Antimicrobial activity, safety and acceptability of formulated ginger-fortified hand sanitizer gel

Abstracts

Hand hygiene remains the most effective means of breaking transmission of most infectious diseases in and out of hospital settings. Hand hygiene could be achieved by different means. However, in recent time, the use of hand sanitizer which could either be a supplement or alternative to hand washing with soap and water has been promoted. The effectiveness of a formulated herbal hand sanitizer was investigated in this study. The herbal (ginger) hand sanitizer was formulated and screened on both bacterial and fungal isolates using different microbiological methods in this study. The skin and eye irritation potential of the sanitizer were conducted on experimental animals. Structured questionnaire was used to test the effects of the product on the skin of consenting human volunteers. The hydro-alcoholic extract of ginger showed a concentration-dependent activity on the test organisms. *Escherichia coli* ATCC 8739 was the most susceptible isolates followed by *Staphylococcus aureus* ATCC 6538. *Serratia marcescens* ATCC 9986 was more resistant to the extract at lower concentrations (0.78 and 1.56 mg/ml). *Aspergillus fumigatus* was the most susceptible out of the three fungi tested followed by *Penicillum chrysogenum*.Herbal hand sanitizer (with weighted effectiveness of 3.82) performed better than commercial hand sanitizer (with weighed effectiveness of 3.78). In the glass beads test, both herbal and commercial sanitizers inhibited the growth of *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739 and *Serratia marcescens* ATCC 9986. Though it caused eye irritation, the herbal sanitizer produced neither skin irritation nor dryness. The formulated hand sanitizer is economical, simple and safe throughout long periods of continued use.

Key words: Sanitizer, ginger, fungi, hand hygiene, pathogens, irritation

INTRODUCTION

The recent epidemic of communicable diseases further highlights the need of maintaining hygiene (Vyaset, 2011). Chassin *et al.* (2015) attributed the low level of hygiene to some factors which include lack of awareness, knowledge of risk and unavailability of hand hygiene facilities among others. Hand sanitizers (HS) are antiseptic products usually applied on hands to reduce the number of viable pathogenic microorganisms without causing any damage to the skin (CDC 2009). Hand sanitizers could either be a supplement or alternative to hand washing with soap and water (Kampf *et al.,* 2002; Kampf and Kramer, 2004). Herbal hand sanitizers made from herbs are natural plant-based alternatives to chemical sanitizers. The active components of medicinal plants are obtained by suitable solvents, such solvents are evaporated away and the residue could be further diluted to prescribed concentrations.

Many plants contain bioactive phyto-compounds that inhibit the proliferation of disease causing microorganisms (Omoya and Akharaiyi, 2012; Akroum *et al.,* 2009), tumor (Khalil *et al.,* 2005), inflammation and necrosis (Omoya and Akharaiyi, 2012; Lin and Huang, 2000) among others. Herbs and spices are very important and useful as therapeutic agents against many pathological infections (Gull *et al.,* 2012). The spices have a unique aroma and flavor which are derived from compounds known as phytochemicals or secondary metabolites (Avato *et al.,* 2000; Melvin *et al.,* 2009).

Ginger (*Zingiber officinale*) is a medicinal plant that has been widely used all over the world, since antiquity, for a wide array of unrelated ailments including arthritis, cramps,

rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases (NPGS/GRIN. 2011; Ali *et al.,*2008). Ginger has direct anti-microbial activity and thus can be used in the treatment of both bacterial and fungal infections (Akintobi *et al.,* 2013; Tan and Vanitha, 2004). Ginger belongs to *Zingiberaceae* family; the *Zingiberaceous* plants have strong aromatic and medicinal properties and are characterized by their tuberous or non-tuberous rhizomes (Chen *et al.,* 2008; Sharma *et al.,* 2010).

