RESEARCH PAPER

An In-Vitro Antimicrobial Study of Swarna Prashana

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ABSTRACT

Swarnaprashana is an ancient Ayurvedic formulation of Lehana, which has been mentioned in Kaumarbhritya for its immunoenhancing activity and the prevention of infectious diseases in children. Although this formulation has long been used, there is a lack of scientific evidence for its antimicrobial properties. The current study aims to assess the in vitro antibacterial activity of Swarnaprashana at varied doses against common newborn infections. The experimental study was conducted in vitro by the agar well diffusion method on Mueller-Hinton agar. Swarnaprashana was prepared by Swarna Bhasma, Madhu (honey), and Ghrita (clarified butter) in aseptic conditions. We tested the antibacterial activity against E. coli (MTCC 2592) and Staphylococcus aureus (MTCC 1430). Test samples included conventional Swarnaprashana in three concentrations (25%, 50%, and 100%) and Swarnaprashana in DMSO in corresponding concentrations. Antimicrobial activity was assessed by the measurement of zones of inhibition in millimetres. Normal Swarnaprashana presented dose-dependent antibacterial activity, with higher inhibition values for Staphylococcus aureus (15 mm at 50% and 23 mm at 100%) than for Escherichia coli (12 mm and 18 mm, respectively). Swarnaprashana, made with DMSO as a solvent, worked well against Staphylococcus aureus, with zones of inhibition of 14 mm at 25%, 18 mm at 50%, and 24 mm at 100% concentration. When DMSO is used as a solvent, it shows better antibacterial activity and diffusion properties. The inhibition zones were 18 mm at 25%, 21 mm at 50%, and 22 mm at 100% concentration against Escherichia coli. The present study establishes that Swarnaprashana has appreciable in vitro antibacterial activity, influenced by concentration, type of microbes, and solvent system. These results justify its traditional use and form a scientific basis for further in vivo and clinical studies.

Keywords: Swarnaprashana, Swarnabhasma, Antimicrobial activity, E. coli, Staphylococcus aureus

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INTRODUCTION

Infectious diseases are still one of the main causes of morbidity and healthcare utilisation in children worldwide[1]. The pediatric population is most vulnerable to microbial infections due to the developmental immaturity of both innate and adaptive immune mechanisms [2]. A newborn child during the neonatal period may acquire *Escherichia coli, Staphylococcus aureus*, *Proteus mirabilis*, and *Salmonella typhi* infections, etc., which are the common pathogens that may cause neonatal septicaemia, meningitis, osteomyelitis, gastroenteritis, and urinary tract infection[3]. *Graha roga* (~complicated infectious disorders) were also prevalent during the ancient period to prevent *Graha roga* in children[4].

In Ayurveda, the ancient system of medicine originating in India, there is a focus on preventive healthcare and the maintenance of immune competence, especially in children, under the specialised branch of *Kaumarabhritya.* In Ayurvedic classical texts, children are said to have *Alpa Bala,* implying susceptibility to diseases, and therefore recommend specific interventions

to enhance *Vyadhikshamatva* (disease resistance)[5]. Among these interventions, *Lehana* formulations are recommended for regular administration during infancy and early childhood to promote physical growth, cognitive development, and immunity. *Swarnaprashana* is one such *Lehana* preparation, traditionally prepared with *Swarna Bhasma* along with *Madhu* (honey) and *Ghrita* (clarified butter). The classical texts, especially Kashyapa Samhita, have explained the beneficial effect of *Swarnaprashan,* administration as follows: enhancing *Medha* (Intellect), *Agni* (Digestion, metabolism), *Balvardhanam* (physical strength), *Ayushyam* (promoting lifespan), *Mangalam* (auspicious), *Punyam*(righteous), *Vrishyam* (fertility), *Varnyam* (enhancement of colour and complexion), *Grahapahan* (protection from evil spirits and microorganisms)[6].

