Evaluation of acute toxicity and phytochemical analysis of *Gliricidia sepium* and *Xylopia aethiopica*extracts

ABSTRACT

Objectives:*Gliricidia sepium* and *Xylopia aethiopica* are frequently utilized in traditional medicine in Ivory Coast. To mitigate potential risks associated with their uninformed use, it is crucial to assess their safety levels.This studywasaimed at evaluating the acute toxicity and analyzing the phytochemicals in ethanolicextracts of *Gliricidia sepium* leaf and *Xylopia aethiopica* fruit.

Materials and Methods:triphytochemistry was carried out using staining and precipitation methods. . While the Metabolite determinations were conducted via spectrophotometry. The assessment of acute toxicity followed the OECD protocol 423, employing female rats of the Wistar strain.

Results: Phytochemical analysis revealed that Ethanolicextracts of *Xylopia aethiopica* fruit (XAF)contains 7.72% polyphenols, 4.09% alkaloids, 0.34% tannins, 0.12% saponins, and 0.001% terpenoids. In comparison, *Gliricidia sepium*leaf (GSL)exhibited levels of 7.49% polyphenols, 3.41% alkaloids, 0.47% tannins, 0.01% saponins, and 0.51% terpenoids. The acute toxicity study demonstrated the absence of mortality and major toxicity issues at maximum doses of 5000 mg/kg body weight.

Conclusion: Ethanolicextracts of XAF and GSL, do not exhibitany acute toxiceffect and theybothcontainsomephytochemicals. This validates the frequent utilization and provides assurance to communities usings these plants.

.***Keywords:*** *Gliricidia sepium, Xylopia aethiopica, phytochemical, acute toxicity*

UNDER PEER REVIEW

1. INTRODUCTION

Medicinal plants continue to be widely embraced worldwide, despite remarkable advances in modern medicine. Their appeal stems from the presence of phytochemical compounds that confer interesting therapeutic properties [1]. In Ivory Coast, two such plants, *Xylopia aethiopica* and *Gliricidia sepium*, are effectively utilized in traditional treatments for various conditions, including malaria, dysentery, ulcers, tumors, and allergic reactions. Research has shed light on the antibacterial, antifungal, and antiviral properties of *Gliricidia sepium* [2]. Similarly, extracts from the fruits of *Xylopia aethiopica* have demonstrated antioxidant, antimicrobial, and antiviral activities [3, 4]. However, the mere presence of these activities raises questions. Are these properties alone sufficient to warrant their incorporation into therapeutic practices? Can these plants be used safely? Accidents associated with the folkloric use of certain medicinal plants have been reported. Notably, *Ephedra sinica* has been linked to numerous cases of cardiovascular events [5], and the traditional use of *Momordica charantia* for treating amenorrhea has led to incidents of vomiting, fainting, hyperthermia, and skin rashes [6]. In Africa, around 30% of drug-related incidents are attributed to the empirical use of mixtures of plant extracts [7]. Consequently, the use of medicinal plants requires caution, necessitating acute toxicity studies before any application to guarantee their safety. The overarching goal of this study is to contribute to the evaluation of the safety level in the use of medicinal plants. Specifically, we aim to assess acute toxicity and measure the secondary metabolites contained in the extracts of GSLand XAF.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant material

The plant material used for this study consists of the leaves of *Gliricidia sepium* and the fruits of *Xylopia aethiopica*. The leaves of *Gliricidia sepium* were harvested during the month of October 2021 at the National Floristic Center (CNF) of Félix Houphouët-Boigny University (figure 1), while the fruits of *Xylopia aethiopica* were harvested in the Azaguié region in Ivory Coast (figure 2). After harvest, the leaves and fruits were carefully washed, cut into small pieces, then dried out of the sun for two weeks before being ground into a fine powder. Plant identification was carried out at the CNF.

Figure 1:*Gliricidia. sepium*Leaves (Jacq.)
Kunth ex Walp Figure 2: *Xylopia. aethiopica*Fruits
(Dunal) A. Rich

2.1.2 Animal material

Rats of the Rattus norvegicus species, Wistar strain weighing between 97 and 140 g and aged 16 weeks were used for this study. They were provided by the animal store of thenormal school superior (Abidjan, Ivory Coast). The animals were housed in plastic cages covered with stainless steel grills. They were acclimatized for a week. The cages contained a litter of wood shavings renewed every two days throughout the experiment.