Hand sanitizers are effective in reducing gastrointestinal illnesses in households (Sandora, *et al.,* 2004), curbing absentee rates in elementary schools and offices(Hammond, *et al.,*2000),reducing illnesses in university dormitories (White, *et al.,*2003) and reducing infections in healthcare settings. Alcohol-based hand sanitizers are recommended as a component of hand hygiene. The use of herbs in breaking the transmission of pathogenic microorganism has not been implored as that of curative and preventive use of herbs. If encouraged it will minimizing the use of chemicals used in hand hygiene that could cause adverse effects.

A good hand sanitizer is that which is economical, simple to use and extremely efficient in operation. It must have a high substantivity effect and safe for long periods of continued use. Also, it should be able to possess antimicrobial activity against a wide range of fungal and bacterial species. This study therefore aimed at formulating an effective hand sanitizer with good quality from a common medicinal plant with no skin and ocular irritation and which also enjoy a high acceptability among prospective users.

MATERIALS AND METHODS

Collection of Plant materials

The fresh form of *Zingiber oficinale* (ginger rhizome) was purchased at Bode Market in Ibadan, Oyo State, Nigeria. The plant was identified in the Herbarium of the Department of Plant Science, Ekiti State University.

Collection of Test Organisms

The test organisms used in this study include four fungi (*Aspergillus fumigatus, Absidia corymbifera* and *Penicillium chrysogenum*), three Gram-positive (*Staphylococcus aureus, Bacillus subtilis* and *Enterococcus*) and two Gram-negative bacteria (*Escherichia coli* and *Serratia marcescens*) isolates. All isolates were collected from the Department of Microbiology, Ekiti State University, Ado-Ekiti.

Preparation of Plant Extract

The method of Olayemi and Opaleye (1999) was adopted for the extraction of the plant. 3kg of ginger was macerated using a blender to which 2500 ml of 95 % ethanol was added for pre-extraction. After five days, the mixture was filtered using Whatman No. 1, filter paper. The extract was evaporated to dryness under reduced pressure at 40°C using a rotary evaporator (Laborota 4000 efficient, Heldolph, Germany). The extract was diluted using 50% dimethyl sulphoxide (DMSO) to give 50 mg/ml stock solution (Taylor *et al.,* 1995). This was then diluted to the required concentration for the bioassay.

Determination of the Antimicrobial Activity of the Plant Extract

Minimum Inhibitory Concentration (MIC)

A different concentration of gingerextract was prepared in solution using 5.0% dimethyl sulphoxide and the extracts were by filtration through a 0.23 µm membrane filter. Filter paper disc (5.75 in diameter) was sterilized in hot air oven for 2 hours at 160 ^{o}C, and then impregnated by different concentrations of extracts. Each of the standardized test organisms was seeded on the sterile Nutrient Agar (Oxoid) aseptically and the paper disc was carefully placed on it. The plate was incubated at 37 ^{o}C for 24 h and the zone of inhibition was observed and measured to the nearest millimeter.

Determination of fungicidal activity

The technique of Nene and Thpilyal (2002) was used to screen for antifungal activity of the plant extract. Sterilized extract was mixed with sterilized Potato Dextrose Agar (PDA) (Oxoid) to achieve a concentration range of 3.125 mg/ml to 25.000 mg/ml. The inoculation was done at the center of each plate with a 5 mm mycelium block from the advancing edge of a five day old culture of the test fungi on PDA. The blocks were placed at the centre of each Petri plate in an inverted position to get greater contact of the mycelium with the culture medium. The inoculated plate was incubated at 25 ^{o}C. Potato Dextrose Agar without extract was also maintained at the same condition to serve as control. After 72 hours of incubation, the diameter of fungi was measured in mm. The percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = [(\text{Control} - \text{Test})/\text{Control}]\text{x}100$$

Formulation of the Herbal Hand Sanitizer

The herbal hand sanitizer was formulated hygienically using: 500 ml of absolute ethanol, 1 g of ginger extract, 200 ml of sterile water, 3g of thickening agent (Zhejiang Xinyong Biochemical Co., Ltd. China.), 1ml of perfume, 10 ml of humectants and 5 ml of glycerin. The formulation was made to guarantee a minimum of 60% alcohol content to improve its effectiveness and serve as in-solution preservative.