Acharya Sushruta has also recommended that newborns consume *Swarna* (gold), *Madhu* (honey), and *Ghrita* immediately after birth, a practice known as *Jatakarma samskara*[7]. It is also employed for the destruction of *Grahroga* (~complicated infectious disorders) as *Jatakarma Samskara*[8].

From a contemporary scientific viewpoint, metallic and mineral-based formulations have garnered interest for their prospective antimicrobial and immunomodulatory properties[9]. Experimental evidence demonstrates that gold-based compounds and gold nanoparticles possess inhibitory effects against a wide array of microorganisms. The suggested mechanisms encompass the disruption of microbial cell membrane integrity, the inhibition of essential enzymatic activity, the induction of oxidative stress, and the immunomodulation of host responses[10]. These observations furnish a scientific basis for assessing traditional gold-containing formulations, such as *Swarnaprashana,* regarding their potential efficacy in infection prevention.

People also think that the adjuvants in *Swarnaprashana* are important for its biological activity. Many people say that honey has antibacterial properties that work against both Gram-positive and Gram-negative bacteria. This is because it has an osmotic effect, an acidic pH, hydrogen peroxide production, and bioactive phytochemicals[11]. Traditionally, *Ghrita,* a lipid-based medium, is thought to be a *Yogavahi,* which makes active ingredients more available and stable. Lipid-based carriers have been shown to help cells take in more of a substance and may help keep antimicrobial action going[12].

A recent in vitro study has demonstrated the antipseudomonal activity of *Swarna bhasma* (150 mcg/µl)[13]. The present study aims to evaluate the antimicrobial activity of *Swarna prashana* in different concentrations against two common pathogenic organisms, e.g., *Escherichia coli* & *Staphylococcus aureus,* responsible for neonatal septicaemia, meningitis, gastroenteritis, and urinary tract infection, etc.

MATERIAL AND METHOD

Trial Drug's Authenticity for The Study:

Swarna Bhasma (premium quality) was purchased from Dhootapapeshwar Pharmaceutical company (Batch no. P240500254, Date of Manufacturing-05/202). *Madhu* (Honey) was procured from Chhattisgarh Herbals. *Ghrita* was prepared at home from cow's milk. The antimicrobial study was conducted on the *Swarnaprashana* after getting approval from the Institute Ethical Committee (IEC).

Preparation Of *Swarna Prashana*:

Initially*, Swarna Bhasma* 100mg was administered in the *kharal*. 12ml of *madhu* was administered. With the pestle, the *Madhu* and *Swarna Bhasma* were churned until a homogenous mixture was prepared. Once the mixture was prepared homogenously, 8 mL Ghrita was administered in the small glass container and was placed in a warm water bath to melt the *Ghrita*. Once the *Ghrita* was melted, it was added gradually to the already existing homogenous mixture in the mortar and churned continuously in a clockwise direction until a homogenous mixture. After 8 hours of continuous churning, once the mixture becomes a thick, viscous mixture, the prepared medicine was carefully packed in a clean container and stored for further use. The preparation was carried out by using sterile vessels, gloves, a cap, and a mask throughout the entire preparation process to avoid any contamination.

Methodology

Bacterial strains for testing:

A total of two strains of bacteria (including gram-negative and gram-positive) were taken for the study, viz. E. coli (MTCC NO 2592), Staphylococcus aureus (MTCC NO 1430).

Preparation of media:

Mueller Hinton agar medium was prepared, sterilised by autoclaving at 121 °C for 15 minutes, and poured into

sterile Petri plates under aseptic conditions. The plates were allowed to solidify.

Mueller–Hinton agar medium

Mueller–Hinton agar medium was used as the standard culture medium for evaluating the antimicrobial activity of the test drug. The medium was prepared according to the manufacturer's instructions under aseptic conditions.

