

Spasmolytic effect of *Psidium guajava* Linn. (Myrtaceae) leaf aqueous extract on rat isolated uterine horns

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Abstract

Globally, primary dysmenorrhoea is one of the most frequent gynaecological disorders in young women. It is associated with increased uterine tone, and exaggerated contractility of uterine smooth muscles. In many rural African communities, a number of medicinal plants, including *Psidium guajava* Linn. (family: Myrtaceae), are used traditionally for the management, control and/or treatment of primary dysmenorrhoea. The present study was, therefore, undertaken to examine the spasmolytic effect of *Psidium guajava* leaf aqueous extract (PGE) on isolated, spontaneously-contracting and oestrogen-dominated, quiescent uterine horns of healthy, young adult, female Wistar rats. Graded, escalated concentrations of PGE (0.5–4.0 mg/ml) produced concentration-dependent and significant inhibitions of the amplitude of spontaneous phasic contractions of the isolated rat uterine horn preparations. In a concentration-related manner, PGE also significantly inhibited or abolished contractions produced by acetylcholine (ACh, 0.5–8.0 μ g/ml), oxytocin (0.5–4.0 μ U), bradykinin (2.5–10 ng/ml), carbachol (CCh, 0.5–8.0 μ g/ml) or potassium chloride (K⁺, 10–80 mM) in quiescent uterine horn preparations isolated from the oestrogen-dominated rats. The spasmolytic effect of PGE observed in the present study lends pharmacological support to the traditional use of 'guava' leaves in the management, control and/or treatment of primary dysmenorrhoea in some rural African communities.

Key words: rat, isolated uterine horns, *Psidium guajava* leaf aqueous extract, spasmolytic effect

Introduction

Globally, primary dysmenorrhoea is one of the most common gynaecological disorders in young women (Doubova *et al.*, 2007). Women with primary dysmenorrhoea have been found to produce higher levels of prostaglandins (PGF_{2 α}) in their endometria, which in turn, increase uterine tones and cause exaggerated uterine muscle contractility (Speroff *et al.*, 1999). A

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number of medicinal plants, including *Psidium guajava* Linn. (family: Myrtaceae), is usually employed traditionally for the management, control and/or treatment of primary dysmenorrhoea in many rural African communities. At present, the Western, conventional treatment of primary dysmenorrhoea depends largely on the administration of non-steroidal anti-inflammatory drugs (NSAIDs) during the painful stages of the menstrual cycle (Dobova *et al.*, 2007). The use of infusions, decoctions and extracts of *Psidium guajava* leaves for the relief of some menstrual disorders associated with abdominal cramps and discomfort has been documented since ancient times (Conway, 2001).

The oestrogenic property of flavonoids is well known. Flavonoids stimulate immature uterine muscles by acting on oestrogen receptors, thereby preventing binding of oestrogens to their receptors on uterine muscles (Revuelta *et al.*, 1997). The pharmacological effects of the flavonoid quercetin on β_2 -adrenoceptors have been described in intact rat adipocytes (Kuppusamy and Das, 1994). The present study was, therefore, undertaken to investigate the effect of *Psidium guajava* leaf aqueous extract (PGE) on rat isolated uterine muscle contractility.

Materials and methods

Ethical considerations

Experimental protocols and procedures used in this study were approved by the Animal Ethics Committee of the University of KwaZulu-Natal, Durban 4000, South Africa; and conform to the “*Guide to the Care and Use of Laboratory Animals in Research and Teaching*” (Published by the Ethics Committee of the University of KwaZulu-Natal, Durban 4000, South Africa).

Plant material

Fresh leaves of *Psidium guajava* Linn. (family: Myrtaceae) were collected from an open grassland on the Westville Campus of the University of KwaZulu-Natal, Durban, South Africa, in March 2008. Identification of the plant material was carried out by the Taxonomist/Curator of the University of KwaZulu-Natal’s (Westville Campus) Botany Department. A voucher specimen (WDHCHIWORORO.1) of the plant has been deposited in the Herbarium of the University’s Botany Department.

