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Anticandidal and Antistaphylococcal Activity of Soap Fortified with *Ocimum gratissimum* Extract

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Abstract: The possibility of producing a highly competitive water-soluble hard soap fortified with *O. gratissimum* extract (Ts) which will inhibit the growth and activity of *Candida* and staphylococci was investigated in comparison to a commercial standard soap (Ss) and the control soap without an extract (Cs). The standard agar well diffusion method and broth micro-dilution assay technique were used for the antimicrobial potency determination of the soap samples and Minimal Inhibitory Concentrations (MIC) respectively. The pH range of the soap samples was 9.8-10.2 ±0.2. Ts had clear forest green coloration as compared to the creamy appearance of Ss and Cs. The moisture content (%), foamability (cm) and total fatty matter (%) values for Ts were 19.7±0.02, 13.4±0.03 and (10.0±0.01), respectively. The inhibitory activity of soap fortified with *O. gratissimum* against all *Candida* sp. and *Staphylococci* was significant over Cs but insignificant towards Ss ($p<0.05$). The sensitivity values (mm) for *C. albicans*, *C. parapsilosis* and the staphylococci to Ts ranged between 9.5±0.02-23.1±0.01, 8.7±0.00-21.5±0.02 and 6.5±0.01-26.5±0.02, respectively, as compared to their low sensitivity to Cs, (6.2±0.01-17.0±0.00), (6.5±0.01-15.8±0.00) and (6.2±0.01-18.0±0.00), respectively. The MIC was 0.0312 and 0.0156 g mL^{-1} for the Ss and Cs and Ts, respectively for all isolates (*Candida* and *staphylococci*). On an overall, Ts was highly comparable with Ss as compared to the Cs in terms of general acceptability, texture and fragrance and other analyzed parameters.

Key words: *Ocimum gratissimum*, *Candida*, *Staphylococcus aureus*, antimicrobial, soap

INTRODUCTION

Soaps are water-soluble or insoluble cleansing agents or detergents made from animal and vegetable fats, oils and greases; or chemically, the sodium or potassium salt of a fatty acid, formed by the saponification interaction of fats and oils with alkali. There are two types of water-soluble soaps: Hard Soaps, which are made from oils like the palm kernel oil that contain a high percentage of saturated acids and are saponified with sodium hydroxide known as lye and soft soaps, which are semi fluid soaps made from oils like the cottonseed oil and they are usually saponified with potassium hydroxide (Ryer, 1969).

Based on formulation and functional chemical constituents, soaps could be categorized as toilet soaps, antiseptic soaps or medicated soaps. The antiseptic and medicated soaps are made to fight pathogenic microbes and other germs due to special chemical additives in them while the toilet ones are made for conventional cleansing purposes. Many of the chemical additives are believed to have undesirable effects on some users (Woolath, 1985), therefore the search for natural additives which are regarded as safe for use. In modern times, the use of soap has become universal in industrialized nations due to a better understanding of the role of hygiene in reducing the population size of pathogenic microorganisms.

In spite of the great advances observed in modern medicine, plants still make an important contribution to health care possibly due to the recognition of the value of traditional medical systems especially in tropical countries. The African basil, *Ocimum gratissimum* L. (Lamiaceae), is naturally used in folk medicine to treat different upper respiratory tract infections, diarrhea, ophthalmic, skin diseases of bacterial and fungal origin, pneumonia, cough and conjunctivitis (Onajobi, 1986; Holetz *et al*., 2003; Adebolu and Salau, 2005; Mbata and Saikia, 2005; Okigbo and Ogbonna, 2006; Nwinyi *et al*., 2009). This perennial sub-shrub or herb grows mostly in southern Nigeria and the wet part of the tropics as it is available in nurseries, with plant dealers and in most homes (Fayemi, 1999). It has a pleasant smell which gives it the common name scent leaf plant.

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Ocimum gratissimum, grown for its essential oil, is known to contain eugenol used for various purposes. Begum *et al.* (1993), Nakamura *et al.* (1999), Mbata and Saikia (2005) and Matasyoh *et al.* (2008) have reported the efficacy of this oil against several strains of *Staphylococcus aureus*, *Klebsiella*, *Shigella flexneri*, *Proteus mirabilis*, *Salmonella enteritidis*, *Escherichia coli*, *Listeria monocytogenes*, *Trichophyton rubrum*, *T. mentagrophytes* and *Candida albicans*. Aside eugenol, linalool, methyl cinnamate, camphor and thymol are other known effective ingredients found in this plant (Matasyoh *et al*., 2008). Other uses of *O. gratissimum* include flavoring, garnishing, seasoning and coloring of food (Mohammed *et al*., 2007) as Edeoga *et al.* (2006) elucidated the importance of this medicinal plant in the pharmaceutical industry. However, there has been no report of the application of *O. gratissimum* extract in soap making.