Materials Required

- Mueller–Hinton agar powder
- Distilled water
- Conical flask
- Measuring cylinder
- Autoclave
- Sterile Petri plates

Method of Preparation

A measured quantity of Mueller–Hinton agar powder (38 g/L) was weighed accurately and suspended in the required volume of distilled water. The mixture was then heated with continuous stirring until the agar was dissolved in water and a clear solution was obtained. The prepared medium was then sterilised by autoclaving at 121 °C under 15 lbs pressure for 15 minutes. After autoclaving, the medium was allowed to cool to a temperature of 45–50 °C. The sterile molten agar was then aseptically poured into sterile Petri plates and allowed to solidify at room temperature.

Inoculation of plates by the lawn culture method

The Mueller–Hinton agar plates prepared in petri plates were inoculated with the lawn culture technique to ensure the even growth of the test organisms.

A fresh bacterial culture (18–24 hours old) was taken, and a suspension was made in sterile normal saline to match the turbidity comparable to a 0.5 McFarland standard. A sterile cotton swab was dipped into the standardised inoculum, and the excess fluid was removed by pressing the swab against the inner wall of the tube.

The Mueller–Hinton agar plate was inoculated evenly by swabbing the entire surface in three directions, rotating the plate approximately 60 degrees after each swabbing to ensure uniform distribution of the bacterial inoculum. Finally, the swab was moved once around the rim of the agar.

After inoculation, the plates were allowed to stand for 5–10 minutes at room temperature to permit absorption of excess moisture before application of the test drug.

Agar well diffusion method

The antimicrobial activity of *Swarnaprashana* was evaluated by the agar well diffusion method using Mueller–Hinton agar medium.

Preparation of Wells

After inoculating the Mueller–Hinton agar plates with the test organism using the lawn culture technique, the plates were allowed to dry for 5–10 minutes at room temperature. Sterile cork borers of 5mm diameter were used to punch uniform wells in the agar at equidistant points. The agar plugs were carefully removed to create wells.

Group I: Normal *Swarnaprashana* -

Normal *Swarnaprashana* was administered without any solvent. Different volumes of the test drug were applied directly placed into the wells as follows;

Well 1: - 1 drop of *Swarnaprashana*

Well 2: - 2 drops of *Swarnaprashana*

Well 3: - 3 drops of *Swarnaprashana*

Well 4: - Control Group (Azithromycin)

A standardized dropper was used to ensure consistency in the volume of the drop.

Group II: *Swarnaprashana* prepared with DMSO (Solvent-based Concentrations)

Swarnaprashana was diluted with Dimethyl Sulfoxide (DMSO) as a solvent to prepare predefined concentrations under aseptic conditions:

Well 1: - 25% concentration

Well 2: - 50% concentration

Well 3: - 100% concentration

Well 4: - Control Group (DMSO Solvent)

A fixed volume of 50–100 µL of each concentration was aseptically placed into the respective wells using a sterile micropipette.

Diffusion and incubation

After loading the test samples, the plates were kept undisturbed at room temperature for 30–60 minutes for the diffusion of the drug into the agar medium. The plates were then incubated at 37 °C for 18–24 hours in an incubator.

Observation and recording of results

After incubation, the plates were observed for the presence of a zone of inhibition around the wells. The diameter of the zone of inhibition was measured in millimetres using a transparent ruler or Vernier calliper and recorded as an indicator of antimicrobial activity.

Preparation of test samples

A) Normal *Swarnaprashana*- (1Drop,2Drop,3Drop)

B) *Swarnaprashana* dissolved in DMSO Solvent were prepared at different concentrations (25%, 50% &100%) using sterile distilled water/DMSO as required.

Preparation of Stock Solution-Initially, 10 ml of *Swarnaprashana* was taken in a sterile test tube, to which 2–3 ml of sterile DMSO (Dimethyl sulfoxide) was added. The mixture was gently mixed and then kept in a warm water bath for 5 minutes to facilitate uniform solubilization.

