Journal of Zhejiang University SCIENCE B
ISSN 1673-1581
http://www.zju.edu.cn/jzus
E-mail: jzus@zju.edu.cn

# Effects of two medicinal plants *Psidium guajava* L. (Myrtaceae) and *Diospyros mespiliformis* L. (Ebenaceae) leaf extracts on rat skeletal muscle cells in primary culture

BELEMTOUGRI R.G.[†1], CONSTANTIN B.[2], COGNARD C.[2], RAYMOND G.[2], SAWADOGO L.[1]

([1]*Laboratory of Physiological Animal, University of Ouagadougou BP 7021, Burkina Faso*)

([2]*Institute of Physiology & Biology Cell, University of Poitiers, France*)

[†]E-mail: rbelemtougri@voila.fr

Received Oct. 4, 2005; revision accepted Oct. 23, 2005

**Abstract:** Crude decoction, aqueous and ethanolic extracts of two medicinal plants (*Psidium guajava* and *Diospyros mespiliformis*), widely used in the central plateau of Burkina Faso to treat many diseases were evaluated for their antagonistic effects on caffeine induced calcium release from sarcoplasmic reticulum of rat skeletal muscle cells. These different extracts showed a decrease of caffeine induced calcium release in a dose dependent manner. Comparison of the results showed that *Psidium guajava* leaf extracts are more active than extracts of *Diospyros mespiliformis* and that crude decoctions show better inhibitory activity. The observed results could explaine their use as antihypertensive and antidiarrhoeal agents in traditional medicine, by inhibiting intracellular calcium release.

**Key words:** *Psidium guajava*, *Diospyros mespiliformis*, Myrtaceae, Ebenaceae, Medicinal plants, Intracellular calcium, Sarcoplasmic reticulum, Caffeine

**doi:**10.1631/jzus.2006.B0056 **Document code:** A **CLC number:** Q958.12$^{+}$2.5

## INTRODUCTION

In Africa, people use natural resources for medicinal purposes. *Psidium guajava* and *Diospyros mespiliformis* are medicinal plants used in tropical and subtropical countries to treat many disorders such as diarrhoea, cough and gastrointestinal disorders.

It was reported that *Psidium guajava* leaf extract has a wide spectrum of biological activities such as anticough, antibacterial, haemostasis (Jaiarj *et al*., 1999; 2000), antidiarrhoeal and narcotic properties (Lozoya *et al*., 1990), and antioxidant properties (Qian and Nihorimbere, 2004).

According to Lutterodt and Maleque (1988) and Meckes *et al*.(1996), the leaf extract is used to treat diarrhoea, abdominal pain, convulsions, epilepsy, cholera, insomnia and has hypnotic effect.

Some studies reported that the leaf extract and its derivative identified as quercetin has effect on the intracellular calcium levels in gastrointestinal smooth muscle (Lutterodt, 1989; Lozoya *et al*., 1990), in cardiac muscle cell (Morales *et al*., 1994; Apisariyakul *et al*., 1999) and in neuromuscular junction (Re *et al*., 1999; Chaichana and Apisariyakul, 1996).

More than twenty identified compounds from *Psidium guajava* leaf have been reported in (Seshadri and Vasishta, 1965; Osman *et al*., 1974; Lutterodt and Maleque, 1988). The major components are: β-selinene, β-caryophyllene, caryophyllene oxide, squalene, selin-11-en-4α-ol (Meckes *et al*., 1996), guaijavarin, isoquercetin, hyperin, quercitrin and quercetin-3-O-gentobioside (Lozoya *et al*., 1994), morin-3-O-α-L-lyxopyranoside and morin-3-O-α-L-arabopyranoside (Arima and Danno, 2002), β-sitosterol, uvaol, oleanolic acid and ursolic acid (Begum *et al*., 2004).

Our recent phytochemical screening of *Psidium guajava* leaf showed tannins in aqueous extract, an-

thocyans, alkaloids, flavonoids, tannins and steroids/terpenoids in ethanolic extract.

Two studies showing that *Diospyros mespiliformis* exhibits antimicrobial activity (Adeniyi *et al*., 1996; Sanogo *et al*., 1998) have been reported. Anthraquinones, tannins, triterpene, saponins, steroids and sugars in the leaf extract of this plant had been reported (Adeniyi *et al*., 1996).

According to our recent phytochemical screening of *Diospyros mespiliformis*, flavonoids, tannins and saponosides were found in aqueous extract, anthocyans, flavonoids tannins and steroids/terpenoids exists in ethanolic extract.

