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ACUTE TOXICITY STUDIES AND PHYTOCHEMICAL SCREENING OF THE BARK OF STEMS AND THE LEAVES OF *ERYTHROXYLUM EMARGINATUM* (*ERYTHROXYLACEAE*)

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ABSTRACT

Erythroxylum emarginatum is a plant used by the population to relieve the pains during arthritis and asthma. In this study we realized the phytochemical screening of the aqueous and ethanolic extracts of the bark of stems and the leaves of *Erythroxylum emarginatum (Erythroxylaceae*) and we studied the general acute toxicity of aqueous and ethanolic of the bark of stems of this plant on Wistar strain rats. We realized chromatographic on thin layer (TLC) to confirm the results of phytochemical screening. The aqueous and ethanolic extracts of the bark of stems and the leaves of this *Erythroxylum emarginatum* revealed the presence of alkaloids, flavonoids, leuco-anthocyanins, polyphenols, polyterpenes, sterols, tannins (catechic and Gallic) and quinones. The presence of these biocompounds was confirmed by TLC. The study of general acute toxicity evaluated on the wistar strain rats, showed that the lethal dose 100 (LD_{100}) situated between [2000; 5000 [mg / kg body weight, the lethal dose 50 (LD_{50}) situated between [300; 2000 [mg / kg body weight and maximal dose tolerated (MDT) was 300 mg / Kg body weight for the aqueous and ethanolic extracts. According to these results the aqueous extract was toxic and the ethanolic extract was weakly toxic.

KEYWORDS: *Erythroxylum emarginatum*, inflammation, phytochemical, lethal dose.

INTRODUCTION

Plants represent for a long time an essential and inexhaustible source of medicine. Today a majority of the world population, more particularly in developing countries, use only the plants as remedies again diseases.[1,2,24] Despite the various solutions offered by pharmaceutical companies, people are moving increasingly towards herbal medicine. Phytotherapy appears as a safer alternative to modern medicine. The search for new most active natural molecules in plants to treat human diseases is therefore necessary to researchers. The major part of the listed plants were not the object of phytochemical study yet[14] and one of the problems bound to the use of medicinal plants stays the determination of the lethal doses 50, 100 and the maximal tolerated dose to avoid possible acute poisonings of the body.[11,22]

Several of these medicinal plants being in Côte d'ivoire, our study concerned *Erythroxylum emarginatum*, a plant belonging to the family of *Erythrolaceae*. The ethnobotanic investigation and the meditative bibliographical data allowed us to know that the leaves of this plant are used in the form of decoction by the populations to relieve the pains during arthritises, and asthma[7, 17] and would abound in many biocompounds.

In order to study the pharmacological activities bound to this plant, we studied the phytochemical composition and the general acute toxicity of the aqueous and ethanolic extracts.

MATERIELS AND METHOD

Collection of plant materials

The bark of stems and the leaves of *Erythroxylum emarginatum* were collected to Kami on 2013 in the region of Bouaflé, a city situated in the western center of

Côte d'ivoire. After identifying at the National Floristic Center at the Félix Houphouët Boigny University, these plant parts were dried at room temperature for two weeks and sprayed with an electric grinder type IKA- MAG®.

Preparation of extracts

The extracts were prepared according to the extraction method used by **Guédé-Guina and al. (1993)**. 1 liter of distilled water was added in 100 grams of the leaves or the bark of stems powder. The obtained mixture was homogenized by means of a magnetic agitator during 24 hours. The homogenate was filtered successively twice on some cotton wool and then once on Whatman paper 185 millimeters of diameter. The collected filtrate was put in steam room at 500°C. The obtained powder constituted the aqueous extract used for the characterization of biocompunds and the preparation of the various concentrations of products for acute toxicity studies. The ethanolic extract was prepared in the same way as the aqueous extract but by using 700 ml of ethanol and 300ml of water.