2.2 Methods

2.2.1 Preparation of the hydroethanolic extract

Each extract was prepared according to the method described by [8]. One hundred grams (100 g) of plant powder were dissolved in one liter of hydroalcoholic solvent comprising 70% ethanol and 30% distilled water. The mixture was then homogenized 10 times at the rate of 2 minutes per revolution using a mixer. The homogenate obtained was drained in a square of fabric then filtered successively three times on hydrophilic cotton and then on Whatman paper (3 mm). The filtrate was evaporated at 45°C in an oven for 48 hours. The dry powder obtained constituted the 70% ethanolic extract.

2.2.2 Calculation of extraction yield

The yield is the ratio of the quantity of extract obtained to the material. It is expressed as a percentage relative to the dry matter (vegetable powder) and calculated according to the following formula:

$$R\ (\%) = M1 \times 100 / M0$$

R: Extract yield expressed as a percentage (%),

M1: Mass of dry extract (g),

M0: Vegetable powder mass (g).

2.2.3 Acute toxicity

UNDER PEER REVIEW

The experiment was conducted in accordance with OECD 423 protocol [9]. One dose was tested: 5000 mg/Kg CP (limit test). A total of 9 female rats, divided into 3 batches of 3 each, were used in the experiment. The animals were fasted the night before experimentation (they were deprived of food). The animals are weighed and the test substance is administered. Administration is made orally at a rate of 1 mL per 100 g body weight, in a sequential process in which three animals are used at each stage. They are then observed for 30 min. Treated animals are observed for 14 days for signs of acute intoxication.

2.2.4 Phytochemical screening

Phytochemical screening consists of detecting the various families of secondary metabolites present in the plant part under study by means of specific characterization reactions. These reactions are based on precipitation or staining by reagents specific to each family of compounds [10]. The phytochemical compounds sought and the reagents used are presented in Table 1.

Table 1: Reagents and tests for characterizing chemical groups

CHEMICAL GROUPS	REAGENTS	REACTIONS
TANINS	Ironchloride (FeCl3)	-blue-black (gallic tannins) -green or blue-green (catechic tannins)
FLAVONOIDS	Cyanidine	pink or red
ALKALOIDS	Mayer Wagner	yellowish-white precipitate brown precipitate
POLYPHENOLS	Ferricchloride	Blackish-bluecoloring
SAPONOSIDES	Agitation	Persistent moss
QUINONES	Borntraegen	Red or purple coloring

2.2.5 Quantitative determination of phytochemical compounds

2.2.5.1 *Determination of alkaloids*

100 mg of plant powder was dissolved in 4 mL ethanol to obtain a stock solution concentrated(MSC) to MSC = 25 mg/mL. The pH of this solution was then measured and maintained between 2 and 2.5 by adding a few drops of hydrochloric acid (HCl). Next, 2 mL of Dragendorff reagent was added to the extract, which was then centrifuged at 7,000 g for 3 minutes. A few drops of Dragendorff were added to the centrifugate to complete the precipitation, then a second centrifugation was carried out, the centrifugate was completely decanted and the precipitate recovered, then washed with ethanol. The filtrate is discarded and the residue treated with 2 ml di-sodium sulfate solution. The brownish-black precipitate formed is then centrifuged. Two drops of di-sodium sulfate are added to the medium to complete precipitation, followed by a second centrifugation. The residue is recovered and dissolved in

UNDER PEER REVIEW

2ml of concentrated nitric acid, heated if necessary. Finally, the solution obtained is diluted by making up to 10ml with distilled water. Then 1ml of this diluted solution is added to 5ml of thiourea, and the absorbance is measured at 435nm against a blank consisting of nitric acid, distilled water and thiourea prepared as the sample [11]. Preparation of the calibration curve.

17 g of atropine powder were dissolved in 17 mL of nitric acid to form the stock solution, concentrated to 1mg/mL. From this stock solution, dilutions were made and topped up with 10 mL distilled water. Subsequently, 1 mL of each dilution was added to 5 mL of 3% thiourea. The absorbance reading (X) was at a wavelength of 435 nm. The alkaloid content was determined by the formula:T % = 100Y/ MSC

