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PHYTOCHEMICAL SCREENING AND ACUTE TOXICITY STUDY OF *XYLOPIA VILLOSA* (ANNONACEAE) BARKS STEMS OF AQUEOUS AND HYDROETHANOLIC EXTRACTS

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ABSTRACT

Xylopia villosa is a plant whose barks stems and ground seeds were used in treatment of cold, headaches, ulcers and boils. This study aims to determine secondary metabolites and acute toxicity parameters of *Xylopia villosa* barks stems of aqueous and hydroethanolic crude extracts in female Wistar rats. The phytochemical screening performed, revealed that both extracts contain alkaloids, polyphenols, leucoanthocyanins, saponins, flavonoids, sterols and polyterpenes. However there are no quinones and tannins (catechin and gallic) in these extracts. With regard to parameters, we obtained 2000 mg / kg body weight (bw), 281.83 mg / kg bw and 275 mg / kg bw indicating successively the lethal dose 100 (LD_{100}), lethal dose 50 (LD_{50}) and tolerated maximum dose with aqueous extract. The hydroethanolic extract of *Xylopia villosa* barks stems administered by intraperitoneal route at dose 5000 mg/kg of bw caused no morbidity and lethality. This result indicated the LD_{50} of hydroethanolic extract is higher than 5000 mg / kg bw. According to Global Harmonized Classification System (SCGH), hydroethanolic extract of this plant is hardly toxic.

KEYWORDS: Phytochemical screening, *Xylopia villosa*, Secondary metabolites, Acute toxicity, lethal dose, Côte d'Ivoire.

INTRODUCTION

Plants are vital to biodiversity and serve primarily to human welfare.[1] They have a cultural importance and economic potential in the food, health care, energy, clothing and housing construction. Relationships between plants and humans have existed for long dates.[2] Medicinal plants are valuable resources for the majority of rural populations in Africa, where more than 80% of these populations use them to ensure their health care.[3] In addition, these plants are invaluable resources for the pharmaceutical industry.[4] However, the use of traditional drugs includes risk such as kidney damage, heart, lung and liver are legion.[5,6,7]

The ivorian flora in 1979 revealed five thousand species including *Xylopia villosa* (*X.villosa*). Xylopia are a large pantropical genus comprising about 150 species of which around thirty are found in mainland tropical Africa and 25 species in Madagascar. *X.villosa* is a tree whose wood, hard and durable enough, is used to make building poles and tool handles.[8] Powder or macerated bark of *X.villosa* is used in traditional medicine to treat various diseases including colds and headaches. The ground seeds are applied on ulcers and boils for healing.[1,8] It produces a monoterpene essential oil whose composition is dominated by sabinene or β-ocimene.[9] However, no phytochemical and toxicological studies of this species exist in the literature. In order to fill items missing, the general objective of this study was to perform a phytochemical screening and acute toxicity study of *X. villosa* barks stems on female Wistar rats. To do this, certain chemical groups such as alkaloids, flavonoids, polyphenols, tannins, saponins, leucoanthocyanins, quinones, sterols and polyterpenes have been sought in aqueous and hydroethanolic total extracts. Toxicological

parameters like the LD_{50}, DL_{100} and tolerated maximum dose were also determined in this study.

MATERIALS AND METHODS

Plant Material

X. villosa barks stems were harvested in June 2014 at the National Floristic Center of Felix HOUPHOUET BOIGNY University where can be found a sample recorded at the number 14712.

Preparation of Extracts

The barks stems of *X. villosa* were dried for four weeks, then made powder using an electric grinder IKA-type MAG®. The extraction of secondary metabolites from the powder *X. villosa* was performed using water and ethanol. 100 grams of powder of *X. villosa* were macerated for 24 hours in 1 liter of 70% ethanol. The macerated obtained was then filtered twice on white cotton and once on Whatman filter paper N°4. The filtrate obtained in 70% ethanol was evaporated to dryness at reduced pressure at temperature of 40°C using a rotary evaporator type Buchi 161 Water Bath. About aqueous extract preparation, 100 grams of *X. villosa* barks stems powder were added to 100 milliliters of boiling distilled water. Just like hydroethanolic preparation, the resulting mixture was filtered twice on white cotton and once on Whatman filter paper N°4. The filtrate obtained is preserved at temperature of 40°C in an oven for drying.

