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PHYTOCHEMICAL SCREENING AND TOXICOLOGICAL STUDY OF AQUEOUS AND ETHANOLIC EXTRACTS OF ENTADA MANNII (FABACEAE)

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ABSTRACT

This study aimed to evaluate the acute toxicity and to carry out the phytochemical study of aqueous and ethanolic extracts of *Entada mannii* (Fabaceae) a plant used in the traditional treatment of diabetes in the south-east of Cote d'Ivoire. The various tests carried out in the context of phytochemical screening was oriented towards the identification of the main chemical groups. To evaluate the toxicity, 60 wistar rats were divided into twenty groups containing three animals in each group. Mortality was observed after 24 hours while animal aspect and behavior changes were noted for 14 days. The acute toxicity study indicates that the extracts of *Entada mannii* (Fabaceae) have no toxicity at a dose of 5000 mg / kg. The phytochemical screening of 2 extracts revealed the following main groups: sterols, polyterpenes, polyphenols, flavonoids, alkaloids, saponosides.

KEYWORDS: Entada mannii**,** acute toxicity, rats, phytochemical screening.

INTRODUCTION

The use of plants for therapeutic purposes is a reality and a well-documented practice in all human civilizations since the dawn of time. In Africa and more particularly in Cote d'Ivoire, the contribution of these plants has been decisive in the management of various diseases.

The importance and the richness of this contribution have increased considerably over the years.[1] It is estimated that more than 80% of the African population uses medicinal plants to heal themselves.[2] Faced with this growing craze, a certain caution should be observed, because the lack of knowledge of the doses of the extracts administered empirically as well as those of their biochemical, pharmacological and toxicological properties expose the user populations to real risks of therapeutic accidents which can sometimes be tragic.[3,4,5,6] In addition, mixtures administered by healers most often contain extracts from several plants, posing an additional risk of uncontrolled interactions.[7,8]

Hence the need to pay special attention to the valuation of this pharmacopoeia by studying the toxicity of plants and doses administered empirically. This study aims to make a contribution in this direction through the realization of phytochemical sorting and the study of the acute toxicity of *Entada mannii* (Fabaceae) a plant used in the traditional treatment of diabetes in the south-east of Cote d'Ivoire.

Given the promising results of pharmacological tests, the determination of the main chemical groups present in this plant and the study of its toxicity are necessary and will serve to better rationalize its use.

MATERIAL AND METHODS

Plant material

The barks of *Entada mannii* (Fabaceae) collected from Agboville (south east of Côte d'Ivoire) were identified by the National Floristic Center of University Felix Houphouet Boigny (Cocody-Abidjan). A voucher specimen of the plant has been deposited in this Center herbarium.

Experimental animals

For these experiments we use rats Wistar. Adult wistar rats (60) of both sexes, 6-8 weeks old, weighing 117-290 g and bred at the Department of Biosciences, University Felix Houphouet-Boigny (Abidjan, Ivory Coast), were used for the experiments. The animals were kept in

standard cages with good ventilation, free access to food and water. Experimental procedures and protocols used in this study were approved by the Ethical Committee of Health Sciences of University Felix Houphouet-Boigny (Ivory Coast-Abidjan). These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals.[9]

Preparation of aqueous extracts of *Entada mannii* (Fabaceae)

Barks harvested were air dried at room temperature (28±1 °C) for one month. The dried barks were ground into fine powder. The powder (20 g) was soaked in 500 mL of distilled water with a blender. The mixture was then filtered through the gauze and a second time on Whatman filter paper (3 MM). Evaporation of the solvent was achieved in an oven at 50 ° C. After drying, we get a greenish powder used to prepare the aqueous extract of *Entada mannii* (Fabaceae) (AEEM).

Preparation of ethanolic extracts of *Entada mannii* (Fabaceae)

The dry bark powder (50 g) obtained previously was soaked in 250 mL of 70% ethanol with a blender. The mixture was then filtered through the gauze and a second time on Whatman filter paper (3 MM). Evaporation of the solvent was achieved in an oven at 40 °C. After drying, we get a greenish powder used to prepare the ethanolic extract of *Entada mannii* (Fabaceae) (EEEM).

Experimental protocol

Phytochemical screening protocol

The dosages of the different major chemical groups were carried out according to the specific methods and adapted to each major group. The secondary metabolites targeted in this study are: sterols, polyterpenes, polyphenols, flavonoids, alkaloids and saponosides.[10,11,12,13,14]

Determination of sterols and polyterpenes

The search for sterols and polyterpenes was carried out by the Liebermann reaction. Five (5) ml of each solution was evaporated to dryness without charring, the residue in a water bath was dissolved hot in 1ml of acetic anhydride. The solution was poured into a test tube. Carefully, 0.5 ml of concentrated sulfuric aid is poured along the wall of the tube. The appearance at the interphase, of a purple ring, turning blue then green indicated a positive reaction. The control trial was performed with cholesterol and sitosterol.[10]

Polyphenol search by ferric chloride reaction

To 2 ml of each solution is added a drop of alcoholic solution of 2% ferric chloride. Ferric chloride caused the presence of polyphenolic derivatives by the appearance of a green color more or less dark. The control was performed with an alcoholic solution of gallic acid.

