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Research Communication

Phytochemical screening and acute toxicity studies of crude ethanolic extract and flavonoid fraction of *Carissa edulis* leaves

Hajara Ibrahim[1]*, Felicia E. Williams[2], Kayode M. Salawu[1], Afiniki M. Usman[3]

[1]Department of Pharmacognosy and Drug Development, University Of Ilorin Nigeria, [2]Department of Clinical Pharmacy and Pharmacy Practice, University Of Ilorin Nigeria. [3]Department of Pharmacognosy and Drug Development, Ahmadu Bello University Zaria, Nigeria.

*Correspondence: Hajara Ibrahim; hajara400@gmail.com; +2348060902839

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ABSTRACT: ***Carissa edulis*** **is used traditionally in Nigeria for the treatment of epilepsy, headache, syphilis, rheumatism and sickle cell anaemia. In this study, phytochemical screening was conducted to determine putative active components of *Carissa edulis*, as well as acute toxicity studies of the ethanolic extract and flavoniod fractions of the leaf. The phytochemical screening of the ethanolic extract of the leaves indicated the presence of carbohydrates, anthraquinones, saponins, tannins, flavonoids and alkaloids. Acute toxicity studies for the ethanolic extract and the flavonoid fraction were carried out using Lorke's method. In the first part of the experiment (phase A), Three groups of mice (n=3 in each group) were intraperitoneally given 10 mg/kg, 100 mg/kg and 1000 mg/kg of body weight concentration of the ethanolic extract and the mice were observed for 24 hours. The same procedure was repeated for the flavonoid fraction. The results showed that administration of 1000 mg/Kg concentrations of the ethanolic extract was fatal, while no death was recorded at the same concentration of flavonoid fraction. On this basis, in the second part of the study (phase B), mice were intraperitoneally administered 600 mg/kg, 1000 mg/kg, 1600 mg/kg and 2900 mg/kg concentration of the ethanolic extract while those for the flavonoid fraction had 1200 mg/kg, 1600 mg/kg, 2900 mg/kg and 5000 mg/kg flavonoid fraction. All mice were observed for 24 hours. The LD_{50} of the ethanolic extract and flavonoid fraction of the *Carissa edulis* leaves was 2154.1 mg/kg which is said to be slightly toxic. The maximum tolerated doses for both ethanolic extract and flavonoid fraction of *Carissa edulis* was 646.23mg/kg. The findings revealed that the leaves of *Carissa edulis* contain carbohydrates, anthraquinones, saponins, tannins, flavonoids and alkaloids that may contribute to its reported medicinal value. The acute toxicity studies suggest that the extract and fraction are slightly toxic. Thus further studies are necessary for full characterization of the active components in order to develop it further for safe medicinal use.**

KEYWORDS: **Phytochemical Screening, Acute Toxicity, Carissa edulis, Herbal Medicines.**

INTRODUCTION

Medicinal plants have been described as any plant part that provide health-promoting characteristics, temporary relief of symptoms or have curative properties (Terry, 2009). The use of herbs as the "earlier medicines" is a universally accepted phenomenon. Knowledge of medicinal plant was passed from one generation to the other verbally, however in the 21st century there is need that such knowledge must be evaluated and documented (Harborne, 1997). Detailed study of the phytochemical constituents of plants has led to the synthesis and improvement in the availability of scarce phytochemicals for pharmaceutical uses and this has ultimately reduced the cost of the product (Nairn, 1980; Serrentino, 1991).

In Africa, the abundant vegetation and high cost of conventional drugs encouraged the use of herbal medicine. Minor ailments in rural areas are commonly treated with local herbs. In Nigeria, several studies have documented the notable advancement in the use of herbal products in traditional medicines (Olaniyi, 1974; Sofowora, 1982; Gbile, 1986; Adjanohoun *et al*., 1991).

Herbal medicine or phytotherapy involves the use of medicinal plant in the management of disease conditions or improving well-being. Certain potent plants possess powerful action (e.g. digitalis) while others exert mild or gentle action (e.g. mint). In all cases, the activities depend on one or more of the phytochemical constituents present in the plant (Sofowora. 1984; Weiss, 2001). Phytochemical constituents present in plants exhibit diversified physiological and pharmacological effects, some of which produce toxic or adverse effects (Downum *et. al*; 1993).

Carissa edulis is a member of the family Apocynaceae. It is found in Northern Nigeria, Mali, Guinea, Ghana, Togo, Madagascar, Somalia, India and China (Ibrahim, 1997). It is commonly called *Cizaki'* or *'Lemun tsuntsu'* among Hausa people of Northern Nigeria, and in Somalia it is called *'adishawel*'.

