

Evaluation of the antiseptic properties of Cassia alata-based herbal soap

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Abstract

In this study, we evaluated the antimicrobial potency of a herbal soap formulated with ethanol extract of *Cassia alata*. The herbal soap exhibited excellent antimicrobial effect in the in vitro studies as well as in the palm-washing studies on volunteers. The antimicrobial activity of the soap is predominantly against Gram-positive and opportunistic yeast. At a reduction time of 5 mins, the herbal soap recorded a significantly ($P < 0.05$) lower mean viable microbial count of 2.12×10^4 cfu/ml (a reduction in microbial load of 94.78%) as against the 4.07×10^5 cfu/ml recorded before the application of the soap. 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. These findings have high medical, industrial and economic significance as extracts of *Cassia alata* could be harnessed in the formulation of medicated soaps.

INTRODUCTION

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. Ethnomedicinally, juice and extracts from leaves of the plant are topically applied as anti-inflammatory and antimicrobial agents, especially in the treatment of skin diseases including eczemas, ring-worms and pruritus (Benjamin, 1980; Benjamin and Lamikanra, 1981; Oliver, 1986; Ayim, 1987; Akinde et al., 1999). These plant materials are either used alone or formulated into local soaps, ointments and creams which are often commercially available. Due to lack of evidence on the efficacy of herbal soap, and the poor aesthetic presentation, these products are mostly patronized by low income group in the local communities in the past. But interestingly, the popularity of herb-based soaps is increasing due to many years of accumulated experience on their efficacy on topical disorders. Currently, there are so many commercial brands of herb-based soaps with good claims of efficacy and are now enjoying increasing patronage. It is therefore important to investigate these soaps to validate the claims and also establish other useful properties which will help in promoting public acceptance and encourage wider usage.

Soaps act as emulsifiers or surfactants, softening the horny-layer of the epidermis and acts as a germicide by enhancing the permeability of microbial envelope thereby disrupting the integrity of microbial cells. Antimicrobial activity of soaps make them useful agent for bathing, laundry, washing, and cleansing of surfaces (Fuerst, 1978; Hugo and Russel, 1983).

The cleansing and germicidal properties of the soapy-plants are comparable to those of the standard soaps, which are salts of higher fatty acids. Crude preparations of soapy plants are able to soften the skin epidermis, enhance greater penetration and cleansing of sores and acne and thereby promote rapid healing and resolution of blemishes.

In this study we evaluated the antiseptic potentials of *Cassia alata*-based herbal soap formulated in our laboratory. *Cassia alata* is known to contain some secondary metabolites like resin, saponin, phenols, flavonoids, anthraquinone glycosides and alkaloids (Akinde et al., 1999). These phytoconstituents are also known to possess surface activity and other soap related properties. In previous studies, *Cassia alata* has been found to possess excellent wound-healing properties (Benjamin and Lamikanra, 1981; Palanichamy et al., 1991) and is also useful in the treatment of eruptive and pustular skins conditions by rubbing crushed fresh leaves on infected area (Akinde et al., 1999).

MATERIALS AND METHODS

MICROORGANISMS

Clinical Isolates of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*, obtained from the standardized stock culture collection of the Department of Microbiology, University of Nigeria, Nsukka were used as test organisms in the antimicrobial tests.

CHEMICALS AND REAGENTS

Culture media used were nutrient agar, NA (Oxoid, England) and Sabouraud dextrose agar, SDA (Oxoid, England). Chemicals used include caustic soda (Stratech Chemicals Industry, Nigeria), ethanol (Wamco Chemical Industry, Nigeria); palm-kernel oil, antiseptic soap® (Jumbo Chemicals, Nigeria).

COLLECTION, IDENTIFICATION AND PROCESSING OF PLANT

The leaves of *Cassia alata* (1kg) were collected in the month of August, 2005 from different mature plant stands in a bush at Nsukka, south-east Nigeria. The collected leaves were identified by Mr Ekekwe formerly a garden staff at the Department of Botany, University of Nigeria, Nsukka. The leaves were air-dried, pulverized and stored in air-tight bottles for the studies.

EXTRACTION

The *Cassia alata* powder (200 g) was macerated in 500 ml of 95% ethanol in a 1 L capacity air-tight conical flask, with occasional agitation, for 24 h after which the mixture was filtered. The ethanol extract obtained was concentrated in vacuo and freeze-dried. The extract was subjected to a preliminary antimicrobial activity, using the agar-well diffusion technique.

FORMULATION OF HERBAL SOAP

The ethanol extract of *Cassia alata* (35.82 g) was incorporated into a soap formulated with Sodium hydroxide, NaOH (1 g) and palm-kernel oil (8.4 g) using the cold saponification process. Briefly, the NaOH (1 g) was weighed into a clean beaker containing 5 ml of distilled water. The extract was incorporated into the hot palm-kernel oil which was then poured into the soda solution in the beaker with continuous stirring with a glass rod until the molten mixture became homogenous. The semi-solid mixture was pored into a mould and allowed to solidify. A commercial sample of an antiseptic soap® was used as a reference product in the study.

ANTIMICROBIAL EVALUATION OF THE HERBAL SOAP

The agar-dilution method (Tilton and Howard, 1987; Baron and Finegold 1990) was employed in the in vitro evaluation. The herbal soap (1 g) was dissolved in distilled water (50 ml) to obtain a 2% suspension. The suspension was vigorously shaken for dissolution of soap, dispersion of foam and for homogeneity. Thereafter, a 1.0 ml portion of the soap solution was added to 20 ml of sterile molten culture media in Petri-dishes and allowed to set. Then a 0.1 ml of standard inoculum of each test organism was streaked on the plates which were then incubated under the standard conditions for the respective test organism. Following incubation, plates were observed for the presence or absence of microbial growth.

