Phytochemical, Toxicological, and Antimicrobial Evaluation of the Hydroethanolic Extract of Calotropis procera (Ait.) Leaves Used in Traditional Medicine in the Maritime Region of Togo: An Experimental Study

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Abstract

Background

Urogenital infections remain a significant global health concern due to their high prevalence and severity. Urogenital infections rank second among community-acquired bacterial infections, with an estimated 150 million cases annually, while the WHO reports over 340 million new cases of genital infections each year. In developing countries, limited access to treatments leads many to rely on medicinal plants. However, antimicrobial resistance has reduced treatment efficacy, increasing the need for alternative therapies. *Calotropis procera* (Ait.), widely used in traditional medicine, possesses various therapeutic properties. Despite its traditional use, few studies have explored its antimicrobial efficacy in the Togolese context. This study aims to evaluate the phytochemical composition, acute toxicity, and antimicrobial properties of the hydroethanolic extract of *Calotropis procera* (Ait.) leaves harvested in the maritime region of Togo.

Methods

Phytochemical screening was conducted using standard protocols. Acute toxicity tests were carried out following Organization for Economic Co-operation and Development (OECD) guidelines 423, and antimicrobial activity was carried out using the agar-well diffusion method against five bacterial and fungal reference and clinical strains commonly implicated in urogenital infections, including *Escherichia coli*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*. Data were analyzed using SPSS version 25. The mean values were reported with standard errors.

Results

Phytochemical screening revealed the presence of terpenoids, alkaloids, and coumarins. Acute toxicity tests showed no adverse effects at doses up to 5,000 mg/kg of body weight (b.w.). However, the extract did not exhibit significant antimicrobial activity against the tested strains involved in urogenital infections.

Conclusion

While the *Calotropis procera* extract demonstrated promising phytochemical and toxicological profiles, its lack of antimicrobial activity suggests limited therapeutic potential for urogenital infections. Future studies should investigate optimized extraction techniques and additional pharmacological properties to enhance therapeutic potential.

Background

Infections, particularly those affecting the urogenital tract, remain a significant global health concern due to their frequency and severity [1]. Among these, microbial infections of the urogenital tract are among the most common [2]. Moreover, urinary tract infections (UTIs) rank second among community-acquired bacterial infections, following respiratory tract infections, with an estimated 150 million cases annually [2,3]. Additionally, the World Health Organization (WHO) reports over 340 million new cases of genital infections each year [4].

As the emergence and re-emergence of these diseases continue worldwide, developing countries are particularly vulnerable [5]. To combat these infections, numerous antimicrobial treatments have been developed [1]. However, access to conventional treatments remains limited due to high costs, making medical care inaccessible to the poorest populations [6]. Consequently, many individuals rely on traditional medicine, primarily plant-based remedies, for their healthcare needs. This practice is supported by the WHO, which estimates that around 80% of the population in these developing countries can be treated using medicinal plants [7–9].

Since prehistoric times, medicinal plants have played a crucial role in human health, serving both nutritional and therapeutic purposes. They are a major source of medicines due to their abundance of secondary metabolites [8,10,11]. Furthermore, these plants represent a valuable heritage for humanity, offering an inexhaustible source of bioactive molecules with diverse biological and pharmacological activities [12,13]. It is estimated that approximately 25% of the world's prescribed medicines come from natural sources, with 60–70% of antibacterial and anticancer compounds derived from these same sources [14].

Nevertheless, the frequent use of synthetic molecules has led to the emergence of multi-resistant microorganisms, thereby reducing the efficacy of certain treatments. As a result, the scientific community is increasingly turning to natural substances, particularly medicinal plants, in the search for new bioactive compounds capable of effectively combating microbial infections while promoting traditional medicine [6]. Indeed, the medicinal activity of plants is largely attributed to the presence of phytochemical or bioactive compounds [15].

Several studies have demonstrated the antimicrobial activity of plant extracts with therapeutic properties [15–18]. Among these, *Calotropis procera* (Ait.), a wild species of the Apocynaceae family, stands out for its traditional use in local cheese-making and the treatment of various diseases such as ulcers and tumors [15]. Additionally, it possesses antihelminthic, anticoagulant, analgesic, anti-inflammatory, purgative, and antipyretic properties and is used in the treatment of leprosy and leukoderma, among other conditions [17].

Despite its widespread use, few studies have explored its antimicrobial efficacy and safety profile in the Togolese context. Therefore, this study aims to evaluate the phytochemical composition, acute toxicity, and antimicrobial properties of the hydroethanolic extract of *Calotropis procera* (Ait.) leaves harvested in the maritime region of Togo.

Materials and methods

Study framework

The study was conducted in 2022 in the maritime region of Togo, with an area of 6,100 km², or 10.78% of the total area of the country and a population of 3,534,991 inhabitants [19]. The study specifically focused on the prefectures of Avé, Zio, Agoé-Nyivé, and Golfe, where the leaves of *Calotropis procera* were collected (Fig. 1). The Maritime region of Togo was chosen due to its high population, reliance on traditional medicine, abundant *Calotropis procera*, health challenges, and available research infrastructure.

