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Phytochemical analysis and acute toxicity of *Colocasia esculenta* leaves for use in livestock

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Abstract
The aim of this study is to promote African pharmacopoeia by analyzing the chemical composition and acute toxicity of the aqueous extract of *Colocasia esculenta* leaves, a plant commonly used in food and traditional medicine. The extract was prepared by macerating the leaves in distilled water and filtering the solution. It was then subjected to qualitative phytochemical screening and acute toxicity testing. Acute toxicity was evaluated in Wistar rats after a single oral administration of 2000 mg/kg body weight, following OECD Guideline 423, with body weight monitored throughout the experiment. Phytochemical analysis revealed the presence of sterols, polyterpenes, polyphenols, catechin tannins, flavonoids, alkaloids, and quinones, while saponosides were absent. The toxicity study showed no signs of toxicity at the tested dose. A slight increase in body weight was observed in treated rats compared to controls, indicating that *Colocasia esculenta* leaves are rich in bioactive compounds and safe for use.

Keywords: *Colocasia esculenta*, phytochemical, acute toxicity

1. Introduction
Colocasia esculenta L. is a tropical herbaceous plant of the Araceae family, widely cultivated in rice fields, lowlands, and marshy areas. It is mainly grown for its tubers, which are used for human consumption in Ivory Coast. *Colocasia esculenta L.* leaves also have nutritional value, as they are a potential source of bioactive compounds and nutrients that can be used in human and animal food [1]. Several studies conducted in West Africa have highlighted the value of these leaves as a source of fiber, protein, and minerals, which can be used in feed for monogastric animals and ruminants [2, 3]. In addition, phytochemical analyses play an essential role in characterizing the secondary metabolites present in taro leaves, which are known for their antimicrobial, antioxidant, and anti-inflammatory properties. Identifying these compounds is essential for evaluating the therapeutic potential of local plant resources. These metabolites can contribute to improving the health and zootechnical performance of animals [4, 5] and reduce the use of antibiotics in livestock farming [6]. From a nutritional standpoint, taro leaves have a significant protein, fiber, mineral, and vitamin content and could be used as an alternative food source in situations where conventional foods are scarce or expensive [7]. However, compounds such as calcium oxalates can have antinutritional or toxic effects when present in high concentrations [8]. Toxicological assessment is essential to ensure their safety. Furthermore, acute toxicity assessment is a crucial step in the scientific validation of the use of taro leaves in animal feed and health. Acute toxicity assessment, in accordance with international recommendations, is an important step in determining tolerable doses and ensuring the safe use of these leaves in animal feed and health [9]. This study therefore aims to characterize the phytochemical composition of *Colocasia esculenta* leaves while assessing their acute toxicity, in order to contribute to their rational use in Ivorian livestock farming systems.

2. Materials and Methods
2.1. Materials
2.1.1. Plant material
Leaves from *Colocasia esculenta* (Araceae) were used as plant material. They were harvested at Nangui Abrogoua University in the municipality of Abobo (Abidjan, Ivory Coast).

2.1.2. Animal material
The animal material consisted of six female albino *Rattus norvegicus* (Muridae) rats of the Wistar strain, aged 6 ± 1 weeks and weighing between 107 and 122 g.

These rats were supplied by the vivarium of Abidjan Higher Normal School, where they were raised at a humidity level of 50 to 55%, in natural light and at a temperature of 24 ± 2°C. They were fed Faci pellets and had free access to water.

2.2. Methods

2.2.1. Method for obtaining powder from *Colocasia esculenta* leaves

After harvesting, the *Colocasia esculenta* leaves were washed with drinking water and then dried on aluminum foil placed on the workbench at laboratory temperature. These leaves were then ground using an IKA A10 Labortechnik (Germany) food processor to obtain powder.

2.2.2. Extraction method

Fifty (50) grams of *Colocasia esculenta* leaf powder were macerated in 1.5 L of distilled water, stirred, and then filtered several times using a cloth and cotton wool to obtain a filtrate. A dry extract in the form of flakes was obtained after drying the filtrate in an oven at a temperature of 50°C.