Determination of antimicrobial property of the formulated hand sanitizer

Finger imprint test of the effect of herbal hand sanitizer

The method of Wood (1982) was used to determine the after-use effect of the herbal sanitizer. Simple random sampling method was used to select the subjects that have given their verbal consent to participate in the study. The subjects include 60 students (30 males and 30 females) of Ekiti State University, Ado-Ekiti. All the subjects reported that they have not used similar products nor beon antibiotic treatment at least two weeks to the study. The subjectswere divided into six groups and the treatment given to each of the groups is stated in Table 1.

The subjects were instructed on how to apply the sanitizers [herbal hand sanitizer was prepared and a commercial sanitizer (Germ-X) was used as a positive control]on their fingers to ensure even distribution and how to gently press the fingers on the surface of the sterile Nutrient Agar opened slightly in a sterile environment. After inoculation the plates were incubated at 37 ^{o}C for 24 h. After these, the growth patterns of the finger imprints were observed and recorded as confluent growth, many growths, few growths and no growth and scored as 1, 2, 3 and 4 respectively.

The weighted effectiveness was calculated as:

$$WE = (\sum Growth\ pattern\ x\ score)/100$$

The sanitizer was classified as very poor (if WE is between 1and 1.4), poor (if *WE* is between 1.5 and 2.0), fair (if *WE* is between 2.5 and 3.0), average (if *WE* is between 3.1 and 3.4) and excellent (if *WE* is between 3.5 and 4.0).

Glass beads test

Five beads (3mm) were sterilized in the autoclave (121 ^{o}C for 15 mins) and carefully introduced into sterile petri dishes containing 100 µl of each of standardized inoculums of the culture of the test bacteria. The plates were covered and gently rocked to ensure all part of the beads was covered with the broth. The excess culture was drained and the beads were also allowed to dry. Each of the beads was dipped into the herbal sanitizer, removed and left for 60 s for the excess sanitizer to drain off. Later, the beads were carefully picked by sterile forceps, separately planted into the sterile Nutrient Agar and incubated at 37 ^{o}C for 24h. The plates were observed for the sign of growth around the challenged beads.

Determination of irritation tests

Animal Care and Management

Three nulliparous and non-pregnant female New Zealand White Rabbits weighing 2.0 to 3.5 kg and between twelve to twenty weeks old were purchased from the Animal House of the College of Medicine, Ekiti State University, Ado-Ekiti. The animals were acclimatized for a period of one week and observed daily to ensure there was no abnormality in their general conditions. They were given identification by numbering the inner surface of the ear with a permanent marker. The animals were housed in individual compartment of wooden rabbit cages under standard laboratory conditions of natural light/dark cycle at room temperature (24.6±4.2 ^{o}C) and humidity (68±28%); fed on standard rabbit feed (Ladokun Feeds, Ibadan, Nigeria) and given water *ad libitum*.Care was taken to ensure the food and drinking water was free from contaminants that could affect the study.

All animals were handled in accordance with the Guidelines for Animal Research as detailed in the NIH Guidelines for the Care and Use of Laboratory Animals (2011).The methodology was designed according to OECD Guidelines for the Testing of Chemicals No. 404 'Acute Dermal Irritation/Corrosion' (2002) and Method B4 Acute Toxicity (Skin Irritation) of Commission Directive 2004/73/EC.

Skin Irritation Test of the Formulated Herbal Hand Sanitizer

The fur on the dorsal trunk of the each of the test animals was sheared approximately 24 hours prior to the testing. The animals were grossly observed to ensure healthy intact epidermis. Three suitable sites were selected on the back of the rabbits. At each test site, 0.5 ml of the test material was introduced under a 2.0 cm X 2.0 cm 4ply cotton gauze patch and placed in position on the shorn skin. Each patch was secured with a strip of surgical adhesive tape. The trunk of the rabbits were wrapped in an elastic bandage for the duration of 1 and 4 hour exposure periods. Animals were returned to their cages after treatment. One patch was removed at 3 minutes, 1 hour and 4 hours after each application. After consideration of the skin reactions produced in the first animal, the remaining two animals were treated with 0.5 ml of the test material. A patch was