2.2.5.2 *Determination of polyphenols*

For the preparation of 20% Na_2CO_3, 10 grams of Na_2CO_3were dissolved in 50 mL of distilled water, then stirred until completely dissolved and allowed to stabilize for at least 1 hour, giving a concentration of 20%Na_2CO_3.To prepare Folin-Ciocalteau (1:10), 5mL of Folin reagent was taken and diluted in 50 mL of distilled water.10 g of extract was weighed and dissolved in 10 mL methanol, to obtain a concentrated stock solution at MSC = 1mg/mL. Subsequently, 1 mL of this solution was removed and added to 1ml FCR. The mixture was vortexed for 6 minutes. After stirring, 1 mL sodium carbonate was added and made up to 10 mL with distilled water. The mixture was incubated in the dark for 1 hour. The abundance reading was taken at 735 nm against a blank prepared in the same way by replacing the extract with distilled water. Preparation of the gallic acid calibration range5 mgof gallic acid was dissolved in 5 mL of distilled water, then stirred to give a solution concentrated to C= 1 mg/mL. A series of dilutions of the gallic acid stock solution was then made with distilled water. Indeed, 1 mL of each dilution was transferred to test tubes (C0 to C10) where each tube received 1ml of FCR (1:10) and allowed to stand for 6 minutes. After this time, 1mL of 20% sodium carbonate was added to each tube. Finally, the whole mixture was incubated in the dark for 1 hour [12]. Absorbance was read at 735 nm. The polyphenol content was determined by the formula:T % = 100Y/ MSC.

2.2.5.3 *Determination of tannins*

4 g of vanillin was dissolved in 100 mL of 96%ethanol, giving a vanillin concentration of 4%. 10 mg of extract was weighed and dissolved in a 10 mL volume of distilled water to obtain a concentrated stock extract solution MSC = 1 mg/mL. Next, 100 µL of extract was taken and transferred to a test tube, then 3 mL of 4% vanillin was added. Finally, 1500 µL of 32% HCl was added to this solution. The mixture was incubated in t against a blank prepared in the same way, replacing the plant extract with distilled water [12]. Preparation of the tannic acid calibration range. 5 mg of tannic acid were dissolved in 5 mL of distilled water and homogenized. From this solution, successive dilutions were made. Half of each tube was transferred to the next tube. Subsequently, 100 µL of each dilution was transferred to test tubes numbered C0 to C10, where each tube received 3 mL of 4% Vanillin. After homogenization, 1500 µL of 32%HCL was added to each tube, then shaken. The mixture was incubated in the dark for 1 hour. Absorbance was read at 500 nm on a spectrophotometer.Tannin content was determined by the formula: T % = 100Y/ MSC.

2.2.5.4 *Determination of saponins*

8 g of vanillin were dissolved in 100 mL of 96° ethanol, giving a vanillin concentration of 8%. 25 mg extract was weighed and dissolved in 1 mL distilled water to obtain a concentrated stock solution MSC = 25 mg/mL extract. Then 100 µL of this solution was taken and transferred to a test tube. Next, 500 µL of 8% vanillin were added to this mixture and placed in an ice-cold water bath, followed by 5 mL of 72% sulfuric acid. The mixture was stirred and incubated at room temperature for 3 min. After this time, the mixture was incubated for a further 10 min at 60°C and then allowed to cool. Absorbance readings were taken at 544 nm against a reagent blank prepared as follows (ethanol + vanillin 8%+ sulfuric acid 72%) [13].The saponin content was determined by the formula: T % = 100Y/ MSC.

2.2.5.5 *Determination of terpenoids*

250 mg dry extract was taken and dissolved in 5 mL distilled water to obtain a concentrated MSC stock solution = 50 mg /mL.1mL of the previously prepared stock solution was taken and transferred to a test tube, then 2 mL of chloroform was added and homogenized, then left to stand for 3 minutes. Next, 200 µL of concentrated sulfuric acid was added and incubated in the dark for 2 hours. After incubation, the reddish-brown precipitate formed was carefully separated from the supernatant by undisturbed decantation. Subsequently, 3 mL of absolute methanol was added and stirred well until

UNDER PEER REVIEW

the precipitate was completely dissolved [14]. The absorbance reading was taken at 538 nm against a blank prepared in the same way, replacing plant extract with methanol. The terpenoid content was determined from the following formula: T % = 100Y/ MSC.

2.2.6 Statisticalanalysis

Statistical tests and graphs were performed using GrapPhad.Prism 9.5.1 software. Results were presented as mean ± errors on the mean. Data were evaluated using ANOVA followed by the Student's t test at the 5% threshold to assess the significance of observed differences.

3. RESULTS

3.1 Extraction yield

The average masses obtained are recorded in Table 2.

Table 2: Yield of Hydroalcoholic Extractions

Plantspecies	Plant Powder Mass (G)	Dry Extract Mass Obtained (G)	Yield(%)
GSL	400	73,84	18,46± 0,5 a
XAF	400	57,78	14,44± 0,12 b

3.2 Acute toxicityassessment

The acute toxicity study indicates that at the dose of 5000 mg / kg bw, rats showed no signs of toxicity 30 minutes after administration of the extracts. There were no changes in mobility or behavior. No mortality was observed. The same observations were made 3 hours and 14 days after administration. The results are shown in Table 3.

