MICROBIOLOGICAL EVALUATION OF *Cassia alata* HERBAL FORMULATED ANTISEPTIC SOAP.

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ABSTRACT: The antimicrobial activity of the pulverized leaves and cold-macerated ethanolic extract of *Cassia alata* was carried out by the agar-well diffusion technique. The ethanolic extract was formulated into a herbal soap by cold-saponification process, and potency evaluated *in vitro* by streaking using agar-dilution technique; and *in vivo* by enumeration of viable counts following killing rate kinetics using the pour-plate technique. The preliminary antimicrobial sensitivity screening of the ethanolic extract and the herbal formulated antiseptic soap respectively showed high antimicrobial activities against the test organisms. The herbal formulated soap exhibited excellent *in vitro* and *in vivo* antimocrobial activities against some species of aucthocthonous skin microflora. Its effect was remarkable against *Staphylococcus aureus*, coagulase-negative *Staphylococcus aureus* (CONSA), *Bacillus subtilis* and opportunistic yeast (*Candida albicans)*. The herbal formulated soap significantly ($P < 0.05$) recorded lower mean viable count (2.12×10^4 cfu/ml), with a corresponding significant ($P < 0.05$) higher mean percentage reduction in viable count (94.78 %), in a death reduction time (DRT) of 5 minutes; as compared to the standard antiseptic soap. The herbal formulated soap was well tolerated, as it was non-irritant on the skin of volunteers.

INTRODUCTION

Plants from almost all the taxonomic groups have been exploited by man from time for various medicinal purposes. This is attributed to the presence of many bioactive compounds (metabolites), enhancing many clinical applications, notably a wide spectum of antimicrobial activity (Berdy, 1982; Sofowora, 1982, 1986 and 1993). From folklores, especially in Eastern Nigeria, many plants with frothing or foaming ability have been variously employed as soaps by both the locals and herbalists in bathing as well as for the treatment and cure of skin and wound infections. Such uses had been informed by the ability of the crude preparations in softening the skin epidermis, greater penetration and cleansing of sores, wounds and abscesses, thereby enhancing rapid healing. These excellent cleansing and antimicrobial properties of the soap-plants rivaled that of the standard soaps, which are salts of higher fatty acids. Soaps are surfactants, which act as emulsifiers softening the horny-layer of the epidermis; and germicidal by enhancing the permeability of microbial envelope; hence frequently used for bathing, cleansing of surfaces as well as in laundry due to their antimicrobial activity (Fuerst, 1978, Hugo and Russel, 1983).

These properties informed the choice of *Cassia alata* for the work. The phytochemistry of the plant is replete with many secondary metabolites like resin, saponin, phenols, flavonoids, anthraquinone glycosides and alkaloids (Akinde *et al;* 1999). 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 (Achara and Chatterjee, 1975; Benjamin, 1980; Benjamin and Lamikanra, 1981; Oliver, 1986; Ayim, 1987; Akinde *et. al.*, 1999). Also, *Cassia alata* possessed excellent wound-healing ability (Benjamin and Lamikanra, 1981: Palanichamy *et. al.*, 1991) as well as being useful in the treatment of eruptive and pustular skins conditions by rubbing crushed fresh leaves on infected areas (Akinde *et. al.*, 1999). This preliminary work was carried out to ascertain and justify the folkloric skin cleansing and antimicrobial efficacy of the plant by its formulation into a herbal soap, and its potency assayed on primary skin pathogens.

MATERIALS AND METHODS

Biologicals

Clinical isolates of *Staphylococcus aureus,* coagulase-negative *Staphylococcus aureus,* (CONSA), *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans,* obtained from the stock culture collection of the Department of Microbiology, University of Nigeria, Nsukka were used as test organisms in the antimicrobial activity tests. Four healthy undergraduates (two males and two females) of the University of Nigeria, Nsukka were used as volunteers.

Culture Media and Chemicals

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

Matured leaves (1 kg) of *Cassia alata,* were randomly collected from the mature plants stands in Nsukka Forest Nigeria, and identified by the taxonomist at the Department of Botany, University of Nigeria, Nsukka. The leaves were air-dried, pulverized and stored in air-tight bottles for the assays.

