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Antioxidant and antimicrobial activities of *Cassia alata* (L.) Roxb. Leaves

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ABSTRACT

Cassia alata is a plant of Caesalpiniaceae family used for the treatment of management infectious diseases. This work aimed at evaluating antioxidant and antimicrobial activities of hydroethanolic extract of *Cassia alata* leaves. The extract was obtained by the maceration of crude leaf powder (hydroethanolic 30:70). The phytochemical screening was focused on the detection of major chemical groups. The total flavonoids contents were studied using the aluminum chloride colorimetry method. The antioxidant capacity was carried out by the phosphomolybdate reduction method and by FRAP method. The antimicrobial activity of the extract was carried out by the diffusion method and the micro dilution. The extract showed the presence of phenolic compounds, flavonoids and reducing compounds. The concentration of total flavonoids of *Cassia alata leaves* was 70.91± 5.66 mg RE/g. The antioxidant activity by the phosphomolybdate reduction method and by the FRAP method were respectively 69.57 ± 4.83 mg AAE/g and 225.5 ± 17.32 mg FSE/g. The extract of *Cassia alata* has bactericidal action on *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* SARM, *Pseudomonas aeruginosa* ATCC 27853 and *Cutibacterium acnes* ATCC 6919 and fungicidal action on *Candida albicans* ATCC 10231. This study shows that *Cassia alata* could be a source of a new antioxidant and antimicrobial agent.

Keywords: *Cassia alata*, antioxidant, antimicobial, bactericidal, fungicidal.

INTRODUCTION

The use of plants to prevent and treat infectious diseases over the years has attracted the attention of scientists around the world (Falodun et al., 2006). Several researches carried out on medicinal plants based on ethnobotanical surveys carried out by local populations have led to the discovery of phytochemical constituents for the purpose of application in the prevention and treatment of diseases. These studies have established a scientific basis for the efficacy of these plants. The development of pathogenic microorganism resistance to most available antimicrobial drugs and the high treatment costs resulting from this resistance have made it important to search for new safe, effective and cost-effective ways to manage infectious

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diseases (Akinpelu and Onakoya, 2006). The therapeutic success of quinine and quinidine isolated from cinchona bark and recently artemisinin from *Artemisia annua* in malaria chemotherapy demonstrates the importance of higher plants as potential sources of new drugs (Igoli et al., 2005).

In fact, one of the most commonly used plants locally to treat superficial fungal infections is *Cassia alata* also called *Senna alata* (Timothy et al., 2012). The Dartrier or Winged Breaker, Senna alata (L.) Roxb. *Cassia alata* is a plant of the Caesalpiniaceae family, or Fabaceae, subfamily Caesalpinioideae, according to phylogenetic classification. *Senna alata* (L.) Roxb. is from South America (Mexico), but it has been planted everywhere for medicinal and ornamental purposes and it is now pantropical (Levy and Lewis, 2011). In many countries, including the majority of tropical African countries, it has become naturalized and is often considered as a weed (Levy and Lewis, 2011). *Cassia alata* is a shrub measuring 3-4 m tall, with compound leaves 50-80 cm long. The leaves are pinnately compound. They have a rounded tip with a slight indentation in the middle. Ornamental foliage that opens in the morning and closes in the evening (Pissang et al., 2016). The flowers are arranged in a vertical column and bloom from the base of the column. Leaves and flowers have a foul smell (Pissang et al., 2016). The fruits are almost straight pods, dark brown turning black. Seeds are spread by water or animals (Pissang et al., 2016). *Senna alata* is used medicinally mainly as a laxative or purgative, as well as in the treatment of skin problems (Hoekou et al., 2016). The laxative effects are generally obtained with the decoction of leaves, which is drunk, and, more rarely, of flowers, roots or stems. Dermatoses treated with *Senna alata* These include ringworm, favus and other fungal infections, impetigo, syphilitic wounds, psoriasis, herpes, chronic lichen planus, scabies, erythema and itching (Levy and Lewis, 2011).

In view of the curative activities of this plant described above, it would be important to test this plant on the repellent microorganisms of the pathologies that are used in order to be able to recommend it with confidence. It is in this perspective that the present study aimed to carry out phytochemical screening, to evaluate the antioxidant power, and the antibacterial and antifungal activities of this plant of the Togolese flora.

MATERIALS AND METHODS

Plant material

The plant material used was *Cassia alata* leaves. The plant organs were collected in May 2022 at Hahotoe in the Maritime Region of Togo. The sample was identified at the Laboratory of Botany and Plant Ecology of the Faculty of Sciences of the University of Lomé where a voucher specimen was deposited in the herbarium under the number TOGO02802.

