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Antimicrobial activity of two medicated soaps on pathogenic bacteria isolated from human skin

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Abstract

Medicated soaps labeled as being "antimicrobial" can be purchased from any supermarket, but the antimicrobial spectrum of activity is rarely mentioned. Thus the *in vitro* antimicrobial activity of two popular brands medicated soaps, Dettol and Lifebuoy was conducted by agar disc diffusion method. Reference bacterial strains were isolated from human skin. The isolated strains (Sample-1 and Sample-2) were characterized by morphologically and biochemically. Both the samples were gram negative, non-motile with positive methyl red, catalase, starch hydrolysis, citrate BSA and EMB test. Their maximum growth was achieved at pH6 and 40°C temperature. Sample-1reported its optimal growth when fructose and yeast extract were used as a carbon and nitrogen source respectively. Whereas glucose and peptone were better carbon and nitrogen sources respectively for Sample-2. These two strains were treated with three different concentrations (50mg/ml, 75mg/ml and 100mg/ml) of Dettol and Lifebuoy soap. Both the soaps showed satisfactory results with all the three concentrations. But their activities were different from each other. Degree of their activities was also increased with the increased concentration. Dettol soap was much stronger than Lifebuoy soap in their antibacterial activity against the two strains having the highest zone of inhibition 10 mm, 15 mm respectively with the concentration of 100 mg/ml. Thus Dettol soap can be recommended for daily use.

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Introduction

Any substances having antimicrobial activity can either kill microbes or inhibit the growth of microbes, which is essential for the prevention of many human diseases and skin infections (Chaudhari, 2016). The largest organ of human body is skin, which accumulates sensory information from the environment and acts as a first line defense of the body (Solanki *et al.*,2011). Many organisms possesses mucosal epithelial layer that acts as protective barrier to the stringent external environment (Cole, 1997). Microbes can be found everywhere such as in water, soil, air, human body and in all life as well as inert things that can cause harmful diseases to human body. Many chemical compounds or group of chemical compounds are used by the people in daily life to remove the harmful microbes such as phenols, alcohols, acids, soaps, ammonium compounds etc. (Bhat *et al.*,2012). Soaps are disinfectants, which are used in cleaning dust, microorganisms, stains etc. General ingredients of soaps are fats and oils and soaps are manufactured through saponification process (Chaudhari, 2016).

Mainly two types of soaps are manufactured such as non-antimicrobial soap and antimicrobial soap or medicated soap (Osborne and Grube,1982).Medicated soaps are very important because they can clean 65-85% germs from human skin. Medicated soaps are incorporated with germicidal ingredients for enhancing the antimicrobial activity of theses soaps. Antimicrobial soaps can eliminate bacteria at a specific concentration and also show bacteriostatic activity. Many herbal soaps are also produced for treating the bacterial infection (Saikia*et al.,* 2006; Solanki *et al.,* 2011). Recently, soaps have been improved industrially into more useful forms with different trade names for the protection and treatment of different skin diseases (Aliyu, 2012).

Two types of bacterial populations are found on human skin; those are resident bacteria and transient bacteria. Resident bacterial species can replicate on the skin of healthy people but transient bacterial species rarely can survive on the healthy human skin (Johnson *et al.*,2002). Some pathogenic bacteria such as *Staphylococcus aureus* (Fluit, 2001), *Bacillus subtilis* and *Pseudomonas aeruginosa* (Higaki *et al.*,2000) can cause skin infection which can be cleaned by antiseptic soaps (Chaudhari, 2016).*Staphylococcus aureus* is a pathogenic bacterial species that is commonly found in the lesions of various skin diseases of humans ((Higaki *et al.*,2000). Medicated soaps are important factors in decreasing transient microbes from human skin and also some high-detergency soap provides emulsification abilities (Farzana *et al.*,2004). Handhygiene is very important for all specially for those people who are associated with health caring and food handling because contamination of pathogenic bacteria can cause many diseases. Antimicrobial ingredients containing medicated soaps can eliminate more microbes from skin than non–antimicrobial soaps (Riaz, 2009). Most of the peoples don't follow the correct technique and duration of hand washing (Lucet *et al.,* 2002).

