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Evaluation of antidiarrhoeal effects of *Psidium guajava* L. (Myrtaceae) aqueous leaf extract in mice

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ABSTRACT

A crude aqueous extract of the leaves of the guava tree, *Psidium guajava* L. (Myrtaceae), were studied for antidiarrhoeal effects, to place one of its traditional medical uses. The extract induced a decrease in the propulsive movements of the intestinal contents in mice. These findings suggested that an aqueous extract of guava leaves may be used as an effective treatment for non-specific diarrhoea in folk medicine.
Keywords: Psidium guajava L. (Myrtaceae); *in vivo* test; guava tree; intestinal motility.

Brazil enjoys a wide environmental and biological diversity, compared to the rest of the world. From the vast array of the Materia Medica of the indigenous system, many plants have been reported to have activity against diarrhoea and thus act as very useful remedies for the alleviation of human suffering (Mukherjee et al., 1998). Diarrhoea is a very common ailment and a national problem in many tropical countries, causing millions deaths of throughout the world annually.

Psidium guajava L. (Myrtaceae), the common guava tree, is widely distributed as a native plant in Latin America, from Mexico to Brazil. The leaves have been used in folk medicine for many years to treat diarrhoea, stomach ache and hepatic problems. *P. guajava* is a medium-sized tree, reaching a height of 8m. To date, phytochemical investigations have been reported on the tannins, flavonoids (Habib, 1986; Padula & Rodriguez-Amaya, 1986; Lutterodt, 1989; Lutterodt, 1992), essential oils (Smith & Siwatibau, 1975; Macleod & Troconis, 1982), proteins (Deo & Shastri, 2003), sesquiterpenoid alcohols and triterpenoid acids (Smith & Siwatibau, 1975; WilsonIII & Shaw, 1978; Begum et al., 2002). *P. guajava* is also a relatively well-studied species with respect to diarrhoea (Lutterodt, 1989; Tona et al., 1998; Gonçalves et al., 2005). The bark, leaves, fruit and root have also been evaluated pharmacologically for the treatment of gastrointestinal diseases (Tona et al., 1998; Rabe & van Staden 1977; Cáceres et al., 1993; Olajide et al., 1999; Lans et al., 2000; Lin et al., 2002; Lozoya et al., 2002). This plant possesses antimicrobial (Gnan & Demello, 1999; Jaiarj et al., 1999; Abdelrahim et al., 2002), antimutagenic (Grover & Bala, 1993) and hypoglycaemic (Roman-Ramos et al., 1995; Oh et al., 2005) properties.

Worldwide, the leading guava producers are Brazil, Colombia, Egypt, India, Mexico, Pakistan, South Africa and Venezuela (Salazar et al., 2006).

The aim of this work is to evaluate the gastrointestinal effects of an aqueous extract of *P. guajava* leaves in order to provide a scientific basis for the popular medical use of these leaves.

Fresh leaves of *P. guajava* were collected in the Medicinal Plant Garden at the School of Pharmaceutical Sciences – UNESP, Araraquara, São Paulo, Brazil, in January 2003 and have been kept in our laboratory for future reference. Botanical identity was kindly authenticated by Dr. LVS Sacramento of the Department of Active Natural Products, School of Pharmaceutical Sciences, São Paulo State University (UNESP) at Araraquara (São Paulo, Brazil) and a specimen of the plant has been deposited in the University Herbarium.

The plant material was air dried and then ground with a five mm diameter mesh. The plant extract was prepared as described by Michelin & Marona (2004). The powdered material was extracted in water by bringing the mixture to the boil, leaving to stand and filtering. The aqueous extract was evaporated in a rotary evaporator and then reconstituted with sterile water (100 mg/mL).

Freshly-prepared extracts were tested for the presence of flavonoids and tannins by thin-layer chromatography (TLC). The analysis was performed on Merck® silica gel 60 F_{254} aluminum plates, which were developed with the following mobile phases:

butanol/acetic acid/water (8:1:1, v/v/v) solution
ethyl acetate / formic acid / water (90 : 5 : 5, v/v/v)
chloroform : methanol : *n*-propanol : water (5:6:1:4, v/v/v/v).

The TLC plates were sprayed either with cerium sulphate, ninhydrin or Dragendorff reagent, and then visualized under UV light at 254 nm and 365 nm.

Mice were obtained from the Animal Facility

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Centre of UNESP. Their experimental treatment conformed to the National Institute of Health (1996).

