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# Comparative Antimicrobial Study of *Samangadi Kwatha* and *Samangadi Arishta* on Selected Diarrhoea Causing Bacteria

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## ABSTRACT

Diarrhoea is the passage of three or more loose or liquid stools per day and more frequently than normal for the individuals. In bacterium-induced diarrhoea, rapid loss of fluids and electrolytes results from inhibition of the normal absorptive function of the intestine as well as the activation of secretary processes. *Atisara*, one of the diseases mentioned in Ayurvedic classics has its cardinal feature as frequency of loose stools which mimic the diarrhoeal symptoms. *Samangadi Kwatha* is one of the formulations mentioned in *Chakradatta* which is indicated in *Sarvatisara*. *Kwatha* should be prepared and used instantly as it has limited shelf life. On the other hand, *Arishta* have prolonged shelf life and also palatable. Considering these factors, present study was planned to compare antibacterial properties of two dosage forms *Samangadi Kwatha* and *Samangadi Arishta* on diarrhoea causing bacteria such as *Salmonella enterica, Shigella flexneri and Escherichia coli.* **Methodology:** In antibacterial study by agar well diffusion method, the zone of inhibition of both the samples were tested against three bacteria namely *Salmonella enterica, Shigella flexneri and Escherichia coli*. The results of antibacterial study are weighed up and scrutinized. **Results:** In the antibacterial activity assessment, *Samangadi Kwatha* exhibited a zone of inhibition against all three organisms at all tested concentrations. However, *Samangadi Arishta* showed zone of inhibition at moderate and higher concentrations.

**Key Words** *Diarrhoea, Samangadi Kwatha, Samangadi Arishta, Antibacterial study*

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## INTRODUCTION

Diarrhoea is a condition characterized by passing of three or more loose or liquid stools per day, occurring more frequently than usual for the individual[1]. It is a significant health concern, particularly in children under the age of five, where it ranks second in leading cause of death. Approximately 525,000 children under the age of five succumb to Diarrhoea each year[2]. This combination results in watery and frequent stools characteristic of the condition. *Atisara*, as mentioned in Ayurvedic classics, is a specific

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disease characterized by the cardinal feature - frequent loose stools, which closely resemble the symptoms of Diarrhoea. It is primarily caused by infections from bacteria, viruses, or parasites affecting the gastrointestinal tract. Among the Diarrhoea inducing bacteria, *Salmonella enterica*, *Shigella flexneri*, and *Escherichia coli* are the most common culprits. Studying the response of these infectious agents to *Ayurvedic* medicines, can significantly contribute to the effective management of *Atisara*. *Samangadi Kwatha*[3] *(SK)* is a significant herbal formulation mentioned in *Chakradatta*, an ancient Ayurvedic text. It holds a particular importance due to its therapeutic indications in *Sarvatisara*. *Kwatha* has limited shelf life but *Arishta* has prolonged shelf life. Hence a modified dosage form was prepared, i.e., *Samangadi Arishta (*SA*)* using *Samangadi Kwatha dravyas* based on *Anukta mana Arishta* reference[4].

The present study is aimed to compare the antimicrobial activity of efficacy of *Samangadi Kwatha* and, *Samangadi Arishta* on the causative organisms of Diarrhoea, namely *Escherichia coli*, *Salmonella enterica*, and *Shigella flexneri*.

**Objectives of the study**

a) To evaluate and compare *in-vitro* antimicrobial activity of *Samangadi Kwatha* and *Samangadi Arishta.*

**METHODOLOGY**

Antimicrobial susceptibility testing plays a crucial role in various aspects of healthcare and disease management. There are various laboratory techniques for determining the in-vitro antibacterial activity of extracts or pure substances. The commonly used methods are the well or disk diffusion and the broth or agar dilution method[5].

**Source of data**

The evaluation of anti-bacterial activity of *SK* and *SA* was carried out in S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi.

**Materials required**

- Test strain – *E. coli, Salmonella enterica, Shigella flexneri*
- Distilled water, saline
- Test tube, Incubator, Laminar air flow
- Graduated micropipettes
- Growth medium- Nutrient agar
- Sample *Samangadi Kwatha* and *Samangadi Arishta*

**Preparation of Nutrient agar media:**

Beef extract (1 g), yeast extract (2 g), peptone (5 g) and Sodium Chloride (5 g) were dissolved in 990 ml of distilled water. The pH was adjusted to 7.2 and the volume was made up to 1000 ml. Finally, 15 g agar was added to the media and autoclaved at 121˚C for 20 minutes.

