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# Evaluation of the effect of the hydroalcoholic extract of *Tanghinia venenifera* Poir. (Apocynaceae) leaves on cardiac activity

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## Abstract

This work aimed to investigate the effect of the hydro alcoholic extract of *Tanghinia venenifera* (Apocynaceae) leaves on experimentally induced failing isolated frog heart. The extract was perfused according to Langendorff retro perfusion method to assess its effects on contractile force, heart rate, and filling time. It was perfused through the sinus venosus to evaluate its effect on cardiac output. At concentration of 0.75 mg/mL, the extract increases the cardiac output from 24.6 ± 0.34 mL/minute to 54 ± 0.69 mL/minute, it also increases the filling time from 0.08 ± 0.002 seconds to 0.22 ± 0.005 seconds. It induces an increase of 0 to 67.12 ± 0.69 % of the contractile force compared to the experimentally induced failed heart ($p < 0.05$). It decreases the heart rate from 43.5 ± 0.173 beats per minute in the absence of the extract to 27.33 ± 0.305 beats per minute in its presence at 0.75 mg/ml ($p < 0.05$). These results demonstrate that the hydro alcoholic extract of *Tanghinia venenifera* improves cardiac output and exerts a cardiotonic effect through positive inotropic, positive lusitropic, and negative chronotropic actions. These effects are typical of cardiac glycosides activities.

**Keywords:** Cardiotonic; Frog; Positive inotropic; *Tanghinia venenifera*

## 1. Introduction

*Tanghinia venenifera*, an endemic species of Madagascar belonging to the Apocynaceae family, has historically been recognized for its marked toxicity. It was first described in 1806 by Aubert du Petit-Thouars and later referred to as *Cerbera tanghin* by William Jackson Hooker in 1837. During the reign of Queen Ranavalona, I (1828–1860), the plant was used in traditional judicial ordeals in Madagascar [1, 2].

The Apocynaceae family is well known for its richness in cardiac glycosides, a class of bioactive compounds with potent effects on myocardial function. These compounds are characterized by a narrow therapeutic index. They are beneficial at controlled doses but can become highly toxic at high concentrations. This dose-dependent toxicity explains why plants rich in cardiac glycosides have historically been both medicinal and poisonous. *Tanghinia venenifera,* endemic to Madagascar, is an example. During the reign of Queen Ranavalona, I (1828–1860), its seeds were used in traditional judicial ordeals ("tangena ordeal"), in which accused individuals were required to ingest a preparation of the plant. Survival was interpreted as proof of innocence, whereas death-likely resulting from acute cardiotoxicity-was taken as evidence of guilt. Several therapeutically important cardiotonic agents originate from this botanical family, including *Nerium oleander* (oleandrin), and *Strophanthus gratus* (ouabain) [3]. However, phytochemical and pharmacological

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investigations have focused on a limited number of genera, leaving numerous species insufficiently studied. Given the strong correlation between phylogeny and secondary metabolite distribution, chemotaxonomic approaches provide a rational framework for identifying novel cardiotonic candidates within this family.

Heart failure, defined as the inability of the heart to maintain adequate systemic perfusion, remains a leading cause of morbidity and mortality worldwide [4]. Although contemporary management strategies emphasize neurohormonal modulation and device therapy, impaired myocardial contractility remains a central pathophysiological determinant, particularly in advanced stages. Cardiotonics exert positive inotropic effects by enhancing myocardial contractility, thereby improving cardiac output, and remain important in the management of heart failure. Cardiac glycosides remain among the most historically significant plant-derived cardiotonic agents. Their mechanism of action is primarily mediated through inhibition of $Na^+/K^+$-ATPase, leading to intracellular $Na^+$ accumulation, reduced activity of the $Na^+/Ca^{2+}$ exchanger, and increased cytosolic $Ca^{2+}$ availability during systole. The resulting enhancement of sarcoplasmic reticulum $Ca^{2+}$ loading enhances myocardial contractility (positive inotropy), while electrophysiological effects contribute to negative chronotropic and dromotropic actions. Despite a narrow therapeutic index, these compounds continue to inform both clinical practice and mechanistic cardiovascular pharmacology [5, 6].

