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Antimicrobial Activity of *Aragwadhadhi kwatha* Prepared by Varying Methods and Proportions against *Staphylococcus aureus*

Nicy Vincent, E. N. Prajeesh Nath, Arun Mohanan and N. V. Ramesh*

Department of Rasashastra and Bhaishajya Kalpana, Amrita School of Ayurveda, Amrita Vishwa Vidyapeetham, Amritapuri, Kollam - 690525, Kerala, India; drramesh.adiga@gmail.com

Abstract

Background: Wound infections, especially those caused by *Staphylococcus aureus*, remain a significant concern in clinical settings due to bacterial infection, antimicrobial resistance, delayed healing, and increased treatment costs. *Ayurveda* offers time-tested remedies for wound care. **Aim:** This study aimed to explore how variations in the preparation of *Aragwadhadhi Kwatha*, specifically the drug-to-water ratio and reduction method, affect its physical, chemical, and antimicrobial properties. **Methods:** Four samples of *Aragwadhadhi Kwatha* were prepared using different proportions of water and reduction levels, following classical *Ayurvedic* guidelines. Each sample was tested for Total Dissolved Solids (TDS), pH, and specific gravity. Antimicrobial activity was evaluated in vitro against *S. aureus* using the agar well diffusion method. **Results:** The study found clear differences in the analytical profiles of all four samples. Sample 3 had the highest TDS, pH, and specific gravity, indicating a more concentrated decoction. However, Sample 4, prepared with a 1:8 drug-to-water ratio and reduced to one-fourth, as mentioned in *Ashtanga Hridaya*, showed the most effective antimicrobial action. This suggests that a balanced method of preparation can yield better therapeutic outcomes, rather than simply increasing concentration. **Conclusion:** The results confirm that preparation techniques play a crucial role in the efficacy of *Ayurvedic* formulations. Sample 4's performance supports the traditional guidelines given in the *Sharangadhara Samhita* for medium-hard drugs. Standardising such classical formulations is essential to ensure quality, consistency, and greater acceptance in both modern clinical practice and scientific research.

Major Findings: The study demonstrated that variation in preparation methods significantly influences both physicochemical properties and antimicrobial activity of *Aragwadhadhi Kwatha*. Among the samples, Sample 4 (1:8 reduced to 1/4) showed the highest antimicrobial activity against *Staphylococcus aureus*.

Keywords: Antimicrobial, *Aragwadhadhi Kwatha, Staphylococcus aureus, Vrana,* Wound

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1. Introduction

Wound infections present a significant and persistent challenge in the realm of healthcare[1]. Proper wound care is essential for reducing the microbial load, eliminating unhealthy slough and necrotised tissue, and maintaining optimal wound moisture levels. Commonly, wound infections are attributed to microorganisms such as *Escherichia coli, S. aureus*, *Proteus mirabilis, Acinetobacter baumannii/haemolyticus* and *Pseudomonas aeruginosa*[2]. Classical *Ayurvedic* texts have long provided a repository of knowledge regarding the use of various herbs and Herbo-mineral combinations for the purification *(Vrana shodhana*) and healing *(ropana*) of wounds[3]. Among these formulations, *Aragwadadhi Kwatha*, mentioned in the *Ashtanga Hridaya* under *Sodhanadhigana Samgrahaniya Adhyaya*, is particularly noted for its efficacy in cleansing wounds[4]. The topical application of

*Author for correspondence

such *Ayurvedic* formulations has demonstrated potential in enhancing wound healing. *Aragwadhadhi Kwatha* has diverse properties, including being antipruritic, anti-microbial, anti-helminthic, anti-pyretic, digestive stimulant, anti-hyperlipidaemic, anti-inflammatory, immuno-modulatory, anti-oxidant, laxative, mucolytic, anti-diabetic, and anti-hyperglycaemic[5].