Considering the need for exploring natural resources as an alternative to industrial antiseptics in soap making, the fact that these natural herbs are easily accessible and very cheap to cultivate or purchase and the quest for solving certain severe dermal infections especially within the tropics, this research was designed. The aims were to ascertain the possibility of producing a highly competitive water-soluble hard soap fortified with *O. gratissimum* extract and further determine its anticandidal and antistaphylococcal activity.

MATERIALS AND METHODS

Plant material and extract preparation: Fresh leaves of *O. gratissimum* were purchased from Mile 12 market, Lagos, Nigeria in May 2008 and identified by a Plant Taxonomist within the department of Chemical and Environmental Sciences, Babcock University, Nigeria. The leaves were washed several times using tap water followed by distilled water and then air dried for two weeks. The dried leaves were pulverized into fine powder and 30 g of the fine powder was weighed into a 500 mL conical flask followed by the addition of 300 mL absolute methanol (Sigma, USA) for total phytochemical extraction. The mixture was stored in a cool dry place for 48 h after which it was filtered through two folds of Whatman No. 1 filter paper. The extract was further concentrated in a vacuum using rotary evaporator (Eyela Rotavap N-1001, Rikakikai Co. Ltd., Tokyo) at 40°C.

Determination of saponification value of PKO: The method and calculations described by Athene (2009) were employed in determining the saponification value of the PKO. About 10 g of Palm Kernel Oil (PKO) was used for this analysis and the value was deduced by dividing the total amount of fat (in grams) by the total amount of lye (in grams).

Production of soap: Two kinds of soap viz., Test soap (Ts) and Control Soap (Cs) were produced following the same steps. Leaf extracts of *O. gratissimum* was incorporated into Ts while Cs was not fortified with the extracts. A commercial antiseptic soap regarded as Standard Soap (Ss) was purchased from a commercial store.

For the soap production, 114 g of PKO was weighed into a 1 L clean glass jar and kept in a cool dry place to adjust to room temperature. Sixty milliliters of overnight refrigerated sterile filtered tap water was poured into a 250 mL conical flask and 28 g of Sodium hydroxide pellets (lye) was slowly added to it with constant stirring until the lye dissolved. This lye-water mix was kept in a cool dry place for 1 h. Following this was the careful addition and homogenization of the lye-water mix with the PKO. The final step involved the addition of 10 g L^{-1} *O. gratissimum* extract to Ts, continuous mixing for 15 min until it became thick. The thickened soap solution was left to solidify overnight.

Analysis of soap samples: The three soaps used in this study-Ts, Cs and Ss-were analyzed for their pH, moisture content, Total Fatty Matter (TFM) and foamability according to the methods described by Ryer (1969). Each test was carried out in triplicates.

Source of test cultures: The test microorganisms used in this study were *Candida albicans* (3 isolates), *C. parapsilosis* (2 isolates), *Staphylococcus aureus* (3 isolates), Methicillin-Resistant *S. aureus* (MRSA) (2 isolates) and *S. aureus* (ATCC 25923). *S. aureus* (ATCC 25923) was obtained from the stock culture deposits of the Department of Biosciences and Biotechnology, Babcock University, Nigeria while the other clinical isolates were from Babcock University Medical Centre and Olabisi Onabanjo University Teaching Hospital, Nigeria. The staphylococcal isolates were from patients with visible skin infections with and without pus. Confirmation of bacterial taxonomy was by series of conventional biochemical and physiological tests which include mannitol, glucose and lactose fermentation, gram staining, catalase and coagulase tests and NaCl tolerance tests as described by MacFaddin (2000), Fobres *et al.* (2002) and Leboffe and Pierce (2002). Comparison of results for species authentication was further made using the Bergey's Manual of Systematic Bacteriology (Sneath *et al*., 1986). The MRSAs were subjected to methicillin and oxacillin antibiotic disk tests

for confirmation. The taxonomy of the *Candida* species were confirmed by the microscopic observations of a simple wet mount as well as germ tube test and capacity to produce chlamydospore on cornmeal agar fortified with Tween 80 polysorbate (Brown, 2005). Nutrient broth served as maintenance medium for all test cultures during this study.

