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Phytochemical Screening and Acute Toxicity of Aqueous Extract of Leaves of *Conocarpus erectus* Linnaeus in Swiss Albino Mice

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ABSTRACT

Mangroves represent areas of high biological productivity and it is a region rich in bioactive substances used in medicine production. *Conocarpus erectus* (Combretaceae) known as button mangrove is one of the species found in mangroves and it is used in folk medicine in the treatment of anemia, catarrh, conjunctivitis, diabetes, diarrhea, fever, gonorrhea, headache, hemorrhage, orchitis, rash, bumps and syphilis. The present study aimed to investigate the acute toxicity of aqueous extract of leaves of *C. erectus* in Swiss albino mice. The plant material was collected in Vila Velha mangroves, located in Itamaracá (PE). The material was subjected to a phytochemical screening where extractive protocols to identify majority molecules present in leaves were used. The evaluation of acute toxicity of aqueous extract of *C. erectus* followed the model of Acute Toxicity Class based on OECD 423 Guideline, 2001. The majority molecules were identified: flavonoids, tannins and saponins. The LD_{50} was estimated at 2,000 mg/kg bw. Therefore, the aqueous extract showed low acute toxicity classified in category 5.

Key words: acute toxicity, aqueous extract, *Conocarpus erectus*, medicinal plants, plants extracts.

INTRODUCTION

Conocarpus erectus L., popularly known as mangrove button belongs to the family Combretaceae and it is found in tropical and subtropical regions around the world (Bandeira 2003). Different parts as leaves, stem, fruits and flowers have antioxidant, anticancer and antimicrobial properties (Abdel-Hameed et al. 2011). In folk medicine, it was reported to astringent, styptic and tonic preventing anemia, catarrh, conjunctivitis, diabetes, diarrhea, fever, gonorrhea, headache, hemorrhage, orchitis, prickly heat, swellings and syphilis (Abdel-Hameed et al. 2013).

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Therefore, the determination of acute toxicity is the first step in toxicological investigations of aqueous extract of *Conocarpus erectus* L., constituting a preliminary pharmacognostic, exploring resources manipulated by the population.

MATERIALS AND METHODS

Plant Materials

Conocarpus erectus L. leaves were collected in mangrove located in Vila Velha, Itamaracá-PE, Brazil, in December 2013. Voucher specimens was identified and deposited at Herbarium of the Federal University of Pernambuco with number UFP 75.457.

Extraction of Conocarpus Erectus Leaves

The extract was prepared by infusion from 260 g of fresh leaves of *C. erectus* L. The material was weighed, ground and the infusion was performed by adding distilled water at 100°C for 30 minutes. The aqueous extract was lyophilized and stored at 5° C. The ratio of extraction was 1.38 %.

Experimental animals

Nine female Swiss albino mice (Mus musculus) were used, aged 11 weeks, weighing on average 38.5 g from the Department of antibiotics from Universidade Federal de Pernambuco (UFPE). The animals were divided into three groups of three animals: control group (G1), treated group with 5,000 mg/kg of aqueous extract of *C. erectus* L. (G2) and treated group with 2,000 mg/kg of aqueous extract of *C. erectus* L. (G3). The animals were kept under normal conditions of temperature and humidity under natural light-dark cycle of 12 hours and given water and diet (Purina) *ad libitum*. Experiments with animals were performed according to the Organization for Economic Cooperation and Development (OECD 2001) and with the approval of the Ethics Committee on Animal Experimentation from UFPE under the number 23076.025194/2012-10.

Phytochemical Test

Phytochemical screening of the aqueous extract of leaves of *C. erectus* L. by infusion through thin layer chromatography was performed. The presence of saponins was performed according to Harbone (1982). Silica gel plates were used 20x20cm (0.25 mm thick) with suitable developers and development system employing chromatographic patterns as shown in Table I (Harbone 1982, Wagner et al. 1984, Wagner 1996) where we investigated the presence of: alkaloids, coumarins flavonoids, triterpenes, saponins and tannins.