Two types of bacterial insusceptibility towards the antiseptics can be found such as intrinsic insusceptibility and acquired insusceptibility.

Some gram negative and mycobacteria are naturally resistant towards disinfectants, which is known as intrinsic insusceptibility. Mutation, transposons and other factors can cause acquired insusceptibility of microbes (Farzana *et al.,* 2004).

The present study was conducted to characterize biochemical and morphological properties of pathogenic bacteria isolated from human skin and investigate the antimicrobial activities of two medicated soaps on these pathogenic bacteria.

Materials and methods

Sample collection

The medicated soap samples used for the study were purchased from standard cosmetics stores of Rajshahi, Bangladesh. The batch numbers, expiry dates and the presence or absences of the manufacturers seal were noted.

Bacterial sample collection

Sterile swab sticks were wetted with sterile peptone and collected sample from the sweat skin such as neck and arm of the student of Department of Genetic Engineering and Biotechnology, University of Rajshahi. Then samples with swab sticks were inoculated in saline water solution. All precautions were taken to minimize cross-contamination during sample collection.

Enrichment of skin bacteria

For enrichment of skin bacteria, 100 ml LB liquid media were prepared and autoclaved at 121°C for contamination free. Then 100µl of bacterial samples were inserted into LB liquid media on the laminar air flow bench and incubated for 16-18 hours at 37°C temperature with shaking 160 rpm to prepare bacterial mixed culture.

Isolation of pathogenic bacterial strains from mixed culture

MacConkey agar medium was used for isolation of gram-negative enteric bacteria which show pathogenic characteristics and causes disease (MacConkey, 1905). Serial dilution methods were used to dilute mixed bacterial samples up to 10 times and spread different diluted suspension on MacConkey agar medium.

Streaking of single colony in LB agar media and preparation of pure liquid culture

After mixed plate culture, colonies were selected according to their morphological characteristics. Single colony was taken into previously prepared agar plate with the help of a sterilized loop and streaked on the agar plate. Then plates were incubated at 37°C for 24 hours. After getting the pure single colonies, they were transferred into LB liquid media for further use.

Morphological and biochemical characterization of isolated bacteria

Morphological and biochemical tests were performed for specific identification of bacteria. Isolated bacteria were characterized by several morphological (gram staining and motility) and biochemical tests (catalase, methyl red test, MacConkey test, Mannitol test, Starch Hydrolysis test, Triple Sugar Iron test, Bismuth Sulfite Agar test, and Eosin Methylene Blue Agar test).

Effect of pH and temperature on bacterial growth

Bacterial growth was influenced by temperature and pH. The effect of pH on bacterial growth was determined by varying the pH (ranging from 4.0-8.0) of the broth in different flasks. For optimum temperature for the growth, the medium was inoculated and incubated at different temperature ranging 25-45°C. After 24 hours of incubation cell density was measured by spectrophotometer at 600 nm.

Effect of carbon and nitrogen sources on bacterial growth

The growth medium was supplemented with different carbon sources viz., sugar, glucose, fructose, dextrose and maltose (at the level of 1%, w/v) and nitrogen sources viz, yeast extract, peptone, urea, ammonium sulfate and sodium nitrate (1 %, w/v) were used for the measuring the best carbon and nitrogen sources for bacterial optimum growth.

Preparation of soap samples

A sterile blade was used to scrap each of the soaps and dissolved in sterile distilled water for making the stock solution with a concentration of 100mg/ml. These stock solutions were then stored in refrigerator for further use. 50 mg/mL, 75 mg/mL, and 100 mg/mL were used in all of our experiments.

Preparation of disc with soap samples

The discs (6 diameters) were prepared by punching the Whatmanfilter paper with the help of punching machine.

These sterilized paper discs were soaked with different concentrations (50 mg, 75 mg, 100 mg/ disc) of each soap sample and keep them at laminar airflow for drying. They were then packed into sterile bottles, corked and stored in the refrigerator for future use in susceptibility test.

Antimicrobial susceptibility testing

Disc diffusion method was used in the antibacterial activity test. Then LB agar plate was prepared and 100µl of bacterial culture was spread by spreader. Dettol and Lifebuoy soap discs with different concentrations were added into spreading plates with standard antibiotic disc (Gentamycin). After overnight incubation at 37°C, then zones were observed on the plate and were measured with the help of mm scale.

