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## EVALUATION OF ANTI-BACTERIAL ACTIVITY OF LAVANGADI VATI AND ITS MODIFIED SUSPENSION FORM WITH HPTLC FINGERPRINTING

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### ABSTRACT

The present study carried out to investigate the antibacterial potential of *lavangadi vati* and its modified suspension dosage form, which is effectively giving in infectious respiratory conditions. The aim of the study is to determine its zone of inhibition and minimum inhibitory concentration against *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus mutans* and standardisation by HPTLC fingerprinting. Antibacterial activity was performed by cup plate method and MIC method as a comparison between the both dosage form against different bacterial strains and results were compared with that of standard, Ciprofloxacin. In case of *lavangadi vati*, the growth of inhibition ranges from 10 to 20mm for all the sensitive bacteria whereas that of *lavangadi vati* suspension ranges from 8mm to 12mm. *Lavangadi vati* shown its minimum inhibitory concentration of 12.5 µg/ml against *K. pneumonia*, 25 µg/ml against *S. aureus*, 12.5 µg/ml against *S. mutans*; whereas *lavangadi vati* suspension shown its minimum inhibitory concentration of 25 µg/ml against *K. pneumonia*, 50 µg/ml against *S. aureus* and 3.12 µg/ml against *S. mutans*. On HPTLC of the suspension, twelve spots were visualized at ultraviolet 254nm, nine spots at ultraviolet 366nm and 7 spots at visible light.

**Keywords:** *Lavangadi vati*, minimum inhibitory concentration, Fourier transform infrared, anti-bacterial, suspension

## INTRODUCTION

In the era of infectious diseases outbreaks, the scientific society constantly in exploration of newer compounds to ward of this disease and to handle the drug resistant strains of microbes. Herbal medicines are a huge compendium of different phytoconstituents which are serving as a double-sided coin as a prevention and cure of disease. Among them *lavangadi vati* is a regularly practising polyherbal formulation with regards to *pranavaha srotovikaras* (respiratory system disorders) especially *kasa* (cough) and *swasa* (asthma) associated with *pratisyaya* (sneezing). This formulation in solid dosage form already proven its antibacterial activity on *haemophilus influenza* performed by well diffusion method [1].

According to WHO respiratory infectious disease ranking first place in ambulatory consultations, hospitalization and death. Seasonal form of appearance and high infective form of bacteria are responsible for major acute respiratory infections, mostly affecting the elderly and paediatric age groups [2]. *Staphylococcus aureus* (gram +ve), *Klebsiella pneumonia* (gram -ve), *Streptococcus mutans* (gram +ve) are bacteria causing upper and lower respiratory tract infections has been analysed for the current study. The phytochemical constituents in a medicine effectively combat or prevent a disease by their

antioxidant effect. In the current study along with *lavangadi vati* and its modified suspension form also has been analysed for the antibacterial activity, since liquid dosage form helps more in bioavailability, easy administration, patient compliance and acceptability compared to solid dosage form for all age groups. Chromatographic fingerprints like HPTLC aid in the identification and authentication of the phytoconstituent concentration found in a herbal medication.

## MATERIALS AND METHODS

### Preparation of *lavangadi vati*

The raw materials are collected from GMP certified KLE Ayurveda Pharmacy, Belagavi and authenticated at AYUSH approved drug testing laboratory B M Kankanawadi Ayurveda Mahavidyalaya, Belagavi. *Lavangadi vati* contain 40gms each of *lavanga*, *maricha*, *vibhitaka* and 120gms *khadira* in fine powder form. It was given *bhavana* (trituration) with 480ml *babbula kashaya* (decoction) till the attainment of pill roll consistency and dried under shade reddish brown coloured tablets were obtained [3].

### Preparation of *lavangadi vati* suspension

For the easiness of preparation *lavangadi vati* was powdered. 20gm each of *lavangadi vati* in powder form, mannitol was mixed in a mortar and pestle and then the Sodium CMC solution (4%) was added and mixed

homogenously to form a colloid form. The preservatives like methyl paraben (0.05 gm), propyl paraben (0.01gm) and sodium benzoate (0.5gm) were added and mixed homogenously after dissolving in 10ml portable water. The *final lavangadi vati* suspension of brown colour was kept in an air tight container.

### Phytochemical screening

Various phytochemical screening was performed using hydroethanolic extract of *lavangadi vati* to detect the presence of phytoconstituents [4].

### Antibacterial assay

*Lavangadi vati* is mainly given for respiratory disorders especially infective cough. Hence organisms responsible for upper and lower respiratory tract infection commonly found in local community like *Klebsiella pneumonia* (ATCC NO-29665), *Staph aureus* (ATCC NO-12598) and *S. mutans* (ATCC NO- 25175) were selected for study. The bacterial strains obtained from Maratha Mandal Dental College; Belagavi were used for evaluating antimicrobial activity. The anti-microbial study done by Agar Well Diffusion Method and MIC Determination (Macro broth dilution method) [5]. Both the method the control drug used was Ciprofloxacin.