Chemicals

Phosphomolybdate, rutin, aluminium chloride, ascorbic acid. sulphuric acid, 2,4,6-tripyridyl-s-triazine (TPTZ).

Microbial strains Tested

The microbial strains used were wild-type reference strains (confirmed by susceptibility testing) that were obtained from the American Type Culture Collection (ATCC). They were *Staphylococcus aureus* (ATCC29213) ; *Staphylococcus aureus* MRSA ; *Pseudomonas aeruginosa* (ATCC27853) *; Cutibacterium acnes* (ATCC6919) and *Candida albicans* (ATCC 10231). They were provided to us by the microbiology laboratory of the National Institute of Hygiene of Lomé (INH).

Hydroethanolic extraction of harvested leaves

The extraction was carried out following the previous work of Hoekou (2016). The hydro-ethanolic extract was obtained by maceration under continuous agitation of 200 g of plant material powder in 2000 mL of ethanol-water mixture (70: 30) at room temperature (25 - 30°C) for 48 hours. The maceration was filtered with Whatman N°1 paper. The filtrate was evaporated with a rotavapor under vacuum at 40°C and then freeze-dried. The dry extract obtained was

weighed for the determination of the yield and then stored in the refrigerator in tubes at 4°C, protected from light, until use. Extraction yield is determined by the following formula:
Yield = (Mass of extract / Mass of plant material)*100.

Phytochemical Screening

Phytochemical tests focused on the detection of major chemical groups (alkaloids, flavonoids, phenolic compounds, saponins, sterols and triterpenes, reducing compounds) by tube reactions. Using standard procedures as described by Harborne (1998). The dry extract was dissolved in distilled water at a concentration of 1 mg/ml for phytochemical testing.

Determination of total flavonoids contents

The total flavonoids contents of hydroethanolic extract of plant material was studied using the aluminum chloride colorimetry method described by Okselni et al. (2018). 1.5 ml dry extract dissolved in distilled water (1 mg/ml), 1.5 ml of 2% aluminum chloride was added and the optical density was measured at a wavelength of 415 nm with a spectrophotometer after 30 minutes at laboratory temperature. This was repeated three times. Flavonoid levels were obtained from the rutting calibration curve and expressed as rutting equivalents per gram of dry matter (mg ER/g).

Antioxidant activity

The Phosphomolybdate Reduction Method

The reduction of phosphomolybdate was carried out according to the method described by Ouadja et al. (2018). To 1 mL of 1 mg/mL extract, 9 mL of the working reagent was added. The whole mixture was placed in a water bath at 95°C for 90 minutes and then the optical density was measured at a wavelength of 695 nm with a spectrophotometer. The reagent consists of 90 ml of 0.6M sulphuric acid, 5 mL of 0.1% sodium hydrogen phosphate and 5 mL of 1% ammonium molybdate. This was repeated three times. Ascorbic acid was used as a standard antioxidant under the same experimental conditions. Results were expressed in milligrams of ascorbic acid equivalent per gram of dry extract (mg AAE/g).

The FRAP Method

The ability to reduce ferric ions was measured using the method described by Kantati et al. (2022). To 3 mL of 1 mg/mL extract, 3 mL of FRAP reagent was added [pH acid buffer = 3.5 (50 mL), 2,4,6-tripyridyl-s-triazine (TPTZ) solution (5 mL) and iron III chloride solution (5 mL)] and the optical density was measured at a wavelength of 695 nm using the spectrophotometer after 10 minutes. This was repeated three times. A calibration line with ferrous sulphate ($FeSO_4$) as the reference molecule was used for the determination of concentrations. The values obtained are expressed as mg equivalent of ferrous sulphate per gram of dry matter (mg FSE/g).

Antimicrobial activity

Antimicrobial tests were performed using the liquid microdilution method coupled with appropriate solid milieu spreading (Anani et al., 2015).

Preparation of extracts

The crude hydroethanolic extract from the leaves of *Cassia alata* was used. The dry extract was dissolved in distilled water to prepare 100 mg/mL solutions and then filtered on a 0.45 µm millipore membrane. The sterility of the extract was verified by inoculating a 100µL aliquot of the extract onto Muller Hinton (MH), chocolate agar (GC) and Sabouraud chloramphenicol agar.

Preparation of Microbial Suspensions

The microbial strains tested were successively transplanted into Muller-Hinton broth for bacteria and Sabouraud broth for yeast and then onto agar (Muller-Hinton and GC for bacteria; Sabouraud chloramphenicol agar for yeast). A 24-hour colony (48 hours for *Cutibacterium acnes* ATCC 6919) of each strain was collected using a sterile loop and inoculated in 10 mL of suitable broth (Muller-Hinton broth for bacteria and Sabouraud broth for yeast). From this suspension, dilutions to the thousandth (10^{-3}) were made. 100 µL of

these dilutions were spread on agar milieu (Muller-Hinton agar for bacteria, Sabouraud chloramphenicol agar for yeast and chocolate agar for *Cutibacterium acnes* ATCC 6919*)* to assess the microbial load of the suspensions before the tests were performed.