The preparation of these formulations, known as *Kwatha,* falls within the broader category of *Panchavidha Kashaya Kalpana*[6]. *Ayurvedic* formulations vary due to traditional practices, regional differences, and practitioner discretion. Establishing a scientifically validated drug-to-water ratio ensures consistent therapeutic benefits across different batches and practitioners. The choice of drug-to-water ratio and reduction technique significantly influences the concentration of active constituents, thereby affecting the antimicrobial efficacy against *S. aureus*. A standardised approach not only enhances the reliability of *Ayurvedic* formulations but also supports their academic, industrial, and clinical applications, ensuring widespread adoption and trust in *Ayurveda*.

The present study aims to investigate the impact of different drug-to-water ratios and reduction techniques on the analytical properties and antimicrobial efficacy of *Aragwadhadhi Kwatha*, with particular reference to its effectiveness against *S. aureus*. The objectives of the study are to prepare four *Aragwadhadhi Kwatha* samples using distinct drug-to-water ratios and reduction methods as described in *Ayurvedic* texts, and to evaluate these samples through analytical assessments, including total dissolved solids, pH, and specific gravity, along with the determination of their antimicrobial activity against *S. aureus.*

2. Materials and Methods

Raw materials for *Aragwadadhi kwatha* were procured from a GMP-certified *Ayurveda* medicine manufacturing unit, Amrita Life, Enterprises Pvt. Ltd, Clappana PO, Vallickavu, Kollam, Kerala, India.

Ethical approval: The conducted research is not related to either human or animal use.

2.1 Ingredients of *Aragwadhadhi Kwatha*[4]

The ingredients of *Aragwadadhi kwatha* were selected based on the description provided in *Ashtanga Hṛdaya*,

Table 1. Table showing ingredients of *Aragwadadhi kwatha*

S. No.	Sanskrit name	Botanical name	Parts used	Quantity
1	*Aragwadha*	*Cassia fistula*	Bark	1 Part
2	*Indrayava*	*Holarrhena antidysenterica*	Seed	1 Part
3	*Patala*	*Stereospermum suaveolen*	Stem bark	1 Part
4	*Kakatikta*	*Leea hirta* Roxb	Whole plant	1 Part
5	*Nimba*	*Azadirachta indica*	Bark	1 Part
6	*Amruta*	*Tinospora cordifolia*	Stem	1 Part
7	*Madhurasa*	*Marsdenia tenacissima*	Root	1 Part
8	*Sruvavriksha*	*Butea monosperma*	Bark	1 Part
9	*Pata*	*Cyclea peltata*	Root	1 Part
10	*Bhunimba*	*Andrographis paniculata*	Whole plant	1 Part
11	*Saireyaka*	*Strobilanthes ciliatus*	Whole plant	1 Part
12	*Patola*	*Trichosanthes cucumerina*	Leaf	1 Part
13	*Karanja*	*Pongamia glabra*	Bark	1 Part
14	*Pootikaranja*	*Holoptelea integrifolia*	Bark	1 Part
15	*Saptachada*	*Alstonia scholaris*	Bark	1 Part
16	*Agni*	*Plumbago zeylanica*	Root	1 Part
17	*Karavella*	*Momordica charantia*	Whole plant/root	1 Part
18	*Bana*	*Tephrosia purpurea*	Root	1 Part
19	*Madanaphala*	*Randia dumetorum*	Fruit	1 Part

Sūtrasthāna, Chapter 15 (*Śodhanādi Gaṇa Saṅgraha Adhyāya*) (Table 1).

2.2 Preparation of Samples

Raw materials were cleaned with water and crushed well to get coarse powder. For each sample 150gm of *Aragwadadhi kwatha choorna* was taken. The drug and water mix was subjected to mild heat (*Madhyama Agni*) with frequent stirring without covering its mouth. The temperature was maintained at 90-95°C (Figure 1). The temperature was maintained at 90-95°C to simulate *Madhyama Agni*, as described in classical *Ayurvedic* texts for *kwātha kalpanā*, which ensures efficient extraction of water-soluble active principles without charring or degradation of the drug. Controlled heating below vigorous boiling prevents thermal degradation of thermolabile phytoconstituents and ensures uniform reduction and reproducibility of the decoction. The decoction was filtered using the same standard filtration technique for all samples to maintain consistency.

