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RESEARCH ARTICLE

# Calcium Antagonistic Activity of *Biophytum petersianum* on Vascular Smooth Muscles of Wistar Rat

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**ABSTRACT**

The whole plant of *Biophytum petersianum* was extracted with a mixture of water – alcohol (1:1) to evaluate its relaxant effect on aorta rings. In isolated Wistar rat tissue, the hydro-ethanolic extract (0.1, 0.25 and 0.5 mg/ml) non-competitively antagonized calcium chloride and high-$K^+$-induced aorta contractions in a concentration-dependent manner. Moreover, the inhibition of noradrenaline–induced contractions in the presence of extract (1 mg/ml) suggests that the extract has an effect on mobilization of intracellular calcium. These results indicate that hypotensive effect of *Biophytum petersianum* may result from inhibition of calcium influx via both voltage-and receptor-operated calcium channels.

**Keywords:** *Biophytum petersianum, Aorta, Calcium antagonistic activity, Hypertension*

*Biophytum petersianum* Klotzsch (Oxalidaceae.) is an annual plant which is distributed widely in tropical areas in Africa, Madagascar and Asia. It has been used in traditional medicine for many purposes. In Mali, the leaves are used in wound healing and for its complement fixing activity [1]. It is reported that aqueous extract of the leaves has hypocholesterolemic [2] and hypoglycaemic [3] effects. It also showed insulinotropic activity in non-diabetic and alloxan-diabetic rabbits [4]. Amentoflavone extracted from the roots of *B. petersianum* showed a selective inhibition of cyclooxygenase (COX)-1/COX-2 [5]. In Togo, the whole plant is reported to have antihypertensive effect and previous studies with hydro-ethanolic extract on guinea-pig and Wistar rat confirmed this indication [6]. Hydro-ethanolic extract of the whole plant is also claimed to stimulate corticosterone and aldosterone secretion in rat without any desensitization phenomenon [7].

Calcium plays an important role in the pathophysiological process of hypertension through the increase of heart activity, mainly by activation of smooth muscle contraction under a variety of circumstances [8]. It's well documented that substances that inhibit calcium release in vascular smooth cells prevent vasoconstriction [9] and are beneficial in the treatment of hypertension.

The antihypertensive effect of *B. petersianum* may be explained by inhibition of calcium release into the inside of vascular smooth cells. The present study was designed to examine the possible effect of hydro-alcoholic extract of *B. petersianum* on intracellular mobilization and on extracellular calcium influx through L-Type channels of rat aortic rings in $Ca^{2+}$-free and high $K^+$ medium.

## MATERIALS AND METHODS

### *Chemicals*

Noradrenaline, Verapamil, Quercetin, Gallic Acid and Sodium carbonate were purchased from Sigma Chemical Co. (St. Louis, MO).

### *Plant material*

Whole plant material was collected in September 2003 in the locality of Kévé (southern region of Togo). A voucher specimen was deposited in the herbarium under the number 1032 in the Department of Botanic, Faculty of Sciences, University of Lomé, Togo.

### *Animals*

Wistar rats of both sex (180-230 g) provided by the Department of Animal Physiology were used in these