Carissa edulis parts are used in ethno-medicine for wide variety of illnesses, such as epilepsy, headaches, chest complaints, gonorrhea, syphilis, rheumatism, rabies and often used as a diuretic (Nedi *et al.,* 2004; Ya'u *et al.* 2008). Other folkloric uses of C. *edulis* include fever, sickle cell anaemia and hernia (Ibrahim, 1997).

Flavonoids are water-soluble polyphenolic compounds, and consist of 6 major subgroups. They are classified chemiclally as chalcones, flavones, flavonols, flavononones, anthocyanins and isoflavonoids. Together with carotenes, flavanoids are responsible for the colouring of fruits, vegetables and herbs. Flavonoids have antioxidant activity, and are receiving increasing interest due to the many health-promoting effects ascribed to them. Some of the activities attributed to flavonoids include: anti-allergic, anti-cancer, antioxidant, anti-inflammatory and anti-viral. The flavonoids quercetin is known for its ability to relieve hay fever, eczema, sinusitis and asthma. Epidemiological studies have illustrated that heart diseases are inversely related to flavonoid intake. It has been shown that flavonoids prevent the oxidation of low-density lipoprotein thereby reducing the risk for the development of atherosclerosis.

This study is design to provide preliminary phytochemical data on the crude ethanolic leaf extract of C. *edulis*, and to assess acute toxicity of the ethanolic extract and flavonoid fraction of the plant's leaf.

MATERIALS AND METHODS

Plants Source

Plant sample was collected in Zaria, Nigeria in May 2012 and was identified at the Herbarium, Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria. A voucher number 900132 was issued. The plant leaves were air-dried, and powdered using pestle and mortar.

Animals

Twenty six Swiss Albino Mice of either sex were obtained from the Animal House of the former Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University Zaria, Nigeria. The animals were maintained in a well-ventilated room, and fed on growers mash. The Departmental Animal Use Committee approved the mice used in this study for the indicated purpose.

Preparation of extracts

A stock of 200 g of powdered plant material was weighed and placed in a conical flask and macerated with 80% petroleum ether for 24 hours. The mixture was filtered and the marc was dried. Solvent extraction of the dried marc was carried out by maceration with 80% ethanol for 72 hours. The sample was then filtered and the filtrate was concentrated in a rotary evaporator. The weight of the concentrated filtrate was determined and recorded.

Table 1: Composition of the extracted fractions from the leaves of *C. edulis*

Fraction	Percentage Yield
Petroleum Ether extract	1.0 g (0.5%)
Ethanol extract (A)	15.2 g (7.6%)
Diethyl Ether fraction (B)	0.2 g (1.32%)*
Aqueous fraction (C)	15 g (98.68)
Saponin fraction (G)	1.0 g (6.58%)
Flavonoid fraction (H)	1.0 g (6.58%)

*Calculated in relation to ethanol extract. Values are from 200 g of powdered leaves

Table 2: Phytochemical constituents Identified in ethanolic extract of the leaves of *C. edulis.*

Constituent	Test	Ethanolic Extract (A)	Ether Fraction (C)	Saponins Fraction (G)	Flavonoids Fraction (I)	Aqueous Fraction (B)
Carbohydrates	Molisch's	+	_	+	+	_
	Fehling's	+	_	+	+	+
Free anthraquinones	Bontrager's	+	+	_	_	_
Combined anthraquinones derivatives	Modified Bontrager's	+	_	_	_	_
Cardiac Glycosides	Keller–Kiliani	+	–	–	–	+
	Kedde's	+	+	_	–	+
Steroids And terpenes	Liebermann–Burchard's	+	+	_	_	_
	Salkwoski's	+	+	_	_	_
Flavonoids	Shinoda	+	+	_	+	_
	NaOH	+	+	_	+	_
	Ferric Chloride	X	X	X	X	X
Saponins	Froth	+	+	+	_	+
Tannins	Ferric Chloride	X	X	X	_	X
	Bromine water	+	_	_		_
	Lead	+	_	_	_	_

Key: +, Present; −, Absent; X, phenolic compound present

Isolation of flavonoids

The ethanolic extract was further subjected to further fractionation to isolate the flavonoid fraction (Harbone, 1997). The concentrate of ethanolic extract (A) was diluted with water, after which the sample was filtered and partitioned with diethylether to give the aqueous fraction (B) and the diethylether fraction (C). The aqueous fraction was further partitioned with water-saturated n-butanol to give the n-butanol fraction (D) and the aqueous fraction (E). The n-butanol fraction was treated with 1% KOH and two fractions emerged i.e. the KOH portion (F) and the n-butanol fraction (G). Fraction G contains the saponins. The KOH fraction was acidified with dilute HCl (1%), and partitioned with water-saturated n-butanol which separated to give the HCl fraction (H) and the n-butanol fraction (I) which was referred to as the crude flavonoid. It was then transferred to an evaporating dish and placed on a water bath to be evaporated to dryness. The resulting product was well collected, packaged and labeled for further use.