Search for flavonoids by the so-called cyanidin reaction

Two (2) ml of each solution was spray dried in a capsule. It was allowed to cool. The residue was taken up with 5 ml of hydrochloric alcohol half. The solution was poured into a test tube. 2 magnesium chips were added. The pink-orange coloring was observed. The addition of 3 drops of isoamyl alcohol intensified this staining confirming the presence of flavonoids. The control was performed with an alcoholic quercetin solution.

Search for tannins

The tannins are divided into two groups

a. Research catechism tannins by Stiasny reagent

Five (5) ml of each extract was evaporated to dryness in a capsule. Fifteen (15) ml of Stiasny reagent was added to the residue. The mixture was kept in a water bath at 80 ° for 30 minutes and allowed to cool. The observation of precipitation in large flakes characterizes the presence of catechin tannins.

b. Search for gallic tannins

The previous solution has been filtered. The filtrate is collected and saturated with sodium acetate. The addition of 3 drops of FecL3 at 2% caused the appearance of an intense blue-black color showing the presence of gallic tannin.[10,14]

Search for free or combined quinones

The Borntraegen reagent (half-diluted ammonia) revealed the free quinoline substances. For the combined quinoline substances it was necessary to carry out a prior hydrolysis. The test consisted of immediately hydrolyzing the solutions to characterize the free and combined total quinoline substances. Two (2) ml of each extract was evaporated to dryness. In a capsule, 5 ml of hydrochloric acid at 1/5, the residue was triturated. The solution is heated for half an hour in a boiling water bath in a test tube. After cooling, the hydrolyzate is extracted with 20 ml of chloroform in a test tube. The chloroform phase collected in another test tube and 5 ml of 1/2 diluted ammonia was added. The appearance of the coloring from red to

purple indicates the presence of quinones. A control trial was performed with a chloroform solution of anthraquinone.[10,13]

Search for alkaloids

The general reagents of alkaloids are: Dragendorff reagent (sodium iodobismuthate reagent) Bouhardat reagent (iodine-iodide reagent) and Valsen-Mayer reagent (potassium iodomercurate reagent). Six (6) ml of each solution was evaporated to dryness in a capsule. The residue was taken up with 6 ml of alcohol at 60°. The alcoholic solution was divided into 3 test tubes. In the first tube was added 2 drops of Dragendorff reagent. The appearance of precipitate or an orange color indicates the presence of alkaloids. In the second tube was added 2 drops of Bouhardat reagent. The appearance of a precipitate or a reddish-brown color indicates the presence of alkaloids. In the third tube was added 2 drops of Valsen-Mayer reagent. The appearance of a precipitate or a cream-white color indicates the presence of alkaloids.

Search for Saponosides

In a test tube 160 mm high and 16 mm in diameter we added 15 ml of the dissolved extract. After vigorous stirring for 10 s and let stand for 10 minutes the persistence of the foam from a height of more than 4 mm indicated the presence of saponosides.

Acute toxicity study protocol

The acute toxicity study was conducted according to OECD Guideline 423 (Organization for Economic Co-operation and Development).[15-19] Sixty (60) rats weighing between 117 and 290 g were divided into 10 lots per extract, for a total of twenty groups. Group 1 rats received the 5 mg / kg / bw dose of the aqueous extract, while Group 2 rats received the 5 mg / kg / bw dose of the same extract to confirm the first result. Group 3 animals received a dose of 50 mg / kg / bw of the aqueous extract. Group 4 rats received the 50 mg / kg / bw dose of this extract to confirm the first result. Group 5 received 300 mg / kg / bw of the aqueous extract while Group 6 received 300 mg / kg / bw of the same extract to confirm the first result. Group 7 rats received the 2000 mg / kg / bw dose of the aqueous extract. Those in group 8 received the 2000 mg / kg / bw dose of the same extract to confirm the first result. Group 9 received the 5000 mg / kg aqueous extract while Group 10 received the 5000 mg / kg / bw of the same extract to confirm the first result.

This experiment was renewed with the ethanolic extract. All these rats were regularly observed for 14 days according to the OECD recommendations, mortality and symptoms of intoxication (clinical signs) were noted.

RESULT AND DISCUSSION

Phytochemical screening

The results of the chemical screening of the aqueous and ethanolic extracts of *Entada mannii* (Fabaceae) are shown in tables 1 and 2. Both extracts contain: sterols, polyterpenes, polyphenols flavonoids, alkaloids and saponins. There is an absence of catechetical tannins, gallic tannins and quinones in the two extracts.

Table 1: Phytochemical screening tests of aqueous and hydroethanolic extracts of Entada mannii.