Table 3: General condition of animals after product administration

Parameters	Control lots	Lots 5000mg /Kg *G. Sepium*	Lots 5000mg /Kg *X. Aethiopica*
Number Of Animals	3	3	3
Mobility	N	N	N
Aggression	A	A	A
Fecal Condition	N	N	N
Vomiting	A	A	A
Tail Condition	N	N	N
Numbers Of Dead	0	0	0

A : Absence, N : Normal

3.3 Phytochemical screening

The identification of the different classes of secondary and primary metabolites making up a plant gives us an insight into the phytochemistry of the medicinal plant. To this end, we carried out phytochemical tests on the two plants studied (*Gliricidia sepium* and *Xylopia aethiopica*) (Table 4).

Table 4: Different groups of compounds detected in hydroalcoholic plant extracts

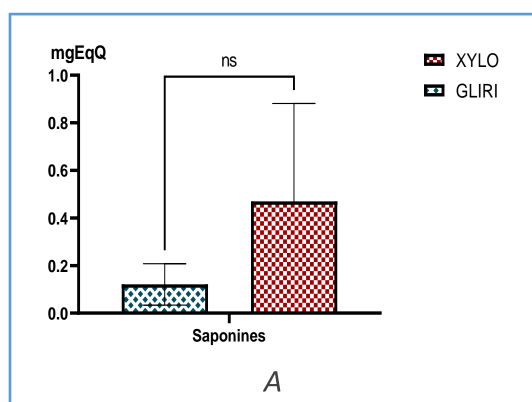
UNDER PEER REVIEW

Extracts	Sterols& Poly Terpenes	Polyphenols			Quinones	Alkaloids		Saponosides
		Flavonoids	Tanins					
			Cat	Gal		B	D	
XAF	+	+	-	-	-	+	+	+
GSL	+	+	+	-	-	+	+	+

(-) : absence ; (+) : présence

3.4 Secondary metabolite assay

Phytochemical assays on both plants showed the presence of different metabolite groups in varying quantities. From the different calibration curves, a straight line equation was determined, enabling us to determine the different quantities of secondary metabolites present in the two plants, as follows: Alkaloids in milligrams of atropine equivalent, polyphenols in milligrams of gallic acid equivalent,tannins in milligrams of tannic acid equivalent, saponins in milligrams of quillaja equivalent, terpenoids in milligrams of linalool equivalent. These values are shown in Figure 3.



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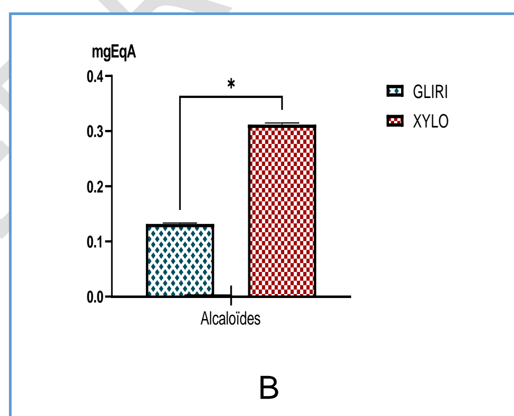

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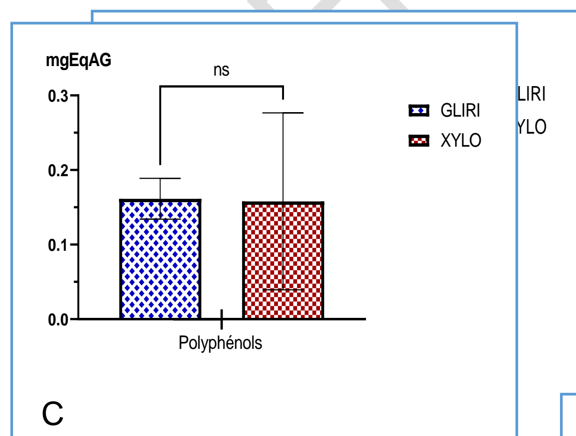

