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Evaluation of the Antibacterial Properties of Aqueous and Methanol Extracts of *Cassia alata*

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ABSTRACT

The antibacterial activity of the aqueous and methanol extracts of sundried leaves of *Cassia alata* was investigated by testing the extracts against some pathogenic *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* using the Agar cup plate method. The Minimum Inhibitory Concentration (MIC) of the aqueous extract against susceptible test organism was determined using the Agar dilution method. The results showed that the plant part can be used to treat infections caused by *S. aureus* and *B. Subtilis* which were susceptible. The *in vitro* findings justify the use of the extract of *Cassia alata* in traditional medicine practice for the treatment of some external skin infections. *Cassia alata* has shown to be a very versatile plant and can be a viable alternative as an antibacterial agent in the future either alone or in combination with other medicinal plants, if formulated into appropriate pharmaceutical dosage forms. Further, scope exists for *in vivo* research studies with preformulation testing, pharmaceutical dosage formulation and development, pharmacokinetics, safety and efficacy in patients.

Key words: *Cassia alata*, methanol and aqueous extracts, antibacterial properties, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*

INTRODUCTION

Plants have been classified as an essential source of medicinal agents for centuries and a huge number of novel drug components have been isolated from natural plant source and their extract used for in traditional medicine (Obeidat *et al.*, 2012).

Cassia alata is one of the most important species of the genus *Cassia* which is rich in anthraquinones and polyphenols. The leaves of *C. alata* have been qualitatively analyzed for the presence of primarily five pharmacologically active anthraquinones: rhein, aloë-emodin, chrysophanol, emodin and physcion as well as the flavonoid, kaempferol (El-Mahmood and Doughari, 2008; Moriyama *et al.*, 2001). The flavonoid, kaempferol has been reported to have anticancer properties (Fernand *et al.*, 2008).

Idu *et al.* (2007) observed that, preliminary phytochemical analysis of *Cassia alata* showed the presence of phenols, tannins, anthraquinones, saponins and flavonoids. Odunbaku and Lusanya (2011) also corroborated this study; they further stated that, the plant also had alkaloids and cardenolides.

Sharma *et al.* (2010) also reported in their study that Preliminary phytochemical screening of alcoholic extract revealed the presence of anthraquinone glycosides, Phenolic compounds; Saponin glycoside and while aqueous extract showed presence of glycosides and Phenolic compounds, Saponin glycoside.

These anthraquinone derivatives are well known to exhibit a variety of biological activities such as antimicrobial, antifungal, antitumor, antioxidant, cytotoxic and hypoglycaemic activities. Other chemicals contained in the plant include saponin which acts as a laxative and expels intestinal parasites. Rhein and chrysophanol are also known to be present in the roots in addition to two other quinone pigments.

Pieme *et al.* (2006) investigated the acute and sub-acute toxicities of hydro-ethanolic extract of leaves of *Cassia alata* on Swiss mice and Wistar albino rat. This study presents strong evidence of the nontoxic effect of the hydroethanolic extract of *Cassia alata*. *Cassia alata* showed some protective effect on hepatocytes and improved liver architecture. These results showed that the use of the extract of *Cassia alata* is safe and explained the extensive utilisation of the plant in traditional medicine. In the light of this, Chukeatirote *et al.* (2007) stated that since ancient time, plant and animal products have been used for treatment of diseases and disorders and that, plants in particular, have been used to treat infections due to its antimicrobial properties.

The effects of water, methanol and chloroform extracts on some pathogenic *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Proteus mirabilis* showed that the plant parts can be used to treat infections caused by these bacteria. *S. aureus*, *S. pyogenes* and *P. mirabilis* were more susceptible while *E. coli* and *P. aeruginosa* were less sensitive.

Uddin *et al.* (2008) observed in their study that methanol extract of *Cassia tora* had strong antioxidant properties and that the results they obtained provided a support for the use of this plant in traditional medicine. They also observed that the methanol and aqueous extracts of *Cassia tora* showed significant antibacterial activities against few gram positive negative bacterial strains.

Pieme *et al.* (2006) observed that, the ethanol-aqueous extract of *S. alata* showed moderate antibacterial and antifungal activity. Also, Sharma *et al.* (2010) stated that Ethanol and aqueous extract of *Cassia alata* had antibacterial activity. Awal *et al.* (2004) also observed that ethanol leave extract of *Cassia alata* had antibacterial properties. They further stated that, ethanol extract of *Cassia alata* has cytotoxic effect against *Artemia* and postulated that *C. alata* can be used for the treatment of cancer cell line in humans.