Given the well-established occurrence of cardiac glycosides within the Apocynaceae family and considering chemotaxonomic principles that relate botanical classification to phytochemical composition, we hypothesized that *Tanghinia venenifera* may possess cardiotonic properties. The present study was designed to investigate the cardiac effects of the hydroalcoholic extract of *T. venenifera* leaves in an experimentally induced failing isolated frog heart model. By quantitatively assessing contractile force, heart rate, filling time, and cardiac output, we aimed to determine whether the extract exhibits a functional signature consistent with cardiac glycoside-like activity.

## 2. Materials and methods

### 2.1. Preparation of the Extract

The leaves of *Tanghinia venenifera* used in this study were collected in July 2022 from the rural commune of Ambila Lemaitso, Atsinanana region, Tamatave province, Madagascar. They were shade-dried in a well-ventilated area at ambient temperature. After drying, they were ground using a BROOK CROMPTON © Series 2000 hammer mill at the Laboratory of General Pharmacology, Pharmacokinetic and Cosmetology (LPGPC), Faculty of Sciences, University of Antananarivo, Antananarivo, Madagascar.

Two hundred grams of the powder were macerated in 1 liter of a hydroalcoholic solvent (ethanol-water, 60:40, v/v), at room temperature, for three days and stirred daily for 10 minutes. After three days, the macerate was filtered through cotton wool, followed by filtration using Whatman filter n°2. The filtrate was then centrifuged at 3000 rpm for 15 minutes. The supernatant was collected and evaporated to dryness under reduced pressure at 80°C using a rotary evaporator (Evapotec®).

### 2.2. Phytochemical screening

A phytochemical screening was performed to identify the major chemical families present in the extract, using the method described by Fong and colleagues (1977). This test is based on colorimetric or precipitation reactions. Specific reagents were employed for the detection of each chemical family [7].

### 2.3. Experimental Animals

Frogs of the species *Hoplobatrachus tigerinus*, weighing between 80 and 150 g, were used in this study. They were purchased from the Analakely market (Antananarivo, Madagascar). Prior to experimentation, the animals were acclimatized for five days in the animal house of the Laboratory of General Pharmacology, Pharmacokinetic and Cosmetology (LPGPC), Faculty of Sciences, University of Antananarivo, Antananarivo, under a 12-hour light/12-hour dark cycle, at a temperature around 20 °C. During this period, the frogs were fed with small crickets and had free access to water.

### 2.4. Preparation of the isolated heart

To isolate the heart, the frog was decerebrated and despinalized. The animal was placed in a dorsal recumbent position on a dissection board. A thoracotomy was performed, and the pericardium was carefully removed. Once the heart was exposed, the tip of the ventricle was ligated using non-stretchable cotton thread. Depending on the experimental test,

either the sinus venosus or the thoracic aorta was catheterized. The heart was then isolated by carefully removing all surrounding tissues.

The isolated heart was perfused via the catheterized vessel with amphibian Ringer solution (Table 1) and mounted on the experimental apparatus [8]. The catheter was connected to a reservoir containing Ringer solution, while the ventricular apex was attached to a writing stylus counterbalanced with a 1 g weight, enabling the mechanical recording of cardiac contractions on graph paper mounted around a rotating drum.

**Table 1** Composition of 1 litre Ringer solution (g/l)

| NaCl (g) | KCl (g) | $CaCl_2$ (g) | Dextrose (g) | $NaHCO_3$ (g) | $H_2O$ (g) |
|---|---|---|---|---|---|
| 6 | 0.14 | 0.12 | 1 | 0.2 | 1000 |

### 2.5. Evaluation of cardiotonic activity

To investigate the effect of *Tanghinia venenifera* extract on cardiac function, the Langendorff method was employed using the isolated frog heart rendered experimentally failing by allowing it to beat spontaneously for an extended period [9]. The extract's effects on contractile force, heart rate, and filling time were assessed by injecting it into the aorta at concentrations ranging from 0.125 to 0.75 mg/mL. Meanwhile, perfusion through the sinus venosus was performed to evaluate its effect on cardiac output, and the contractions were recorded on a rotating drum.