100% concentration- 2 ml of stock solution

50% concentration- 1 ml of stock solution +1 ml of sterile DMSO

25% concentration - 0.5 mL of stock solution +1.5 mL of sterile DMSO

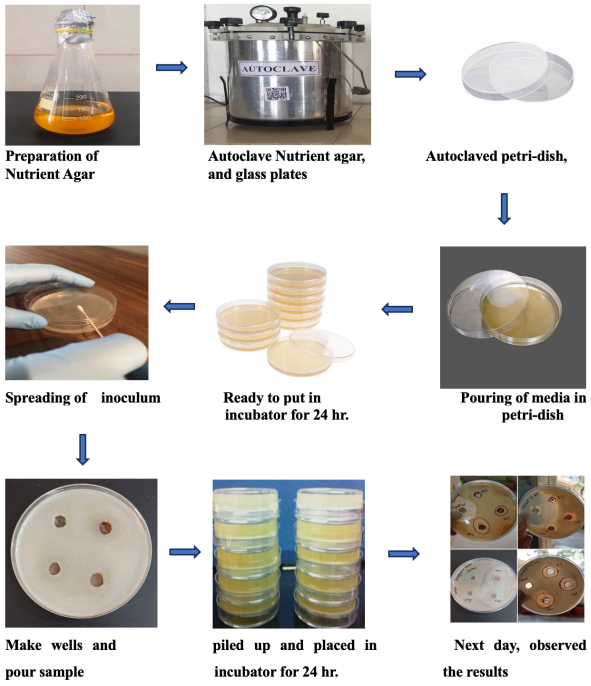

Figure 01: agar well diffusion method

Results

NORMAL *SWARNAPRASHANA* Concentration (Drop)	Antimicrobial activity zone of inhibition (mm).	
	Staphylococcus aureus	*Escherichia coli*
25% (1Drop)	MI	MI
50% (2Drop)	15	12
100% (3Drop)	23	18

MI (Minimal inhibition)

SWARNAPRASHANA PREPARED WITH DMSO SOLVENT Concentration (Drop)	Antimicrobial activity zone of inhibition (mm).	
	Staphylococcus aureus	*Escherichia coli*
25% (1Drop)	14	18
50% (2Drop)	18	21
100% (3Drop)	24	22

Normal *Swarnaprashana*

Figure – 02 *Staphylococcus aureus*

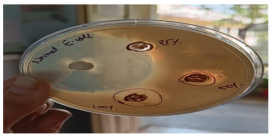

Figure – 03 *Escherichia coli*

***Swarnaprashana* Prepared with Dmso Solvent**

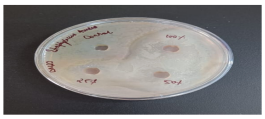

Figure – 04 *Staphylococcus aureus*

Figure – 05 Escherichia *coli*

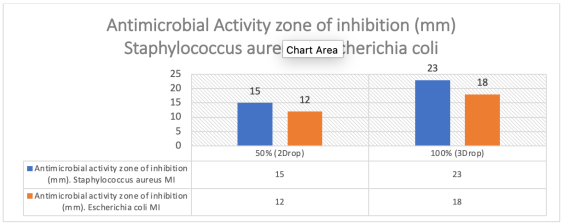

Table 01- Normal *Swarnaprashana*

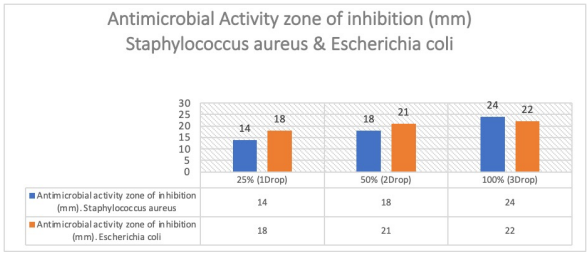

Table 02- *Swarnaprashana* prepared with DMSO Solvent

DISCUSSION

Previous studies [14-16] have shown the antimicrobial activity of gold nanoparticles (AuNPs). To date, no antimicrobial studies have been conducted on *Swarnabhasma* at different concentrations. However, one study was carried out to evaluate the effect of *Swarnabhasma* on Pseudomonas aeruginosa at a concentration of 150 µg/ µl[17]. In contrast, the present study has been done in predefined three concentrations of *Swarnaprashana,* that is, (25%, 50% &100% Concentrations) to know the antimicrobial effect on E. coli (MTCC NO 2592) and Staphylococcus aureus (MTCC NO 1430).

