*ejpmr, 2024, 11(7), 542-552*

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

# PHYTOCHEMICAL INVESTIGATION AND IN VITRO ANTHELMINTIC ACTIVITY OF PSIDIUM GUAJAVA

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**Article Received on 23/05/2024** **Article Revised on 13/06/2024** **Article Accepted on 03/07/2024**

## ABSTRACT

*Psidium guajava*, a tropical fruit widely distributed in subtropical and tropical regions, has been recognized for its medicinal properties in traditional medicine systems. This study aimed to conduct a comprehensive phytochemical investigation of *P. Guajava* extracts and evaluate their potential anthelmintic activity through in vitro assays against parasitic worms. Phytochemical analysis of the extracts revealed a diverse array of bioactive compounds, including flavonoids, phenolics, tannins, terpenoids, and alkaloids. These compounds have been associated with various pharmacological activities, including antioxidant, antimicrobial effects. The presence of such phytochemicals suggests the potential therapeutic value *of Psidium guajava* in treating various ailments. Subsequently, in vitro assays were conducted to assess the anthelmintic activity of *Psidium guajava* extracts against parasitic worms. The extract was studied in different concentrations (100, 200 and 300mg/ml). The anthelmintic activity *P. Guajava* extract in lower concentration is not significant, in higher concentration extract 200 and 300 mg/ml exhibited 53.96±1.820 and 42.14±1.91minutes paralysis time respectively when compared to the standard Albendazole which was 43.97±1.63 minutes. The death time recorded was 70.91±1.16 and 66.09±4.15 minutes respectively in comparison to the standard which was recorded to be 61.17±7.25 minutes. Effectiveness of the extract *P. Guajava* was inversely proportional to the time for paralysis (vermifuge) and death (vermicidal) of the worms. This suggests that *P. Guajava* extracts may interfere with the metabolic pathways or structural integrity of the parasites, leading to their expulsion or death. The findings of this study highlight the potential of *P. Guajava* as a natural source of anthelmintic agents. Additionally, the safety and toxicity profile of *P. Guajava* extracts should be investigated to ensure their suitability for therapeutic use.

**KEYWORDS:** *Psidium guajava*, phytochemical investigation, anthelmintic activity, parasitic worms, in vitro assays.

## INTRODUCTION

### *Psidium Guajava*

- Guava is of numerous trees and shrubs of the genus Psidium (family Myrtaceae) native to tropical America. The term "guava" appears to derive from Arawak Guayabo "guava tree", via the Spanish guayaba.
- Guava's believed to have originated from Mexico or Central America. It is now very popular in Asian countries and is also increasingly available in American countries, particularly after its health benefits have been revealed.
- The common types of guava include apple guava, yellow fruited cherry guava, strawberry guava, and red apple guava.
- It is mostly eaten raw (ripe or semi-ripe) or consumed in the form of juice, jams, and jellies.
- The common guava has a fruit with a yellow skin and white, yellow, or pink flesh.[1]
- Guavas are known for their sweet and tangy flavour and many uses, but there's much more to this fruit than meets the eye.
- Many consider it a "magical" fruit because of its array of nutrients and medicinal uses.[2]

### Plant Description[3]

Psidium guajava is a shrub or small tree usually growing 1-6 m tall, but occasionally reaching 10 m in height. The older stems are covered in a smooth, light reddish-brown, bark that peels off in flakes. This sometimes gives the trunks a mottled appearance, because the newly revealed bark is somewhat greenish-brown in colour. Younger stems are greenish in colour, hairy (pubescent), and somewhat four-angled (quadrangular). 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 apices) 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.

The flowers are usually borne singly in the upper leaf forks (axils). These flowers are about 25 mm across and are borne on a hairy stalk (pubescent peduncle) 1-2.5 cm long. Each flower has four or five green sepals (6-15 mm long) that are fused together at the base and four or five white petals (10-20 mm long). They also have large numbers (200-250) of small white stamens (6-10 mm long) and a style (6-12 mm long) topped with a stigma.

