

# Antimicrobial Activity of Medicated Soaps Commonly Used By Dar es Salaam Residents in Tanzania

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## Mwambete and Lyombe: Medicated Soaps' Antimicrobial Profiles

An *in vitro* evaluation of the anti-microbial activity of medicated soaps was conducted using ditch-plate and hand washing techniques. Strains of reference microbes namely *Candida albicans* (ATCC90028), *Staphylococcus aureus* (ATCC25923), *Pseudomonas aureginosa* (ATCC27853) and *Escherichia coli* (ATCC25922) were tested at three different soaps' concentrations (1.0, 4.0 and 8.0 mg/ml). A total of 16 medicated soaps were assayed for their antimicrobial efficacy. Of these, 13 were medicated and 3 non-medicated soaps, which served as control. Ciprofloxacin and ketoconazole were employed as positive controls. Label disclosure for the soaps' ingredients and other relevant information were absorbed. The most common antimicrobial active ingredients were triclosan, trichloroxyleneol and trichlorocarbanilide. ANOVA for means of zones of inhibition revealed variability of antimicrobial activity among the medicated soaps. Positive correlation ( $r=0.318$ ;  $P<0.01$ ) between zones of inhibition and soaps' concentrations was evidenced. Hand washing frequencies positively correlated with microbial counts. Roberts<sup>®</sup> soap exhibited the largest zone of inhibition (34 mm) on *S. aureus*. *Candida albicans* was the least susceptible microbe. Regency<sup>®</sup> and Dalan<sup>®</sup> exhibited the least zone of inhibition on the tested bacteria. Protex<sup>®</sup>, Roberts<sup>®</sup>, Family<sup>®</sup> and Protector<sup>®</sup> were equally effective ( $P<0.01$ ) against *S. aureus*. In conclusion, majority of the assayed medicated soaps have satisfactory antibacterial activity; though lack antifungal effect with exception of Linda<sup>®</sup> liquid soap. The hand washing technique has proved to be inappropriate for evaluation of soaps' antimicrobial efficacy due to presence of the skin microflora.

**Key words:** Antimicrobial activity, ditch-plate and hand washing techniques, medicated soaps

Soaps and other cleansing agents have been around for quite long time. Archeological findings during the excavation of ancient Babylon revealed a soap-like material in clay cylinders. Inscriptions on the cylinders indicate that fats were boiled with ashes, which is a method of making soaps. Likewise, a medical document from about 1500 B.C. shows that Egyptians combined animal and vegetable oils with alkaline salts to form a soap-like material used for treating skin diseases, as well as for washing<sup>[1]</sup>. Moses, in the Bible, gave the Israelites detailed laws concerning personal cleanliness. He also related cleanliness to health and religious purification. People were instructed to wash their clothes and bath in water. Nowadays, disinfection, decontamination, antiseptis/sanitization, and sterilization just naming a few, there are terms that describe processes of cleaning by either using soaps/detergents or other cleaning agents<sup>[1]</sup>. Numerous cleaning agents are available in the market, which are presented in various forms with distinct formulations. Triclosan, trichlorocarbamide and p-chloro-m-xyleneol (PCMX/chloroxyleneol) are the commonly used antibacterials in medicated soaps. These are generally, only contained at preservative level unless the product is clearly marked as antibacterial, antiseptic, or germicidal<sup>[2]</sup>.

Scrubbing body or hands, particularly with soaps, is the first line of defense against bacteria and other pathogens that can cause colds, the flu, skin infections and even deadly communicable diseases<sup>[3,4]</sup>. Conceptually, many people consider that an antimicrobial potion of soaps is effective

at preventing communicable diseases. But now researchers highlight that too much of it can have the opposite effect-spreading diseases/infections instead of preventing them<sup>[5,6]</sup>. Overutilization of medicated soaps might result in antimicrobial resistance and even rendering an individual more vulnerable to microbial attacks such as opportunistic skin infections<sup>[7,8]</sup>.

