

hand hygiene (Ray et al., 2010). Fingernails contain the most bacteria present on human hands. These bacteria are extremely easy to transfer and may reside on person's hand when they touch currency notes, coins, doorknob and other things. Paper currency is frequently used as the exchange of goods and services. It may be possible that currency notes act as an environmental vehicle for the transmission of pathogenic microorganisms and may contribute to the transmission of different diseases.

Hand washing has long been recognized as an important procedure in preventing the transmission of disease (Larson, 2001). Soaps have important role in eliminating microbes as it removes and kills bacteria and other pathogenic microorganisms.

According to a study done by Osborne and Grube antibacterial soaps can remove 65%85% bacteria from human skin (Osborne and Grube, 1982). Herbal soaps are prepared by adding dried medicinal herbs (or flowers and stems of herbs) into soap base to make it more effective.

This study has been investigated to observe antimicrobial activity of different herbal soaps on commonly isolated microbes from currency notes and human nails.

Materials and Methods:

Sample collection

Note sample and nail sample were collected from people in Patna in the month of December. Nail sample was collected from the hand of random rickshaw puller by rubbing sterile wet cotton swab previously dipped in peptone water (1%). Both were then kept in labeled sterile bags separately.

Isolation of bacteria

Currency note sample was aseptically placed in 10ml sterile broth using forceps and shaken vigorously for 10 min both clockwise and anticlockwise to dislodge the microorganisms in the broth. Sample dilution was prepared using this broth

and spreading on Nutrient Agar (N.A.) plate was done with dilution 10^{-5} and 10^{-7} . Spreading by direct swab method on N.A. plate was done for nail sample. Plates were kept at 37°C for 24-48 hours for incubation.

Sub-culturing the isolates on different media

Isolates from note sample were subcultured on MacConkey media and Eosin methylene blue (EMB) plates. Isolate from nail sample was cultured on EMB media and Mannitol salt agar (MSA) by spreading technique. Isolated colonies were given code for identification after sub-culturing. For note sample, the isolates were marked as N1 and N2. For nail sample, the isolate was marked as NA.

Preparation of soap sample

250 mg and 500 mg of Himalaya tulsi, Medimix, Patanjali neem and Khadi naturals Lemongrass were mixed separately in 1ml of autoclaved distilled water to prepare the soap concentrations of 250 mg/ml and 500 mg/ml of each soap sample (Chaudhari, 2016).

Preparation of sterile disc

Sterile discs of diameter 4mm Whatman's No. 1 of 70mm filter papers were incorporated into vials containing soap samples. It was allowed to stand for the period of 1-2 hours for complete absorption. Then discs were removed from soap solution and allowed to dry in an oven at 25°C (Chaudhari, 2016).

Antimicrobial assay

Agar disc diffusion method published by the Clinical and Laboratory Standards Institute (CLSI) for bacteria was used for antimicrobial assay. Isolate N1, N2 and NA were inoculated on Mueller-Hinton agar (MHA) plates by spread plate technique. Prepared sterile discs of all soap samples were aseptically transferred directly onto the surface of plates with the help of a sterile forceps (Chaudhari, 2016). Plates were incubated at 37°C for 24-48 hours to examine the zone of inhibition formed by

each isolate in the presence of disc of soap samples at the concentration 250 mg/ml and 500 mg/ml.

Results and Discussion:

In our study, we have considered one tulsi containing soap, two neem containing herbal soaps and one lemongrass containing soap at two different concentrations i.e., 250mg/ml and 500mg/ml. N.A. media plates were inoculated with the dilution 10^{-5} and 10^{-7} of note sample by spreading technique and cultural characteristics of distinct colonies were noted (Table 1) and it was sub-cultured on Eosin Methylene Blue (EMB) agar on which no growth was observed. On MacConkey media, one of the isolates from dilution 10^{-5} and 10^{-7} were inoculated and cultural characteristics were noted, named as N_1 for 10^{-5} and N_2 for 10^{-7} (Table 3). Nail sample was inoculated on N.A. plates by direct swab method and cultural characteristics were noted (Table 2). The isolate from N.A. media was sub-cultured on EMB media and Mannitol Salt Agar (MSA). No bacterial colony was observed on EMB media and cultural characteristics of colony observed on MSA media was noted and named as N_A for nail sample (Table 4). Efficacy of sample herbal soaps at concentration 250 mg/ml and 500 mg/ml against isolate N_1 , N_2 and N_A were observed by performing disc agar diffusion method. Diameter of zone of inhibition were noted (in mm) for N_1 (Table 5), N_2 (Table 6) and N_A (Table 7). Graph comparing zone of inhibition for Himalaya (Fig. 1), Medimix (Fig. 2), Patanjali (Fig. 3) and Khadi naturals (Fig. 4) have been depicted.

