

Pharmacognostic and Pharmacological Screening of *Psidium guajava* Stem Extract for its Analgesic Potential

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Abstract

Context: The search for biologically active compounds from natural source has always been of great interest to researchers looking for new source of drugs useful in infectious diseases. Higher plants have played a vital role as the source of important therapeutic agents. **Objective:** The present investigation was aimed to find novel analgesic agent from herbal origin. For the purpose, *Psidium guajava* stem extracts was screened for its analgesic potential. **Materials and Methods:** Animal model of acetic acid induced writhing was followed. Three different extracts were used to study the activity. **Results:** The methanolic extract at the dose tested was shown to possess analgesic activity. The significant reduction in acetic acid-induced writhings suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of prostaglandins (PGs) and other endogenous substances. **Discussion:** The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics. In general acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, PGs bradykinins and substance P, which stimulate nerve endings. Local peritoneal receptors are postulated to be involved in the abdominal constrictions response. **Conclusion:** It can be concluded that the crude extract of stem of *P. guajava* have given positive results for analgesic activity. These medicinal herbs may afford lead compounds which could be beneficial for future drug development.

Key words: Standardization, *psidium guajava*, analgesic

INTRODUCTION

Before the introduction of modern medicines, disease treatment was entirely managed by herbal remedies. It is estimated that about 80% of the world population residing in the vast rural areas of the developing and under developed countries still rely mainly on medicinal plants. Medicinal plants are the only affordable and accessible source of primary health care for them, especially in the absence of access to modern medical facilities. Studies reveal that there are more traditional medicine providers than the allopathic providers especially in the rural areas.^[1]

Psidium guajava is a low evergreen tree or shrub 6–25 feet high, with wide-spreading branches and square, downy twigs, is a native

of tropical America. The branches and stems are usually crooked and have a smooth surface.^[2] The sapwood is light brown and the heartwood is reddish brown, hard, heavy, and strong. Leaves are opposite, oblong or elliptic, margin entire, glabrous above, prominent nerved and pubescent beneath.^[3]

Guava is rich in tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fiber

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Received: 11-02-2018

Revised: 20-03-2018

Accepted: 05-04-2018

and fatty acids. Guava fruit has higher content of Vitamin C than citrus and contains appreciable amounts of Vitamin A as well. Guava fruits are also a good source of pectin-a dietary fiber. The leaves of guava are rich in flavonoids in particular quercetin. Much of guava's therapeutic activity is attributed to these flavonoids. The bark of guava tree contains considerable amount of tannins (11–27%).^[4] Leucocyanidin, luectic acid, ellagic acid, gallic acid, amritoside with new pentacyclic triterpenoids guajanoic acid, oleanolic acid and ursolic acid have been recently isolated from the leaves of guava.

Extracts from apple guava (leaves or bark) are implicated in therapeutic mechanisms against cancer, bacterial infections, inflammation and pain.^[5,6] Essential oils from guava leaves display in vitro anticancer activity.^[7] Guava leaves are used in folk medicine as a remedy for diarrhoea and guava bark is used as an astringent and antimicrobial agent. Traditionally Guava leaves are used as antidiabetic agent.^[8]

As this plant has multifarious biological activities and used traditionally to treat various ailments, therefore, it must be authenticated and standardized to avoid any kind of adulteration. Taking in view its importance this study was designed to evaluate pharmacognostic parameters and its pharmacological evaluation for analgesic potential.

MATERIALS AND METHODS

Plant material

The stems of *P. guajava* were collected from herbal garden of Lovely Professional University, Phagwara. Plant materials were authenticated at Regional Research Institute (Ayurvedic), Bangalore and a voucher specimen no. NADRI/BNG/SMP/Drug Authentication/2010- 11/644) has been retained at the Department of Pharmacognosy and Phytochemistry, Lovely Professional University, Phagwara, Punjab.

Experimental animals

Swiss Albino mice were purchased from National Institute of Pharmaceutical Education and Research, Mohali. The experimental protocol was approved by the Institutional Animal Ethics Committee.

