Journal of Advanced Pharmacy Education & Research
2: 177-183 (2011) ISSN 2249-3379

Thin layer chromatographic profiling and evaluation of analgesic activity of *Psidium guajava* leaf extracts in mice

Jaydeep Sarkar[1], Sujoy Pal[1], Sanjib Bhattacharya[2*], Moulisha Biswas[1]

1. Bengal Institute of Pharmaceutical Sciences, Kalyani, Nadia 741235, West Bengal, India.
2. Bengal School of Technology (A College of Pharmacy), Delhi Road, Sugandha, Hooghly 712102, West Bengal, India.

*Corresponding author: sakkwai@yahoo.com

ABSTRACT:

Psidium guajava L. (Myrtaceae), commonly known as guava in English, is an evergreen small tree grown in tropics and subtropics and also cultivated commercially in India for its consumable fruits. The present study assessed the different solvent extracts of *P. guajava* leaf for thin layer chromatography (TLC) and also evaluated their analgesic potential by acetic acid induced writhing assay in Swiss albino mice. The chloroform extract yielded maximum spots in TLC, followed by methanol and petroleum ether extracts respectively. The entire test extracts exhibited significant analgesic activity. The methanol extract was found to be the most potent followed by the chloroform and petroleum ether extracts respectively. The present preliminary study demonstrated marked analgesic activity of *P. guajava* leaf in Swiss mice.

Key words: Analgesic, *Psidium guajava*, writhing, leaf.

INTRODUCTION:

Psidium guajava L. (Myrtaceae), an evergreen small tree, commonly known as guava in English, is an important food crop and medicinal plant indigenous to South America and grown in tropical and subtropical countries, and widely used like food and in folk medicine around of the world. It is commercially cultivated in India for its consumable and palatable fruits. A number of metabolites in good yield and some have been shown to possess useful biological activities belonging mainly to phenolic compounds, flavonoids, carotenoids, terpenoids and triterpenes. Extracts and secondary metabolites of this plant, particularly those from leaves and fruits possess useful pharmacological activities. *P. guajava* is mainly known for its antispasmodic and antimicrobial properties

in the treatment of diarrhoea and dysentery. It has also been used extensively as an oral hypoglycaemic agent. Several pharmacological studies have demonstrated the ability of this plant to exhibit antioxidant, hepatoprotection, anti-allergic, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, antitussive, antidiabetic, antiinflamatory and antinociceptive activities, supporting its traditional uses. [1, 2]. However, the planar chromatographic profile and analgesic assessment of petroleum ether, chloroform and methanol extract from *P. guajava* leaf are still not reported. Therefore, in the present investigation we attempted these studies on the leaf extracts of *P. guajava* grown in India.

MATERIALS AND METHODS:

Plant material: The mature leaves of *Psidium guajava* L. (Myrtaceae), were collected during November 2010 from Nadia, West Bengal, India. The plant material was taxonomically identified by Dr. P. Venu, Scientist F, Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen (CNH/32/2011/Tech.II/424) was maintained in our research laboratory for future reference. The plant material was shade-dried with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40, and stored in an air-tight container.

Preparation of plant extracts: The dried powdered material (350 g) was defatted with petroleum ether (60-80°C), the percentage extractive value was 3.72% w/w. The defatted powder material thus obtained was further extracted with chloroform and methanol for 72 h in a percolator. The solvent was distilled off in reduced pressure and resulting semisolid mass was vacuum dried using rotary flash evaporator to yield a solid residue and the percentage extractive values were accordingly 5.09% w/w and 7.87% w/w respectively. The preliminary phytochemical analysis was performed for all three extracts to identify the phytoconstituents present in the extracts. [3]

Chemicals: Acetyl salicylic acid (aspirin) and glacial acetic acid from Sigma-Aldrich Chemical Corp. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade obtained commercially.

Experimental animals: Adult male albino mice of Swiss strain weighing 20 ± 2 g were procured from registered breeders (Rita Ghosh & Co., Kolkata, India) and maintained under standard laboratory conditions (temperature 25 ± 2°C with dark and light circle

14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The mice were acclimatized to laboratory condition for 10 days before commencement of the experiment. All experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee (Reg. no. 367001/C/ CPCSEA).