TABLE I
Metabolites eluting system developers and used for photochemical screening of the aqueous extract of *Conocarpus erectus*.

METABOLITES	SYSTEM ELUTION	REVEALING	REFERENCES
Alkaloids	AcOEt-HCOOH-AcOH-H_2O (100:11:11:27v/v)	Dragendoff	Harbone 1982
Coumarins	Éter-tolueno-AcOH 10% (50:50:50v/v)	KOH – ETOH 10%	Harbone 1982
Flavonoids	AcOEt-HCOOH-AcOH-H_2O (100:11:11:27v/v)	Difenilborilo-xiietilamina	Harbone 1982
Saponins	AcOHt-HCOOH-AcOH-H_2O (100:11:11:27v/v)	Vanilina Sulfúrtica	Harbone 1982
Tannins	CHCl3-CH3OH-H_2O (65:30:05v/v)	Cloreto Férrico 1%	Wagner 1996
Triterpenes	AcOEt-HCOOH-AcOH-H_2O (100:0.5:0.5:0.5v/v)	Lieberman/Burchard	Wagner et al. 1984

Toxicological Analysis

To establish the LD_{50} (median lethal dose) was used the methodology of according with Acute Toxic Class Method (OECD 2001) for acute toxicity test of single dose (Guideline 423). In this acute toxicity study, the initial dose of 5,000 mg/kg bw was used due to known information about toxicity of the plant (Abdel-Hameed et al. 2013). The next tested dose 2,000 mg/kg b.w. was selected depending on the mortality observed in the first 24 hours of exposure, and finally was estimated toxicological category, according to the specifications of OECD (2001). The control group received distilled water orally with corresponding dose to the body weight of the animal.

RESULTS

Phytochemical Screening

The aqueous extract of *C. erectus* L. showed the presence of flavonoids, tannins and saponins and absence of alkaloids, coumarins, and triterpenes (Figure 1).

Toxic Signs and Behavioral Analysis

The Toxic Signs and Behavioral Analysis were performed according to Malone (1977). The toxic effects of aqueous extract of *C. erectus* L. in mice are shown in Table II. The G1 group did not show behavioral changes. In G2 the animals showed stimulants effects as: increased respiratory frequency, piloerection, stereotyped movement, tail erection, sweep, vibrissae movement and tail contraction; depressant effects as: dyspnea, change gear and prostration; and others effects such as: photophobia, spasms, contortion abdominal, abdominal distension, reflux, escape reaction, cyanosis, muzzle edema, pallor and vocal tremor. In G3 group the animals showed stimulants effects as: increased respiratory frequency, piloerection, stereotyped movement, fine tremors, erection tail, sweep, movement vibrissae, attack posture and lifting upper train; depressant effects as: change gear and prostration; and others effects such as: photophobia, spasms, contortion abdominal, abdominal distension, reflux, exploratory behavior, escape reaction, muzzle edema, pallor and vocal tremor.

Acute Toxicity

In group treated with 5,000 mg/kg (G2), the first animal dosed survived, then two further animals were dosed and both died. The dosing processed at 2,000 mg/kg (G3) and 100% of animals survived. Therefore, the LD_{50} of the aqueous extract of *C. erectus* is estimated in 2,000 mg/kg, being classified according to the Globally Harmonized System for

