https://doi.org/10.31925/farmacia.2023.2.20 **ORIGINAL ARTICLE**

THE EFFECT OF EXTRACTION METHOD OF *VERNONIA AMYGDALINA* DELILE. LEAVES ON CARDIOTONIC EFFECT

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Abstract

Vernonia amygdalina Delile leaves contain secondary metabolites that prevent heart disease. This study aims at demonstrating the cardiotonic effect of various ethanolic extracts obtained with simplicia powder of African leaf. In this direction, we tested the effect of African leaf ethanolic extract on normal rat heart isolates and determined its effect on heart contractility and heart rate. The results showed that the highest cardiac glycoside content in the ethanolic extract obtained by reflux extraction was 30.05 ± 0.13 mg/g. In an inotropic test, the same extract with a concentration of 1 mg/mL increased contractility by 74.02%, and regarding the chronotropic test, an 22.68% increase was observed. African leaf ethanolic extract (from *Vernonia amygdalina* Delile), obtained using reflux method, with a 1 mg/mL concentration, exhibited positive inotropic and chronotropic activity.

Rezumat

Frunzele de *Vernonia amygdalina* Delile conțin substanțe benefice în prevenirea patologiilor cardiace. Acest studiu își propune să demonstreze efectul cardiotonic al diferitelor extracte etanolice obținute din pulbere de frunze africane. În acest sens, am testat efectul extractului etanolic asupra cordului izolat de șobolan și am determinat efectul său asupra contractilității și frecvenței cardiace. Rezultatele au arătat că cel mai mare conținut de glicozide cardiotonice din extractul etanolic, obținut prin refluxare, a fost de 30,05 ± 0,13 mg/g. Efectul inotrop al extractului cu o concentrație de 1 mg/mL a crescut contractilitatea cu 74,02%, iar efectul cronotrop a prezentat o creștere de 22,68%. Extractul etanolic obținut prin refluxarea frunzelor africane (din *Vernonia amygdalina* Delile), având o concentrație de 1 mg/mL, a prezentat efecte inotrop și cronotrop.

Keywords: *Vernonia amygdalina* Delile, cardiotonic, inotropic, chronotropic

Introduction

Cardiotonic activity is the ability to increase the efficiency and contraction of the heart muscle, which leads to increased blood flow in all tissues in the body. People with heart arrhythmias and heart failure need these activities to keep their hearts beating. Cardiotonic activity is closely related to inotropic and chronotropic effects. Inotropic effects play a role in myocardial muscle contractility in the heart, while chronotropic effects play a role in heart rate [1]. These effects can be produced by secondary metabolites that interact with their target receptors to produce cardiotonic activity.

Cardiotonic activity is associated with an increase in the strength of the contraction of the heart muscle (myocardium). As the force of myocardial contraction increases, the amount of blood leaving the left ventricle with each contraction also increases. As the amount of blood leaving the left ventricle increases, cardiac output (the amount of blood leaving the left ventricle with each contraction) also increases [2].

Glycosides can be bound to secondary metabolites and have different pharmacological effects, for example, phenolic glycosides, coumarin glycosides, chromone glycosides, flavonoid glycosides, anthraquinone glycosides, saponin glycosides, cardiac glycosides, cyanogenic glycosides and thioglycosides. These compounds have different activities, and it is necessary to pay attention to the side effects of these glycoside compounds. Cardiac glycosides are natural secondary metabolites that have a pharmacological effect on the heart muscle in small doses. The cardiotonic effect was well known in the Ancient Egypt and has been used in the treatment of heart disease. The cardiac glycoside compounds consists of a 5β-cyclopentane aglycone nucleus, 14β androstane-3β, 14 (cyclopentane

perhydrophenanthrene), and a sugar moiety (often oligosaccharides) at the C-3β position.

In several studies regarding the cardiotonic activity, several secondary metabolites identified in plant extracts have this activity. Flavonoids and glycosides are secondary metabolites that are often targets for cardiotonic activity testing [3-6]. In addition, these two secondary metabolites show an effect on cardiac muscle contraction [7-12].