**Preparation of the inoculum:**

*Escherichia coli* (MTCC 42), *Salmonella enterica* (MTCC 3231) and *Shigella flexneri* (MTCC 1457) was procured from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh. Loopful of 48h old culture from the slants was transferred to

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sterile saline and mixed well to prepare a homogenous inoculum (Figure 1 & 2).

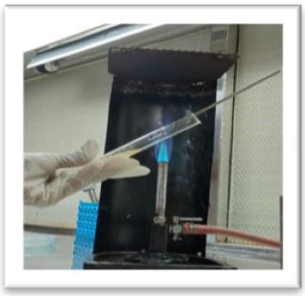

**Figure 1** Preparation of inoculum

**Figure 2** Dilution of inoculum in saline

**Well diffusion method:**

After cooling to 45-50º C, 20 ml of medium were placed into each sterile petriplates (Figure 3). One ml of the inoculum of strain was immediately poured to the plate and spun to ensure even dispersion (Figure 4). A sterile borer was used to drill the wells (Figure 5). The antibiotic and samples were administered into the wells (Figure 6). Plates were incubated overnight at 37º C and inspected after 48 hours. Samples of *Samangadi Kwatha* and *Samangadi Arishta* were taken in - 25 µl, 50 µl, 75 µl, 100 µl, 125 µl concentrations, Control (Distilled water) - 50 µl, Standard (*Ampicillin*) 1mg /1ml – 20 µl, 30 µl, 30 µl respectively. The results were assessed based on the zone of inhibition (ZOI) around the lower (25µ), moderate (50µ & 75µ) and higher (100µ & 125µ) concentrations of drug.

## RESULTS

*Samangadi Kwatha* showed antibacterial activity at the different volumes used against *Escherichia coli*, *Salmonella enterica* and *Shigella Flexneri* (Figure 7, 8 & 9), whereas *Samangadi Arishta* showed antibacterial activity at the different volumes used except at 25 µl against *Escherichia coli*, *Shigella flexneri* and except at 25µl and 50µl against *Salmonella enterica* (Figure 10, 11 & 12). The result of ***in*** *vitro* antibacterial study of *Samangadi Kwatha* and *Samangadi Arishta* against *E. coli*, *Salmonella enterica* and *Shigella flexneri* is given in (Table 1, 2 and 3).

**Table 1** *In vitro* antibacterial activity of *Samangadi Kwatha* and *Samangadi Arishta* against *E. coli*

| Sample | Volume | ***Samangadi Kwatha*** ZOI – (Radius in mm) | | ***Samangadi Arishta*** ZOI - (Radius in mm) | |
|---|---|---|---|---|---|
| **Concentrations** | 25 µl | 6 | 0 | 0 | 6 |
| | 50 µl | 7 | 5 | 6 | 7 |
| | 75 µl | 7 | 6 | 8 | 7 |
| | 100 µl | 8 | 7 | 8 | 8 |
| | 125 µl | 9 | 7 | 8 | 9 |
| **Control (DD water)** | 50 µl | 0 | 0 | 0 | 0 |
| **Standard (*Ampicillin*) 1mg / 1ml** | 20 µl | 15 | 7 | 7 | 15 |

**Table 2** *In vitro* antibacterial activity of *Samangadi Kwatha* and *Samangadi Arishta* against *Salmonella enterica*

| Sample | Volume | ***Samangadi Kwatha*** ZOI – (Radius in mm) | | ***Samangadi Arishta*** ZOI - (Radius in mm) | |
|---|---|---|---|---|---|
| **Concentrations** | 25 µl | 5 | 6 | 0 | 0 |
| | 50 µl | 7 | 7 | 0 | 0 |
| | 75 µl | 8 | 7 | 5 | 5 |
| | 100 µl | 9 | 8 | 6 | 6 |
| | 125 µl | 9 | 8 | 7 | 7 |

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| | | | | | |
|---|---|---|---|---|---|
| **Control (DD water)** | 50 µl | 0 | 0 | 0 | 0 |
| **Standard (*Ampicillin*) 1mg / 1ml** | 30 µl | 7 | 7 | 8 | 8 |