applied to the back of each rabbit and was allowed to remain in contact with the skin for a period of 4 hours. A depilatory produced by Softsheen-Carson® was used as a positive control. The test sites were then observed at 1 hour, 24, 48 and 72 hours following the removal of the patches for evidence of primary irritation and scored according to the Draize scale (Draize *et al.,* 1944). Cage-side observations for general condition, appearance and demeanor were made daily. The body weights of the animals were measured daily.

Ocular irritation test of the formulated herbal hand sanitizer

A little quantity(0.1 ml) of the hand sanitizer was carefully instilled into the cupped conjunctiva sac of the right eye of each rabbit following which the eyelids were gently held together for one second and then massaged for 30 seconds. The left eye served as the negative control. The readings were performed at 1hour, 24, 48, 72 hours and seven days after the application, and the corneal, iris and conjunctiva alterations were graded according to the Draize scale (Draize *et al*. 1944). The scores were processed differently to decide if the formulated herbal sanitizer was irritant or not and/or to grade the severity of the irritation.

Administration of Questionnaire

A well-structured questionnaire was given alongside a bottle of the formulated hand sanitizer to a total number of 20 randomlyselected subjects recruited in this study. The aim and the objectives of the study were disclosed to them and those that consented to participate were recruited. Subjects that have participated in any part of this study earlier, on antibiotics, have wound in the fingers and on similar products were excluded.The subjects include 12 females and 8 males with age range of 20-39 years old (n=18) and 40-59 years old (n=2) drawn from the university community. The questionnaire sought to test the knowledge of respondents on the use of hand sanitizer (in 6 questions), the effect of the herbal hand sanitizer after application for 15 days (17 questions) and the product satisfaction as it affects hand hygiene practices (4 questions). The questionnaire also assessed the demographic characteristics of the subjects.

Statistical Analyses

Statistical analysis was done using SPSS (version 17) to determine frequency distribution, analysis of variance (ANOVA) and Dunn's Multiple Comparisons Test. The level of significance was set at 0.05.

Ethical Clearance

The protocol for this work and the ethical clearance was also sought from the Research Governing body of the Faculty of Science, Ekiti State University.

RESULTS

The activity of the extract on the test organisms was concentration dependent. The extract has the highest activity against *E. coli* ATCC 8739 followed by *Staph. aureus* ATCC 6538. Compared to *Ent. faecalis* ATCC 29212 and *B. subtilis* KZN, *S. marcescens* ATCC 9986 was more resistant to

the extract at low concentrations (0.78 and 1.56 mg/ml) (Table 2). The susceptibility of *Ent. faecalis* ATCC 29212 to the extract differs significantly at $\rho<0.01$ from the susceptibility of *E. coli* ATCC 8739.

As shown in Table 3, *A. fumigatus* was the most susceptible of the three fungi, followed by *P. chrysogenum.* Also, it is clear that the effect of the extract was concentration dependent. At $P<0.05$, the percentage inhibition of all the three fungi tested was significantly different from one another. The effectiveness of the extract on *A. fumigatus* was most pronounced at the 96th hour of exposure. At the 48th and 72nd hours of exposure, *A. corymbifera* and *P. chrysogenum* inhibition were at the peak (Table 3).The commercial hand sanitizer (germ-X) when used after washing with bland soap had a better sanitizing effect than the herbal hand sanitizer. On the other hand, the herbal hand sanitizer performed better than the commercial hand sanitizer on unwashed hand. It recorded a weighted effectiveness of 3.82 while that of commercial hand sanitizer with 3.78 (as shown in Table 4). Considering the glass beads method, both herbal and commercial sanitizers inhibited the growth of *Staph. aureus* ATCC 6538, *E. coli* ATCC 8739 and *S. marcescens* ATCC 9986. The herbal sanitizer performed better on *B. subtilis* KZN than germ-X as reported in Table 7.

