INTRODUCTION

Infectious diseases are a major public health problem around the world and particularly in Africa. They are responsible for 45 percent of deaths in countries with low income and almost a premature mortality in the world [1]. Among infectious diseases, bacterial infections are for 70% of cases of mortality caused by microorganisms [2]. Other infectious agents involved include viruses and fungi. The toxinegines fungi that grow on food and develop mycotoxins which ingestion in a contaminated food can cause poisoning to the consumer [3].

Abusive and uncontrolled use of synthetics products meant the emergence of bacteria and fungi resistant to many antibiotics and antifungals conventional [4]. Beyond infectious diseases, the harmful effects of oxidative stress on human health are a serious problem of public health [5]. Free radicals, such as the superoxides, the peroxide of hydrogen and hydroxyl ions, and Singlet oxygen, are produced in larger quantities than the antioxidants in oxidative stress in the body and this imbalance is the cause of cell damage and chronic diseases [6]. It is useful to supplement the diet by antioxidant compounds.

Traditional medicine used since the millennium by people is very useful to treat many diseases [7]. According to WHO’s estimation, about 80% of African rely on traditional medicine for their medical care [8]. Currently, about 25-30% of all the drugs available for the treatment of diseases are derived from natural products [9]. Many ethnobotanical surveys in Benin and in several other countries of the sub-region show that many plants are traditionally used to treat different infectious diseases and many other chronic diseases. However, the therapeutic effects of these plants are only based on a purely empirical basis, most often without any scientific evidence.

Crateva adansonii is a plant species of the family Caparaceae, traditionally used to treat skin infections [10]. The organs used are leaves, roots, and sometimes the bark. Some scientific research questions stem from the traditional therapeutic use of Crateva adansonii. i) What is the chemical compound involved in the pharmacological properties of C. adansonii? ii) These pharmacological properties vary according to the organs (leaf and bark)? iii) Are the extracts of this plant toxic? In this direction, the study aims to evaluate the phytochemistry, anti-radical, antimicrobial properties and cytotoxicity of C. adansonii.

MATERIALS AND METHODS

Chemicals and reagents

The solvents such as ethanol, hexane, ethyl acetate were purchased from Qualikens Fine Chem Pvt. Ltd., (Yonkers). Pin: 391340 India). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), were obtained from Fluka (Neu-Ulm, Germany). Folin-Ciocalteu reagent, gallic acid were from sigma-aldrich (St. Louis, MO 63103 USA). Potato Dextrose Agar-PDA, Mueller Hinton agar, and broth were purchased from HiMedia Laboratories Pvt. Ltd, India. All other materials, chemicals, and reagents used in the experiment were of analytical grade quality.

Plant material collection

The leaves and bark of C. adansonii were collected in the township of Porto-Novo. These organs were air-dried in the laboratory at 25-30°C for 15 d and then ground in powder using grinder Retsch type SM 2000, 1430, lpm. The smooth powder was used to make the extracts and qualitative phytochemical screening.

Qualitative phytochemical screening

The phytochemical profiling to assess the secondary metabolites of C. adansonii leaves and bark was done according to Houghton and Rahman [11].

ABSTRACT

Objective: This study aims to search the secondary metabolites of Crateva adansonii (leaves and bark) and evaluate some biological activities (antimicrobial, antioxidant and cytotoxicity’s property) of these extracts.

Methods: The phytochemical screening was made with standard method. Agar diffusion method was used for antibacterial activity coupled with Minimum Inhibitory Concentration (MIC) and Bactericidal (MBC) determination. The antifungal test was performed by the mycelial development reduction method. DPPH method was used to evaluate the extracts antioxidant activity. Artemia salina larvae were use as support to evaluate the toxicity of the extracts.

Results: The results of the phytochemical screening reveal the presence of polyphenols, triterpenoids, and glycosides in the both organs (leaves and bark) of C. adansonii. All the extracts have low activity on the reference strains with a larger diameter of 17±00 mm (S. oralis). Only the ethanolic and hydroethanolic leaf’s extracts inhibited respectively 20% and 10%, of the clinical strain S. aureus. The ethyl acetate leaf’s extract shows the best antifungal activity (89,19%) with A. clavatus. All extracts present dose-dependent antiradical properties and are non-toxic for the cells of Artemia salina. C. adansonii bark extracts had the best reducing power (23,80±6, 137 mmol/g EqAA EAA) of the DPPH radical.