Krishnan *et al.* (2010) observed that acetone and Methanol extracts of *Cassia spectabilis* were very effective on *Bacillus subtilis*.

Reezal *et al.* (2002), opined that *C. alata* has been proven to be effective against *C. albicans* growth culture by using the ethanol and aqueous barks extracts. They further stated that Miconazole when compared to the *Cassia alata* barks aqueous and ethanol extract on *C. albicans* growth, showed only a slight differences between them. 30 mg mL⁻¹ Miconazole with 18 mm inhibition zone and 30 mg mL⁻¹ of barks aqueous and ethanol extracts with inhibition of 16 mm and 14 mm. This proved that this plant has potential to be exploited as a natural source of antifungal remedy in the future. With an increase of discs concentration, the extracts might produce at least the same or better effects than Miconazole.

Esimone *et al.* (2008) observed that there was an excellent effect of the *in vitro* study of the antimicrobial potency of herbal soap formulated with ethanol extract of *Cassia alata*. They stated that the antimicrobial activity of the soap is predominantly against Gram-positive and opportunistic

yeast. The herbal soap formulated with *Cassia alata* demonstrated high potency against common pathogens of the skin and therefore a potential excipient in the production of antiseptic soaps. They observed that the findings have high medical, industrial and economic significance as extracts of *Cassia alata* could be harnessed in the formulation of medicated soaps.

Oladunmoye *et al.* (2007) observed in their study that ethanol extract of *Cassia hirsuta* inhibited the activities of some microorganisms by altering their genome. They further stated that the extract can be mutagenic and also possess antimicrobial activities against pathogenic bacteria.

Abubacker *et al.* (2008) stated that aqueous extract of *Cassia alata* can be used as potential antifungal agent. They observed that the aqueous extract of *Cassia alata* had effect on *Aspergillus flavus*, *A. parasiticus*, *Fusarium oxysporum* and *Candida albicans*.

Cassia alata traditional usage varies greatly in different places and countries. This plant is well known to treat external infections mostly the skins. The leaves, however, shown to be the choice for treatment rather than the flowers, barks and seeds. Throughout the tropics, the leaf juice is collected and found to be useful in treating skin diseases. Whereas, an infusion of the roots is used to treat rheumatism and as a strong laxative (Reezal *et al.*, 2002).

Plants have been classified as an essential source of medicinal agents for centuries and a huge number of novel drug components have been isolated from natural plant source and their extract used for in traditional medicine (Obeidat *et al.*, 2012).

Historically, plants have provided a good source of anti-infective agents with the compounds which are highly effective instruments in the fight against microbial infections (Idu *et al.*, 2007).

Plants with different medicinal properties have been employed by traditional medical practice for the treatment of different disease conditions. In eastern Nigeria, some plants which have frothing or foaming ability have been employed as soap for bathing and for treatment of skin and wound infections (Esimone *et al.*, 2008).

Cassia alata is known to have laxative properties. Elujoba *et al.* (1989). Traditionally, tea are made from the leaves and taken as a treatment for constipation and intestinal worms. The leaves are pounded and rubbed on the skin to cure eczema and ringworm (Pieme *et al.*, 2006) and white-spot fungal skin infections (Palanichamy and Nagarajan, 1990). The leaves contain a fungicide, chrysophanic acid which is a common ingredient in soaps, shampoos and lotions (Ajose, 2007; Villasenor *et al.*, 2002). The leaves are boiled with water and simmered before applied over the infected area 2 times a day due to wound healing properties. In treatment for ringworm, usually, the leaves are crushed and made into paste. Then it is spread upon the affected parts. For treatment of eczema, the infected part is washed repeatedly with strong decoction of the leaves and flowers. In cases of bronchitis and asthma, decoction of the leaves and flowers in herpetic constitutions administered repeatedly taken during the day relieves dyspnoeal oppression and promotes expectoration. The medicine also acts on the bowels slightly and increases the secretion of urine.