The fruit is either rounded (globose), egg-shaped (ovoid) or pear shaped (pyriform) and turns from green to yellowish in colour as it matures. These berries (2.5-10 cm long) are crowned with the remains of the persistent sepals (calyx lobes) and have a juicy pink, white or yellowish coloured pulp containing numerous seeds. The seeds are yellowish in colour and kidney-shaped (reniform). Both planted and wild trees are used for fruit which aids their spread.

**Common Names**[4]

Guava is known as various names in various regions of the world. The common names of *Psidium guajava* include.

**Table 1: common names of Psidium Guajava.**

| | |
|---|---|
| **Arabic** | Guwafah |
| **Bengali** | Piara |
| **Brazil** | Araca |
| **Cambodia** | Trapaeksruk |
| **Chinese** | Fan shiliu |
| **English** | Apple guava |
| **French** | Gouyave |
| **Germany** | Guavenbaum |
| **India** | Amarood; jamba |
| **Portuguese** | Goiaba |
| **Thailand** | Farang |
| **Philippines** | Bayabas |
| **Spanish** | Guayaba |

**Taxonomical Classification**[5]

**Table 2: Taxonomical classification of *Psidium Guajava*.**

| | |
|---|---|
| **Kingdom** | Plantae |
| **Subkingdom** | Tracheobionta |
| **Division** | Magnoliphyta |
| **Class** | Magnoliopsida |
| **Sub-class** | Rosidae |
| **Order** | Myrtales |
| **Family** | Myrtaceae |
| **Genus** | Psidium |
| **Species** | Psidium guajava |

**Ethnomedical Uses**[6,7,8,9,10]

*Psidium guajava* fruit (Guava) is an ethnomedicine. It has special importance in the traditional system of medicine. In 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.

**Table 3: Ethnomedical uses of Psidium Guajava.**

| S. No | Plant part | Compound | Ethnomedicinal uses |
|---|---|---|---|
| **1** | **Seed** | Glycosides, carotenoids and phenolic compounds | Antimicrobial activity |
| **2** | **Bark** | Phenolic | Strong antibacterial activity, stomach ache and antidiarrheal activity |
| **3** | **Leaves** | Phenolic, flavonoids, gallic acid, catechin, epicatechin, rutin, naringenin, kaempferol | Antioxidant, anti-inflammatory, antispasmodic, anticancer, antimicrobial, anticonservative, and neuropathic activity |
| **4** | **Skin** | Phenolic | Improvement of food absorption |

| | | | |
|---|---|---|---|
| **5** | **Pulp** | Ascorbic acid, carotenoids (lycopene, β-carotene, β cryptoxanthin) | Antioxidant, anti-hyperglycaemic |

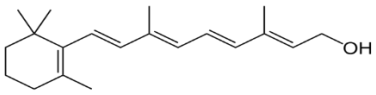
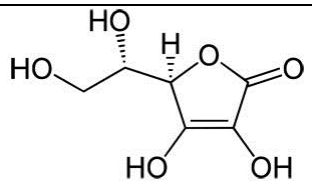
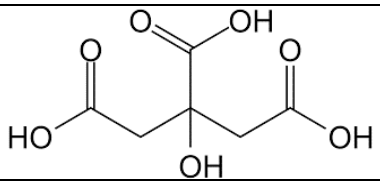
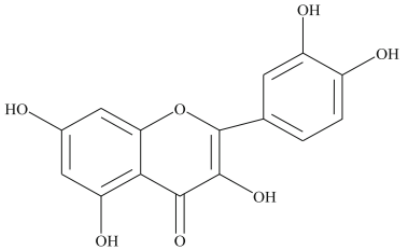
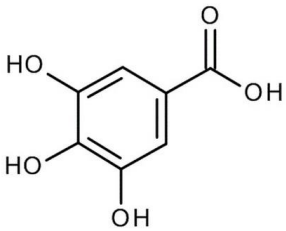
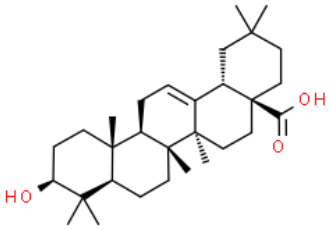
**"Fig. 1": Psidium Guajava tree.**