Table 6 shows the absence of erythema, eschar and edema formation on the rabbit skin on exposure to the herbal hand sanitizer at time interval of 3 minutes, 4 hours, 24 hours, 48 hours and 72 hours respectively. As seen in Table 7, there was no reaction in the cornea and iris of the rabbit's eyes on exposure to the herbal hand sanitizer at time interval of 3 minutes, 1 hour, 24 hours, 48 hours and 72 hours respectively. Plate 1 shows the reactions of the eyes and skin of the experimental animals on exposure to the herbal sanitizer. Conversely, there was severe eye reaction on the conjunctivas of the selected animals at the same time intervals. Six (30%) of the subjects reported that they used the herbal hand sanitizer for 1-2 times daily while fourteen (70%) reported that they used herbal hand sanitizer for 3-5 times daily as shown in Table 8.

DISCUSSION

Human hands harbour microorganisms ranging from normal flora to pathogenic species (Cogen *et al.,* 2008). Human skin provides optimum growth conditions for most disease causing organisms and also the opportunistic pathogens. These bacteria evidently could develop resistance to the cleaning agents, thus contributing to their persistence in an ecosystem (CDC, 2008). The use of hand sanitizer is one of the means of reducing the microbial load present on the hands (Mayo Clinic, 2014).

Ginger has direct anti-microbial activity and thus can be used in the treatment of bacterial infections (Tan and Vanitha, 2004). Several reports have been published that describe the antibacterial and antifungal properties of different herbs and spices (Gull *et al.,* 2012). However, there is little information on the exact mechanism of their antimicrobial action (Yin *et al.,*2002; Gur *et al.,*2006; Pattaratanawadee *et al.,* 2006; Yusha'u *et al.,* 2008; Oskay *et al.,* 2009; Belguith *et al.,* 2010; Poeloengan *et al.,* 2011; Gull *et al.,* 2012). The antimicrobial activity of medicinal

plants is due to specific phytochemicals or essential oils present in them (Avato *et al.,* 2000; Gull *et al.,* 2012).

This study revealed the microbial effect of ginger on the different bacterial and fungal tested at different concentrations ranging from 10mg/ml to 50mg/ml. The extract had the highest zone of inhibition on *E. coli* followed by *S. aureus* while *B. subtilis* showed the least susceptibility. These findings compare well with the findings of Omoya and Akharaiyi (2012) and Nguyen *et al.* (2014), who reported maximum antimicrobial activity of ginger extract against *E. coli,* and slightly low inhibitory effect on *B. subtilis.*

Ginger extracts have been reported to exhibit antifungal activity against *A. fumigatus, Fusarium* sp and *Alternaria* sp (Zahra *et al.* 2009; Yahaya *et al.*, 2012). Ficker *et al.* (2003) also isolated and identified the antifungal phytocompound from ginger. The factors responsible for the high susceptibility of the test organisms to ginger extract are not exactly known but may be attributed to the secondary metabolites and bioactive compounds in higher plants (Stewart *et al.,* 1991).

The formulated herbal hand sanitizer completely inhibited the growth of microorganisms on agar medium. Also, the herbal hand sanitizer exhibited a high antimicrobial efficacy in inhibiting the growth of all the test organisms when used on glass bead. This results agrees well with the study of Onyeagba *et al.* (2004), Pankaj *et al.* (2012) and Kamrul *et al.*(2014) which showed the good antimicrobial activity of ginger extract against food borne pathogens. The goal of hand hygiene is a sufficient reduction of microbial load on the skin and consequently break the disease transmission route (Omogbai *et al.,* 2011). It is easier to prevent the hands from contamination than to decontaminate already soiled hands. The critical density of microorganisms on the hands needed for the spread of pathogens depends on the type and duration of contact, the type of microorganism, the individual's resident flora and their colonization resistance (Chassin *et al.,* 2015).