(Source: adt-france-togo, 2019)

Plant material

Healthy leaves of *Calotropis procera* (Ait.) (Fig. 2) were collected in June 2022 from the prefectures of Avé, Zio, Agoé-Nyivé, and Golfe, located in the Maritime Region of Togo. The plant species was formally identified by Dr. Hodabalo KAMOU, a botanist at the Laboratory of Botany and Plant Ecology, Faculty of Sciences, University of Lomé. A voucher specimen was deposited in the herbarium of the same institution under the reference number TG02210. The collected leaves were washed, air-dried at 25°C in the laboratory, powdered, freeze-dried, and stored in airtight flasks for experimental use.

(Source: Photo TCHAKONDO, 2022)

Animal material

Male and female albino rats of the Sprague Dawley strain, with average weights of 162 g for males and 146 g for females, respectively, were used for toxicity testing. The animals were obtained from the animal facility of the Faculty of Sciences, University of Lomé.

All experimental procedures involving animals were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the Faculty of Sciences, University of Lomé. Although no formal approval number was issued, the study was conducted in accordance with institutional guidelines, the Animal Experimentation and Health Act, and the European Council Directive 86/609/EEC of 24 November 1986 for the protection of animals used for experimental and other scientific purposes [20].

Microbial strains tested

The microbial strains used were wild-type reference strains (confirmed by susceptibility testing) from the American Type Culture Collection (ATCC) and clinical strains isolated from urogenital infections. These strains were chosen due to their clinical relevance in urogenital infections. They were *Escherichia coli*

(ATCC 25922, clinical isolate 1555), *Neisseria gonorrhoeae* (clinical isolate 0993), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25922, clinical isolate 29213), *Candida albicans* (ATCC 10231, clinical isolate 1134). They were provided by the bacteriology laboratory of the National Institute of Hygiene of Lomé (INH).

Hydroethanolic extraction of harvested leaves

Hydroethanolic extraction followed the method described by Agbankpe *et al*. in 2016 [21]. Two hundred (200) grams of powdered leaves were macerated in 4 L of ethanol-water (70:30 v/v) for 72 hours with continuous stirring. The mixture was filtered with Whatman No. 1 paper, concentrated using a rotary evaporator and freeze-dried to obtain the dry extract. The dry extract was weighed to determine the extraction yield and then stored in the refrigerator in tubes at 4°C, protected from light, until use. The extraction yield is determined by the following formula:

$$\text{Yield } (\%) = \left(\frac{\text{Extract Mass}}{\text{Plant Material Mass}}\right) * 100 \text{ [18]}$$

This method is commonly employed in pharmacognostic studies to assess extraction efficiency.

Phytochemical screening

A qualitative phytochemical analysis of the extract at a concentration of 1 mg/ml was performed using standard methods [22,23]: Phenolic compounds were identified by iron trichloride, alkaloids by the Dragendorff test, flavonoids by the Shibata test, terpenoids by the Salkowski test, saponins by the foam test, coumarins by the ultraviolet lamp (UV) fluorescence test, and free reducing sugars by the Fehling test. All tests were done in comparison with a control.

Acute toxicity tests

The acute toxicity tests were conducted following the Organization for Economic Co-operation and Development (OECD) guidelines 423 [24]. The male and female rats were divided into three batches A, B and C of three rats, respectively. They were fasted for 24 hours before treatment. The weight of the animals was measured before each treatment. Oral gavage was chosen as the route of administration. A stock solution of the extract of the phytomedicine was obtained from the dissolution of 10 g of products in 50 ml of physiological water (NaCl 0.9%), that is, a concentration of 200 mg/ml. From this stock solution, different dilutions were carried out to obtain concentrations corresponding to the doses of 2500 mg/kg and 5000 mg/kg body weight (b.w.) of the extract of the leaves of *C. procera*, respectively. These doses correspond to batches B and C respectively. The control group representing batch A received only physiological water (NaCl 0.9%). After treatment, rats were observed individually for the first 30 minutes and regularly for the first 24 hours, with special attention for the first 4 hours. They were then observed daily for 14 days after the administration of the extracts. The clues looked for were

changes in skin, hair, somatomotor activity and behaviours such as: tremor, convulsion, salivation, diarrhoea, lethargy, sleep and coma. The weight of each rat was determined every day at the same time for 14 days. Lethality has been assessed [25].

Antimicrobial activity

Antimicrobial activity was assessed using the agar-well diffusion method, as described by Yala *et al.* (2016) [6]. This method consists of inoculating target microorganisms into a culture medium. Wells are then made in agar, where extracts are added. The inhibition zones around the wells indicate the antimicrobial efficacy of the extracts tested. The results can be used to determine the susceptibility of microorganisms to the extract studied at different concentrations.