Phytochemical screening

Chemical tests for the screening and identification of bioactive chemical constituents in barks stems of *X. Villosa* study were carried with extracts prepared using the standard procedures.[10,11,12]

Test for alkaloids

Alkaloids were detected by Dragendorff and Bouchardat reagents. 6 mL of each plant extract of the two extracts were evaporated on a sand bath. The residue of each extract is taken up in 6 mL of alcohol (60°) and the alcoholic solution thus obtained was distributed in two tubes test. In the first tube were added 2 drops Dragendorff reagents (aqueous solution of iodo-bismuth potassium). The appearance of a precipitate or an orange color indicates the presence of alkaloids. In the second tube, are added 2 drops of Bouchardat reagent (aqueous solution of iodine-iodide). The appearance of a reddishbrown color indicates their presence.

Test for phenols

2 mL of each extract was added a drop of alcohol solution of ferric chloride (2%). The appearance of a blackish-blue or darker or lighter green color indicated the presence of phenolic compounds.

Test for flavonoids

2 mL of each plant extract are evaporated in a capsule, without carbonizing the residue. After cooling, the residue is taken up 5 mL of hydrochloric alcohol (obtained by mixing 10 mL of 96° ethanol,10 mL of distilled water and 10 mL of concentrated hydrochloric acid) diluted 2 times in a test tube. It is added 2 to 3 magnesium shavings (exotherm). This gives a pink-orange or purple. The addition of 3 drops isoamyl alcohol intensifies a pink-orange or purple, indicating the presence of flavonoids. The control is performed with the alcoholic solution of quercetin.

Test for tannins

Detection of catechin tannins

5 mL of each extract are evaporated. The dry residue was added 15 mL of reagent Stiasny (10 mL of 40% formalin added 5 mL of hydrochloric acid (HCl) concentrate). The mixture was kept in a water bath at 80°C for 30 minutes. It is cooled under running water. The observation of large flake precipitate characterizes catechin tannins.

Test for gallic tannins

The solution containing the flakes is filtered and the filtrate collected is then saturated with sodium acetate. To the mixture, 3 drops of ferric chloride 2%. The appearance of an intense black-blue color indicates the presence of gallic tannins.

Test for sterols and polyterpenes

They were detected by the reaction of Liebermann. 5 mL of each of the two extracts were evaporated on a sand bath. The residue was dissolved in 1 mL of hot acetic anhydride; we added 0.5 mL of concentrated sulphuric acid. The appearance at the interphase of a purple ring, turning blue to green indicated a positive reaction.

Test for leucoanthocyanins

2 mL of each extract were evaporated. After cooling, the residue was added 5 mL of hydrochloric acid and 1 mL of isoamyl alcohol. The solution was heated for 15 minutes in a water bath at 80°C for 30 minutes. The appearance of a cherry-red or purple characterizes the presence of leucoanthocyanins.

Test for quinones

Identification of quinones was used Borntraeger reagent (ammonia diluted 2 times) that allows the detection quinone substances. Evaporated to dryness in a capsule 2 mL of each plant extract. The residue was mixed in 5 mL of hydrochloric acid (HCl) diluted 1/5. The solution is in a boiling water bath for half an hour in a test tube. Then cooled in a cold water stream and the hydrolyzate is extracted with 20 mL of chloroform in a test tube. The chloroform phase was collected in a test tube and mixed with 1/2 mL of dilute ammonia 2 times. The appearance of a color ranging from red to purple indicates the presence of quinones.

Test for saponins

10 mL of each plant extract were put into a test tube of 160 mm height and 16 mm in diameter. This was stirred vigorously test tube for 10 seconds. The foam height is measured after 10 minutes resting. A height of more than

1 cm of foam, indicates the presence of saponins. The saponins may also be demonstrated by the persistence of the foam.

Thin layer chromatography (TLC)

The support used in this study is a plate dimension of silica gel aluminum 20 × 20cm (Silica gel 60 F_{254}). Four mixtures of compounds have been used as standards:

- Scopolamine, atropine for alkaloid compounds,
- Rutin for phenolic compounds,
- Lupeol for terpene compounds,
- Escin for saponins

Experimental Animals

The female Wistar rats of average weight 120.60 ± 0.87 grams, aged ten (10) weeks, were used to assess the acute toxicity of aqueous and hydroethanolic extracts of *X. villosa.* They were provided from *Ecole Normale Supérieure* (ENS) in Abidjan – Côte d'Ivoire and fed by pellets. Two weeks before the experiment, they were transferred and acclimatized in the animalery of the Faculty of Pharmaceutical and Biological Sciences of the Felix Houphouet Boigny University.