The current in vitro experiment assessed the antibacterial properties of *Swarnaprashana* against Staphylococcus aureus and Escherichia coli with varying concentrations

and solvent media. The results obtained in this study clearly indicate the presence of substantial antibacterial properties in *Swarnaprashana,* which vary according to microbial species, concentration, and solvent media used. Normal *Swarnaprashana* had strong antibacterial properties against Staphylococcus aureus, with zones of inhibition of 15 mm at 50% concentration and 23 mm at 100% concentration. The dose-dependent increase in antibacterial properties confirms the fact that the greater the concentration of *Swarnaprashana*, increase the inhibitory effect on microbial growth. The heightened sensitivity of the Gram-positive pathogen S. aureus may be ascribed to its less complex cell wall structure, which lacks a lipopolysaccharide outer membrane, thereby enabling the infiltration of bioactive compounds found in *Swarnaprashana.*

Conversely, zones of inhibition against Escherichia coli were somewhat smaller, measuring 12 mm at 50% concentration and 18 mm at 100% concentration. The natural resistance mechanisms of Gram-negative bacteria, such as the outer membrane that prevents the diffusion of antibacterial agents, can account for the decreased susceptibility. Previous research has shown that conventional metals and herbal substances are more effective against Gram-positive bacteria than Gram-negative bacteria.

Swarnaprashana made with the solvent DMSO had good antibacterial activity against Staphylococcus aureus, with zones of inhibition of 14 mm at 25% concentration, 18 mm at 50% concentration, and 24 mm at 100% concentration. It showed zones of inhibition of 18 mm at 25%, 21 mm at 50%, and 22 mm at 100% concentration against Escherichia coli. This shows that the solvent makes the antibacterial activity stronger.

The enhanced antibacterial efficacy of the DMSO-based *Swarnaprashana* formulation may be attributed to the superior solvent characteristics of DMSO, which can enhance the solubility, stability, and diffusion of active compounds across the agar layer and bacterial cell membrane. DMSO is known to be a good solvent that can help compounds get through biological membranes, which makes antimicrobial agents more available to the body.

The gradual increase in the diameter zone with higher concentrations in the DMSO-based *Swarnaprashana* formulation could account for the concentration-dependent antibacterial activity. It is important to note that the DMSO formulation has shown more activity against E. coli than normal *Swarnaprashana*, even at lower concentrations. This means that the choice of solvent is very important in in vitro antimicrobial studies.

Role of *Swarnabhasma* in Antimicrobial Activity-

Swarnabhasma is thought to be the most important constituent in *Swarnaprashana,* which plays a major role in the antimicrobial activity. In this present research, the antibacterial potentiality of *Swarnaprashana* against Staphylococcus aureus and Escherichia coli could be attributed to some extent by the presence of *Swarnabhasma*. It has been proven that gold compounds and gold nanoparticles possess antimicrobial activity by disrupting the cell membrane of microbes, inhibiting metabolic enzymes, and generating oxidative stress in microbial cells.