Conclusion: C. adansonii is therefore an active principle source for the development of drugs to antimicrobial and antioxidant activities.

Keywords: Screening phytochemical, Antimicrobial activity, Anti-radical activity, Benin

Original Article

PHYTOCHEMISTRY AND BIOLOGICAL ACTIVITIES OF CRATEVA ADANSONII EXTRACTS

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Antimicrobial activity

Aqueous and water-ethanol extracts

According to the method described by Dah-Nouvlessounon et al. [4], 50 g of powder of the leaves and the bark of Crateva adansonii, have respectively been macerated for 72 h at room temperature in 500 ml of each solvent (distilled water for aqueous extract and distilled water/ethanol in 30:70 proportion for hydro-ethanol extract). The resulting homogenate was filtered three times on hydrophilic cotton and once on paper Wattman N 1. This filtrate was then dried at 45 °C in a fume hood for aqueous extract and concentrated using rotavapor for hydro-ethanol extract. The concentrate was put in the oven at 40 °C until total evaporation. The resulting powder was the extracts.

Successive extraction with ethanol, hexane and ethyl acetate

The extraction method used is an adaptation to the protocol used by Sanogo et al. [12]. 50 g of powder of the leaves and the bark of Crateva adansonii, have respectively been macerated for 72 h at room temperature in 500 ml of ethanol 96°. The mixture was filtered, and the half was evaporated. After adding 25 ml of distilled water and 50 ml of hexane to the second half, the upper organic phase was recovered and evaporated to obtain hexanic extract. To the lower phase, 50 ml of ethyl acetate was added, after 30 min the upper phase is recovered, evaporated and dry to obtain the ethyl acetate extract. The yield of each extract was determined.

Antimicrobial activity

Tested microorganisms

These microorganisms include: 14 references strains (Staphylococcus aureus ATCC 29213, Staphylococcus epidermidis, T22695, Micrococcus luteus, Streptococcus oralis, Enterococcus foecalis ATCC 29212, Pseudomonas aeruginosa, Proteus mirabilis A24974, Proteus vulgaris A25015, Escherichia coli ATCC 25922, Candida albicans MMH, Aspergillus parasiticus CMBB20, Aspergillus ochraceus CMBB91, Aspergillus fumagatus CMBB99, Aspergillus clavatus NCPT 97 and 10 Staphylococcus strains, isolated from Buruli ulcer wounds.

Sensitivity test (antibiogram)

This test was evaluated using the disk diffusion method inspired from that described by Henry et al. [13]. Indeed, 1 ml of bacterial culture (adjusted to 0.5 McFarland standard) was used to flood a Petri dish containing Mueller-Hinton agar (Bio-Rad, France). Two to four sterile disks (6 mm) are deposited in the Petri dish previously flooded of bacterial culture under aseptic conditions. This dish was inoculated with 30 μl of tested extract. For each extract, the experiment is duplicated, and negative control is performed with the solvent in place of the extract. The dishes are then kept at room temperature 15-30 min before being incubated at 37 °C during 24 h and 48 h. The inhibition diameters were measured using a scale after incubation times of 24 h and 48 h.

Determination of minimum inhibitory concentration (MIC)

The method of macro dilution with visual observation previously describe by Dah-Nouvlessounon et al. [4] was used. First, the extracts were diluted in sterilized distilled water to the highest concentration of 20000 μg/ml and then nine dilutions were performed to obtain the concentrations of 10000 μg/ml, 5000 μg/ml, 2500 μg/ml, 1250 μg/ml, 625 μg/ml, 312.5 μg/ml, 156.25 μg/ml, 78.12 μg/ml and 39.06 μg/ml in screw caped. To 1 ml of the above concentrations was added 1 ml of the bacteria inoculum (10° UFC) to obtain 2 ml as a final volume. Culture medium without samples and others without microorganisms were used in the tests as a control. Tubes were incubated at 37 °C for 18-24 h and growth was indicated by turbidity. The MIC is the lowest concentration of the compound at which the microorganism tested does not demonstrate visible growth (turbidity).

