

Chemical component and acute toxicity study of *Erythrococca anomala* (Euphorbiaceae)

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ABSTRACT :

Erythrococca anomala (Juss. ex. Poir) Pain (Euphorbiaceae) is a medicinal plant widely used in sub-saharan Africa. It is popular against certain diseases such as malaria, arthritis, rheumatism and toothache. However, there are no data on its phytochemical and biological profile, hence the importance of this study is to search for chemical groups of this plant and to determine the toxicological parameters that justify its use in the traditional medicine. Standard characterization methods and thin layer chromatography (TLC) were used for the phytochemical screening. The acute toxicity study of *Erythrococca anomala* was performed according to the guideline OECD 423 using Wistar rats. Phytochemical screening revealed the presence of polyphenols, alkaloids, catechol tannins, saponins, leucoanthocyanins, flavonoids, polyterpenes and sterols which could justify the biological and pharmacological properties of this herb. The acute toxicity study of the extracts, administered intraperitoneally in Wistar rats, was used to determine the 50% lethal dose (LD₅₀) value to be 741.31 mg/kg of body weight (BW), 100% lethal dose (LD₁₀₀) corresponding to 2000 mg/kg BW and maximum tolerated dose (MTD) to 700 mg/kg BW. These toxicological data allow us to qualify *Erythrococca anomala* at very low toxic hence its importance in the traditional use against malaria and multifaceted pain.

Keywords: *Erythrococca anomala*, Phytochemical screening, Secondary metabolites, Acute toxicity, Côte d'Ivoire.

INTRODUCTION:

Erythrococca anomala (Juss. ex Poir.) Prain (Euphorbiaceae), thorny shrub and dioecious, up to 3m high, is a nutritional and medicinal plant in sub-Saharan Africa. In Côte d'Ivoire, leaf powder, alone or mixed with that of *Psychotria peduncularis* is used by friction against malaria and meningitis.^{1,2} In Cameroon, the decoction or maceration of leaves, laxative and purgative, is taken to expel tapeworms or treat dental pain while in Nigeria the bark is used against arthritis and rheumatism. The fruit pulp and root bark are also used as a tonic against asthenia.³ *E. anomala* is used successfully in Africa on the basis of experiences of populations. In order to rationalize its use, we have initiated this study to search for the major chemical groups and assess to the acute toxicity of aqueous and hydroethanolic 70% extracts of *Erythrococca anomala*. Both lethal doses 50% (LD₅₀) and 100% (DL₁₀₀) and the maximum tolerated dose (MTD) are also determined.

MATERIAL AND METHODS

Plant material

The plant material consists of leaves, roots and stem bark of *Erythrococca anomala* harvested in the Yakassé-mé area in the Department of Adzopé and identified at the Centre National de Floristique of Félix Houphouët Boigny University where a sample of this plant has been kept (OAT -ErAn).

Animals

Adult Wistar rats weighing between 117.0 and 120.8 g were used for evaluation of the total acute toxicity of *Erythrococca anomala* extracts using intraperitoneal method. These rats were from the pet store of the Ecole Normale Supérieure d'Abidjan (ENS Côte d'Ivoire) and fed by pellets. Two weeks before the experiment, they were transferred and acclimatized to the animal facility of the Faculty of Pharmaceutical and Biological Sciences of Félix Houphouët Boigny University.

Extract preparations

The aqueous extract was prepared from 100 grams of organ powder (leaf, root or stem bark) of *Erythrococca anomala* in 1 L of boiling distilled water for fifteen minutes. The ob-

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tained solution was filtered through cotton wool and then under vacuum with Whatman paper filter No. 4. The collected filtrate was dried in an oven at 40 ° C. The obtained powder was used to get the crude total aqueous extract of *Erythrococca anomala*.

The method of Guédé-Guina ⁴ was used to obtaining the hydroethanolic 70% extract of *Erythrococca anomala*. One liter of hydroethanolic solution 70% (EtOH/H₂O, 70:30) and 100 g of *Erythrococca anomala* organ powder were used for this purpose. The resulting mixture was homogenized using a magnetic stirrer for 24 hours. The solution was filtered through cotton wool and then under vacuum in the same conditions as previously. The filtrate collected was concentrated in a rotarvapor and then dried in an oven at 40 ° C. The obtained powder constituted the hydroethanolic 70% extract of *Erythrococca anomala*.