Several traditional uses of *Cassia alata* have been discovered in several places. In Indo China and Philippines Island, the leaves are considered most effective against herpes and the wood in decoction is used as a mild purgative. In Guinea, the pounded fresh leaves are applied or rubbed on to all kind of skin affections. In the Gold Coast, the leaves are applied to dhobey itch, craw-craw and ringworm on the forehead or on the skin. This is one of the most effective amongst native medicine. The leaves are also boiled and drunk by women to hasten the delivery of children.

This study investigates the antibacterial activity of the aqueous and methanol extracts of sundried leaves of *Cassia alata* by testing the extracts against selected bacteria.

MATERIALS AND METHODS

Plant materials: *Cassia alata* leaves were collected from the premises of the Federal Government College in Warri, Delta State-Nigeria and authenticated at the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos. The collected leaves were cleaned of unwanted foreign materials, cut up into small pieces and dried in sunlight for a week, ground and weighed. The dried material was coarsely milled, packed into a brown paper bag and stored at room temperature in the laboratory until used.

Chemicals and reagents: Sterile petri dishes, Mueller Hinton agar and Sabouraud Dextrose Agar (SDA) were purchased in Lagos. Cup borer, diameter 6 mm, Hot air oven, Cooling incubator C1-10S (REMI Instruments Ltd., Mumbai, India), Incubator (Astell Hearson, England), Uniscope SM801A Laboratory Water bath (Surgifield medicals, England), Vertical Heating pressure steam sterilizer LDZX-30FB (Labnet international Inc, Woodbridge, USA), Mettler P1210 balance (GallenHorup) was provided by the Department of Pharmaceutics and Pharmaceutical Technology, University of Lagos.

Extraction of plant materials

Methanol extraction: The leave material was soaked in a Winchester bottle with methanol for 48 h (maceration). The extract was concentrated using a Buchi V-801 rotary evaporator at 35°C.

Aqueous extraction: The leave material was boiled with water in a 2000 mL (2 L) of pyrex beaker at 60°C in a water bath for an hour twice, filtered and concentrated on a water bath at 70°C.

The coarsely milled leaves and stem bark of *Cassia alata* were extracted separately using water and methanol as solvents. About 140 g of the powdered sample was continuously extracted with a particular solvent by use of a Soxhlet extraction apparatus for 24 h. The extracts were filtered and concentrated to dryness under reduced pressure and controlled temperature (50-55°C) to obtain solvent-free semisolid extracts. The solvent-free semisolid extracts obtained were used for the antimicrobial studies.

Test microorganism and growth media: The microorganisms (Gram-positive bacteria: *Bacillus subtilis*, *Staphylococcus aureus*, Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*) used for the study were obtained from the stocks of the Pharmaceutical Microbiology laboratory of the Department of Pharmaceutics and Pharmaceutical technology, Faculty of Pharmacy, University of Lagos.

Assay of organism: The bacterial strains were grown and maintained on Mueller Hinton agar at 37°C. All bacteria were first cultivated in their various diagnostic media and then subcultures into nutrient Agar plates. This was to remove the effects of inhibitory agents and indicators in the diagnostic media. All bacteria were sub cultured into various sterile nutrient broths in universal bottles and incubated overnight. Using sterile saline in a universal bottle, the culture suspension was calibrated to about 10^8 CFU mL⁻¹ using sterile Pasteur pipette. All standardized assay organisms were kept for the assay.

Antibacterial activity evaluation: The antibacterial activity of aqueous and methanol extracts of the leaves of *Cassia alata* at concentrations of 50, 100, 150 and 200 mg mL⁻¹ were determined using the cup plate method. A molten Mueller Hinton agar stabilized at 45°C was seeded with 0.1 mL of a 24 h broth culture of the test organism (*B. subtilis*, *E. coli*, *P. aeruginosa* and *S. aureus*) containing approximately 10⁸ CFU mL⁻¹ in a sterile petri dish and allowed to set. Wells of 6 mm diameter were created with a sterile cork borer and filled to about three-quarters full with solutions of the aqueous and methanol extracts of the leaves of *Cassia alata*. The plates were pre-incubated for 1 h at room temperature to allow for diffusion of the solutions and then incubated for 24 h. The zones of inhibition were measured (mean, n = 2). Streptomycin and propylene glycol were used as positive and negative controls, respectively.

Statistical analysis: The experiments were run in duplicate and the zones of inhibition were determined and recorded (mean, n = 2). The different effects of methanol and aqueous extract of *Cassia alata* extracts on the test organisms were analyzed using ANOVA.