Determination of minimum bactericidal concentration (MBC)

Referring to the results of MIC test, all tubes showing no microorganism growth were identified. Each tube is inoculated into a Petri dish containing MH agar and incubated at 37 °C for 24 h. The lowest concentration of the extract in which the microorganism did not grow on solid medium is considered as Minimum Bactericidal Concentration.

Evaluation of antifungal activity

This test was evaluated by a culture method inspired of those described by Dohou et al. [14]. All extracts (2000 mg/ml) have been previously dissolved in sterile distilled water.1 ml was mixed with 10 ml of agar Potato Dextrose Agar-PDA hot then sunk in Petri dishes. Extracts were then tested with a single concentration of 20 mg/ml. After solidification of the Medium, a disc (6 mm diameter) contaminated on one of its surfaces by the pathogen (fungal strain) was placed in the center of petri plate. A control (control) was placed by a monoculture of the previously described as fungal strain at the center of the agar PDA (10 ml without extract). Each test is performed twice. Plates were incubated at 25±1 °C. After 5 days of incubation, the diameter of the mycelium was measured. The inhibition percentage was determined using the formula

\[
\text{Antioxidant activity test: method of DPPH}
\]

DPPH scavenging effect of C. adansonii extract was assessed by the method described by Kim et al. [15]. With slight modifications. A volume of 1.5 ml of the extract solution (100/μg/ml) was added to 3 ml of the methanolic solution of DPPH (0.4 mg/ml). The mixture was placed in a dark area at room temperature for 15 min, and then the absorbance was measured at 517 nm against the blank (1.5 ml of methanol and 3 ml of DPPH at 10 μg/ml). Radical scavenging activity was determined using a calibration curve (R² = 0.99) with ascorbic acid (0-10 mg/ml).

Quantitative phytochemical screening of the bark and leaves extracts

Full phenolics content

Total polyphenols were determined by using adapted Folin-Ciocalteu method as described by Singleton et al. [16]. Briefly, the methanolic solution of each extract (10 mg/ml) was diluted to 1/100 with distilled water. 125μl of this solution was then mixed with 625 μl of Folin-Ciocalteu reagent (10%). After 5 min, 500 μl of aqueous sodium carbonate (Na2CO3 75 μl/g) were added. After 2h of incubation in the dark at the room temperature, the absorbances were measured in triplicate at 760 nm against a blank (0.5 ml Folin-Ciocalteu and 1 ml of Na2CO3) with a spectrophotometer (BIOMATES 3S). The total phenolics content was determined using the plotted standard calibration curve with gallic acid (0-10 mg/ml).

Cytotoxic activity of the extracts

The cytotoxic effect of the extracts was evaluated according to an adaptation of the method described by Farshori et al. [17]. The tests are carried out twice on 72 h larvae of Artemia salina. Briley, a test was constituted of 16 A. salina larvae in a 2 ml solution containing 1 ml of the extract tested concentration and 1 ml of the sea. The number of surviving larvae is counted after incubation at room temperature (24 h) and the DI50 is calculated using the regression line obtained from the surviving larvae in function of the extracts concentration representation.

Statistical analysis

The results of the experience were expressed as mean±standard deviation. Data were analyzed using Student Newman and Keuls (SNK) test and MINITAB (version 17) with the software SAS 9.2. p values less than 0.05 (p<0.05) were considered significant.