Our study shows how effective herbal soaps are against isolates derived from nail and note sample when cultured in laboratory. Currency notes which are used in exchange of goods have emerged as a potential carrier of pathogen due to direct contact and transfer from one hand to another as it. Isolation of pathogenic microorganisms on currency notes indicates that there are chances of transmission of pathogen among people (Mate et al., 2018). Study by Kelechi Ukaegbu-Obi in

Umudike showed that a human finger nail harbors a broad diversity of pathogenic microorganism (Kelechi Ukaegbu-Obi, 2017). In a study by Mohsen Hashim Risan, highest contamination of *Bacillus* spp. was isolated from finger nails. Moreover, the highest prevalence in male were (16 isolates) and percentage of bacteria isolated 61.53 % while in female were (10 isolates) and percentage of bacteria isolated 38.46% (Risan, 2017).

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 Adebisi, 1981). 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 (Igbeneghu, 2013). In a study by Zeeshan Afsar, when plant extract showing maximum antimicrobial activity were combined with prepared soap and hand sanitizer formulations and then tested for antimicrobial activity, exhibited zones of inhibition ranging from 18 to 26 mm which was far better than the zones of inhibition of individual extracts pointing this enhancement of antimicrobial properties towards the synergistic effect or total sum of effects produced by the combinations of extracts (Afsar, 2016).

Cultural characteristics of the isolates on the nutrient agar plates.

Table 1. Showing cultural characteristics of the isolates from note sample at dilution 10^{-5} and 10^{-7}

Dilution	Cultural Characteristics		
	Form	Elevation	Margin
10^{-5}	Circular	Flat	Entire
	Circular	Raised	Entire
	Circular	Flat	Entire
10^{-7}	Circular	Raised	Entire

Table 2. Showing cultural characteristics of the isolates from nail sample

Colony no.	Form	Elevation	Margin
1.	Circular	Convex	Entire
2.	Irregular	Flat	Filamentous

Table 3. Showing cultural characteristics of the isolates from note sample on MacConkey media.

Isolate code	Colony characteristics					
	Form	Elevation	Pigmentation	Margin	Opacity	Surface
N1	Circular	Raised	Dark pink without sheen	Entire	Opaque	Mucoid
N2	Circular	Convex	Pink without sheen, purple at center	Cur led	Opaque	Mucoid

Table 4. Showing cultural characteristics of the isolates from nail sample on Mannitol salt agar (MSA) media.

Isolate code	Colony characteristics						
	Form	Elevation	Colour	Margin	Surface	Position	Opacity
NA	Circular	Elevated	White	Entire	Mucoid	On the media	Opaque

Antimicrobial susceptibility profiles of herbal soaps

Table 5. Diameter of Zone of Inhibition (mm) on N₁ by soap samples

Soap concentrations (mg/ml)	Himalaya Tulsi	Medimix	Patanjali Neem	Khadi Natural Lemon grass
250	6.5	6	7	5
500	12	9	9	10

Table 6. Diameter of Zone of Inhibition (mm) on N₂ by soap samples

Soap concentrations (mg/ml)	Himalaya Tulsi	Medimix	Patanjali Neem	Khadi Natural Lemon grass
250	0	4.2	4.5	0
500	4.3	8	10	7

Table 7. Diameter of Zone of Inhibition (mm) on N_A by soap samples

Soap concentrations (mg/ml)	Himalaya Tulsi	Medimix	Patanjali Neem	Khadi Natural Lemon grass
250	0	0	0	0
500	4.2	6	10	8

Graph comparing zone of inhibition (in mm)