*Psidium guajava* and *Diospyros mespiliformis* are used to treat many diseases, especially hypertension and diarrhoea in the central plateau of Burkina Faso ([1]Nacoulma-Ouédraogo, 1996). Indeed, in developing countries, hypertension is now considered as a cause of mortality in adults. In spite of the presence of known antihypertensive modern medicines in the pharmaceutical market, among medicinal plants used to treat this disease are *Psidium guajava* and *Diospyros mespiliformis*. Few of these plants are biologically and chemically investigated in order to determine their effectiveness and active constituents. Some reports indicated that the extracts of these plants could interfere with calcium influx and/or calcium release from an intracellular store. So the study was done in mammalian skeletal muscle cells in culture (Belemtougri *et al*., 2001). Skeletal muscle cells have well characterized calcium release processes which could be the target of these plant components.

The present paper reports the inhibition of calcium release from sarcoplasmic reticulum by these plant leaves extracts on rat skeletal muscle cells. This could indicate one mechanism underlying their actions.

## MATERIALS AND METHODS

### Plant collection

Fresh leaves of *Psidium guajava* and *Diospyros mespiliformis* were collected from Gampéla (Burkina Faso, West Africa) in July 1997. The plants were authenticated by Prof. Millogo-Rasolodimby, Department of Botany, University of Ouagadougou. The herbarium specimens are deposited in this Department.

### Preparation of plant extracts

Crude decoctions were prepared from the shade dried leave powder. Six grams of leaf powder of each plant were macerated in deionized water for 24 h at room temperature and then boiled for 10 min to mimic the traditional preparation methods. After cooling, the resulting extract was filtered through Whatman n° 2 filter and evaporated to give crude extracts.

Forty grams of leaf powder of each plant were successively extracted with ethanol and deionized water to give ethanolic and aqueous extracts (Belemtougri *et al*., 2001).

On the other hand, the results of screening the compounds summarized in Tables 1 and 2 were obtained according to [2]Samaté (2001). Briefly 1 g of each plant leaf powder was macerated in 10 ml of chlorhydric acid or 5% phosphoric acid. The alkaloids were detected using Mayer and Dragendorff method followed by thin layer chromatography. Steroids/terpenoids, phenolic components (tannins and flavonoids) were determined using Liebermann-Burchard method. The infusion previously prepared becomes blue when it is mixed with ferric chloride indicating the presence of tannins while it is red when mixed with vanillic chloride, indicating the presence of flavonoids.

### Cell culture

Primary cultures of rat skeletal muscle cells were initiated from neonatal progenitors. These myogenic cells were obtained by trypsinisation of small muscle pieces of 1 to 2 day-old rat hindlimbs (Cognard *et al*., 1993a). Briefly, the cells were maintained for 96 h in growth medium (Ham F12, Gibco) supplemented with 10% fetal calf serum (Boehringer, Mannheim, Germany), 10% heat-inactivated horse serum (Gibco) and 1% antibiotics: (Penicillin G 100 U/ml, Sigma and Streptomycin 50 U/ml, Sigma).

---

[1]Nacoulma-Ouédraogo, O.G., 1996. Plantes médicinales et pratiques médicales traditionnelles au Burkina Faso: cas du plateau central. Thèse de Doctorat Es Sciences Naturelles, Université de Ouagadougou, tome II, p.285.

[2]Samaté, A.D., 2001. Compositions chimiques d'huiles essentielles extraites de plantes aromatiques de la zone soudanienne du Burkina Faso: valorisation. Thèse de Doctorat Es Sciences Physiques, Université de Ouagadougou, p.164.

The growth medium was exchanged for a fusion medium constituted by Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% heat-inactivated horse serum and 1% antibiotics. This medium exchange was considered as time zero to age differentiated cells in culture.

Experiments were carried out on 3 to 5 day-old myotubes after induction of fusion, at stages exhibiting well characterized calcium release processes.

**Intracellular free calcium measurements**

Intracellular free calcium was measured with the fluorescent dye Indo-1/AM (Sigma Chemical Co., St. Louis, MO, USA). Cells were loaded with 3 μmol/L of the lipophilic form of Indo-1 (dissolved in dimethylsulfoxide) diluted in control solution. The cells were incubated for 45 min in the dark at room temperature, washed with control solution and incubated 10 min at 37 °C to obtain complete de-esterification of the probe. Fluorescence was recorded at room temperature using an OSP100 CA microscopic photometry device (Olympus).

Excitation of Indo-1 was set in UV range (350 nm) by means of a xenon lamp. Fluorescence emission was acquired through a dichroic filter (455 nm) by means of two photomultiplier tubes with two band pass filters at 405 nm for the emission of the calcium bound form and at 485 nm for the calcium free form fluorescence of the probe.