Bioassay: The standard agar well diffusion method described by Perez *et al.* (1990) and broth micro-dilution assay technique as specified by the British Standard Specification No. 541 and National Committee for Clinical Laboratory Standards (NCCLS, 2000) were used for the antimicrobial potency determination of the soap samples and minimal inhibitory concentrations (MIC), respectively. For the initial susceptibility testing of the isolates to the soap samples, 10.0, 7.5, 5.0, 2.5 and 1.0 g of each sample soap was weighed and dissolved separately in test tubes containing 10 mL of sterile distilled water to give 100, 75, 50, 25 and 10% (w/v) concentrations of each soap solution. This gave a total of 15 soap concentrations based on the 3 soap samples. From each prepared concentration, 20 μL soap solution was aseptically transferred into a 5 mm bored well in a solidified Muller-Hilton Agar plate which has been inoculated with the test isolate via pour plate technique. Plates were kept for 30 min before incubation at 37°C for 24 h. The extent of susceptibility was recorded as clear zones of inhibition around the wells.

For the MIC determination, a 50% (w/v) concentrated soap solution was prepared as described above. Six test tubes containing sterilized 5 mL double strength nutrient broth were then serially diluted with 5 mL of the prepared 50% (w/v) soap solution. This design gave final soap concentrations that were half of initial concentrations ranging from 50 to 1.56% (w/v). One milliliter of standardized inoculum were then introduced aseptically into each diluted tube and incubated at 37°C for 24 h. Negative (N) and positive (P) control tubes were set in place and the N contained freshly prepared soap sample concentration while P contained the corresponding test isolate culture. MIC was recorded as the lowest dilution which inhibited the growth of test isolate as expressed by lack of visible turbidity of solution. All experiments were in triplicates.

Statistical analysis: The significant differences in mean values were determined by the Duncan Multiple Range Test (DMRT). The p-values of <0.05 were considered statistically different.

RESULTS

Analysis of soap samples: The soaps produced, Ts and Cs, were alkaline in nature (pH 9.8 and 10.2, respectively) as Ts had clear forest green coloration while Cs appeared creamy (Table 1). The commercial soap, Ss, was also alkaline (pH 9.9) and creamy in appearance. The saponification value of the PKO used in making Ts and Cs was 4.05. For the moisture content (%), Ts had a value of 19.7±0.02 as compared to Cs (28.0±0.00), both of which were higher than Ss. Ts had a foamability value (cm) of 13.4±0.03 as compared to the Cs (9.9±0.01). On an overall, Ts was highly comparable with Ss as compared to the Cs in terms of general acceptability, texture and fragrance (data not shown) and pH, total fatty matter and foamability.

Inhibitory activity of sample soaps against test isolates: The inhibitory activity of *O. gratissimum* fortified soap against all *Candida* sp. was significant over Cs ($p<0.05$) (Table 2). The sensitivity values (mm) for *C. albicans* and *C. parapsilosis* to Ts ranged between 9.5±0.02-23.1±0.01

Table 1: Analysis of soap

Parameters	Control soap	Test soap	Standard soap
pH	10.2±0.2	9.8±0.2	9.9±0.2
Moisture content (%)	28.0±0.00	19.7±0.02	14.1±0.00
Total fatty matter (%)	1.8±0.03	10.0±0.01	4.0±0.01
Foamability (cm)	9.9±0.01	13.4±0.03	16.3±0.03

