuma*. The response was concentration-associated in the reported dataset: increasing extract concentration was accompanied by reductions in both PT and DT.

The aqueous extract showed shorter PT and DT values than the petroleum ether extract at each corresponding concentration. At 100 mg/mL, its PT and DT approached the values reported for the Albendazole standard, although the standard remained faster for both endpoints. These findings indicate activity in the experimental model but do not by themselves establish clinical efficacy in humans or therapeutic equivalence to Albendazole. The source document associates the observed activity with plant phytoconstituents. The qualitative screening table reports high levels of saponins, tannins, phenolics and carbohydrates in the aqueous extract, while the petroleum ether extract was recorded as negative for the listed constituents. Because the discussion section contains a different description of detected constituents, the original laboratory observation sheets should be checked before final publication.

The acute toxicity results reported in the source document showed no mortality or morbidity up to 2000 mg/kg in the described acute study. This

finding is supportive of the reported experimental safety observation, but it should not be interpreted as proof of safety in humans or as evidence for chronic toxicity, reproductive safety or clinical tolerability.

6. CONCLUSION:

Within the limits of the reported in vitro model, petroleum ether and aqueous extracts of *Psidium guajava* demonstrated anthelmintic activity against *Pheretima posthuma*, with activity increasing across the tested concentration range. The aqueous extract produced shorter paralysis and death times than the petroleum ether extract at corresponding concentrations. Further work should identify the active constituents, investigate mechanisms of action, confirm findings using appropriately controlled and replicated experiments, and evaluate pharmacological and toxicological profiles before any clinical application is considered.

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