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(RESEARCH ARTICLE)

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Phytochemical analysis and cardiotonic activity of hydroalcoholic extract of *Petchia erythrocarpa* (Vatke) Leeuwenb. (APOCYNACEAE) in isolated frog heart

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Abstract

The Apocynaceae family is known for its richness in cardiotonic glycosides. This study aimed to investigate the cardiotonic activity of the hydroalcoholic extract from the leafy stems of *Petchia erythrocarpa* on experimentally induced heart failure using isolated frog heart. Cardiac contraction force, heart rate, diastolic filling time, and cardiac output were evaluated.

The extract was perfused in the isolated heart at concentrations ranging from 0.15 to 0.9 mg/mL. Within this range of concentration, the extract significantly increased cardiac contraction force by 18 to 73 % ($p < 0.05$), the cardiac output from 0.51 ± 0.07 to 0.91 ± 0.05 mL/s ($p < 0.05$), the diastolic duration from 0.13 ± 0.003 to 0.24 ± 0.009 s ($p<0.05$), while it decreases the heart rate from 44.5 ± 1.1 to 31.3 ± 0.6 beats/min ($p < 0.05$). These results indicate that the extract possesses positive inotropic and lusotropic effects and a negative chronotropic effect, confirming its potential as a cardiotonic agent.

Keywords: Cardiotonic; Frog; Isolated heart; *Petchia erythrocarpa*

1. Introduction

Cardiotonic agents are drugs that enhance the contractile force of the heart, thereby improving cardiac output. They can be classified according to their mechanism of action. Cardiac glycosides, such as digoxin and digitoxin, act by inhibiting the Na^+/K^+-ATPase pump, which increases intracellular calcium and strengthens myocardial contraction. β_1-adrenergic agonists, including dobutamine and dopamine, stimulate β_1-receptors to elevate cAMP levels and enhance calcium influx during depolarization. Phosphodiesterase III inhibitors, such as milrinone, prevent cAMP degradation, producing a positive inotropic effect [1]. More recently, calcium sensitizers, like levosimendan, have been developed to increase myofilament sensitivity to calcium without raising intracellular calcium concentrations [2]. Together, these agents are used in the management of heart failure and certain cardiac rhythm disorders, offering different therapeutic strategies to improve cardiac performance.

Medicinal plants have long been recognized as a rich source of cardiotonic agents, particularly cardiac glycosides. Several species, including *Digitalis purpurea*, *Strophanthus gratus*, and *Petchia erythrocarpa* (Vatke) Leeuwenberg (Apocynaceae), produce naturally occurring glycosides that enhance myocardial contractility by increasing intracellular calcium via inhibition of the Na^+/K^+-ATPase pump. These plant-derived compounds remain clinically relevant for the

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management of heart failure and certain arrhythmias, offering therapeutic benefits that complement synthetic inotropes. The study of traditional medicinal plants continues to provide novel molecules with positive inotropic and lusitropic effects, highlighting the potential of ethnopharmacology in cardiovascular drug discovery [3].

Petchia erythrocarpa is an endemic species to Madagascar and the Comoros. It is locally known as "*Tandrokosy*" in the southeastern region of Madagascar. It is a shrub that can reach up to 10 m in height with white latex [4]. In Madagascar, its bark decoction is used for the treatment of hepatitis, malaria, stomach disorders, and diarrhoea. Leaf decoction is traditionally used to manage hypertension and gastric disorders [5].

Since *P. erythrocarpa* belongs to the Apocynaceae family, which is known to be rich in cardiac glycosides, we hypothesized that it may possess cardiotonic properties. Despite its various traditional uses, no pharmacological studies on its cardiac activity have yet been conducted on this species. To test our hypothesis, the effect of its leaves hydro alcoholic extract on cardiac contractile force, heart rate, diastolic filling time, and cardiac output were evaluated on isolated frog heart.

2. Materials and methods

2.1. Plant Material

Wild plants of *Petchia erythrocarpa* were collected from the southeastern part of Madagascar (district of Vangaindrano). A voucher specimen was identified at the herbarium of the Botany Section at the Botanical and Zoological Park of Tsimbazaza, Antananarivo, Madagascar.

2.2. Extract preparation and phytoscreening

Leaves of *P. erythrocarpa* were dried under shade, at room temperature, in an aerated room. The dried leaves were ground, and the powder was macerated in a mixture of ethanol: water (60:40, v/v), at room temperature for 4 days. The macerate was filtered on hydrophile cottonwool and on Whatman filter paper n° 2. The filtrate was centrifuged at 3000 rpm, for 10 minutes. The supernatant was collected and evaporated to dryness under pressure, using a rotating evaporator at 80 °C.