Results are means of triplicate analyses with standard deviations

Table 2: Zones of clearance (mm) of the three soaps on some *Candida* species

	Concentrations (g mL^{-1}) of soaps				
Isolates	0.1	0.25	0.5	0.75	1.0
***C. albicans* (OS_{10})**					
Ts	13.0±0.00^a	14.5±0.02^a	20.0±0.00^a	23.1±0.01^a	21.5±0.02^a
Cs	11.0±0.00^b	12.0±0.00^b	15.1±0.01^b	16.4±0.02^b	17.0±0.00^b
Ss	13.1±0.02^a	15.1±0.01^a	20.7±0.00^a	22.7±0.03^a	21.1±0.00^a
***C. albicans* (OS_{22})**					
Ts	10.2±0.01^a	12.0±0.00^a	16.5±0.02^a	19.1±0.01^a	18.5±0.00^a
Cs	6.2±0.01^b	8.0±0.00^b	10.1±0.01^b	12.4±0.02^b	13.0±0.00^b
Ss	10.2±0.00^a	12.7±0.02^a	16.0±0.00^a	19.7±0.03^a	19.5±0.02^a
***C. albicans* (OS_9)**					
Ts	10.5±0.02^a	11.5±0.02^a	14.0±0.00^a	18.4±0.00^a	17.5±0.02^b
Cs	7.1±0.00^b	10.5±0.00^b	11.5±0.02^b	14.0±0.00^b	14.7±0.00^a
Ss	10.3±0.01^a	11.5±0.00^a	14.1±0.00^a	19.1±0.01^a	18.0±0.00^b
***C. parapsilosis*(OS_4)**					
Ts	8.7±0.00^a	13.0±0.00^a	16.5±0.02^a	20.0±0.00^a	19.0±0.00^a
Cs	6.5±0.01^b	8.0±0.00^b	11.0±0.00^b	15.1±0.00^b	15.8±0.00^b
Ss	9.1±0.00^a	13.1±0.01^a	16.3±0.01^a	19.8±0.03^a	18.7±0.03^a
***C. parapsilosis*(OS_7)**					
Ts	9.0±0.00^a	11.1±0.01^a	15.5±0.02^a	21.5±0.02^a	17.5±0.02^a
Cs	6.5±0.01^b	9.7±0.03^b	10.5±0.00^b	14.2±0.01^b	15.5±0.00^b
Ss	8.9±0.03^a	11.0±0.00^a	15.7±0.03^a	20.9±0.03^a	18.1±0.01^a

Mean values with standard deviation labeled with similar alphabets showed no significant differences ($p>0.05$) along the same column. Ts: Test soap; Cs: Control soap; Ss: Standard soap

Table 3: Zones of clearance (mm) of the three soaps on some *staphylococci*

	Concentrations (g mL^{-1}) of soaps				
Isolates	0.1	0.25	0.5	0.75	1.0
***S. aureus* (OS_{12})**					
Ts	8.6±0.00^a	11.2±0.00^a	18.1±0.01^a	20.5±0.00^a	26.5±0.02^a
Cs	6.8±0.00^b	8.5±0.00^b	11.2±0.00^c	15.1±0.01^b	17.0±0.00^c
Ss	9.1±0.02^a	11.5±0.02^a	14.0±0.00^b	19.7±0.00^a	19.9±0.00^b
***S. aureus* (OS_{15})**					
Ts	8.1±0.03^a	10.1±0.00^a	13.0±0.00^b	19.0±0.00^a	24.7±0.03^a
Cs	6.7±0.01^b	7.0±0.00^b	9.0±0.02^c	13.1±0.01^b	15.0±0.00^b
Ss	8.9±0.02^a	10.1±0.01^a	14.9±0.03^a	18.7±0.03^a	24.7±0.00^a
***S. aureus* (OS_5)**					
Ts	7.7±0.02^a	9.5±0.00^a	12.3±0.01^a	19.5±0.00^a	23.0±0.00^a
Cs	7.2±0.00^a	7.8±0.00^b	10.0±0.00^b	15.1±0.01^b	19.3±0.01^b
Ss	8.0±0.00^a	8.9±0.00^a	13.3±0.01^a	19.9±0.03^a	23.5±0.00^a
MRSA (BM_2)					
Ts	6.5±0.00^a	9.8±0.02^a	12.5±0.00^a	18.0±0.03^a	21.8±0.00^a
Cs	6.2±0.01^a	7.4±0.00^b	10.5±0.02^b	13.1±0.01^b	18.0±0.00^c
Ss	7.0±0.02^a	9.5±0.02^a	13.3±0.01^a	17.8±0.00^a	19.8±0.03^b
MRSA (BM_9)					
Ts	6.5±0.00^a	9.0±0.00^a	13.0±0.00^a	17.7±0.03^a	22.0±0.00^a
Cs	6.4±0.01^a	7.2±0.00^b	9.5±0.02^b	13.1±0.01^b	15.0±0.00^b
Ss	6.8±0.02^a	9.1±0.01^a	13.6±0.02^a	17.1±0.00^a	21.5±0.00^a
***S. aureus* (ATCC25923)**					
Ts	9.0±0.00^a	13.1±0.01^a	18.0±0.00^a	19.0±0.00^a	22.5±0.00^a
Cs	7.2±0.01^b	11.0±0.00^b	14.5±0.02^b	16.3±0.01^b	18.0±0.00^b
Ss	9.5±0.02^a	12.5±0.02^a	18.5±0.00^a	20.0±0.00^a	22.7±0.02^a