**Experimental solutions**

The experiments were performed in a control solution containing (mmol/L): NaCl 130; KCl 5.4; $CaCl_2$ 2.5; $MgCl_2$ 0.8; Glucose 5.6; HEPES 10 and adjusted at pH 7.4 with Tris.

The test solutions were made by adding caffeine (final concentration 10 mmol/L) (caffeine solution) or adding 1 mg/ml to 10 mg/ml of the plant extracts to caffeine solution (caffeine+*Psidium* or *Diospyros* solution). They were added to the control solution just before experiments. Rapid changes of the solutions in the surrounding of interrogated cells were achieved by means of a home made microperfusion system.

**Statistical analysis**

Data analysis was performed by means of Software Prism 3.0 (GraphPad Software, San Diego, CA, USA) and Origin 5.0 (Microcal Software Inc., Northampton, MA, USA). Results of the experiments were expressed as means±*SEM* and paired or unpaired Student's *t*-test was used to test for significance difference between the means. $P<0.05$ was considered significant.

RESULTS

Different extracts of *Psidium guajava* and *Diospyros mespiliformis* failed to display any activity on rat skeletal muscle cells when applied alone (data not shown). This indicated their ineffectiveness on resting calcium in skeletal muscle cell. Then we used caffeinic solution to investigate some activity on intracellular calcium release from sarcoplasmic reticulum (SR). Caffeine was applied in order to directly stimulate calcium release through ryanodine receptor channels (Cognard *et al*., 1993b). Application of caffeine (10 mmol/L) on myotubes induced $Ca^{2+}$ release from SR. For each cell 10 mmol/L caffeine solution was applied and the response was considered as control (Fig.1a) and another was interro-

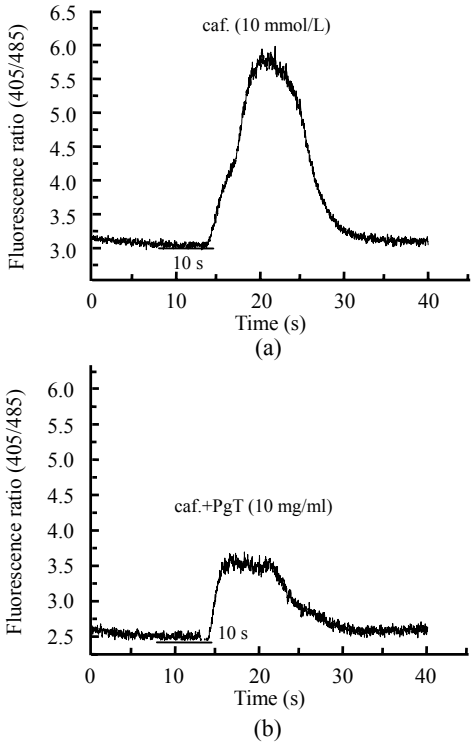


**Fig.1 Effects of caffeine (a) and caffeine plus *Psidium guajava* crude leaf extract (b) on intracellular calcium release from sarcoplasmic reticulum**

gated with caffeine+plants extracts. As illustrated in Fig.1b and Fig.2b, crude extracts of *Psidium guajava* and *Diospyros mespiliformis* at 10 mg/ml decreased the amplitude of $Ca^{2+}$ release from SR compared with the controls. This indicated that the extracts have significant antagonistic effect on caffeine sensitive $Ca^{2+}$ release from SR.

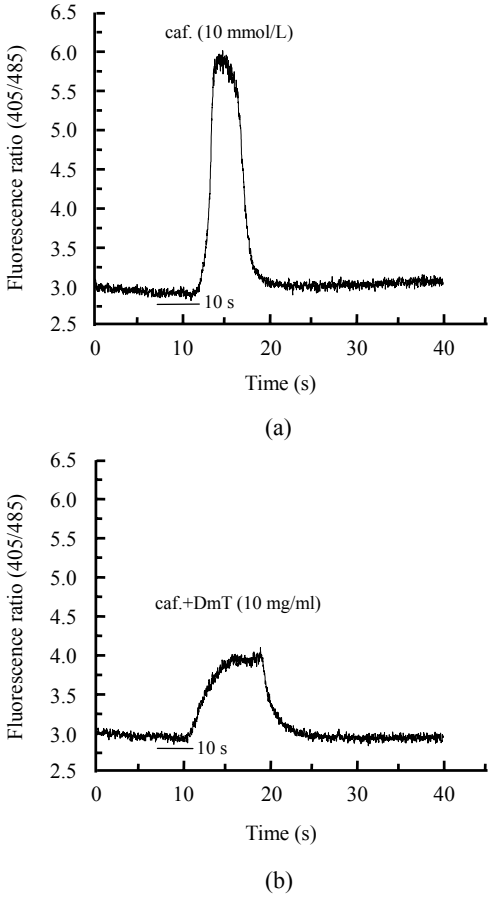


**Fig.2 Effects of caffeine (a) and caffeine plus *Diospyros mespiliformis* crude leaf extract (b) on intracellular calcium release from reticulum sarcoplasmic**

Different extracts of the two plants showed inhibition on intracellular calcium release in dose dependent manner; the crude decoctions were the most active. At 10 mg/ml, crude decoction of *Psidium guajava* (Fig.3a) was more active than that of *Diospyros mespiliformis* (Fig.3b).

