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THE ANTIMICROBIAL ASSESSMENT OF SOME NIGERIAN HERBAL SOAP.

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Abstract

Twenty samples of herbal soaps were evaluated for their antimicrobial activity against bacteria and yeast of significance in skin infections with the aim to provide some justification for the continued use of the soaps in the management of superficial skin infections. All the soaps were found to possess antimicrobial activity in a concentration and organism dependent manner. The soaps were more active against the gram positive organisms than the Gram negative organisms while none of the soaps had activity against the tested yeasts. Only 35% of the soaps were appropriately packaged with adequate directions for use and storage. The study showed that the tested soaps possessed antimicrobial properties and they can contribute to the treatment and management of skin infections caused by bacteria if well prepared with the appropriate plant materials to target specific causative organisms and packaged with appropriate directions for use and storage.

Keywords: Antimicrobial activity; Skin infections; Herbal soaps; Medicinal plants.

Introduction

Skin infections occur frequently in hot and warm humid climate that is characteristic of the tropics and many developing countries. They are very common in childhood worldwide and between 49 - 80.4% of African school children are affected (Dagnew *et al*., 1991). Among Nigerian primary schools pupils, the prevalence rate is about 40.4% (Oyedeji *et al*., 2006). Skin infections are also common in adolescent and young adult males (El-Said 2001; Tan 2007). These infections have been reported to be a major problem in Tanzania where about 34.7% of the rural populations have skin diseases ((Mollel, 1994; Satimia *et al*., 1998).

Most skin infections are caused by fungi, *Staphylococcus* species and *Streptococcus* species (Darmstadt 2000; Dryden 2009; Backeshwain *et al*., 2011) and they are treated with various antibacterial and antifungal agents in orthodox medicine. In the developing countries, herbal remedies for skin care are prepared from a variety of plant parts such as leaves, stem, root, bark, sap or fruit and scientific evidence supporting the use of some of such plants have been provided by many workers (Mukherjee and Suresh 2000; Ferro *et al*., 2003; Kareru *et al*., 2008). These medicines are administered topically and may be applied in the form of cream, lotion, gel, sap, solvent extract, ointment or soaps (Semkina, 2005; Esimone *et al*., 2008; Nebedum *et al*., 2009a). Soaps are a very common vehicle for application of these medicinal plants for external use in the treatment of skin diseases (Ajaiyeoba *et al*., 2003; Ahmed *et al*, 2005; Ajose, 2007; Eje *et al*., 2009; Kareru *et al*., 2010).

Locally manufactured soap is made from lye obtained from ash of burnt cocoa husks, plantain peels, palm wastes, wood and other plant debris and is known to have some antimicrobial properties (Lamikanra and Allwood, 1977; Lamikanra and Adebiyi, 1981; Moody *et al*., 2004). The Nigerian market is flooded with different types of herbal soaps for the treatment of a variety of skin infections but most of them have not been evaluated for their antimicrobial activity. The assessment of these soaps for their antimicrobial properties will give information on the activity of the soaps. This study was therefore carried out to assess the antimicrobial properties of these soaps against common skin pathogens and as such justify their continued use in the management of various skin infections.

Materials and Methods

Collection of herbal soaps

Twenty brands of herbal soaps (two samples each) were purchased from herb sellers and stores in five different markets in Osun and Oyo state located in Western Nigeria. These samples were purchased in their original packages and taken to the laboratory. The samples were coded A- T. Brands A - K as well as S and T were presented without any indication of constituents and the sellers or producers were unwilling to disclose the herbal constituents. Brands L– R had the lists of their herbal constituents indicated as shown in Table 1.

Preparation of soap sample discs

Soap suspensions at concentrations range of 2-50% w/v were prepared using sterile distilled water. Sterile filter paper disc (Whatman No 1, with diameter 6 mm) were then soaked with 2 drops of each test concentration of herbal soaps. The discs were allowed to dry at room temperature.

Test organisms

Clinical strains of *Staphylococcus epidermidis, Staphylococcus capitis, Staphylococcus aureus, Bacillus* species, *Pseudomonas aeruginosa*, *E. coli, Candida albicans* and *C. pseudotropicalis* obtained from the Pharmaceutical Microbiology laboratory of Obafemi Awolowo University were employed in the investigation.