Preparation of Psidium guajava extract

One kilogramme of fresh *Psidium guajava* leaves was air-dried under shade for a period of 2 weeks at room temperature ($26 \pm 1^\circ\text{C}$). The dried leaves were milled into fine powder in a commercial Waring blender, macerated in distilled water for 48 hours (with occasional shaking) at room temperature ($26 \pm 1^\circ\text{C}$), and extracted twice, on each occasion with 2.5 l of distilled water. The combined aqueous extract solubles were filtered and concentrated under reduced pressure in a rotary evaporator at 60°C . Freeze-drying and solvent elimination of the resulting aqueous extract finally gave 36.56 g (*i.e.*, 3.656% yield) of a light brown, powdery, crude *Psidium guajava* leaf aqueous extract (PGE). Without any further purification, aliquot portions of the plant’s crude extract residue were weighed and dissolved in distilled water (at room

temperature) for use on each day of our experiments.

Animals

Healthy, young adult, female Wistar rats (250–300 g) were used. The animals were kept and maintained under conventional laboratory conditions of temperature, humidity and light, and allowed free access to food (standard pellet diet) and drinking tap water *ad libitum*. All the animals were fasted for 16 h, but still allowed free access to drinking water, before the commencement of our experiments. The rats were euthanased by halothane inhalation. Euthanased rats were quickly placed on a dissecting table, and their abdomens were opened by midline incisions, following which the two uterine horns were quickly removed from the animals. The two uterine horn muscle segments were cleaned free from fat and connective tissues, and trimmed. In some of the experiments, the normal female rats used were pre-treated (*i.e.*, primed) with stilboesterol (0.1 mg/kg subcutaneously) 20–24 hours prior to euthanasia and harvesting of uterine horns (in order to induce oestrous). Vaginal smears were taken immediately before sacrifice in order to ascertain that the animals were in oestrous.

Effect of PGE on rat isolated uterine horns

Each isolated uterine horn preparation was suspended under an applied resting tension of 1.0 g in a 30-ml Ugo Basile organ-bath containing De Jalon's physiological solution (DJS) of the following composition, in mM: NaCl, 154., KCl, 5.63., NaHCO₃, 5.95., CaCl₂, 0.65., and glucose, 2.77; pH adjusted to 7.4. The bathing DJS was maintained at 32 ± 1°C and continuously aerated with carbogen (*i.e.*, 95% O₂ + 5% CO₂ gas mixture). The tubular uterine horn segments were kept in Ugo Basile organ-baths for 45–60 min, during which time the bathing DJS was changed every 15 min. After the equilibration period, the uterine horn preparations were challenged with graded concentrations of PGE (0.5–4.0 mg/ml), and/or reference drugs used, at different times. PGE and/or reference drug solutions used were added to the bath-fluid sequentially. Bath-applied PGE and/or reference drug concentrations used were repeated (where necessary) after washing out the previous PGE or reference drug concentrations 4–5 times, and subsequently allowing the tissues to rest for 5–10 min, or until its tone returned to baseline level. In order to make allowance for changes in tissue sensitivity, two isolated uterine horn preparations from the same animal were always set up at a time; one used as 'control' and the other one used as 'test' (*i.e.*, PGE- or reference drug-treated) preparation. 'Control' uterine horn strips were only treated with distilled water equivalent to the volume/s (0.1–0.8 ml) of bath-applied PGE or reference drug/spasmogen solutions used. Each 'test' uterine horn preparation was used for one concentration-response curve only. Under the same experimental conditions, the possible role of β_2 -adrenoceptor stimulation in PGE-induced uterine muscle relaxation was examined by preincubating the uterine horn preparations with propranolol (10⁻⁶ M) for 20 min before exposing the muscles to different concentrations of PGE.

In some experiments, agonist drug- and spasmogen-induced contractions of uterine horns isolated from stilboesterol-primed, oestrogen-dominated, non-pregnant rats were elicited by sequential, exogenous additions of either acetylcholine (ACh, 0.5–8.0 μ g/ml), oxytocin (0.5–4.0 μ U), bradykinin (2.5–10 ng/ml), carbachol (CCh, 0.5–8.0 μ g/ml), or potassium chloride (K⁺, 10–80

mM) to the bath-fluid, followed, 30 sec later, by 4–5 times washouts. Maximal muscle tensions developed by the high concentrations of the agonist drug and spasmogens used were similar, and vary between 2.8 and 3.1 g. PGE-induced decreases in the agonist drug- or spasmogen-provoked muscle contractions were regarded as inhibitory effects of the plant's extract.