Statistical analysis

All experiments were carried out in triplicates and the results are presented as the mean of three independent observations. Graphs were prepared using GraphPad Prism Software version 8.0 (GraphPad Software, San Diego, CA, USA)

Results

Morphological and biochemical characterization of isolated bacterial strains

On the basis of colony size and shape, only two (Sample-1 and Sample-2) colonies were selected and streaked from the MacConkey agar plates (Fig.1).

Sample-1 was gram-negative, aerobic,non-motile, with positive methyl red, catalase, starch hydrolysis, citrate BSA and EMB test. On the other hand, Sample-2 was gram negative, non-motile, with negativemannitol and TSI test (Table 1).

Table 1. Biochemical characteristics of the isolated samples.

No.	Test name	Results	
		Sample-1	Sample-2
01	Gram test	Gram negative	Gram negative
02	Motility test	_	_
03	Methyl Red test	+	+
04	Catalase test	+	+
05	MacConkey test	+	+
06	Mannitol test	_	_
07	Starch Hydrolysis test	+	+
08	TSI test	Gas- , H_2S-	Gas- , H_2S-
09	Citrate test	+	+
10	Bismuth Sulfite Agar (BSA) test	+	+
11	Eosin Methylene Blue (EMB) agar test	+	+

Optimization of pH

Both the samplesshowed a wide range of pH tolerance (pH4.0-8.0) capacity but maximum growth was achieved at pH 6.0 (Fig.2). Above and below this pH, the growth was lower.

Effect of temperature

Bacterial growth was recorded at different temperatures ranging from 25 to 50°C revealed that the isolates showed maximum growth at 40°C (Fig.3).

In this experiment bacterial strains showed better growth from 35-45°C, but 40°C was found to be the most effective temperature for growth of both the samples. Above and below this temperature, the growth was lower (Fig. 3).

Table 2. Zone of inhibition (mm) on both the bacteria strains by Dettol soap.

SL. No.	Dose (mg/ml) 100 µl/disc	Zone of inhibition (mm)	Gentamycin zone of inhibition (mm)	Resistant pattern
Sample-1	50	10±0.16	20±0.15	Resistant
	75	11±0.12		Intermediate
	100	11±0.16		Intermediate
Sample-2	50	10±0.12	17±0.14	Resistant
	75	11±0.17		Intermediate
	100	15±0.18		Susceptible

Note: Resistant=<10 mm; Intermediate =10-15 mm; Susceptible=>15 mm.

Effect of carbon sources

Different carbon sources had both stimulating and inhibitory effects on bacterial growth. Sample-1 reported maximum growth with fructose while Sample-2 showed maximum growth with glucose (Fig.4).

Effect of nitrogen sources

Among the various nitrogen sources tested, yeast extract was found to be the best nitrogen source for Sample-1 and peptone for Sample-2 (Fig.5).

Table 3. Zone of inhibition (mm) on both the bacteria strains by Lifebuoy soap.

SL. No.	Dose (mg/ml) 100 µl/disc	Zone of inhibition (mm)	Gentamycin zone of inhibition (mm)	Resistant pattern
Sample-1	50	05±0.08	14±0.14	Resistant
	75	07±0.09		Resistant
	100	09±0.1		Resistant
Sample-2	50	08±0.07	20±0.12	Resistant
	75	08±0.09		Resistant
	100	10±0.12		Resistant

Note: Resistant=<10 mm; Intermediate =10-15 mm; Susceptible=>15 mm.

Anti-bacterial activity assay

From table 1 and 2, it was clearly found that the inhibition zones of these soaps (Dettol and Lifebuoy) were significantly different from each other. 100mg/ml had the stronger inhibition zones as compared to 75mg/ml and 50 mg/ml in both the cases for both the samples. For Sample-1, inhibition zones produced by Dettol soap was 10 mm,11mm and 11mm whereas for Sample-2 it was 10mm,11mm and 15 mm (Table 2) respectively for three concentrations (50 mg/ml, 75mg/ml and 100 mg/ml). The inhibition zones produced by Lifebuoy soap was 5mm, 7mm and 9mm (for Sample-1) and 8 mm, 8mm and 10 mm (for Sample-2) respectively for the same concentrations. Between these two soaps, Dettol soap was more effective than Lifebuoy soap for both the samples.