The effect of the soap on the human skin surfaces was evaluated on the outer palms of the healthy volunteers. Four healthy undergraduates (two males and two females) of the University of Nigeria, Nsukka provided informed consent and were used for the topical test according to our institutional ethical protocol. The skin of the palm was swabbed into sterile normal saline in Bijou bottles before washing with the soap. After washing with the test and standard soaps, the outer-palms were flooded with 1ml of standard inoculum of *Staphylococcus aureus* (108 cfu/ml) for 1 h. Thereafter, the surfaces were swabbed into sterile normal saline in separate Bijou bottles. Aliquots from the respective treatments were cultured and counted on agar plate count using the pour-plate technique. The killing rate was then determined by the enumeration of viable counts.

RESULTS AND DISCUSSION

In the preliminary antimicrobial sensitivity screening, the ethanol extract of *Cassia alata* showed excellent activity against the test organisms (Table1). Many of these organisms are natural flora of the skin and also known etiologic agents of several skin and mucous membranes infections of man. In previous studies, the antimicrobial activity of *Cassia alata* extract has been attributed to the presence of some active secondary metabolites and high acid values due to the hydrolysis of esters, as well as to the abundance of phenolic compounds (Acharya and Chatterjee, 1975; Rai, 1978; Rai and Obayemi, 1978; Smith and Ali, 1979; Ayinde et al., 1999)

Figure 1

Table 1: Preliminary antimicrobial activity of ethanol extract of

Test microorganism	Antimicrobial activity
	IZD (mm) \pm SEM
<i>Staphylococcus aureus</i>	18.5 \pm 0.55
<i>Escherichia coli</i>	13.4 \pm 1.00
<i>Bacillus subtilis</i>	16.4 \pm 0.45
<i>Pseudomona aeruginosa</i>	12.8 \pm 0.33
<i>Candida albicans</i>	8.2 \pm 0.67

The *Cassia alata*-based herbal soap demonstrated an excellent antimicrobial activity against the tested microbial skin flora (Table 2). The activity against *Staphylococcus aureus* is of significant interest. The skin carries large numbers of bacteria flora, mainly Gram-positive picked up from the various objects with which it comes in contact. Of these natural flora, *Staphylococcus aureus* commonly found on the hands, face and in deep layers of the skin is perhaps the most widely encountered and very undesirable. *Staphylococcus aureus* is ubiquitous and are not easily eliminated especially in the deeper skin layers, sweat gland, sebaceous gland, and the hair-follicles by routine washing and scrubbing even with some antiseptic soap (Fuerst, 1978; Hugo and Russel, 1983, Rosenberg and Cohen, 1983; Singleton; 1987). Thus, the potency of the *Cassia alata* herbal soap against *Staphylococcus aureus* is very remarkable and could be harnessed in the containment of the organism implicated as the commonest etiologic agent of boils, carbuncles, breast abscess, infantile-impetigo (Fuerst, 1978).

Figure 2

Table 2: antimicrobial evaluation of herbal formulated Soap

Test micro organism	Antimicrobial activity	
	Herbal soap	Antiseptic soap
<i>Staphylococcus aureus</i>	-	-
<i>Bacillus subtilis</i>	-	-
<i>Candida albicans</i>	-	+

- = absence of microbial growth; + = presence of microbial growth

The herbal soap was not tested against Gram-negative bacteria, since they are not auchthchonus skin flora. However, they are only encountered when the normal Gram-positive bacteria are depleted by antibiotic application, other non-physiological conditions such as the spillage from the

gastrointestinal tract (GIT) onto the skin around the anal and genital regions as well as on soles of the feet (Rosenberg and Cohen, 1983). The herbal soap was active against the spore-forming *Bacillus subtilis*. This organism is usually encountered by contacts with the materials handled (especially soil) and are mostly harmless, but occasionally could become opportunistic causing infections especially of the eyes (Fuerst, 1978).

The activity of the herbal soap against *Candida albicans* which normally inhabits part of the respiratory, gastrointestinal and female genital tracts is also important (Fuerst, 1978, Hugo and Russel, 1983; Rosenberg and Cohen, 1983). In this study, the herbal soap inhibited the growth of *Candida* cells. *Candida albicans* is known to be inherently resistant to many antimicrobial agents. Although it is a natural human body flora, it also causes some opportunistic infections in debilitated, immuno-compromised patients as well as patients on prolonged antibiotics and immunosuppressants (Hugo and Russel, 1983).

Even though the human skin cannot be made absolutely free of bacteria, the *Cassia alata*-based soap exhibited high antimicrobial potency against *Staphylococcus aureus* (Table3). The organism is the most widely encountered and undesirable auchthchonus normal skin flora difficult to be totally removed. At a reduction time of 5 mins, the herbal soap recorded a significantly ($P < 0.05$) lower mean viable count of 2.12×10^4 cfu/ml (a reduction in microbial load of 94.78%) as against the value for the control treatment. In this study, the herbal soap formulated with *Cassia* demonstrated high potency against skin flora and indicates the potential of the plant as excipient in the production of antiseptic soaps for combating skin infections especially in the tropics. These findings have high economic, industrial and medical significance.

Figure 3

Table 3: Effect of *Cassia alata*-based herbal soap on viable cells on the palms of volunteers

Treatment	Mean ∇ viable cell count \pm SEM (cfu/ml) $\times 10^4$	Mean percentage reduction in viable cell count (%)
Before soap application	40.7 \pm 0.76	-----
<i>Cassia alata</i> herbal soap	2.12 \pm 0.85	94.78 \pm 1.82
Antiseptic soap®	3.25 \pm 0.61	91.88 \pm 1.63

∇ Viable counts measure in triplicates of four volunteers after a reduction time of 5 minutes.

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