The major chemical groups in the hydro alcoholic extract were determined using a technic based on colouring and precipitation reactions as well as by ultra-violet light examinations, according to the methods described in the literature [6].

2.3. Experimental Animals

Frogs (*Hoplobatrachus tigerinus*) weighing between 80 and 100 g were used. They were bought at local market and kept in a humid area in the animal house of Laboratory of General Pharmacology, Pharmacokinetic and Cosmetology of the Sciences Faculty, University of Antananarivo, for 3 weeks. The frogs were given free access to food which consisted of small locusts. Animal procedures were conducted with strict adherence to the University of Antananarivo, Sciences Faculty Animal Ethic Committee's Guide for the Care and Use of Laboratory Animals.

2.4. Preparation of the isolated heart

The isolation of the frogs' hearts was done according to the standard procedure. Briefly, the frog was decerebrated and demodulated, then fixed supine on a board. The heart was exposed by removing the skin, the thoracic muscle, and the pericardium. A small incision was made in the aorta to introduce the cannula filled with ringer solution according to Langendorff's method [7]. A ligature was made around the cannula while lifting the tip of the ventricle and isolated heart, while getting rid of the surrounding tissue.

2.5. Evaluation of the extract effect on isolated heart activity

The isolated heart was mounted on Langendorff's apparatus, which permits delivery of Lock-Ringer solution (g / L: $CaCl_2$: 0.24; NaCl: 9; KCl: 0.42; $NaHCO_3$: 0.5; dextrose: 1.0) [8] at a constant rate of 3ml/min at room temperature. The tip of the ventricle with the help of a fine thread was tied to the free limb of Sterling's heart lever which was fixed to a stand, the tension was adjusted at 1 g by altering the height of the lever.

The isolated heart was perfused for 5 min with Ringer's solution until a regular cardiogram was recorded on a kymograph paper wrapped around a rotating drum driven by a motor at the rotation speed of 1.25 mm/s. Amplitude of

heart contraction was noted, the heart rate was determined, and the duration of diastole was recorded. Heart failure was experimentally induced by leaving the heart beating until maximal reduction of the contraction amplitude. At that point, extract was directly injected in the cannula at increasing concentration. The hydro alcoholic extract of the leaves of *Petchia erythrocarpa* was prepared by dissolving it in calcium free Ringer solution, and injected in the cannula at concentrations from 0.15 to 0.9 mg/ml. After each injection, the heartbeat was recorded and the parameters of the cardiac activity were evaluated [9]

2.6. Evaluation of the extract effect on isolated heart output

The heart was mounted to the apparatus according to a modified Langendorff's method, by inserting the cannula in the sinus vein. The extract at different concentrations was injected into the cannula. Aortic outflow was collected, and cardiac output was measured volumetrically to determine cardiac output (mL/min) [9,10].

2.7. Statistical analysis

The results are expressed as mean ± SEM and were analysed using Microsoft Office LTSC Excel 2021. One-way ANOVA and post ANOVA t-test were performed to compare the groups, p value < 0.05 was considered significant.

3. Results

3.1. Phytochemical results

Extraction with ethanol-water (60:40), 200 g of plant material, at room temperature for 3 days gives 26 g of hydro alcoholic extract corresponding to 13 % extraction yield. Phytochemical screening revealed a high content of reducing sugars, steroids, terpenes, and cardiac glycosides; a moderate content of alkaloids; and a low content of anthocyanins and leucoanthocyanins (Table 1).

Table 1 Major chemical group in hydro alcoholic extract of *P. erythrocarpa*

Chemical group	Relative Content
Reducing sugars	+ + +
Steroids and triterpenes	+ + +
Cardiac glycosides	+ + +
Polysaccharides	+ +
Alkaloids	+ +
Anthocyanins	+
Leucoanthocyanins	+

3.2. Effect of the extract on cardiac contractile force

Injected through the cannula connected to the aorta, the extract increased the amplitude of cardiac contraction, in effect - concentration dependent. In the exhausted heart, contraction amplitude was 14 ± 0.04 % compared to the normal contraction. In the presence of the extract at concentrations ranging from 0.15 to 0.9 mg/mL, a significant increase in height of contraction from 18 ± 0.06% to 73 ± 0.05 % is observed with an EC_{50} of 0.58 mg/mL ($p < 0.05$) (Figure 1). These results demonstrate that the extract enhances cardiac contractile force, indicating a positive inotropic effect.