Preparation of culture media

Mueller Hinton (MH) agar medium was used for antibacterial testing due to its standard application in antibiotic susceptibility testing, with the exception of *Neisseria gonorrheae*, for which Polyvitex enriched chocolate agar was used as it supports its fastidious growth requirements. Sabouraud agar, supplemented with chloramphenicol, was used for *Candida albicans* to inhibit bacterial contamination and support fungal growth. MH medium was prepared by dissolving 38 g of powder in 1 liter of distilled water. Sabouraud medium was prepared by dissolving 45.5 g of powder in 1 liter of distilled water. All media were autoclaved at 121°C for 15 minutes and then poured into sterile 90 mm diameter Petri dishes (cans) to obtain a thickness of 4 mm of agar. After drying at room temperature (about 25°C), the cans were kept in the refrigerator until biological tests.

Strain revitalization

Bacterial and fungal strains have been revitalized by seeding them on the appropriate media. Mueller-Hinton agar for *S. aureus, E. coli, P. aeruginosa*, with the exception of *N. gonorrheae*, for which chocolate agar (CA) was used, and Sabouraud-chloramphenicol medium for *Candida albicans*. The cans were incubated at 37°C for 24 hours. For *N. gonorrheae*, incubation was carried out in a humid atmosphere enriched with carbon dioxide (CO_2).

Preparation of inoculi

Bacterial and fungal inoculi were prepared by taking a colony from the revitalized culture and suspending it in a sterile tube containing saline (0.09% NaCl). The density of the suspension has been adjusted to match the turbidity of a 0.5 Mac Farland solution.

Preparation of solutions

One gram (1 g) of the lyophilized hydroethanolic extract was dissolved in 5 mL of distilled water to obtain a stock solution at a concentration of 200 mg/mL. Dilutions to 1/2 and 1/4 were then performed

to obtain concentrations of 100 mg/ml and 50 mg/ml respectively. The sterility of the solutions was verified by filtration on a 0.45 µm membrane, followed by inoculation on a non-selective culture medium.

Susceptibility tests

Mueller-Hinton (MH) agar was flooded with 2 mL of bacterial inoculum of *S. aureus, E. coli* and *P. aeruginosae*, adjusted to 0.5 Mac Farland, while chocolate agar and Sabouraud-chloramphenicol medium were used for *N. gonorrheae* and *C. albicans, respectively.* Excess inoculum was removed, and the cans were dried for 15 minutes at room temperature under aseptic conditions. Cupules of 6 mm in diameter were then made in the agar, and 50 µl of the extracts in different concentrations were deposited in them. The dishes were incubated at 37°C for 24 hours for the conventional germs and in a CO_2 rich atmosphere for *N. gonorrheae*. Distilled water was used as a negative control, and ciprofloxacin was used as a positive control. Antimicrobial activities were assessed by measuring, using a caliper, the diameters of the inhibition zones induced by extracts, antibiotics or antifungals, and each test was performed in duplicate.

Data analysis

Data collected were analyzed using SPSS version 25 (calculation of percentages, means, standard deviations, and standard errors). The mean values were reported with standard errors. The difference between the means was considered statistically significant at the 5% level ($p < 0.05$).

Results

Phytochemical screening

Hydroethanolic extraction of *C. procera* leaves yielded 21.38 g dry matter from 200 g plant material, giving an extraction yield of 10.69%. The extract had a pH of 6.76, a gummy consistency and a brown color. Phytochemical analysis revealed the presence of terpenoids, alkaloids and coumarins in different proportions. Phenolic compounds were detected in trace amounts, while saponins, tannins, flavonoids and reducing sugars were absent (Table 1).

Table 1
Phytochemicals of the hydroethanolic extract of *Calotropis procera* leaves

Compounds sought	Results obtained
Alkaloids	+
Coumarines	+
Terpenoids	+
Phenolic compounds	+/-
Saponines	-
Tannins	-
Flavonoids	-
Reducing compounds	-
Legend: +: Presence; -: Absence; +/-: Trace	

Acute toxicity tests

The effect of the acute toxicity of the hydroethanolic extract at 2500 and 5000 mg/kg on the body weight (b.w.) of rats is illustrated in Figs. 3 and 4. For weight stability, both male and female rats showed no significant deviation in body weight trends compared to controls, even at the highest tested dose (5000 mg/kg). Observation of treated animals showed no mortality or evidence of morbidity from the 2500 mg/kg dose to the 5000 mg/kg dose (tremor, convulsion, salivation, diarrhea, lethargy, sleep, and coma). The body index monitored daily showed no significant difference between the treated rats and the control. According to OECD 423 guidelines for acute toxicity, the LD50 of this extract is greater than 5000 mg/kg and is classified in category 5 (LD50 treatments between 2000 and 5000 mg/kg).