The safety of the formulated hand sanitizer is of great importance. A good hand sanitizer must be safe to use. Using the Draize method of classification (1944), the herbal hand sanitizer was classified as non-irritant to skin but severely irritant to the eyes. These properties of the formulated herbal hand sanitizer conform well to the standard for healthcare antiseptic products recommended in professional and national guidelines in 2002 by Center for Diseases Control and Prevention (CDC, 2002).

Compliance to hand hygiene has been reported to break the cycle of transmission of pathogens both in the hospital and at home (Pessoa-Silva *et al.,* 2007; Burnett, 2009; Nicol *et al.,* 2009). Application of sanitizer could therefore be very useful and accepted to play a significant role in reduction of bio-burden on the hand and as well as prevents irritation due to constant hand washing (Kampf *et al.,* 2005).

The overall assessment of the questionnaire in determination of the efficacy of the formulated herbal hand sanitizer shows a positive result as over 70% of the respondent's confirm this in respect to each of the questions asked. There was no adverse reaction in the formulated herbal hand sanitizer and probably ginger may have contributed to the lack of adverse reactions

(lack of skin drying or irritation) as hand washing removes lipids from the hand; which is responsible for skin drying or irritation (Kampf *et al.,* 2005; Akintobi *et al.,*2013).

CONCLUSION

From this study, the formulated herbal hand sanitizer shown to be a promising cosmetic hygiene products as it is characteristically reduce microbial load and mayserve as an alternative to hand washing with soap and water. Herbal hand sanitizer has no adverse skin reaction on individual when used (at least for two weeks of this study). Its use could also aid compliance to hand hygiene among household at home or health workers in the hospitals since they do not easily cause skin irritation and or dryness.

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Table 1: The treatment and distribution of human volunteer subjects

Treatment	**Sex**	
	Male	**Female**
Washed hand (using bland soap) with herbal hand sanitizer	5	5
Unwashed hand with herbal hand sanitizer	5	5
Washed hand (using bland soap) with commercial hand sanitizer	5	5
Unwashed hand with commercial hand sanitizer	5	5

Washed hand (using bland soap)	5	5
Unwashed hand	5	5

Table 2: The antibacterial activity of the hydroalcoholic extract of ginger (zone of inhibition in mm)

Test Organisms	**Concentrations (mg/ml)**				
	0.78	**1.56**	**3.125**	**6.25**	**12.50**
Ent. faecalis ATCC 29212	4.32±0.72[a]	4.31±0.02[a]	5.67±0.26[a]	5.70±1.73[a]	5.96±0.23[a]
B. subtilis KZN	4.32±0.31[b]	5.21±1.00[b]	6.06±0.09[b]	6.10±1.94[b]	6.43±1.94[b]
Staph. aureus ATCC 6538	6.13±1.49	8.44±0.23	8.27±1.62	8.64±2.56	10.15±2.91
E. coli ATCC 873	6.52±0.63[ab]	8.86±1.02[ab]	10.28±2.93[ab]	10.69±1.94[ab]	13.23±2.83[ab]
S. marcescens ATCC 9986	4.32±0.88	4.31±0.37	9.39±1.05	8.02±1.63	9.26±0.25

[ab]=Data showing significant difference at $p<0.01$

Table 3: Percentage inhibition of test fungi at different concentrations (mg/ml) of ginger

Time (hours)	**Test Fungi**											
	Aspergillus fumigatus				***Absidia corymbifera***				***Penicillium chrysogenum***			
	25.00	**12.50**	**6.26**	**3.125**	**25.00**	**12.50**	**6.26**	**3.125**	**25.00**	**12.50**	**6.26**	**3.125**
48	50.00	21.05	12.50	8.33	54.24	22.55	33.33	16.67	41.67	40.75	33.33	15.79
72	63.26	32.63	16.67	8.14	51.00	30.83	20.39	14.27	51.01	47.58	38.76	11.58
96	68.42	34.48	29.41	10.53	44.92	29.41	7.79	12.38	36.84	36.84	21.05	6.90
120	57.89	20.97	27.83	-1.05	20.97	34.78	7.33	2.64	34.21	22.63	20.00	1.21
144	53.33	30.51	18.28	-10.34	37.92	21.41	2.91	-0.03	48.28	18.72	17.24	-1.69