At 10 mg/ml the action of different extracts of *Psidium guajava* could be classified as follows: crude decoction>aqueous extract>ethanolic extract; but those of *Diospyros mespiliformis* were: crude decoction>ethanolic extract>aqueous extract. At the same

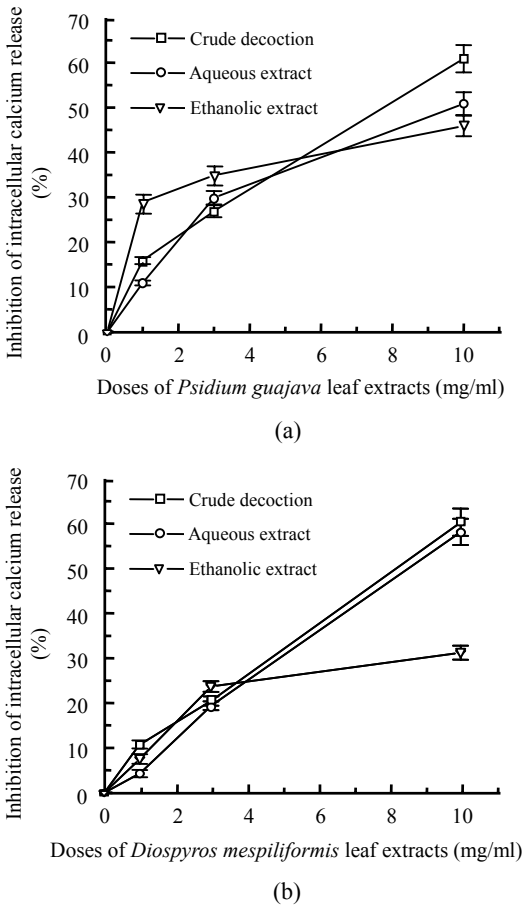


**Fig.3 Percentage inhibition of intracellular calcium release from sacroplasmic reticulum by different leaf extracts of *Psidium guajava* (a) and *Diospyros mespiliformis* (b) at different doses**

dose crude decoction of *Psidium guajava* showed 61% of inhibition with $IC_{50}$≈7.65 mg/ml and that of *Diospyros mespiliformis* showed 51% with $IC_{50}$≈8.84 mg/ml. However, ethanolic extract of *Diospyros mespiliformis* showed $IC_{50}$≈9.23 mg/ml but the other extracts of the two plants showed $IC_{50}$ more than 10 mg/ml.

It was interesting to observe that at low doses (3 mg/ml) ethanolic extract of *Psidium guajava* (Fig.3a) and aqueous extract of *Diospyros mespiliformis* (Fig.3b) were more active than other extracts.

The extract process used in this study yielded 7.7515 g of ethanolic extract and 1.2501 g of aqueous extract in *Psidium guajava* leaf extract of forty grams dry powder. Table 1 summarizes the different components of the different extracts of *Psidium guajava*. The same procedure yielded 5.40 g of ethanolic ex-

tract and 1.328 g of aqueous extract in *Diospyros mespiliformis* leaf extract of forty grams dry powder. Table 2 summarizes the different components of the different extracts of *Diospyros mespiliformis*.

Ten mg/ml crude decoctions of *Psidium* and *Diospyros* showed 61% and 51% inhibition of intracellular calcium release, aqueous extracts exhibited 51% and 29% of inhibition and the last ones, ethanolic extracts showed 46% and 54% inhibition of calcium release from sarcoplasmic reticulum.

Considering the effectiveness of the different extracts, we can conclude that crude decoctions are more active than other extracts.

## DISCUSSION

We examine here the effects of different leaf extracts of *Psidium guajava* and *Diospyros mespiliformis* on the caffeine induced intracellular calcium release from sarcoplasmic reticulum of rat skeletal muscle cells. Our different extracts failed to show any effect on resting calcium activity. This suggests no effects of crude extracts on transporters involved in handling of basal calcium activity. We utilized caffeine which is known to induce calcium release from SR following activation of ryanodine receptors (Zimanyi and Pessah, 1994). This release was measured by means of a fluorescent dye Indo-1/AM in this study.