Antimicrobial activity

Tables 2 and 3 present the antimicrobial activity of the hydroethanolic extract of the leaves of *Calotropis procera*. Hydroethanolic extract showed no significant inhibitory activity against the bacterial or fungal strains tested, even at the highest concentration of 200 mg/ml (Tables 2 and 3; Figs. 5 and 6). In contrast, the positive control (ciprofloxacin) showed areas of inhibition ranging from 18 mm to 44 mm for bacterial strains, and nystatin showed areas from 31 mm to 32 mm for *Candida albicans.*

Table 2
Results of antibacterial activity

Bacterial strains	Diameter of the zones of inhibition (mm) as a function of concentration			
	200mg/ml	100mg/ml	50mg/ml	CPX Antibiotic 20 mg/ml
Escherichia coli ATCC 25922	-	-	-	44 ± 1,00
Escherichia coli 1555	-	-	-	18 ± 1,00
Neisseria gonorhoeae 0993	-	-	-	20 ± 1,00
Pseudomonas aeruginosa ATCC 27853	-	-	-	27,5 ± 0,25
Staphylococcus aureus ATCC 25922	-	-	-	38 ± 0,00
Staphylococcus aureus 29213	-	-	-	25 ± 0,25
Legend: (-) = Absence of inhibition; CPX = Ciprofloxacin				

Table 3
Results of antifungal activity

Fungal strains	Diameter of the zones of inhibition (mm) as a function of concentration			
	200mg/ ml	100mg/ml	50mg/ ml	Antifungal Nystatin 20mg/ml
Candida albicans ATCC 10231	-	-	-	32 ± 0,00
Candida albicans 1134	-	-	-	31 ± 1,00
Legend: (-) = Absence of inhibition				

Discussion

This study evaluated the phytochemical composition, acute toxicity, and antimicrobial properties of the hydroethanolic extract of *Calotropis procera* leaves, traditionally used in the Maritime region of Togo to treat urogenital infections. The results of the phytochemical screening performed on the leaves of *C. procera* revealed the presence of terpenoids, alkaloids and coumarins. These findings align with the work of Mainasara *et al*. (2012) in Nigeria [26], which also highlighted the presence of traces of tannins and flavonoids in the hydroethanolic extract. These bioactive compounds are known for their pharmacological potential, including their antimicrobial and anti-inflammatory properties. Some studies report that all parts of *Calotropis procera* contain cardenolides, alkaloids, saponins, terpenoids,

coumarins, tannins and flavonoids obtained with different extraction methods and types [27–29]. Thus, this difference from our results could be explained first and foremost by the type of extraction and then by hydroclimatic and edaphic factors. These factors are of paramount importance in plant biosynthesis.

The acute toxicity test of the hydroethanolic extract indicated an LD50 greater than 5000 mg/kg, classifying it as category 5 (low toxicity) according to OECD Guidelines 423. This suggests that the extract is safe at high doses when administered orally. Our results on toxicity are similar to those of Sarkodie *et al*. (2022) in Ghana who found that hydroethanolic extract from the leaves of *Calotropis procera* did not cause death or symptoms of poisoning up to the oral dose of 4800 mg/kg (b.w.) [30]. Similarly, Kinda *et al*.(2019) in Burkina Faso reported that the 5 000 mg/kg (b.w.) dose for leaf extract had no effect on treated mice [31]. However, other studies have reported toxic effects associated with different parts of the plant, emphasizing the need for further toxicological investigations. For that, *C. procera* plant is one of the few plants that is not fed to animals due to its toxicity [32,33].

Regarding antimicrobial activity, the hydroethanolic extract exhibited no significant inhibitory effect against tested bacterial and fungal strains associated with urogenital infections. These findings are consistent with those of Mainasara *et al*.(2012) in Nigeria whose hydroethanolic extracts of *C. procera* leaves did not show a significant effect against E. *coli, S. aureus and P. aeruginosa* tested at 120 mg/ml compared to tetracycline [26]. However, Contrary to our results, Saddiq *et al*.(2022) in Saudi Arabia, using the agar-well diffusion method, tested the ethanolic extract of *C. procera* at the concentration of 10 mg/ml that was active on *E. coli, S. aureus and C. albicans* with inhibition diameters of 21.96 mm, 18.66 mm and 21 mm, respectively [34]. The difference between our results and the literature could be explained by the difference in chemical compounds at the organ level related to edaphic and climatic conditions. Indeed, plants rich in saponins have immunological and anti-inflammatory properties. Similarly, tannins have been reported to have antibacterial potential due to their basic character that allows them to react with proteins to form stable water-soluble compounds, thereby killing bacteria by directly damaging their cell membrane [26]. The Antimicrobial power of plants is thought to depend on phenolic compounds and flavonoids [34,35]. Thus, the absence of these chemical compounds in our extracts would be at the origin of their ineffectiveness with regard to the germs tested. Although the hydroethanolic extract of C. procera did not exhibit antimicrobial activity in this study, its phytochemical composition suggests potential pharmacological applications beyond antimicrobial properties.