Antimicrobial test:

The standard agar diffusion method recommended by CLSI (2006) was employed. Two distinct colonies of each test organism were taken from a 24-hour agar culture and were suspended in 10 ml sterile distilled water in test tubes using a sterile loop. The suspension was thoroughly mixed

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with a spin mixer and then adjusted to 0.5 McFarland Standard. The suspension was applied in duplicates to the surface of over-dried Mueller Hinton agar (Oxoid) using sterile swab sticks. The inoculated plates were incubated at 37^{o}C for 20 minutes for acclimatization and

Table 1: Herbal components of soaps tested

Soap code	Constituents
A-K	Undisclosed
L	Aloe vera, camwood extract, cocoa pod ash solution, honey, palm kernel oil, palm bunch ash solution and shea butter
M	Aloe vera, camwood extract, cocoa pod ash solution, honey, palm kernel oil, palm bunch ash solution and shea butter
N	Aloe vera, camwood extract, cocoa pod ash solution, honey, palm kernel oil, palm bunch ash solution and shea butter
O	Aloe vera, avocado oil, honey, lime juice and palm kernel oil
P	Aloe vera, honey and palm kernel oil
Q	Aloe vera, camwood extract, cocoa pod ash solution, honey, palm kernel oil, palm bunch ash solution, shea butter and lime juice
R	Aloe vera, camwood extract, cocoa pod ash solution, honey, palm kernel oil, palm bunch ash solution, shea butter and lime juice
S-T	Undisclosed

Table 2: Inhibitory activities of soap samples 1-10

Soap Sample	Conc. %w/v	Organisms / Zones of inhibition (mm)												
		Sc1	Sc2	Sc3	Se1	Se2	Sa1	Sa2	Sa3	Bs	Ps	Ec	Ca	Cp
1	10	-	-	-	-	9	7	12	22	7	-	7	-	-
	20	9	-	9	-	10	8	28	25	8	-	7	-	-
	30	10	-	9	-	11	8	32	27	8	-	8	-	-
	40	10	8	10	-	11	10	34	27	10	-	9	-	-
	50	10	9	12	-	11	10	36	29	12	-	9	-	-
2	10	8	-	-	-	8	9	20	23	7	-	-	-	-
	20	10	7	-	-	10	9	20	25	9	-	-	-	-
	30	10	9	-	-	10	9	22	28	9	-	-	-	-
	40	10	9	9	-	11	12	15	30	9	-	-	-	-
	50	11	9	12	7	12	20	28	32	12	-	7	-	-
3	10	8	-	-	-	11	8	20	26	8	-	7	-	-
	20	10	7	-	7	11	8	22	28	8	-	7	-	-
	30	10	7	-	7	12	10	24	30	10	-	7	-	-
	40	11	9	-	7	12	10	24	32	12	7	7	-	-
	50	13	11	-	9	14	12	25	34	14	7	7	-	-
4	10	15	9	7	-	10	8	10	18	-	-	-	-	-
	20	18	11	12	-	10	12	10	22	7	-	-	-	-
	30	20	16	12	9	12	14	11	22	8	-	8	-	-
	40	22	16	14	10	12	16	12	25	10	-	9	-	-
	50	22	16	15	11	12	17	12	30	10	-	10	-	-
5	10	8	7	-	-	8	7	15	15	-	-	-	-	-
	20	8	7	-	-	8	8	15	17	-	-	-	-	-
	30	8	7	-	-	10	8	17	20	8	-	-	-	-
	40	10	7	-	-	13	10	20	24	8	-	-	-	-
	50	10	7	-	-	15	12	24	28	8	-	-	-	-
6	10	8	8	-	-	13	12	13	14	8	-	-	-	-
	20	10	8	-	-	13	12	15	16	10	-	-	-	-
	30	10	8	-	7	16	15	16	19	10	-	-	-	-
	40	12	8	-	7	17	15	16	19	11	-	-	-	-
	50	12	9	-	7	17	17	17	20	11	-	7	-	-
7	10	8	7	-	-	7	7	12	16	9	-	-	-	-
	20	8	9	-	-	11	10	16	18	9	-	7	-	-
	30	8	12	-	-	15	14	20	19	10	-	7	-	-
	40	8	16	10	-	20	22	25	20	12	-	9	-	-
	50	8	21	10	7	26	24	28	23	16	-	9	-	-
8	10	12	10	8	-	10	8	9	14	9	-	-	-	-
	20	14	12	10	-	13	10	10	18	10	-	-	-	-
	30	16	12	12	-	16	10	12	24	10	-	-	-	-
	40	18	12	13	11	20	12	13	24	10	-	-	-	-
	50	20	15	15	11	24	12	13	27	10	-	-	-	-
9	10	12	7	9	-	10	7	7	16	-	-	-	-	-
	20	14	11	9	-	12	11	9	19	-	-	-	-	-
	30	14	12	10	-	15	11	11	20	-	-	-	-	-
	40	16	12	12	-	17	16	12	23	-	-	-	-	-
	50	17	14	14	-	20	17	12	25	-	-	-	-	-
10	10	13	10	10	-	10	9	9	20	-	-	-	-	-
	20	13	10	12	-	12	12	12	23	7	-	-	-	-
	30	15	14	15	-	16	13	12	24	7	-	-	-	-
	40	16	14	15	-	18	17	13	26	8	-	-	-	-
	50	18	16	15	-	20	20	14	30	8	-	-	-	-