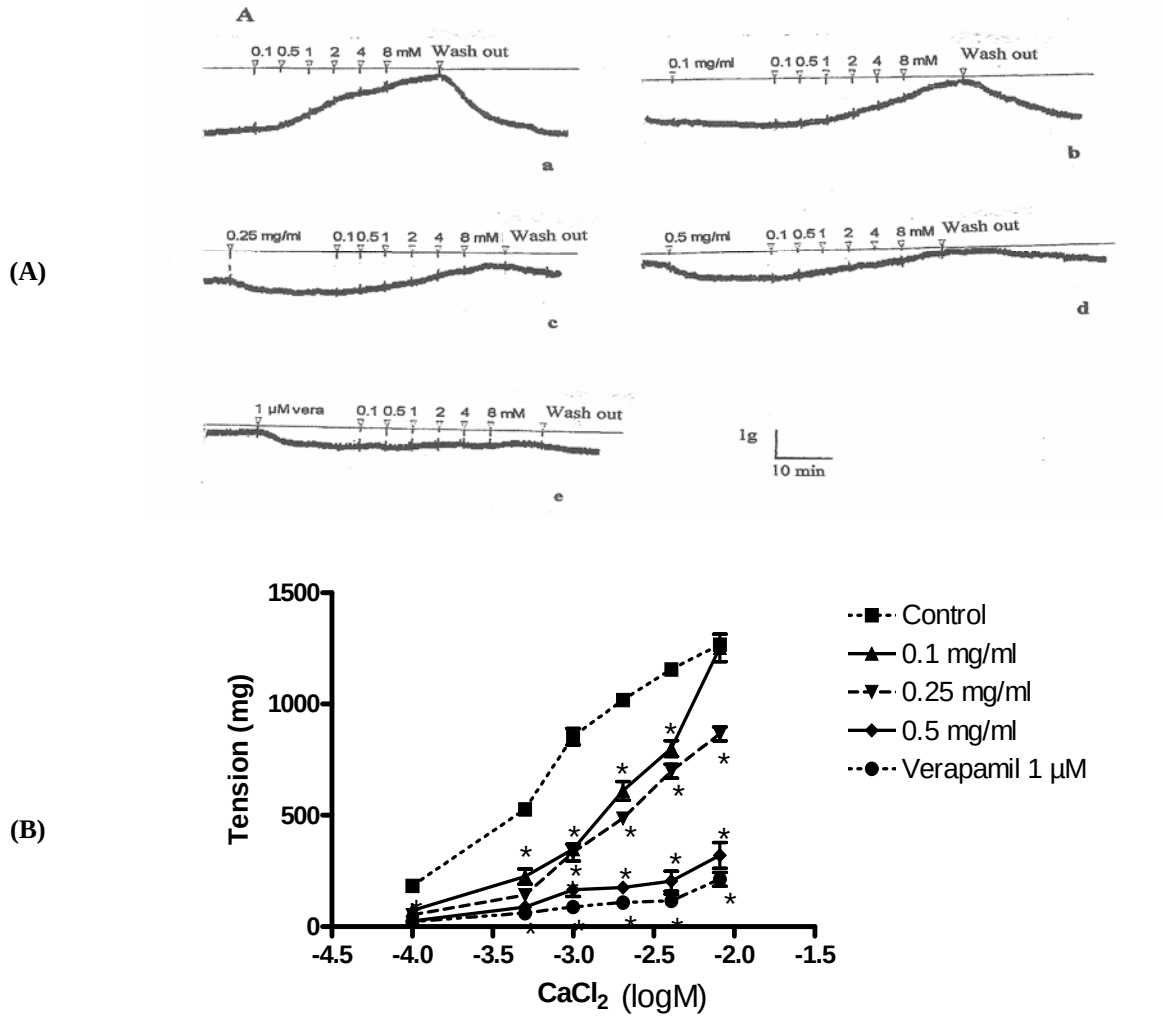


**Fig. 1**. Muscle tension produced by cumulative concentrations of calcium chloride in the absence and presence of hydro-alcoholic extract of *B. petersianum* in rat thoracic aorta. **A:** representative traces of the effect of the cumulative calcium chloride concentrations on rat isolated aorta in a high $K^+$ medium without calcium in the absence (a) and in the presence of the 0.1 mg/ml (b), 0.25 mg/ml (c) and 0.5 mg/ml (d) extract and 1µM verapamil (e). **B:** Effects of the plant extract on the contraction induced by calcium chloride in rat thoracic aorta. Each point represents the mean of 5 preparations. Vertical bars indicate the S.E.M. * $p < 0.001$, when compared with control value.

experiments. They were kept under standard environmental conditions with free access to food and water.