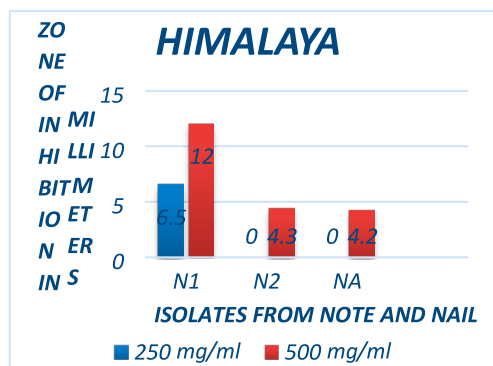


Fig. 1. Himalaya soap activity against different isolates

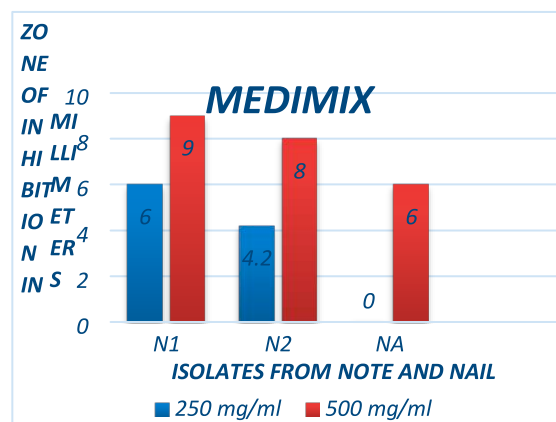


Fig. 2. Medimix soap activity against different isolates

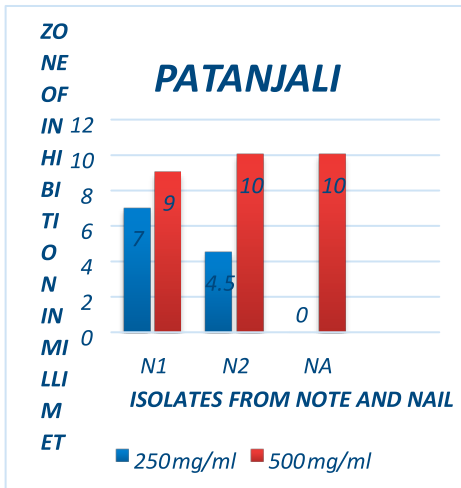


Fig. 3. Patanjali soap activity against different isolates

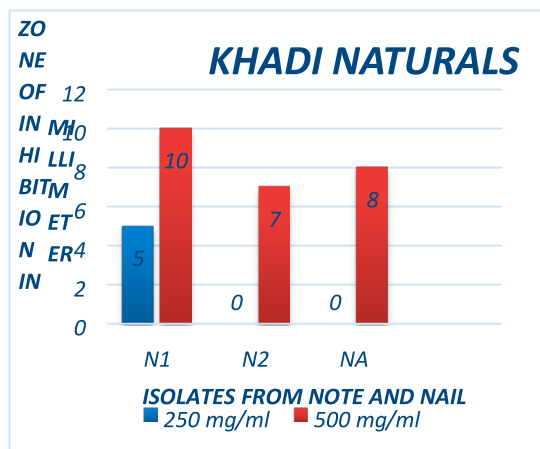


Fig. 4. Khadi naturals soap activity against different isolates

Conclusion :

Present study of antibacterial activity of herbal soaps indicates that soap containing different form of herbs like neem, tulsi, lemongrass which have already shown promising results against bacteria even at low concentration from work by other researchers and in our study too they have exhibited their antibacterial property in the form of soap by forming zone of inhibition against the isolates from note and nail sample. The activity also

varies as isolate of the sample changes. In every case as the concentration of soap sample is increased from 250 mg/ml to 500 mg/ml, values of diameter of zone of inhibition had increased indicating that dilute sample has been somehow less effective. Isolating microbes from note and nail sample indicates that we carry lots of microorganisms with us and exchange of these microorganisms via direct contact is very frequent. Washing hands with soaps is an effective way to reduce microbial load and hence its transfer. Herbal soaps also benefit by causing less harm to skin due to presence of herbal extracts. In future more study can be performed by studying the constituents of these soaps and adding some variations to deal with antimicrobial resistance-the most problematic issue in the field of medicine.

Acknowledgment :

This research was funded by Basic Scientific Research. We are thankful to our Principal Dr. Sister M. Rashmi A.C. giving us an opportunity to pursue research under the BSR scheme of UGC. Patna Women’s College, Patna for their constant support and guidance.

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