Limits and future directions

This study provides valuable information on the phytochemical and toxicological properties of *C. procera*. However, its limitations include the use of a single extraction method and the focus on a limited range of biological activities. Future studies should explore different solvents or advanced extraction techniques, such as supercritical fluid extraction, to improve the yield and efficiency of bioactive compounds. In addition, the investigation of other pharmacological properties, including anti-inflammatory and antioxidant activities, and the performance of in vivo antimicrobial studies will be

essential to assess the bioavailability and therapeutic efficacy of active compounds. These efforts will enable the full therapeutic potential of this plant to be exploited.

Conclusion

This study evaluated the phytochemical composition, acute toxicity and antimicrobial properties of the hydroethanolic extract of *Calotropis procera* leaves, traditionally used in the maritime region of Togo to treat urogenital infections. Phytochemical screening revealed the presence of terpenoids, alkaloids and coumarins, while acute toxicity tests showed a high margin of safety, with no adverse effects observed at doses of up to 5,000 mg/kg body weight. However, the extract showed no significant antimicrobial activity against the bacterial and fungal strains tested, suggesting limited efficacy in the treatment of urogenital infections. This finding highlights the need to further explore other extraction methods and biological activities, such as anti-inflammatory or antioxidant properties. In summary, while *C. procera* has promising phytochemical and toxicological profiles, its application as an antimicrobial agent requires further research.

Abbreviations

ATCC
American Type Culture Collection
b.w.
Body weight
CA
Chocolate agar
INH
National Institute of Hygiene of Lomé
LD50
Median lethal dose
MH
Mueller-Hinton
OECD
Organization for Economic Co-operation and Development
OMS
Organisation Mondiale de la Santé
SPSS
Statistical Package for Social Sciences
WHO
World Health Organization

Declarations

Ethics approval and consent to participate

The study was conducted following ethical guidelines for animal experimentation and research. The animals used in the study were treated according to bioethics guidelines, including compliance with the Animal Experimentation and Health Act and the European Council Directive of 24 November 1986 on the protection of animals used for scientific purposes.

Consent to publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors' contributions

Conception and design of the study: Samadou TCHAKONDO, Namtande BOLETI, Akouavi Emefa GAMAYIZOME, Efui Holaly GBEKLEY; Data collection and processing: Samadou TCHAKONDO, Namtande BOLETI, Akouavi Emefa GAMAYIZOME, Tchilabalo BOUYO; Supervision: Efui Holaly GBEKLEY, Tchadjobo TCHACONDO; Data analysis, interpretation and writing: Samadou TCHAKONDO, Efui Holaly GBEKLEY, Tchadjobo TCHACONDO; Manuscript Review: All authors have reviewed and approved the final version of this manuscript.

