International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 792-797 www.ijprajournal.com ISSN: 2456-4494

Diversified Antimicrobial potency of Gourakshan product against Biofilm Producing Organisms

Amar M.Deshmukh[1], Swati N.Zodpe[2]
1. Department of Microbiology, Shri ShivajiCollege of Arts, Commerce and Science, Akola.
2. Assistant Professor and Head Department of Microbiology Shri Shivaji Collage Akola

Date of Submission: 05-05-2024 Date of Acceptance: 15-05-2024

ABSTRACT

The current study was carried out to check the activity of Gorakshan product against Biofilm producing organisms. Total 20 clinical samples were collected during study and further processed for isolation and identification. The frequently encountered organisms from clinical samples are S.mutans, E.coli, P. aeruginosa, S.typhiandS.aureus. The biofilm producers were confirmed on the basis of Congo red agar method and Tube method.S.mutansandS.typhi were found to be biofilm producers. In further study antimicrobial activity of Gorakshan product was checked against biofilm producers. Snanadivilayan, Cow urine and Panchgavyasoap was found to be effective in controlling the organism.

Keywords: Cow urine, Snanadivilayan, Angrajsoap, Panchgavya soap and Maraham, Oral sample and Clinical sample

I. INTRODUCTION

India is the land of traditions with its roots in ancient science directly linking social rituals and scientific reasons behind them in India, a cow is called Gau Mata and Kamdhenu due to its nourishing nature like a mother. Kamdhenu is the nature of the sacred cow who believed accomplish desired things. Panchgavya is treasure of health benefits and medicinal properties. The Ayurvedic system of medicine has described the significance of using cow milk, ghee, urine, dung, and curd each of which is termed 'gavya' (i.e., obtain from 'Gau' means cow) for the treatment of various diseases. Each product possesses different components and uses for human health Agriculture and other purposes. Panchgavya has been derive from two words, 'panch' meaning five and gavya meaning obtained from 'Gau' means cow (Bajaj et al.,2021).

For millennia, the cow has been central to Indian economy, life and culture. There are innumerable references-Vedic and subsequent, to the sacred significance of cow. The benefits of cow have been described at length in relation to Agriculture, Environment, Health, Economy and Spiritual progress. The socio-political issues surrounding cow as a sacred animal have raised acrimonious debates. But such harangues should not prevent us from an open-minded enquiry into the medicinal value of cow products. To those who have any aesthetic repugnance to such a domain of study need to be reminded of the current major developments in the human gut microbiome and successful faecal transplants against the infections with Clostridium difficile (Raut and Vaidya, 2018).

II. MATERIAL AND METHODS

Collection of products–

Product like Distilled cow urine, Soaps, Maraham and Snanadivilayanare obtained from General store of local market, Akola.

Collection of sample –

Oral infections samples such as dental caries, dental plaque and periodontal disease were collected from MSB Dental Clinic by using a sterile swab and urine and blood samples were collected from GMC Akola by using a EDTA tube.

Isolation and Identification–

Using the swabbing technique, dental swab and urine samples and blood samples were inoculated on Nutrient agar.

The inoculation was then let to incubate for 24 hrs at 37°C. After isolation the isolates were processed for confirmation by morphological study.

On the basis of staining procedure morphology of isolates were observed. The confirmation of isolates was done by Biochemical Characterization in which sugar fermentation test and IMViC test was done.

On the basis of Cultural Morphology and Biochemical Characterization the isolates were tentatively confirmed (Bergey's Manual of Determinative Bacteriology, 1939).

On the basis of microscopic examination the isolates were confirmed i.e. S.aureus, Escherichia coli, S.mutans, Salmonella typhiand Pseudomonas aeruginosa.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 792-797 www.ijprajournal.com ISSN: 2456-4494

The isolates were streaked on selective medium such as S.aureusgrow on Mannitol salt agar, Escherichia coli grow on EMB agar, S. mutansgrow on Salivarius agar, Salmonella typhigrow on Bismuth Sulfite agar and for Pseudomonas aeruginosa grow on Citrimide Agar and observed the colony.

Biofilm production –

The confirm isolates were study for the biofilm production with the help of Congo Red agar and tube method there is three isolates were not produced biofilm i.e. E. coli, S. aureus and P. aeruginosa and remaining two isolates were produced biofilm i.e. S. mutans and S. typhi. This biofilm producing isolates were use for the Antimicrobial activity test.