Thin layer chromatographic studies: Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel G as stationary phase. The mobile phases, results and chromatograms are depicted in Figures 1-3.

Analgesic evaluation: acetic acid-induced writhing test: Swiss albino mice were divided into five groups (n = 6). Group I received acetic acid (1% v/v, 10 ml/kg b.w., i.p.) and writhing reflex was noted for the period of 15 minutes. Group II received aspirin (100 mg/kg b.w. p.o.) Groups III, IV and V received the petroleum ether, chloroform and methanol extracts at the doses of 200 mg/kg b.w., p.o. respectively. Thirty minutes after aspirin and extracts administration, group II to V received acetic acid (1% v/v, 10 ml/kg b.w., i.p.) and writhing reflex was noted for the period of 15 min. [4, 5] The mean writhing scores in each group were calculated and expressed the percentage of protection using the following formula:-

(Control mean - Treated mean/ Control mean) ×100 %

Statistical analysis: The data are represented as mean ± standard error of mean (SEM). Degree of significance was assessed by Student's '*t*' test.

RESULTS AND DISCUSSION:

Preliminary phytochemical studies showed the presence of steroids in the petroleum ether extract; triterpenoids in the chloroform extract; and steroids, glycosides, phenolic compounds, tannins, carbohydrates, saponins in the methanol extract.

Among the various methods for separating plant constituents, the thin layer chromatographic procedure is the one of the most commonly used techniques of general application. [6] Thin layer chromatography (TLC) involves the separation of mixtures of organic compounds on thin layers of adsorbents that are usually coated on glass, plastic, or aluminum sheets; and this particular technique is the easiest, cheapest and

most widely used method for the characterization of natural products and their preparations. [7] The chloroform extract yielded maximum spots in TLC, followed by methanol and petroleum ether extracts respectively (Figures 1-3). All of these TLC profiles may serve as characteristic fingerprint of *P. guajava* leaf. These data would therefore be suitable for monitoring the identity and purity of the plant material and for detecting adulterations and substitutions. [8]

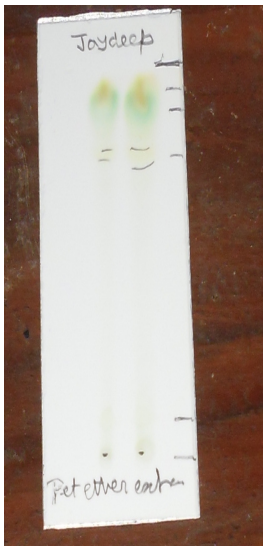

Fig. 1. TLC profile of the pet. ether extract of *P. guajava* leaf. Solvent system: benzene: chloroform: ethyl acetate (4: 3: 3). R_f values: 0.10, 0.74, 0.87, 0.93.

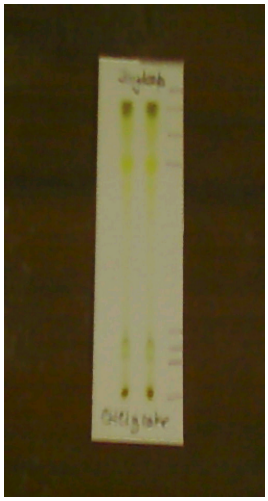

Fig. 2. TLC profile of the chloroform extract of *P. guajava* leaf. Solvent system: benzene: chloroform: ethyl acetate (3: 4: 3). R_f values: 0.10, 0.15, 0.21, 0.73, 0.84, 0.93.

Fig. 3. TLC profile of the methanol extract of *P. guajava* leaf. Solvent system: ethyl acetate: methanol (7: 3). R_f values: 0.34, 0.44, 0.50, 0.68, 0.84.