### *Preparation of extract*

The powder of the whole plant (100 g) was macerated during 72 hours in water/ethanol (50% v/v) solution, at room temperature with occasional stirring. After filtration, the filtrate was evaporated in a vacuum rotavapor at 50°C (yield 12 %). The extract was dark brown and soluble in distilled water.

### *Total phenols determination*

The phenolic content in the hydro-alcoholic extract of *Biophytum petersianum* was measured by Folin-Ciocalteu reagent using gallic acid as standard [10]. Briefly, we dissolved the plant extract in methanol/distilled water (v/v, 1:1) to achieve a concentration of 1mg/ml. About 500µl of this solution was mixed with 5 ml of Folin-Ciocalteu reagent (1:10 ml distilled water), and 4 ml of sodium carbonate ($Na_2CO_3$, 1 M). The mixture was incubated at room temperature for 15 minutes and the absorbance was measured at 765 nm against a methanol blank using a spectrophotometer (Sequoia–Turner, Model 340). Total phenolic content was determined using a six point standard curve of Gallic acid (0–250 mg/L). All tests were carried out in triplicate. Data were converted into milligram gallic acid equivalents per gram of *Biophytum petersianum* extract (GAE/g of BPE).

### *Total flavonoids determination*

Aluminium chloride colorimetric method described by Pourmorad et *al.* [10] was used to determine flavonoid content in plant extract. The extract was dissolved in methanol to achieve a final concentration of 10 mg/ml. About 500 µl of this solution was mixed with 1.5ml of