**Table 3** *In vitro* antibacterial activity of *Samangadi Kwatha* and *Samangadi Arishta* against *Shigella flexneri*

| **Sample** | **Volume** | ***Samangadi Kwatha* ZOI – (Radius in mm)** | | ***Samangadi Arishta* ZOI - (Radius in mm)** | |
|---|---|---|---|---|---|
| **Concentrations** | 25 µl | 6 | 6 | 0 | 0 |
| | 50 µl | 7 | 7 | 6 | 6 |
| | 75 µl | 8 | 8 | 8 | 8 |
| | 100 µl | 8 | 8 | 9 | 9 |
| | 125 µl | 8 | 9 | 10 | 10 |
| **Control (DD water)** | 50 µl | 0 | 0 | 0 | 0 |
| **Standard (*Ampicillin*) 1mg / 1ml** | 30 µl | 10 | 10 | 12 | 11 |

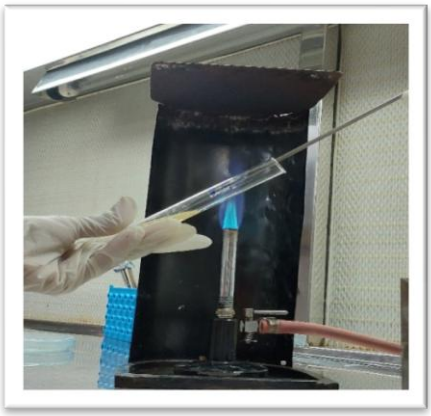

**Figure 3** Preparation of nutrient agar plate

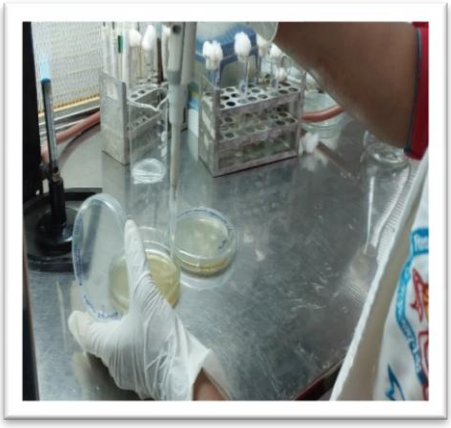

**Figure 4** Addition of inoculum

**Figure 5.** Creating wells in petriplates

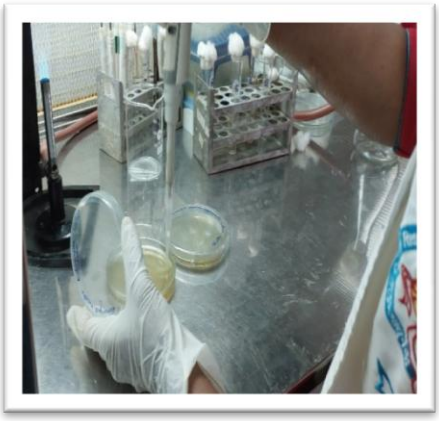

**Figure 6.** Addition of the test drugs in wells

## DISCUSSION

Diarrhoea is a condition characterized by the frequent passing of loose or liquid stools, typically three or more times per day. It is a major health issue, especially among children under the age of five and is recognized as the second leading cause of death in this age group. The primary cause of Diarrhoea is infection, often resulting from the bacteria, viruses, or parasites that affect the gastrointestinal tract. Effective management of Diarrhoea involves identifying the specific causative agent and providing appropriate treatment, which may

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include rehydration therapy, antimicrobial agents and supportive care.