RESULTS

Qualitative phytochemical screening

The leaves and bark contain different secondary metabolites (table 1). The tannins (catechiques and Gallic), triterpenoids and reducing compounds are intensely present in the leaves than the bark. However, the alkaloids, anthocyanins, the coumarins, the tannins (catechiques and Gallic), triterpenoids and reducing compounds are intensely present in the leaves than the bark. The leaves and bark contain different secondary metabolites (table 1). The tannins (catechiques and Gallic), triterpenoids and reducing compounds are intensely present in the leaves than the bark.
Antibacterial activities of ethanolic, hydroethanolic extracts, and the bark extracts showed inhibition of clinical strains of Staphylococcus aureus, Pseudomonas aeruginosa, P. mirabilis, Enterococcus faecalis, S. epidermidis, Pseudomonas vulgaris, S. oralis, and Candida albicans, with reference strains inhibitory diameters obtained as mean±SD. Each value is the mean of two assays.

| Bacterial strains | Leaves ethanolic extract | Leaves aqueous extract | Leaves residual ethanolic extract | Leaves ethyl acetate extract | Bark ethanolic extract | Bark aqueous extract | Bark residual ethanolic extract | Bark ethyl acetate extract |
|-------------------|--------------------------|------------------------|----------------------------------|-----------------------------|------------------------|----------------------|-----------------------------|---------------------------|
| S. aureus         | 17±0.02                  | 14±0.01                | 12±0.04                          | 9±0.01                      | 5±0.08                 | 12±0.04              | 11±0.03                    | 14±0.01                   |
| Ps. aer           | 13±0.02                  | 10±0.01                | 11±0.03                          | 8±0.01                      | 5±0.01                 | 11±0.03              | 10±0.01                    | 14±0.01                   |
| P. mir            | 17±0.02                  | 14±0.01                | 12±0.04                          | 9±0.01                      | 5±0.08                 | 12±0.04              | 11±0.03                    | 14±0.01                   |
| M. lut            | 17±0.02                  | 14±0.01                | 12±0.04                          | 9±0.01                      | 5±0.08                 | 12±0.04              | 11±0.03                    | 14±0.01                   |
| S. epidermidis    | 17±0.02                  | 14±0.01                | 12±0.04                          | 9±0.01                      | 5±0.08                 | 12±0.04              | 11±0.03                    | 14±0.01                   |
| P. vulgaris       | 17±0.02                  | 14±0.01                | 12±0.04                          | 9±0.01                      | 5±0.08                 | 12±0.04              | 11±0.03                    | 14±0.01                   |
| S. oralis         | 17±0.02                  | 14±0.01                | 12±0.04                          | 9±0.01                      | 5±0.08                 | 12±0.04              | 11±0.03                    | 14±0.01                   |
| P. mirabilis      | 17±0.02                  | 14±0.01                | 12±0.04                          | 9±0.01                      | 5±0.08                 | 12±0.04              | 11±0.03                    | 14±0.01                   |
| E. faecalis       | 17±0.02                  | 14±0.01                | 12±0.04                          | 9±0.01                      | 5±0.08                 | 12±0.04              | 11±0.03                    | 14±0.01                   |
| E. coli           | 17±0.02                  | 14±0.01                | 12±0.04                          | 9±0.01                      | 5±0.08                 | 12±0.04              | 11±0.03                    | 14±0.01                   |
| C. albicans       | 17±0.02                  | 14±0.01                | 12±0.04                          | 9±0.01                      | 5±0.08                 | 12±0.04              | 11±0.03                    | 14±0.01                   |

Table 1: Composition in a secondary metabolite of the leaves and bark of *C. adansonii*

| Secondary metabolites | Composition |
|-----------------------|-------------|
|                       | Leaves      | Bark        |
| Alkaloids             | -           | +           |
| Tannins               | +++         | +           |
| Tannins catechines    | +++         | +           |
| Gallic tannins        | +++         | +           |
| Flavonoids            | +           | ++          |
| Anthocyanins          | -           | -           |
| Leuco-anthocyanins    | +           | +           |
| Coumarins             | -           | -           |
| Quinoniques derivatives| +         | +           |
| Triterpenoids         | +++         | ++          |
| Steroids              | -           | -           |
| Cardenolides          | -           | -           |
| Cyanogeniques derivatives| -        | -           |
| Mucilages             | ++          | +++         |
| Glycosides            | -           | -           |
| Anthraceniques free   | -           | -           |
| Cyanogeniques derivatives| -        | -           |
| Power (IM)            | 3/10        | 2/10        |
| Reducing compounds    | +++         | ++          |
| Anthraceniques free   | -           | -           |
| Glycosides to GR      | -           | -           |
| C-glycosides         | ++          | ++          |
| Muclages              | +++         | +++         |

+++: Very intense; ++: Intense; +: Very intense; -: Negative

**Crataeva adansonii** extract yields

The leaves aqueous extract presents the highest yield (16.7%) while the lowest (0.66%) was obtained with the leaves hexanic extract (table 2).