2.2.3. Phytochemical tests

Phytochemical tests revealed the major phytochemical groups found in Colocasia esculenta leaves. The different compounds sought were polyphenols, sterols, polyterpenes, flavonoids, tannins, quinones, alkaloids, and saponosides. Polyphenols were detected using a ferric chloride reaction, which involves adding a drop of a 2% alcoholic ferric chloride solution (2 mL of the solution, consisting of extract and distilled water) to observe the appearance of a blue-black color. This color indicates the presence of polyphenols in the tested solution.

The Liebermann reaction revealed the presence of sterols and polyterpenes. The principle of this reaction is to observe the appearance of a purple ring, turning blue then green in a solution containing these two compounds after evaporating 5 mL of solution (extract + distilled water) in a capsule on a sand bath. Next, dissolve the hot residue obtained in 1 mL of acetic anhydride, then pour 5 mL of concentrated sulfuric acid down the side of the tube.

The reaction to cyanidin revealed the flavonoids in the aqueous extract of Colocasia esculenta leaves. For this purpose, 2 mL of the solution (extract + distilled water) was evaporated to dryness on a sand bath in a capsule. After cooling, the residue was taken up with 5 mL of hydrochloric alcohol diluted by half, and the solution was poured into a test tube. Two drops of magnesium were then added, producing a pink-orange or purplish color. The addition of three drops of isomylic alcohol, which intensifies this color, confirms the presence of flavonoids.

With regard to the presence of catechin tannins, Stiasny's reagent was used. Five (5) mL of solution (extract + distilled water) were evaporated to dryness in a capsule, then 15 mL of Stiasny's reagent (30% formaldehyde, concentrated HCl at 1/0.5) were added to the residue. The mixture obtained was kept for 30 min in a water bath at 80°C and cooled under running water. The observation of a coarse flake precipitate characterizes catechin tannins. In addition, the previous solution was filtered and the filtrate was collected and then saturated with sodium acetate. The addition of 3 drops of 2% FeCl3 causes the appearance of an intense blue-black color indicating the presence of gallic tannins.

Borntrager's reagent (ammonia diluted to half strength) was used to detect free quinone substances. For combined quinone substances, prior hydrolysis (1/5 HCl) was performed. The test consisted of immediately hydrolyzing the solutions to characterize the total quinone substances. After dry evaporation of 2 mL of the solution (extract + distilled water) in a capsule, the residue was triturated in 5 mL of 1/5 hydrochloric acid. The solution was placed in a test tube and heated in a boiling water bath for half an hour. his solution was cooled under running cold water and the hydrolysate was extracted with 20 mL of chloroform in a test tube. The chloroform phase was then collected in another test tube and 0.5 mL of half-strength ammonia solution was added. The appearance of a red to purple color indicates the presence of quinones. Alkaloids have the property of combining with heavy metals (iodine, bismuth, mercury, and tungsten) to form precipitates. Dragendorff's reagent and Bouchardat's reagent were used to detect the alkaloids. Six milliliters of the solution (extract + distilled water) were evaporated to dryness in a capsule. The residue obtained was taken up with 6 mL of 60° alcohol and divided into two test tubes. Two drops of Dragendorff's reagent were added to the first tube, and the appearance of a precipitate or orange color indicated the presence of alkaloids. Two drops of Bouchardat's reagent were added to the second tube, resulting in the formation of a reddish-brown precipitate, which indicated a positive reaction. The detection of saponosides is based on one of their physical properties. Agitating solutions containing saponosides produces a persistent foam. For this reason, 2 mL of the solution (extract + distilled water) was placed in a test tube, which was then shaken for 30 seconds and left to stand for 15 minutes. The persistence of foam more than 1 cm high after resting is characteristic of the presence of saponosides.