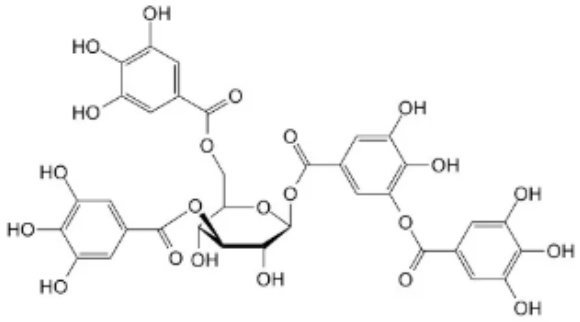
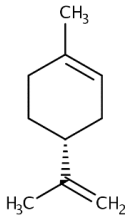
**"Fig 2": Psidium Guajava fruit.**

**Chemical Composition**

The guava fruit contains vitamin A, C, iron, phosphorus and calcium. It has more vitamin C than the orange. The fruit contains saponin, oleanolic acid, lyxopyranoside, arabopyranoside, guaijavarin, quercetin and flavonoids.[11,12,13] Ascorbic acid and citric acid are the major ingredients of guava that play important role in anti-mutagenic activity.[14] The skin of fruit contains ascorbic acid in very high amount; however, it may be destroyed by heat. The strong pleasant smell of fruit is credited to the carbonyl compounds.[13] Guava fruit contains terpenes, caryophyllene oxide and p-selinene in large quantity which produce relaxation effects.[15] The flavonoid content is higher in the methanolic extract of the guava.[16] There are 41 hydrocarbons 25 esters, 13 alcohols and 9 aromatic compounds in guava.[17] Titratable acidity and the total soluble solids are present in fruit.[18] Guajadial is also present in guava.[19] Essential oil is present in leaves which contain α-pinene, limonene, β-pinene, isopropyl alcohol, menthol, terpenyl acetate, caryophyllene, longicyclene and β-bisabolene. Oleanolic acid is also found in the guava leaves.[20] Leaves have high content of limonene about 42.1% and caryophyllene about 21.3%.[21] Leaves of guava have a lot of volatile compounds.[22,23] The bark includes 12–30% of tannin and one source declares that it includes tannin 27.4%, or polyphenols, resin and the crystals of calcium oxalate. Tannin is also present in roots. Leukocyanidins, gallic acid and sterols are also present in roots. Carbohydrates with salts are present in abundance. Tannic acid is also its part.

**Table 4: Chemical Composition of *Psidium Guajava.***

| S. No | Name of the Structure | Structure |
|---|---|---|
| 1. | Vitamin A |  |
| 2. | Vitamin C (ascorbic acid) |  |
| 3. | Citric acid |  |
| 4. | Quercetin |  |
| 5. | Gallic acid |  |
| 6. | Oleanolic acid |  |

| | | |
|---|---|---|
| **7.** | **Tannic acid** |  |
| **8.** | **Limonene** |  |