Table 4: Effectiveness of hand sanitizer using finger imprint test

Treatment	**Growth Pattern**				**Score**	
	CG	**MG**	**FG**	**NG**	**WE**	**Remarks**
Washed hand (using bland soap) with herbal hand sanitizer	1	12	13	74	3.60	Excellent
Unwashed hand with herbal hand sanitizer	0	1	16	83	3.82	Excellent
Washed hand (using bland soap) with commercial hand sanitizer	1	8	12	79	3.69	Excellent
Unwashed hand with commercial hand sanitizer	0	7	8	85	3.78	Excellent
Washed hand (using bland soap)	24	29	31	16	2.39	Fair
Unwashed hand	30	52	17	1	1.89	Poor

Key: **CG** = Confluent growth, **MG** = Many growth, **FG =** Few growth, **NG =** No growth, WE=Weighted effectiveness

Table 5: Glass beads test on efficacies of the formulated herbal hand sanitizer on test organisms

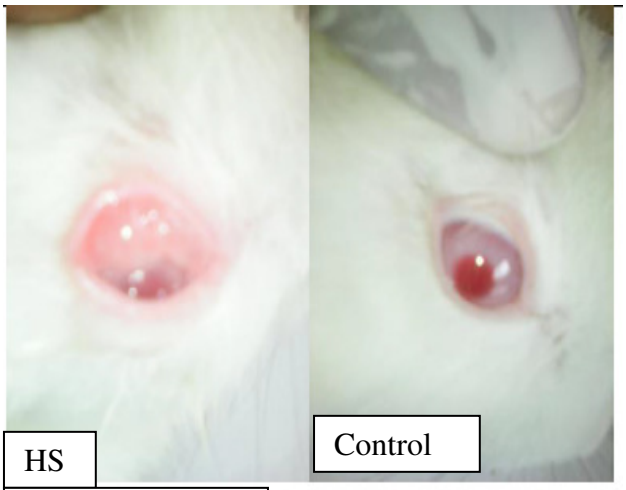
Organisms	**Test**				**Control**	
	Herbal hand sanitizer		**Commercial Hand sanitizer (germ-X®)**		**Sterile Distilled water**	
	Growth	**No growth**	**Growth**	**No growth**	**Growth**	**No growth**
Ent. faecalis ATCC 29212	1	4	1	4	5	0
B. subtilis KZN	1	4	2	3	5	0
Staph. aureus ATCC 6538	0	5	0	5	5	0
E. coli ATCC 8739	0	5	0	5	5	0
S. marcescens ATCC 9986	0	5	0	5	5	0

Table 6: Assessment of skin irritation test score of formulated herbal hand sanitizer

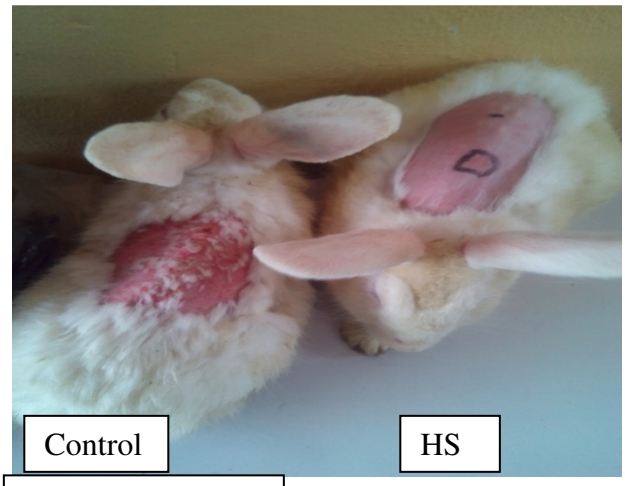
Skin reaction	Observation time	Individual Score- Rabbit Number (Test Group)				Individual Score- Rabbit Number (Control Group)			
		A	B	C	Total	A	B	C	Total
Erythema and eschar formation	3 minutes	0	0	0	0	0	0	0	0
	4 hours	0	0	0	0	0	0	0	0
	24 hours	0	0	0	(0)	0	0	0	(0)
	48 hours	0	0	0	0	0	0	0	0
	72 hours	0	0	0	(0)	0	0	0	(0)
Edema formation	3 minutes	0	0	0	0	0	0	0	0
	4 hours	0	0	0	0	0	0	0	0
	24 hours	0	0	0	(0)	0	0	0	(0)
	48 hours	0	0	0	0	0	0	0	0
	72 hours	0	0	0	(0)	0	0	0	(0)