Phytochemical screening

***(a)* Colorimetric method**

Large families of secondary metabolites have been sought in the plant following the conventional characterization methods.[4, 11, 15, 16, 21]

Test for Alkaloids

The extract was warmed and 1% hydrochloric acid was added. The mixture was allowed to stand for two minutes and was filtered. A small amount of dragendorff's reagent was then added. The solution was then observed for a reddish-brown colour with a high turbidity when it was added.

Test for Phenol

To 1ml of the leaf extract and 1ml of tuber extract 2ml of distilled water was added followed by a few drops of 10% ferric chloride. Formation of blue or black colour indicates the presence of phenols.

Test for Flavonoids

To the extract, two drops of ammonia (NH3) was added and observed for colour change.

Test for Tannins

Two milliliters of water and few drops of 1% ferric chloride were added to 1mL of extract contained in a test tube. The appearance of a blue, blue-black or black coloration indicates the presence of gallic tannins, the green or dark green coloration shows the presence of catechic tannins.

Test for Steroids

Few drops of concentrated tetraoxosulphate acid (H_2SO_4) was added in drops to the extract and was observed for a colour change.

Test for Leucoanthocyanins

One milliliter of extract was added to 5mL of hydrochloric alcohol. The preparation was heated in the water-bath for15minutes. A cherry red or purplish coloration meant the presence of leucoanthocyanins.

Test for Quinones

An aliquot of extract was dissolved in 5mL of diluted HCl (1/5) and heated in a boiling water bath for 30minutes, and then extracted with 20mL of $CHCl_3$ after cooling. To the organic phase was added 0.5mL of 50% NH4OH diluted solution. The positivity of the reaction was indicated by a red to violet indicates a positive reaction.

Test for Saponins

The extract solution was shaken with 5 ml distilled water and heated to boiling point and was observed for frothing formation.

***(b)* Thin layer chromatography (TLC)**

The support used in this study is an aluminum plate of silica gel 20×20cm (Silica gel 60 F_{254}). A single system of migration was used for the realization of the TLC, it was the n-butanol / glacial acetic acid / water (50/10/40). This method was used by Békro Y A and al, on 2007 with modifications [5]. Three mixtures of compounds were used as standards: sparteine-atropine-reserpine-quinine- codeine for alkaloid compounds, gallic acid-quercetine-rutine for the phenolic compounds, linalool-digitoxin-betacaroten for terpenic compounds.

The visualization of the tasks was made by an UV leading lamp (Fisher Bioblock Scientific®) for 254 nm and for 365 nm and the revelators were the regent of dragendorf for alkaloid compounds, the regent of Folin-Ciocalteu for the phenolic compounds, sulphuric anisaldehyde for terpenic compounds, the chloride of aluminum ($AlCl_3$) for flavonoids, the aqueous (10%) solution of ferric chloride ($FeCl_3$ / H_2O) for tannins, the regent of Borntrager for coumarins and quinones. After the visualization, the plates were photographed and we calculated the migration front of compounds and compared to the standards migration.

Animal material

The experimental animals used to evaluate the general acute toxicity of the aqueous and ethanolic extracts of *Erythroxylum emarginatum* were Wistar strain rats of average weight 90 ± 0,31 grams and aged 10 weeks supplied by the pet shop of the Superior teachers training college (ENS) of the Félix Houphouët Boigny University. The rats were placed in plastic cages containing shavings of wood renewed every 3 days. They were transferred and acclimatized during approximately 4 weeks to the pet shop of the pharmaceutical and biological sciences department of Félix Houphouet Boigny University.

The rats were put on an empty stomach 24 hours before administration of the extracts.

Evaluation of the acute toxicity

The study of acute toxicity was realized according to the guideline of the OECD 423 (Organization of Cooperation and Economic developments).[18] The different administered solutions were prepared the day before. We established with the aqueous extract four (4) lots of three (3) rats with a lot witness and for the ethanolic extract five (5) lots of three (3) rats with a lot witness.

The rats of the lot witnesses received 0,5ml from physiological solution NaCl (0,9%).