Determination of antimicrobial potency

The microdilution technique in 96-well microplates was used to determine the Minimum Inhibitory Concentrations (MIC) and the Minimum Bactericidal Concentrations (MBC) or Fungicidal (MFC) in order to derive the antimicrobial potency (AP) of the extracts (Anani et al., 2015). A 100 µL aliquot of Muller-Hinton broth was deposited in all but the first wells of the microplate. 100 µL of the stock solution of the extracts (100 mg/ml) were deposited in these first and second wells. The mixture of the contents of the second wells was homogenized and then half dilutions were carried out by taking 100 µL of the solution each time. At the end of the dilutions, the concentrations of the extract obtained are: 100; 50 ; 25 ; 12.5 ; 6.25 and 3.125 mg/ml. Then 100 µL of microbial suspension was added to the contents of each well. The trials were carried out in triplicata. The microplates were incubated at 37°C for 24 hours (48 hours for *Cutibacterium acnes ATCC 6919*. Macroscopic observations of the various wells were made to determine the MIC. The MIC of the extract is the smallest of the concentrations of the extract that does not show visible growth of the microorganism tested by the bare eye. The Minimum Bactericidal Concentration (MBC) or Fungicide (MFC) was determined by spreading the contents of all wells with an extract concentration greater than or equal to the MIC on agar milieu (Muller-Hinton agar for bacteria, Sabouraud chloramphenicol agar for yeast and chocolate agar for *Cutibacterium acnes ATCC 6919*). Colonies were counted after incubation of the media at 37°C for 24 hours (72 hours for *Cutibacterium acnes ATCC 6919*) and the specified MBC. The lowest concentration of the contents of the well without culture after spreading corresponds to the minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) (99.99% inhibition of the starting inoculum). The MBC/MIC (or MFC/MIC) report has made it possible to specify the modality of action of the extracts (Fauchère, 2002). If the MBC/MIC ratio is less than or equal to 2, the substance is said to be bactericidal (or fungicid) (Fauchère, 2002). On the other hand, if it is greater than 2, the substance is said to be bacteriostatic (or fungistatic) (Fauchère, 2002).

Data analysis

The data collected were analysed (calculation of percentages, means and standard deviations) with Microsoft's Excel spreadsheet, version 2019. Differences between results were considered significant at the 5% threshold (p-value < 0.05).

RESULTS

Extract yield

The hydroethanolic extract of *Cassia alata* leaves had a greyish color; its pH was 5.53 and there was a yield of 9 %.

Phytochemical Screening

The extract from the leaves of *Cassia alata* showed the presence of phenolic compounds, flavonoids and reducing compounds, but revealed the absence of alkaloids, saponins and triterpenes (Table 1).

Total flavonoids contents

The concentration of total flavonoids of *Cassia alata* leaves expressed as rutting equivalent per gram of dry extract was 70.91± 5.66 mg RE/g.

Antioxidant activity

The antioxidant activity of the extract by the phosphomolybdate reduction (PR) method and by the FRAP method are presented in the table below (Table 2).

Antimicrobial activity

The results indicated that the hydroethanolic extract of *Cassia alata* (L.) Roxb. inhibited the *in vitro* growth of all tested germs to varying degrees. The results are reported in Table 3.

The MIC of crude hydroethanolic extract from the leaves of *Cassia alata* (L.) Roxb. ranged between 3.125 mg/mL and 50 mg/mL while MBC ranged from 6.25 mg/mL to 100 mg/mL (Table 3). The extract of *Cassia alata* (L.) Roxb. presented bactericidal action on *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* SARM, *Pseudomonas aeruginosa* ATCC 27853 and *Cutibacterium acnes* ATCC 6919 (MBC/MIC ratio = 2) and fungicidal action on *Candida albicans* ATCC 10231 (with MFC/MIC = 2).

Figure 1 : *Cassia alata* (L.) Roxb.plant.

Table 1: Phytochemical screening of *Cassia alata* hydroethanolic extract.

	***Cassia alata* (L.) Roxb.**
Alkaloids	-
Phenolics	+
Saponins	-
Triterpenes and sterols	-
Flavonoids	+
Reducing compounds	+

Legend: +: Positive; -:Negative

Table 2: Results of antioxidant activity by the phosphomolybdate reduction (PR) method and by the FRAP method.