**APPLICATIONS/TREARMENT[24,31]**

**Table 5: Pharmacological activities of *Psidium Guajava*.**

| S.No. | Pharmalogical effect | Pharmalogical activity | References |
|---|---|---|---|
| 1. | Antioxidant activity | With the extraction of guava and 65% ethanol with the conc.0.47 g/l, it shows the effects of scavenging hydroxy radicals and it inhibits lipid peroxidation. | Wang *et al.*, 2007 |
| 2. | Antidiabetic activity | With the extraction of guava and methanol with the conc. of 0.2-1.0 ml, the dosage is depending upon the percentage of inhibition activity against the α-amylase enzyme. | Manikandan *et al.*, 2013 |
| 3. | Antibacterial activity | The 75% methanol/acetone and guava extract with the conc. of 5.0 and 2.0 mg/ml, it shows the effects of antibacterial activity against on *E. coli*, *S. typhimurium*, and *S.multocida*. | Puntawong *et al.*, 2012 |
| 4. | Antifungal activity | With the use of hexane 50 mg/ml, it shows the effects of antifungal activity against *Trichophyton rubrum*, *T. tonsurans*, *Candida parapsilosis* and *C. albicans*. | Abdelrahim *et al.*, 2002 |
| 5. | Antimalarial activity | The solution of aqueous 10-20 mg/ml, fever "teas" are constructed with the leaves as an active component. They are also present in the pot plant that is used to make the steam medicine for malaria. Antimicro-bials, flavonoids, and terpenoids are included in the stem bark extract, which has been demonstrated to be effective for the treatment of malaria. | Nundkumar *et al.*, 2002 |
| 6. | Antihypertensive activity | Using water, extraction of guava and ethanol with the conc. of 0.6-2.0 g/kg (according to body wgt), it shows against the antihypertensive activity by controlling the levels of blood pressure from the beginning to the end of the experiment. | Gutierrez *et al.*, 2008 |
| 7. | Anticancer activity | With the extraction of guava and essential oil (<1.6 mg/day), it has been demonstrated that the *p. guajava* leaf has antiprostate cancer activity. In a xenograft mouse tumour model, it reduced the prostate specific antigen serum concentrations as well as the distant metastasis. | Chen *et al.*, 2010 |
| 8. | Antiobesity property | Guava leaves are utilized 200 mg/kg of body weight in diabetic rats. As a result of poor carbohydrate metabolism, GLs reduced blood glucose levels and encouraged oral glucose tolerance, both of which are necessary to avoid weight loss. The insulin levels stabilised as a result of increased hexokinase and G6PDH activity and decreased gluconeogenic enzyme and glucose-6-phosphatase activity. | Vinayagam *et al.*, 2018 |

## MATERIALS

The plant of Psidium guajava leaves were collected from the medicinal garden of Deccan school of pharmacy Hyderabad, Telangana, India. Fresh are authenticated by Dr. Rafiuddin Naser Associated Professor, Department of Botany, Moulana Azad college of Aurangabad, (M.S) (PCOG.H-217). Then the leaves were dried under shade separately (room temperature). Air dried plant materials were ground in to powder and the plant materials were then used for further investigations. For anthelmintic study adult earthworm pheritimaposthuma (earth worm) of nearly equal size were collected from the local area.

## METHODS

Around 250gm of leaves of *Psidium Guajava* were Collected, washed in running tap water and then rinsed

with distilled water, cutted and was subjected to drying at room temperature for about two weeks in open air. The dried Leaves were powdered using mixer grinder and passed through sieve no 22. The coarse powder was extracted with Methanol in Soxhlet's apparatus at a temperature not exceeding 40°C for 96 hours. The extract was concentrated under reduced pressure in rotary evaporator to yield a crude semi-solid mass percentage of yield. After drying the extracts was used for Phytochemical, chromatography and in-vitro pharmacological screening.


**"Fig 3": Showing Soxhlet apparatus for extraction of *Psidium Guajava.***

**PRELIMINARY PHYTOCHEMICAL SCREENING[32,37]**

Phytochemical analysis of the different extract of leaves were carried out using standard methods. The plant materials were checked for the presence of various active constituents, like carbohydrates, protein, glycosides, phytosterols, flavonoids, amino acids, terpenes, and alkaloids (Table-3).

**Chemicals:** Water, alcohol, 70% alcohol, extracts, Reagents: mayer's, wagner's, fehling's, bendict's, dragendroff's, hager's, sulphuric acid, 10% ammonia, pyridine, sodium nitroprusside, acetic anhydride, ferric chloride, 5% and 10% lead acetate, 10% ammonium hydroxide solution, Conc. $H_2SO_4$, α – Napthol.

**Determination of Total Phenolic Compounds**

The methanolic extract of 0.5ml *Psidium Guajava* was prepared and Gallic acid in a series of concentrations 2,4,8,16 and 32 µg/ml.

To the extract in the test tube add 2.5 ml Folin- ciocalteu reagent and 2.5 ml NaCO3. And make up the volume up to 10 ml with distilled water.

Incubate the test tubes for 25 minutes at 25°C
Absorbance is measured under UV at 760 nm
And a graph is plotted.

**Determination of Total Flavonoids**

Weigh accurately 1.0 ml of plant extract and the standard Rutin is prepared in different series of concentration like 100, 200, 300, 400 and 500 µg/ml.

To the extract in the test tube Add 3 ml of methanol 0.2 ml of 10% AlCl3, 0.2 ml of 1M potassium acetate and make up the volume with distilled water.