The observed results showed that the different extracts tested were dose-dependently effective in the inhibition of calcium release induced by caffeine. The extracts of *Psidium guajava* were more active than those of *Diospyros mespiliformis*. The crude decoctions were more effective than other extracts. This difference could be due to several factors: polarity and chemical nature of the components, differential extraction, partition and quantity of active ingredients present in the different extracts. The crude decoctions appeared to contain the total water soluble active compounds which have synergistic effects on inhibition of calcium release. It suggested that one or several compounds could inhibit ryanodine-sensitive calcium release channels from SR.

The chemical composition of these plants reported in literature showed the presence of various components, especially tannins, flavonoids (quercetin) and triterpenoids (Osman *et al*., 1974). These results are in a good agreement with our phytochemical screening.

The decrease in the calcium release from SR to myoplasm could be explained by interactions of the extracts with different elements involved in the regulation of calcium release, for example the ryanodine receptors. Our extracts could increase $Mg^{2+}$ in the cytoplasm and it is well known that this ion has powerful inhibitory effect on calcium release in skeletal muscle (Lamb, 2000). It was also reported that extracts of *Psidium guajava* could block the L-type calcium membrane channels (Conde-Garcia *et al*., 2003). Here, this pathway was not explored since depolarizing stimuli were not used, but direct activation of calcium release from SR.

Flavonoids existing in the extracts could play an

**Table 1 Summary of results after chemical screening of *Psidium guajava* leaf extracts**

| Extracts | Anthocyans | Anthracenosides | Alkaloids | Flavonoids | Quinones | Tannins | Steroids/ Terpenoids | Saponosides |
|---|---|---|---|---|---|---|---|---|
| Aqueous extract | − | − | − | − | − | + | − | − |
| Ethanolic extract | + | − | + | + | − | + | + | − |
| Dichloromethanolic extract | − | − | + | − | − | − | + | − |

+: Presence; −: Absence

**Table 2 Summary of results after chemical screening of *Diospyros mespiliformis* leaf extracts**

| Extracts | Anthocyans | Anthracenosides | Alkaloids | Flavonoids | Quinones | Tannins | Steroids/ Terpenoids | Saponosides |
|---|---|---|---|---|---|---|---|---|
| Aqueous extract | − | − | + | − | − | + | − | + |
| Ethanolic extract | + | − | + | − | − | + | + | − |
| Dichloromethanolic extract | ND | − | − | ND | ND | − | + | ND |

+: Presence; −: Absence; ND: Not determined

important role and are phenolic compounds widely distributed in the plant kingdom and have several pharmacological properties such as spasmolytic (Havsteen, 1983; Capasso *et al*., 1991) and antidiarrhoeal (Tona *et al*., 1999) activities. Flavonoids have been reported to have free radical scavenger properties (Bharani *et al*., 1995). van Acker *et al*.(1995) reported that flavonoids are antioxidants found usually in plants, fruits and vegetable and are known to be excellent scavengers of free radicals and accordingly used in the treatment of vascular endothelial damage and diseases of vascular wall involving inflammation. According to Lutterodt (1989), Morales *et al*.(1994), Lozoya *et al*.(1994) and Tona *et al*.(1999), some flavonoids isolated from *Psidium guajava* leaves exhibited spasmolytic and antispasmodic activities. It was also shown that flavonoids inhibited certain mammalian enzyme systems such as PKC and PLC and then could reduce $Ca^{2+}$ release in cytoplasm and lead to muscle relaxation.

It has been reported that quercetin, the main flavonoid of *Psidium guajava* leaves had many pharmacological activities. According to Alikaridis (1987) and Yuting *et al*.(1990), quercetin isolated from *Ilex* species has free radical scavenging activity and lipid antiperoxidation activity, and may relax intact aortic rings (Muccillo Baisch *et al*., 1998).

Formica and Regelson (1995) reported that quercetin exhibited antioxidant and spasmolytic effects. It induced relaxation of cardiovascular smooth muscle (Duarte *et al*., 1993; Formica and Regelson, 1995) which could lead to lower blood pressure in vivo. This justifies its use in the treatment of hypertension in the central plateau of Burkina Faso. It has been shown that quercetin has relaxant effects on vascular and intestinal smooth muscle (Duarte *et al*., 1993; Morales *et al*., 1994).

Furthermore, quercetin showed inhibition on skeletal muscles contraction (Chaichana and Apisariyakul, 1996; Apisariyakul *et al*., 1999).

According to Re *et al*.(1999), quercetin isolated from *Psidium guajava* leaf induced reduction of presynaptic molecular activity by modulating the cytosolic calcium concentration in mouse neuromuscular junction.

These biological activities of flavonoids and especially those of quercetin showed that flavonoids could play an important role in the inhibition of calcium release from SR.

