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SHORT COMMUNICATION

Comparative *In Vitro* Antibacterial Activity of *Mridwikadi Leham* and *Mridwikadi* Syrup Against Selected Respiratory Pathogens

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Abstract

Background: Respiratory infections have been on the rise and are a significant global health concern. *Mridwikadi Leham* (ML) is an *Ayurvedic* formulation used to treat respiratory conditions. **Aim:** This study aims to formulate a syrup from ML and compare ML and *Mridwikadi* Syrup (MS) antibacterial activity against respiratory pathogens like *Klebsiella pneumoniae* and *Staphylococcus aureus.* **Method:** Ethanol extracts of both ML and MS were tested for their antibacterial activity against *K. pneumoniae* and *S. aureus* using the disc diffusion method. The physicochemical properties of the extracts were also evaluated. **Result:** Both ML and MS demonstrated significant antibacterial activity against *K. pneumoniae* and exhibited a zone of inhibition with a diameter of 28mm. Against *S. aureus*, the syrup extract (MS) showed a zone of inhibition with a diameter of 30mm, while the *leham* extract (ML) had a zone of inhibition with a diameter of 25mm. **Conclusion**: The study provides initial evidence that ML and MS have antibacterial effects against *K. pneumoniae* and *S. aureus*. These findings suggest that these *Ayurvedic* formulations may hold promise as potential treatments for respiratory infections. Further research, including clinical studies, is needed to confirm the efficacy of the syrup (MS) in treating coughs and respiratory infections in humans. This study aims to understand *Ayurvedic* remedies in the context of respiratory infections. It opens the door for further investigations and potentially developing new treatments for these common and sometimes severe health issues.

Keywords: *In Vitro* Antibacterial Activity, *Klebsiella pneumoniae, Mridwikadi Leham, Mridwikadi* Syrup, *Staphylococcus aureus*

1. Introduction

Ayurvedic pharmaceuticals play a pivotal role in advancing global acceptance and the evolution of Ayurveda. To ensure the accessibility, palatability, safety, cost-effectiveness, and extended shelf life of *Ayurvedic* products, modern innovation and novel dosage forms are imperative. In light of these considerations, a compelling need exists to transform traditional *Ayurvedic* formulations into contemporary dosage forms. This transformation must adhere to stringent quality standards and undergo rigorous quality control assessments.

Upper Respiratory tract Infections (URIs) rank among the top three outpatient diagnoses nationwide[1]. URIs encompass a range of viral and bacterial infections that result in self-limiting inflammation and swelling of the upper airways, often accompanied by cough[2]. Notably, some causative agents of URIs include *K. pneumoniae* and *S. aureus. K. pneumoniae*, a gram-negative anaerobic bacterium, is ubiquitously found in natural environments such as soil, water, and various surfaces. It also colonizes different mucosal surfaces, including the upper respiratory tract[3]. *S. aureus* is a gram-positive bacterium that causes pulmonary

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infections like pneumonia, empyema, etc. *Kasa* (cough) is one of the presentations of respiratory tract disease. *Pittaja Kasa* presents with excessive yellowish mucus, indicating that the infection has penetrated more deeply into the respiratory system, as is seen in pneumonia. *Mridwikadi Leham* is mentioned in *ashtanga hridayam Kasa*chikitain the context of *paitika Kasa*[4]. Keeping this in view, *mridwikadi* syrup and *leham* forms were prepared in the present study and subjected to further analysis.

2. Materials and Methods

2.1 Sample Preparation

The ingredients of *Mridwikadi Leham* and syrup were collected from a GMP-certified pharmacy 'Amrita Life'. The voucher specimen was preserved for further analysis. The samples were prepared at the Amrita School of Ayurveda. Ingredients and the quantity used for *Mridwikadi Leham* and syrup are mentioned in Table 1.

2.1.1 Preparation of Mridwikadi Leham

Powdered *Piper longum*, dried *Vitis vinifera* and sugar were added into a mixer and made into a powder. The powder was ground in the motor by gradually adding honey to gain a soft homogeneous paste. After that, the *leham* was transferred to a glass container.