Incubate for 25 minutes
Absorbance is observed in UV at 420nm
Plot the graph.

**ANTHELMINTIC ACTIVITY**

Adult earthworms pheretimaposthuma were collected from the local area of gunupur, odisha. The length of the pheretimaposthuma is 6±1 cm. The earthworms were washed with normal saline and divided into different group, each group containing three earthworms. The standard solution (Albendazole) and different extracts were freshly prepared before performing the experiments. Then different concentrations (100, 200,300 mg/ml) of test sample of extracts (Methanol) were prepared and poured into different Petri dishes. The pheretimaposthuma were transferred one by one into the petri dishes.

The observations were made for the time taken to paralysis and death of individual worms were noted. The time taken for paralysis was noted when any movement of worm could not be observed. Death was included

when the worms lost their motility followed by white secretions and fading away of their body colour. Statistical analysis of the data was expressed as mean ± standard error mean (SEM).

## RESULTS

The preliminary phytochemical investigation revealed that the presence of different phytoconstituents like carbohydrates, protein, glycosides, phytosterols, flavonoids, amino acids, terpenes, and alkaloids, etc (Table-3). The presence of above phytoconstituents may be responsible to show a potent anthelmintic activity.

The peak anthelmintic activity exhibited by the Methanolic extract at highest concentration (300 mg/ml) which takes 42.14±1.91minutes paralysis and 66.09±4.15 minutes for death of the worms. Potency of the extract was inversely proportional to the paralysis (vermifuge) and death (vermicidal) time of the worms. Different extract of *Psidium guajava* possess several medicinal properties, has been extensively researched for various pharmacological properties.

**Table 6: Phytochemical screening of extracts of *Pisidium Guajava* ('+' present '-' absent).**

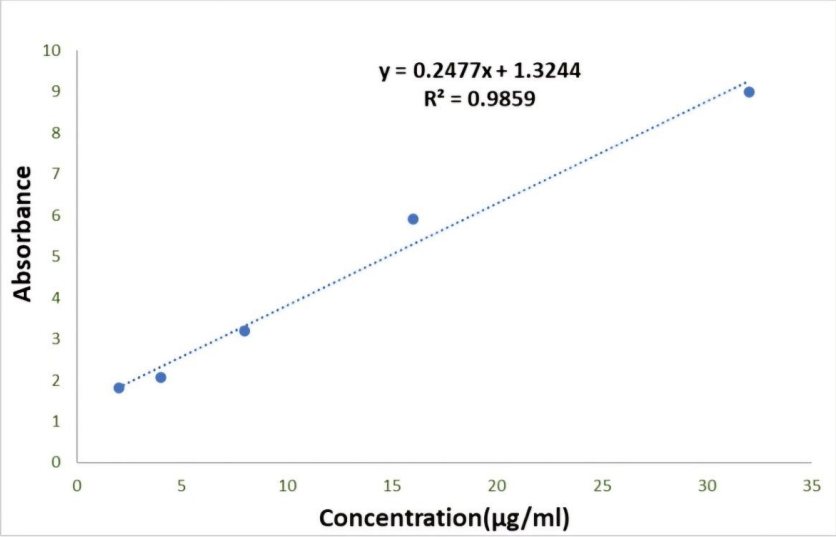
| S. No | Test for Phytoconstituents | Methanol extract |
|---|---|---|
| 1 | Alkaloids | + |
| 2 | Carbohydrates | + |
| 3 | Glycosides | + |
| 4 | Phyto sterols | + |
| 5 | Isoflavones | + |
| 6 | Amino acid & Proteins | + |
| 7 | Triterpenoids | + |

**Table 7: Estimation of Total phenolic content of methanolic leaf extract of P. guajava.**

| S. No | Concentration | Absorbance | | | Mean±SD |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | |
| 1 | 2 | 1.791 | 1.83 | 1.82 | 1.814±0.020257 |
| 2 | 4 | 2.06 | 2.09 | 2.06 | 2.070±0.017321 |
| 3 | 8 | 3.185 | 3.191 | 3.192 | 3.189±0.003786 |
| 4 | 16 | 5.905 | 5.913 | 5.91 | 5.909±0.004041 |
| 5 | 32 | 8.997 | 9 | 9.001 | 8.999±0.002082 |
| | Unknown Concentration | 2.65 | 2.8 | 2.96 | 2.47±0.447996 |

Total phenolic content of the alcoholic extract of P. guajava was determined by using the Folin-Ciocalteu reagent and were expressed as GAE per gram of plant extract. The total phenolic contents of the extract were calculated using the standard curve of gallic acid **(y = 0.2477x + 1.3244 $R^2$ = 0.9859)** As shown in figures. The alcoholic extract of P. guajava was found to contain total phenolic value of **2.47±0.44799 mg GAE/g** of gallic acid.