Standardization

Powder microscopy

The powder of stems of *P. guajava* was stained with respective reagents such as phloroglucinol, hydrochloric acid, iodine solution and ruthenium red. The sample was mounted on glass slide and observed under the microscope ($\times 10$ and $\times 40$) to determine the type of cells, phloem fibres and lignified tissues. The prepared slides were scanned in NIKON eclipse 90 i scanner.^[9]

Determination of moisture content

Karl Fischer titration

The titration beaker was filled with 20 ml methanol. Then the stopcock of the automatic Karl Fischer burette was opened slowly so that Karl Fischer reagent from the bent tube was added dropwise to the methanol in the beaker. With addition of every Karl Fischer reagent drop, the pointer swung to right side and came back again to its zero position. The solenoid valve kept on switching off and on until complete moisture in the methanol was removed. At the end point, the meter pointer remained on the right side. The reading on the Karl Fischer burette was noted and Karl Fischer factor was calculated as:

$$\text{Karl Fischer factor} = \frac{15.66}{\text{Karl Fischer ml on the burette}}$$

A specified quantity (50 mg) was weighed and added to the methanol in the beaker. The start titration button was pressed and the sample was titrated.^[10] The moisture content was calculated by the formula:

$$\text{Moisture content} = \frac{100 \times \text{Karl Fischer reagent reading} \times \text{KF Factor}}{\text{Weight of sample (mg)}}$$

Extraction

Successive solvent extraction [Figure 1] was performed on small scale with coarsely dried powdered plant material (50 g). The dried powdered stems were successively macerated with acetone, methanol and distilled water respectively for 48 h. The extracts were filtered through Whatman filter paper. The extracts after filtration were evaporated to dryness under reduced pressure and made to a specified volume.

Analgesic activity

Animals used

Swiss albino mice of either sex weighing 25–40 g breed in National Institute of Pharmaceutical Sciences and Research, Mohali were used.

Housing

The animals were housed in the animal house in groups of five animals each in clean polyacrylic cages. The animals were housed under 12 h light and dark cycle with an average temperature $25 \pm 2^\circ\text{C}$ and humidity 55–65%.

Diet

The mice were fed with commercial pelleted animal feed supplied by M/S Hindustan Lever Ltd., Bangalore, India and free access of water.

Extracts used

Acetone, methanol and aqueous extracts of stem of *P. guajava* were subjected to evaluate the analgesic activity.

Evaluation of analgesic activity

Twenty five healthy mice were divided into ten groups for each extract, each group consisting of five animals. The allocation of animals to various groups was done as given in Table 1. Dried extracts were dissolved in distilled water and 2.5% Tween 80. Each animal of different groups was given the mentioned dose of extracts with oral administration and acetic acid was given i.p.

Animal model

Acetic acid induced writhing model was used for activity determination.

Procedure

The *P. guajava* stem extracts, Tween 80 in saline (control) or Acetylsalicylic acid will be administered orally to the animals after 12 h of fasting. 60 min after extract administration, 1% (v/v) acetic acid (dose of 10 ml/kg) will be injected (i.p.).^[11] Writhings that occurred between 5 and 15 min. after acetic acid administration will be counted. Acetylsalicylic acid (ASA-150 mg/kg orally) will be used as positive control. Significant reduction in the number of writhings compared to the control (that received tween 80 in saline) will be considered as analgesic response.^[12]

Statistical analysis

Each group consisted of 5 animals. The data was expressed as mean \pm standard error of mean. All the extracts were compared with control group using one way analysis of variance followed by Dunnet test. $*P < 0.05$ was considered statistically significant.