Phytochemical screening

The phytochemical study was performed from the aqueous and 70% hydroethanolic extracts of *Erythrococca anomala* using standard reactions and thin layer chromatography (TLC). The phytochemical screening was to characterize the chemical groups in the total plant extracts and likely to possess biological activities. Thus, chemical groups such as alkaloids, polyphenols, flavonoids, polyterpenes, tannins and saponins were searched in the extracts of leaves, roots and stem bark of *Erythrococca anomala* using the methods described by Trease and Evans.⁵ Confirmation of the presence of chemical groups was performed by TLC (Alufolien 60F254 Merck) according to the methods described by Wagner *et al.*⁶ In general, the same chemical reagents were used as revealing reagents. The Fast Blue B (FBB) and ferric chloride have also been used, giving the spots corresponding to Indian brown for flavonoids. For polyterpenes and sterols, the sulfuric vanillin was used giving a polychrome plate after heating at 100 ° C. The revelation of saponins was made from the characteristic spots, after spraying and heating the plate, corresponding to the violet color compared to the reference (the escin). Furthermore, the detection of alkaloids was achieved by spraying the Dragendorff reagent giving orange spots. For cardiotoxic compounds, they were revealed through Kedde reagent that gave purple pink spots.

Acute toxicity study

Acute toxicity study was performed according to the guideline of the Organization for Economic Cooperation and Development (OECD 423).⁷ Sodium chloride (NaCl) 0.9% was used for the preparation of different concentrations of *Erythrococca anomala* extracts. The concentrations of aqueous and 70% hydroethanolic extracts were prepared taking into account the rate body weight (BW) and the amount of product to be injected was expressed in mg/kg BW. The average weight of the rats was 118.9 ± 0.08 grams. The rats were fasted for 24 hours before administrating the different doses of *Erythrococca anomala* extracts. For an injectable dose of 100 mg/kg BW, an amount of 1 mL of saline extract solution corresponding to 11.89 mg/mL of extract need to be inject to each rat.

Aqueous extract

Twelve rats were divided into 4 groups including a control group (group 1). Each rat of the control group received 1 ml of 0.9% NaCl by intraperitoneal method. Rats of lots 2 to 4 have received various concentrations of aqueous extract by intra-peritoneal route starting with the maximum dose of 5000 mg/kg BW according to:

- Lot 2 → dose of 5000 mg/kg BW
- Lot 3 → dose of 2000 mg/kg BW
- Lot 4 → dose of 300 mg/kg BW

Thus, the animals treated were observed for 14 days with a special focus during the first 24 hours in order to meet the clinical signs and possible deaths in each lot.

Hydroethanolic extract

Doses of 2000 and 5000 mg/kg WB were used. Thus, 9 rats average weight of 118.9 g were divided into 3 lots with a control group (group 1). Each rat in the control group received 1 mL of 0.9% NaCl intraperitoneally while the rats of lots 2 and 3 received the various concentrations of hydroethanolic 70% extract of *Erythrococca anomala* intraperitoneally:

- Lot 2 → dose de 2000 mg/kg WB,
- Lot 3 → dose de 5000 mg/kg WB.

RESULTS

The phytochemical screening revealed that the chemical groups contained in the aqueous and hydroalcoholic extracts of leaves, roots and bark of *Erythrococca anomala*. These chemical groups were polyphenols, leucoanthocyanins, alkaloids, saponins, flavonoids, tannins, polyterpenes and sterols. TLC allowed the confirmation of these chemical groups. Flavonoids were particularly found as genin and glycosyl. However, there is no cardiotoxic compound in the extracts (**Table 1**). In general, all aqueous extracts contained alkaloids, flavonoids, polyphenols, saponosides, leucoanthocyanins, polyterpenes and sterols. However, that of leaves seems to be the richest in alkaloids, flavonoids and polyphenols. Furthermore, the saponins, the leucoanthocyanins, polyterpenes and sterols were present in the roots. Gallic and catechin tannins were absent from these extracts. For hydroethanolic extracts, we found catechin tannins in addition to previous chemical groups. Alkaloids, polyphenols, leucoanthocyanins, polyterpenes and sterols were present in roots, leaves and stem bark. However, there were no saponins and gallic tannins in these hydroalcoholic extracts.

