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COMPARATIVE STUDY OF THE VASODILATORY EFFECT OF THE HEXANIC, DICHLOROMETHANIC, ETHYL ACETATE, BUTANOLIC AND AQUEOUS EXTRACTS OF *LUDWIGIA OCTOVALVIS* (JACQ.) P.H.RAVEN (ONAGRACEAE)

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ABSTRACT

This study aimed to compare vasodilation activity of different extracts of *Ludwigia octovalvis* prepared using hexane, dichloromethane, ethyl acetate, n-butanol, and water. *In vitro* tests were performed using isolated aorta of guinea pig pre contracted with norepinephrine. The results of these tests indicate that only the ethyl acetate, n-butanol and aqueous extracts possess vasodilatory activity, with respective EC_{50} values of 0.45, 0.32 and 0.81 mg/ml. N-butanolic extract is the most active. According to these results, the vasodilator molecules of *Ludwigia octovalvis* are spread in the ethyl acetate, n-butanol and aqueous extracts, but the most active ones would be in the n-butanolic extract.

KEYWORDS: *Ludwigia octovalvis*, vasodilation, isolated aorta, guinea pig.

INTRODUCTION

Hypertension is an important public health problem. It is a risk factor for the development of cardiovascular and kidney diseases.[1,2] Cardiac complications are responsible for high rates of mortality, causing approximately 17 million deaths per year.[3]

Since blood pressure depends on cardiac output and peripheral blood vessels resistance, many drugs are used to manage blood pressure levels in hypertensive patients, such as diuretics and beta-adrenergic blockers which reduce the cardiac output, and vasodilators which reduce peripheral resistance such as renin inhibitor, angiotensin converting enzymes (ACE) inhibitors, angiotensin receptors blockers, calcium channel blockers, α-adrenergic blockers.[4,5]

However, the high cost of antihypertensive pharmaceutical drugs, their non-availability and inaccessibility, especially in rural areas, push patients to seek alternative approaches, such as herbal remedies, for their treatment of hypertension and other diseases. For example, in Madagascar, people use decoction prepared with the aerial part of *Catharantus roseus* (Apocynaceae),[6] *Diodia* sp. (Rubiaceae), *Lantana camara* Linn. (Verbenaceae), *Cajanus indicus* (Fabaceae),[7] to treat high blood pressure. According to the results of the ethnobotany survey that we have conducted in the southern part of the highland of Madagascar, *Ludwigia octovalvis* is also used to treat this disease. The aim of this work is to justify that traditional use of this plant and identify later a molecule responsible of its therapeutic activity.

MATERIALS AND METHODS

Plant material

Plant material used in this work was collected from Ambositra, in the "Haute Matsiatra" Region in the southern highland of Madagascar. This species was identified at the Department of Botany, Botanical and Zoological Park of Tsimbazaza (Antananarivo), and a voucher specimen was deposited at the botany department of this institution.

Extracts' preparation

Plant was dried under shade, at room temperature, in an aerated room, for 6 weeks. Dried materiel was ground using an electric grinder BROOK CROMPTON Série 2000®. The powder was afterwards macerated in ethanol-water (60:40 v/v) for 3 days at room

temperature. The macerate was then filtrated on Whatman paper n°2. Filtrate was centrifuged for 10 min at 4000rpm in a centrifuge (RHEOTOR ADB Type 10/57®). The clear supernatant was collected and evaporated to dryness in a rotative evaporator under vacuum Büchi® at 60°C.

Dried hydro alcoholic extract was dissolved in water. Liquid-liquid partitioning extraction method was performed on aqueous phase starting with less polar solvents, hexane, dichloromethane and ethyl acetate, followed by solvents with increasing polarity and n-butanol.[8] A phytochemical screening was performed to detect the principal secondary metabolites in each extract and the remaining aqueous extract.[9]

Evaluation of vasodilation activity of the different extracts

Experimental animals

Guinea pig of either sex, of age 8–12-month, weighing 300-350 g were used for the experiments. They were housed at the animal house, Pharmacology Dept., Faculty of Sciences, University of Antananarivo, Madagascar. All tests were performed in accordance with the guidelines for the care of laboratory animals of the Faculty of Sciences Animal Ethics Committee, and the study was approved under (Reg. no.32-2020). The cycle of light and darkness in their house was (12:12hr) and the temperature was 22±2°C. Animals were fed with standard laboratory diet and had water *ad libitum* and were fastened 12 hours prior to the test.