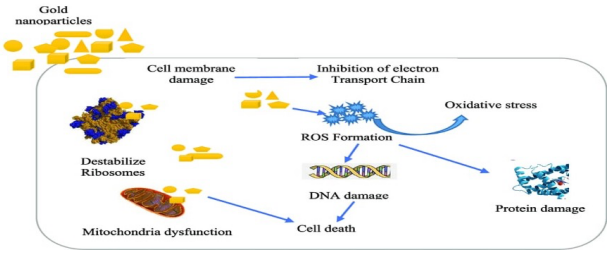

Figure – 06 Mode of Action of *Swarnabhasma*

The dose-dependent increase in zones of inhibition in the study corroborates the hypothesis that elevated concentrations of *Swarnabhasma* contribute to enhanced antibacterial activity. Staphylococcus aureus is more sensitive than Escherichia coli, which is in line with the resistance mechanisms of Gram-negative bacteria. This suggests that *Swarnabhasma* may work better with the cell walls of Gram-positive bacteria. Moreover, the augmented antibacterial efficacy of *Swarnaprashana* formulated with DMSO indicates that enhanced dispersion and penetration of *Swarnabhasma* particles affect antimicrobial characteristics.

Ayurveda classifies *Swarnabhasma* as Krimighna, Ojovardhaka, and Rasayana. This means that *Swarnabhasma* is connected to its ability to stop the growth of microbes and boost the body's defences. So, *Swarnabhasma* is an important part that helps make Swarnaprashana work as an antimicrobial[18].

Role of *Madhu* in Antimicrobial Activity-

Madhu (honey) is well known for its natural ability to kill bacteria, and it is expected to make *Swarnaprashana* much more effective at doing this. *Madhu's* ability to kill germs is thought to come from a number of factors, including high osmolarity, acidic pH, the production of hydrogen peroxide, and the presence of bioactive

compounds like flavonoids and phenolic acids. These traits work together to stop bacteria from growing and living.

In this study, *Madhu* may function as an antimicrobial agent and a synergistic carrier, potentially augmenting the overall inhibitory efficacy of *Swarnaprashana* against S. aureus and E. coli. The comparatively elevated antibacterial efficacy against Gram-positive bacteria may be attributed to their susceptibility to osmotic stress and peroxide-induced damage. Madhu is also known to help active ingredients spread through agar media better, which may be because it makes the zones of inhibition bigger.

Ayurvedically, Madhu is classified as *Yogavahi, Krimighna*, and *Lekhana*, suggesting its ability to potentiate the action of associated drugs while manifesting its own antimicrobial effects[19].

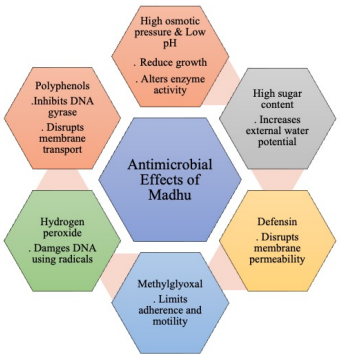

Figure – 07 Antimicrobial Effects of *Madhu*

Role of *Ghrita* in Antimicrobial Activity-

Ghrita (clarified butter) is a major lipid carrier in *Swarnaprashana* and has a supportive but crucial role in antimicrobial action. Although *Ghrita* alone may not possess potent direct antibacterial activity in vitro, its role as a lipid carrier is of prime importance in enhancing the stability, dispersion, and penetration of the active principles, such as *Swarnabhasma* and phytoconstituents.

In the present study, *Ghrita* may have contributed significantly to the uniform distribution of *Swarnabhasma* particles, thereby helping in the consistent antimicrobial activity at different concentrations. Lipid carriers have been recognised to enhance membrane permeability, which may help in the transport of active principle across the bacterial cell membrane. This action may be of prime importance in the context of Gram-negative bacteria, as indicated by the enhanced antimicrobial activity in solvent-assisted formulations.

From an Ayurvedic standpoint, *Ghrita* is characterised as *Sanskaranuvarti, Yogavahi*, and *Balya*, which highlights its capacity to carry and improve the therapeutic properties of drugs when processed with it. In this case, *Ghrita* is not entirely a primary antimicrobial substance but functions as a critical adjuvant that optimises the antimicrobial efficacy of the formulation[20].