2.1.2 Preparation of Mridwikadi Syrup

The *draksha* (dry raisin) was rinsed and transferred into a vessel that contained 960 ml of water. This was boiled and reduced to 120 ml by adding a fine powder of *pippali* at the final stage. After filtration, 65% of sugar[5] was added, and volume was adjusted to the previous level by mild heat, and again filtration was done. At last, honey was added to the lukewarm solution and stored in a glass container at room temperature.

Table 1. Ingredients and their quantity used in *leham* and syrup

Ingredients	Botanical name	Part used	*Leham*	Syrup
Draksha	*Vitis vinifera*	Fruit	60g	60g
Pippali	*Piper longum*	Fruit	15g	15g
Sugar	*Saccharum officinarum*	Stalk	96g	78g
Honey	-	-	85g	85g

2.2 Analysis

Basic physicochemical characteristics of the finished product were analysed in the Quality Control Lab of Rasa Shastra and Bhaishajya Kalpana, Amrita School of Ayurveda. An antimicrobial study was conducted at the Amrita Centre for Advanced Research in Ayurveda (ACARA).

- Physicochemical parameters like total solid content (Brix), pH, LOD, specific gravity, and total sugar were evaluated for both formulations. The pH value of a liquid was determined using a pH meter. LOD was done using the Shimadzu moisture balance apparatus. Brix value was measured using a Hand Refractometer. Specific gravity is directly noted using a hydrometer.
- Antimicrobial study was conducted using the disc diffusion method and the samples taken were the ethanolic extract of *leham* and syrup. Nutrient agar media plates were prepared, and the plate was inoculated with the test microorganism (bacteria) by spread plate method. Agar wells were made approximately 10mm in diameter, and samples under test were filled and marked on the concerned wells. The samples under test were placed so that the antibacterial zone of the added drug was easily visible on the agar surface and distributed evenly so that they were no closer than 24 mm from each other, centre to centre. The plates under test were incubated for 24 hours at 37°C inside a bacteriological incubator. After incubation, plates under test were examined. The resulting zones of inhibition were uniformly circular with a confluent lawn of growth. The diameters of the zones of complete inhibition were Measured and noted.

3. Results

The time required for syrup preparation was 1 hour 15 minutes, while the time required for preparing *leham* was 3 hours 10 minutes. The yield of *leham* and syrup was 291g and 275g, respectively.

3.1 Organoleptic Characters

The organoleptic characters of *leham* and syrup are illustrated in Table 2.

3.2 Physicochemical Analysis

The physicochemical analysis of *leham* and syrup is illustrated in Table 3.

Table 2. Organoleptic characters of *leham* and syrup

Organoleptic characters	*Leham*	Syrup
Colour	Dark brown	Reddish brown
Odour	Pleasant	Pleasant
State	Semi-solid	Liquid
Taste	Sweet and acrider	Sweet and less acrid

Table 3. Physico-chemical characters of *leham* and syrup

Physico-chemical	*Leham*	Syrup
pH (5%aq solution)	4.75	4.81
LOD at 110°C	1.06	-
Brix	82	66
Specific gravity	-	1.320
Total sugar (%)	52.79	52.38

Table 4. Zone of inhibition (diameter) of the syrup and *leham* extract

Plate	Zone of Inhibition (diameter) Syrup extract	Zone of Inhibition (diameter) *Leham* extract
Plate 1 - *K. pneumoniae*	28mm	28mm
Plate 2 - *S. aureus*	30mm	25mm

3.3 Antibacterial Study

Figure 1 and Figure 2, depict the antibacterial study findings for *leham* and syrup against *S. aureus* and *K. pneumoniae*. Table 4 summarises the zones of inhibition exhibited by both *leham* and syrup formulations towards *S. aureus* and *K. pneumoniae*.

4. Discussion

4.1 Organoleptic Characters

The *leham* was dark brown with a pleasant odour, sweet and acrider in taste, whereas the syrup was sweeter and less acrid, making it more palatable than *leham*. The liquid state of syrup is more convenient to intake than the semi-solid *leham*, especially in children; this makes the syrup more acceptable.

