International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

THE CARDIOACTIVE SCREENING OF THE EXTRACT FROM THE BARK OF *STROPHANTHUS CUMINGII* A.DC. (APOCYNACEAE) USING ISOLATED FROG HEART

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ABSTRACT

Strophanthus cumingii A. DC. (Apocynaceae) is one of two endemic *Strophanthus* species in the Philippines the bark of which is used as an arrow poison [1]. *Strophanthus* species used for this purpose are known to contain cardiac glycoside strophanthin in the bark and seeds [2]. This study determined the cardioactivity of the bark extract *S. cumingii* and its sub-fractions on isolated frog hearts. The hexane fraction was the most cardioactive with a maximum of 30.58% increase in the force of contraction and 38.24% increase in the frequency of contraction. The non-polar fraction of the crude extract from the bark of *S. cumingii* elicited a positive inotropic and negative chronotropic effect on the isolated frog hearts.

Keywords: Cardioactivity, Strophanthin, Inotropic, Chronotropic

INTRODUCTION

Cardiovascular diseases (CVDs) remain to be the leading cause of non-communicable disease deaths as of 2014 globally [3]. Among the other types of CVDs, congestive heart failure is the third leading cause of mortality [3]. Congestive heart failure (CHF) is a condition where the heart does not pump enough blood to meet the need of the body tissues [4]. Despite the availability of agents that are used in the treatment of CHF, the mortality rate due to CHF increased annually. Thus, this research has been pursued to explore new and natural sources of agents for the treatment of the said condition.

S. cumingii is one of two endemic species of *Strophanthus* in the Philippines. The bark of the plant is utilized by the locals as arrow-poison for hunting wild animals [2]. Plants belonging to the genus *Strophanthus* are known to contain toxic alkaloids and the cardiac glycosides strophanthin. The cardiac glycosides are found in the seeds and bark [1] and are used as arrow poisons responsible for the paralyzing effects. Strophanthin and other cardiac glycosides are employed in the CHF treatment [4]. The cardioactivity can be correlated to strophanthin that may be present in the bark of *S. cumingii*.

MATERIALS AND METHODS

Collection and Authentication: The plant samples were collected in the mountainous areas of Sitio Lauingen, Santo Domingo, Ilocos Sur, Philippines and authenticated at the Botany Division of the Philippine National Museum.

Extraction: The bark of *S. cumingii* was air-dried, percolated in 80% methanol and concentrated under vacuo at 40^{o}C using a Rotary Evaporator. The aqueous methanolic extract was partitioned between solvents of increasing polarities. Four fractions were obtained namely hexane (SC-H), dichloromethane (SC-D), n-butanol (SC-B) and water (SC-W).

Cardioactive Bioassay: The cardioactive bioassay was done using the ADI Powerlab® organ bath set-up. Isolated hearts of frog were hooked on one end inside the organ bath and the other end suspended by a thread attached to a transducer connected to the ADI Powerlab®. The organ bath chamber was maintained with fresh frog ringer's solution and a constant flow of carbogen (5% carbon + 95% oxygen). The force of contraction (in grams) and the time interval between each contraction (in seconds) were measured.

The baseline contraction of each isolated heart was read and recorded by the software (ADI Labchart v8®) for 30 seconds prior to the first administration of each test extract. A 200µL of each test extract, (or g-strophanthin or the vehicle control Di-methyl-O-sulfoxide) was added 12 times, maintaining a 30-second interval for each administration. The percentage increase in the force of contraction of all isolated hearts, as well as the changes in the time interval were computed using the value of individual baseline contraction.

RESULTS

The cardioactive bioassay using isolated frog hearts showed that the crude methanolic extract elicited a small increase in the force of contraction relative to the baseline; nonetheless the extract maintained the force of contraction of the hearts until the sixth addition. Among the four (4) fractions, the hexane fraction (SC-H) elicited the highest cardioactivity with a mean of 20.2467% increase in contraction relative to the baseline (Table I).