Tube Method

A loopful of the isolated bacteria from overnight cultured media was inoculated in each glass tube containing 10 ml of trypticase soy broth with 1% glucose. The inoculated tubes were then incubated at 37°C. After incubation for 24 hours, tubes were emptied and washed with phosphate buffer saline and left to dry. Crystal violet (0.1%) was used to stain the dried tubes for 15 minutes. Excess stain was then removed by washing the tubes with deionized water. The tubes were then dried in inverted position and examined for biofilm production. Presence of a visible film lining the bottom and the wall of the tube indicated positive result for biofilm production while formation of a stained ring at the air-liquid interface was an evidence of a negative result (Sultan et al., 2019)

Congo Red Agar Method

Congo red agar is a specially prepared medium composed of brain heart infusion (BHI) broth (37 g/l) supplemented with sucrose (50 g/l), agar No 1 (10 g/l) and Congo red (0.8 g/l). We prepared a concentrated aqueous solution of the Congo red stain that was then autoclaved at 121ºC for 15 minutes. Finally it was added to the autoclaved BHI agar with sucrose at 55ºC. Prepared CRA plates were inoculated with the isolated uropathogens and aerobically incubated at 37°C for 24 hours. Appearance of black dry crystalline colonies on the CRA plates indicated biofilm production while the colonies of biofilm non-producer remained pink or red colored. (Sultan et al., 2019)

Antimicrobial activity Test –

Antimicrobial activityof the soaps was carried out by agar diffusion technique firstly 10 gm of soaps extract was prepared by adding 100 ml of distilled water. It was prepared in a conical flask for the further use and remaining product like Distilled cow urine, Snanadivilayanand Maraham are directly used. The Molten Muller Hinton agar was inoculated with 100 µL of standardized test organisms and holes were bored equidistantly with a sterile cork borer of 6 mm in diameter. The bottom was sealed with a drop of agar and filled with different concentrations of the soap solutions (OladosuPeters O. et al., 2018).

And the plates were incubated at 37°C for 18 to 24 hrs. Post incubation plates were observed for zone of inhibition around the wells, measured and recorded using transparent meter rule.

III. RESULTS AND DISCUSSION

During the described work, a total of 20 clinical samples were collected from different pathology labs and 4 hospitals such as Government Medical College, Akola, MSB Dental Clinic, Akola. All the samples were collected under sterile conditions by wearing gloves and mask. A sterile tubes was used for sample collection (Table 1).

The samples were carried in the Microbiolgy Laboratory of Shri Shivaji College of Arts, Commerce and Science, Akola. Further it is processed for isolation and identification of bacteria. Isolation was done by inoculating samples on various selective media such as EMB Agar, Pseudomonas Isolation Agar, Bismuth Sulfite Agar, Mannitol Salt Agar and Mitis Salivarious Agar. The Cultural and Morphological characters were studied.

The obtained isolates were further checked for Biofilm formation ability of that organism. The confirmation Biofilm producer was done on the basis of the Test performed were Congo Red Agar Method and Test Tube Method. On the basis of test performed S. mutansandS. typhi were found to be biofilm producers whereas P. aeruginosa,S. aureus, E. coli are non-biofilm producing bacteria.

LoimarantaVuokkoet al., (2020) showed that both xylitol and erythritol inhibited real-time biofilm formation of the used S. mutans strains in the presence of 1% sucrose, but the sensitivity ofthe strains to polyols differed. The polyol-induced inhibition of the real-time biofilm formation was only partly explained by a decrease in the number of viable S. mutans cells or the amount of polysaccharides in the biofilms.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 792-797 www.ijprajournal.com ISSN: 2456-4494

The ability of P. aeruginosa to form biofilm in wound has confirmed on the result of Harrison Balestraet al., (2003), who reported that biofilm formation by certain pathogens such as P. aeruginosa can sometimes be rapid and the presence of such organisms in wounds could lead to the development of biofilms within a period of 24 to 48 hours after colonization. Our results were accordance to the above results. (Table 2)

Further study was continued with the antimicrobial activity of different Gourakshan Product against biofilm producing organism was also checked. Angaraj soap and Marham shows very less zone of inhibition against isolates. So they were considered as Resistant whereas Panchagavya Soap shows zone of inhibition observed was 20 mm for S. mutans and 16mm for S. typhi. Similar zone of 21mm for Snanadivilayanagainst S. mutans and zone of 17mm against S. typhi was observed. Cow urine was best showing zone of 24 mm and 22.3 mm for S. mutans and S. typhi respectively. Both products were found to be effective remedy in controlling the infectious microflora. (Table 3 Graph 2)

Chaudhari Varsha, 2016 reported that the results of the zone of inhibitions using the organisms showed that there were significant differences ($P<0.05$) on the various microorganisms used for the study. Staphylococcus aureus have more zone of inhibition (42mm) while Bacillus have zone of inhibition (30mm).