Mean values with standard deviation labeled with similar alphabets showed no significant differences ($p>0.05$) along the same column. Ts: Test soap; Cs: Control soap; Ss: Standard soap

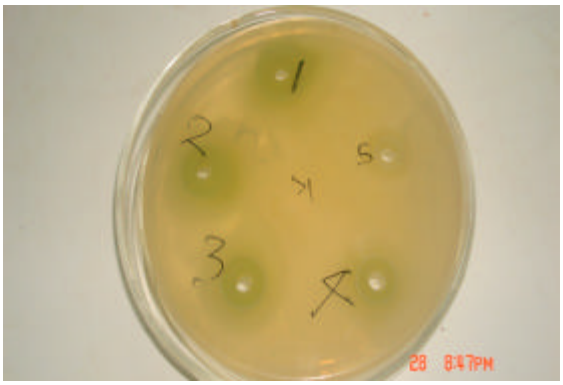

Fig. 1: Inhibition zones of concentrations of the soap fortified with *O. gratissimum* extracts against *C. parapsilosis* (OS_7). 1 = 1.0 g mL^{-1}, 2 = 0.75 g mL^{-1}, 3 = 0.5 g mL^{-1}, 4 = 0.25 g mL^{-1}, 5 = 0.1 g mL^{-1}

and 8.7±0.00-21.5±0.02, respectively, as compared to their low sensitivity to Cs, (6.2±0.01-17.0±0.00) and (6.5±0.01-15.8±0.00), respectively. Also, the activity of the Ts and Ss increased progressively with an increase in the concentration of soap tested up to the 0.75 g mL^{-1} soap concentration after which a reduction in activity was recorded at 1.0 g mL^{-1} concentration.

Ts exhibited a significant inhibitory activity over Cs ($p<0.05$) against the staphylococci (Table 3). Their

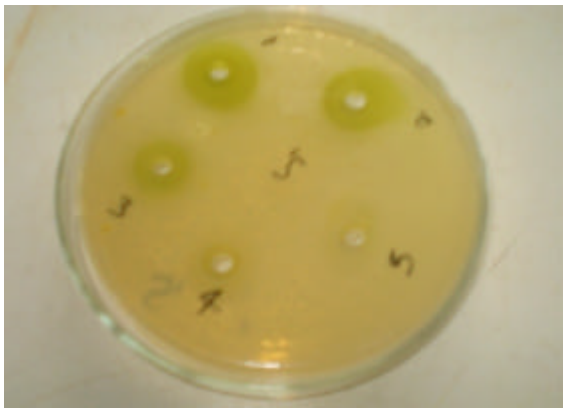

Fig. 2: Inhibition zones of concentrations of the soap fortified with *O. gratissimum* extracts against *S. aureus* (OS_{15}). 1 = 1.0 g mL^{-1}, 2 = 0.75 g mL^{-1}, 3 = 0.5 g mL^{-1}, 4 = 0.25 g mL^{-1}, 5 = 0.1 g mL^{-1}

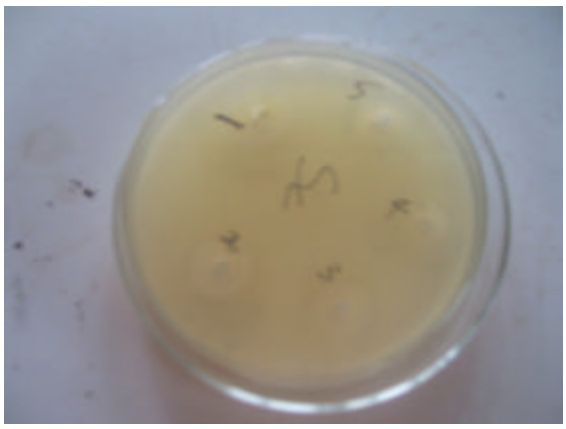

Fig. 3: Inhibition zones of concentrations of the control soap against *S. aureus* (OS_5). 1 = 1.0 g mL^{-1}, 2 = 0.75 g mL^{-1}, 3 = 0.5 g mL^{-1}, 4 = 0.25 g mL^{-1}, 5 = 0.1 g mL^{-1}

