TRIPHYTHOCHEMISTRY AND STUDY OF ACUTE TOXICITY OF AQUEOUS AND HYDROETHANOLIC EXTRACTS OF *PAVETTA CORYMBOSA* LEAVES (Rubiaceae).

Comment [et1]: the title as written has no meaning, we propose to instead write phytochemistry and evaluation of the acute toxicity of aqueous and hydroacetonic extracts of***PAVETTA CORYMBOSA*** **LEAVES (Rubiaceae).**

Abstract

Pavettacorymbosa (Rubiaceae) is a plant widely known in sub-Saharan Africa in the field of traditional medicine.It is used empirically against certain pathologies such as malaria, diabetes, hemorrhages and typhoid fever.However, there is no scientific data on its phytochemical and biological profile, so this study was carried out to research the chemical groups contained in the leaves of this plant and determine the toxicological parameters which would justify its use in traditional medicine.Several standard characterization methods were used for phytochemical screening of aqueous and70% hydroethanolic extractsof Pavettacorymbosa leaves.The study of the acute toxicity of the two types of extracts from the leaves of this plant was carried out according to the OECD guideline 423 using Wistar rats.Phytochemical screening indicated the existence of polyphenols, saponosides, quinones, flavonoids, polyterpenes and sterols in the two types of extracts of this plant, which would justify its biological and pharmacological effects.The study of the acute toxicity of these two types of extracts, administered intraperitoneally to Wistar rats, showed no mortality of the rats.These toxicological results allow us to qualify the leaves of *Pavettacorymbosa* as being non-toxic, hence the interest in its use in traditional medicine against certain pathologies.

Comment [et2]: these statements are not proven, we suggest you review the literature on the plant you will find many studies on phytochemistry, as well as the biological potential of this plant

Comment [et3]: give ideas about the methods used for the characterization

Comment [et4]: these observations are not enough to reach to this conclusion. You must go deeper

Keywords: *Pavettacorymbosa*, phytochemical screening, acute toxicity, chemical groups.

Introduction

The *Pavettacorymbosa* shrub is a medicinal plant from sub-Saharan Africa which reaches approximately four (04) meters high.In Burkina Faso, Benin and Togo, the powder is used successfully for the treatment of diabetes, salmonellosis and hemorrhages[1].In Ivory Coast, a decoction of the leaves is used against malaria [2].Based on people's experiences, these same sheets are used in the case of open wounds [3].To justify its traditional use, this study was

Comment [et5]: leaves or sheets? English has to be strengthen

initiated with the aim of searching for the major chemical groups and evaluating the acute toxicity of aqueous and hydroethanolic extracts of *Pavettacorymbosa* leaves.

Comment [et6]: Aliyu and al, 2017 already revealed the presence of different metabolites in the lipophilic extract of the plant for instance

I. Material and Methods

I.1.Plant material

The plant material consists of *Pavettacorymbosa* leaves collected in the Grand-Bassam area and identified at the National Floristic Center of Félix HouphouëtBoigny University, in Ivory Coast.

Comment [et7]: Does the plant have a voucher number? Give it and precise the name of the Professor or the personal the made the authentication

I.2.Animal material

Adult female ratsof the Wistar variety with average weights between 120 and 130g were fasted for 24 hours then used for the acute toxicity test by intraperitoneally route of aqueous and 70% hydroethanolic extracts of *Pavettacorymbosa* leaves.These spleens were provided by the animal store of the Faculty of Pharmaceutical and Biological Sciences of Félix Houphouët-Boigny University.

I.3.Preparation of the two types of extracts

The aqueous extract was prepared from 100 grams of *Pavettacorymbosa* leaves powder in 1 L of boiling distilled water for ten minutes.The mixture obtained was filtered through cotton, then under vacuum with the Whatman No.4 paper filter.The filtrate obtained was dried in an oven at 40°C which constituted the crude total aqueous extract of *Pavettacorymbosa*.As for the 70% hydroethanolic extract, the Guédé-Guina[4] method was used.Thus 100 g of *Pavettacorymbosa* leaves powder was used for this purpose.The resulting mixture was homogenized using a magnetic stirrer for 24 hours.The solution was filtered through cotton then under vacuum under the same conditions as above.The filtrate obtained was concentrated on a rotary evaporator then dried in an oven at 40°C.The residue obtained constituted the 70% hydroethanolic extract of *Pavettacorymbasa*.

I.4.Phytochemical study

The phytochemical study of the aqueous and70% hydroethanolic extracts of *Pavettacorymbosa* leaves was carried out using standard reactions for the characterization of chemical groups.These included chemical groups such as polyphenols, flavonoids, polyterpenes, quinones, saponosidestannins and alkaloids using the methods described by Trease and Evans[5].The detection of sterols and polyterpenes was carried out using the

Liebermann reaction.The characterization of compounds belonging to the polyphenol group was carried out by the reaction with ferric chloride.The flavonoids were revealed by the cyanidrin reaction.The presence of tannins is highlighted by the iron trichloride solution.The appearance of a dark green or blue-green color indicates the presence of tannins.The general characterization of the alkaloids was carried out using the Dragendorff reagent.The search for saponosides was based on the property of aqueous solutions containing saponosides to foam after stirring.The presence of quinones was confirmed by a change to yellow following the addition of a few drops of NaOH.