2.2.4. Acute toxicity using the OECD method in rats

The acute toxicity of the extract was evaluated in accordance with Guideline 423 of the Organization for Economic Cooperation and Development [9]. The rats were selected according to the recommendations described in paragraphs 11 and 12 of the aforementioned directive, then divided into two groups of three animals each. The study focused on young, healthy, nulliparous, and non-pregnant rats aged 8 to 10 weeks and weighing between 110 and 120 g. Before the extract was administered, the animals were fasted overnight prior to the test, while maintaining free access to water. A limit test was then carried out. After the fasting period, the rats were individually identified and weighed, then a single dose of 2000 mg/kg body weight of aqueous extract of *Colocasia esculenta* leaves was administered orally using a gastric tube. The rats were again deprived of food for 3 to 4 hours. After administration of the extract, the rats were observed individually at least once during the first 30 minutes, then regularly during the first 24 hours. Particular attention was paid during the first four hours after administration, then daily observations were made for a period of 14 days. The animals were examined at least twice a day for any signs of toxicity or behavioral changes. The parameters observed included changes in the coat, skin, mucous membranes, and eyes, as well as alterations in respiratory and circulatory functions, somatomotor activity, and general behavior. Attention was also paid to the possible occurrence of signs such as tremors, salivation, convulsions, diarrhea, lethargy, drowsiness, and coma. In addition, the rats were weighed every two days during the 14-day observation period.

3. Results and Discussion

3.1. Results

3.1.1. Phytochemical testing of *Colocasia esculenta* leaves:

Table 1 lists the various secondary metabolites found in

Colocasia esculenta leaves following phytochemical analysis. Sterols and polyterpenes, polyphenols, flavonoids, tannins, alkaloids, and quinones were detected in the aqueous extract of *Colocasia esculenta* leaves. In addition, no saponosides were found.

Table 1: Phytochemical composition of *Colocasia esculenta* leaves

Secondary metabolites	Results
Sterols and polyterpenes	+
Polyphenols	+
Flavonoids	+
Catechin tannins	+
Gallic tannins	+
Quinones	+
Alkaloids (BOUCHARDAT)	+
Alkaloids (DRAGENDORFF)	+
Saponins	-

+: Presence
-: Absence

3.1.2. Acute toxicity of *Colocasia esculenta* leaves

At a single dose of 2000 mg/kg body weight, the aqueous extract of Colocasia esculenta leaves did not cause any signs of toxicity in rats (Table 2). In addition, a slight increase in body weight was observed in treated rats compared to controls (Figure 1). According to the OECD classification, the lethal dose that can cause death in half of the rats (LD_{50}) is greater than 5000 mg/kg body weight.

Table 2: Parameters observed after treatment of the rats

Observation	30 mn		4 hours		24 hours		48 hours		1 week		2 week	
	C	T	C	T	C	T	C	T	C	T	C	T
Skin and fur	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Mucous membrane	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N
Lethargy	No	No	No	No	No	No	No	No	No	No	No	No
Sleep	N	N	N	N	N	N	N	N	N	N	N	N
Coma	No	No	No	No	No	No	No	No	No	No	No	No
Convulsion	No	No	No	No	No	No	No	No	No	No	No	No
Diarrhea	No	No	No	No	No	No	No	No	No	No	No	No
Mobility	N	N	N	N	N	N	N	N	N	N	N	N
Respiratory rate	N	N	N	N	N	N	N	N	N	N	N	N
Heart rate	N	N	N	N	N	N	N	N	N	N	N	N
Dying	No	No	No	No	No	No	No	No	No	No	No	No
Mortality	No	No	No	No	No	No	No	No	No	No	No	No

C: Control (distilled water);
T: Treated (2000 mg/kg body weight of aqueous extract of *Colocasia esculenta* leaves);
N: Normal; no apparent signs different from controls
No: no signs observed