Phytochemical screening of *Carissa edulis* ethanolic leaf extract and other fractions

The plant extract was subjected to qualitative chemical screening in order to identify the various classes of phytochemical constituents using the standard methods of Trease and Evans (Evans, 2009). The extracts were screened for carbohydrates, tannins, glycosides, terpenes and steroids, flavonoids, alkaloids and anthraquinones.

Acute toxicity studies

The lethal dose (LD_{50}) was determined using the method of Lorke (1983). The method consisted of two phases. In the first stage, three groups of three mice each were injected intraperitoneally (IP) with the *Carissa edulis* ethanolic extract at doses of 10, 100 and 1000 mg/kg body weight and observed for signs of toxicity and death within 24 hours. In the second stage, four groups of one mouse each were treated with four more specific doses of the extract based on the result of the stage 1. The same procedure was repeated for the flavonoid fraction. The LD_{50} value and the Maximum Tolerated Dose (MTD) were calculated as follows:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality,
D_{100} = Lowest dose that produced mortality.

Maximum Tolerated Dose (MTD) = 30% of LD_{50}

RESULTS

Table 1 shows the composition of the extracted fractions from the leaves of *C. edulis*. The percentages shown reflect quantitative values of the different fractions obtained from fractionating the primary extract.

The phytochemical screening of the samples indicated the presence of carbohydrates, anthraquinones, tannins, flavonoids, cardiac glycosides, steroids and terpenes in the ethanol extract (Table 2). These were fractionated into the various solvents (diethyether and n-butanol).

The primary data of the acute toxicity studies in the two phases of the experiments are shown in Tables 3 and 4. The LD_{50} and the Maximum Tolerated Dose (MTD) obtained using the formula above were 2154.1 mg/kg and 646.23 mg/kg respectively. The same values were obtained for the ethanolic extract and the flavonoid fraction.

DISCUSSION

The phytochemical screening of the ethanolic extract of the leaves of C. *edulis* indicated the presence of carbohydrates, anthraquinones, saponins, tannins, flavonoids and alkaloids. Of these, carbohydrate is a primary metabolite while other compounds detected are secondary metabolites. The presence of these secondary metabolites is responsible for the plant's anti-inflammatory, analgesic, anti-oxidant, anti-convulsant and anti-microbial activities (Nedi *et al,* 2004; Ya'u *et al,* 2008*;* Ibrahim, 1997). Flavonoids are large class of

compounds with a lot of therapeutic activities including antioxidant, antiviral, analgesics anti-inflammatory and anticancer properties (Ibrahim, 1997). These properties are under current investigation to fully understanding the molecular basis for their actions.

Table 3: Acute toxicity of the ethanolic leaf extract of *C. edulis*

Phase I		
Group	Dosage (mg/kg)	Mortality
1	10	0/3
2	100	0/3
3	1 000	1/3

Phase II		
Group	Dosage (mg/kg)	Mortality
1	600	0/1
2	1 000	0/1
3	1 600	0/1
4	2 900	1/1

Table 4: Acute toxicity of the flavonoid leaf extract of *C. edulis*

Phase I		
Group	Dosage (mg/kg)	Mortality
1	10	0/3
2	100	0/3
3	1 000	0/3

Phase II		
Group	Dosage (mg/kg)	Mortality
1	1 200	0/1
2	1 600	0/1
3	2 900	1/1
4	5 000	1/1

The ethanolic extract and flavonoid fraction of *C. edulis* gave an LD_{50} of 2154.1 mg/kg, which is classified as slightly toxic according to the description given by Lorke (1983). LD_{50} values less than 1000 mg/kg body weight is considered toxic; while those greater than 5000 mg/kg body weight have negligible toxic effect. The maximum tolerated doses of the ethanolic extract and flavonoid fraction gave the same valus of 646.23 mg/kg. While the calculated LD_{50} values are the same for both ethanolic extract and flavonoid fraction, it appears that the flavonoid fraction might be safer, since no death was observed in the animals treated with this fraction at phase 1 of the study.

Conclusion

Our analyses confirm that the ethanolic leaf extract of *Carissa edulis* showed the presence of carbohydrates, saponins, cardiac glycosides, anthraquinones, tannins, flavonoids, steroids and alkaloids. The acute toxicity studies also indicate that the ethanolic extract and flavonoid fraction of the leaf extract of *Carissa edulis* are slightly toxic with an LD_{50} of 2154.1 mg/kg. Further studies on the acute toxicity of other fractions of *Carissa edulis* are in order to ascertain its full safety.

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