Significant differences were observed in the zone of inhibition in all types of antiseptic and herbal soaps used for the study. These results are in correlation of our results. We found S. mutans and Salmonella typhi having significant zone of inhibition against the Panchgavya Soap and Snanadivilayan(Gaurakshan Products).(Table 3)

Table No 1: Isolates obtained from various clinical samples

Sr No	Sample	Isolate obtain
1	A1	E.coli, S. mutans,
2	A2	S. mutans, S. aureus
3	A3	S. mutans, E.coli
4	A4	E. coli, S. mutans
5	A5	S.mutans, S. typhi
6	A6	S.aureus, P. areuginosa
7	A7	E.coli, S. typhi
8	A8	S. typhi
9	A9	S.aureus, P. areuginosa
10	A10	S. aureus, E.coli,
11	A11	S.aureus, P. areuginosa
12	A12	S.mutans, S. typhi,
13	A13	S. typhi P. areuginosa
14	A14	S.aureus, P. areuginosa
15	A15	E.coli, S. mutans,
16	A16	S. mutans, S.aureus, P. areuginosa
17	A17	S. typhi P. areuginosa
18	A18	S.mutans, S. typhi
19	A19	S. typhi, E.coli,
20	A20	S. typhi P. areuginosa

Table 2 : Frequency Distribution of Bacteria Found in various samples

Sr.No.	Name of organisms	No. of Isolates (out of 40)	Percentage
1	Escherichia coli	7	17.5
2	Staphylococcus aureus	7	17.5
3	P. aeruginosa	8	21
4	S. mutans	9	23
5	Salmonella typhi	9	23

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 792-797 www.ijprajournal.com ISSN: 2456-4494

