Vasorelaxant effects of Psidium guajava L. (Myrtaceae) aqueous leaf extract on rat aorta

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Abstract
Psidium guajava is widely used in the central plateau of Burkina Faso to treat many diseases among which hypertension. Aqueous leaf extract of this plant was evaluated on wistar rat aorta precontracted by Norepinephrine (NE) or 5-hydroxytryptamine (5-HT). The results showed endothelium-independent vasodilation. The plant extract relaxed NE and 5-HT precontracted rat aortic rings in a dose dependent manner. The plant extract shifted towards right concentration response curves induced by NE and 5-HT in a non-parallel manner. This suggests that vasorelaxation effects were likely via voltage dependent calcium channel (VDCC) and receptor-operated calcium channel (ROCC), and probably via inhibition of intracellular calcium release from calcium store. These results seem to confirm its use in hypertension traditional treatment.

Keywords: P. guajava, sarcoplasmic reticulum, hypertension, vasorelaxation, endothelium-independent

Introduction
Arterial hypertension is a common and progressive disorder that poses a major risk for cardiovascular and renal diseases. Hypertension is an important public health problem and its treatment and control varies considerably among the countries. In Africa, people used natural resources for medicinal purposes. In developing countries, hypertension is now considered as a cause of mortality in adults because of their lifestyle. In Burkina Faso its prevalence is estimated to 40% of cardiovascular diseases [1]. Among the natural resources, plants are used to treat many disorders such as hypertension. Psidium guajava is one of the plants used to treat this disease. Psidium guajava is native of tropical America and has been naturalized in tropical countries of the world. The ripe fruits which are rich in vitamin C, are consumed fresh or industrialized as jam or juice. All the parts of the plant are used by healers to treat various human ailments such as cough, bronchitis, diarrhoea, hypertension and other gastro-intestinal diseases [2, 3]. Phytochemical studies resulted in the isolation and identification of various compounds among which terpenoids, flavonoids and tannins are reported [4, 5]. Pharmacological studies showed a broad spectrum of effects, mainly antidiarrheic [6-8], antispasmodic [9-11], spasmyloytic [11], inotropic [12] and antioxidant [13-15] effects. Some studies have shown that P. guajava extracts induce contractile [16] and relaxant [17] effects on rat isolated aortic rings.
Recent ethnomedical studies show that P. guajava is used through the world for the treatment of many diseases such as anti-inflammatory, diabetes [8], hypertension, caries, wounds, pain relief and reducing fever [5]. Antibacterial properties [18-21], anti-infectious diarrhoea effect [22] and hepatoprotective activity [23] have been reported. In South Africa, the leaf of P. guajava is used to treat diabetes mellitus and hypertension [24, 25]. P. guajava is employed as food, mainly the ripe fruit rich in ascorbic acid is used as juice, jelly nectar or concentrated [26]. Two triterpenoids, 20 β-acetoxy-2α,3β-dihydroxyurs-12-en-28-oic acid (guava noice-acid) and 2α-3β-dihydroxy-24-p-z-coumaroyloxyurs-12-en-28-oic acid (Guava comaric acid) are isolated from the fresh leaves of P. guajava [5].
In this study, we investigated whether Psidium guajava leaf extract which is used to treat hypertension would exert relaxation of isolated arteries in vitro and to explore the underlying mechanism responsible for its medicinal use to treat this disease.
Materials and Methods

Plant collection
Fresh leaves of Psidium guajava were collected around Ouagadougou. This plant was identified by the herbarium of the University Joseph KI-ZERBO where a specimen was kept under the number ID 17909 and sample number 6911.

Preparation of plant extracts
Aqueous decoctions were prepared from the shade dried leaves. Forty grams of leaves powder of the plant were macerated in one liter (1 L) of 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 what man n°2 and freeze-dried for 24 h and then lyophilized to give brown powder which was utilized for experiments.

Animals
Male Wistar rats (250 – 300 g) were used in this study. The animals were fed with standard diet and were kept at 24 °C ± 2 and submitted to a 12 h light/dark cycle with free access to food and water. The study protocols and ethical issues were approved by ethical committee of Faculty of University Joseph KI-ZERBO.