Plant organ	Extracts	Sterols and polyterpenes	Polyphenols	Flavonoids	Tanins catechetical
Entanda mannii (Bark)	Aqueous	+	++	++	-
	Ethanolic	++	++	++	-

++ : abundantly present - : absence

+ : present

Table 2: Phytochemical screening tests of aqueous and hydroethanolic extracts of Entada mannii.

Plant organ	Extracts	Gallic tannins	Quinones	Alkaloids	Saponosides
Entada mannii (Bark)	Aqueous	-	-	+	+
	Ethanolic	-	-	+	NT

++ : abundantly present - : absence

+ : present NT : no tested

The results of the phytochemical screening indicate the presence of various chemical groups in the different extracts studied. Thus, it has been noticed that the extracts contain many chemical groups sought in different proportions: sterols, polyterpenes, polyphenols flavonoids, alkaloids and saponins with the exception of catechic tannins, gallic tannins and quinones. Several studies have shown the benefits of these compounds found in the bark of *Entada mannii*. For example, alkaloids, flavonoids, anthocyanins and leucoanthocyanins have antioxidant potency. They promote the regeneration of tissues, reduce the permeability of blood capillaries and increase their resistance to hemolysis[10]. Alkaloids, sterols and terpenes

are compounds that exhibit various activities in plants and have beneficial effects in humans and animals[20] Moreover, the presence of many of these compounds has been demonstrated in several plants from the Ivorian and African pharmacopoeia in general.[21,22,23]

This is how Anthony and al.[24] showed that the methanolic extract of the bark of *Entadrophragama angolense* contained: alkaloids, flavonoids, cardiac glycosides, saponins and terpenoids.[25] Phytochemical screening of aqueous and ethanolic extracts of *Morinda morindoides* leaves revealed the presence of: sterols, polyterpenes, flavonoids polyphenols, alkaloids, saponins, tannin catechins, gallic tannins and quinones.[26]

Acute toxicity

Administration of the aqueous and ethanolic extract of *Entada mannii* did not cause behavioral changes or death in these animals. Clinical signs of acute intoxication such as agitation, aggression, body twisting, convulsions and diarrhea were not observed during the study period (24 hours). However at doses of 5000 (mg / kg Pc), the animals exhibited accelerated breathing, difficult movement and convulsions.

The results of rat mortality according to the different doses of aqueous extract and hydroethanolic administered to the rats, are presented in tables 3 and 4.

Table 3: Variation of the mortality according to the different doses of the aqueous extract Entanda mannii.

LOTS	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6	Lot 7	Lot 8	Lot 9	Lot 10
Number of rats per batch	3	3	3	3	3	3	3	3	3	
Injected doses (mg / kg/pc)	5	5	50	50	300	300	2000	2000	5000	5000
Number of dead animals	0	0	0	0	0	0	0	0	0	0

Table 4: Variation of mortality according to the different doses of the ethanolic extract Entanda mannii.

LOTS	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6	Lot 7	Lot 8	Lot 9	Lot 10
Number of rats per batch	3	3	3	3	3	3	3	3	3	3
Injected doses (mg / kg/pc)	5	5	50	50	300	300	2000	2000	5000	5000
Number of dead animals	0	0	0	0	0	0	0	0	0	0

During the 14 days following the observation, there was no observed mortality even at the 5000 mg / kg body weight dose. All animals were found normal and there was no significant behavioral change until the end of the observation period. This study made it possible to determine the toxicological parameters of the aqueous and ethanolic extract administered by gavage. The estimated LD50 value greater than 5000 mg / kg Pc classifies the aqueous and ethanolic extracts of *Entanda mannii* as low toxicity substance[27] The OECD 423 method used does not refer to the precise value of the LD50, but determines the category of the Globally Harmonized GHS Classification System of the extract[15] The absence of death observed at different doses makes it possible to classify the extracts in category 5 of the GHS. In this category, the LD50 is estimated to be greater than 5000 mg / kg body weight. The maximum tolerated doses of the extracts which are confused here with the LD50 are much higher than the doses necessary to have pharmacological effects.

As a result, the doses used in this study could be tolerated by the body and could be used without harm in humans.

CONCLUSION

From the present study, the phytochemical study of the aqueous and ethanolic extracts of *Entada mannii* indicated the presence of the main major chemical groups such as: sterols, polyterpenes, polyphenols flavonoids, alkaloids and saponins in both extracts. However we notice the absence of catechic tannins, gallic tannins and quinones. Furthermore the results of toxicity studies allowed to classify the two extracts among the little or slightly toxic substances. The maximum tolerated doses of both extracts 5000 (mg / kg Pc) are well above the doses needed to have pharmacological effects. These two substances therefore offer interesting safety margins. However, in addition to the present toxicity study, the evaluation

of the biological tolerance by the assay of certain biochemical and haematological parameters in the animals is also necessary for a better rationalization of the use of this plant.

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ONFLICT OF INTERESTS

The authors claim that there is no conflict of interest.

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