One type of medicinal plant that is nutritious for health is African leaves (*Vernonia amygdalina* Del.). African leaves have properties for curing various diseases and are very easy to grow, making them easy to find. African leaves contain flavonoids, tannins, saponins, and terpenoids that kill parasites that cause malaria, anti-amoeba, anti-tumour and antimicrobial. In addition, African leaves have benefits for diabetes, diarrhoea and malaria; they stabilize blood pressure, help cure insomnia, help prevent stroke, prevent cancer and prevent heart disease [13]. However, previous studies show that the plant has very high K^+ concentrations compared to other minerals, and K^+ ion causes hyperpolarization of the myocardial cells, hence leading to reduced force and rate of heart contractility. However, there is limited scientific information on its effects on reducing the rate and force of the heart contraction and its utilization by local communities and traditional herbalists in the management of sustained high blood pressure (hypertension).

Materials and Methods

Reagents and Chemicals

The chemical and reagents used were 3,5 dinitrobenzoic acid (Sigma Aldrich), distilled water (Merck), ethanol (Merck), sodium hydroxide (Merck), sodium chloride (Merck), kalium chloride (Merck), sodium bicarbonate (Merck), magnesium sulphate heptahydrate (Merck), potassium hydrogen sulphate (Merck), calcium chloride (Merck), glucose (Merck).

Preparation of Extract

The preparation of simplicia powder was carried out at the Pharmacognosy Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara, Indonesia. A total of 2 kg of fresh leaves (*Vernonia amygdalina* Delile.) were cleaned of dirt by washing them one by one with clean running water, then drained, weighed and then dried by drying in a drying cabinet (± 50ºC). Africa leaves are considered dry (simplicia) when they are brittle (crushed into pieces), then powdered using a blender and weighed the dry powder (simplicia powder). Simplicia powder is stored in a well-closed container protected from sunlight, heat, and humidity. Simplicia powder of *Vernonia amygdalina* Delile leaves (500 g) was extracted using ethanol absolute with maceration, percolation, reflux and soxhletation method [14-16].

Phytochemical Screening

A standard phytochemical screening procedure was used to identify the phytochemical compounds present in the ethanolic extract of African leaves. Alkaloids were tested with Dragendorrf, Mayer and Bouchardat; flavonoids with Mg and HCl; saponins with distilled water and HCl; tannins with $FeCl_3$; glycosides with the Molisch test; cardiac glycosides by performing the Keller-Kiliani test; and steroids and terpenoids by Liebermann's test [17-21].

Determination of Cardiac Glycoside Levels

The Kedde reaction, a colorimetric technique for determining unsaturated pentacyclic lactones (cardiac glycosides), was carried out using cardiac glycoside levels 3,5-dinitrobenzoic acid (3% solution of 3,5-dinitrobenzoic acid in ethanol with a 1:1 mixture with 2M NaOH solution in distilled water). 100 µL of the mixture was mixed with 150 µL of digoxin in ethanol at various concentrations (3.125, 6.25, 12.5, 25, 50, 100 µg/mL). The test solution's optical density (OD) was measured at a wavelength of 540 nm with a UV-Vis spectrophotometer. Levels were measured by plotting the absorbance obtained with a calibration curve obtained from digoxin as standard [20].

Inotropic and Chronotropic Effects of African Leaf Ethanol Extract

The effect of African leaf ethanolic extract on normal rat heart isolates and determine the effect of heart contractility and heart rate. The rats were anesthetized first with ketamine at a 50 mg/kg bw dose intraperitoneally. Then heparin was given at a dose of 5000 IU/kg bw intraperitoneally. Rat hearts were isolated and placed in Petri dishes containing frozen Krebs-Henseleit physiological solution (NaCl 6.9 g; KCl 0.35 g; $NaHCO_3$, 2.1 g; $MgSO_4x7H_2O$ 0.29 g; KH_2SO_4 0.16 g; $CaCl_2$ 0.28 g; glucose 1 g; pH 7.4) and fed with carbogen (95% O_2 + 5% CO_2) mixture. The heart is cleaned of fat and pericardium. After cleaning, the heart was hung on a Langendroff apparatus and filled with Krebs-Henseleit solution at a 20 mL/minute rate and kept flowing with carbogen. The ventricular part of the heart is connected to a transducer that will record the movement of the heart muscle. After achieving a stable condition, the heart isolates were given an ethanolic extract of African leaves, and the effects of contractions and pulses on the heart were observed through the recording delivered by the transducer [22]. The treatment was repeated three times.