On the other hand, regardless of a wide-spread availability of the so-called medicated soaps; a number of communicable infectious and food-borne diseases as well as poor-hygienic conditions-related health problems are rampant. This can partially be explained by the fact that, occasionally some of these antimicrobial consumer products could have insufficient quantities of antimicrobials. It seems to be more of a marketing phenomenon. Unfortunately, in the long-run may adversely affect the consumers, because overuse of these agents can ascribe to the emergence of drug-resistant microorganisms<sup>[7-9]</sup>. This instigated us to embark on evaluation of the antimicrobial effects of the so-called medicated soaps.

A total of 16 different brands of the most commonly used medicated soaps were randomly collected / purchased from shops, drug stores, and pharmacies in Dar es Salaam City during a 3 months study-period. These were subjected to the below described antimicrobial activity tests. Using a wax marker, a Nutrient agar-plate (Roth, German) was divided in four sectors and labeled 1 through 4. Firstly, fingers were rubbed over sector 1 prior to washing hands. Secondly, using a scrub brush, soap, and water,

hands were scrubbed for about 2 min, and then the fingers were rubbed over sector 2. The second step was repeated for sectors 3 and 4. This technique was performed twice: The first process was conducted in open-air on laboratory bench while the second was aseptically carried out in laminar flow cabin. The aim was to rule out contamination of the agar plates by microorganisms present in the air, which could have been settled onto the agar plates by gravitational force. One un-inoculated agar plate served as negative control. Microbial counts were performed following an incubation of the agar plates for 12 to 42 h at 37°.

Three concentrations (1, 4, and 8 mg/ml) of each brand of soap were prepared by dissolving in sterile distilled water by vigorous shaking till a homogenous suspension was obtained. Then a 30 µl aliquot of the soap suspension was deposited into a 5-mm well made with a sterile cork-borer into Mueller-Hinton or Saboraud's dextrose agars (Roth, German) that was preceded by inoculation of strains of reference microorganisms, *Pseudomonas aeruginosa* (ATCC27853), *E. coli* (ATCC25922), and *Staphylococcus aureus* (ATCC25923), and *Candida albicans* (ATCC90028) respectively. Antibiotic discs (profloxacin and ketoconazole) (Sigma-Aldrich, USA) were also incorporated as positive controls, while non-medicated soaps such as Mbuni®, Linda® and Imperial® were incorporated as negative control. All prepared soap suspensions were stored under the same condition until use. Zones of inhibition (ZI) were measured and recorded following an overnight

incubation and 42 h at 37° for bacteria and the fungus, respectively.

All above procedures were performed twice in duplicate for consistency of results, and therefore the resultant numerical data are expressed as mean. Statistical data analysis (for means and variance) was carried out using a computer package SPSS version 16 (Chicago, IL). Differences of means of ZI among the samples were analyzed by the T-test. Significance level was set at  $P < 0.05$ .

Of 16 assayed samples, 13 were medicated antibacterial soaps and one sample each of a cloth washing soap (Mbuni®), a liquid hand washing soap (Linda®) with density of 1.02 g/ml and a regular bathing soap (Imperial®). Out of 16 assayed soaps, 10 disclosed antimicrobial active ingredients (AAIs) while only 3 disclosed expiry dates. The most commonly used AAIs were triclosan/irgasan DP300, trichloroxylenol and trichlorocarbanilide (Table 1).

