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COMPREHENSIVE ANTIMICROBIAL EFFICACY OF GOURAKSHAN PRODUCT AGAINST BIOFILM FORMING ORGANISMS

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ABSTRACT

This study aimed to assess the efficacy of Gorakshan product against biofilm-producing organisms. A total of 20 clinical samples were collected and processed for isolation and identification of microorganisms. Predominantly encountered organisms included S. mutans, E. coli, P. aeruginosa, S. typhi, and S. aureus from clinical samples. Biofilm-producing capability was confirmed using Congo red agar and Tube methods, with S. mutans and S. typhi identified as biofilm producers. The antimicrobial activity of Gorakshan product, specifically Snanadivilayan, Cow urine, and Panchgavya soap, demonstrated effectiveness in controlling these organisms.

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

INTRODUCTION

India, renowned for its rich traditions, deeply intertwines ancient science with social rituals, particularly venerating the cow as "Gau Mata" and "Kamdhenu" due to its nurturing essence akin to a mother. Kamdhenu, the mythical sacred cow, is believed to fulfill desires. Panchgavya, a composite of cow-derived products like milk, ghee, urine, dung, and curd collectively termed 'gavya' from 'Gau' (cow)—holds significant medicinal properties and health benefits according to Ayurvedic medicine. Each product serves diverse purposes in human health, agriculture, and beyond. The term "Panchgavya" originates from 'panch,' meaning five, and 'gavya,' derived from 'Gau' [1].

Throughout millennia, the cow has played a central role in Indian life, culture, and economy, with numerous Vedic and subsequent references highlighting its sacred status and benefits. The cow's contributions span agriculture, environmental sustainability, health, economic prosperity, and spiritual growth. While socio-political debates continue regarding the cow's sacred status, it is crucial to objectively explore the medicinal potential of cow-derived products. Despite any initial reluctance, modern advancements such as studies on the human gut microbiome and successful fecal transplants combating infections like Clostridium difficile underscore the relevance of such investigations [2].

MATERIALS AND METHODS

Collection of Products: Products such as distilled cow urine, soaps, Maraham, and Snanadivilayan were procured from a local general store in Akola.

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Collection of Samples: Oral infection samples including dental caries, dental plaque, and periodontal disease were collected from MSB Dental Clinic using sterile swabs. Urine and blood samples were collected from GMC Akola using EDTA tubes.[3]

Isolation and Identification: Using the swabbing technique, dental swabs, urine samples, and blood samples were inoculated onto Nutrient agar plates.

The inoculated plates were then incubated at 37°C for 24 hours. After incubation, the isolated colonies were subjected to morphological studies for identification.

Morphology of the isolates was observed based on staining procedures. Confirmation of the isolates was carried out through biochemical characterization, including sugar fermentation tests and IMViC tests [4].

Based on cultural morphology and biochemical characterization, the isolates were tentatively identified according to Bergey's Manual of Determinative Bacteriology (1939).

Microscopic examination further confirmed the identity of the isolates, namely S. aureus, Escherichia coli, S. mutans, Salmonella typhi, and Pseudomonas aeruginosa.

Streaking and Observation: The isolates were streaked onto selective media: S. aureus on Mannitol salt agar, Escherichia coli on EMB agar, S. mutans on Salivarius agar, Salmonella typhi on Bismuth Sulfite agar, and Pseudomonas aeruginosa on Citrimide Agar. Colonies were observed for growth characteristics [5].

Biofilm Production: Confirmed isolates were assessed for biofilm production using Congo Red agar and the tube method. Among the isolates, E. coli, S. aureus, and P. aeruginosa did not produce biofilms, while S. mutans and S. typhi did. These biofilm-producing isolates were selected for antimicrobial activity testing [6].