Acute toxicity

After administrating the hydroethanolic 70% extract of *Erythrococca anomala* to the rats at 2000 and 5000 mg/kg BW, there was no significant change in the behavior of these rats and no mortality was recorded during 14 days. However with the aqueous extract, various clinical signs were observed in the first 24 hours after administration of the extract (**Table 2**). Deaths have also been reported during the first 24 hours at 2000 and 5000 mg/kg BW. It was only at the dose of 300 mg/kg BW that no death was recorded during 14 days (**Table 3**). Thus, the data in **Table 2** allowed to represent the evolution of mortality of rats based on dose of leaves aqueous extract of *Erythrococca anomala* injected (**Figure 1**).

Table 1. Phytochemical screening results

			Alkaloids	Flavonoids	Polyphenols	Polyterpenes sterols	Saponins	Leuco-antho-cyanins	Tannins	Cardiotonic compounds
TUBES	Stem bark	AE	++	-	-	+	++	+	-	ND
		HE	-	-	-	+	-	-	-	ND
	Leaves	AE	++++	+++	+++	+	++	+++	-	ND
		HE	++	++	++	+	-	++	-	ND
	Roots	AE	+	+	-	++	+++	++++	-	ND
		HE	+	++	++	++	-	++++	+	ND
TLC	Stem bark		+	+	+	+	ND	ND	ND	-
	Leaves		++	++	++	++	ND	ND	ND	-
	Roots		+	+	+	+	ND	ND	ND	-

AE: Aqueous extract HE: Hydroethanolic extract ND: Not detected

- Absence ; + low presence ; ++ Average presence ; +++ High presence

Table 2. Clinical signs observed during 24 h after leaves extracts injection

Signes cliniques	Aqueous extract dose injected (mg/kg BW)				Hydroethanolic extract dose injected (mg/kg BW)		
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 1	Lot 2	Lot 3
	0	300	2000	5000	0	2000	5000
Constrictions abdominales	-	-	+	+	-	-	-
Immobilité	-	-	+	+	-	-	-
Respiration accélérée	-	-	+	+	-	-	-
Paralysie des membres postérieurs	-	-	+	+	-	-	-
Alimentation	+	+	-	-	+	+	+

+: Presence of sign -: Absence of sign

Table 3. Rat mortality versus dose of leaves extract injected

	Aqueous extract				Hydroethanolic extract		
	Lot 1 (Control)	Lot 2	Lot 3	Lot 4	Lot 1 (Control)	Lot 2	Lot 3
Rat number	3	3	3	3	3	3	3
Injected doses (D) (mg/kg BW)	0	300	2000	5000	0	2000	5000
LogD	-	2.47	3.30	3.69	-	3.30	3.69
Number of dead rats	0	0	3	3	0	0	0
(%) mortality	0	0	100	100	0	0	0