I.5. Acute toxicity study

The acute toxicity of the two types of extracts was carried out according to the guideline of the Organization for Economic Cooperation and Development (OECD 423) [6]. The 0.9% sodium Dchloride (NaCl) solution was used for the preparation of different concentrations of *Pavettacorymbosa*extracts. The concentrations of aqueous and 70% hydroethanolic extracts were prepared taking into account the average weight of the rats and the quantity of products (mg/kg BW). The average weight of the rats was 120.9 ± 0.07g. The animals were fasted for 24 hours before administration of different doses of *Pavettacorymbosa* extracts and NaCl solution. For an injectable dose of 100 mg/kg body weight, 1 mL amounts of solution of each extract and saline were injected into each lot of rats. Thus lot 1 which is the control received the 0.9% NaCl solution. Lots 2, 3 and 4 respectively received doses of 300, 2000 and 5000 mg/kg of aqueous extract of *Pavettacorymbosa*. Furthermore, lots 5, 6 and 7 received doses of 300, 2000 and 5000 mg/kg of 70% hydroethanolic extract of the plant. The animals thus treated were subjected to continuous observation for 14 days with particular attention during the first 24 hours in order to note the clinical signs and mortalities of each lot.

Comment [et8]: Does the country have an ethical committee for the use of laboratory animals? You must give the reference of the authorization of this committee

II.Results

II.1. Triphytochemistry

Triphytochemistry revealed the presence of several secondary metabolites in the aqueous extracts of *Pavettacorymbosa* leaves;these are sterols and polyterpenes, polyphenols, flavonoids and saponosides.As for the hydroethanolic extract, in addition to the chemical groups mentioned above, there is the presence of quinones **(Table 1).**

Table 1: Triphytochemistry of aqueous and hydroethanolic extracts of *pavettacorymbosa*

Extracts	Sterols and polyterpenes	Polyphenols	Flavonoids	Tannins	Quinones	Alkaloids	Saponosids
Aqueous	+	++	++	-	-	-	++
Hydroéthanolic	++	++	++	-	+	-	+

+ : Presence

- : Absence

II.2.acute toxicity

After administration of aqueous and70% hydroethanolic extracts of *Pavettacorymbosa* toadults female rats at doses of 300, 2000 and 5000 mg/kg body weight, there was no significant change in the behavior of the latter.In addition, no mortality was recorded during the 14 days of observation **(Table 2).**

Table 2: Clinical signs and mortality observed following the administration of different types of extracts

	AqueousExtract			**hydroethanolicExtract**		
Doses injected (mg/kg) bw	300	2000	5000	300	2000	5000

Abdominal constrictions	-	-	-	-	-	-
Immobility	-	-	-	-	-	-
Breathing accelerated	-	-	-	-	-	-
Paralysis of members	-	-	-	-	-	-
Animals feeding	+	+	+	+	+	+
mortality	0	0	0	0	0	0

+ : Correct diet

- : Absence of signs

III.Discussion

Phytochemical exploration highlighted the presence of certain chemical compounds in the aqueous and hydroethanolic extracts of *Pavettacorymbosa* leaves.The main active compounds identified are flavonoids, saponosides, polyphenols, quinones, polyterpenes and sterols.The presence of these metabolites indicates that the two types of extracts express biological activities.Numerous studies indicate that flavonoids have anti-inflammatory properties capable of regulating the functioning of the immune system[7, 8].Furthermore, many flavonoids are capable of reducing the production of oxygen species[9.Among antioxidants, polyphenols are likely to react with most reactive oxygen species[10].Likewise, flavonoids are likely to react with most reactive oxygen species[11].Regarding mortality, no deaths were observed at doses of 300, 2000 and 5000 mg/kg bw of aqueous and hydroethanolic extracts of *Pavettacorymbosa* leaves.The non-toxic nature of the hydroethanolic extract could be explained by the absence of tannins and alkaloids in said extract.The absence of tannins and alkaloids in the hydroethanolic extract would be linked to the presence of ethanol in the hydroethanolic mixture used as solvent;because tannins only dissolve in water[11].The non-toxic nature of the aqueous extract of *Pavettacorymbosa*leaves would be due to the fact that the combination consisting of *Pavettacorymbosa*powder and distilled water could not promote the extraction of toxic substances stored in the mucilage cells[12].The globally harmonized classification system (GHS) according to OECD 4237 classifies aqueous and hydroethanolic extracts of *Pavettacorymbosa* leaves in category 5 and defines them as non-toxic substances according to the Hodge and Sterner scale[13]inrats.

Conclusion

This study allowed the characterization of certain chemical compounds in aqueous and hydroethanolic environments of *Pavettacorymbosa* leaves.The main active compounds present were flavonoids, polyphenols, quinones, polyterpenes and sterols.The presence of these metabolites could confer on this plant certain biological activities of important pharmacological interest.The study of the acute toxicity of *Pavettacorymbosa* leaves made it possible to classify this plant in the category of non-toxic substances.In view of the toxicological results, the leaves of *Pavettacorymbosa* can be considered a great hope in the treatment of pathologies such as malaria, diabetes, salmonellosis and hemorrhages.

References

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