Sc1= *Staph. capitis* strain 1; Sc2= *Staph. capitis* strain 2; Sc3= *Staph. capitis* strain 3; Se1=*Staph epidermidis* strain 1; Se2=*Staph epidermidis* strain *2;* Sa1= *Staph aureus* strain 1; Sa2= *Staph aureus* strain 2; Sa3= *Staph aureus* strain 3; Bs= *Bacillus* species Ps= *Pseudomonas aeruginosa;* Ec= *E. coli*; Ca=*Candida albicans;* Cp= *Candida pseudotropicalis.*

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Table 3: Inhibitory activities of soap samples K-T

Sample	Conc. %w/v	Organisms / Zones of inhibition (mm)												
		Sc1	Sc2	Sc3	Se1	Se2	Sa1	Sa2	Sa3	Bs	Ps	Ec	Ca	Cp
K	10	13	13	8	13	12	11	12	11	13	7	-	-	-
	20	13	13	10	13	14	11	13	12	14	8	-	-	-
	30	13	13	11	14	15	14	15	15	14	8	7	-	-
	40	14	13	11	14	15	14	16	17	16	8	7	-	-
	50	15	15	12	15	18	14	18	20	16	10	8	-	-
L	10	11	11	9	10	12	11	16	14	15	-	-	-	-
	20	14	11	10	14	12	11	17	15	16	8	-	-	-
	30	15	11	10	14	16	13	17	19	18	10	7	-	-
	40	19	13	11	15	16	13	17	20	22	12	7	-	-
	50	20	14	15	17	20	16	20	22	26	14	8	-	-
M	10	10	7	7	8	7	7	13	12	12	7	-	-	-
	20	11	10	8	9	9	10	13	12	14	9	-	-	-
	30	11	11	10	12	14	15	15	17	15	10	-	-	-
	40	11	12	11	12	15	15	15	15	18	12	-	-	-
	50	11	15	11	13	15	16	20	15	20	12	-	-	-
N	10	-	9	7	9	8	8	12	10	-	-	-	-	-
	20	7	9	7	9	10	8	13	12	10	-	-	-	-
	30	7	10	10	10	10	9	13	12	11	-	-	-	-
	40	8	12	10	10	10	14	16	15	16	-	-	-	-
	50	9	14	10	11	12	15	16	18	19	-	-	-	-
O	10	-	8	-	7	10	-	7	11	9	-	-	-	-
	20	-	8	8	7	10	7	10	12	9	-	-	-	-
	30	11	11	8	8	10	7	10	12	10	-	-	-	-
	40	15	13	8	9	10	9	11	12	12	7	-	-	-
	50	17	13	10	10	12	12	12	13	12	7	-	-	-
P	10	9	10	8	9	10	9	13	12	8	-	-	-	-
	20	12	10	9	9	11	11	14	17	8	-	-	-	-
	30	15	11	10	10	13	11	14	17	10	8	-	-	-
	40	18	12	12	12	14	13	17	20	12	11	-	-	-
	50	20	12	13	12	14	15	17	23	16	14	-	-	-
Q	10	9	10	7	9	9	12	12	13	10	9	-	-	-
	20	11	11	9	9	11	16	12	14	12	10	-	-	-
	30	14	11	11	12	13	15	15	16	13	10	-	-	-
	40	17	13	12	13	14	15	15	20	13	13	-	-	-
	50	19	13	13	15	16	16	17	22	15	15	-	-	-
R	10	10	9	10	-	9	7	10	19	-	-	-	-	-
	20	14	9	10	-	11	8	10	22	-	-	-	-	-
	30	16	11	11	-	14	11	11	24	-	-	-	-	-
	40	20	12	13	7	16	11	11	24	-	-	-	-	-
	50	24	14	13	7	16	14	12	25	-	-	-	-	-
S	10	18	10	-	-	10	10	10	20	-	-	-	-	-
	20	20	11	7	7	12	11	10	24	-	-	-	-	-
	30	22	13	12	9	15	13	13	24	-	-	-	-	-
	40	23	14	12	9	17	16	13	26	-	-	-	-	-
	50	25	16	13	9	17	16	14	28	-	-	-	-	-
T	10	14	-	10	-	8	8	12	16	9	-	-	-	-
	20	16	8	13	-	9	10	12	16	9	-	-	-	-
	30	16	10	13	9	2	11	13	20	10	-	-	-	-
	40	18	12	15	10	14	12	14	20	10	-	-	-	-
	50	20	12	17	11	15	13	15	22	11	-	-	-	-