**Antibacterial activities of C. adansonii**

The bacterial strains sensitivity varies according to the organs and extracts types (table 3). Indeed, some extracts such as: leaves aqueous, leaves hexanic, leaves residual ethanolic extracts, as well as the aqueous bark extract, do not have inhibitory effect with reference strains. Besides, leaves water-ethanolic and ethyl acetate extracts inhibit the growth of 20% of the reference strains while the leaves ethanolic extract is active on 40% of these strains. For the bark extracts, water-ethanolic extract and ethanol extract are respectively inhibiting 20% and 30% of the reference strains. Generally, reference strains have a very low sensitivity to the leaves and bark extracts at 100 mg/ml (table 3). The larger inhibitory diameter zone (17±0.02 mm) for active extracts was recorded with the leaves ethyl acetate extract against the growth of *Streptococcus oralis*. Besides, the lowest (3±0.18 mm) was obtained with the bark ethanolic extract (Escherichia coli). The inhibitory diameter zone of active extracts does not vary (*p > 0.05*) in the time (24 and 48 h).

| Organ | Type of extract | Yield (%) |
|-------|----------------|-----------|
| Leaves. Aqueous extract | 16.7 |
| Leaves. Water-ethanolic extract | 10.78 |
| Leaves. Extract | 3.98 |
| Leaves. Hexanic extract | 0.66 |
| Leaves. Ethyl acetate extract | 0.88 |
| Leaves. Residual ethanolic extract | 0.74 |
| Bark. Aqueous extract | 8.13 |
| Bark. Water-ethanolic extract | 2.4 |
| Bark. Extract | 2.5 |

Table 3: Inhibitory diameters zones of the different extracts

| Organ | Type of extract | S. aureus inhibitory diameters (mm) | Ps. aer inhibitory diameters (mm) | P. mirabilis inhibitory diameters (mm) | M. lut inhibitory diameters (mm) | S. epidermidis inhibitory diameters (mm) | P. vulgaris inhibitory diameters (mm) | S. oralis inhibitory diameters (mm) | E. faecalis inhibitory diameters (mm) | E. coli inhibitory diameters (mm) | C. albicans inhibitory diameters (mm) |
|-------|----------------|------------------------------------|-----------------------------------|----------------------------------------|----------------------------------|----------------------------------------|-------------------------------------|-------------------------------------|-----------------------------------|---------------------------------|----------------------------------|
| Leaves Aqueous | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| water-ethanolic | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Extract | 12±0.12 | 0 | 0 | 5±0.04 | 12.5±0.08 | 9±0.17 | 0 | 0 | 0 | 0 | 0 |
| Ethyl acetate | 0 | 0 | 0 | 0 | 14±0.3 | 0 | 17±0.02 | 0 | 0 | 0 | 0 |
| Hexanic | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Bark Aqueous | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| water-ethanolic | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Extract | 0 | 0 | 0 | 0 | 0 | 3±0.18 | 0 | 13±0.08 | 0 | 3±0.33 | 0 |

S. aureus: Staphylococcus aureus, Ps. aer: Pseudomonas aeruginosa, P. mir: Proteus mirabilis, M. lut: Micrococcus luteus, S. epidermidis: Staphylococcus epidermidis, P. vul: Proteus vulgaris, S. oralis: Streptococcus oralis, E. faecalis: Enterococcus faecalis, E. coli: Escherichia coli, C. albicans: Candida albicans, Values of inhibition diameter are obtained as mean±SD. Each value is the mean of two assays (*n = 2)*

Regarding the clinical strains of *Staphylococcus aureus*, only the leaves ethanolic, hydroethanolic extracts, and the bark ethanolic showed antibacterial activity. The inhibitory rate varies according to the extract. Indeed, the leaves ethanolic extract inhibit 20% of the growth

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of the clinical strain, while the leaves water-ethanolic extracts and the bark ethanolic extract inhibit each one 10% of the strains (table 4).

