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Research Article

COMPARATIVE ANTIBACTERIAL ACTIVITY OF *BHARANGYADI YOGA* VARIANTS W.S.R. TO *SALMONELLA TYPHI*

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ABSTRACT

Salmonella typhi, the causative organism of typhoid fever, remains a major global public health concern. Despite the availability of conventional antibiotics, the emergence of antibiotic-resistant strains of *Salmonella typhi* poses a serious challenge in combating this infectious pathogen. As a result, the exploration of alternative therapeutic options, such as traditional herbal remedies, has gained prominence. *Bharangyadi Yoga* is a combination of specific herbal ingredients, used in traditional medicine system to address various types of fever and related ailments as mentioned in *Sahasrayogam*, has to be prepared and administered in the form of *Kashaya*. In vitro antibacterial activity of *Bharangyadi Kashaya* and *Bharangyadi Arka* was carried out by well diffusion method with different concentrations against *Salmonella typhi* and the area of inhibition of microbial growth is measured. Moderate zone of inhibition was observed in *Arka*, while no zone of inhibition was seen in any of the concentration of *Kashaya*. The antibacterial effectiveness of *Bharangyadi Arka* can be attributed to its volatile and other aqueous extract content, enhanced solubility and release of volatile active constituents. In this study, *Arka* is established to have better antibacterial activity compare to *Kashaya* as a new dosage form. However, Negative antibacterial result of *Kashaya* does not indicate lack of therapeutic effectiveness.

INTRODUCTION

In the quest for effective and safe treatment options against infectious diseases, traditional medicinal systems have played a crucial role in providing valuable insights into herbal remedies with potential therapeutic properties. *Ayurveda*, an ancient Indian system of medicine, is renowned for its utilization of diverse medicinal plants in the formulation of herbal preparations to combat various ailments. Among these formulations, "*Bharangyadi Yoga*" has garnered significant attention for their historical use and potential health benefits.

Salmonella typhi, the causative agent of typhoid fever, remains a major global public health concern, particularly in regions with inadequate sanitation and hygiene conditions.[1] Despite the availability of conventional antibiotics, the emergence of antibiotic-resistant strains of *Salmonella typhi* poses a serious challenge in combating this infectious pathogen. As a result, the exploration of alternative therapeutic options, such as traditional herbal remedies, has gained prominence.

Bharangyadi Yoga is a combination of specific herbal ingredients, used in traditional medicine system to address various types of fever and related ailments as mentioned in *Sahasrayogam*, has to be prepared and administered in the form of *Kashaya*.[2]

Kashaya and *Arka*, derived from Ayurvedic principles, are two distinct herbal formulations. *Bharangyadi Kashaya* is prepared through the decoction of aqueous extract process using specified herbal ingredients.[3] However, its short shelf life and large dosage pose challenges for patient's compliance. While *Bharangyadi Arka* is the herbal distillate of water-based extract derived through a unique process

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in *Arkayantra*.[4] It exhibits a longer shelf life[5], requires smaller dose. [6]

The present study aims to conduct an investigation of *Bharangyadi Yoga* variants - *Bharangyadi Kashaya* and *Bharangyadi Arka*, with a focus on their comparative antibacterial activity against *Salmonella typhi*.

MATERIALS AND METHODS

Materials required for the study such as, Test strain – *Salmonella typhi*, Growth medium – Nutrient agar, Test sample – *Bharangyadi Kashaya* and *Bharangyadi Arka*, Positive control **-** Ampicillin, Negative control - Distilled water, Sterile Test tubes, Petri plates, Sterile graduated micropipettes of 25µl, 30µl, 50µl, 75µl and 100µl, Incubator, Laminar air flow chamber and Hot air oven.

Method followed

Bharangyadi Kashaya (BHK)[3] and *Bharangyadi Arka* (BHA)[4] was prepared by following the general method of preparation as mentioned in classical texts.

Table 1: Ingredients of *Bharangyadi Yoga*[2]

S. no	Ingredients	Botanical source	Part used	Quantity
1	*Bharangi*	*Clerodendron serratum*	Root	1 part
2	*Parpata*	*Fumaria parviflora*	Whole plant	1 part
3	*Shunthi*	*Zingiber officinale*	Rhizome	1 part
4	*Vasa*	*Adhatoda vasica*	Leaf, Stem	1 part
5	*Pippali*	*Piper longum*	Fruit	1 part
6	*Bhunimba*	*Andrographis paniculate*	Whole plant	1 part
7	*Nimba*	*Azadirachta indica*	Stem bark	1 part
8	*Guduchi*	*Tinospora cordifolia*	Stem	1 part
9	*Musta*	*Cyperus rotandus*	Tubers	1 part
10	*Dhanvayas*	*Fagonia cretica*	Whole plant	1 part

The investigation into the comparative in vitro anti-bacterial activity of *Bharangyadi Kashaya* and *Bharangyadi Arka* against *Salmonella typhi* was conducted using the well diffusion method with different concentrations at the S.D.M. Centre for Research in Ayurveda and Allied Sciences in Udupi.