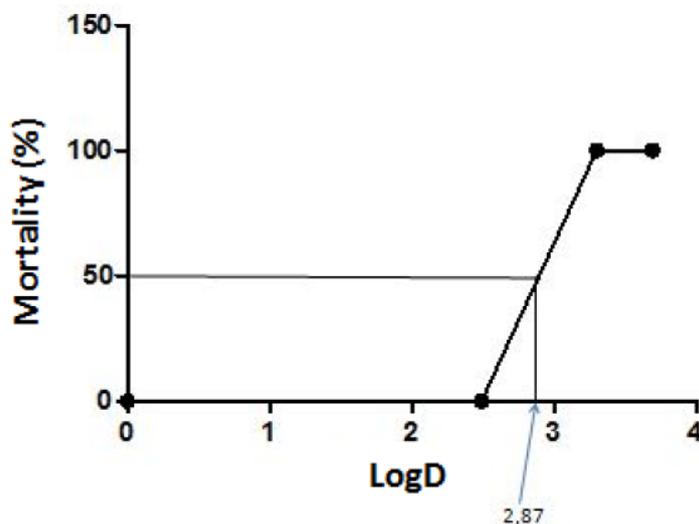


Figure 1. Curve of rat mortality versus dose of leaves aqueous extract injected

Toxicological parameters

Method OECD 423 used is not intended to determine the precise value of the LD₅₀ but direct it within a specific range in order to find it in the category of the Globally Harmonized Classification System (GHS). The curve of rat mortality percentages versus logarithm of injected dose was used to determine the LD₅₀ value. The value of 741.31 mg/kg BW was obtained for aqueous extract of *Erythrococca anomala* leaves (Figure 1). For the hydroethanolic extract leaves, LD₅₀ value was greater than 5000 mg/kg BW.

DISCUSSION

The phytochemical study of aqueous and hydroalcoholic extracts of *Erythrococca anomala* revealed that this plant contained alkaloids, polyphenols, flavonoids, saponins, polyterpenes and sterols. The rich of these extracts of *Erythrococca anomala* in active chemical compounds could explain its use in traditional therapy. Indeed, this plant material contained a large number of molecules that have multiple interests in industry, food, cosmetics and dermatopharmacy. These compounds including alkaloids, polyphenols, tannins, flavonoids and polyterpenes. Many studies suggested that flavonoids have anti-inflammatory properties that could modulate the immune system.⁸⁻¹⁰ Moreover, many flavonoids were able to reduce the production of oxygen species.⁸ Among the antioxidants, many authors have highlighted the important role of polyphenols.¹¹ Flavonoids were likely to react with the most reactive oxygen species.¹² Other pharmacological effects are attributed to alkaloids such as analgesic, anticholinergic, antimalarial, antihypertensive, antitussive, central stimulant, depressant cardiac, diuretic, anti-tumor and sympathomimetic.¹³ Tannins allowed healing, sealing of the skin and mucous membranes, and promoted vasoconstriction of small vessels.¹⁴ The saponins have expectorant activity by making some foaming mucosal inflammatory bronchi to facilitate expectoration. In addition, they were powerful haemolysing agents. They also had sweetening properties and were widely used in food.¹⁵

The acute toxicity study has allowed the determination of the toxicological parameters of the aqueous extract of leaves of *Erythrococca anomala* administered intraperitoneally. There are the maximum tolerated dose (MTD, 700 mg/kg BW), the 50% lethal dose (LD₅₀, 741.31 mg/kg BW) and 100% lethal dose (LD₁₀₀, 2000 mg/kg BW). Concerning the hydroethanolic 70% extract of the leaves, no death was observed at 2000 and 5000 mg/kg BW. This difference in toxicity may be due to the fact that the whole (leaf powder + distilled water) heated to boiling for 15 minutes allowed the dissolution of a larger quantity of toxic substances stored in the cell mucilages, especially saponin^{4,15} that were almost absent in the hydroethanolic extract. However, these toxicological data allowed to classify, according to the GHS, the aqueous extract of *Erythrococca anomala* leaves in category 4 and define the toxicity of the substance as low according to Hodge and Sterner Scale.¹⁶ According to the same scale, the 70% hydroethanolic extract of the leaves was practically non-toxic.

CONCLUSION

This study allowed us to realize the characterization of certain chemical compounds in aqueous and ethanolic extracts of *Erythrococca anomala*. The main active compounds identified were flavonoids, alkaloids, leucoanthocyanins, saponins, tannins catechin, polyphenols, polyterpenes and sterols. The presence of these compounds could confer to this plant certain biological activities. The study of acute toxicity of aqueous extracts and hydroethanolic 70% of the leaves of *Erythrococca anomala* has allowed the determination of the toxicological parameters such as the maximum tolerated dose, the 50% lethal Dose and 100% lethal Dose. This study also identified the low toxicity and virtually non-toxic characters of aqueous and hydroethanolic extracts of the leaves, respectively. In view of the toxicological data, the leaves of *Erythrococca anomala* may be considered a great hope in the treatment of ailments such as malaria, dysentery, meningitis, dental pain, arthritis and rheumatism.

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