Drugs used
Norepinephrine bitartrate, (NE) and 5-hydroxytryptamine (5-HT) were purchased from Sigma. The aqueous extract of Psidium guajava (P. guajava), NE and 5-HT were dissolved in deionized water. All solutions were freshly prepared on the day of the experiment.

Experiments
The animals were killed by cervical dislocation. The thoracic aorta was immediately isolated and put in Krebs solution and cleaned of adherent connective tissue and cut into rings (3 – 4 mm length). Each ring was suspended in organ bath between two parallel stainless hooks. One hook was fixed while the other was connected to a force transducer for the isometric tension. The organ bath contained 5 ml Krebs’ solution at 37°C, bubbled with carbogen (95% O₂ and 5% CO₂). The Krebs’ solution contained the following composition (mM): NaCl, 120; KCl, 4.7; CaCl₂, 2.5; MgCl₂, 1.2; NaHCO₃, 15; KH₂PO₄, 1.2; D-glucose, 11; Hepes, 10; PH = 7.4.

The rings were stretched progressively to a basal tension of 2 g and allowed to equilibrate for at least 90 min, during which time the bath solution was replaced with prewarmed solution every 15 min to protect against interfering metabolites [27]. The presence or lack of functional endothelium was examined by demonstrating the presence or absence of relaxation induced by Ach 100 µM on aortic rings precontracted with 1 µM NE. Aortic rings showing an Ach induced relaxation ≤ 10%, indicative of an effective removal of the endothelium were utilized for experiments. After a washout and an equilibration period of 60 min, the aortic rings returned to the basal level and the experiments can begin.

For relaxant effects of the plant extract evaluation, two protocols previously used in our laboratory) [28-30] were used. Aortic rings were precontracted, first, by a single concentration of Norepinephrine (NE) (1 µM), 5-hydroxytrytamine (5-HT) (100 µM) and then different increasing cumulative concentrations of Psidium guajava were added cumulatively to the organ bath; second, rings were precontracted with increasing cumulative concentrations of NE or 5-HT. In this case, pre-treatment with the plant extract was performed 20 min before the administration of NE or 5-HT. In control conditions, the first concentration response to NE or 5-HT was almost identical to the second one.

Statistical analysis
Relaxant effects were expressed as percentage relaxation from NE, 5-HT precontraction levels. Statistical analysis was done using Graph Pad Prism (Graph Pad Software, San Diego, CA, USA). Concentration – response curves were analysed by nonlinear regression. Data were shown as mean ± SEM. Statistical significance was estimated by Student’s t-test. A less than 0.05 p value was considered as significant.

Results
Vasorelaxation effect of P. guajava on rat aortic rings precontracted by NE or 5-HT.

The aqueous extract of P. guajava has no activity on rat aorta basal tone when applied to the organ bath. Then we utilized the spasmogenic substances, NE and 5-HT, to research some antagonistic effects on rat aorta.

P. guajava concentration-dependently relaxed the without endothelium aortic rings precontracted by NE or 5-HT (figures 1A & 1B, respectively).

Fig 1: Effects of aqueous extract of P. guajava on endothelium denuded aortic rings precontracted with NE (1 µM) (A) or precontracted with 5-HT (100 µM) (B).
In both cases the relaxation was 100%. Then with NE the results show an IC50 = 135 μg/ml (figure 1A) and an IC50 = 69.59 μg/ml (figure 1B) for 5-HT.

Effects of *P. guajava* on the NE and 5-HT induced concentration-contraction curves.

NE and 5-HT both induce sustained contraction of aortic rings in a concentration-dependent manner. *P. guajava* was added to the baths before cumulative addition of NE or 5-HT. In this case *P. guajava* inhibited the NE or 5-HT induced vasoconstriction and concentration-dependently shifted the concentration contractile curves towards right on a nonparallel manner with decrease of the maximum level (figures 2A & 2B).

![Fig 2: Inhibitory effects of *P. guajava* on the contractions induced by NE as control (A) and 5-HT as control (B) in endothelium denuded aortic rings. Results are presented as means ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001.](http://www.phytojournal.com)

*P. guajava* at 30 μg/ml and 100 μg/ml concentrations inhibited more the contractile effect of NE (figure 2A) than that of 5-HT (figure 2B). In that last case, at 30 μg/ml, *P. guajava* leaf extract shifted the concentration contractile curve of 5-HT towards right but not decrease the maximum level contraction.