Principle:

The antimicrobial agent is poured in wells that diffuses in the agar medium according to different concentrations and inhibits the growth of the microbial strain. The area of inhibition of microbial growth is measured.

The procedure is divided into three steps,

i. Preparation of Nutrient agar medium
ii. Preparation of inoculum
iii. Well diffusion method and measuring of the zone of inhibition

Preparation of Nutrient agar medium:

Beef extract (1gm), yeast extract (2gm), peptone (5gm) and sodium chloride (5gm) were dissolved in 990ml of distilled water. The pH was adjusted to 7.2 and the volume was made up to 1000ml.

Finally, 15gm agar was added to the media and autoclaved at 121°C for 20 minutes.

Preparation of Inoculum

Salmonella typhi (MTCC 733) was procured from Microbial Type Cultural Collection and Gene Bank (MTCC), IMTECH, Chandigarh.

Loopful of 48 hours old culture from the slants was transferred to sterile saline and mixed well to prepare a homogenous inoculum.

Well diffusion method

The nutrient agar media was cooled to around 45-55°C and 20ml volume of it, was poured into sterile petri plates [Fig.1]. 1ml of the inoculum was immediately added to the plate and swirled for uniform distribution [Fig.2]. Then a total of 7 wells were bored using a sterile borer [Fig.3]. In 5 wells the sample - BHK with varying concentrations of 25µl, 50µl, 75µl, 100µl, and 125µl were dispensed and the other 2 wells were dispensed with 50µl of control (distilled water) and 30µl of standard (ampicillin). The same method was applied to other petri dish utilizing the sample – BHA [Fig.4].

The whole procedure was carried out under aseptic conditions in a laminar air flow unit. The process is repeated for duplicated petri plates and all four plates were incubated overnight at 32°C temperature and observed for 24 hours. On the next day the observation was made for the presence of a zone of inhibition around the wells in both the petri plates and the average reading was considered as the result.

RESULT

The findings from the in-vitro assessment of antibacterial efficacy of *Bharangyadi Kashaya* [Fig.5] and *Bharangyadi Arka* [Fig.6] against *Salmonella typhi*, conducted on distinct petri plates respectively, are presented below.

Table 2: Zone of inhibition of BHK against *Salmonella typhi*

Sample	Volume	Zone of inhibition – (Radius in mm)	
Bharangyadi Kashaya	25 µl	0	0
	50 µl	0	0
	75 µl	0	0
	100 µl	0	0
	125 µl	0	0
Control (Distilled water)	50 µl	0	0
Standard (Ampicillin 1mg/ml)	30 µl	15	15

Table 3 - Zone of inhibition of BHA against *Salmonella typhi*

Sample	Volume	Zone of inhibition – (Radius in mm)	
Bharangyadi Arka	25 µl	0	0
	50 µl	5	5
	75 µl	6	6
	100 µl	6	6
	125 µl	7	7
Control (Distilled water)	50 µl	0	0
Standard (Ampicillin 1mg/ml)	30 µl	15	15