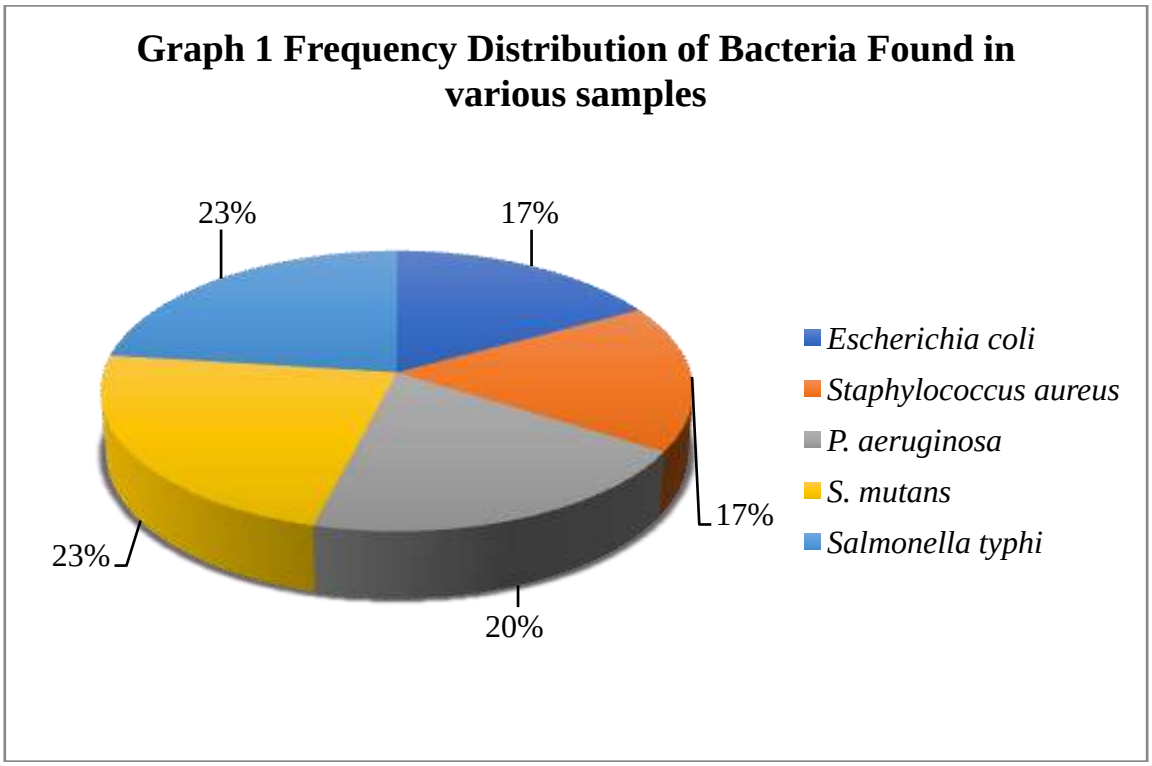
Table 3 : Biofilm formation

Sr.No.	Name of organisms	Biofilm Test
1	S. mutans	Positive
2	P. aeruginosa	Negative
3	Staphylococcus aureus	Negative
4	Salmonella typhi	Positive
5	Escherichia coli	Negative

Key:- Positive Test = Biofilm Producers, Negative Test = Non-Biofilm Producers

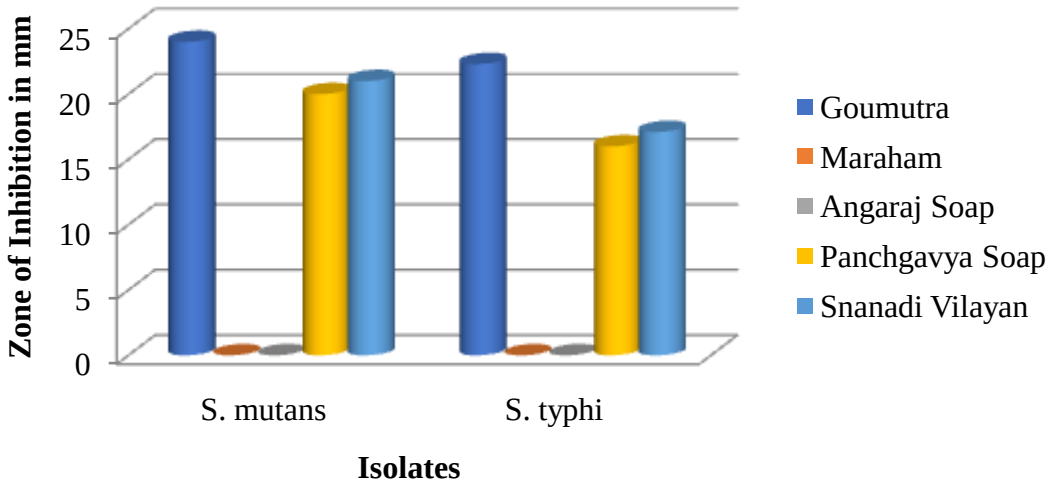
Table 4: Antimicrobial Activity of different Gorakshan Products against clinical isolates (Biofilm Producers) obtained.

Sr. No.	Isolates	Zone of inhibition (in mm)				
		Goumutra	Maraham	Angaraj Soap	Panchgavya Soap	SnanadiVilayan
1	S. mutans	**24.0**	**R**	**R**	**20.0**	**21.0**
2	S. typhi	**22.5**	**R**	**R**	**16.0**	**17.1**



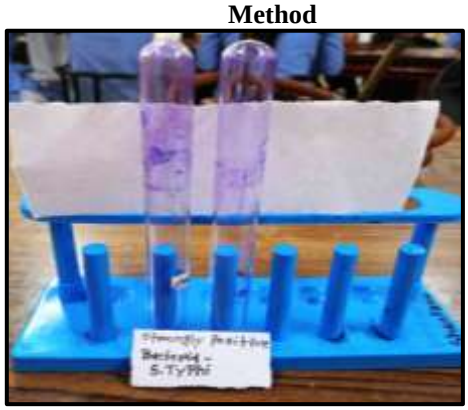
International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 792-797 www.ijprajournal.com ISSN: 2456-4494

Graph 2 : Antimicrobial Activity of different Gorakshan Products against clinical isolates (Biofilm Producers) obtained.