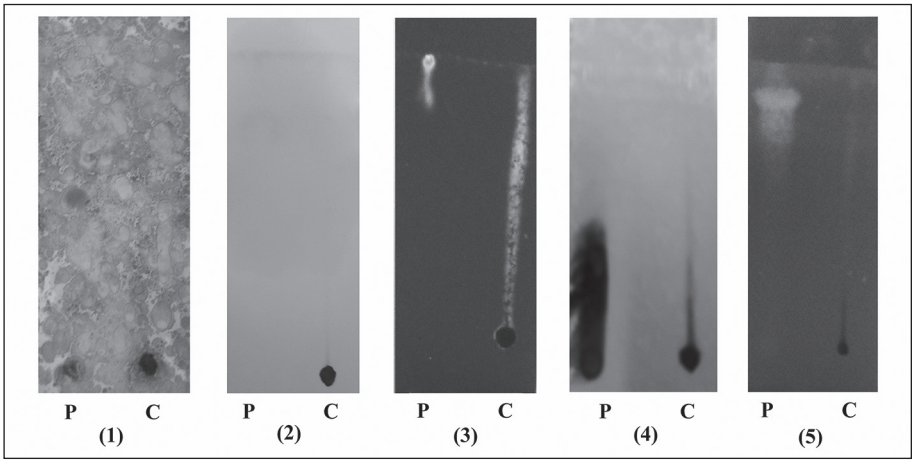

Figure 1 - Chromatogram of Alkaloids, Coumarins, Flavonoids, Tannins and Triterpenes.

TABLE II

Parameters related to toxic signs and behavioral analysis in swiss albino female mice assessed during sixty minutes after the oral administration of the aqueous extract of *C. erectus* l. in doses of 2,000 mg/kg bw and 5,000 mg/kg bw.

PARAMETERS	DOSAGE					
	2,000 mg/kg			5,000 mg/kg		
	CM	RM	DM	CM	RM	DM
STIMULANTS						
Increased Respiratory Frequency	+	++	+++	-	-	+++
Piloerection	++	++	++	-	+++	+++
Exophthalmia	-	-	-	-	-	-
Stereotyped movement	+++	+++	++	+++	+++	++
Fine tremors	-	-	+	-	-	-
Tail **e**rection	+	+	+	+	+++	-
Sweep	+	+++	+	+	++	+++
Vibrissae **m**ovement	+	+++	+	++	++	+
Agitation	-	-	-	-	-	-
Tail contraction	-	-	-	-	+	-
Fail curling	-	-	-	-	-	-
Attack posture	+	++	-	-	-	-
Heels	-	-	-	-	-	-
Lifting upper train	+	-	-	-	-	-
DEPRESSANT						
Decreased respiratory frequency	-	-	-	+	-	-
Dyspnea	-	-	-	-	-	+++
Prostration	+++	++	++	+++	++	+++
Change gear	++	++	+	+	-	+++
Lowering hindquarters	-	-	-	-	-	-
OTHERS						
Photophobia	+++	+++	++	-	+++	+++
Spasms	++	+	++	++	+	+++
Fecal excretion	-	-	-	-	-	+
Ciarrhea	-	-	-	-	-	-
Diurese	-	-	-	-	-	-
Contortion abdominal	+	-	-	-	-	+
Abdominal distension	-	+	-	-	+	+
Reflux	+++	+++	+++	-	-	+++
Exploratory Behavior	+	-	+	-	-	-
Escape Reaction	+++	+	+	+++	+	+
Aggressiveness	-	-	-	-	-	-
Cyanosis	-	-	-	+	+++	+
Muzzle edema	+	+	+	++	-	+++
Petechiae	-	-	-	-	-	-
CHANGE: Depression x Shake	-	-	-	-	-	-
Pallor	-	+	+	-	++	+++
Vocal tremor	-	-	+	+	+	+++
DEATH	-	-	-	-	+	+

- = no effect + = low effect ++ = moderate effect +++ = high effect (Malone 1977).

Classification and Labeling of Chemicals (2015) as low toxicity and belonging to category 5 according to the criteria of the experimental protocol adopted.

DISCUSSION

The phytochemical investigation shows that aqueous extracts of *Conocarpus erectus* revealed presence of flavonoids, tannins and saponins and absence of alkaloids, coumarins, and triterpenes.