Extraction and Antimicrobial Activity of Extract

Twenty grams of *Cassia alata* powder was macerated in 200 ml of 95 % ethanol contained in a 500 ml capacity air-tight conical flask for 24h, with occasional shaking, after which the mixture was filtered. The ethanolic extract obtained was concentrated in vacuo and freezed-dried. The extract was preliminary evaluated for antimicrobial activity, using the agar-well diffusion method (Tilton and Howard, 1987; Baron and Fine gold, 1990).

Herbal Soap Formulation

Ethanolic extract of *Cassia alata* (35.82 g), Sodium hydroxide, NaOH (1 g), palm-kernel oil (8.4 g) as excipients where formulated into a soap (10 g), using the cold saponification process. One gram of NaOH was weighed into a clean beaker containing 5 ml of distilled water. The concentrations of the excipients were added to the solution with continuous stirring, using a glass rod for homogeneity of the molten mixture. The semi-solid mixture was poured into a mould and allowed to solidified. A standard antiseptic soap was used as the standard.

Antimicrobial Evaluation of Herbal Formulated Soap

The *in vitro* antimicrobial activity of the herbal formulated soap was evaluated by a modification of the standard agar-dilution method. The formulated soap (1 g) was dissolved in distilled water (50 ml), to obtain a 2 % suspension. The suspension was vigorously shaken for homogeneity by dispersion of foam; and 1.0 ml added to 20 ml of sterile molten culture media, in petri-dishes and allowed to set. Thereafter, 0.1 ml standardized inoculum of the test organisms were streaked on the plates; and cultures respectively incubated under the standard conditions for the test organisms. Following incubation, plates were observed for the presence or absence of growth. In the *in vivo* evaluation, the outer palms of the volunteers before application of the soap were swabbed into sterile normal saline in Bijou bottles. After washing

with the soap, the outer-palms were flooded with 1 ml of standardized inoculum of *Staphylococcus aureus* (10^8 cfu/ml) for 1 h. After 5 mins, the surfaces were swabbed into sterile normal saline in separate Bijou bottles. Aliquots from the respective treatments were cultured on plate count agar, using the pour-plate technique for the determination of killing rate by the enumeration of viable counts.

RESULTS AND DISCUSSION

The preliminary antimicrobial sensitivity of ethanolic extract of *Cassia alata* (Table 1) indicated excellent activity against the test organisms. 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 this study, the enhanced antimicrobial activity of *Cassia alata* extract may be attributed to its metabolites or bioactive compounds (Ayinde *et. al.* 1999), high acid values due to the hydrolysis of esters (Rai, 1978, Rai and Obayemi, 1978); Smith and Ali, 1979, Rai and Obayemi, 1983) as well as the abundant presence of phenolic compounds Rao *et al.* (1975), Acharya and Chatterjee (1975); Smith and Ali (1979).

Table 1**:** Preliminary antimicrobial activity of ethanolic extract of *Cassia alata*

		Antimicrobial Activity
Test Organisms	*C. alata* Extract	Inhibition zone diameter, IZD (mm)
Staphylococcus aureus	+	18.5
Coagulase-negative Staphylococcus aureus	+	14.6
Escherichia coli	+	13.4
Bacillus subtilis	+	16.4
Pseudomonas aeruginosa	+	12.8
Candida albicans	±	8.2

a = MIC of extract; + = Growth inhibition: ± = Partial growth inhibition

The *Cassia alata* formulated soap demonstrated excellent antimicrobial activity against the tested aucthocthonous skin microflora (Table 2).

Table 2: *In vitro* antimicrobial evaluation of herbal formulated soap

	Antimicrobial Activity[a]	
Test Organism	Herbal Soap	Antiseptic Soap (Control)
Staphylococcus aureus	+	+
Coagulase-negative Staphylococcus aureus	+	+
Bacillus subtilis	+	+
Candida albicans	+	±