	PR (mg AAE/g)	**FRAP (mg FSE/g)**
***Cassia alata* (L.) Roxb.**	69.57 ± 4.83	225.5 ± 17.32

Legend: mg AAE/g: mg equivalent of ascorbic acid per gram of extract, mg FSE/g : mg equivalent of ferrous sulphate per gram of extract.

Table 3: Action of crude hydroethanolic extract from *Cassia alata* (L.) Roxb. leaves on microorganisms.

Microbial strains	Hydroethanolic extract of *Cassia alata* (L.) Roxb.			
	MIC (mg/mL)	MBC or MFC (mg/mL)	MBC/MIC or MFC/MIC	Types of activity
Staphylococcus aureus ATCC 29213	12,5	25	2	Bactericidal
Staphylococcus aureus MRSA	12,5	25	2	Bactericidal
Pseudomonas aeruginosa ATCC 27853	50	100	2	Bactericidal
Cutibacterium acnes ATCC 6919	3,125	6,25	2	Bactericidal
Candida albicans ATCC 10231	3,125	6,25	2	Fungicidal

MIC: Minimum inhibitory concentration in mg/mL; MBC: Minimum bactericidal concentration in mg/mL

DISCUSSION

Microorganisms are responsible for infections of the skin. For this, the antimicrobial activities of the extract *by Cassia alata* (L.) Roxb. has been evaluated. The results show that this extract has bactericidal and fungicidal activities on the microorganisms tested. The extract could therefore treat Infections including folliculitis, acne and skin fungus. Antimicrobial activities on Gram-negative bacteria could be beneficial on people with folliculitis. Anti *Cutibacterium acnes* is very good at treating acne. Antifungal activity can be used for the treatment of cutaneous mycoses. Similar studies have shown that crude extract from the leaves of *Cassia alata* (L.) Roxb. is active against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans* (El-Mahmood and Hamuel, 2008; Pissang et al., 2016). On the other hand, the work of (Makinde et al., 2007) shows that the alcoholic extracts from the leaves of *Cassia alata* do not have significant activity on *Staphylococcus aureus.* This difference could be explained by the solvent methanol used. The results of the work by Chomnawang et al. (2005) also revealed an activity of *Cassia alata* (L.) Roxb. on *Cutibacterium acnes ATCC 6919;* They worked on the antimicrobial effect of Thai herbal medicines against acne-inducing bacteria. Other extracts of Togolese flora have shown antimicrobial activities on the microorganisms responsible for acne, in particular *Cutibacterium acnes* (Kombate et al., 2022).

In order to investigate the nature of the major phytochemical groups that will be at the origin of these activities, phytochemical screening was carried out. The results show the presence of phenolic compounds and flavonoids. These results are consistent with the work of Pissang et al. (2016) including phytochemical screening of the hydroethanolic crude extract of *Cassia alata* (L.) Roxb. also revealed in the presence of flavonoids, an absence of alkaloids and saponins. On the other hand, phytochemical screening of *Cassia alata* performed by El-Mahmood and Hamuel, (2008) revealed the presence of alkaloids, tannins, saponins, phenols, flavonoids, anthraquinones and cardiac glycosides. This difference could be explained by the fact that they used other solvents for extraction, including distilled water, methanol, and chloroform. These compounds are known to be biologically active and therefore justify the antioxidant power (Sarkar et al., 2014) and the antimicrobial activities of *Cassia alata* (L.) Roxb. These secondary metabolites have antimicrobial activity through different

mechanisms. For example, tanins have been shown to form irreversible complexes with the proline-rich protein (Shimada, 2006) resulting in the inhibition of cellular protein synthesis (Quinlan et al., 2002). Similarly, flavonoids have been shown to exhibit antimicrobial, anti-inflammatory, analgesic, antiallergic, cytostatic, and antioxidant properties (Hodek et al., 2002). The presence of these secondary metabolites in the leaves of *Cassia alata* (L.) Roxb. justify its various biological activities. The antioxidant and antimicrobial activities observed in the present by this work could justify its use in traditional medicine.

Further investigation would help for more comprehension of these activities and may help to promote Enhanced Traditional Medicines.

Conclusion

This study shows that *Cassia alata* could be a source of a new antioxidant and antimicrobial agent. The hydroethanolic extract have bactericidal and fungicidal activities. Further research is needed to isolate, characterize and identify the bioactive constituents responsible for the observed activity.

AUTHORS' CONTRIBUTIONS

Conception : KFM, EHG, BK; Design, data collection and processing : KFM, AD, KA; Supervision : KM, DSK, AD ; Analysis, interpretation and writing : KFM, AD, KM, DSK; Review and final revision approval : all authors.

COMPETING INTERESTS

The authors declare that they have no competing interests on this work.

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