Other lots of rats received concentrations of aqueous and ethanolic extracts of *Erythroxylum emarginatum* according to the middle weight of the lots of rats (**table I**). The administration by intra-peritoneal way was retained for the injection of the solutions with sterile syringes. The various dilutions obtained from the two extracts were respectively 50mg / kg, 300mg / kg and 2000mg / kg of body weight for the aqueous extract and 50mg / kg, 300mg / kg, 2000mg / kg and 5000mg / kg for the ethanolic extract.

The quantity of extract of *Erythroxylum emarginatum* to inject was 100mg for 1000g of body weight of animal and the volume of solution to be injected was 1ml by animal.

The handled animals were submitted to a continuous observation for 24 hours to count the deaths and note the clinical signs observed for every lot.[19]

The parameters of acute toxicity were the Maximal Dose Tolerated (MDT), the Lethal Dose for 50% of effect (LD_{50}) and the Lethal Dose for 100% of effect (LD100). The MDT is an estimated value, and the values of the LD_{50} and the LD_{100} were obtained from the curve of TREVAN[23] given by the percentage of mortality of rats according to the decimal logarithm of administered doses.[20]

RESULTS AND DISCUSSION

Phytochemical Screening

The phytochemical screening showed that the bark of stems and the leaves of *Erythroxylum emarginatum* extracts contain alkaloids, flavonoids, polyphenols, tannins (catechic and Gallic tannins)**(Fig.1)**, saponins, polyterpenes, steroids, leuco-anthocyanins and quinones **(table 1)**. The chromatographic plates confirmed the presence of these biocompounds and revealed another biocompound such the coumarins which have an anti-inflammatory power[13] (**Table 2) (Fig.2)**. The phytochemical screening of the aqueous and ethanolic extracts of bark of stems and leaves of *Erythroxylum emarginatum* indicated that the bark of stems is richer in alkaloids and flavonoids than the leaves which are usually used to treat arthrisis and asthma (**Table1**). The bark of stems and the leaves ethanolic extract of *Erythroxylum emarginatum* lacked tannins (catechic and gallic) and saponins (**Table1**). A study led by Nishiyama and al on 2007, showed that the leaves of *Erythroxylum emarginatum* lacked alkaloids.[17] Contrary to this study, our work showed the presence of small quantities of alkaloids in leaves.

Evaluation of the acute toxicity

After the injection of the various aqueous and ethanolic extracts we observed clinical signs and deaths in the different lots of rats (**Table 3 and 4**). The projections made on the curve of TREVAN allowed to determine the parameters of acute toxicity (**fig.3**).[6, 10] The study of the acute toxicity of the aqueous and ethanolic extracts of *Erythroxylum emarginatum* allowed observing certain clinical signs to the rats which received doses of these extracts. These clinical signs are function of doses administered, so we observed, the sleep, the shiver, the lack of appetite, the accelerated breath, the abdominal constriction and the paralysis of the posterior members, these signs were also observed by Djyh GB and al. on 2010 during the evaluation of the acute toxicity of the total aqueous extract of barks of *Mansonia altissima* (bois bété) to mice.[9] For the same dose of extract, the aqueous extract is more toxic than the ethanolic extract. So with a dose of 2000mg / kg of body weight of animal, we obtained 100% of mortality for the aqueous extract against 66,66% of mortality for the ethanolic extract. The various tests of toxicity on aqueous extracts and ethanolic of *Erythroxylum emarginatum* allowed us to obtain the following results, the maximal tolerated dose was 300mg / kg of body weight for both extracts and the lethal dose 50 was 1939,40mg / kg of body weight for the ethanolic extract and 1727,27mg / kg of body weight for the aqueous extract. The lethal dose 100 was 5000mg / kg of body weight for the ethanolic extract and 2000mg / kg of body weight for the aqueous extract. In toxicology, it is known that a pharmacodynamic substance which the LD50 is meanwhile situated 500 in 5000 mg / kg of body weight is moderately toxic.[8] According to this classification, the aqueous and ethanolic extracts of *Erythroxylum emarginatum* administered by intra-peritoneal way are moderately toxic.