The findings from the hand wash technique show the time-dependent microbial counts; that is an increase of microbial counts with increase of hand washing frequencies. A relatively substantial difference in microbial counts was exhibited by Mbuni® and Protex® on sectors 3 and 4 with respect to rest of the soaps, which correspond to time-interval of 4 and 6 min, respectively following exposure to the medicated soaps (Table 2). Results from the incubation of medicated soap's suspensions (30 µl) with the strains

**TABLE 1: LABEL DISCLOSURE OF THE ASSAYED MEDICATED SOAPS**

Brands	Active ingredient	Expiry date	Indication	Manufacturer
Meditex	Chloroxylenol	None	Bactericide	Mukwano Industries Tanzania Ltd
Duru	None	None	Bactericide	Murzar oil mills Ltd
Protex	Trichlorocarbanilide 0.25% and triclosan 0.20%	08/2009	Bactericide	Colgate Palmolive (Pvt.) Ltd
Lifebuoy	Trichroloocarbanilide 0.06%, triclosan 0.02%	06/2010	Bactericide	Unilever Kenya Ltd
Rungu	Irgasan DP 300	None	Bactericide	HB worldwide Ltd
Roberts	Irgasan DP 300	None	Bactericide	PZ Cussons East Africa Ltd
Family	Trichroloocarbanilide, Irgasan DP 300	None	Bactericide	G and B soap Industries Ltd
Dettol	Chloroxylenol 0.5% Trichroloocarbanilide 0.5%	09/2010	None	Reckitt Benkiser East Africa limited
Protector	Irgasan DP 300	None	None	Showerlux Industries Ltd
Regency neem	Neem	None	Antiseptic	Neem Africa Ltd
Dalan	None	None	Bactericide	
Imperial	None			
Linda	None			SH Amoni Interprise
Mbuni	None			
Liquid soap	None	None	Antiseptic, antibacterial and germicidal effect	Ravino Industries
Tetmesol	Monosulfiram 5% w/w	None	Scabicide	Nicholas Piramal India Ltd / Shelly's Pharmaceuticals.

of reference microorganism-inoculated-agar plates depict a variability of antimicrobial efficacy (Tables 3 and 4). A statistical analysis (ANOVA) also revealed significant inter-group differences with regard to antimicrobial efficacy ( $F=10.313$ ;  $df= 2$ ;  $P=0.0001$ ). A positive correlation ( $r=0.318$ ;  $P<0.01$ ) between ZI and

the tested soaps' concentrations was observed (Tables 3 and 4). Protex<sup>®</sup>, Roberts<sup>®</sup>, Family<sup>®</sup> and Protector<sup>®</sup> were equally effective ( $P<0.05$ ) against *S. aureus* (Table 3). Regency and Dalan<sup>®</sup> exhibited the least ZI on the tested bacteria (Table 4). *C. albicans* was the least susceptible showing very small ZI at the highest assayed concentration (8 mg/ml) as depicted on both Tables 3 and 4.

**TABLE 2: ANTIMICROBIAL EFFECTS OF MEDICATED SOAPS ASSAYED BY HAND WASHING TECHNIQUE**

Brand	Sector			
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>
Meditex	+	++	+++	+++
Duru	+	++	+++	+++
Protex	+	++	+	+
Lifebuoy	+	++	+++	+++
Rungu	+	++	+++	+++
Roberts	+	++	+++	+++
Family	+	++	+++	+++
Dettol	+	++	+++	+++
Protector	+	++	+++	+++
Regency neem	+	++	++	++
Dalan	+	++	++	++
Tetmesol	+	++	+++	+++
Linda	+	++	+++	+++
Mbuni	+	+++	++	-
Liquid soap	+	++	+++	+++
Imperial	+	++	+++	++

(-) no growth, (+) <10; (++) <100; (+++) >100 microbial counts/sector.