The result of standard gallic acid obtained is **y = 0.2477x + 1.3244 $R^2$ = 0.9859.**

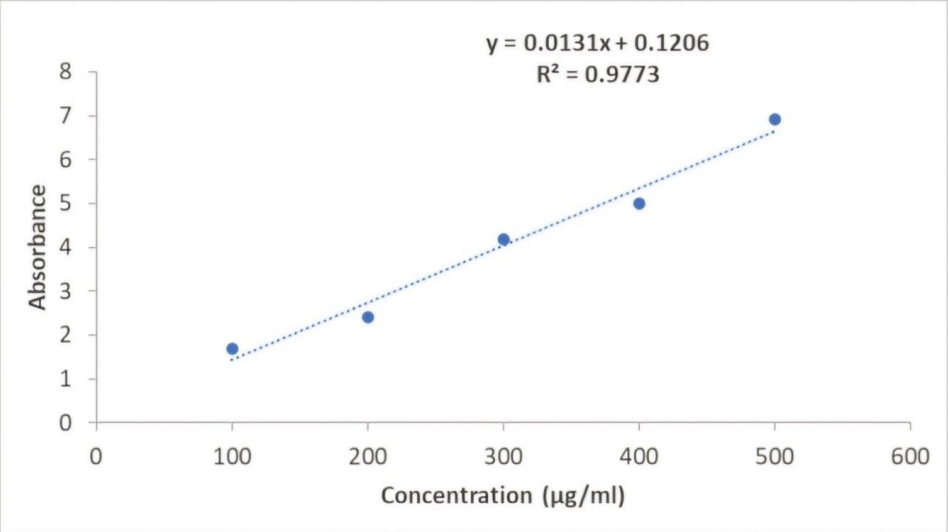



**Graph 1: Graph showing Total phenolic content of *P. guajava.***

The total flavonoid content of alcoholic extract of P. guajava was determined using AlCl3 method. A standard curve of quercetin at different concentrations was obtained and the flavonoid content of the alcoholic extract was extrapolated and calculated as shown in figure. The total flavonoid content of alcoholic extract of P. guajava was calculated as **147.48±1.685mg QE/g extract. R2 = 0.9773.**

**Table 8: Estimation of Total Flavonoid content of methanolic leaf extract of *P. guajava.***

| S. No | Concentration | Absorbance | | | Mean±SD |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | |
| 1 | 100 | 1.695 | 1.68 | 1.697 | 1.691±0.009 |
| 2 | 200 | 2.38 | 2.396 | 2.415 | 2.397±0.018 |
| 3 | 300 | 4.185 | 4.184 | 4.198 | 4.189±0.008 |
| 4 | 400 | 5.004 | 5.005 | 4.994 | 5.001±0.006 |
| 5 | 500 | 6.915 | 6.923 | 6.925 | 6.921±0.005 |
| | **Unknown Concentration** | 145.6 | 148 | 148.85 | 147.48±1.685 |