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Figures

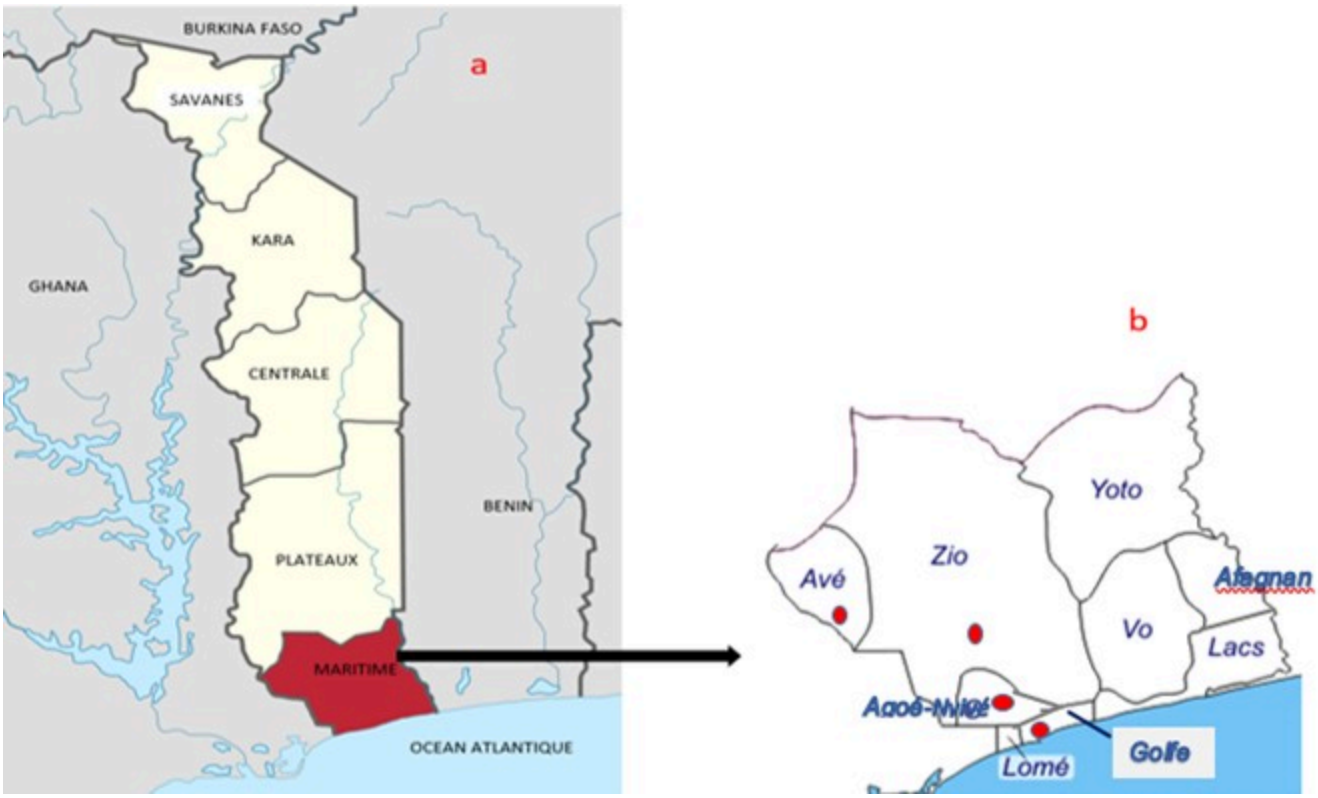

Figure 1

Map of Togo(a); map of the maritime region (b) / *O*: Study areas

(Source: adt-france-togo, 2019)

Figure 2

Fresh (a) and dried (b) leaves of *Calotropis procera* (Ait.)

(Source: Photo TCHAKONDO, 2022)

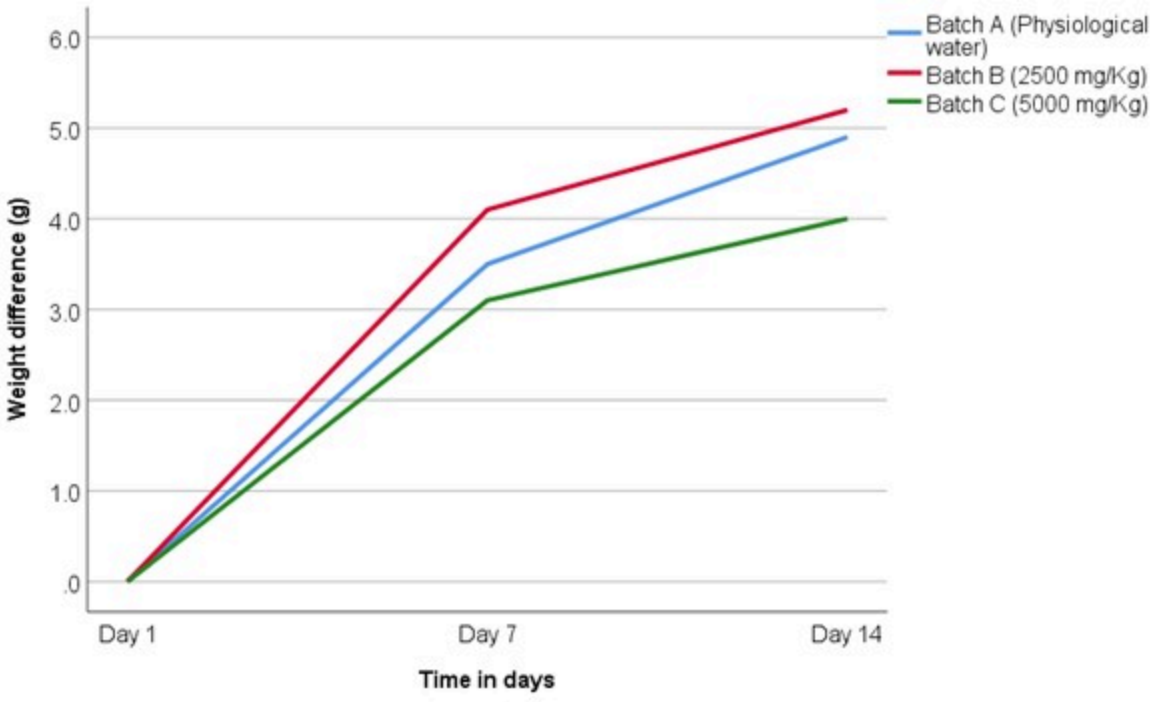

Figure 3

Effect of the extract on body weight in male rats at 2500 and 5000 mg/kg (b.w.)