Discussion

The antihypertensive drugs mediate their effects through relaxation of arteriolar smooth muscle resulting in decreasing peripheral vascular resistance [31].

Our results show that the plant extract right shifted the NE and 5-HT curves with suppression of the maximum effect. These results point a non-competitive inhibition. Removal of functional endothelium from the aortic rings used did not abolish relaxation of the rings precontracted by NE or 5-HT. This result suggests involvement of Endothelium Derived Relaxing Factor (EDRF)-independent vasodilation mechanisms.

The results indicated also that the action of *P. guajava* extract was directly on vascular smooth muscle cells to induce vasorelaxation. Vascular smooth muscle cells contraction required Ca2+, which can provide through receptor-operated Ca2+ channels (ROCCs), voltage-dependent Ca2+ channels (VDCCs) [32] and release of Ca2+ from sarcoplasmic reticulum through inositol trisphosphate (IP3) and ryanodine receptors respectively.

*P. guajava* extract reduced concentration dependently the agonist induced vasoconstrictions, NE and 5-HT via α1-adrenoceptors and 5-HT receptors respectively. NE, a α1-adrenoceptor agonist, induces aortic rings contraction by Ca2+ influx through ROCCs and by release of Ca2+ from sarcoplasmic reticulum [33]. Indeed, NE induces activation of phospholipase C (PLC) to produce IP3 and diacylglycerol (DAG) which both activate protein kinase C (PKC). IP3 induces Ca2+ release from sarcoplasmic reticulum by opening IP3 receptors [34, 35].

5-HT induces vascular smooth muscle contraction by increasing the level of intracellular calcium of two manners: i) influx of extracellular Ca2+ and ii) release of Ca2+ from sarcoplasmic reticulum by opening 5-HT2A receptors localised in rat aortic rings [36].

Our results suggest that the plant extract induces vasorelaxation by inhibiting IP3 and/or ryanodine receptors to release Ca2+ from sarcoplasmic reticulum. The extract can also activate Ca2+-ATPase to facilitate the entry of cytoplasmic Ca2+ into sarcoplasmic reticulum. This last hypothesis needs further investigation.

On the other hand, the plant extract can activate K+ channels localised in vascular smooth muscle cells. Indeed, Quest et Cook [37] reported that K+ channel activators produce smooth muscle relaxation by opening K+ channels that results an increase in K+ efflux and decrease in intracellular calcium. Calcium channels blockers and K+ channels openers are for the treatment of hypertension because of their action to induce vascular smooth muscle relaxation. Considering the present results, it is possible to suggest that *P. guajava* leaf extract could exert antihypertensive action in vivo and could justify its utilization in the management of hypertension by rural populations in Burkina Faso.

The previous screening of the plant extract showed flavonoids and tannins as the main components [38]. Many investigators reported that flavonoids contained in *P. guajava* leaf extract induce relaxation in rat isolated aorta and porcine coronary artery [39, 40]. Other studies suggest that flavonoids possess free radical scavenging and antioxidant properties which can...
explain their vasorelaxation properties [41] and these properties are endothelium-independent [39]. The flavonoids are also reported to inhibit entry of extracellular calcium and then induced vasorelaxation [42] and/or inhibiting intracellular calcium release [43]. It can be suggested that our extract induced relaxation by inhibiting influx of extracellular calcium and/or inhibiting intracellular calcium release from its stores in the vascular smooth muscle cells. Tannins are reported to reduce blood pressure [43] and can contribute to vasorelaxation. Many investigators [44-46] have reported that tannins isolated from plants play an important role in rat aortic rings relaxation. But these results cannot be compared with our results because they have demonstrated the involvement of endothelium in the vasorelaxation.

Conclusion

P. guajava leaf extract possessed an endothelium-denuded vasorelaxation which provides a rationale for the use of this plant in traditional medicine for hypertension treatment. Further studies are in progress to isolate the main component(s) responsible for this action.

References

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