Table I. Percent Increase in the Force of Contraction of the Isolated Frog Hearts

Code	Mean Percent Increase in Contraction
Crude Methanolic Extract	-2.0075%
SC-H	20.2467%
SC-D	-10.245%
SC-B	-10.245%
SC-W	-15.558%
g-Strophanthin	67.8483%
Di-methyl-O-sulfoxide (DMSO)	-20.9525%

SC-H = S. cumingii A. DC. hexane fraction
SC-D = S. cumingii A. DC. dichloromethane fraction
SC-B = S. cumingii A. DC. butanol fraction
SC-W = S. cumingii A. DC. water fraction

The time interval (in seconds) was also measured and correlated to the chronotropic effect of *S. cumingii*. Chronotropic effect is defined as the ability to affect the heart rate, either to increase or decrease the rate [5]. In the ADI Labchart v8® tracing, the time interval between each contraction correlates to the chronotropic effect. A positive % deviation signifies a longer time interval in between each contraction which results to a decreased frequency of contractions per unit time and heart rate. The percent deviation (% increase) in the time interval in between each contraction was computed in relation to the baseline. The percent deviation was correlated to chronotropic effect.

A 13.51% mean increase in the time interval in contraction was observed on the hearts administered with the crude methanolic extract. Among the fractions, it was the SC-H that elicited the highest increase in the frequency of contraction with 38.24%. The reference standard g-strophanthin significantly increased the time interval in between contraction of the isolated hearts where a 45.07% mean increase was recorded. Table II summarizes the percent increase in the time interval in between contraction for all the test groups.

Table II. Percentage Increase in the Contraction Interval of the Isolated Frog Hearts

Code	Mean Percent Increase in Time interval
Crude Methanolic Extract	13.51%
SC-H	38.24%
SC-D	23.38%
SC-B	5.66%
SC-W	3.08%
g-Strophanthin	45.07%
Di-methyl-O-sulfoxide (DMSO)	1.38%

SC-H = S. cumingii A. DC. hexane fraction
SC-D = S. cumingii A. DC. dichloromethane fraction
SC-B = S. cumingii A. DC. butanol fraction
SC-W = S. cumingii A. DC. water fraction

Statistical Analysis: The percentage increase in the force of contraction of the isolated frog hearts in the cardioactive bioassay was tested using One-way Analysis of Variance (One-way ANOVA) at a 95% confidence interval. The analysis showed that the contraction among the fractions was significant with the f-test at 110.229 (P-value $0.000 < 0.05\ \alpha$), this means that the values were not the same in all the treatments. Based on Least Significant Difference (LSD) post-hoc analysis, the cardioactivity of all the four fractions, including the crude methanolic extract, and negative control are significantly different to the positive control. On the other hand, SC-H, crude methanolic extract and the positive control were significantly different to negative control. Thus, based on the mean of their increase in the force of contraction, SC-H was the most active as compared to the positive control.

DISCUSSIONS

Previous researchers have isolated the cardiac glycoside of related *Strophanthus* species on different fractions. Kingston et al. has isolated a cardenolide from the methanolic extract of the leaf and bark of *S. bovinii* (Apocynaceae)[6]. Beentje has mentioned that the majority of the cardiac glycosides were isolated on the semi-polar fraction and some on the non-polar fractions of the seed extract of *S. gratus* and *S. eminii*[7]. The results of this cardioactive bioassay implied that the cardioactive constituents of *S. cumingii* bark is non-polar. Although most of the cardiac glycosides are semi-polar [8], the hexane could have extracted the steroidal aglycone of the cardiac glycoside. Ouabagenin, the aglycone portion of g-strophanthin has been proven to elicit similar positive inotropic effect despite the absence of the sugar component [9].

The present study observed that the bark of *S. cumingii* elicited a positive inotropic and negative chronotropic effect on isolated frog hearts similar to the activities of other cardiac glycosides [10]. Palad (2012) noted similar positive inotropic and negative chronotropic effects of the crude methanolic extract of the stems and flowers of *S. cumingii* on intact frog heart [11]. The cardiotoxicity study of the leaf extract of *S. cumingii* on intact turtle hearts has shown a more significant positive inotropic effect compared to the leaf extract of *Alstonia scholaris* [12]. According to Encinas, *et al.* (2014) the contractile effect of the leaf extract from *S. cumingii* was not dependent on concentration, and this observation was also noted in the present study [13].

CONCLUSIONS

The present study confirmed that the bark of *S. cumingii* has a positive inotropic and negative chronotropic effect on the isolated hearts of frog. The cardioactive effect of the plant sample is similar to other species of the genus *Strophanthus.*

ACKNOWLEDGEMENT

The authors present their utmost gratitude to the Graduate School of the University of Santo Tomas and the Research Center for Natural and Applied Sciences (RCNAS) for the assistance and support.

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