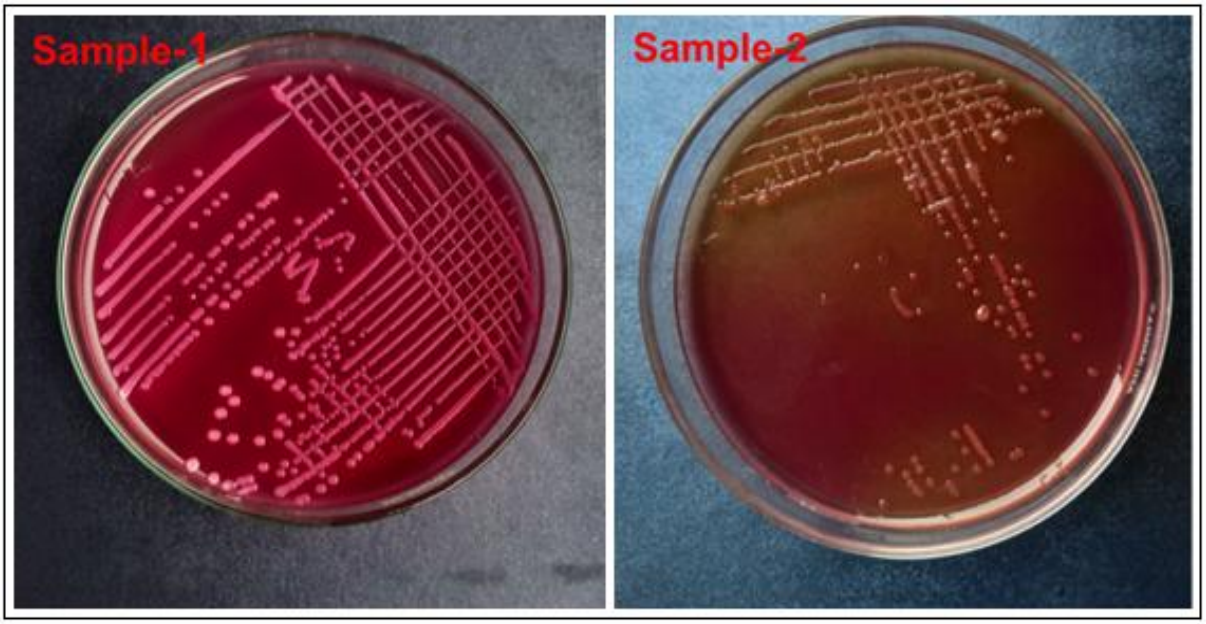

Fig. 1. Screening of pathogenic bacteria from human skin. MacConkey agar media was used for the screening of bacterial strains.

Discussion

The soaps are cleaning agents routinely used for cleaning purposes and removing germs present on the surface of skin. Soaps generally disrupt the microbial cell membrane and disrupt cells proteins. The choice of soaps varies from person to person but it should be

effective against disease causing microbes present on the skin. But prolonged use of these soaps could lead to the development of microbial resistance and allergic reactions to skin.

In this study, two pathogenic bacterial strains (Sample-1 and Sample-2) achieved their optimum growth at pH 6 and 40°C temperature. So, the strains were considered mesophilic in nature (Ventosa*et al.*, 1998). Sample-1 showed maximum growth when fructose and yeast extract were used as a carbon and nitrogen sources respectively. In the presence of glucose (carbon source) and peptone (nitrogen source), Sample-2 showed its maximum growth.