2.2.1 Sample 1

Sample 1 was prepared as per *Sushruta Samhita*[7] and *Ashtanga Samgraha Indu* commentary[8], 150 g (1 part) of *Aragwadhadi Kashaya Choorna* was boiled with 2400 ml (16 parts) water and reduced to 600 ml (1/4 part). The total preparation time was 2 h and 25 min.

2.2.2 Sample 2

Sample 2 was prepared as per *Bhavaprakasha*[9] and *Sharngadhara Samhita*[10], 150 g (1 part) of the *Aragwadhadi Kashaya Choorna* was boiled with 2400 ml (16 parts) of water and reduced to 300 ml (1/8 part). The total preparation time was 2 h and 40 min.

2.2.3 Sample 3

Sample 3 was prepared as per *Sushruta Samhita* (Dalhana commentary)[7], 150 g (1 part) of the drug was boiled with 2400 ml (16 parts) of water and reduced to 150 ml (1/16 part). The total preparation time was 3 h and 5 min.

2.2.4 Sample 4

Sample 4 was prepared as per *Ashtanga Samgraha*[11] and *Ashtanga Hrudaya*[12], 150 g (1 part) of the drug was boiled with 1200 ml (8 parts) of water and reduced to 300 ml (1/4 part). The total preparation time was 1 h and 5 min.

Four samples were prepared with different drug-to-water ratios and reduction techniques, as shown in Table 2.

2.3 Analysis of Sample

Analytical tests were conducted to determine the Total Dissolved Solids (TDS), pH and specific gravity of 4 samples. An analytical study was carried out to know the particular chemical configuration of the final product and to point out the physicochemical changes and effects of different *Samaskaras* (*Agni Sannikarsha*) during the pharmaceutical processing. The TDS or Brix content is assessed using a hand refractometer, while specific gravity is determined through a hydrometer. For pH analysis, a digital pH meter was employed after calibration.

2.4 Antimicrobial Activity

Antimicrobial activity of four samples was assessed against *S. aureus* using the agar well diffusion method[13], conducted for 3 different trials.

Materials required for antibacterial activity were Nutrient Agar (Merck, Germany), Saboraud Dextrose Agar (Merck, Germany), Micropipettes, Autoclaved



Figure 1. Preparation of 4 samples of *Aragwadadhi kwatha.*

Pipette tips, Petri plates, Conical Flask, L-rod, Spirit, Bunsen burner, Autoclaved MilliQ water, Discard beaker, Autoclave microcentrifuge tubes.

Prepare Nutrient Agar (NA) media plates and Saboraud Dextrose Agar (SDA) plates, inoculate plates with the test microorganism (50 µl bacteria and fungi inoculum from previously prepared bacterial broth) by the spread plate method. Make agar wells of approximately 10mm in diameter and fill them by adding the samples under test to the concerned wells. Each well should be made in such a way that the antibacterial zone of the drug added is easily visible in the agar surface and distributed evenly so that they are no closer than 24 mm from each other, centre to centre. Incubate the NA plates under test for 24 h at 37°C and SDA plates for 48 h inside a bacteriological incubator. After incubation, examine all plates under test. The resulting zones of inhibition will be uniformly circular with a confluent lawn of growth. Measure the diameters of the zones of complete inhibition, including the diameter of the control zone.

Table 2. Table showing the preparation of 4 samples in different drug water ratio and different reduction techniques

Sample name	Drug	Water	Water reduction
Sample 1	150 gm (1part)	2400 ml (16 parts)	600 ml (1/4 part)
Sample 2	150 gm (1part)	2400 ml (16 parts)	300 ml (1/8 part)
Sample 3	150 gm (1part)	2400 ml (16 parts)	150 ml (1/16 part)
Sample 4	150 gm (1part)	1200 ml (8 parts)	300 ml (1/4 part)

3. Results

3.1 Physicochemical Parameters

The analytical study of these samples revealed variations in Total Dissolved Solids (TDS), pH levels, and specific gravity as shown in Table 3.