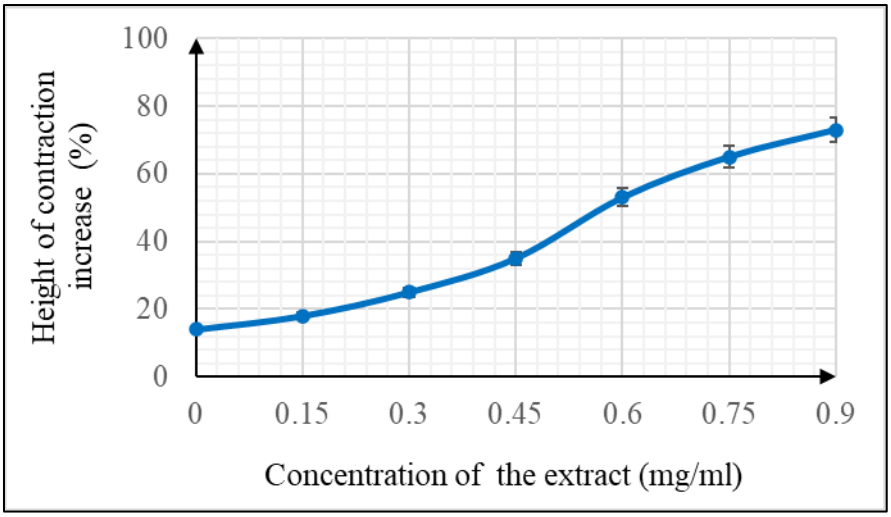

Figure 1 Variation in the height of contraction of the experimentally induced failing heart in the presence of the extract injected into the aorta, using Langendorff method ($\bar{x} \pm \bar{\sigma}$; n = 6; $p < 0.05$)

3.3. Effect of the extract on heart rate

Injected through the cannula connected to the aorta, the extract decreased heart rate in a concentration-dependent manner. In the absence of the extract, the heart rate of the failing heart was 44.5 ± 1.1 beats/min. It reduces to 40.23 ± 0.7, 38.33 ± 0.6, 36 ± 0.5, 35.35 ± 0.7, 33.23 ± 0.5, and 31.33 ± 0.6 beats/min in the presence of the extract at concentrations ranging from 0.15, 0.3, 0.45, 0.6 and 0.9 mg/mL ($p < 0.05$) (Figure 2). These results indicate that the extract exerts a negative chronotropic effect.

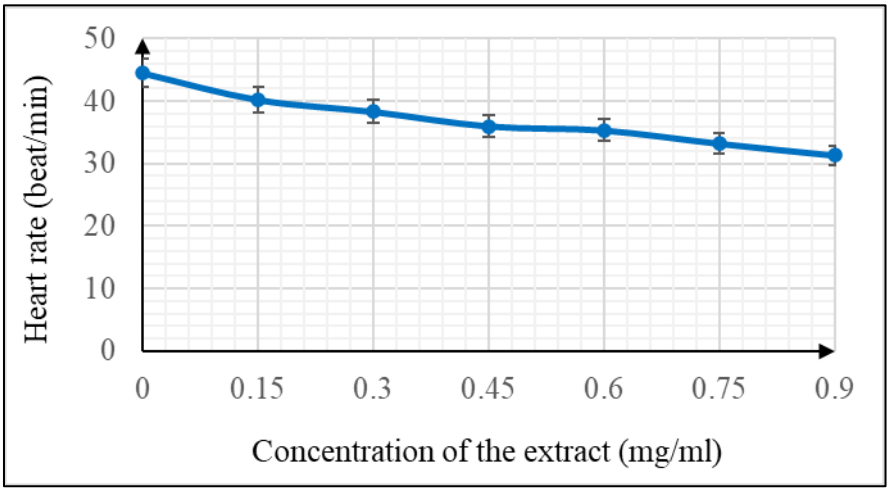

Figure 2 Variation in the heart rate of the experimentally induced failing heart in the presence of the extract injected into the aorta at different concentration according to Langendorff method ($\bar{x} \pm \bar{\sigma}$; n = 6; $p < 0.05$).

3.4. Effect of the extract on filling time

Injected in the aorta according to the retro perfusion of Langendorff, the extract increases the duration of diastole in a concentration-dependent manner. The diastolic duration of the experimentally induced failing heart is 0.13 ± 0.003 seconds, versus 0.14 ± 0.004, 0.16 ± 0.004, 0.17 ± 0.003, 0.20 ± 0.005, 0.21 ± 0.004, and 0.24 ± 0.009 seconds in the presence of the extract at concentrations ranging from 0.15 to 0.9 mg/mL (Figure 3).