Results and Discussion

Phytochemical Screening

The phytochemical screening of simplicia powder and African leaf extract was carried out to obtain information on the secondary metabolite class. Phytochemical screening was carried out on the triterpenoid/steroidal compounds, alkaloids, tannins, saponins, flavonoids

and glycosides. The results of the phytochemical screening of simplicia and extracts of African leaves can be seen in Table I. The results of phytochemical screening showed that the secondary metabolites contained in African leaf simplicia and extracts were flavonoids, glycosides, cardiac glycosides, steroids, saponins, and tannins.

Total Cardiac Glycosides African Leaf Ethanol Extract

The results of determining the total cardiac glycoside content of the ethanolic extract of African leaves are shown in Table II

Table I

Results of the phytochemical screening of simplicia and ethanolic extract of African leaves

No	Secondary Metabolite	Simplicia	Method			
			Maceration	Percolation	Reflux	Soxhletation
1	Alkaloids	-	-	-	-	-
2	Flavonoids	+	+	+	+	+
3	Glycoside	+	+	+	+	+
4	Steroids/triterpenoids	+	+	+	+	+
5	Saponins	+	+	+	+	+
6	Tannin	+	+	+	+	+
7	Cardiac glycoside	+	+	+	+	+

(+) positive: contains a group of compounds; (-) negative: does not contain a group of compounds

Table II

Cardiac glycoside levels from ethanolic extract of African leaves

No.	Extraction Method	Level (mg cardiac glycoside/g extract) ± SD
1.	Maceration	19.58 ± 0.49
2.	Percolation	23.46 ± 0.56
3.	Reflux	30.05 ± 0.13
4.	Soxhletation	14.69 ± 0.41

The highest cardiac glycoside content in ethanol extract with the reflux extraction method was 30.05 ± 0.13 mg/g, the lowest cardiac glycoside content in ethanol extract with the Soxhletation method was 14.69 ± 0.41 mg/g. The solvent's level of polarity strongly influences the solubility of glycosides. The more polar the solvent, the higher the concentration of cardiac glycosides in the extract. The presence of glycosides (sugar groups) in the structure of cardiac glycosides affects the polarity, and also the cardenolide groups increase the polarity of cardiac glycosides, while steroids in the structure of cardiac glycosides reduce the polarity of the compounds, so that in this case, the ethanol solvent in the extract is the best solvent to obtain high levels of cardiac glycosides [23]. Digoxin and digitoxin are the most widely clinically used cardiac glycosides. Cardiac glycosides have inhibitory activity against NKA enzymes in the myocardium so that they can increase heart contraction (positive inotropic).

African Leaf Ethanol Extract Cardiotonic Activity Test

Testing cardiotonic activity using the Langendorff device, the parameters that can be seen in this tool are inotropic and chronotropic effects on the heart.

Inotropic Test

The inotropic test is used to determine whether cardiac isolates exhibit an increase in contractility. The results of the inotropic test on cardiac isolates are presented as percentages (%) that produce contractility increase. The results of the inotropic test on rat heart isolate that treat with digoxin, and African leaf extract can be seen in Table III.