The analgesic efficacy of *P. guajava* leaf extracts was evaluated by acetic acid induced writhing method in mice to assess peripheral (non-narcotic) type of analgesic activity. [8] Acetic acid induced writhing is chemically induced nociception by intraperitoneal injection of dilute acetic acid solution to mice. The chemical agents can produce nociceptive reactions in mice. Intra-peritoneal injection of phenyl para quinone, bradykinin or dilute acetic acid (1-3% v/v) produces pain reaction that is characterized as writhing response. Constriction of abdomen, turning of trunk (twist) and extension of hind limbs (at least one) are considered as writhing reaction to chemically induced pain. [9, 10]

Acetic acid induced writhing test is known as a visceral pain model nociception. Several mediators like kinins, acetylcholine, substance P, calcitonin-gene-related peptide and prostaglandins (PG) take part in visceral pain model nocicepotion and transmission of the nociception from the viscera. In this test both central and peripheral analgesics are detected. Analgesics of narcotic (central) e. g. morphine, pentazocin, pethidine etc and non-narcotic (peripheral) type, e. g. aspirin, ibuprofen, indomethacin etc. can inhibit the writhing response in mice. [9-11]

Based on the results obtained from the present study, it can be inferred that all the test extracts had effective peripheral analgesic actions. The methanol extract was found to

be the most potent followed by the chloroform and petroleum ether extracts respectively (Table 1). The present preliminary study confirms marked analgesic activity of *Psidium guajava* leaf which may be due to presence of multitude of constituents as revealed by TLC profile.

Table 1. Analgesic effect of *P. guajava* leaf extracts on acetic-acid induced writhing in mice.

Treatment	Dose	Number of writhing	% Inhibition
Acetic acid (1% v/v)	10 ml/kg	52.83 ± 1.400	-
Acetic acid + Aspirin	100 mg/kg	17.66 ±1.606*	66.57
Acetic acid + Pet. Ether extract	200 mg/kg	22.27 ± 1.383*	57.84
Acetic acid + Chloroform extract	200 mg/kg	18.15 ± 1.351*	65.64
Acetic acid + Methanol extract	200 mg/kg	15.09 ± 1.971*	71.43

Values are mean ± SEM (n = 6). *p < 0.001 when compared to control.

REFERENCES:

1. Sanda K.A., Grema H.A., Geidam Y.A., Bukar-Kolo Y.M. Pharmacological aspects of *Psidium guajava*: An update. Int. J. Pharmacol. 2011; 7: 316-324.
2. Gutiérrez R.M., Mitchell S., Solis R.V. *Psidium guajava*: a review of its traditional uses, phytochemistry and pharmacology. J. Ethnopharmacol. 2008; 117: 1-27
3. Kokate CK (1994): Practical Pharmacognosy. 4th Edition. New Delhi. Vallabh Prakashan. pp. 107-112.
4. Kostar R., Anderson M., de Beer E.J. Acetic acid for analgesic screening. Fed. Proc. 1959; 18: 412.
5. Bhattacharya S., Nagaich U. Assessment of anti-nociceptive efficacy of *Costus speciosus* rhizome in Swiss albino mice. J. Adv. Pharm. Tech. Res. 2010; 1: 34-40.
6. Kokate C.K., Purohit A.P., Gokhale S.B. Pharmacognosy. 34th ed. Nirali Prakashan: Pune, 2006.

7. Stahl E. Apparatus and general techniques. In: Stahl E., editor. Thin Layer Chromatography, A Laboratory Handbook. 2nd ed. Springer-Verlag: Berlin, Heidelberg, New York, 1969, 52-85.
8. Ghosh A.K., Bhattacharya S. Planar chromatographic studies on *Abies webbiana* leaves. Int J Chem. Tech. Res. 2009; 1: 807-814.
9. Vogel H.G. Drug Discovery and Evaluation, Pharmacological Assays. 2nd ed. Springer Verlag: Berlin, Heidelberg, 2002.
10. Kulkarni S.K. Hand Book of Experimental Pharmacology. 3rd ed. Vallabh Prakashan: New Delhi, 1999.
11. Parkes M.W., Pickens J.T. Conditions influencing the inhibition of analgesic drugs of the response to intra peritoneal injections of phenylbenzoquine in mice. Brit. J. Pharmacol. 1965; 25: 81-87.