### 2.6. Data Presentation and Analysis

The results are expressed as mean ± standard error of the mean ($\bar{x} \pm \bar{\sigma}$). Comparisons between means were performed using Student's t-test with Microsoft Office LTSC Excel 2021. Differences between two means were considered statistically significant for $p < 0.05$.

## 3. Results

### 3.1. Extraction Yield

From 200 g of plant material, 23 g of dry extract was obtained, corresponding to an extraction yield of 11.5 %. The extract was brown and bitter.

### 3.2. Phytochemical screening results

Phytochemical screening of the hydroalcoholic extract of *Tanghinia venenifera* revealed the presence of reducing sugars, glycosidic heterosides, steroids, and triterpenes in high concentrations. Anthocyanins and leucoanthocyanins were present in moderate amounts (Table 2).

**Table 2** The major secondary metabolites in hydro alcoholic extract of *T. venenifera* leaves

| Familles chimiques | Teneur |
|---|---|
| Reducing sugar | + + + |
| Cardiac glycosides | + + + |
| Steroids et triterpenes | + + + |
| Anthocyanins | + + |
| Leucoanthocyanins | + + |

### 3.3. Effect of the extract on cardiac contractile force

When injected into the thoracic aorta of the experimentally failing isolated frog heart, the *Tanghinia venenifera* extract increased cardiac contraction amplitude in a concentration-dependent manner. The extract induced an increase in contractile force of 67.12 ± 0.69 % compared to the amplitude of failed heart contraction, at concentration of 0.75 mg/mL, with a $CE_{50}$ of 0.63 mg/mL ($p < 0.05$) (Figure 1). These results indicate that the extract enhances cardiac

contraction, demonstrating its positive inotropic effect. At concentrations of 0.875 mg/mL and above, the amplitude of contraction decreased to 43.5 ± 0.519 %, indicating a toxic effect of the extract.

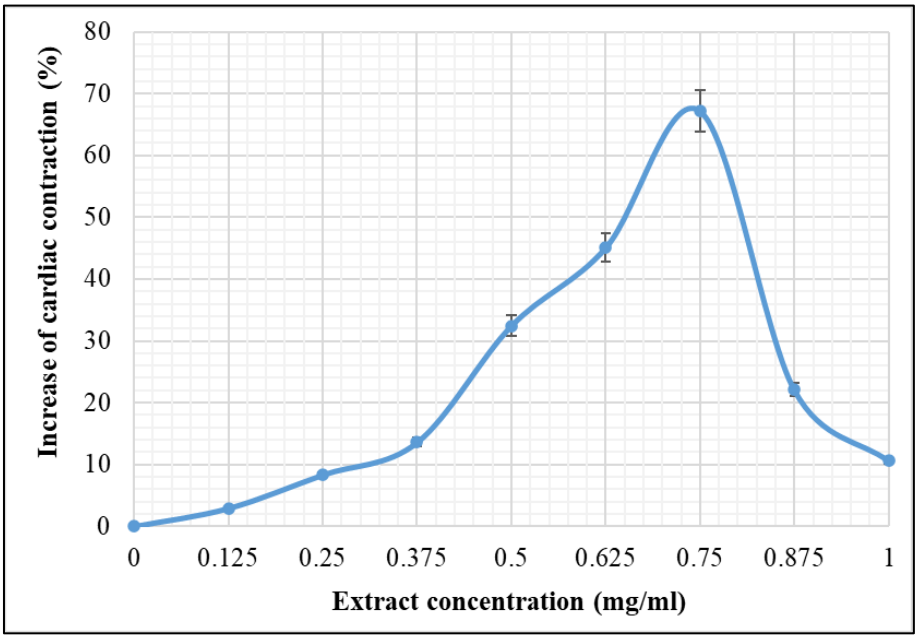


**Figure 1** Variation of the increase of the amplitude of contraction of the experimentally failing isolated frog heart in the presence of the extract, administered non-cumulatively via injection into the thoracic aorta ($\bar{x} \pm \bar{\sigma}$; n = 10; $p < 0.05$)