Drugs

The following reference drugs were used: acetylcholine chloride, carbachol chloride, potassium chloride (Sigma Chemical Co.), oxytocin (Parke-Davis), bradykinin (Sandoz), and stilboesterol (Kirby Pharmaceuticals). All drugs were dissolved and/or diluted in distilled water on each day of our experiments. Drug concentrations quoted in the text refer to final organ-bath concentrations.

Data analysis

Experimental data obtained are expressed as means (\pm standard errors of the means, SEM). Distilled water-induced 'control' means were used as baseline values. The differences in responses among the different groups were analysed for statistical significance by using One-Sample Student's *t*-test, followed by Dunnett's post-hoc test. In all cases, values of $P < 0.05$ were taken to imply statistical significance.

Results

Effect of PGE on rat isolated uterine horns

Isolated uterine horns harvested from normal (non-stilboesterol-primed) female rats exhibited spontaneous phasic contractions periodically, with a mean amplitude of 2.10 ± 0.3 g ($n=8$). PGE (0.5–4.0 mg/ml) raised the basal tone and reduced the amplitude of phasic contractions, in a concentration-dependent manner. Figure 1 shows a typical trace obtained with a uterine horn, while Fig. 2 summarizes the relaxant effects of PGE on uterine horn preparations. The IC_{50} value of the inhibitory effects of PGE was 0.45 ± 0.04 mg/ml ($n=8$). The inhibitory effects of PGE were reversed by washing out PGE for 4–5 times, and subsequently allowing the uterine tissue to rest for 5–10 min. Preincubation of the uterine strips with propranolol (10^{-6} M) did not modify the relaxant effects of PGE (0.5–4.0 mg/ml) on the uterine horn preparations (data not shown).

Effects of PGE on spasmogen-induced contractions of uterine horns isolated from stilboesterol-primed, oestrogen-dominated, non-pregnant rats

Uterine horns taken from stilboesterol-primed, oestrogen-dominated, non-pregnant (normal) rats were always quiescent and devoid of spontaneous, rhythmic contractions. A range of PGE (0.5–4.0 mg/ml) inhibited or abolished contractions produced by acetylcholine (ACh), oxytocin, bradykinin, carbachol (CCh) or high-potassium solutions (K^+ , 10–80 mM), in a concentration-related manner, in the isolated, oestrogen-dominated, uterine horn muscles. Figure 3 summarizes the inhibitory effects of PGE on CCh- and K^+ -induced uterine horn contractions ($n=6-8$).

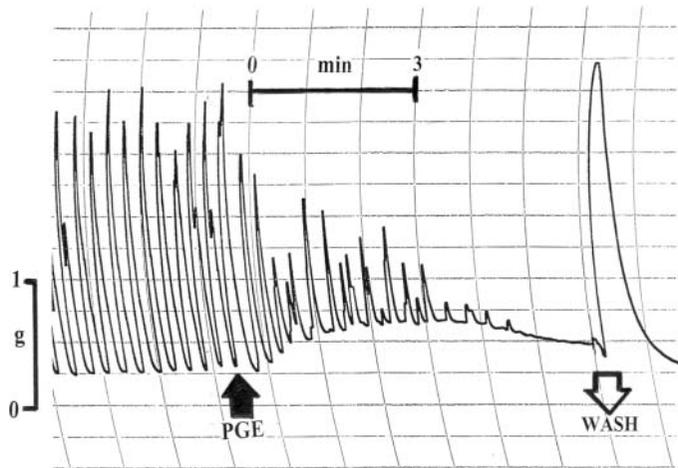


Fig. 1. Effect of *Psidium guajava* leaf aqueous extract (PGE) on a rat isolated uterine horn. PGE (4.0 mg/ml) was added to the bath-fluid at the left-hand-side, upward-pointing solid arrow, and washed out 4–5 times at the adjacent right-hand-side, downward-pointing open arrow.