RESULTS

Standardization

Powder microscopy

Powdered microscopy of *P. guajava* stem was done. Small amount of fine powder of stem of *P. guajava* was taken,

Table 1: Allocation of animals for analgesic activity

| Group No. | Group name | Dose (mg/kg) |
|-----------|------------------------------|--------------|
| 1. | Control group (vehicle) | - |
| 2. | Standard group (Aspirin) | 150 |
| 3. | Test group-acetone extract | 200 |
| 4. | Test group- methanol extract | 200 |
| 5. | Test group- aqueous extract | 200 |

stained and examined under the microscope, lignified fibres, xylem vessels, phloem fibres and calcium oxalate crystals [Figure 2] were observed. Tran verse section of stem was also observed [Figure 3]. It shows thick walled outer layer covered with thick cuticle. Outer layer also shows unicellular trichomes. Cambium layer is present beneath the outer layer. Fibres and vessels are lignified. Medullary rays are thick walled and lignified. Centre is occupied by pith consists of oval, parenchymatous cells. Crystals are abundant.

Moisture content

KF factor = 15.66/KF ml on the burette

KF factor = 15.66/0.8=31.32

Moisture content = $\times 100$ KF reagent reading \times Factor/Weight of sample (mg)

It was observed that moisture content of *P. guajava* stem in case of Karl Fischer method was found to be 25.5%.

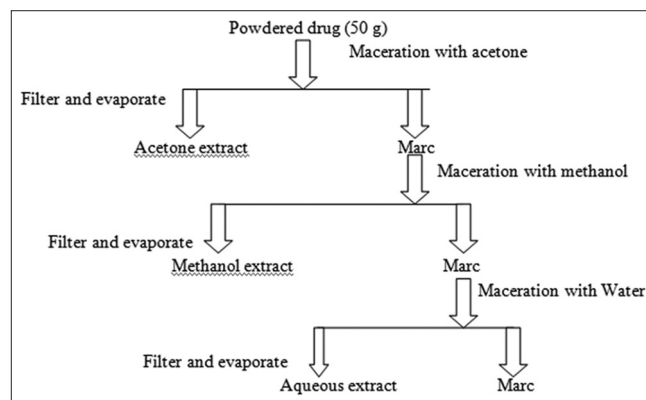


Figure 1: Scheme of extraction^[14]

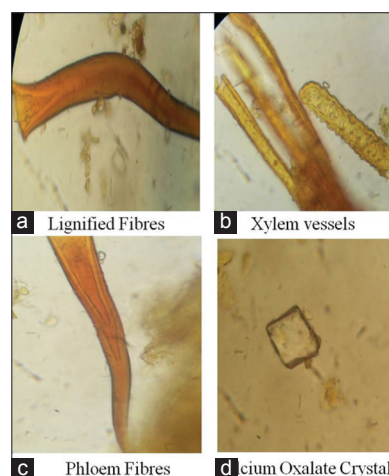


Figure 2: Powder microscopy characters of *Psidium guajava* stem extracts. (a) Lignified fibres, (b) xylem vessels, (c) phloem fibres, (d) calcium oxalate crystals

Table 2: Effect of *P. guajava* on acetic acid induced writhings in mice

| Treatment | Dose mg/kg | Writhings | Inhibition (%) |
|------------------|------------|--------------|----------------|
| Control (saline) | - | 42.8±1.241 | - |
| Standard-aspirin | 150 | 10.4±0.509** | 75.7 |
| Acetone extract | 200 | 29.8±0.916** | 30.37 |
| Methanol extract | 200 | 15±1.049** | 64.95 |
| Aqueous extract | 200 | 21.4±0.509** | 50 |

Each value represents mean±SEM, $n=5$. Statistical significant test with control was done by one-way ANOVA followed by Dunnet test through INSTAT software. ** $P<0.01$. *P. guajava*: *Psidium guajava*, ANOVA: Analysis of variance, SEM: Standard error of mean

Extraction

The plant material (50 g) was successively macerated with acetone (300 ml), methanol (300 ml) and water (300 ml) at room temp. for 48 h.