### 3.4. Effect of the Extract on Heart Rate

Injected in a non-cumulative manner into the thoracic aorta of the experimentally failing isolated frog heart, *Tanghinia venenifera* extract decreased heart rate in a concentration-dependent manner. It caused a reduction from 43.5 ± 0.173 beats per minute for the failing isolated frog heart to 27.33 ± 0.305 beats per minute at a concentration of 0.75 mg/mL ($p < 0.05$) (Figure 2). These results indicate that the extract possesses a negative chronotropic effect. At concentrations of 0.875 mg/mL and above, the heart rate starts increasing.

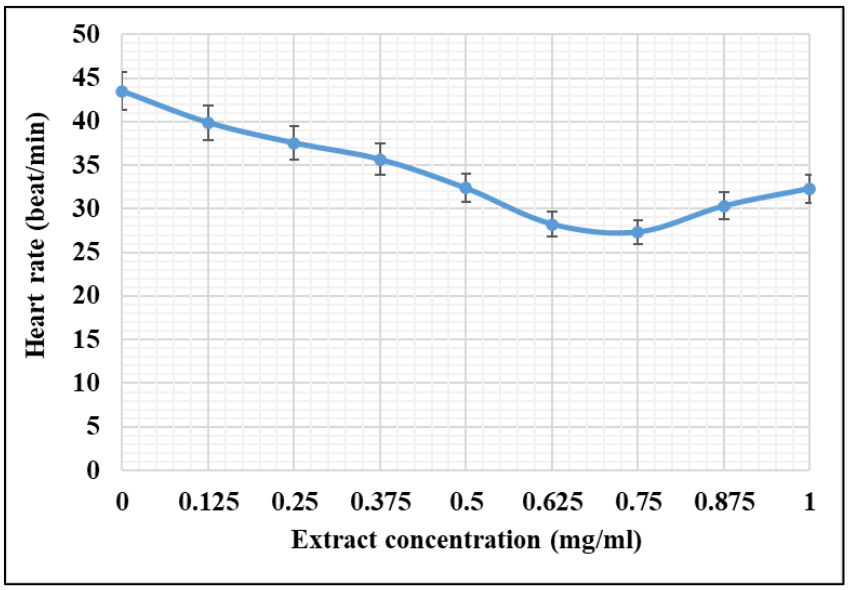


**Figure 2** Variation in the heart beat of the experimentally failing isolated frog heart in the presence of the extract, injected non-cumulatively via the aorta ($\bar{x} \pm \bar{\sigma}$; n = 10; $p < 0.05$)

### 3.5. Effect of the extract on filling time

When injected in a non-cumulative manner into the thoracic aorta of the experimentally failing isolated frog heart, T. *venenifera* extract increases the duration of diastole, in a concentration-dependent manner. In the absence of the extract, diastolic duration was 0.08 ± 0.002 seconds, whereas in the presence of the extract at 0.75 mg/mL, it increased to 0.22 ± 0.005 seconds ($p < 0.05$) (Figure 3). These results indicate that the extract prolongs diastole, thereby enhancing ventricular filling time, consistent with a positive lusitropic effect. At concentrations of 0.875 mg/mL and above, diastolic duration decreased to 0.11 ± 0.002 seconds, reflecting the toxic effect of the extract.