Evaluation of the vasodilatory effect of the different extracts

Aorta Preparation

Animals were anesthetised and then killed by cervical dislocation. A thoracotomy was then performed. After pulmonary and cardiac resection, thoracic aortic was dissected from the inferior diaphragm to the aortic base.

Isolated aorta was cleansed from the attached connective tissue and was placed in a petri dish containing Kreb's Henseleit solution at room temperature and aerated with carbogen. The aorta was cut into rings of about 3mm length. The aortic ring was then inserted into a 3 ml organ bath containing Kreb's Henseleit solution at 37°C and aerated with carbogen.[10]

Aortic ring was mounted on an isometric transducer Statham Gould © under a tension of 2 g, connected to a digital recorder amplifier SIGMA Monitor. The changes in aortic dilatation tone were recorded in grams in the computer.

The isolated organ was equilibrated for 90 minutes in organ bath, which was renewed every 15 minutes. Once stabilized, 10^{-3} M norepinephrine was injected in the bath to check its viability and to sensitise it. To assure that the endothelium is intact, 10^{-3}M of acetylcholine was injected in the bath.[11] Then it was rinsed, and left to stabilize for 30 minutes, during which the bath was renewed twice.

Vasodilation Activity

After the equilibrium period, the aortic ring was contracted with 10^{-3} M norepinephrine until it reached maximal contraction, considered as 100%. At this moment, the extract was injected in the bath in a cumulative manner until maximal relaxation. This test was repeated six times, and the result of changes in smooth muscle tone was expressed in percentage of aortic tone.[12] Percentage of aortic contractile tone is the value of aortic tone after injection of extract divided by the aortic tone after norepinephrine administration, considered as 100%.

Statistical Analysis

The results were analysed using Microsoft Excel 2019 and GraphPad Prism software (version 7.0). Data obtained in the different spots were compared. The statistical significance of differences was assessed using one-way ANOVA followed by Student test. Differences with value < 0.05 were significant. Data were presented as mean ± sem.

RESULTS

Injected in the bath containing the isolated aorta pre contracted with norepinephrine, the hexane and dichloromethane extracts did not relax the organ, while the ethyl acetate, n-butanol and aqueous extracts provoke 100% relaxation of the organ pre contracted with norepinephrine. At the concentration of 0.125 mg/ml, aqueous extract does not relax the organ, while ethyl acetate and n-butanolic extract provoke respectively 11 ± 0.4% and 18.14 ± 0.7%. The organ is completely relaxed in the presence of ethyl acetate, n-butanolic and aqueous extracts at the concentrations of 0.875, 0.625 and 1.25 mg/ml ($p<0.05$) (Figure 1). These results show that ethyl acetate, n-butanolic and aqueous extracts possess vasodilatory activity, and n-butanolic is the most active.