4.2 Physico-chemical Characters of *Leham* and Syrup

Both the preparation has acidic pH indicates a stable product and inhibits microbial growth, ensuring more

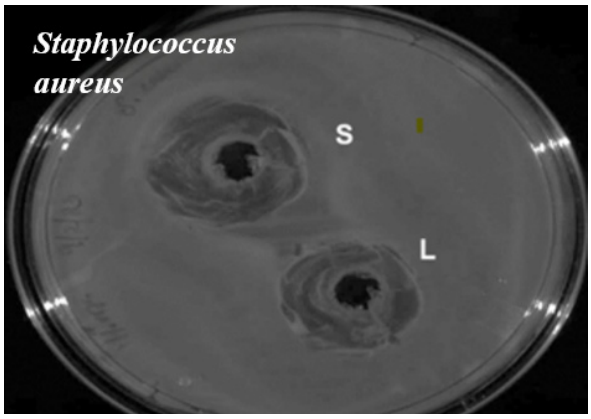

Figure 1. Antibacterial activity of *Leham* (L) and Syrup (S) extract in *Staphylococcus aureus*.

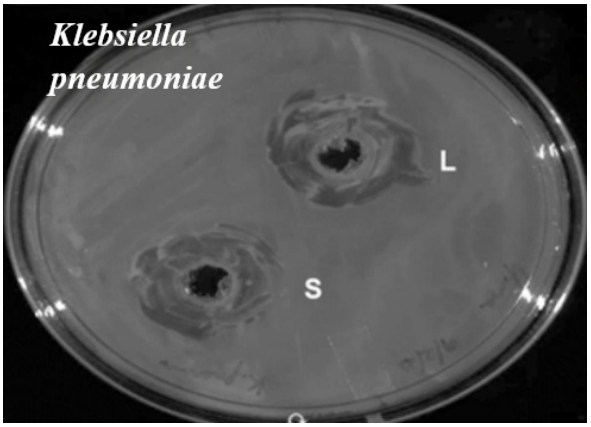

Figure 2. Antibacterial activity of *Leham* (L) and Syrup (S) extract in *Klebsiella pneumoniae*.

shelf life. The *leham* has a good LOD value, which implies the product is stable. *Leham* shows a higher brix value than syrup, indicating more concentration in *leham*. The specific gravity of syrup seems to be within the limit. Total sugar estimation helps to know the amount of total sugar in the sample or monitors added sugar levels. *Leham* shows a slight increase in total sugar content than syrup and both were found to be within normal limits.

4.3 Antibacterial Study

The *leham* and syrup showed the same antibacterial effect against the bacteria *K. pneumoniae*, and the syrup extract showed more effect on bacteria *S. aureus* than the *leham* extract. The observed difference in antibacterial efficacy between the syrup and *leham* extracts against *S. aureus* could be attributed to some factors. Firstly, the syrup extract might contain elevated

concentrations of specific antibacterial components that exhibit heightened potency against *S. aureus*, compared to the *leham* extract. Additionally, differences in the extraction procedures employed during syrup preparation, particularly the application of moderate heat, could contribute to enhanced extraction of active constituents, there by augmenting the antibacterial activity of the syrup extract.

5. Conclusion

The transformation of *Mridwikadi Leham* into syrup form has addressed the challenges related to taste and patient acceptance, particularly in pediatric cases. This innovative approach not only facilitates consumption but also maintains the high quality and efficacy of the medicine. Including 65% sucrose in the syrup formulation and its high osmotic pressure acts as a natural preservative, enhancing shelf life while preventing the growth of bacteria, fungi, and mold.

Moreover, our study has provided compelling evidence of the antibacterial properties of both *Mridwikadi Leham* and the newly developed syrup. Specifically, they exhibited antibacterial activity against *K. pneumoniae* and *S. aureus*, two significant pathogens associated with respiratory infections.

However, it is essential to underscore that this study represents a preliminary investigation stage. Further research is warranted to validate the syrup's efficacy in treating cough-related conditions. Clinical studies will be instrumental in confirming its therapeutic benefits and establishing its role as a valuable addition to managing respiratory ailments. These future studies will contribute significantly to the broader field of *Ayurvedic* medicine and its potential to address contemporary healthcare challenges.

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