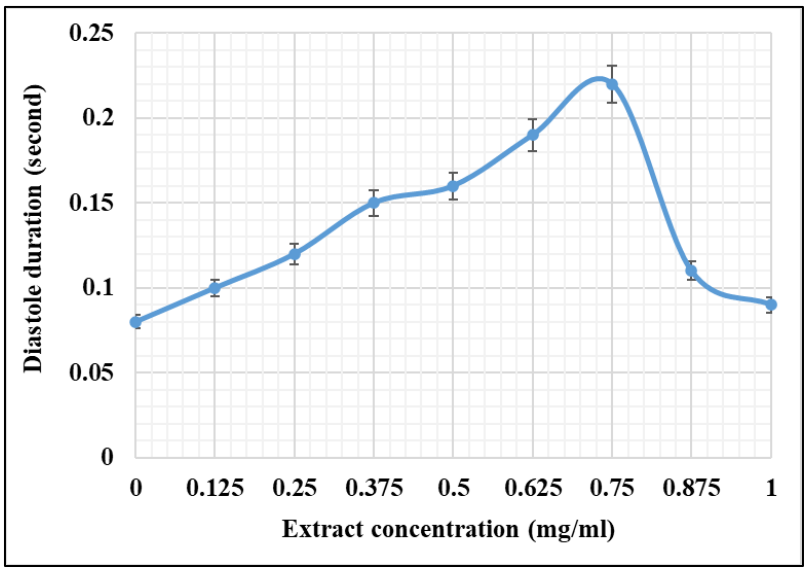


**Figure 3** Variation in diastolic duration of the experimentally failing isolated frog heart in the presence of the extract, administered non-cumulatively via injection into the aorta ($\bar{x} \pm \bar{\sigma}$; n = 10; $p < 0.05$)

### 3.6. Effect of the Extract on Cardiac Output

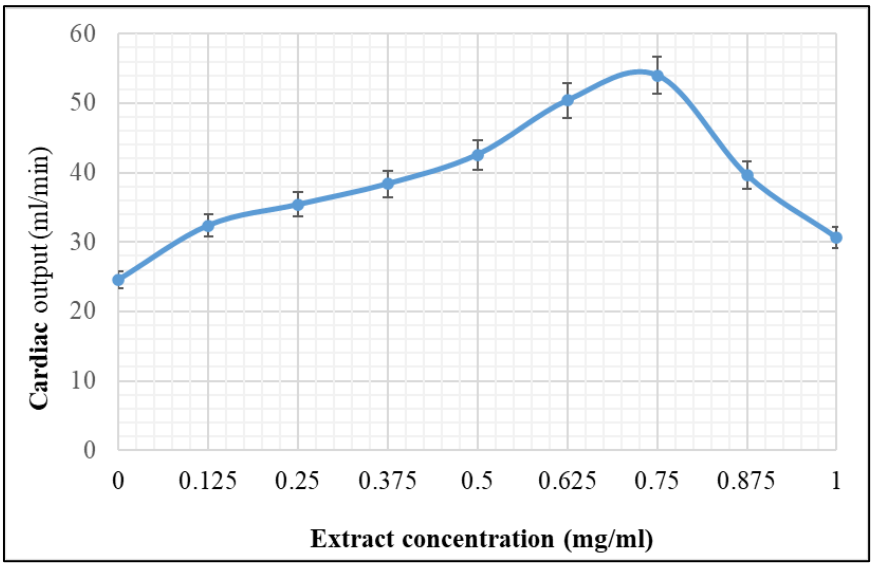


**Figure 4** Variation in cardiac output of the experimentally failing isolated frog heart in the presence of the extract administered non-cumulatively ($\bar{x} \pm \bar{\sigma}$; n = 10; $p < 0.05$)

When injected, in a non-cumulative manner, in the experimentally failing isolated frog heart, *T. venenifera* extract increased cardiac output, in a concentration-dependent manner. Before extract administration, cardiac output of the experimentally failing isolated heart was 24.6 ± 0.34 mL/minute, whereas in the presence of the extract, it increased up to 54 ± 0.69 mL/minute at concentration of 0.75 mg/mL ($p < 0.05$) (Figure 4). These results indicate that the extract

enhances cardiac output. At concentrations of 0.875 mg/mL and above, cardiac output decreased to 39.6 ± 0.11 mL/minute.