sensitivity values (mm) to Ts ranged between 6.5±0.01-26.5±0.02 as compared to their low sensitivity to Cs, (6.2±0.01-18.0±0.00). Figure 1 and 2 reveal that there was visible significant concentration-dependent increase in zones of inhibition of Ts against *C. parapsilosis* (OS_7) and *S. aureus* (OS_{15}), respectively over Cs tested against *S. aureus* (OS_5) (Fig. 3). The MIC was 0.0312 g mL^{-1} (3.125%) and 0.0156 g mL^{-1} (1.56%) for the Ss and Cs and Ts, respectively for all isolates (*Candida* and *Staphylococci*).

DISCUSSION

The pH range of the three tested soap samples (9.8-10.2 ±0.2) are in agreement with recommendations of the National Soap Directory (2009) which clearly states that cured soap should have a pH between 8 and 10. This alkaline pH range permits the soap a better reaction with the skin to prevent burning of the skin. The moisture content value for Ts indicated a moderate rate of solubility in terms of economic value and consumer satisfaction while the foamability value indicated that Ts would lather better than Cs. These physical characteristics of our own soap fortified with *O. gratissimum* extracts placed it at a competitive edge with the Ss, a quality needed for a marketable product.

Delost (1997) has on record the association of *C. parapsilosis* with subcutaneous infections including otitis externa and nail infections while Cheng *et al.* (2009) elucidated more on the association of *S. aureus* with boils, hence the relevance of the production of a potent soap capable of inhibiting these microbes. The remarkable significant differences in susceptibility values and MIC recorded for the soap fortified with *O. gratissimum* extract over Cs against both *Candida* and staphylococci have been attributed to the herbal extract in Ts.

The susceptibilities of both groups of test microbes are in line with the report of Nwosu and Okafor (2009), Nakamura *et al.* (1999) and Lopez *et al.* (2005) who suggested that *O. gratissimum* essential oil has a high antistaphylococcal and antifungal activity. Akinyemi *et al.* (2005) and Nwinyi *et al.* (2009) also recorded a high level of inhibition of *S. aureus* (MRSA and non MRSA, respectively) by the ethanolic extract of *O. gratissimum* and a progressive increase in inhibitory capacity of the extract as the extract concentration increased; this we also recorded for the tested staphylococci regardless of the fact that our extract is a methanolic one. Therefore it may be logical if we say that ethanol and methanol as extraction solvents for *O. gratissimum* active ingredients release the certain bioactives (flavonoids, alkalids, tannins and saponins) that interfere work against the bacteria (Nwinyi *et al.*, 2009). The progressive increase of inhibitory capacity of Ts against the *Candida* isolates up to 0.75 g mL^{-1} soap concentration and subsequent reduction in activity at 1.0 g mL^{-1} concentration may not be appropriately explained.

Based on previous reports of *O. gratissimum* activity which has been mostly on either the crude extract or essential oil, we reason with Adebolu and Salau (2005) who reported that the steamed extract inhibited bacteria almost at the same rate as the extracted oil. This may then imply that the essential oil of this herb, though not extracted for this work, also has a great role to play in the inhibitory activity exhibited by the soap fortified with the herbal extract since we did not defat the leaves prior to extraction.

Soaps and detergents are known to disrupt microbial cell membranes and possibly denature cellular proteins while flavonoids in *O. gratissimum* extracts confer antioxidant property to the extract thereby scavenging for superoxide anions in the cells that come in contact with the extract (Robak and Gryglewski, 1988; Ramanthan *et al.*, 1989). Although we did not study the mechanism of action by which the extract inhibited the microbes, we agree to the logical findings stated above and hereby suggest that there could have been an interference with cell wall and membrane synthesis which further led to collapse of the cell wall and consequent disruption of overall cellular mechanism.

Considering the recorded success in making this simple, affordable and consumer-friendly herbal soap, we may further look into the commercial preparation of the dermal oil from this herb. This will serve as a complement to the soap for individuals plagued with infections such as boils, carbuncles, athlete's foot and other types of ringworm. This is one of the few reports on the application of *O. gratissimum* extract in soap making, a need for our society today since we are going from the laboratory to the greens.

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