a = tested at MIC; + = Growth inhibition; ± = Partial growth inhibition

Of significant interest, is the activity against *Staphylococcus aureus.* The skin carries large numbers of bacteria, mainly Gram-positive picked up from the various objects with which it comes in contact. Of these natural flora staphylococci mainly, *Staphylococcus aureus* and coagulase-nagative *Staphylococcus aureus* commonly found on the hands, face and in deep layers of the skin are perhaps the most widely encountered and extremely undesirable. This is due to their ubiquity and perhaps antimicrobial resistance; hence cannot be totally eliminated especially in the deeper layers, sweat and sebaceous glands, as well as hair-follicles by routine washing and scrubbing even with antiseptic soaps (Fuerst, 1978, Hugo and Russel, 1983; Rosenberg and Cohen, 1983 and Singeleton, 1987). Thus, the potency of the formulated herbal soap, against these staphylococci which are the primary and common etiologic agents of boils, carbuncles, breast abscess, infantile-impetigo, peritonitis,etc (Fuerst, 1978; Sewell *et al.*, 1982; Christensen *et al.*, 1983, Einsenberg *et al.*, 1987; Harmory and Parisi, 1987) is highly desirable in medicare and could be promising in the containment of these ubiquitous organism.

The formulated herbal soap was not tested against Gram-negative bacteria, since they are not aucthocthonous skin flora. However, they are only encountered when the normal Gram-positive bacteria are depleted by prolonged antibiotic application, other unphysiological conditions as well as 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 formulated herbal soap was active against the spore-forming *Bacillus subtilis.* This harmless skin flora encountered due to contacts with materials such as soil, may occasionally becomes an opportunistic pathogen, causing eye-infection, septicemia, empyema, etc (Fuerst, 1978; Rosenberg and Cohen, 1983; Silman *et al.,* 1987; Singleton, 1987 and Tuazon *et al.,* 1987). Also of interest, is the sensitivity of *Candida albicans* to the formulated herbal soap. *Candida albicans* which normally forms part of the respiratory, gastrointestinal and female genital tracts infections (Fuerst, 1978, Hugo and Russel, 1983; Rosenberg and Cohen, 1983), is inertly resistant to many antimicrobial agents. Though a natural human body flora, it apparently causes some opportunistic infections in debilated and immunocompromised patients through dissemination in the bloodstream, especially in the cases of lymphoma, immunosuppression and during antibiotic applications (Fuerst, 1978; Hugo and Russel, 1983; Konemann *et al.,* 1994; Calderone, 2000 and Pappers, 2000).

Even though the human skin cannot be made absolutely free of bacteria, the *Cassia alata* formulated soap demonstrated high potency against *Staphylococcus aureus* (Table 3).

Table 3: *In vivo* antimicrobial evaluation of herbal formulated soap against *Staphylococcus aureus*

	Viable counts (cfu/ml)[b]			% reduction in viable count	
Volunteers	Before Herbal soap application	After Herbal soap application	Control	Herbal soap	Control
1.	4.2×10^5	3.0×10^4	4.0×10^4	92.86	90.48
2.	4.8×10^5	1.5×10^4	2.8×10^4	96.87	94.17
3.	4.3×10^5	2.7×10^5	3.5×10^4	93.72	91.86
4.	3.0×10^5	1.3×10^4	2.7×10^4	95.66	91.00
Mean	40.7×10^5	2.12×10^4	3.25×10^4	94.78	91.88

b = viable count after a DRT of 5 mins.

The staphylococci are the most widely encountered and undersirable aucthocthonous normal skin flora, difficult to be totally removed, thereby causing many skin infections as previously discussed. At the minimal death reduction time (DRT) of 5 mins, the herbal formulated soap recorded a significant ($p<0.05$) lower mean viable count of 2.12×10^4 cfu/ml, corresponding to a significant ($p<0.05$) mean higher reduction in microbial load (94.78 %) as against those of the control. The antimicrobial lethality of the formulated soap compared with that of the control, could be attributed to its metabolites and chemical constituents, as previously discussed.

CONCLUSION

From the foregoing reports, *Cassia alata* formulated soap demonstrated high potency against normal skin flora. This may justify the folkloric claim of the used of *Cassia alata* and other soapy plants for bathing as active skin-infection treatment. This indicates the potential and efficacy of the plant in the production of potent pharmaceuticals to combat the myriads of skin infections especially in the tropics. These findings have high economic, industrial and medical significance.

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