**Figure 7.** ZOI in *SK* in *E. coli*

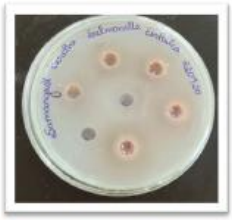

**Figure 8.** ZOI in *SK* in *Salmonella enterica*

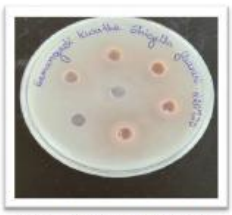

**Figure 9.** ZOI in *SK* in *Shigella flexneri*

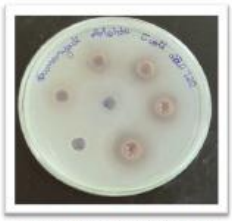

**Figure 10.** ZOI in *SA* in *E. coli*

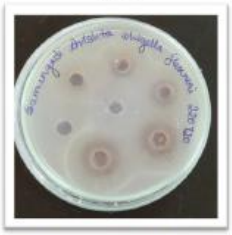

**Figure 11.** ZOI in *SA* in *Shigella flexneri*

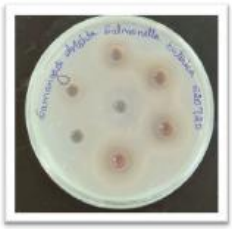

**Figure 12.** ZOI in *SA* in *Salmonella enterica*

The *Samangadi Kwatha* formulation mentioned in *Chakradatta* for the treatment of *Atisara* consists of nine ingredients. These ingredients are *Samanga (Lajjalu), Ativisa, Musta, Vishwa, Hribera, Dhataki, Kutaja Twak, Indrayava* and *Bilwa Phala Majja*. The ingredients of the *Samangadi Kwatha* formulation mentioned in *Chakradatta* possess various therapeutic properties that contribute to their effectiveness in treating Diarrhoea like *Deepana, Pachana, Sangrahi, Krimighna, Vishagna, Atisarahara, Rakta Stambaka, Stambaka, Shulahara etc.*

In this study, the focus was on bacteria induced Diarrhoea, specifically targeting strains of bacteria known to be common causative agents, namely *Salmonella, Shigella* and *Escherichia coli* (E. coli). These bacterial species were selected as they are frequently associated with diarrheal infections and have significant clinical relevance.

The antibacterial study of *SK* and *SA* utilized the Agar well diffusion method, which is a commonly employed technique for evaluating the antimicrobial activity of plant extracts. Nutrient agar was chosen as the agar medium for this study. Nutrient agar is a type of growth medium that provides the necessary nutrients for the growth of a wide range of bacteria. It contains various essential nutrients that support bacterial growth. The agar well diffusion method involved inoculating agar plates with the respective bacterial strains, creating wells in the agar and introducing samples of SK and SA into the wells. The plates were then incubated under suitable conditions and the zone of inhibition around the wells were measured. The zone of inhibition provides an indication of the antimicrobial activity of the samples against the tested bacteria.

The comparison of the zone of inhibition between SK and SA in the antibacterial study revealed that SK exhibited a zone of inhibition against all three tested organisms at all concentrations.

The effectiveness of SK in all tested concentrations can be attributed to its high aqueous extract content. Aqueous extracts may have a greater amount of active compounds, which can contribute to their antimicrobial effectiveness. On the other hand, SA showed zone of inhibition at moderate and higher concentrations. No zone of inhibition was shown at lower concentrations.

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Despite the higher antibacterial potential of SK, the study highlighted the advantages of SA in terms of extended shelf life and improved palatability. These factors are important considerations in clinical practices. Although SA may have a relatively lower antibacterial efficacy compared to SK, its prolonged shelf life and enhanced taste make it a promising option for use in clinical settings.

While the reduced microbiological results obtained for *Samangadi Arishta* compared to *Samangadi Kwatha* may suggest a lower antimicrobial activity, it does not necessarily indicate a lesser clinical efficacy. Clinical effectiveness of a formulation is influenced by various factors beyond its direct antimicrobial activity, such as bioavailability and pharmacokinetics in human subjects. *Samangadi Arishta* may exhibit clinical effectiveness despite its lower antimicrobial activity due to other unexplored factors, such as synergistic interactions with the body's immune system, modulation of host-microbe interactions, or additional therapeutic properties beyond antimicrobial effects. Therefore, further research is needed to understand and explore the various factors contributing to the clinical efficacy of *Samangadi Arishta*.

## CONCLUSION

In the antibacterial study, comparing *Samangadi Kwatha* and *Samangadi Arishta*, it was observed that *Samangadi Kwatha* exhibited a zone of inhibition against all three tested organisms at all concentrations. Conversely, *Samangadi Arishta* exhibited a zone of inhibition at moderate and higher concentrations, showing antibacterial action against the examined pathogens. However, no zone of inhibition was seen at lower doses, suggesting that *Samangadi Arishta's* antibacterial action may be concentration-dependent. To establish the clinical efficacy of *Samangadi Arishta*, clinical trials are necessary. These studies will provide a more comprehensive understanding of its therapeutic potential and effectiveness in the management of specific diarrheal conditions.

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