Comparative Discussion: Individual Ingredients vs *Swarnprashna* Formulation-

1. Synergistic Activity in Combination-

The combination formulation may demonstrate increased or prolonged antimicrobial activity compared to *Swarnabhasma* or *Ghrita* alone. This suggests a synergistic effect of *Madhu,* which possesses immediate antibacterial activity; *Swarnabhasma,* which may possess potential immunomodulatory and nanoparticle-mediated effects; and *Ghrita,* which may possess stabilising and bioavailability-improving effects.

2. Synergistic Mechanism of Action

The addition of honey may provide an unfavourable condition for bacterial growth, and the nano-gold particles may also disrupt microbial cell structure or metabolism. *Ghrita* may aid in the better dispersion of particles and may also aid in sustained release. Therefore, the synergistic effect of the combination may be more effective than the individual components.

3. Enhanced Diffusion and Stability

In agar well diffusion experiments, honey may aid in better diffusion of the formulation, which may increase the zone of inhibition measured compared to testing *Swarnabhasma* or *Ghrita* separately.

4. Validation of Traditional Claim

Ayurvedic texts describe *Swarnprashna* as a prophylactic and immunostimulatory drug in pediatric patients. The antimicrobial activity of the combination formulation validation for the traditional claim.

5. Advantage of the Combination Formulation Over the Single Drug

Although *Madhu* possesses potent direct antibacterial activity, the combination formulation possesses a broader therapeutic index because of the combined antimicrobial activity.

CONCLUSION

The present in vitro experiment proves that *Swarnaprashana* has strong antibacterial properties against Staphylococcus aureus and Escherichia coli, and

this depends on the concentration, type of microorganism, and solvent system used. There was also a dose-dependent increase in antibacterial properties, and higher concentrations showed larger zones of inhibition. Staphylococcus aureus was found to be more sensitive than E. coli, and this could be due to the differences in the cell wall structure of Gram-positive and Gram-negative bacteria.

Swarnaprashana, prepared with DMSO as a solvent, showed strong antibacterial properties, especially against E. coli, and this emphasises the importance of selecting the appropriate solvent system for in vitro experiments. The findings suggest that improved diffusion and penetration properties can significantly increase antimicrobial properties.

The antibacterial property can be ascribed to the synergistic effect of the formulation ingredients. *Swarnabhasma* may be considered as the primary antimicrobial agent, which inhibits the metabolism and cell wall damage of bacteria, and *Madhu* has natural antibacterial properties and a synergistic effect, and *Ghrita* acts as a good carrier, which increases the stability and delivery of antimicrobial ingredients.

IMPLICATION

The antimicrobial property of *Swarnaprashana* can be beneficial for opting for the rational use of *Swarnaprash* at birth in children intended to prevent infectious disorders caused by *Escherichia coli* & *Staphylococcus aureus,* responsible for the neonatal septicaemia, meningitis, gastroenteritis, and urinary tract infection, etc.

LIMITATIONS

- The present study was conducted as an in-vitro experimental study; hence, the outcome may not specifically represent the in-vivo antimicrobial property of *Swarnaprashana* in the human body.
- Only two bacterial strains (Escherichia coli and Staphylococcus aureus) were considered in the study. Thus, the antimicrobial activity against a broader spectrum of gram-positive and gram-negative microorganisms could not be assessed.
- The antimicrobial activity was measured only by the agar well diffusion method, which is primarily a diffusion-based inhibition test and does not provide any information regarding the minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC).
- Standard antibiotics were used only for comparative analysis; however, a detailed quantitative analysis of efficacy between *Swarnaprashana* and standard antimicrobial agents was not performed.

FUTURE SCOPE

- The present in-vitro data will serve as a basis for designing in-vivo studies to evaluate the therapeutic efficacy and safety of *Swarnaprashana* in animal models and human subjects.
- Future studies may be designed to include a broader spectrum of microorganisms, such as gram-positive, gram-negative bacteria, and fungi, to evaluate the broad-spectrum antimicrobial properties of *Swarnaprashana.*

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