Generally, antimicrobial soap could be any cleaning soaps to which AAIs have been added. These chemicals kill bacteria and other microorganisms, though they are not effective at deactivating viruses just like any other kind of soaps. Soaps are intended for reduction of the inoculum sizes of both pathogenic and non-pathogenic microorganisms; the latter include the normal flora. Of these, two types are well known viz. resident flora that are the normal flora of the skin and other human body parts, and transient flora that are usually picked up from objects or other human beings<sup>[10]</sup>. Thus, it is routine practice to wash hands prior to eating, after examining a patient and before surgery, in order to remove some potentially harmful transient flora as well as reduce a number of resident flora, which might cause

**TABLE 3: ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF MEDICATED SOAPS ON TEST MICROBES**

Brand	Conc. (mg/ml)	Mean diameter zones of inhibition (mm)			
		<i>P. aureginosa</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
Meditex	1.0	9.3	10.0	5.5	-
	4.0	11.8	14.0	8.5	-
	8.0	15.0	18.3	12.2	5.5
Duru	1.0	9.8	8.5	10.2	-
	4.0	15.8	11.7	13.5	-
	8.0	19.5	14.5	16.5	-
Protex	1.0	12.3	11.2	20.5	-
	4.0	17.7	15.0	26.3	6.5
	8.0	22.0	18.7	32.2	8.0
Lifebuoy	1.0	8.33	6.5	9.8	-
	4.0	11.7	9.5	14.2	-
	8.0	14.3	12.5	18.2	-
Rungu	1.0	10.0	5.8	9.3	-
	4.0	16.5	9.7	12.2	-
	8.0	20.5	14.8	15.0	-
Roberts	1.0	14.0	16.5	27.0	-
	4.0	20.0	18.8	30.0	5.7
	8.0	24.2	22.5	34.0	8.5
Family	1.0	8.4	18.0	22.0	-
	4.0	12.0	22.0	25.8	-
	8.0	17.7	25.0	29.2	6.5
Protector	1.0	6.5	15.2	20.5	-
	4.0	9.5	17.5	23.5	-
	8.0	12.7	20	26.5	6.0

(-) denotes no inhibition zone; ND-not done

**TABLE 4: ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF MEDICATED SOAPS ON TEST MICROBES**

Brand	Conc. (mg/ml)	Mean diameter zones of inhibition (mm)			
		<i>P. aureginosa</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
Dettol	1.0	10.8	10.0	6.0	-
	4.0	15.3	14.3	9.0	-
	8.0	19.0	18.0	11.8	-
Regency neem	1.0	5.0	5.4	-	-
	4.0	7.7	7.0	6.0	5.3
	8.0	10.7	9.5	9.0	7.5
Dalan	1.0	-	5.0	5.5	-
	4.0	5.3	6.8	9.0	-
	8.0	7.5	9.0	9.7	-
Tetmesol	1.0	7.5	7.2	8.0	-
	4.0	11.0	9.75	13.0	-
	8.0	14.2	13.3	16.8	5.5
Mbuni	1.0	-	6.5	-	-
	4.0	10.3	11.0	9.0	-
	8.0	18.0	17.5	11.6	11.0
Imperial	1.0	6.0	-	7.6	-
	4.0	11.0	9.5	12.0	7.0
	8.0	15.6	13.4	15.6	9.6
Liquid soap	1.0	8.4	9.0	10.5	-
	4.0	10.7	10.6	7.4	6.5
	8.0	12.0	11.5	8.5	8.4
Linda	1.0	9.0	12.0	9.7	12.4
	4.0	12.6	12.3	11.2	14.5
	8.0	14.5	13.0	12.0	20.5
Ketaconazole	15 µg/disc	ND	ND	ND	12.5
Ciprofloxacin	5 µg/disc	26.5	32.0	28.7	ND

(-) denotes no inhibition zone; ND-not done

opportunistic infections<sup>[11,12]</sup>.

An elevation of microbial counts with increase of frequencies of hand washing, observed in this study could be ascribed to further exposure of the resident flora, which usually are fewer than transient floras and reside beneath them. Majority of the assayed medicated soaps have demonstrated satisfactory antimicrobial effect, particularly the antibacterial activity, hence buttressing the label information disclosure that they rather possess antibacterial than antimicrobial effects. Presumably, the observed variability in antimicrobial activity is due to difference of AAI contents, type of formulations, and repeated uses of the agents, which might have made the microorganisms resistant. Tetmesol is primary used for its scabidical effect, however exhibited a moderate antimicrobial activity, which to great extent is attributed to monosulfiram within its formulation<sup>[13]</sup>. The notable antimicrobial effects exhibited by Linda<sup>®</sup> could be largely attributable to presence of undisclosed broad-spectrum preservatives than its liquid state<sup>[11,14]</sup>.