Table 7: Assessment of ocular irritation test score of formulated hand sanitizer

Skin reaction	Observation time	Individual Score- Rabbit Number (Test Group)				Individual Score- Rabbit Number (Control Group)			
		A	B	C	Total	A	B	C	Total
Cornea	3 minutes	0	0	0	0	0	0	0	0
	4 hours	0	0	0	0	0	0	0	0
	24 hours	0	0	0	0	0	0	0	0
	48 hours	0	0	0	0	0	0	0	0
	72 hours	0	0	0	0 0	0	0	0	0 (0)
Iris	3 minutes	0	0	0	0	0	0	0	0
	4 hours	0	0	0	0	0	0	0	0
	24 hours	0	0	0	0	0	0	0	0
	48 hours	0	0	0	0	0	0	0	0
	72 hours	0	0	0	0 0	0	0	0	0 (0)
Conjunctiva	3 minutes	0	1	1	2	0	0	0	0
	1 hour	1	1	1	3	0	0	0	0
\	24 hours	1	1	1	3	0	0	0	0
	48 hours	1	1	1	3	0	0	0	0
	72 hours	1	0	1	2 (26)	0	0	0	0 (0)



Eye irritation test



Skin irritation test

Key: HS = Herbal sanitizer

Plate 1: The result of eye and skin irritation tests of the herbal hand sanitizer on the test rabbit.

Table 8: Assessment of subjects' response on the quality of the formulated hand sanitizers

Questions	Reponses	
	Yes	**No**
Do you receive formal training on how to use hand sanitizer?	20	0
Do you find the hand sanitizer as a quick and easy product to use?	20	0
Do you wear jewellery when applying hand sanitizer?	17	3
Is hand rubbing more rapid for hand cleansing than hand washing?	18	2
Do you use any antibiotics for the last four (4) weeks?	2	18
Do you trust this hand sanitizer to deliver effective hand hygiene?	20	0
Does it cause skin dryness more than repeated hand washing with soap and water?	4	16
Does it cause stinging skin irritation?	0	20
Does it cause more allergy and skin intolerance?	0	20
Does it cause stinging of the hands due to pre-existing skin irritation?	0	20
Does it cause cracking of the skin?	0	20
Does it cause piling of the skin?	0	20
Does it affect the colour of the nail?	0	20
Does it moisturize?	18	2
Does it cause excessive sweating of the palm?	0	20
Does it cause crinkling of the eye?	18	2
Do you perceive the effect after long period of usage?	19	1
Does it cause any involuntary action (e.g. sneezing) when used?	0	20
Does it cause watering of the eye	19	1
Are you pleased with the aroma or odour of the sanitizer?	18	2
How much (in Naira) are you willing to pay for 50 ml bottle which will last for 2-3 weeks upon regular use?		
Less than 500	6	
500-600	8	
700-800	6	
1000 and above	0	
Apart from the germ killing property of the hand sanitizer, what other properties would you like in the product?		
Fragrance	2	
Moisturizing agent	2	
Convenient and attractive packaging	16	
If you were to change one thing about the product to improve hand hygiene, what would it be?		
Fragrance	2	
Moisturizing agent	2	
Convenient and attractive packaging	16	
Please rate your satisfaction with the hand hygiene product		
Not at all satisfied	0	

Averagely satisfied	8
Extremely satisfied	12