The method used in the present biological assay was described by Janssen & Jageneau (1957), Wong & Way (1981), Michelin & Marona (2004) and Salgado et al. (2005). Twenty adult female Albino Swiss mice (*Mus domesticus domesticus*), weighing 24-30 g, were randomly selected and housed in polypropylene cages (30 x 20 x 13 cm) under standardized conditions (21 ± 1°C with a 12 h reversed light-dark cycle and relative humidity 50-60%) for 10 days before performing the experiment. They had free access to water and normal commercial laboratory diet (Purina, Brazil). On the day of the test 20 animals were randomly divided into two groups of 10 mice each. They were weighed and deprived of food, with free access to water. After three hours of food deprivation the animals in groups A and B received orally by gavage 1000 mg/kg body weight of *P. guajava* extract and 0.9% NaCl sterile solution, respectively. Ninety minutes after administering the extracts, 0.3 mL of a 5% charcoal suspension in 10% aqueous suspension of charcoal powder was administered to each animal orally. The animals were sacrificed 45 minutes later and the abdomen opened. The distance moved by the charcoal meal from the pylorus toward the caecum was measured by dissecting the intestine and expressed as a percentage of the total distance from pylorus to caecum for each animal.

This protocol was approved by the Research Ethical Committee of the School of Pharmaceutical Sciences of São Paulo State University (UNESP), Araraquara, São Paulo, Brazil.

The results were expressed as the mean ± S.D. Statistical significance was performed using a Student's *t*-test. A difference was taken to be significant at $P < 0.05$.

In this study, the anti-diarrhoeal effect of the aqueous extract from *P. guajava* leaves, which was selected on the basis of its popular use, was investigated using *Mus musculus* as *in vivo* model. Ninety minutes after administration by gavage of the *P.guajava* extract, significant diminution of intestinal motility was observed in the mice, suggesting that the aqueous extract from *P. guajava* leaves could have beneficial effects on diarrhoea.

The time spent to eliminate the charcoal plug and the standard deviation are shown in Table 1. This feature may explain the use of *P. guajava* as a non-specific antidiarrhoeal agent in traditional medicine.

Intestinal diseases are one of the main causes of death among infants particularly in developing countries. A second important aspect is the large number of plant extracts which are used for their anti-diarrhoeal activity. These two facts make it important to identify and evaluate commonly available natural drugs as an alternative to currently used anti-diarrhoeal drugs. Readily available methods in the literature can be used to characterize and identify many natural products.

Tannins, phenolic compounds and flavonoids were all detected in a preliminary phytochemical screening of the dried extract of guava leaves. These results are confirmed in the literature. Gonçalves et al. (2005) also described flavonoids and tannins. Interestingly, Lozoya et al. (2002) demonstrated that the flavonoid quercetin and its glycosides may be the active compounds.

Various plants with such activities continue to be widely used in the treatment of diarrhoea and may act by several mechanisms, mediated mainly by either μ or d-opioid receptors on enteric nerves, epithelial cells, and muscle. This includes effects on intestinal motility (μ receptors), intestinal absorption (d- receptors) or both (μ and d-receptors). Some agents are preferred to other agents because of their limited ability to penetrate the central nervous system (CNS) (Jafri & Pasricha, 2001).

Each animal received 1000 mg/kg by the oral route. Our results suggest that *P. guajava* aqueous extract showed lowest percentages in small intestine distance. In addition to providing promising new leads in the ongoing search for new drugs, the data analysis in this study has also suggested how future pharmacological investigation and ethnobotanical literature can be useful in guiding research.

The results of this research reveal that the aqueous leaf extract of *P. guajava* contains pharmacologically active substances(s) with anti-diarrhoeal properties, revealed in their inhibitory effect on gastrointestinal propulsion.

According to Bae et al. (2000), some substances, such as flavonoids, from herbal drugs can reach the small intestine without being processed and are then metabolised by the intestinal microflora in the large intestine.

Table 1. Effect of 1000 mg/kg of *P. guajava* aqueous extract on gastrointestinal motility in mice.

Treatment	Distance of charcoal (cm) ± S.D.
Control (saline 0.9%)	77.52 ± 8.97
P. guajava extract (1000 mg/kg)	64.08 ± 8.50*

Values are mean ± S.D., n = 10 (per *group); *P* < 0.05 *vs.* Control, Student's *t*-test

Thus, forthcoming experiments are planned to isolate and identify the bioactive substances in this herbal medicine.

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RESUMO

Avaliação de atividade antidiarréica de extrato aquoso de folhas de Psidium guajava L. (Myrtaceae) *em camundongos*

O extrato aquoso bruto de folhas de *Psidium guajava* L. (Myrtaceae) foi estudado quanto às suas atividades antidiarréicas, a fim de racionalizar o seu uso na medicina tradicional. As amostras causaram a diminuição nos movimentos intestinais em camundongos. Estes resultados sugerem que o extrato aquoso de folhas de *P. guajava* pode ser utilizado na medicina tradicional no tratamento de diarréias não específicas.

Palavras-chave: Psidium guajava L. (Myrtaceae); *In vivo*; goiabeira; motilidade intestinal.

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