**Antifungal activity of *C. adansonii* extracts**

Fig. 1 shows that all the extracts present antagonism to the growth of the four fungal strains. The power of Mycelial development reduction varies \( p<0.01 \) according to the extracts. The SNK test (Student-Newman-Keuls) helped to build homogeneous groups of extract regarding the percentage of inhibition. The bigger inhibitory percentage (89.19%), was obtained with the leaves ethyl acetate extract on *A. clavatus*, while the lowest (20.69%) was obtained by the leaves water-ethanolic extract with *A. parasiticus* strain (fig. 1).

**Total polyphenols content**

Polyphenols content varies according to the extracts and the plant organs (table 5). The leaves ethyl acetate extract has a high content (344.74 µg EAG/100 mg) of total polyphenolic compounds while the leave hexanic extract recorded the lowest one (65.81 EAG µg/100 mg).

**C. adansonii anti-radical properties**

The anti-radical power of *C. adansonii* extracts with DPPH radical is expressed by the equivalent of Ascorbic acid (table 6). The biggest content (23.8044±0.137 mmol EAA/g) was obtained with the bark ethanolic extract. This extract presents the largest reducing power of the DPPH radical. The leave ethanolic extract presents the lower content (11.8271±0.008 mmol EAA/g).
Aspergillus tamarii but active with 100% and 25%). The best inhibition percentage (89, 19%) was recorded with hexanic extract, leaves and bark water-ethanol extracts (between 0 and 25%).

Table 7 shows a variation of the DL50 from the regression line obtained from the representative curve in the number of surviving larvae based on the concentration of extracts. All the extracts have a DL50 greater than 0.1 mg/ml (Table 7).

### DISCUSSION

Biological activities attributed to a plant depend on its composition in bioactive compounds. Indeed, it was noted in this study a variation in the presence of various secondary metabolites groups according to the plant organs (leaves and bark). Most of the identified compounds are intensely present in the leaves and bark.

However, some secondary metabolites as alkaloids are absent in *C. adansonii* leaves and bark. These results are comparable to those of Agbanke et al. [18] Tsado et al. [19] who have highlighted the presence of alkaloids in the *C. adansonii* leaves. This difference could be due to some factors such as the type of soil, the location of the plants, the harvest of bodies. The absence of cyanogenic derivatives, in the both organs, mitigates the risk of extracts toxicity because cyanogenic derivatives are the causes of toxicity due to the production of cyanide ions which manifest through the massive poisoning resulting from ingestion of cyanogenic derivatives to acceleration and amplification of respiratory rate, respiratory depression, dizziness, headache, consciousness disorder, coma, etc. [23].

All the used solvents do not have the same extraction capacity because the yields vary according to the solvents. The variability of yields could be explained by the ability of extracting of each solvent which depends firstly on the solvent affinity with the phyto-molecules and on the other hand the polarity of the solvent [4]. Unlike yields of extract that we have achieved, Nounagnon et al. [24] have found better yields (23.78%) with water-ethanol mixture compared with the one obtained with water (9.36%). Similarly, Agbanke et al. [18] have found a yield of 12.7% with water and 14.3% with the water-ethanol mixture. These differences could be due to several reasons including the stage of maturity of the collected leaves [25], the extraction process, conditions of drying of the plant organs [26], the degree of purity of the used solvents.