Acute toxicity study

Acute toxicity study by intraperitoneal route was performed as per Organization for Economic Cooperation and Development (OECD) - 423 guidelines (OECD, 2001). The animals were divided in 6 groups (n=3) and were fasted overnight prior to drug administration. Following the period of fasting, the animals were weighed and the test substance was administered. The control group (group 1) received 0.9% NaCl. The groups 2, 3 and 4 received respectively aqueous extract of *X. villosa* in the doses of 2000, 300 and 50 mg/kg of bw by intraperitoneal route. The groups 5 and 6 received respectively 70% alcohol extract of *X. villosa* in the doses 2000 and 5000 mg/kg bw by intraperitoneal route. The treated animals were observed for 14 dayswith particular attention during the first 24 hours in order to announce clinical signs and possible deaths in each group.

RESULTS

Phytochemical screening

The phytochemical screening showed that the extracts of *X. villosa* contain alkaloids, flavonoids, polyphenols, leucoanthocyanins, sterols and polyterpenes. However there are no quinones and tannins (catechin and gallic tannins) in both total extracts. The aqueous extract contains saponins while hydroethanolic extract does not contain(**Table 1**).

Table 1: Phytochemical screening of *X. villosa* barks stems

Phytochemical compounds	*X. villosa* bark stems	
	Aqueous extract	Hydroethanolic extract
Alkaloids Dragendorff	+ +	+
Alkaloids Bouchardat	+ +	+
Polyphenols	+	+ +
Flavonoids	+	+ +
Catechin tannins	-	-
Gallic tannins	-	-
Sterols and polyterpenes	+ +	+
Leucoanthocyanins	+	+ +
Quinones	-	-
Saponins	+ +	-

(+) = presence (++) = abundance (-) = absence

TLC

After the migration of different samples on chromatographic plates, some phytocompounds (**Table 2**) were identified after dipping the plates in specific developers (alkaloid compounds, phenolic compounds, terpene compounds, saponins).

Table 2: Characterization of the compounds present in *X. villosa* barks stems on TLC

phytochemical compounds	*X. villosa* barks stems			
	Solvents	Witnesses	Revealing	Results of TLC
Alcaloids	$CH_2Cl_2/CH_3OH/NH_4OH$ (95:5 :0,1)	Scopolamine Atropine	Dragendorff	+
Alcaloids	$CH_2Cl_2/CH_3OH/NH_4OH$ (95:5 :0,1)	Scopolamine Atropine	Bouchardat	+
Flavonoids (glycosyl)	AcoEt/HCOOH/CH_3COOH/H_2O (100 :11 :11 :27)	Rutin	$FeCl_3$	+
Flavonoids (genin)	AcoEt /$CHCl_3$ (60 : 40)	Rutin	$FeCl_3$	+

Polyphenols	AcoEt /$CHCl_3$ (60 : 40)	Rutin	Fast Blue B	+
polyterpenes	C_6H_{14}/ AcoEt (50 :50)	Lupeol	H_2SO_4/C_2H_5OH	+
Saponins	$CHCl_3/CH_3OH/H_2O$ (65 :50 :10)	Escin	Anisaldehyde	+

(+): presence

Clinical signs and death

After intraperitoneally administering of ethanolic extract of barks stems of *X. villosa* to rats at doses of 2000 and 5000 mg / kg bw, there was no significant change in the behavior of these last. Moreover, no mortality was recorded during 14 days of observation. However, after administering of the aqueous extract of *X. villosa* to the rats, different clinical signs were observed in the first 24 hours (**Table 3**). Deaths were also observed during the first 24 hours at doses of 300 and 2000 mg / kg bw. Single dose of 50 mg / kg bw of the aqueous extract which caused no death. The data in **Table 4** allowed drawing the mortality curve of aqueous extract (**Fig. 1**).

Table 3: Clinical signs observed during 14 days after injection of aqueous extract of *X. villosa*

Clinical signs	Witnesses	Aqueous extract			Hydroethanolic extract	
	Group 1 (0 mg/kg bw)	Group 2 (2000 mg/kg bw)	Group 3 (300 mg/kg bw)	Group 4 (50 mg/kg bw)	Group 5 (2000 mg/kg bw)	Group 6 (5000 mg/kg bw)
Tremor	-	+	+	-	-	-
Immobility	-	+	-	-	-	-
Hindlimb paralysis	-	+	-	-	-	-
Rapid breathing	-	+	+	-	-	-
Abdominal constrictions	-	+	-	-	-	-
Sleep	+	-	+	+	+	+
Self-powered	+	-	+	+	+	+

+ : Signs of; — : Absence of signs

Table 4: Aqueous total extract doses of *X. villosa* administered for determination of parameters of acute toxicity