Table III

The inotropic result on rat heart isolate that treat with digoxin and African leaf extract

No.	Group	Baseline pressure (mm/Hg) ± SD	Pressure After administration of extract (mm/Hg) ± SD	Increase in cardiac contractility (%)
1.	Digoxin 0.25 mg/mL	24.89 ± 5.65	124.50 ± 16.4	80.00
2.	Maceration 1 mg/mL	27.43 ± 2.54	87.61 ± 5.98	68.69
3.	Percolation 1 mg/mL	24.71 ± 3.31	80.31 ± 6.41	69.23
4.	Reflux 1 mg/mL	25.61 ± 2.56	98.61 ± 8.67	74.02
5.	Soxhletation 1 mg/mL	26.98 ± 2.98	88.42 ± 7.53	69.48

Table III shows the percent increase in cardiac contractility of rats that given ethanol extracts and digoxin. Administration of digoxin increased contractility by 80.00%, and the ethanol extract with the reflux extraction method at a concentration of 1 mg/mL increased contractility by 74.02%, and in the ethanol extract with the maceration extraction method at a concentration of 1 mg/mL, there was an increase in contractility of 68.69%. Increasing the concentration of the extract increased the percent contractility of the rat heart [24-25]. Positive inotropes influence the heart such that the heart's contractility and pumping

power increase and the heart's performance is enhanced. African leaves contain vernonioside compounds that are structurally similar to cardiac glycosides, which have a steroid nucleus, lactone groups, and sugar groups. Cardiac glycosides have NKA enzyme inhibitory activity, increasing Ca^{2+} levels in cardiac muscle cells and increasing contraction. The presence of other metabolites in the extract may affect the increase in cardiac contractility activity.

Chronotropic Test

The chronotropic test is carried out to see an increase in heart rate in isolates. The results of the chronotropic test on cardiac isolates were in the form of a percent (%) increase in heart rate. The results of the chronotropic test on rat heart isolate given digoxin and African leaf extract can be seen in Table IV.

Table IV

The chronotropic result on rat heart isolate that treat with digoxin and African leaf extract

No.	Group	Baseline pressure (Beats/minute) ± SD	Pressure After administration of extract (Beats/minute) ± SD	Increase in heart rate (%)
1.	Digoxin 0.25 mg/mL	249.89 ± 14.10	328.54 ± 15.34	23.59
2.	Maceration 1 mg/mL	276.65 ± 15.21	336.89 ± 13.22	17.88
3.	Percolation 1 mg/mL	266.41 ± 15.45	325.67 ± 13.89	18.19
4.	Reflux 1 mg/mL	258.71 ± 14.34	334.61 ± 14.76	22.68
5.	Soxhletation 1 mg/mL	278.54 ± 13.98	351.76 ± 14.75	20.81

Table IV shows an increase in heart rate in each treatment group. The highest increase in heart rate occurred in the group with the reflux extraction at a concentration of 1 mg/ml, with a percent increase of 22.68%. Cardiac glycosides are specific compounds that act on the heart muscle, increasing the excitability of the heart rate and cardiac contractility. The aglycone of the glycoside is sometimes referred to as cardiac genin. Over the past few years, the aglycones of these glycosides have been the subject of chemical constituents that can influence the activity of these glycosides. The aglycones of cardiac glycosides are steroids. The steroid core in cardiac glycosides is a steroid derivative in the form of cyclopenantrene, which has an unsaturated lactone ring at the C17β atom. The activity of cardiac glycosides to increase heart rate and cardiac contraction depends on the sugar groups and lactone rings present in the constituent components of these compounds. Medicinal plant extracts containing cardiac glycosides can be prepared from various plant parts, including roots, stems, leaves, bark, seeds, fruits and flowers. Cardiac glycosides, also known as cardiotonic steroids, have a long history of medicinal applications, including digoxin, even though some are highly toxic to humans and animals. The terms cardiac glycosides and such compounds as digitalis are used interchangeably to refer to steroids or steroid glycosides that have a characteristic positive inotropic effect on the heart via dose-dependent inhibition of Na, K, -ATPase [22-25]. The activity of this glycoside compound is clinically useful as a treatment for controlling heart rate in patients with arterial fibrillation.

Conclusions

Using the reflux method and a 1 mg/mL concentration on cardiac isolates, the ethanolic extract of African leaf (*Vernonia amygdalina* Delile) showed both inotropic and chronotropic activity.

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Conflict of interest

The authors declare no conflict of interest.

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