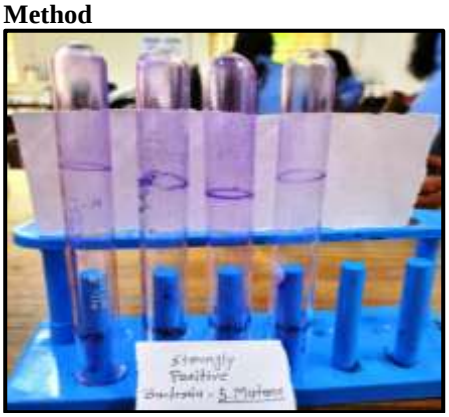


Biofilm Producing BacteriaS. typhi by Plate Method

Biofilm Producing BacteriaS. mutansby Plate Method

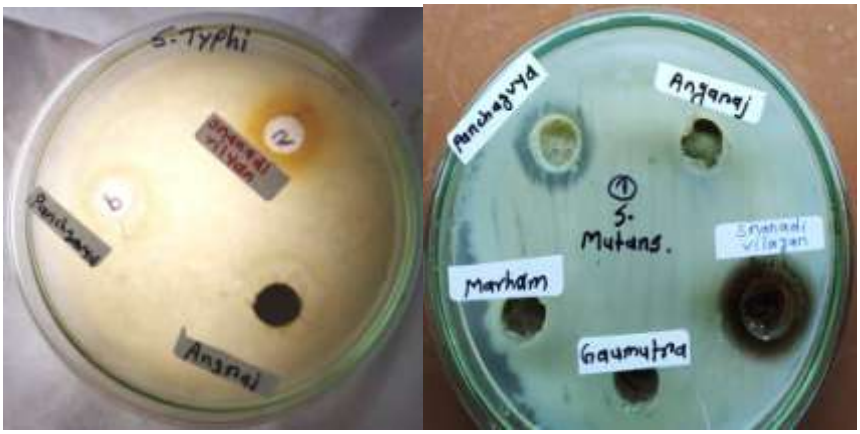


Biofilm Producing Bacteria S. typhi by Tube Method



Biofilm Producing Bacteria S. mutans by Tube Method

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 792-797 www.ijprajournal.com ISSN: 2456-4494



Antimicrobial Activity of Gorakshan products products against biofilm producing organism

IV. CONCLUSION

The conclusion drawn from the study are –

1) The cow products studied during the present work was found to have high antibacterial activity.
2) As compare to other cow product tested Snanadivilayan was found to be best.
3) Least activity was shown by ointment prepared by using cow product.

REFERENCES:

[1]. Bajaj, K. K., Chavhan, V., Raut, N. A., & Gurav, S. (2022). Panchgavya: A precious gift to humankind. Journal of Ayurveda and integrative medicine, 13(2), 100525.

[2]. Bergey, D. H. (1939). Determinative bacteriology. Baltimore: Williams &Wilkins, 556.

[3]. Oladosu, P. O., Umar, Y. A., Salawudeen, A., Izebe, K., Adamu, M. T., & Aboh, M. (2018). Antibacterial activity of soaps indigenously made in Gombe Metropolis, Nigeria. Journal of Natural Remedies, 122-130.

[4]. Raut, A. A., & Vaidya, A. D. (2018). Panchgavya and cow products: A trail for the holy grail. Journal of Ayurveda and Integrative Medicine, 9(1), 64-66.

[5]. Sultan, A. M., & Nabiel, Y. (2019). Tube method and Congo red agar versus tissue culture plate method for detection of biofilm production by uropathogens isolated from midstream urine: Which one could be better?. African journal of clinical and experimental microbiology, 20(1), 60-66.

[6]. Loimaranta, V., Mazurel, D., Deng, D., &Söderling, E. (2020). Xylitol and erythritol inhibit real-time biofilm formation of Streptococcus mutans. BMC microbiology, 20, 1-9.

[7]. Harrison-Balestra, C., Cazzaniga, A. L., Davis, S. C., & Mertz, P. M. (2003). A Wound-Isolated Pseudomonas aeruginosa Grows a Biofilm In Vitro Within 10 Hours and Is Visualized by Light Microscopy. Dermatologic surgery, 29(6), 631-635.

[8]. Chaudhari, V. M. (2016). Studies on antimicrobial activity of antiseptic soaps and herbal soaps against selected human pathogens. Journal of Scientific and Innovative Research, 5(6), 201-204.