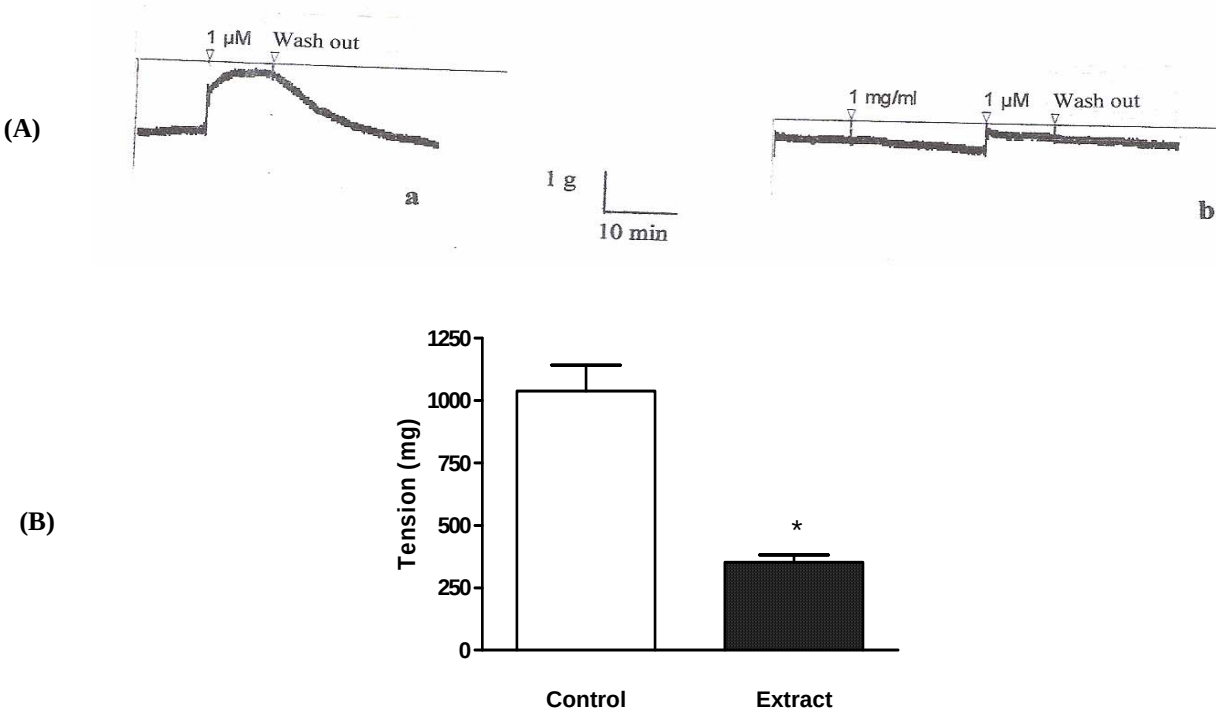


**Fig. 2**. Effect of noradrenaline (1 µM) on rat thoracic aorta in $Ca^{2+}$ -free, high $K^+$ (60 mM) solution in absence (control) and presence of hydro-alcoholic extract of *B. petersianum* (1 mg/ml). The extract was added 20 min prior to drug addition. **A:** representative traces of the opposing effect of extract on noradrenaline-induced contraction in a depolarized medium without calcium in the absence (a) and in presence (b) of 1 mg/ml extract **B:** Effects of the plant extract on the contraction induced by adrenaline in rat thoracic aorta. Each point represents the mean of 6 preparations. Vertical bars indicate the S.E.M., * $p <$ 0.001 when compared with control value.

methanol, 100µl of aluminium chloride (0.1 g/ml), 100µl of sodium acetate (1 M) and 2.8 ml of distilled water. After 30 minutes incubation at room temperature, the absorbance was measured at 415 nm against methanol blank with a spectrophotometer. Quercetin was chosen as standard and six points standards curve (0 – 1000 µg/ml) was used for the determination of total flavonoid content. All tests were carried out in replicate (n = 4). The data were converted into milligram quercetin equivalents per gram of *Biophytum petersianum* extract (QE/g of BPE).

### *Aortic preparation*

The rats were sacrified under ether anesthesia. Thoracic aorta was then removed and immediately placed in cold Krebs-Henseleit solution. Composition of the Kreb's solution in mM was as follows: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $KH_2PO_4$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11.0. The aorta was cleaned of adherent tissue and cut into 5 mm rings, taking care not to damage the endothelium. Each ring was fixed vertically between triangular stainless steel hooks (SA-068, UGO BASILE) under a resting tension of 2 g in 10 ml bath filled with the Kreb's solution and maintained at 37°C. The bath solution was continuously oxygenated and the rings were allowed to equilibrate for 90 minutes before the start of the experiments. Initially, all rings were contracted twice with KCl (60 mM) to confirm the viability of vessels before performing the experimental protocols. Isometric tension change was measured with TDS105A Variable Force transducer (BIOPAC SYSTEM, MODEL MP100, HAVARD APPARATUS) coupled to a computer (Acq Knowledge III software).

### *Effects of B. petersianum on extracellular $Ca^{2+}$ influx through voltage-sensitive channels*

Aortic ring depolarized and contracted by $Ca^{2+}$ was chosen as the model to investigate the effects of *B. petersianum*'s hydro-ethanolic extract on calcium influx through voltage-sensitive channels, as previously described by Hof et *al.* [9]. The rings were exposed to calcium-free high-potassium depolarizing solution (NaCl of Kreb's solution was replaced by 60 mM of KCl). Vessels were allowed to equilibrate for at least 30 minutes before drug addition and the solution was changed twice at 15-minute intervals. Then, cumulative concentration–response curves to $CaCl_2$ (0.1–8 mM) were determined in the absence (control) or in the presence of extract (0.1; 0.25 and 0.5 mg/ml) or verapamil (1µM), which was used as the reference drug.

### *Effects of B. petersianum on the release of intracellular calcium*

The method of Dar et a*l.* was used [11]. Briefly, the contraction was initiated with noradrenaline (1 µM) in calcium-free high $K^+$ physiological solution. Mean am-

plitude of contraction induced by noradrenaline was compared to the contraction obtained in the presence of extract (1 mg/ml) which was introduced 20 minutes prior to noradrenaline addition.