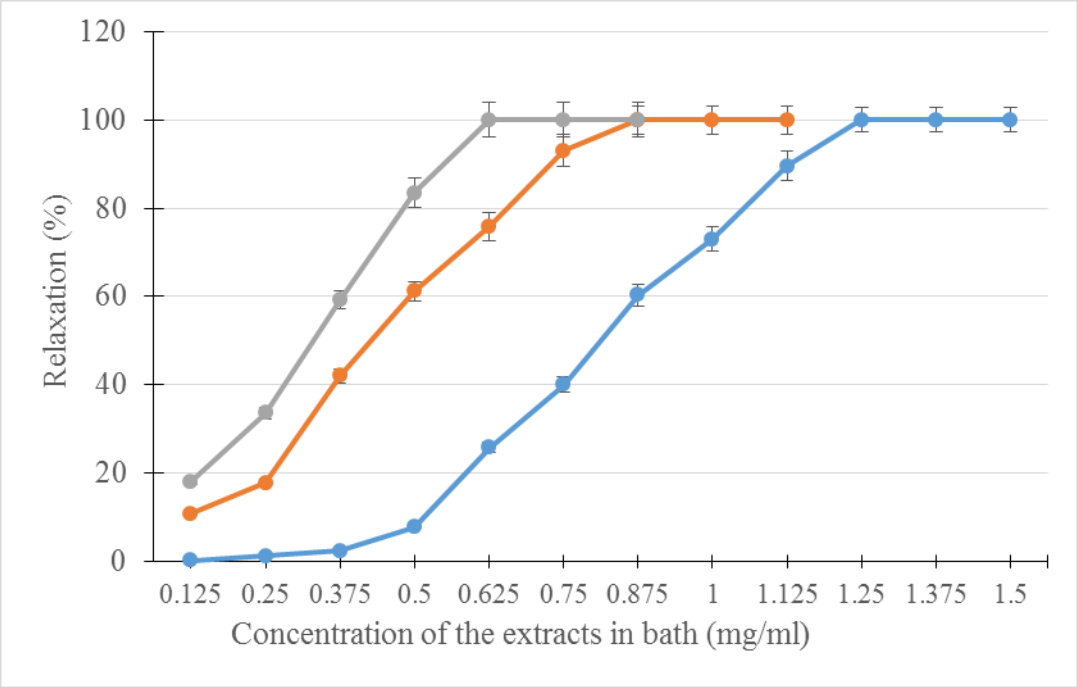

Figure 1: Relaxation of isolated aorta of guinea pig contracted with norepinephrine at the concentration of 10^{-3}M in the bath, in the presence of n- butanolic (▬), ethyl acetate (▬) and aqueous extract (▬) injected in the bath in a cumulative manner ($\bar{x} \pm \bar{\sigma}$; n= 6 ; p < 0.05).

Calculation of EC_{50} using linear regression method indicates that the EC_{50} of n-butanolic extract is the lowest, followed by that of ethyl acetate and then aqueous extract. They are respectively equal to 0.32, 0.45 and 0.81 mg/ml ($p<0.05$) (Table 1). These results demonstrate once again, that the n-butanolic extract contains more active compounds than the other two.

Table 1: EC_{50} values of ethyl acetate, n-butanolic and aqueous extracts of *Ludwigia octovalvis.*

Extracts	Ethyl acetate	n-butanolic	Aqueous
EC_{50} (mg/ml)	0.45	0.32	0.81

Principal secondary metabolites in the different extracts

Phytochemical analysis performed on the different extracts shows that the three active extracts contain phenolic compounds and flavonoids; on top of those n-butanol and aqueous extracts contain tannins and saponins, while ethyl acetate contains terpenoids. For the three common metabolites, n-butanolic extract contains more flavonoids (Table 2).

Table 2: Principal secondary metabolites in the different extracts.

Extracts	Hexane	Dichloromethane	Ethyle acetate	n-Butanol	Aqueous
Tannins	-	-	-	++	+++
Saponins	-	-	-	++	+++
Terpenoids	+++	++	+	-	-
Steroids	+++	++	-	-	-
Phenolc compounds	-	-	++	+++	+
Flavonoids	-	-	++	+++	+
Polysaccharides	-	-	-	++	+++
Anthocyanins	-	-	-	-	+

DISCUSSION

This work aimed to evaluate and compare the vasodilatory activity of three extracts of *Ludwigia octovalvis* with the view to isolate vasodilatory compounds from this plant.

The results that we obtained show that all three extracts possess vasodilatory effect. But according to their EC_{50}, n-butanolic extract is the most active, because it has the least EC_{50}. It probably has a high amount of vasodilatory molecules or it contains more powerful vasodilatory molecules. Considering the results of phytochemical

screening performed on the three effective extract, we advance a hypothesis that the active molecules might be flavonoids or phenolic compounds. In 2005, Yan and Yang isolated 13 molecules from *Ludwigia octovalvis:* beta-sitosterol, oleanolique acid, 2alpha-hydroxy ursolic acid, tormentic acid, daucosterol, maltol, luteolin, quercetin, apigenin, brevifolinecarboxylate of méthyl, gallic and ellagic acid, and 3, 4, 8, 9, 10-pentahydroxydibenzo [b, d]pyran-6-one.[13] Or luteolin and quercetin are flavonoids with vasodilation activity.[14,15] From these literature data, one can advance an hypothesis that it is most probable that the vasodilatory activity of this plant could be due to flavonoids. Further investigation to isolate active compounds from the butanolic extract would enable us to determine a vasodilator molecule from this plant and elucidate its mechanism of action.

CONCLUSION

The results obtained from this work show that the ethyl acetate, n-butanolic and aqueous extracts of *Ludwigia octovalvis* contain active vasodilatory compounds. Most of them may be in the n-butanolic extract, or the most powerful vasodilator molecules are in this extract. Further investigation of this extract to isolate one active vasodilator molecule, is envisaged.

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