Figures

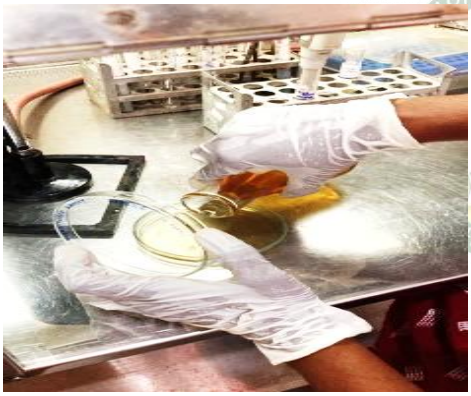

Fig. 1 Pouring of Nutrient Agar Media

Fig. 2 Addition of prepared inoculum

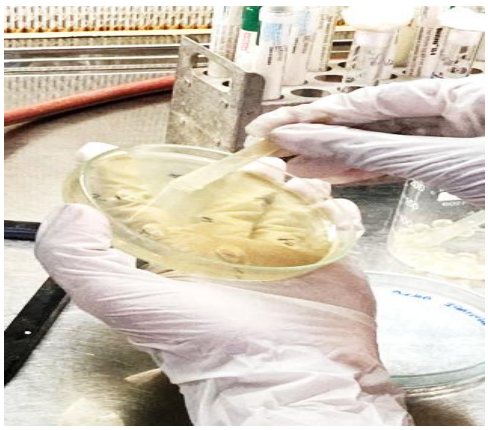

Fig. 3 Wells preparation

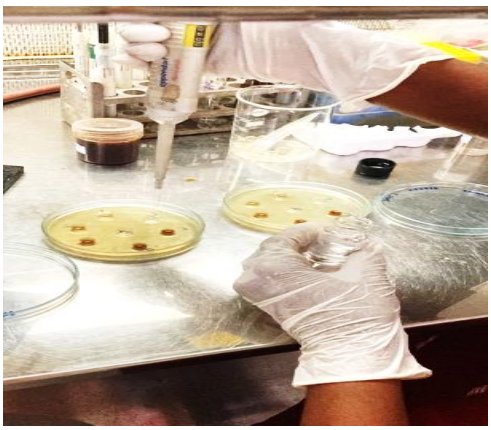

Fig. 4 Dispensing of samples

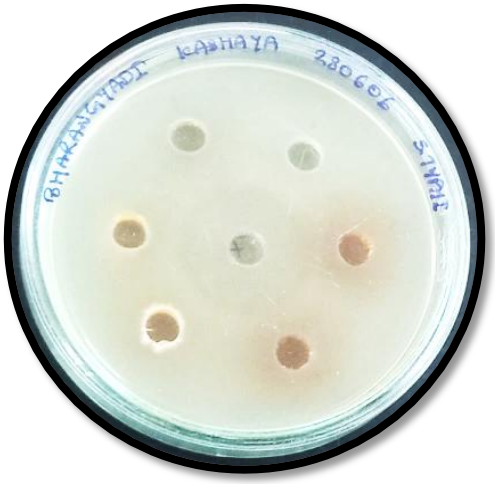

Fig. 5 Antibacterial activity of BHK

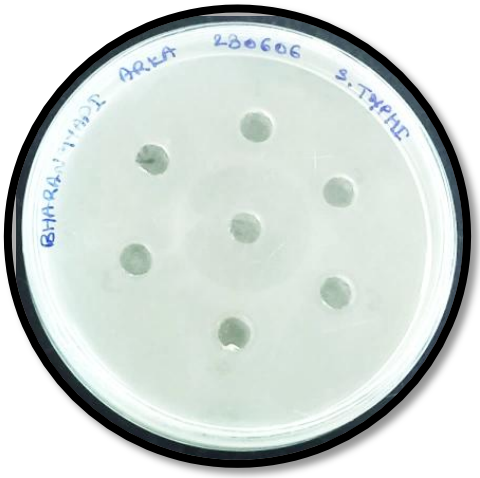

Fig. 6 Antibacterial activity of BHA

DISCUSSION

An antibacterial study involves a systematic scientific investigation or experiment designed to evaluate the activity or efficacy of a substance, such as a drug, extract, or compound, in inhibiting or killing bacteria. The size of the zone of inhibition serves as an indication of the extent of antimicrobial activity demonstrated by the samples against the tested bacteria.

In the current antibacterial study, a comparison of the zone of inhibition between BHK and BHA showed that BHA exhibited a significant zone of inhibition against *Salmonella typhi* organisms at all 50µl, 75µl, 100µl and 125µl concentrations except for 25µl. On the other hand, BHK did not show a zone of inhibition at any concentrations.

The effectiveness of BHA in all tested concentrations can be attributed to its volatile and other aqueous extract content. aqueous extracts generally possess higher solubility, leading to the release of a greater amount of active compounds. This enhanced solubility and release of volatile active constituents contribute to the antibacterial effectiveness of BHA, making it a promising candidate for combating bacterial infections.

While the microbiological results showed reduced antimicrobial activity for BHK compared to BHA, it is important to note that clinical efficacy cannot be solely determined based on these results. Clinical effectiveness depends on a multitude of factors beyond direct antimicrobial activity. Elements like bioavailability, pharmacokinetics, and patient-specific factors also play crucial roles. Although BHK may exhibit lower antimicrobial activity, it could still possess clinical effectiveness due to unexplored factors. These may include potential synergistic interactions with the body's immune system, modulation of host-microbe interactions, or additional therapeutic properties beyond antimicrobial effects.

In addition to its antibacterial potential, the study emphasized several advantages of BHA, making it a promising option in clinical practices. One significant advantage is its extended shelf life, ensuring that the formulation remains stable and effective over an extended period. Furthermore, BHA requires a lower dosage compared to other alternatives, making it more cost-effective and convenient for patients. Lastly, the improved palatability of BHA enhances patient compliance and acceptance, contributing to better treatment outcomes. These factors collectively position BHA as a favourable choice for clinical use, offering both efficacy and practical benefits.

Efforts made to elucidate the potential mechanisms of action of these formulations, we seek to provide scientific evidence for traditionally used and its new dosage form in combating infectious diseases. However, further research and clinical trials are warranted to validate and fully understand the therapeutic benefits of *Bharangyadi Arka* in combating infectious diseases.

CONCLUSION

Based on the study's findings, it can be concluded that *Bharangyadi Arka* demonstrates more potent antibacterial activity compared to *Bharangyadi Kashaya* against *Salmonella typhi*. The enhanced antibacterial efficacy, prolong shelf life, lower therapeutic dose and easier administration of *Bharangyadi Arka* make it a promising candidate for further exploration and potential use in the management of bacterial infections, particularly those caused by *Salmonella typhi*.

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