On the other hand, tannins existing in aqueous and ethanolic extracts of these two plants could also play an important role. It has been reported that they have free radical scavenger properties (Bharani *et al*., 1995) and antioxidant action (Simeray *et al*., 1982; Yoshizawa *et al*., 1987).

Tannins have been reported to have several pharmacological activities such as spasmolytic activity in smooth muscle cells (Tona *et al*., 1999).

According to Owen and Johns (1999), tannins have protein-binding properties and can interfere with many substances. It was suggested that tannins could reduce the intracellular calcium level by a decrease in the calcium inward current and/or by activation of the calcium pumping system (Chiesi and Schwaller, 1994). Zhu *et al*.(1997) reported that tannins inhibited calcium channels and induced muscle relaxation.

With regard to these last properties, it can be suggested that tannins could act with proteins involved in the regulation of ryanodine receptors and partly block calcium release from SR in our assays.

Few studies have been reported on *Diospyros mespiliformis* biological activities. Antibacterial activity has been shown (Adeniyi *et al*., 1996; Sanogo *et al*., 1998). Other *Diospyros* species showed mainly antimalarial (Likhitwatayawuid *et al*., 1999), antiinflammatory (Recio *et al*., 1995), hypotensive (Funayama and Hikino, 1979) and oral antimicrobial (Cai *et al*., 2000) activities.

## CONCLUSION

The results reported here showed that different leaf extracts of *Psidium guajava* and *Diospyros mespiliformis* inhibited caffeine induced calcium release from sarcoplasmic reticulum in a dose dependent manner. These results could explain their traditional use to treat many diseases. More investigations are in progress to isolate active compounds and vascular smooth muscle experiments will be done to verify their possible relaxant effects.

## ACKNOWLEDGEMENT

Thanks are due to Prof. Samaté, Prof. Mil-

logo-Rasolodimby and F Mazin for their helpful collaboration.

## References

Adeniyi, B.A., Odelola, H.A., Oso, B.A., 1996. Antimicrobial potentials of *Diospyros mespiliformis* (Ebenaceae). *Afr. J. Med. Sci.*, **25**:221-224.

Alikaridis, F., 1987. Natural constituents of *Ilex* species. *J. Ethnopharmacology*, **20**:121-144. [doi:10.1016/0378-8741(87)90084-5]

Apisariyakul, A., Chaichana, N., Takemura, H., 1999. Dual effects of quercetin on contraction in cardiac and skeletal muscle preparations. *Research Communications in Molecular Pathology and Pharmacology*, **105**:129-138.

Arima, H., Danno, G.I., 2002. Isolation of antimicrobial compounds from guava (*Psidium guajava* L.) and their structural elucidation. *Biosci. Biotechnol. Biochem.*, **66**:1727-1730. [doi:10.1271/bbb.66.1727]

Begum, S., Hassan, S.I., Ali, S.N., Siddiqui, B.S., 2004. Chemical constituents from the leaves of *Psidium guajava*. *Natural Product Research*, **18**:135-140. [doi:10.1080/14786410310001608019]

Belemtougri, R.G., Constantin, B., Cognard, C., Raymond, G., Sawadogo, L., 2001. Effects of *Sclerocarya birrea* (A. rich) hochst (anacardiaceae) leaf extracts on calcium signalling in cultured rat skeletal muscle cells. *J. Ethnopharmacology*, **76**:247-252. [doi:10.1016/S0378-8741(01)00248-3]

Bharani, A., Ganguly, A., Bhargava, K.D., 1995. Salutary effect of *Terminalia arjuna* in patients with severe refractory heart failure. *Int. J. Cardiol.*, **49**:191-199. [doi:10.1016/0167-5273(95)02320-V]

Cai, L., Wei, G.X., van der Bijl, P., Wu, C.D., 2000. Namibian chewing stick, *Diospyros lycioides*, contains antibacterial compounds against oral pathogens. *J. Agric. Food Chem.*, **48**:909-914. [doi:10.1021/jf9909914]

Capasso, A., Pinto, A., Mascolo, N., Autore, G., Capasso, F., 1991. Reduction of agonist-induced contractions of guinea-pig isolated ileum by flavonoids. *Phytother. Res.*, **5**:85-87.

Chaichana, N., Apisariyakul, A., 1996. Cholinergic blocking effect of quercetin. *Thai J. Pharmacol.*, **18**:1-15.