Usually, consumers buy and utilize antimicrobial products to stay healthy, with an intention to protect themselves from potentially harmful organisms. However, they often fail to consider the inherent risks in both, the chemical exposure that they voluntarily subject themselves to and the potential increase in antibiotic-resistant pathogens in the environment. Regular soaps and water have worked for centuries and there is no scientific evidence that this combination has lost its efficacy<sup>[15]</sup>. Moreover, alcohol based hand rubs have extensively been used in the hospital environment as an alternative to antimicrobial/antiseptic soaps, which provide a better skin tolerance as compared to antiseptic soap due to the moisturizing and softening agents in the formulation. Hand rubs have also demonstrated to have more effective microbiological properties as compared to antiseptic soaps<sup>[12,16]</sup>. Therefore, overutilization of antimicrobial soaps may spell to development of resistance to microbicides. Several studies have purported to show a relationship between the use of triclosan (or other antimicrobial soaps) and antibiotic resistance<sup>[5-7]</sup>. In theory, the use of microbicides in consumer products could

select for microbial strains which also are resistant to clinically important antibiotics. This, in turn, could exacerbate the problem of clinical antibiotic resistance and make treatment of microbial infections even more difficult.

Although bacterial susceptibility to antibiotics is fairly well characterized, currently the relevance of a change in the minimum inhibitory concentrations of antiseptics or disinfectants is unknown<sup>[6]</sup>. Therefore, failure of bactericides to kill clinically isolated bacteria, can be associated with alterations of their antibiotic susceptibility profiles<sup>[11]</sup>. Overuse of chemicals like triclosan has been suggested to cause sensitive bacteria to evolve resistance to its antibacterial actions. Should any antibiotic be discovered that works similarly to triclosan, this antibiotic's effectiveness to combat infections will be reduced since people will be harboring resistant bacteria as result of using soaps containing triclosan<sup>[9,17]</sup>. Contradictory findings on the usefulness of applications of medicated soaps have been reported. Researchers have shown that simply washing thoroughly with plain soap is sufficient to reduce bacteria and, further, is effective against viruses, thus questioning advantages of using antibacterial soaps<sup>[12,16,17]</sup>. Other studies have found that soaps containing AAIs remove more bacteria than simply washing with plain soap and water<sup>[18,19]</sup>.

This study has revealed that most of the assayed medicated soaps have satisfactory antibacterial activity, though lack antifungal effect with exception of Linda<sup>®</sup> liquid soap. Regency-neem<sup>®</sup> would be expected to be very active on *C. albicans* because of the reported both antifungal and antibacterial effects of neem (*Azadirachta indica*)<sup>[20-23]</sup>. Similarly, presence of triclosan/irgasan DP 300 in some of these products (Protex<sup>®</sup>, Rungu<sup>®</sup>, Lifebouy<sup>®</sup>) that is reported to have inhibitory effect on microbial lipid synthesis, would have exhibited stronger antimicrobial effect<sup>[24,25]</sup>. The quantity of this AAI in these products is doubtful. The hand washing technique has proved to be inappropriate for evaluation of soaps' antimicrobial efficacy because of presence of the transient and resident flora of the skin. Because of the observed medicated soaps' antimicrobial effects it is recommended that irrational and long-time usage of these products should be discouraged. It is also important that during development of topical antimicrobial products, a multidimensional approach

be adopted. This will ensure that the resultant product is designed for the specific needs of the market and that those needs are met. Ultimately, the product is more likely to have a long, useful, and profitable utilization.

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