Minimum inhibition concentration (MIC): The Agar dilution technique was used to determine the Minimum Inhibition Concentration (MIC) of the extract of *Cassia alata* against the test organisms. A stock concentration of 500 mg mL⁻¹ of extract was prepared by dissolving 15 g of extract in 30 mL of propylene glycol. Ten working concentration were subsequently prepared from the stock.

Ten different concentration of the extract was used for this determination ranging from 0.625-320 mg mL⁻¹. Sterile petri dishes containing varying volumes of extract and molten agar (total volume 20 mL in each petri dish) depending on the concentration of extract intended were inoculated with 0.2 mL of the test organisms previously diluted to contain approximately 10⁵ CFU mL⁻¹ for bacteria and 10⁵ CFU mL⁻¹ for fungi. A plate without an extract and another without a test organism were used as controls. The plates were incubated at 37°C for 24 h and observed for growth. The experiments were conducted in duplicate. The plate with the lowest concentration of the extract which showed no growth after incubation was taken and recorded as the MIC.

RESULTS AND DISCUSSION

Crude methanol and aqueous extract of leaves and barks from *Cassia alata* were tested for selected bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*).

All the crude extracts inhibited the growth of *S. aureus* and *B. subtilis*, though to varying degrees (Table 1, 2) as indicated by the zones of inhibition. The extract did not show any activity against *E. coli* and *Pseudomonas* which are gram negative bacteria contrary to observations of El-Mahmood and Doughari, (2008). The activity of the extract also varied between solvents with the aqueous extracts demonstrating the highest activity against all the susceptible bacteria as revealed on Table 2 similar to observations of Roy *et al.* (2006) and the results showed that the extracts demonstrated a concentration-dependent antibacterial activity with higher concentrations of 150 and 200 mg mL⁻¹ showing greater zones of inhibition than lower concentrations of 50 and 100 mg mL⁻¹. High MIC values are indication of low activity while low MIC values are indication of high activity. In this study, *S. aureus* and *B. subtilis* had low MIC values thus suggesting higher activity against the corresponding organisms (Table 3).

Table 1: *In vitro* antibacterial activity of methanol extract of *Cassia alata* (zone of inhibition in mm)

Bacteria species	50 (mg mL ⁻¹)	100 (mg mL ⁻¹)	150 (mg mL ⁻¹)	200 (mg mL ⁻¹)
<i>S. aureus</i>	15.00	17.00	19.00	20.00
<i>B. subtilis</i>	12.00	14.00	15.00	16.00
<i>E. coli</i>	0.00	0.00	0.00	0.00
<i>Pseudomonas</i>	0.00	0.00	0.00	0.00

Table 2: *In vitro* antibacterial activity of aqueous extract of *Cassia alata* (Zone of inhibition in mm)

Bacteria species	50 (mg mL ⁻¹)	100 (mg mL ⁻¹)	150 (mg mL ⁻¹)	200 (mg mL ⁻¹)
<i>S. aureus</i>	16.50	18.00	20.00	22.00
<i>B. subtilis</i>	12.50	13.00	14.00	17.00
<i>E. coli</i>	0.00	0.00	0.00	0.00
<i>Pseudomonas</i>	0.00	0.00	0.00	0.00

Table 3: Minimum inhibitory concentration (MIC) profile of *Cassia alata* against test organisms (mg mL⁻¹)

Microorganisms	0.625	1.25	2.50	5.00	10.00	20.00	40.00	80.00	160.00	320.00
<i>S. aureus</i>	+	+	+	+	-	-	-	-	-	-
<i>B. subtilis</i>	+	+	-	-	-	-	-	-	-	-

CONCLUSION

This study has shown that, crude extracts of *C. alata* possessed reasonable activity against some bacteria and has high potential as antibacterial agent. This finding is in line with Pieme *et al.* (2008) and Krishnan *et al.* (2010). This finding provides an insight into the usage of these plants in traditional medicine for the treatment of common skin disorder since the common bacterial skin infections are usually caused by *Staphylococcus* and *Streptococcus* species which are gram positive organisms. This plant can be locally sourced since it grows well in any Nigerian soil. However, the effect of this plant against a wider range of bacteria and fungi and toxicological studies of the extracts is recommended.

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