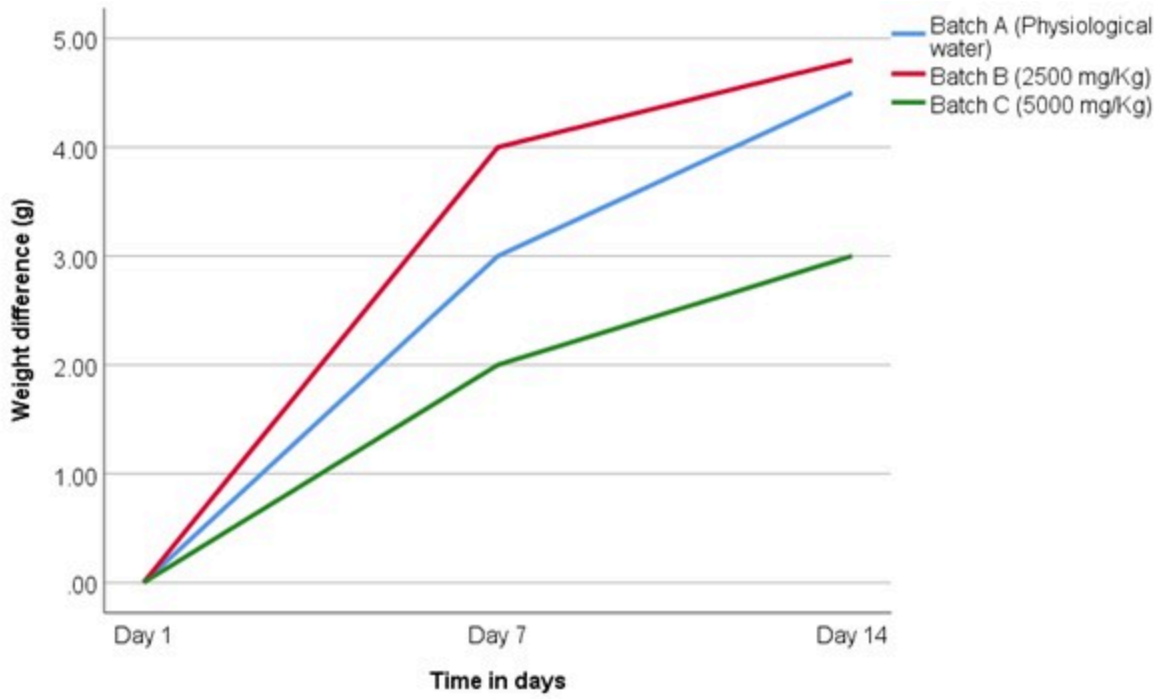

Figure 4

Effect of the extract on body weight in female rats at 2500 and 5000 mg/kg (b.w.)