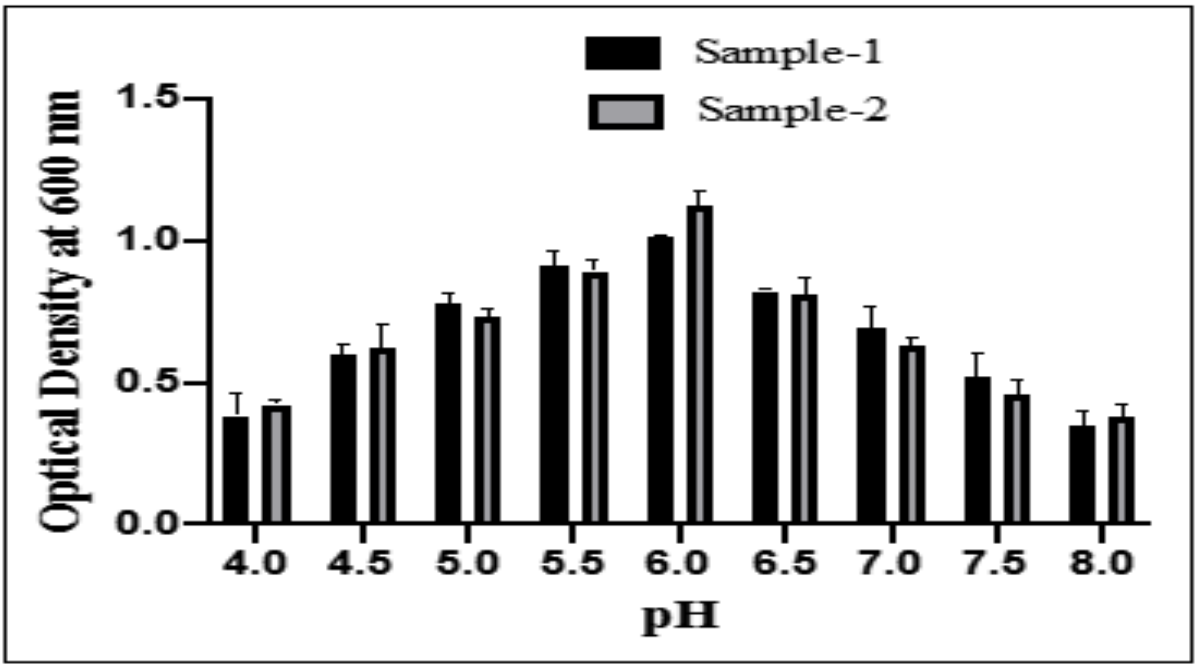

Fig. 2. Impact of pH on bacterial growth. The flasks incubated at different pH (4.0-8.0). Error bars presented mean±standard deviation of triplicates of three independent experiments.