Sc1= *Staph. capitis* strain 1; Sc2= *Staph. capitis* strain 2; Sc3= *Staph. capitis* strain 3; Se1=*Staph epidermidis* strain 1; Se2=*Staph epidermidis* strain *2;* Sa1= *Staph aureus* strain 1; Sa2= *Staph aureus* strain 2; Sa3= *Staph aureus* strain 3; Bs= *Bacillus* species Ps= *Pseudomonas aeruginosa;* Ec= *E. coli*; Ca=*Candida albicans;* Cp= *Candida pseudotropicalis*

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Table 4: Antimicrobial activity of samples (10%W/V) compared with reference antiseptic soap (10%W/V).

Organism	No. (%) of herbal soap		
	Less active than reference soap	As active as reference soap	More active than reference soap
S. capitis 1	8 (40)	2 (10)	10 (50)
S. capitis 2	8 (40)	2 (10)	10 (50)
S. capitis 3	12 (60)	3 (15)	5 (25)
S. epidermidis 1	14 (70)	1(5)	5 (25)
S. epidermidis 2	6 (30)	3 (15)	14 (70)
S. aureus 1	16 (80)	2 (10)	2 (10)
S. aureus 2	7 (35)	6 (30)	7 (35)
S. aureus 3	0 (0)	2 (10)	18 (90)
P. Aeruginosa	0 (0)	16 (80)	4 (20)
Bacillus spp.	16 (80)	1(5)	3 (15)
E. coli	20 (100)	0 (0)	0 (0)
C. albicans	20 (100)	0 (0)	0 (0)
C. pseudotropicalis	20 (100)	0 (0)	0 (0)

Table 5: Presentation of herbal soap samples

Quality	Number of samples	Percentage
Label	7	35
Direction for use and storage	7	35
List of constituents	7	35
Adequate packaging material	7	35
NAFDAC registration number	7	35
Expiry date	7	35

growth of the inoculums. The soap discs were then applied in duplicates equidistant to one another on the inoculated plates. The plates were refrigerated at 4^{o}C for 30 minutes to ensure adequate diffusion of the soap and all test plates were incubated at 37^{o}C for 18 -24 hours. At the end of incubation period the diameter of zones of inhibition around each disc was measured in mm and the mean of duplicate experiments were recorded. Similar procedure was carried out for the antifungal test but test fungi were picked from Sarbouraud Dextrose Agar (Oxoid) plates, suspended in water and swabbed on over-dried Sarbouraud Dextrose Agar plates. The incubation was at 25^{o}C for 5 days. An antiseptic soap containing triclosan 0.6%w/w was used as a reference antiseptic soap.

Results

The herbal soaps exhibited antimicrobial activity against the tested organisms in a concentration dependent manner. The degree of inhibition varied with the species of organisms as well as the strain as shown in Tables 2 -3. The Gram positive cocci were the most susceptible to the soaps and the yeasts were not inhibited by any of the tested herbal soaps. As shown in Table 4, some of the soaps had antimicrobial activity comparable with that of the reference antiseptic soap against the tested organisms. Only 35% of the soaps could be described as adequately packaged or presented as shown in Table 5.

Discussion

Soaps aid in general body hygiene by physical removal of microorganisms adhering lightly to the skin. The act of washing or scrubbing the body with the soap is expected to lead to a reduction in the microbial load on the skin and this can contribute to a reduction in the incidence of skin infections. Apart from this physical removal of organisms on the skin, the achievement of therapeutic effect of an herbal soap can be due to direct antimicrobial activity on microorganisms present on the skin (Lamikanra and Adebiyi, 1981). These include pathogens of importance in skin and wound infections and commensals implicated in opportunistic infections of the skin in the immunocompromised.