Chiesi, M., Schwaller, R., 1994. Reversal of phospholamban-induced inhibition of cardiac sarcoplasmic reticulum $Ca^{2+}$-ATPase by tannin. *Biochemical and Biophysical Research Communications*, **202**:1668-1673. [doi:10.1006/bbrc.1994.2126]

Cognard, C., Constantin, B., Rivet-Bastide, M., Imbert, N., Besse, C., Raymond, G., 1993a. Appearance and evolution of calcium currents and contraction during the early post-fusional stage of rat skeletal muscle cells developing in primary culture. *Development*, **117**:1153-1161.

Cognard, C., Constantin, B., Rivet-Bastide, M., Raymond, G., 1993b. Intracellular calcium transients induced by different kinds of stimulus during myogenesis of rat skeletal muscle cells studied by laser cytofluorimetry with Indo-1. *Cell Calcium*, **14**:333-348. [doi:10.1016/0143-4160(93)90054-A]

Conde-Garcia, E.A., Nascimento, V.T., Santiago-Santos, A.B., 2003. Inotropic effects of extracts of *Psidium guajava* L. (guava) leaves on the guinea pig atrium. *Braz. J. Med. Biol. Res.*, **36**:661-668.

Duarte, J., Pérez-Vizcaíno, F., Zarzuelo, A., Jiménez, J., Tamargo, J., 1993. Vasodilator effects of quercetin in isolated rat vascular smooth muscle. *Eur. J. Pharmacol.*, **239**:1-7. [doi:10.1016/0014-2999(93)90968-N]

Formica, J.V., Regelson, W., 1995. Review of the biology of quercetin and related bioflavonoids. *Food Chem. Toxicol.*, **33**:1061-1080. [doi:10.1016/0278-6915(95)00077-1]

Funayama, S., Hikino, H., 1979. Hypotensive principles of *Diospyros kaki* leaves. *Chem. Pharm. Bull (Tokyo)*, **27**:2865-2868.

Havsteen, B., 1983. Flavonoids, a class of natural products of high pharmacological potency. *Biochem. Pharmacol.*, **32**:1141-1148. [doi:10.1016/0006-2952(83)90262-9]

Jaiarj, P., Khoohaswan, P., Wongkrajang, Y., Peungvicha, P., Suriyawong, P., Saraya, M.L.S., Ruangsomboon, O., 1999. Anticough and antimicrobial activities of *Psidium guajava* Linn. leaf extract. *J. Ethnopharmacology*, **67**:203-212. [doi:10.1016/S0378-8741(99)00022-7]

Jaiarj, P., Wongkrajang, Y., Thongpraditchote, S., Peungvicha, P., Bunyapraphatsara, N., Opartkiattikul, N., 2000. Guava leaf extract and topical haemostasis. *Phytother. Res.*, **14**:388-391. [doi:10.1002/1099-1573(200008)14:5<388::AID-PTR638>3.0.CO;2-K]

Lamb, G.D., 2000. Excitation-contraction coupling in skeletal muscle: comparison with cardiac muscle. *Clinical and Experimental Pharmacology and Physiology*, **27**:216-224. [doi:10.1046/j.1440-1681.2000.03224.x]

Likhitwatayawuid, K., Dejadisai, S., Jongbunprasert, V., Krungkrai, J., 1999. Antimalarials from *Stephania venosa*, *Prismatomeris sessiliflora*, *Diospyros montana* and *Murraya siamensis*. *Planta Med.*, **65**:754-756.

Lozoya, X., Bercerril, G., Martinez, M., 1990. Intraluminal perfusion model of in vitro guinea pig ileum as a model of study of the antidiarrheic properties of the guava (*Psidium guajava*). *Arch. Invest. Med. (Mex.)*, **21**:155-162.

Lozoya, X., Meckes, M., Abou-Zaid, M., Tortoriello, J., Nozzolillo, C., Amason, J.T., 1994. Quercetin glycosides in *Psidium guajava* L. leaves and determination of spasmolytic principle. *Arch. Med. Res.*, **25**:11-15.

Lutterodt, G.D., 1989. Inhibition of gastrointestinal release of acetylcholine by quercetin as a possible mode of action of *Psidium guajava* leaf extracts in the treatment of acute diarrhoeal diseases. *J. Ethnopharmacology*, **25**:235-247. [doi:10.1016/0378-8741(89)90030-5]

Lutterodt, G.D., Maleque, A., 1988. Effects on mice locomotor activity of a narcotic-like principle from *Psidium guajava* leaves. *J. Ethnopharmacology*, **24**:219-231. [doi:10.1016/0378-8741(88)90155-9]

Meckes, M., Calzada, F., Tortoriello, J., González, J.L., Martinez, M., 1996. Terpenoids isolated from *Psidium guajava* hexane extract with depressant activity on central

nervous system. *Phytother. Res.*, **10**:600-603. [doi:10.1002/(SICI)1099-1573(199611)10:7<600::AID-PTR918>3.0.CO;2-7]

Morales, M.A., Tortoriello, J., Meckes, M., Paz, D., Lozoya, X., 1994. Calcium-antagonist effect of quercetin and its relation with the spasmolytic properties of *Psidium guajava* L. *Arch. Med. Res.*, **25**:17-21.