Table 1: Phytoconstituents of the aqueous and ethanolic extracts of bark of stems and leaves of *Erythroxylum emarginatum*

Sr. No	phytoconstituents	Aqueous extract		ethanolic extract	
		Barks	Leaves	Barks	Leaves
1	Alkaloids Dragendof	+ + +	+ +	+ + +	+ +
2	Alkaloids Bouchardat	+ + +	+ +	+ + +	+ +
3	Polyphenols	+ + +	+ + +	+ + +	+ + +
4	Flavonoids	+ + +	+	+ + +	+
5	Catechic tannins	+ +	+ + +	-	-
6	Gallic tannins	-	+ + +	-	-
7	Polyterpenes and sterols	+	+	+ +	+ + +
8	Leucoanthocyanins	+ + +	+ +	+ +	+
9	Quinones	+ + +	-	+ + +	-
10	Saponins	+ +	-	-	-

-: Absence; +: Weak presence; + +: Average presence; + + +: Strong Presence

Table 2: Characterization of the phytoconstituents present in the bark of stems and the leaves extracts of *Erythroxylum emarginatum* on TLC

Sr. No	Mobile phase: n-butanol / glacial acetic acid / water (50/10/40)			
	Revelators used	Visible	UV/254-365 nm	Currently detected phytoconstituents
1	Sulfuric Anisaldehyde	-	Blue-purple, brown	Terpenes
2	Reagent of Folin-Ciocalteu	-	Blue, yellow	Polyphenols
3	$AlCl_3$	yellow	Blue, yellow green	Flavonoids
4	Reagent of Dragendorf	Orange	Orange	Alkaloids
5	Reagent of Borntrager	-	Blue-purple	Coumarins
6	Reagent of Borntrager	-	Blue	Quinones
7	$FeCl_3/H_2O$	Brown	Green-brown	Tannins

Table 3: Clinical Signs observed during the first 24 hours after injection of the aqueous and ethanolic extracts of *Erythroxylum emarginatum*

Sr. No.	Clinical signs	Aqueous extract of *Erythroxylum emarginatum*				Ethanolic extract of *Erythroxylum emarginatum*				
		Lot 1 (witness)	Lot 2 (2000 mg/kg BW)	Lot 3 (300 mg/kg BW)	Lot 4 (50 mg/kg BW)	Lot 1 (witness)	Lot 2 (5000 mg/kg BW)	Lot 3 (2000 mg/kg BW)	Lot 4 (300 mg/kg BW)	Lot 5 (50 mg/kg BW)
1	Abdominal constrictions	-	x	-	-	-	x	x	-	-
2	Immobility	-	-	-	-	-	-	-	-	-
3	Feed	x	-	x	x	-	x	x	-	-
4	Accelerated respiration	-	x	-	-	-	x	x	-	-
5	Paralysis of the hind limbs	-	-	-	-	-	x	-	-	-
6	Tremor	-	x	-	-	-	x	-	-	-
7	Sleep	-	x	x	x/-	-	x	x	x/-	-

X: Presence of signs; -: Absence of signs; **x/-**: some have this sign
BW: Body Weight

Table 4: Acute toxicity parameters determination

Sr. No.	Plant extracts	Aqueous extract of *Erythroxylum emarginatum*				Ethanolic extract of *Erythroxylum emarginatum*				
1	Lots of rats	Lot 1	Lot 2	Lot 3	Lot 4	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5
2	The number of rats used	3	3	3	3	3	3	3	3	3
3	Doses injected (mg/kg of body weight)	0	2000	300	50	0	5000	2000	300	50
4	logarithms of the doses injected	0	3,30	2,47	1,69	0	3,69	3,30	2,47	1,69
5	Number of dead rats	0	3	0	0	0	3	2	0	0
6	Percentages (%) of mortality	0	100	0	0	0	100	66,67	0	0