Tube Method: A loopful of isolated bacteria from overnight cultures was inoculated into glass tubes containing 10 ml of trypticase soy broth with 1% glucose. Tubes were then incubated at 37°C. After 24 hours, tubes were washed with phosphate-buffered saline, dried, stained with 0.1% crystal violet for 15 minutes, washed again, and dried. Presence of a visible film indicated positive biofilm production [7].

Congo Red Agar Method: Congo Red agar plates were prepared with brain heart infusion broth supplemented with sucrose and Congo red stain. Isolated uropathogens were inoculated onto these plates and incubated aerobically at 37°C for 24 hours. Black, dry crystalline colonies indicated biofilm production, while non-biofilm producers remained pink or red [8].

Antimicrobial Activity Test: The antimicrobial activity of the soaps was evaluated using the agar diffusion technique. Soap extracts were prepared by adding 10 gm of soap to 100 ml of distilled water, while Distilled cow urine, Snanadivilayan, and Maraham were used directly.

Muller Hinton agar was inoculated with standardized test organisms, and wells were bored and filled with different soap concentrations. Plates were then incubated at 37°C for 18 to 24 hours, and zones of inhibition around the wells were measured using a transparent meter rule.

RESULTS AND DISCUSSION

During the study, a total of 20 clinical samples were collected from various pathology labs and hospitals including Government Medical College, Akola, and MSB Dental Clinic, Akola. All samples were collected under sterile conditions using gloves and masks, and sterile tubes were employed for collection (Table 1).

The samples were transported to the Microbiology Laboratory of Shri Shivaji College of Arts, Commerce, and Science, Akola, where they underwent isolation and bacterial identification. Isolation was performed by inoculating samples onto selective media such as EMB Agar, Pseudomonas Isolation Agar, Bismuth Sulfite Agar, Mannitol Salt Agar, and Mitis Salivarius Agar. Cultural and morphological characteristics of the isolates were examined.

Subsequently, the isolated bacteria were evaluated for their ability to form biofilms. Confirmation of biofilm producers was conducted using the Congo Red Agar Method and the Test Tube Method. Based on these tests, S. mutans and S. typhi were identified as biofilm producers, while P. aeruginosa, S. aureus, and E. coli did not produce biofilms[9].

Loimaranta et al. (2020) demonstrated that both xylitol and erythritol inhibited real-time biofilm formation of S. mutans strains in the presence of 1% sucrose. However, the sensitivity of the strains to these polyols varied, and the inhibition of biofilm formation was partly attributed to reduced viable S. mutans cells or polysaccharide amounts in the biofilms.

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

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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

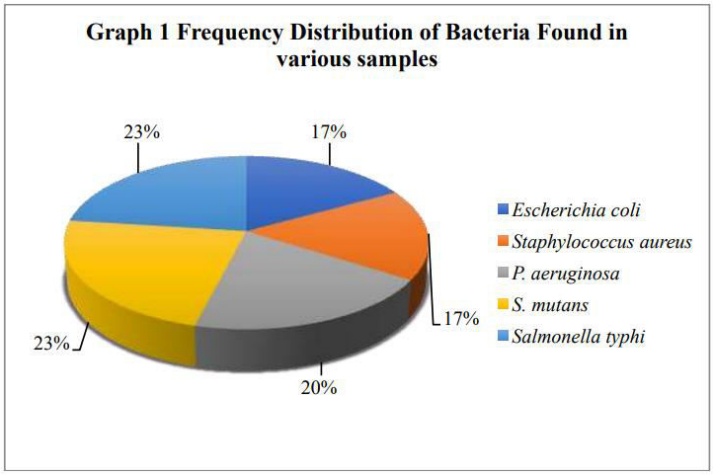

Figure 1: Antimicrobial Activity of Gorakshan products products against biofilm producing organism

CONCLUSION

The conclusions drawn from this study are as follows:

1. The cow products investigated in this study exhibited potent antibacterial activity.
2. Among the tested cow products, Snanadivilayan demonstrated the highest efficacy.
3. The ointment formulated using cow products showed the least activity compared to other tested formulations.

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