The low antibacterial activity was observed with the tests extracts. The low antimicrobial power of the leaves aqueous and water-ethanol extracts had already been mentioned by Agbanke et al. [18], at 20 mg/ml of reference strains and strains isolated from diarrheal infections. Besides, the leaves ethyl acetate extracts have proved very active throughout the fungal strains. According to the scale of Reyes [27] for antifungal activity, the leaves ethyl acetate extract is very active, the same for the bark aqueous extract which is very active with *A. ochraceus*, and *A. parasiticus* (between 75 and 100%) but active with *A. fumigatus* and *A. clavatus* (between 50 and 75%). Some strains are resistant to some extract such as: leaves hexanic, extract, leaves and bark water-ethanol extracts (between 0 and 25%). The best inhibition percentage (89, 19%) was recorded with the leaves ethyl acetate extract. Kpolli et al. [28] have shown that ethyl acetate extracts a large number of polyphenolic compounds that are also known for their antifungal properties. Similarly, Dah-Nouwlesounon et al. [4] have shown that among the organic extracts of *Cola acuminata* and *Garcinia kola* nuts ethyl acetate present best antifungal activity with *Fusarium verticillioides*, *Aspergillus tamarii* and *Penicillium citrinum* strains. Beyond antimicrobial activities, *C. adansonii* extracts also present interesting antiradical’s properties that vary according to the extract type. The ethanol extract of the bark presents the strongest reducing power of the radical DPPH. Similar results are obtained by Moshi et al. [29] which have obvious activities in vitro antioxidant of the hydro-ethanol extracts of leaves of *C. adansonii*. Strong anti-radical power of the extracts could be explained by the richness in secondary metabolites of *C. adansonii* including polyphenols.

The toxicity test results show that all the extracts used are non-toxic at a concentration of 20 mg/ml. Indeed, the DL50 of all the extracts are greater than 0.1 mg/ml. According to the correlation grids associating the degree of toxicity with the DL50 [30], the used *C. adansonii* extracts are therefore non-toxic at 20 mg/ml to larval cells. The test according to the *Artemia salina* model used is a preliminary screening to determine not only the degree of toxicity of a product but also the presence of potential anti-cancer compounds.

Table 7: Dose lethal 50 and the various extracts of *Crateva adansonii* regression coefficients

| Plant organ | Types of extracts | LD50 (mg/ml) | Regression coefficient (R²) |
|-------------|------------------|--------------|----------------------------|
| Leaves      | Aqueous          | 5.70         | 0.7671                     |
|             | Water-ethanolic  | 6.63         | 0.8408                     |
|             | Ethanol          | 4.40         | 0.9175                     |
|             | Hexanic          | 7.41         | 0.7604                     |
|             | Ethyl acetate    | 7.41         | 0.741                      |
| Bark        | Aqueous          | 5.85         | 0.8243                     |
|             | water-ethanolic  | 5.06         | 0.7144                     |
|             | Ethanol          | 6.37         | 0.8329                     |

Larval cytotoxicity

Table 7 shows a variation of the DL50 from the regression line obtained from the representative curve in the number of surviving larvae based on the concentration of extracts. All the extracts have a DL50 greater than 0.1 mg/ml (Table 7).

### CONCLUSION

At the end of the biological activities, we retain a low antibacterial activity at a dose of 100 mg/ml, variable according to the extracts, an antifungal power considerable at a dose of 20 mg/ml on fungi of the genus *Aspergillus*, and anti-radical potential. This plant is, therefore, an active principle source for the development of drugs to antimicrobial and antioxidant activities, especially the cytotoxicity tests revealed its non-toxic nature towards *Artemia salina* larvae. The results show that *C. adansonii* is a medicinal plant with therapeutic properties and partly justify the traditional uses of this plant.

However, our work should be enhanced for better use of the medicinal plant. It will be among other things of: quantify the other secondary metabolites out of polyphenols by Spectrophotometric dosage to better categorize the potential active ingredients; expand antibacterial and antifungal to a larger number of bacterial and fungal strains, confirm the antioxidant activity by other methods and activity, optimize the toxicological aspect complementing cytotoxic in vivo toxicity tests.

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### AUTHORS CONTRIBUTIONS

Baba-Moussa Lamine: conceived and coordinated the experiments and the manuscript writing, Nounagnon S. Martial, and Dah-Nouwlesounon Durand: conceived and designed the experiments, performed the experiments, analyzed the data and writing and editing the manuscript. N’TCHA Christine and Legba Boris: analyzed the data and prepared the initial draft of the manuscript and helped in the experiment part. Baba-Moussa Farid and Adolphe Adjahoun: Thoroughly rechecked, reviewed the manuscript and put necessary efforts to improve the quality of the manuscript.
CONFLICT OF INTERESTS
The authors declare that they have no conflict of interests.

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