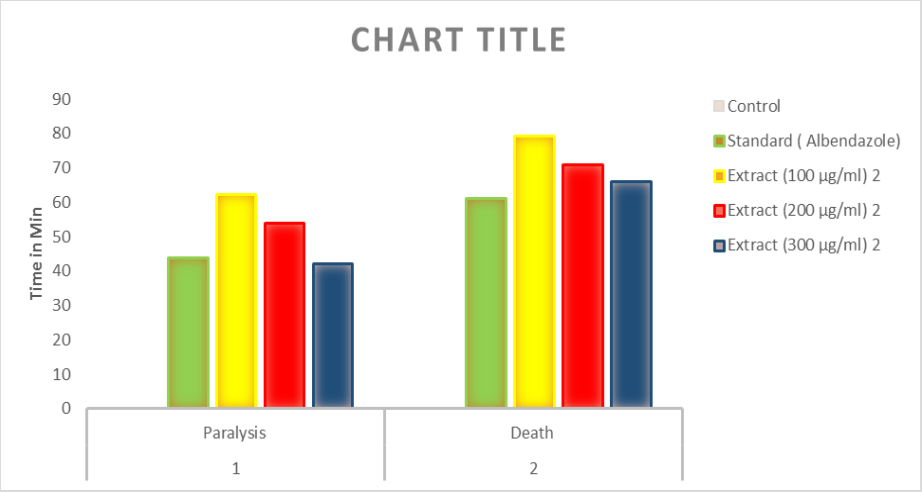

**Graph 2: Graph showing Total Flavonoid content of *P. guajava.***

**Table 9: Paralysis and death time *P.guajava* extract and Albendalzole.**

| S.No | Category | Control | Standard (Albendazole) | Extract (100 µg/ml) 2 | Extract (200 µg/ml) 2 | Extract (300 µg/ml) 2 |
|---|---|---|---|---|---|---|
| 1 | Paralysis | 0 | 43.97 | 62.17 | 53.96 | 42.14 |
| 2 | Death | 0 | 61.17 | 79.34 | 70.91 | 66.09 |


**"Fig 4": Showing anthelmintic activity of P. Guajava extract.**

P. Guajava (100mg/ml) P. Guajava(200mg/ml) P. Guajava (300mg/ml).




**Graph 3: Anthelmintic activity of *P. guajava* extract and Albendazole.**

## DISCUSSION

Phytochemical investigation and in vitro anthelmintic activity studies on Psidium guajava, commonly known as guava, provide valuable insights into its medicinal potential.

Phytochemical investigation involves the systematic analysis of plant constituents to identify bioactive compounds. In the case of Psidium guajava, researchers have identified several phytochemicals such as flavonoids, tannins, phenols, and essential oils. These compounds are known for their antioxidant, antimicrobial, and anthelmintic properties.

The extract was studied in different concentrations (100, 200 and 300mg/ml). The anthelmintic activity P. Guajava extract in lower concentration is not significant, in higher concentration extract 200 and 300 mg/ml exhibited 53.96±1.820 and 42.14±1.91minutes paralysis time respectively when compared to the standard Albendazole which was 43.97±1.63 minutes. The death time recorded was 70.91±1.16 and 66.09±4.15 minutes respectively in comparison to the standard which was recorded to be 61.17±7.25 minutes. Effectiveness of the extract P. Guajava was inversely proportional to the time for paralysis (vermifuge) and death (vermicidal) of the worms. This suggests that *Psidium guajava* extracts may interfere with the metabolic pathways or structural integrity of the parasites, leading to their expulsion or death.

The anthelmintic potential of Psidium guajava makes it a promising candidate for the development of natural treatments for helminth infections in both humans and animals. Its efficacy in vitro suggests that further research, including in vivo studies and clinical trials, could validate its use as an alternative or adjunct therapy to conventional anthelmintic drugs.

## CONCLUSION

In conclusion, the phytochemical investigation and in vitro anthelmintic activity of Psidium guajava highlight its potential as a valuable natural resource for combating parasitic infections. The diverse array of bioactive compounds found in guava, including flavonoids, phenolics, tannins, terpenoids, and alkaloids, contribute to its anthelmintic properties. These compounds have demonstrated efficacy against various species of parasitic worms in controlled laboratory settings.

However, while these findings are promising, further research is needed to fully understand the mechanisms of action behind Psidium guajava's anthelmintic activity and to assess its safety and efficacy in clinical settings. Additionally, exploring the synergistic effects of different phytochemicals present in guava could enhance its anthelmintic potency and minimize the risk of drug resistance.

Overall, Psidium guajava represents a natural and sustainable alternative for combating parasitic infections, offering potential benefits for human and animal health. Further investigation and validation through clinical trials are necessary steps towards harnessing the full therapeutic potential of this plant species.

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