Phytochemical studies of the methanol extract and n-hexane of leaves of *Conocarpus erectus* indicated the presence of triterpenes in n-hexane extract and absence of saponins in the methanol extract (Bandeira 2003).

This fact reinforces the proposal by Gobbo-Neto and Lopes (2007) of the need for studies that assess the differences in chemical composition between organs of the same plant, different times of collection, different cultivation environments and even different forms of nutrition plant used. The secondary metabolites represent a chemical interface between plants and the surrounding environment, so their synthesis are often affected by environmental conditions such as rainfall, U.V. radiation, atmospheric composition, circadian rhythm, plant age and temperature.

Reports about phytochemical evaluations of aqueous extract of leaves of *C. erectus* were not found in the literature and it makes difficult a comparison of the results with others using the same conditions.

Studies on extracts of leaves of *C. erectus* L. showed antimicrobial, antioxidant, anticancer, hepatoprotective and astringent properties (Bandeira 2003, Abdel-Hameed et al. 2011, 2013, Shohayeb et al. 2013). However, pharmacological proprieties can be associated with phytochemical compounds found in this study such as flavonoids which have antioxidant, anti-inflammatory and hepatoprotective properties (Lopes et al. 2000) saponins, related to antimicrobial and anti-inflammatory activities (Müller 2006) and tannins with antimicrobial and astringent activities (Monteiro et al. 2005).

After oral administration of the substance, significant changes were observed in the behavioral parameters of mice, mainly in central nervous system, autonomic nervous system and somatomotor activity.

However, it is possible that the saponins could elevate the toxic effects due to its emollient or stimulant properties (Akah and Offiah 1992).

The evaluation of acute toxicity is a methodology widely used to identify and classify substances as to their ability to cause acute damage to living organisms, in high doses, especially anatomical-pathological and lethal injuries, and can offer assistance to establish security parameters along with other toxicity data to human health (Valadares 2006, Zatta et al. 2009).

For vegetables, the method is useful for identifying the toxicity that may exhibit and minimize the misconception of the population into believing that natural products are devoid of toxic or adverse effects (Cunha et al. 2013)

Information about lethal doses of *C. erectus* L. is known in literature (Abdel-Hameed et al., 2013). According to the guide of OECD, Guideline 423 (OECD 2001), when available information about the tested plant suggests that mortality is unlikely at the highest starting dose level (2,000 mg/kg), then a limit test should be conducted.

The present study concerning to investigation of toxicity of the aqueous extracts of *C. erectus* leaves revealed low acute toxicity, classified in category 5 and LD_{50} estimated at 2,000 mg/kg bw.

Our results do not corroborate those obtained by Abdel-Hameed (2013) who studied leaves, stems, flowers and fruits of *C. erectus* L. and showed that methanol extracts did not induce mortality in Swiss albino mice at doses from 500 mg/kg to 5,000 mg/kg intraperitoneally during 24h of observation, then the extracts did not show acute toxicity with LD_{50}> 5,000 mg/kg.

Differences on route of administration, time of observation or solvent used to prepare the extract may result in unintentional adverse effects on experimental animals and confounded results. According to Turner et al. (2011), specific considerations for delivery of substances to animals are numerous and include factors such as absorption, distribution, metabolism and excretion of therapeutic or chemical agents; route, volume, and frequency of administration; duration of treatment; pH, stability, homogeneity, and osmolality of the substance to be administered; selection of vehicle or solvent for delivering substances that cannot be administered in a solid or particulate state; solution preparation. Some of those aspects could have influenced in divergences of results.

However, further studies should be conducted to perform the histomorphometry of the liver and kidneys from the animals to check possible pathological changes.

CONCLUSIONS

Chemical Constituents identified in the aqueous extract of leaves of *Conocarpus erectus* L. species were flavonoids, saponins and tannins. The extract was classified as belonging to category 5 with a low toxicity. The LD_{50} of the aqueous extracts *Conocarpus erectus* L. was estimated at 2,000 mg/kg body weight.

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