3.1.1 Total Dissolved Solids (TDS)

Sample 1 recorded the lowest TDS value of 2.4, indicating minimal dissolved solid concentration. Sample 2 showed an increased TDS of 3.4, reflecting higher solute extraction. Sample 3 had the highest TDS at 3.8, suggesting a maximum concentration likely due to extensive reduction. Sample 4 presented a moderate TDS of 2.8, indicating balanced solute extraction.

Table 3. Table showing results of physicochemical parameters

Sample name	TDS	pH	Specific gravity
Sample 1	2.4	5.71	1.008
Sample 2	3.4	6.09	1.014
Sample 3	3.8	6.40	1.018
Sample 4	2.8	5.96	1.012

Table 4. Zone of inhibition (mm) of *Aragwadhadhi Kwatha* samples against *S. aureus*

Sample name	Sample 1	Sample 2	Sample 3	Sample 4
Trial 1	NIL	NIL	13 mm	15 mm
Trial 2	NIL	NIL	13 mm	16 mm
Trial 3	NIL	12 mm	14 mm	16 mm
Mean ± SD	0.00 ± 0.00	4.00 ± 6.93	13.33 ± 0.58	15.67 ± 0.58

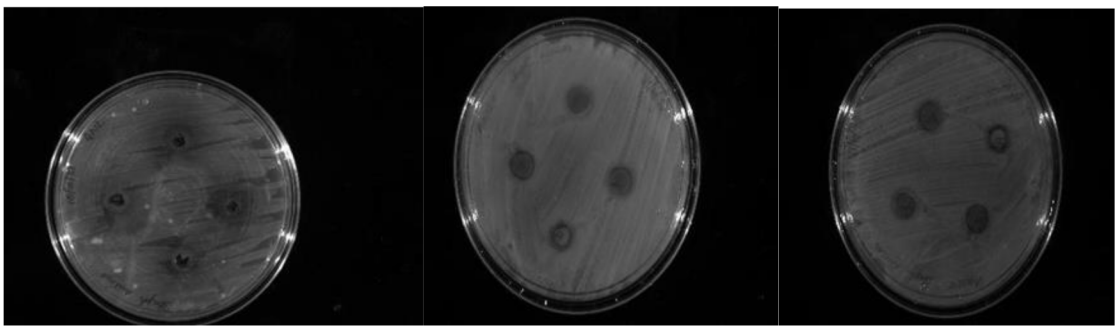


Figure 2. Zone of inhibition of the samples for 3 trials of antimicrobial activity against *S. aureus*. **(a).** Trial 1, **(b).** Trial 2, **(c).** Trial 3.

3.1.2 pH

Sample 1 had the lowest pH of 5.71, making it the most acidic, which may affect stability and microbial resistance. Sample 2 showed a slightly higher pH of 6.09, nearing neutrality. Sample 3 recorded the highest pH at 6.40, indicating the least acidic nature, possibly due to increased alkaloidal or mineral content. Sample 4 had a pH of 5.96, reflecting moderate acidity, potentially favourable for topical use and antimicrobial action.

3.1.3 Specific Gravity

Sample 1 had a specific gravity of 1.008, indicating a lighter and less concentrated formulation. Sample 2 increased to 1.014, suggesting a denser decoction. Sample 3 showed the highest specific gravity at 1.018, aligning with its highest TDS and pH, reflecting maximum concentration. Sample 4 recorded 1.012, indicating a moderately dense and well-balanced preparation.

3.2 Antimicrobial Activity

While analysing antimicrobial activity, Sample 4 (drugs to water ratio was 1:8 and reduced to 1/4), mentioned in *Ashtanga Hridaya*, exhibited the highest antimicrobial activity against *S. aureus*, followed by sample 3, suggesting that the choice of drugs to water ratio and reduction technique can impact the decoction's antibacterial properties.