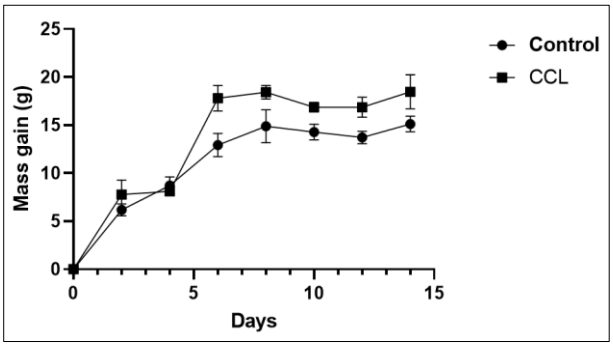

Fig 1: Change in body weight of rats after administration of a single dose of 2000 mg/kg body weight of aqueous extract of *Colocasia esculenta* leaves

CCL: Treatment with 2000 mg/kg body weight of aqueous extract of *Colocasia esculenta* leaves.

3.2. Discussion

With the exception of saponosides, sterols, and polyterpenes, polyphenols, flavonoids, tannins, alkaloids, and quinones were present in the aqueous extract of Colocasia esculenta leaves following phytochemical analysis. This phytochemical diversity confirms the biological potential of this plant and corroborates previous work on taro. Indeed, Agyare *et al*. [10] and Eleazu *et al*. [11], In similar studies, the presence of polyphenols, flavonoids, and alkaloids has been reported in hydroalcoholic and aqueous extracts of *C. esculenta* leaves. Similarly, Odebiyi and Sofowora [4] confirmed the presence of alkaloids, tannins, and polyphenols in aqueous extracts of tropical food plants, including *C. esculenta*. In contrast, Njintang *et al*. [7] observed an absence or low content of sterols and terpenes in certain taro extracts obtained using different extraction protocols, suggesting that the phytochemical composition depends heavily on the soil, the plant's stage of maturity, and the solvent used. Furthermore, some of the secondary metabolites identified are known for their pharmacological properties. Flavonoids have anti-inflammatory, anti-allergic, and analgesic activities in mammals [12, 13]. They also exert significant antioxidant activity by inhibiting the oxidation of LDL cholesterol induced by free radicals [14, 15]. Some flavonoids also have hepatoprotective effects and promote liver cell regeneration [16]. Alkaloids, on the other hand, are known for their antioxidant properties [17] and anti-inflammatory, particularly in animals [18]. Similarly, polyphenols and polyterpenes are known for their antibacterial and anti-inflammatory effects, helping to protect the body against infections and oxidative stress [19]. Phytosterols are widely recognized for their cholesterol-lowering effects and their beneficial role in improving zootechnical performance in farm animals. The tannins detected also have significant biological interest. They are recognized for their antioxidant activities [20], antiviral, antifungal, and antibacterial [21, 22]. They also have anti-inflammatory properties [23] and immunostimulants [24], thus strengthening the animals' immune defenses. However, despite this richness in phytochemical compounds, a thorough toxicological evaluation remains essential in order to popularize the use of this plant in animals. The results of the acute toxicological study showed that oral administration of the aqueous extract of C. esculenta leaves to rats did not result in any mortality at a single dose of 2000 mg/kg body weight. According to the classification system of the Organization for Economic Cooperation and Development [9], this extract is considered unclassified or belonging to category 5, with an $LD_{50} \geq 5000$ mg/kg body weight. It is therefore considered non-toxic. These results are consistent with those of Delongeas *et al*. [25], according to which any substance with an LD_{50} greater than 5 g/kg body weight is considered non-toxic. The good tolerance observed in rats could be attributed to the beneficial chemical composition of the plant, which is corroborated by the increase in body weight gain in treated rats, suggesting a favorable nutritional and physiological effect.

4. Conclusion

At the end of this study, it should be noted that phytochemical analysis of *Colocasia esculenta* leaves revealed a rich chemical composition with the presence of alkaloids, flavonoids, polyphenols, polyterpenes, sterols, quinines, and

tannins. In addition, the acute toxicity study of the extract at a single dose revealed its non-toxicity when administered orally.

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