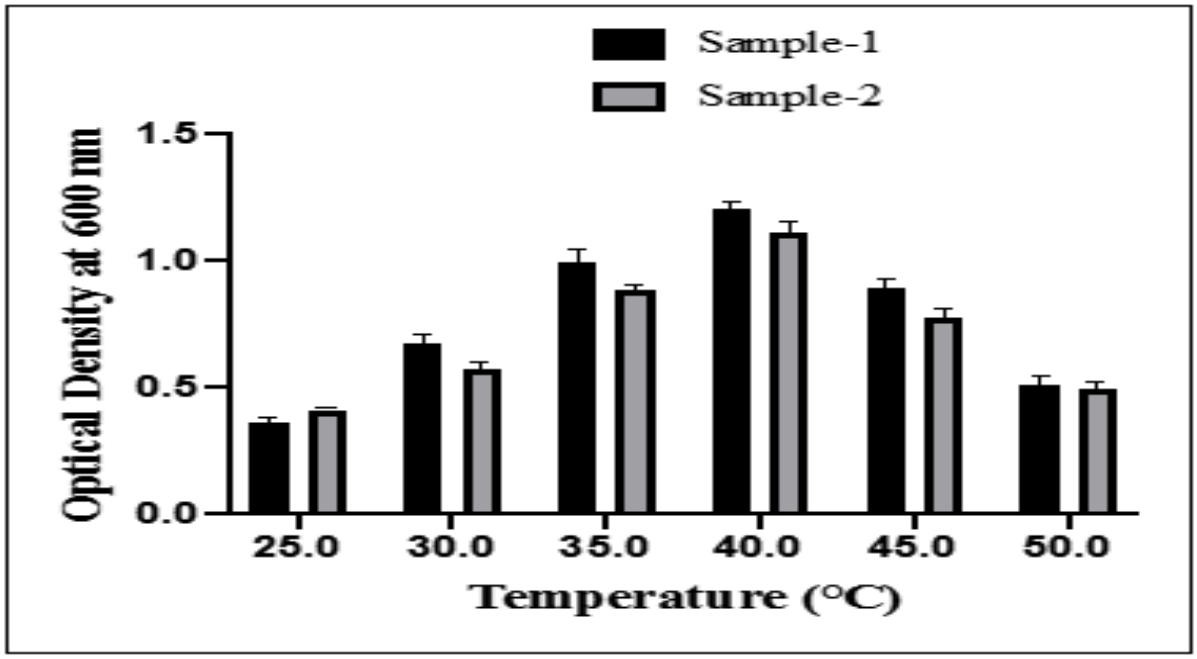

Fig. 3. Effect of temperature on on bacterial growth. Error bars presented mean±standard deviation of triplicates of three independent experiments.

Results obtained from the experimental data revealed that most of the studied antiseptic soaps have antimicrobial activity, though to varying degrees as indicated by the inhibition of the growth pattern of the isolates. Varied levels of effectiveness by soaps were observed against the isolated skin flora pathogens.

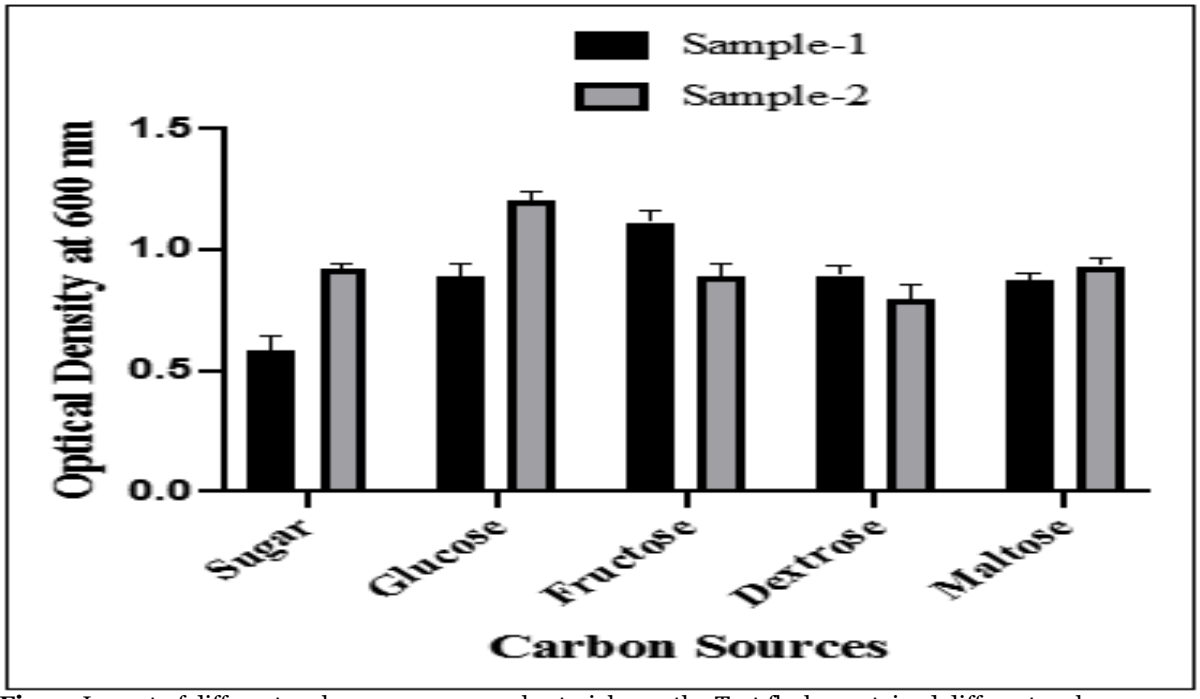

Fig. 4. Impact of different carbon sources on on bacterial growth. Test flasks contained different carbon sources in the medium at a level of 1% (w/v). Error bars presented are mean±standard deviation of triplicates of threeindependent experiments.