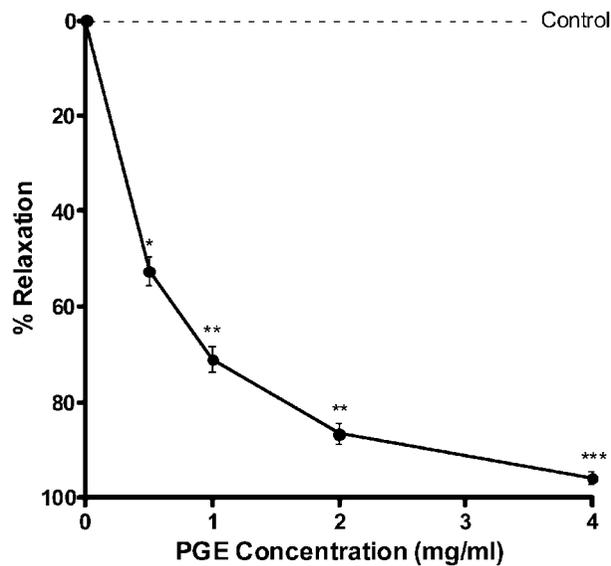


Fig. 2. Concentration-effect curve of PGE (0.5–4.0 mg/ml) on contractile amplitudes of spontaneously-contracting rat isolated uterine horns. Each point represents the mean of 6–8 observations, while vertical bars denote standard errors of the means (SEM). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ versus distilled water-treated controls.

Discussion

The findings of the present study indicate that *Psidium guajava* leaf aqueous extract (PGE, 0.5–4.0 mg/ml) inhibited or abolished the amplitude of spontaneous phasic contractions of

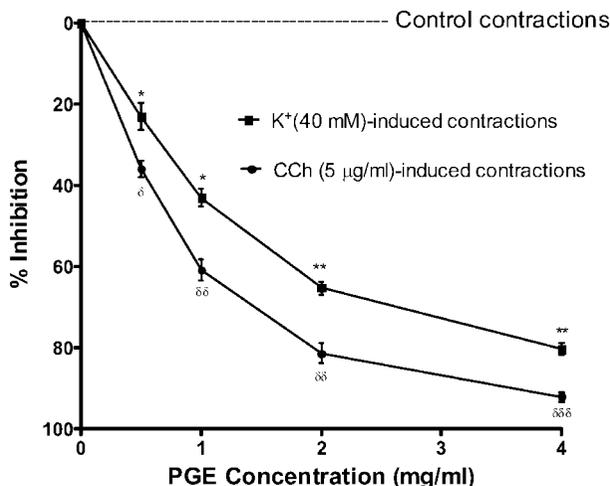


Fig. 3. Concentration-effect curves of PGE (0.5–4.0 mg/ml) on potassium chloride (K^+ , 40 mM) and carbachol (CCh, 5.0 $\mu\text{g/ml}$)-induced contractions of oestrogen-dominated, quiescent uterine horn preparations. Values presented are means of 6–8 observations, while vertical bars denote standard errors of the means (SEM). *, $P < 0.05$; **, $P < 0.01$ for PGE against K^+ -induced control contractions; δ , $P < 0.05$; $\delta\delta$, $P < 0.01$; $\delta\delta\delta$, $P < 0.001$ for PGE against carbachol-induced control contractions.

uterine muscles, as well as the contractions elicited by agonists and spasmogens in oestrogen-dominated, quiescent uterine horn preparations. Phytochemical analysis of *Psidium guajava* leaf extract by a number of investigators (Vargas *et al.*, 2006; Gutiérrez *et al.*, 2008) have revealed the presence of flavonoids which include quercetin and its derivatives (guajaverin, isoquercitrin, hyperin, quercitrin, avicularin), morin and its derivatives (morin-3-O- α -L-lixopyranoside and morin-3-O- α -L-arabopyranoside), rutin, myricetin, luteolin and kaempferol. The flavonoids and terpenoids present in *Psidium guajava* leaf extract have been reported by some investigators to have antispasmodic effects on smooth muscle preparations, and to inhibit inflammation through prostaglandin-synthesis enzymatic system (Dobova *et al.*, 2007). In a study by Revuelta *et al.* (1997), quercetin and kaempferol relaxed rat isolated uteri precontracted with potassium chloride. Thus, it is not unreasonable to speculate that quercetin, kaempferol and other flavonoids present in PGE might have contributed, at least in part, to the uterine relaxant effects observed in the present study.