The zone of inhibition of the samples is as given in Table 4, and Figure 2 shows three trials of 4 samples' antimicrobial activity against *S. aureus* using the agar well diffusion method.

4. Discussions

The study highlights the significant influence of analytical variations on the properties of *Aragwadhadhi Kwatha* emphasises the role of the herb-to-water ratio and reduction technique in determining its physical, chemical, and antimicrobial characteristics. The findings demonstrate that modifications in these parameters result in measurable differences in TDS, pH, and specific gravity, which directly impact the formulation's stability and bioactivity.

One of the most noteworthy findings of the study is related to the antimicrobial efficacy of the prepared samples. Specifically, sample 4, which was prepared with a drug-to-water ratio of 1:8 and reduced to 1/4, exhibited the highest antimicrobial activity against *S. aureus*, reinforcing the idea that standardising these parameters is crucial for optimising therapeutic outcomes. This highlights the importance of specific variations in preparation methods, as they can significantly influence the antibacterial properties of the *Aragwadadhi kwatha*.

The observed variations suggest that the method of reduction plays a critical role in concentrating active constituents, which may enhance the decoction's pharmacological potential. These insights underscore the necessity of establishing a reproducible and evidence-based approach for the preparation of *Aragwadhadhi Kwatha*, ensuring consistency, reliability, and broader clinical applicability in *Ayurvedic* practice.

The study also validates the principles described in *S. Samhita* regarding the relationship between the hardness of drugs, the amount of water used, and the reduction ratio in *kwatha* preparation. According to the *S. Samhita*, for medium-hard drugs, the recommended ratio is 8 times water, reduced to 1/4. Since *Aragwadhadhi Kwatha* is composed of medium-hard drugs, the study findings support this classical guideline, confirming its relevance in modern *Ayurvedic* pharmaceutics. Furthermore, the observed variations suggest that the reduction technique plays a crucial role in concentrating active constituents, potentially enhancing the decoction's pharmacological efficacy.

These findings imply that the preparation process is not uniform, and subtle changes can lead to variations in the therapeutic efficacy of the final product. This underscores the importance of a systematic and standardised approach to pharmaceutical preparation, especially in the context of classical *Ayurvedic* dosage forms like *Kwatha Kalpana*. The study emphasises the critical need for standardisation in various aspects of *Ayurvedic* formulations. Standardisation is essential for ensuring consistency and reproducibility in academics, research, clinical practice, the pharmaceutical industry, and regulatory authorities.

5. Conclusion

Findings in this study revealed that variations in water content and reduction techniques significantly impact

the analytical properties and antimicrobial efficacy of *kwatha Kalpana*. The study reinforces the critical importance of preserving *Ayurvedic* knowledge while aligning it with modern scientific methodologies, paving the way for a more effective and evidence-based approach to healthcare.

Investigating the antimicrobial activity against a broader range of wound pathogens would enhance the clinical relevance of the study. Transitioning from laboratory experiments to clinical trials would help determine the effectiveness of *Aragwadhadhi Kwatha* in actual wound management. Further research can focus on standardisation methods for *Ayurvedic* formulations, which can improve their adoption in clinical practice.

5.1 Limitations of the Study

The study focused exclusively on the antimicrobial activity against *S. aureus*. It would be valuable to broaden the scope to include other common wound pathogens for a more comprehensive assessment. The study's limitation lies in its exclusive assessment of the topical antimicrobial activity of *Aragwadhadhi Kwatha*, while this formulation can also be utilised both internally and externally.

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Author Contribution

Conceptualisation: Nicy and Arun; Methodology, Nicy and Arun; Software, Nicy; Validation, Ramesh and Prajeesh; Data curation, Nicy; Writing- original draft preparation, Nicy; Review and editing, Prajeesh and Nicy; Supervision, Ramesh. All authors have read and agreed to the published version of the manuscript.