Muccillo Baisch, A.L., Johnston, K.B., Paganini Stein, F.L., 1998. Endothelium-dependent vasorelaxing activity of aqueous extracts of *Ilex paraguariensis* on mesenteric arterial bed of rats. *J. Ethnopharmacology*, **60**:133-139. [doi:10.1016/S0378-8741(97)00140-2]

Osman, A.M., Younes, M.E., Sheta, A.E., 1974. Triterpenoids of the leaves of *Psidium guajava*. *Phytochemistry*, **13**:2015-2016. [doi:10.1016/0031-9422(74)85152-6]

Owen, P.L., Johns, T., 1999. Xanthine oxidase inhibitory activity of northeastern North American plant remedies used for gout. *J. Ethnopharmacology*, **64**:149-160. [doi:10.1016/S0378-8741(98)00119-6]

Qian, H., Nihorimbere, V., 2004. Antioxidant power of phytochemicals from *Psidium guajava* leaf. *J. Zhejiang Univ. Sci.*, **5**(6):676-683. [doi:10.1631/jzus.2004.0676]

Re, L., Barocci, S., Capitani, C., Vivani, C., Ricci, M., Rinaldi, L., Paolucci, G., Scarpantonio, A., Leon-Fernandez, O.S., Morales, M.A., 1999. Effects of some natural extracts on the acetylcholine release at the mouse neuromuscular junction. *Pharmacological Research*, **39**:239-245. [doi:10.1006/phrs.1998.0433]

Recio, M.C., Giner, R.M., Manez, S., Gueho, J., Julien, H.R., Hostettmann, K., Rios, J.L., 1995. Investigations on the steroidal anti-inflammatory activity of triterpenoids from *Diospyros leucomelas*. *Planta Medica.*, **61**:9-12.

Sanogo, R., Crisafi, G., Germanò, M.P., de Pasquale, R., Bisignano, G., 1998. Evaluation of Malian traditional medicines: screening for antimicrobial activity. *Phytother. Res.*, **12**:S154-S156. [doi:10.1002/(SICI)1099-1573(1998)12:1+<S154::AID-PTR281>3.0.CO;2-T]

Seshadri, T.R., Vasishta, K., 1965. Polyphenols of the leaves of *Psidium guajava*: quercetin, guaijiaverin, leucocyanidin and amritoside. *Phytochemistry*, **4**:989-992. [doi:10.1016/S0031-9422(00)86281-0]

Simeray, J., Chaumont, J.P., Bevalot, F., Vaguette, J., 1982. Les propriétés antifongiques des cistacéas et plus particulièrement de *Cistus laurifolius* L.: rôle des tannins non hydrolysables. *Fitoterapia*, **53**:45-48.

Tona, L., Kambu, K., Mesia, K., Cimanga, K., Apers, S., de Bruyne, T., Pieters, L., Totte, J., Vlietinck, A.J., 1999. Biological screening of traditional preparations from some medicinal plants used as antidiarrhoeal in Kinshasa, Congo. *Phytomedicine*, **6**:59-66.

van Acker, S.A.B.E., Tromp, M.N.J.L., Haenen, G.R.M.M.M., van der Vijgh, W.J.F., Bast, A., 1995. Flavonoids as scavengers of nitric oxide radical. *Biochem. and Biophys. Res. Communications*, **214**:755-759. [doi:10.1006/bbrc.1995.2350]

Yoshizawa, S., Horiuchi, T., Fujiki, H., Yoshida, T., Okuda, T., Sugimura, T., 1987. Antitumor promoting activity of (−)-Epigallocatechin gallate, the main constituent of tannin in green tea. *Phytother. Res.*, **1**:44-47.

Yuting, C., Rongliang, Z., Zhongjian, J., Yong, J., 1990. Flavonoids as superoxide scavengers and antioxidants. *Free Radical Biology and Medicine*, **19**:19-21. [doi:10.1016/0891-5849(90)90045-K]

Zhu, M., Phillipson, J.D., Greengrass, P.M., Bowery, N.E., Cai, Y., 1997. Plant polyphenols: biologically active compounds or non-selective binders to proteins? *Phytochemistry*, **44**:441-447. [doi:10.1016/S0031-9422(96)00598-5]

Zimanyi, I., Pessah, I.N., 1994. Pharmacology of Ryanodine-Sensitive $Ca^{2+}$ Release Channels. *In*: Handbook of Membranes Channels, p.475-494.