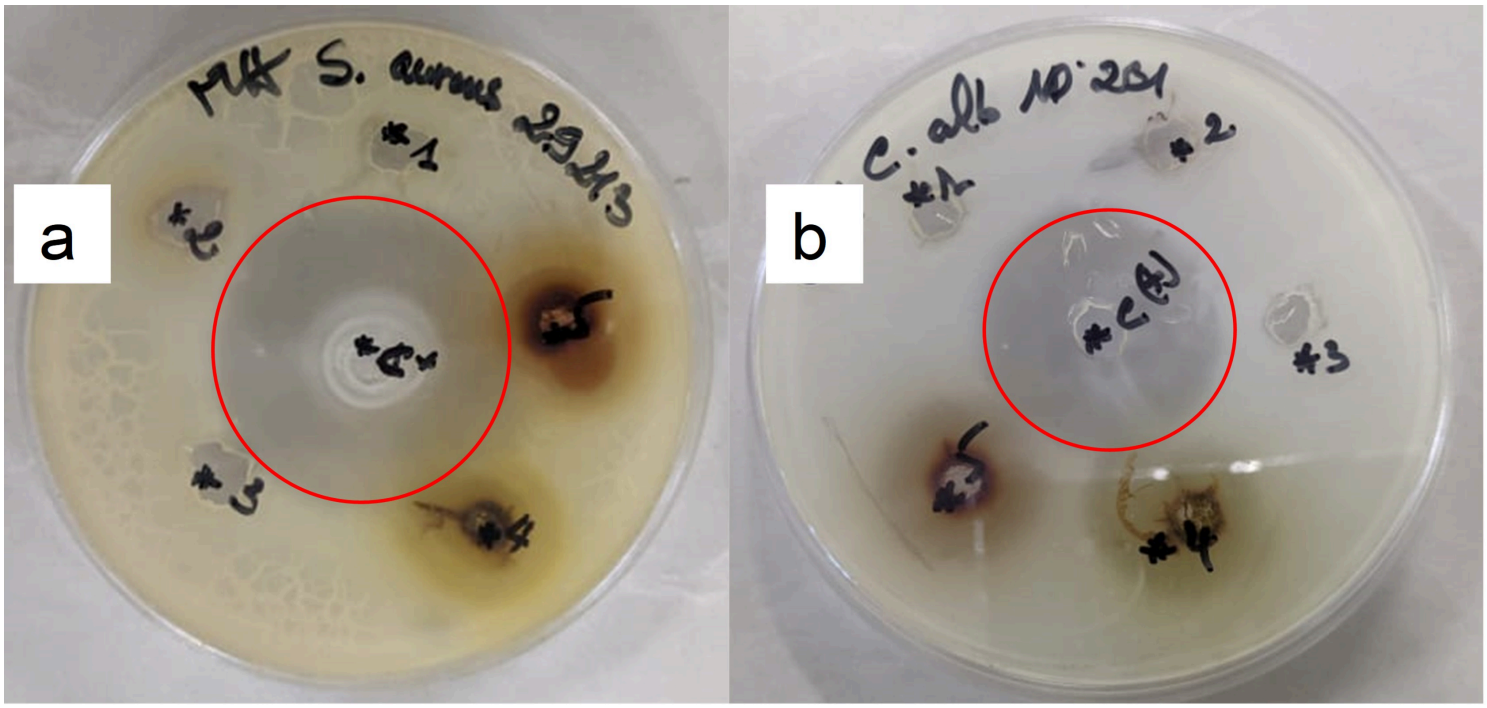

Figure 5

Culture media showing antimicrobial activity of positive controls (Ciprofloxacin and Nystatin) against *S. aureus* (a) and *C. albicans* (b

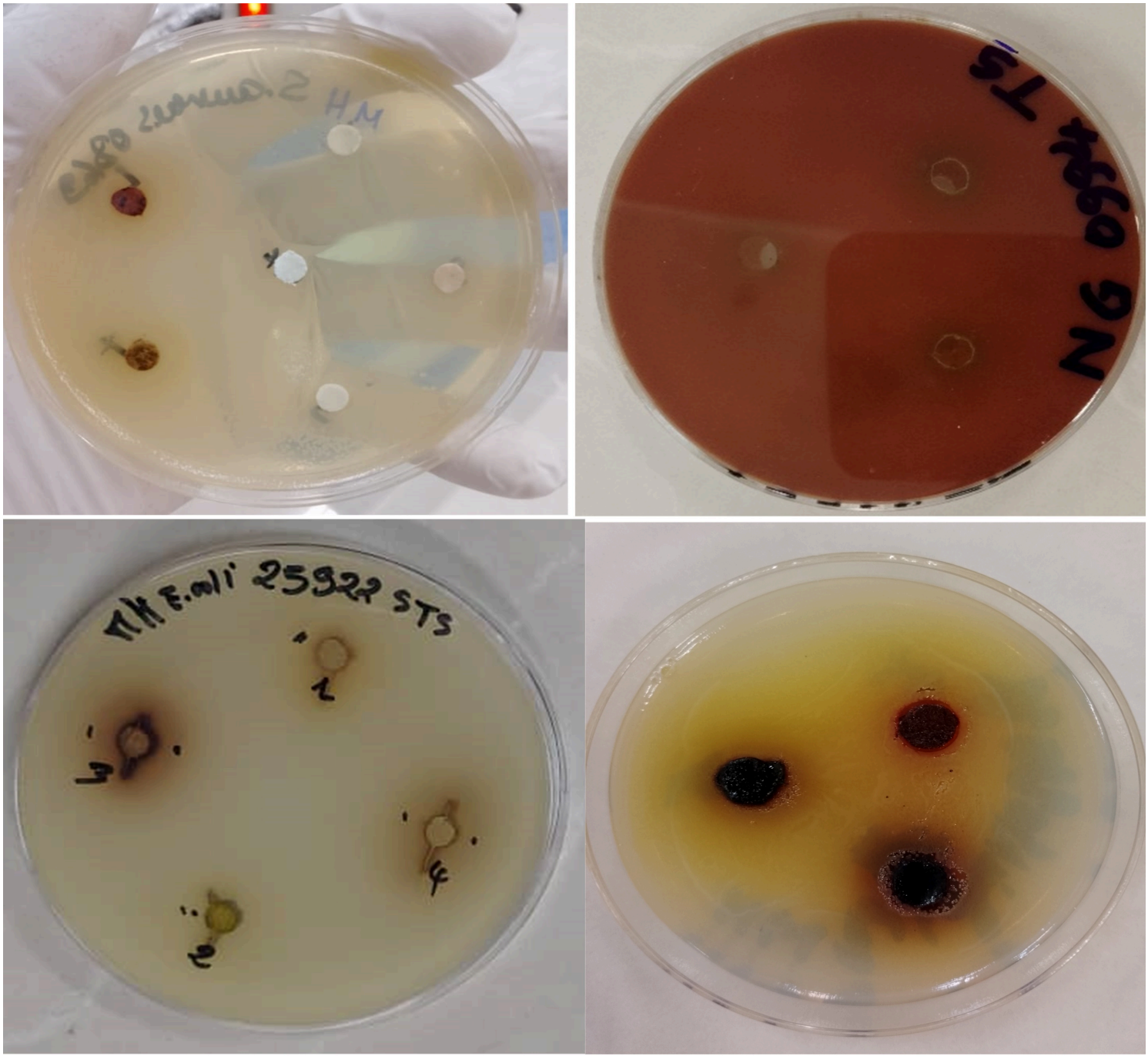

Figure 6

Culture media showing lack of antimicrobial activity of *calotropis procera* extract against tested microorganisms