## 4. Discussion

The present investigation aimed to evaluate the hydroalcoholic extract of *Tanghinia venenifera* leaves on cardiac activity. It was administered in experimentally failing isolated frog heart, using Langendorff method. Our results demonstrate that it exerts concentration-dependent cardiotonic effects. At concentrations between 0.125 and 0.75 mg/mL, the extract increased contractile force, prolonged diastolic duration, reduced heart rate, and enhanced cardiac output, reflecting improved mechanical performance of the compromised myocardium. At higher concentrations (≥0.875 mg/mL), deleterious effects were observed, including reduced contractility, arrhythmias, and decreased cardiac output, consistent with the narrow therapeutic window typical of cardiac glycosides [10].

The positive inotropic effect is likely mediated through $Na^+/K^+$-ATPase inhibition, which increases intracellular $Na^+$ and indirectly elevates cytosolic $Ca^{2+}$ by reducing $Na^+/Ca^{2+}$ exchanger activity. In ventricular myocytes, this increased $Ca^{2+}$ availability enhances sarcoplasmic reticulum calcium release, leading to stronger contractions [11]. This mechanism aligns with previous observations in glycoside-rich Apocynaceae species and other plant-derived cardiac glycosides. ABDULLAH S. SHATOOR conducted a study on the cardiotonic effect of the aqueous extract of the whole plant of *Crataegus aronia* syn. *Azarolus* (L) on the isolated rabbit heart, which yielded results similar to those obtained in the present test [12].

*T. venenifera* extract also exerted a negative chronotropic effect, reflecting direct action on pacemaker cells. In these cells, $Na^+/K^+$-ATPase inhibition slows depolarization and can prolong repolarization, thereby lengthening the cardiac cycle and reducing heart rate. Additionally, the ionic alterations in pacemaker and atrioventricular tissues can slow impulse conduction, further contributing to bradycardia. In the absence of autonomic innervation on isolated heart, these effects are purely electrophysiological and demonstrate the extract's direct influence on cardiac excitability [13].

The prolongation of diastolic duration observed indicates improved ventricular relaxation, a positive lusitropic effect that enhances end-diastolic filling and preload. Together with increased contractility, this mechanism underlies the observed increase in cardiac output, consistent with classical principles of cardiac physiology [14]. Conversely, at higher concentrations, excessive intracellular $Ca^{2+}$ and disrupted $K^+$ gradients destabilize repolarization and resting potential in both pacemaker and cardiomyocytes, leading to arrhythmias and impaired contractile function, illustrating the extract's cardiotoxic potential at higher concentrations [15].

Phytochemical analysis confirmed the presence of glycosidic heterosides, steroids, and triterpenes, supporting the mechanistic basis for the observed cardiotonic effects. The results validate the chemotaxonomic rationale for selecting Apocynaceae species as potential sources of bioactive cardiac agents and align with ethnobotanical reports of *T. venenifera's* potent cardiac activity.

## 5. Conclusion

The hydroalcoholic leaf extract of *Tanghinia venenifera* leaves exerts concentration-dependent positive inotropic, positive lusitropic, and negative chronotropic effects on experimentally induced failing isolated frog heart through direct electrophysiological actions on pacemaker and cardiomyocytes. While demonstrating promising cardiotonic potential, its toxicity at higher concentrations highlights the need for isolation of active constituents, detailed mechanistic studies, and careful dose optimization for therapeutic application. Overall, this study demonstrates the value of a chemotaxonomy-guided approach for identifying bioactive cardiotonic compounds and positions *T. venenifera* leaves as a promising candidate for further pharmacological and phytochemical investigation.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

The authors declare no conflict of interest.

*Statement of ethical approval*

Prior to the start of the experiments, ethical approval for animal use was obtained from the Ethics Committee of the Faculty of Science, University of Antananarivo, under approval register CEA/FS-UA/Registre n°13/2025

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