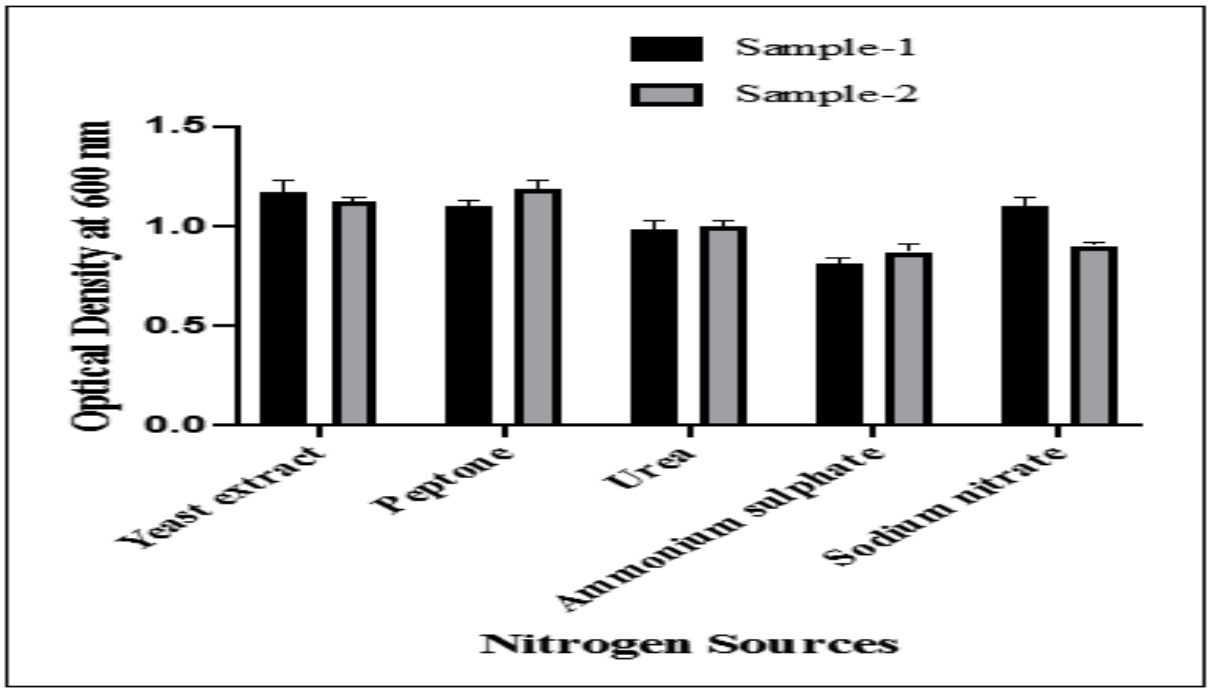

Fig. 5. Impact of different nitrogen sources on bacterial growth. Test flasks contained different nitrogen sources in the medium at a level of 1% (w/v). Error bars presented are mean±standard deviation of triplicates of three independent experiments.

The efficacy of the medicated soapswas assayed using the disc agar diffusion method. Though the assayed medicated soaps had demonstrated satisfactory antimicrobial effects, Dettolsoap was found to be most effective against Sample-1 and Sample-2having the highest zone of inhibition (10 mm, 15 mm) respectively in comparison to Lifebuoy soap. Nwambete and Lyombe (2001) reported that Dettol, Lifebuoy and Tetmosol had inhibitory activities against *E. coli*and *S. aureus* at lower concentrations than that tested in this work. Lifebuoy and Dettol were also reported to have inhibitory effects against *E. coli* and *S. aureus* and also against *Pseudomonas aeruginosa* (Ferozeet *al.,* 2014). Obi (2014) also worked on antibacterial activities of some medicated soaps and showed that Dettol and lifebuoy soap had also antibacterial activities against some human pathogens. Similar results were also achieved by Olakunle*et al.* (2019) and Abbas *et al.* (2016). Medicated soap contain triclosan, trichlorocarbanilide and p-chloro-in-xylenol (PCMX/xylenol) and can remove 65 to85% of bacteria from human skin (Osborne and Grube, 1982; Larson*etal.*,1989).

Conclusion

The present study suggested that the choice of soap should be that which is effective against disease causing bacteria in a small amount. This study proved that the two soaps had antibacterial activity against the isolated two bacterial strains but Dettol soap wasthe effective against the two isolates thus Dettol soap should be the first choice for daily use.

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