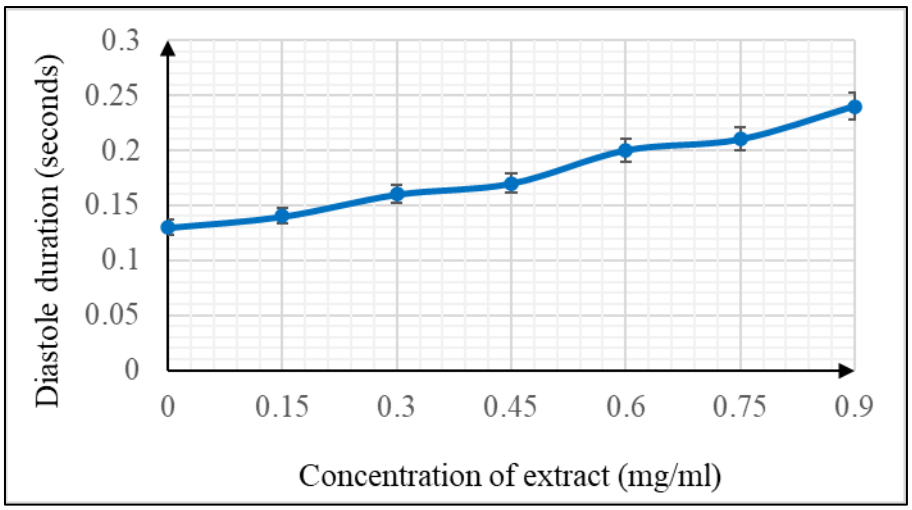

Figure 3 Variation in the duration of diastole of the experimentally induced failing heart in the presence of the extract injected into the aorta according to Langendorff method ($\bar{x} \pm \bar{\sigma}$; n = 6; $p < 0.05$)

3.5. Effect of the extract on cardiac output

When injected through the cannula connected to the sinus, the extract increases cardiac output in a concentration-dependent manner. The output of the experimentally induced failing heart is 0.51 ± 0.07 mL/sec, versus 0.59 ± 0.08, 0.65 ± 0.08, 0.71 ± 0.06, 0.75 ± 0.09, 0.88 ± 0.07, and 0.91 ± 0.05 mL/sec in the presence of the extract at concentrations ranging from 0.15 to 0.9 mg/mL ($p < 0.05$) (Figure 4).

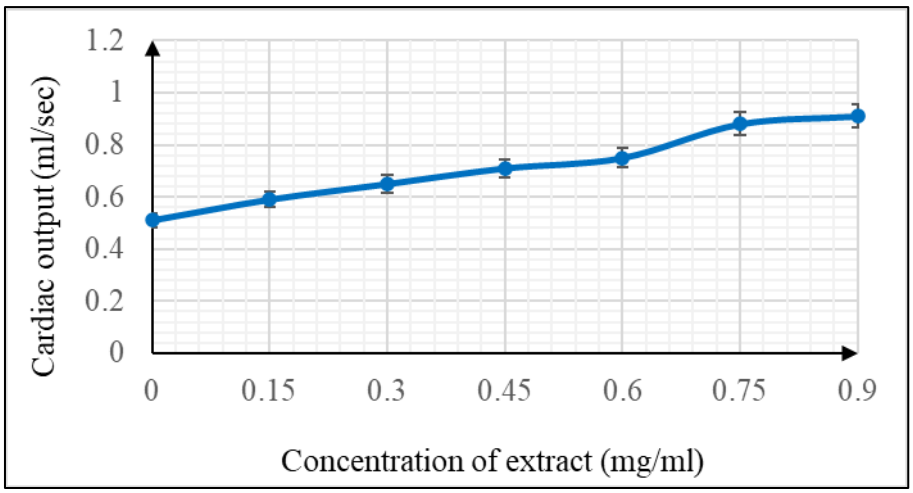

Figure 4 Variation in the cardiac output of the experimentally induced failing heart in the presence of the extract injected into the venous sinus ($\bar{x} \pm \bar{\sigma}$; n = 6; $p < 0.05$)

4. Discussion

The present study was undertaken to assess the cardiotonic activity of the hydroalcoholic extract of *P. erythrocarpa* leaves. It was carried out in isolated heart frog, using Langendorff method. Contraction force, heart rate, filling time and cardiac output were evaluated. Our results show that the extract possesses significant cardiotonic activity in an experimentally induced failing heart model. Extraction with ethanol–water (60:40, v/v) yielded 13 %, indicating an efficient recovery of polar and moderately polar constituents. Such a yield is consistent with the solubilization of glycosides, alkaloids, sugars, and certain terpenoid compounds, which were confirmed by phytochemical screening.