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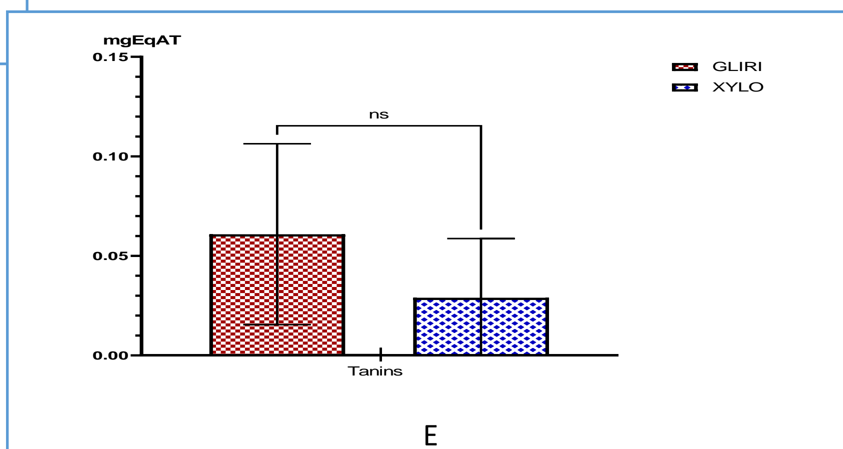

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Figure 3: Phytochemical composition

A: Saponins, B: Alkaloids, C: Polyphenols, D: Terpenoids, E: Tannins

4. DISCUSSION

Our study focused on assessing the safety profile and identifying secondary metabolites within two distinct plant extracts. The extraction yields indicated a notable contrast ($p < 0.05$) between the hydroalcoholic extraction of GSL(18.46 ± 0.5) and that of XAF(14.44 ± 0.12). This disparity highlights *GSL's* higher yield and its richness in metabolites extracted through the ethanol-water mixture (70/30). Thus, the choice of extraction solvent significantly influences the rate of metabolite extraction from these plants [15].
During the 14-day oral administration study, no signs of toxicity emerged. Subsequently, based on this analysis, both studied plants fall into category 5, denoting their non-toxic nature when orally administered [16].
The results of phytochemical tests unveiled the presence of sterols, polyterpenes, saponins, alkaloids, flavonoids, polyphenols, and catechin tannins in the studied medicinal plants, with varying intensities. Additionally, these findings align with previous research [4, 17].The presence of phenolic compounds like flavonoids and tannins, acknowledged for their antiviral properties [18, 19, 20], validates the historical usage of these plants against viral diseases. Furthermore, the presence of saponins in our plants signifies potent antiviral activity, showcasing surfactant, antifungal, antibacterial, and antiviral properties [21]. Comparison of polyphenol content revealed no significant differences between *Xylopia. aethiopica* fruit and *Gliricidia. sepium* leaf, respectively (7.72±0.5; 7.49±0.03). These results are consistent with previous studies [4], which also highlighted the presence of polyphenols in the aqueous extracts of *Gliricidia. sepium* and *Xylopia. aethiopica*. Studies [22] suggest that polyphenols possess antioxidant capabilities. Similarly, tannins and saponins exhibited no significant difference between the two extracts. However, there was a notable difference in alkaloid content ($p < 0.05$), with *Xylopia aethiopica* fruits (4.09±0.01) demonstrating a higher alkaloid content than *Gliricidia sepium* leaves (3.41±0.05) [23]. The presence of various compounds, including phenolic compounds, saponins, alkaloids, and flavonoids, underscores the potential effectiveness of these plants against viral diseases, in accordance with previous findings [18, 19, 20, 21]. Lastly, terpenoids exhibited a significant difference ($P= 0.05$), with higher content in *Gliricidia sepium*leafthan in *Xylopia aethiopica*fruit. To summarize, these two plants, abundant in polyphenols and alkaloids, are likely to possess antioxidant and antiviral properties, aligning with previous research [17].

5. CONCLUSION

This study involved two plants from the Ivorian pharmacopoeia. Oral toxicity tests on extracts of these plants revealed no mortality at a dose of 5000 mg/kg body weight. Consequently, the LD50 of both plants is higher than this dose, indicating high tolerance. The identification and quantification of chemical compounds enabled us to characterize the major groups present in both plants, including flavonoids, catechic tannins, polyphenols, saponosides, alkaloids and terpenoids. This in-depth analysis of chemical constituents reinforces our understanding of the pharmacological properties of these medicinal plants.The results of this study confirm the safe use of both plants at high doses. The information obtained on the chemical composition provides a solid basis for the valorization of these plants in the context of traditional Ivorian medicine. These results encourage further research at the histological (anatomical) and molecular levels.

UNDER PEER REVIEW

ETHICAL APPROVAL

All authors hereby declare that the "principles of laboratory animal protection have been followed.

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UNDER PEER REVIEW

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