Clinical signs	Witnesses	Aqueous extract			Hydroethanolic extract	
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Number of rats used	3	3	6	3	3	3
Injected doses (mg/kg bw)	0	2000	300	50	2000	5000
Logarithm of injected doses	0	3.30	2.47	1.69	3.30	3.70
Number of dead rats	0	3	4	0	0	0
Death percentage (%)	0	100	66.66	0	0	0

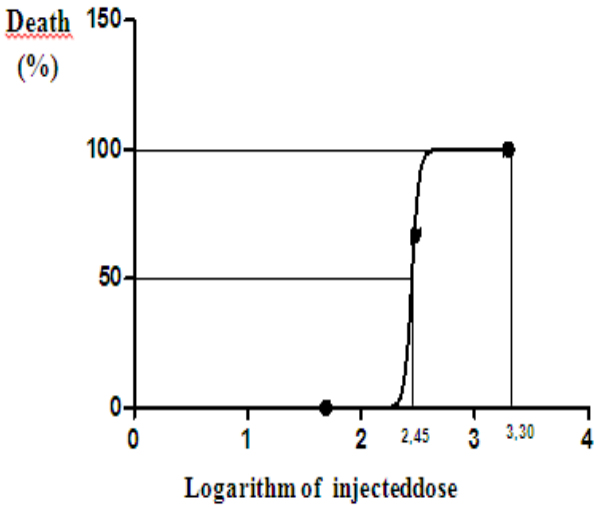

Figure 1: Mortality curve rats according logarithm dose of the aqueous extract of *X. villosa*

Toxicological parameters obtained: In this study, toxicological parameters of aqueous extract obtained are:
- The tolerated maximum dose = 275 mg / kg bw,
- The lethal dose 50 (LD_{50}) = 281.83 mg / kg bw,
- The lethal dose100 (LD_{100}) = 2000 mg / kg bw.

About hydroethanolic extract, these toxicity values are beyond 5000 mg / kg bw.

DISCUSSION

The phytochemical screening revealed an absence of tannins and quinones in the both total extracts of *X. villosa* barks stems. However, it revealed the presence of alkaloids, flavonoids, polyphenols, leucoanthocyanins, sterols and polyterpenes in *X. villosa* barks stems. The aqueous extract contains saponins while hydroethanolic extract does not contain any. These results agree with those obtained with *Xylopia aethiopica*.[13,14] The absence of quinones in *X. villosa* barks stems is in conformity with the work of Yemoa *et al.*, (2008). For the first time,

the presence of leucoanthocyanins is observed in the genus Xylopia.

The acute toxicity study with aqueous extract of *X. villosa* barks stems allowed observing different clinical signs and dead to 300 and 2000 mg / kg bw. The toxic nature of the aqueous extract of *X. villosa* barks stems is linked to the large amount of alkaloids and saponins. Indeed, alkaloids are among the most potent toxic as well as saponins and glycosides. They are often mixed in the same plant and their effects are diverse and multiple nature.[15] This acute toxicity study also obtains toxicological parameters of the aqueous extract of *X. villosa* administered intraperitoneally. These are the tolerated maximum dose (275 mg / kg bw), LD_{50} (281.83 mg / kg bw) and LD_{100} (2000 mg / kg bw). According to Hodge and Sterner (1943), aqueous extract of *X. villosa* is moderately toxic. This toxicity is due to the dissolution of a large amount of toxic substances contained in the cells of mucilages during boiling. These are the alcaloïds and especially saponins that provide the toxic nature in the genus Xylopia.[14,16]

The hydroethanolic extract of *X. villosa* barks stems administered by intraperitoneal route at doses up to 5000 mg/kg bw caused no morbidity and lethality. That means by this route, the LD_{50} is higher than this dose. According to Global Harmonized Classification System (SCGH), substance whose dose of 5000 mg / kg bw was not lethal is Category 5. The ethanolic extract of *X. villosa* is hardly toxic. This could be explained by the absence of saponins in this extract.

CONCLUSION

Phytochemical screening performed on the aqueous and hydroethanolic extracts of *X. villosa* barks stems showed that the plant contains various chemical groups (alkaloids, sterols, polyterpenes, polyphenols, leucoanthocyanes, saponosides and flavonoids as genin and glycosylated). These compounds would be responsible to *X. villosa* biological activity. The acute toxicity study of aqueous and hydroethanolic extracs of X. *villosa* has determined the moderately and hardly toxic characteristics respectively of aqueous extract and hydroethanolic extract. This phytochemical screening and acute toxicity study help to broaden the field of *X. villosa* study.

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