Analgesic activity

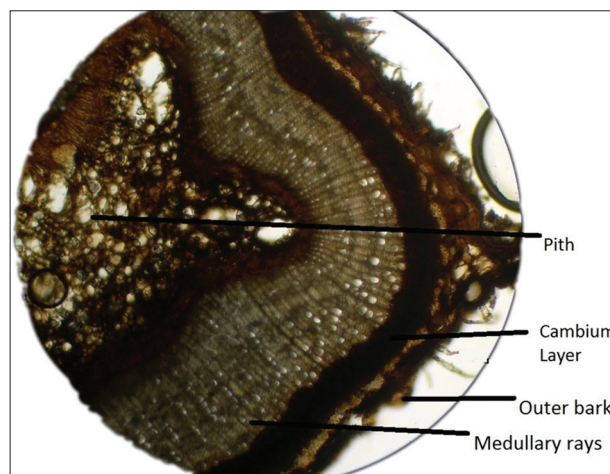
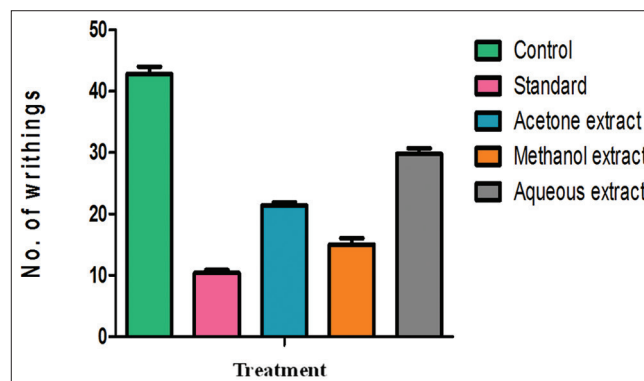
The effect of acetone, methanol and aqueous extracts of *P. guajava* on writhing response in mice is shown in Table 2. The results of present study reveal the analgesic activity of acetone, methanolic and aqueous extracts at a dose of 200 mg/kg p.o. The extracts showed significant inhibitory activity on the writhing response induced by acetic acid when compared to that of control. Methanol extract was found to be more efficacious among all extracts. Dose of 200 mg/kg of the acetone, methanol and aqueous extracts of *P. guajava* could block the writhing response by 30.37%, 64.95% and 50% respectively [Figure 4].

DISCUSSION

Recently there has been a shift in universal trend from synthetic to herbal medicine, which we can say “Return to Nature.” Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments.^[13]

Pharmacognostical study is the study of the structural, physical, chemical and sensory characters of crude drugs of animals, plants and mineral origin. The search for biologically active compounds from natural source has always been of great interest to researchers looking for new source of drugs useful in infectious diseases. Higher plants have played a vital role as the source of important therapeutic agents.

The present investigations were aimed at standardization, preliminary phytochemical investigations, developing thin-layer chromatography profiles and pharmacological study of the stem of *P. guajava*. Phytochemical screening of the crude extract revealed the presence of tannins, flavonoids and alkaloids. Polyphenolic compounds like flavonoids, tannins and phenolic acids commonly found in plants have

**Figure 3:** Transverse section of *Psidium guajava* stem**Figure 4:** Comparative effect of different extracts of *Psidium guajava* stem on acetic-acid induced writhings in mice

been reported to have multiple biological effects including antioxidant activity. Flavonoids present in the plant extract, as evident from phytochemical screening may be responsible for the antioxidant action. Antioxidant compounds may function as free radical scavengers, initiators of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation. Phenolic compounds and flavonoids are major constituents of most of the plants reported to possess antioxidant and free radical scavenging activity. Therefore, search for natural antioxidant source is important.

The methanolic extract at the dose tested was shown to possess analgesic activity. The abdominal constriction

response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics. In general acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, prostaglandins (PGs), bradykinins and substance P, which stimulate nerve endings. Local peritoneal receptors are postulated to be involved in the abdominal constrictions response. The significant reduction in acetic acid-induced writhings suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of PGs and other endogenous substances.

CONCLUSION

From the above, it can be concluded that the crude extract of stem of *P. guajava* have given positive results for analgesic activity. This activity was evaluated from crude extracts. So, further work can be done on isolation and characterization of the components which are responsible for these activities. Moreover, these medicinal herbs may afford lead compounds which could be beneficial for future drug development.

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Source of Support: Nil. **Conflict of Interest:** None declared.