The assessment of the antimicrobial properties of the herbal soaps in this study showed that the soaps possessed antimicrobial activity against the bacteria tested in a concentration dependent manner indicating that the herbs or plant parts that have been used in the preparation of these soaps had antimicrobial principles. Apart from the concentration dependent activity, the inhibitory action was also organism dependent. Majority of the soaps were active against the Gram positive organisms than the gram negative organisms and fungi. The Gram positive organisms especially the gram positive cocci including *S. aureus, S. epidermidis*, and *S. capitis* were inhibited to a large extent by most of the herbal soaps tested. This is of significance as skin infections such as acne, impetigo, furuncles and carbuncles are caused by this group of Gram positive organisms (Kumar *et al*., 2007; Dryden 2009) and the use of these soaps against such infections as indicated by the sellers of these soaps is justified by the results of this study. However, this organism-dependent activity is not usually considered as important by the local producers of herbal soaps who in most cases portray their products as active against all kinds of skin diseases or infections irrespective of causative organisms. About 75% of the soaps were found to be inactive against the two Gram negative organisms *E. coli* and *Pseudomonas aeruginosa* neither was there any activity against the yeasts tested. This lack of activity against the Gram negative organisms could be as a result of the impermeable nature of the Gram negative cell to most antimicrobials

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(Lamikanra, 2010) and more importantly the antimicrobial principles in the soaps tested. *Pseudomonas aeruginosa* is notably notorious for its resistance to most antimicrobial agents (Strateva and Yordanov, 2009) and intensified research to discover new and effective agents against this organism is very important. Fungal skin infections are very common in the tropics due to the high atmospheric humidity and temperatures with infections spreading quickly where there is over-crowding. A lot of the available synthetic antifungal agents are expensive and beyond the reach of people with low socio-economic status, who usually are the ones with skin infections (Oyedeji et al., 2006; Sanuth and Efuntoye, 2010). None of the soaps tested showed activity against the yeasts tested indicating the need for intensified search for antifungal plants for incorporation into Nigerian herbal soaps. Omobuwajo et al., (2011) as well Oladele et al., (2009; 2011) have done some work in this regard.

The observed antimicrobial properties of the soap tested could be linked to the ingredients used in the preparation of the soap in only 35% of the soaps tested (soap samples L to R). The other 65% of the soaps had no information on their constituents. The soaps with the greater activity among this 35% were those with more combination of antimicrobial herbs. Samples L, M, N, Q and R had more antimicrobial herbs than samples O and P and were found to be more active against the tested organisms. The differences in the zones of inhibition produced by the different soaps having the same constituents suggest that there are differences in the quantity of each ingredient in each of the soap. The quantity of each of these ingredients could however not be ascertained since the manufacturers did not disclose this on their labels. The plant materials in the preparation included palm kernel oil, Aloe vera, honey, cam wood, lime juice, palm bunch ash, cocoa pod ash, avocardo oil and shea butter as ingredient in the soaps. These ingredients have established antimicrobial activities. Honey has been shown to possess antimicrobial properties against many organisms especially those involved in wound and skin infections (Efem, 1992; Molan, 1992; Molan, 2001) while the properties of Aloe vera as an antimicrobial has been documented by Agarry et al. (2005) and Nebedum et al. (2009b). The activity of different parts of lime fruit as antimicrobial has also been reported by Aibinu et al. (2007). The extracts of Pterocarpus stems (Camwood) have been shown to possess antimicrobial activities by Ebi and Ofodile (2000) while the leaves are reported to be medicinally useful in the treatment of superficial skin infections such as eczema (Gills, 1992).

This activity-ingredient link was possible in the herbal soaps from the manufacturers whose products could be described as well packaged or presented (samples L – R). They had labels, direction for use and storage, list of constituents, NAFDAC (The National Agency for Food and Drug Administration and Control) registration number as well as expiry dates. The absence of good packaging in the remaining 65% of the soaps increases the risk of exposure of the products to environmental microbial contamination especially fungi which may render the soap samples less active in the treatment of skin infections. Very few of the samples tested were prepared using moulds that imparted definite shapes on them and were packaged in small cardboard boxes. Majority were prepared without the use of definite mould or packaging style hence they appeared shapeless and were wrapped in irregular or shapeless polythene sheets. This absence of adequate direction for use and poor presentation or packaging may discourage acceptance of such herbal soaps by the more enlightened people in the Nigerian society. Hence, there is a need to improve the packaging style of the Nigerian herbal soaps prepared by the local manufacturers.

The results of this study, therefore, show that herbal soaps from Nigeria possess antimicrobial properties and they can contribute significantly to the treatment and management of skin infections caused by bacteria if they are well prepared with the appropriate combination of plant materials to target specific causative organisms and packaged aesthetically with appropriate directions for use and storage.

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