Uterine smooth muscle contraction is mediated mainly via increased intracellular Ca^{2+} and is accomplished by excitation-contraction coupling mechanisms (Zhang *et al.*, 2005). A high K^+ medium could depolarize the cellular membrane of uterine smooth muscle (Bolton, 1979; Zhang *et al.*, 2005). Moreover, it is well-known that K^+ -induced contraction in smooth muscles is due to an increase in Ca^{2+} influx through voltage-operated Ca^{2+} channels (Kaya *et al.*, 2002; Borrelli *et al.*, 2006). It has been suggested that any substance that inhibits high K^+ -induced contractions of smooth muscles is a blocker of Ca^{2+} influx (Gilani *et al.*, 2005). It is also accepted that carbachol (CCh)-induced contractile responses following receptor activation require an increased intracellular Ca^{2+} which is brought about by Ca^{2+} influx through L-type Ca^{2+} channels,

and Ca^{2+} release from intracellular calcium stores (Tanovic *et al.*, 2000). It has been reported that quercetin might be antagonizing the inward calcium membrane current, perhaps by blocking the L-type calcium membrane channels, leading to a decrease in smooth muscle contractile force (Lozoya *et al.*, 1994). However, in another study, it has been reported that quercetin is an activator of L-type calcium channels (Saponara *et al.*, 2002). These contradictory reports probably suggest that the spasmolytic activity of quercetin might be mediated via other mechanisms other than by antagonizing calcium membrane channels.

The finding that low to high concentrations of PGE inhibited or abolished contractions produced by ACh-, oxytocin-, bradykinin-, carbachol- and K^+ in oestrogen-dominated uterine horn preparations in a concentration-related manner, probably suggests that PGE-induced uterine horn relaxations are unlikely to be mediated through β_2 -adrenoceptor stimulation, and that the mechanisms involved in the inhibitory effects of PGE on rat isolated uteri are likely to be complex. The spasmolytic effect of PGE could, therefore, be mediated via a combination of several mechanisms, possibly including inhibition of (i) voltage dependent calcium (Ca^{2+}) channels, (ii) cyclic adenosine monophosphate (cAMP) dependent mechanisms, (iii) muscarinic cholinergic and/or oxytocin receptor-mediated mechanisms, and so forth. However, the failure of propranolol to modify uterine relaxant effect of PGE strongly suggests that PGE-induced uterine relaxation is probably not mediated via β_2 -adrenoceptor activation. In a study by Revuelta *et al.*, (1997), an inhibitor of cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA) and protein kinase C (PKC), N-p-tosyl-1-phenylalanine-chloromethyl-ketone (TPCK), significantly antagonised the uterine relaxation elicited by quercetin and kaempferol; thus suggesting that quercetin and kaempferol increase intracellular cAMP. The elevated intracellular cAMP would thus appear to contribute towards the relaxation of the uterus. The mechanism of cAMP increase in rat uterus by flavonoids could be brought about by inhibition of cAMP phosphodiesterase (Ferrell *et al.*, 1979; Ortmann *et al.*, 1979; Landolfi *et al.*, 1984). In addition, the possibility that PGE- or flavonoid-induced relaxations of the uterus might result from inhibition of protein kinases, such as myosin light chain kinase, and possibly other kinases involved in Ca^{2+} -sensitizing mechanisms, including protein kinase C, still remains an attractive option. Taken together, however, the findings of the present study appear to suggest that the uterine relaxant effect of PGE is probably mediated through a non-specific, spasmolytic mechanism.

The combined anti-inflammatory and analgesic effects described earlier for this plant's extract (Gutiérrez *et al.*, 2008), and the antispasmodic effect of the plant's extract observed in the present study, support the clinical efficacy and use of *Psidium guajava* leaf in the treatment of dysmenorrhoea and other uterine spasmodic disorders. Based on our laboratory, experimental findings and the clinical observations of Doubova *et al.*, (2007), leaf extract of *Psidium guajava* could be recommended as a natural therapeutic alternative to non-steroidal anti-inflammatory drugs (NSAIDs) in the management, control and/or treatment of primary dysmenorrhoea.