### *Statistical analysis*

Data are expressed as the mean ± S.E.M and *n* indicates the number of animals. Statistical analysis was based on analysis of variance (ANOVA) followed by Fisher's LSD test using Systat (Version 11.0). Differences were considered to be significant when $p < 0.05$.

## RESULTS

### *Total phenolic and flavonoid content in Biophytum petersianum*

The results showed that the total phenolic and flavonoid in *B. petersianum* are respectively 205 mg GAE/g of BPE and 97.5 mg QE/g of BPE.

### *Effect of hydro-alcoholic extract of B. petersianum on Calcium channels activity*

In $Ca^{2+}$-free, high $K^+$ (60 mM) solution, the cell membrane of aortic smooth muscle was depolarized, but the lack of $Ca^{2+}$ entry in vascular smooth muscle cells prevented vasoconstriction in this high $K^+$ solution. Cumulative addition of calcium chloride (0.1–8 mM) caused an increase in contraction of the rat aorta (fig. 1). The maximal tension attained at 8 mM $Ca^{2+}$ (1266.6 ± 8.8 mg) was considered as 100 %. When the aortic rings were treated with extract at 0.1; 0.25 and 0.5 mg/ml (20 minutes prior to $CaCl_2$ addition), the KCl-induced contraction was rightward shifted in non-parallel manner. The plant extract at 0.5 mg/ml significantly reduced the maximal tension induced by calcium chloride (74.8%, $p < 0.001$) which was comparable to that of verapamil at 1µM (83.2 %).

To evaluate the effect of *B. petersianum* on the release of intracellular calcium, noradrenaline-induced contraction was measured in presence of extract. Phasic contraction of rat aortic rings induced by noradrenaline in the absence and in the presence of plant extract was 1038 ± 104 mg and 351.66 ± 30.3 mg respectively (fig. 2), showing that contraction maximal amplitude induced by noradrenalin was significantly reduced ( $p < 0.001$) by the hydro-alcoholic extract of *B. petersianum*.

## DISCUSSION

The major finding in this study was that the hydro-alcoholic extract of *B. petersianum* acts as a non-selective calcium antagonist. Our study demonstrated that pre-treatment with the plant extract prevents, in a concentration-dependent manner, the aortic contractile response to high $K^+$. It's well documented that the contractions of smooth muscles which are induced by high $K^+$, are dependent of calcium entry into cell through voltage-operated calcium channels [12,13]. This suggested that the plant extract might interfere with $Ca^{2+}$-sensitive voltage channels in aortic smooth muscle probably by inhibiting $Ca^{2+}$ influx into the cell.

Noradrenaline–induced responses in free-$Ca^{2+}$, high $K^+$ medium, moreover, were significantly reduced in presence of 1 mg/ml of plant extract, suggesting extract also affected the release of $Ca^{2+}$ from intracellular stores.

We have quantified compounds in the extract of *B. petersianum*, such as total phenolic and flavonoid. These findings are consistent with those of Lin et *al.* [14] and Bucal et *al*. [15] who found biflavone and flavonoid, and phenolic compounds from *Biophytum petersianum* (syn. *Biophytum sensitivum*) respectively. Several studies have revealed that phenolic compounds and flavonoids have calcium antagonist activity, and vasodilatory actions [11, 16-19]. However, further investigations are necessary to identify these compounds in *B. petersianum* and to confirm their calcium antagonistic effect.

In summary, the present study demontrates that the hydro-ethanolic extract of *Biophytum petersianum* has calcium antagonistic activity on rat isolated aorta rings. The vasorelaxant effect of *B. petersianum* may be involved in its hypotensive effect. Further experiments are necessary to elucidate the active principles and their possible mechanism of action.

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