The abundance of cardiac glycosides is particularly noteworthy, as this class of compounds is well recognized in Apocynaceae family.

In the experimental failing heart, *P. erythrocarpa* hydro alcoholic extract improves cardiac contractile force in a concentration-dependent manner. This marked enhancement confirms a positive inotropic effect. The concentration dependency of the effect indicates a pharmacologically relevant action rather than a nonspecific stimulant effect. The increase in intracellular calcium availability likely underlies this response [11]. Since phytochemical screening revealed the presence of cardiotonic glycosides in the extract, the observed increase in contractility may be attributed to inhibition of the Na^+/K^+-ATPase pump at the cell membrane, as well as reduced reuptake of calcium ions into the sarcoplasmic reticulum [12]. Inhibition of the Na^+/K^+-ATPase pump leads to increased intracellular sodium concentration. This alteration reduces the activity of the Na^+/Ca^{2+} exchanger, thereby promoting intracellular calcium accumulation and enhancing myocardial contractility. In addition, Randrianavony (1993) demonstrated that an elevation in intracellular calcium concentration also promotes the release of calcium from the sarcoplasmic reticulum, thereby further increasing cardiac contractile force [13]. Thus, the improvement in cardiac contractility induced by the extract is likely a consequence of increased intracellular calcium levels as result of Na^+/K^+-ATPase inhibition by the cardiotonic glycoside present in the extract.

In addition to its positive inotropic activity, the extract produced a significant, concentration-dependent reduction in heart rate. The observed negative chronotropic effect suggests modulation of cardiac electrophysiological properties. Cardiac glycosides are known to prolong the refractory period and slow atrioventricular conduction through Na^+/K^+-ATPase inhibition, resulting in bradycardia [14]. Moreover, these findings are like those obtained with tanghinin, a cardiotonic glycoside studied by Randimbivololona and Rakotomanga (1990) [15]. The simultaneous presence of positive inotropy and negative chronotropy is characteristic of clinical effect of glycoside cardiotonic agents, as it improves cardiac efficiency by enhancing stroke volume [16, 17].

The extract also significantly prolonged diastolic duration, indicating a positive lusotropic effect. Prolongation of diastole increases ventricular filling time, thereby improving preload and optimizing stroke volume according to the Frank–Starling mechanism. This effect is particularly beneficial in heart failure, where impaired relaxation and reduced filling contribute to decreased cardiac output. The increased diastolic time observed in this study may be secondary to heart rate reduction, but it may also reflect improved myocardial relaxation dynamics associated with better calcium handling [18].

Consistent with the combined inotropic and lusitropic effects, cardiac output increased significantly in the presence of the extract. The concentration-dependent rise of the cardiac output demonstrates a substantial functional improvement in the failing heart model. Since cardiac output is determined by heart rate and stroke volume, the increase observed despite bradycardia underscores the dominant contribution of enhanced contractility and improved ventricular filling [19]. This profile resembles that of established cardiotonic agents, such as digoxin, which improve hemodynamic performance while limiting excessive tachycardia [20].

These findings demonstrate that the hydroalcoholic extract of *P. erythrocarpa* exerts positive inotropic, negative chronotropic, positive lusitropic, and overall cardiotonic effects in an isolated failing heart model. The pharmacological activity observed is most plausibly attributed to the presence of cardiac glycosides, although synergistic contributions from alkaloids, triterpenes, and other constituents cannot be excluded.

Nevertheless, some limitations should be acknowledged. The study was conducted in an isolated frog heart preparation, which does not account for systemic neurohumoral influences. Furthermore, the extract is a complex mixture, and the specific active compounds responsible for the observed effects remain to be isolated and characterized. Future studies should focus on bioassay-guided fractionation, molecular characterization of the active principles, evaluation of toxicity profiles, and *in vivo* validation in heart failure models.

5. Conclusion

This study shows that the hydroalcoholic extract of *P. erythrocarpa* exhibits marked cardiotonic activity, evidenced by increased myocardial contractility, reduced heart rate, prolonged diastolic duration, and enhanced cardiac output. Since this species belongs to Apocynaceae family and the high content of cardiac glycosides identified in the phytochemical screening, the cardiotonic activity of the extract is most likely attributable to these glycosidic compounds.

Compliance with ethical standards

Acknowledgments

All authors have contributed according to their speciality to the work.

Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Statement of ethical approval

Procedures involving animals and their care were conducted in conformity with the institutional guidelines of the Sciences Faculty of Antananarivo Animal Ethics Committee and conforms to the provisions of the Declaration of Helsinki in 1995, under registration n° CEA/FS-UA/Registre n°12/2025.

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