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References

- Bolton, T.B. (1979). Mechanism of action of transmitter and other substances on smooth muscles. *Physiol. Rev.* **59**: 606–718.
- Borrelli, F., Capasso, F., Capasso, R., Ascione, V., Aviello, G., Longo, R. and Izzo, A.A. (2006). Effect of *Boswellia serrata* on intestinal motility in rodents: inhibition of diarrhoea without constipation. *Br. J. Pharmacol.* **148**: 553–560.
- Conway, P. (2001). *Tree Medicine—a comprehensive guide to the healing power of over 170 trees*. Judy Piatkus Ltd.
- Doubova, S.V., Morales, H.R., Hernández, S.F., Martínez-García, M.C., Ortiz, M.G., Chávez-Soto, M.A., Arce, E.R. and Lozoya, X. (2007). Effect of a *Psidium guajavae* folium extract in the treatment of primary dysmenorrhea: a randomized clinical trial. *J. Ethnopharmacol.* **110**: 305–310.
- Ferrell, J.E., Sing, P.D.C., Loew, G., King, R., Mansour, J.M. and Mansour, T.E. (1979). Structure-activity studies of flavonoids as inhibitors of cyclicAMP phosphodiesterase and relationship to quantum chemical indices. *Mol. Pharmacol.* **16**: 556–568.
- Gilani, A.H., Bashir, S., Janbaz, K.H. and Shah, A.J. (2005). Presence of cholinergic and calcium channel blocking activities explains the traditional use of *Hibiscus rosasinensis* in constipation and diarrhoea. *J. Ethnopharmacol.* **102**: 289–294.
- Gutiérrez, R.M.P., Mitchell, S. and Solis, R.V. (2008). *Psidium guajava*: a review of its traditional uses, phytochemistry and pharmacology. *J. Ethnopharmacol.* **117**: 1–27.
- Kaya, T.T., Kokhan, G., Soydan A.S., Arpacik, M. and Karadas, B. (2002). Effects of nimesulide and pentoxifylline on decreased contractile responses in rat ileum with peritonitis. *Eur. J. Pharmacol.* **442**: 147–153.
- Kuppusamy, U.R. and Das, N.P. (1994). Potentiation of β_2 -adrenoceptor agonist-mediated lipolysis by quercetin and fisetin in isolated rat adipocytes. *Biochem. Pharmacol.* **47**: 521–529.
- Landolfi, R., Mower, R.L. and Steiner, M. (1984). Modification of platelet function and arachidonic acid metabolism by bioflavonoids: structure-activity relations. *Biochem. Pharmacol.* **33**: 1525–1530.
- Lozoya, X., Meckes, M., Aboud-Zaid, M., Tortoriello, J., Nozolillo, C. and Amason, J.T. (1994). Quercetin glycosides in *Psidium guajava* Linn. leaves and determination of a spasmolytic principle. *Arch. Med. Res.* **25**: 11–15.
- Ortmann, R., Nutto, D. and Waldmeyer, J. (1979). Phloretin and related compounds inhibit agonist stimulated cAMP accumulation in cultured cells of CNS origin. *Biochem. Pharmacol.* **28**: 2357–2361.
- Revuelta, M.P., Cantabrana, B. and Hidalgo, A. (1997). Depolarization-dependent effect of flavonoids in rat uterine smooth muscle contraction elicited by CaCl_2 . *Gen. Pharmacol.* **29**: 847–857.
- Saponara, S., Sgaragli, G. and Fusi, F. (2002). Quercetin as a novel activator of L-type Ca^{2+} channels in rat tail artery smooth muscle cells. *Br. J. Pharmacol.* **135**: 1819–1827.
- Speroff, L., Glass, R.H. and Kase, N.G. (1999). *Clinical gynaecologic endocrinology and infertility*, Lippincott Williams and Wilkins, Baltimore, pp. 566–567.
- Tavonic, A., Jimenez, M. and Fernandez, E. (2000). Lack of effect of nitric oxide on KCl-, acetylcholine- and substance P-induced contractions in ileal longitudinal muscle of the rat. *Life Sci.* **67**: 531–541.
- Vargas, A.D., Soto, H.M., Gonzalez, H.V.A., Engleman, E.M. and Martinez, G.A. (2006). Kinetics of accumulation and distribution of flavonoids in 'guava' (*Psidium guajava*). *Agrociencia* **40**: 109–115.
- Zhang, W.W., Li, Y., Wang, X.Q., Tian, F., Cao, H., Wang, M.W. and Sun Q.S. (2005). Effects of magnolol and honokiol derived from traditional Chinese herbal remedies on gastrointestinal movement. *World J. Gastroenterol.* **11**: 4414–4418.