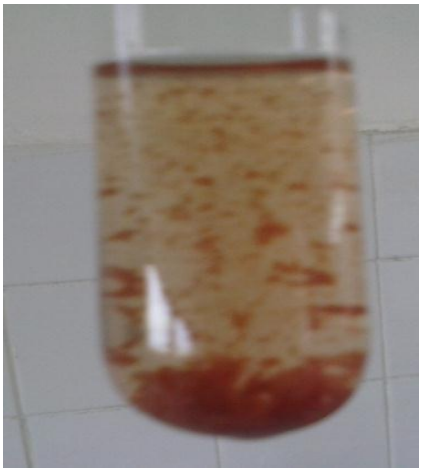

Fig. 1: Catechic tannin characterization in the leaves aqueous extract of *Erythroxylum emarginatum*

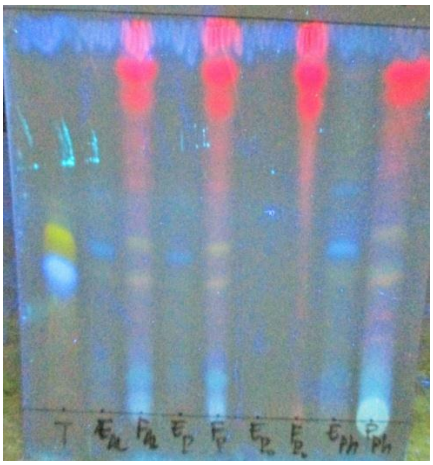

Fig.2: Characterization of flavonoids present in the bark of stems and leaves extracts of *Erythroxylum emarginatum* on thin layer chromatography

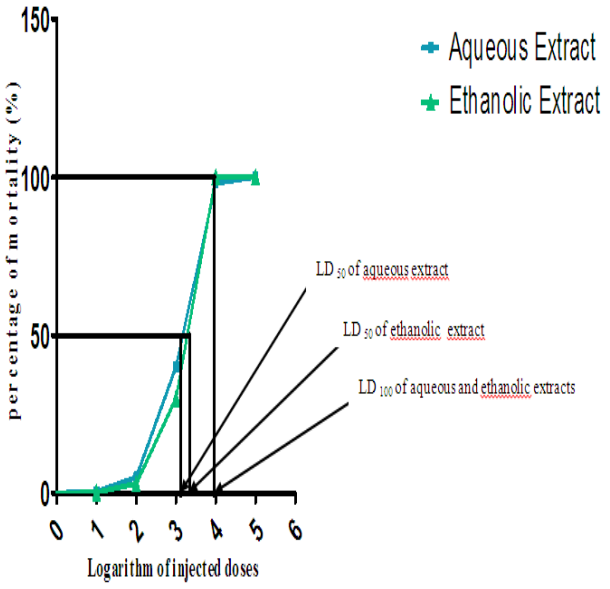

Fig.3: Curves of the evolution of the mortality of rats depending on the doses of the aqueous and ethanolic extracts of *Erythroxylum emarginatum*

CONCLUSION

The present work allowed us to know the phytochemical composition of the aqueous and ethanolic extracts of bark of stems and leaves of *Erythroxylum emarginatum*.

The methods used for this phytochemical sceening (colorimetric method and the TLC) showed that *Erythroxylum emarginatum* is rich in polyphenols and alkaloids in leaves and bark of stems. However the content in polyphenols is more important in the bark of stems than in the leaves. The study of the acute toxicity of *Erythroxylum emarginatum* showed that the aqueous and ethanolic extracts administered by intra-peritoneal way are moderately toxic. The aqueous extract turns out more toxic than the ethanolic extract for the same administered doses. The knowledge of the toxicological parameters of *Erythroxylum emarginatum* is going to allow its use without risk for the populations for the management of diseases. The presence in large quantities of some phytocompounds such as alkaloids, polyphenols and flavonoids allows us to understand it use by the populations to relieve the pains during arthritises and asthma. We shall envisage during our work, to study the anti-inflammatory and antioxidant activities of this plant.

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