### Agar Well Diffusion Method

The brain heart infusion agar plates were used after being warmed to room temperature

The colonies are moved from the tubes to the plates using a loop or a wipe.

Within 15 minutes of correcting the inoculum to a McFarland 0.5 turbidity standard, extra inoculum was removed by rotating a sterile cotton swab on the tube wall above the liquid and the entire surface of the agar plate was swabbed three times. Steer clear of causing aerosols by not contacting the Petri plate's sides. Before drilling wells, the inoculated plate was allowed to stand for at least 3 minutes but no more than 15 minutes. Stock solution made by weighing 10 mg of sample separately and dissolving it in 1 mg of Dimethyl Sulfoxide. We heated a hollow tube with a 5 mm diameter. Press it onto the infected Agar plate above and take it off right away by forming a well in the plate. Similarly, create five wells on each plate. 75, 50, 25, 10, 5, and 5 ml of stock solution were added to each well using a micropipette. One other well-designed control drug, ciprofloxacin, is located on one side of the petri dish containing both *lavangadi vati* and *lavangadi vati* solution. After applying the chemical, the plates were incubated for 15 minutes. Plates should be stacked no higher than five high and inverted. Then, it was placed in an incubator for 18 to 24 hours at 37°C. Only when the growing grass is confluent or almost confluent are plates read. Holding the measuring gadget, the inhibitory zone's diameter is measured to the nearest whole millimetre.

**MIC Determination (Macro broth dilution method)**

Nine dilutions of each drug using BHI (Brain Heart Infusion) are required for the MIC determination. The initial tube contained 380 microliters of BHI (Brain Heart Infusion) broth with 20 microliters of medication added. The following 9 tubes each received 200 microliters of BHI (Brain Heart Infusion) broth for dilutions. Then 200 microliters were transferred from the first tube to the first tube that contained 200 microliters of BHI (Brain Heart Infusion) broth. This was regarded as a  $10^{-1}$  dilution. To create a  $10^{-2}$  dilution, 200 microliters from the tube with  $10^{-1}$  dilution were transferred to the second tube. For each medication, the serial dilution process was repeated up to a  $10^{-9}$  dilution. 5 microliters of the needed organisms' stock cultures were taken and added to 2 millilitres of BHI (Brain Heart Infusion) broth. Each serially diluted tube received 200 microliters of the mentioned culture suspension. After 24 hours of incubation, the tubes were checked for turbidity, and the results were recorded.

**High Performance thin layer chromatography (HPTLC) of *lavangadi vati* & suspension**

For HPTLC, 2g of alcoholic extract of *Lavangadi vati* and suspension were extracted with 25ml of distilled water on a boiling water bath for 25 min, filtered and concentrated. Chromatography was

performed by spotting extract and formulation on precoated silica gel aluminium plate 60F254(5cm x 10 cm with 250 $\mu$ m thickness) using Camag linomat IV sample applicator and 25  $\mu$ l Hamilton syringe. The sample in the form of bands length 8.0 mm, spotted 15mm from the bottom, 20mm apart, at a constant application rate 15 $\mu$ l/s through nitrogen aspirator. Plates were developed mobile phase consisting of Toluene: Ethyl acetate: Acetic acid (8:2:0.1)). subsequent to the development, HPTLC studies were carried out. 10  $\mu$ l of the test solution was applied on aluminium plate precoated with silica gel 60 F254 of 0.2 mm thickness and plate was developed in Toluene: Ethyl acetate: Acetic acid in a ratio of 8:2:0.1. The plate was dried and scanned at 254nm, 366nm and visible light. Densitometric scanning was performed on CAMAG TLC scanner with winCATS software [6].

**RESULTS****Phytochemical screening**

Phytochemical examination of hydro-alcoholic extract of *lavangadi vati* and found the presence of carbohydrate, reducing sugar, pentose sugar, hexose sugar, protein, tannin, alkaloid, anthraquinone glycoside, saponin, steroid, flavonoid, monosaccharide and amino acid as described in literatures [7, 8].

**Antibacterial activity**

The antibacterial activity assessed in terms of zone of inhibition and minimum inhibitory concentration. The results are presented in the (Tables 1 to 2). The antibacterial activity of both samples linearly increased with increase in concentration of *lavangadi vati* and *lavangadi vati* suspension(( $\mu\text{g/ml}$ ). Antibacterial study done by Cup plate/Agar well diffusion method depends on the diffusion of compound in the medium and in Macro Broth Dilution /MIC Method depend on the direct interaction between compound and bacteria (Figure 1-4).

### HPTLC Finger printing

HPTLC has become the most potent technique in fingerprint analysis approach for quality control of herbal medicines because of its simplicity, flexibility and reliability. The qualitative, semi-quantitative and quantitative phytochemical analysis of the herbal drugs and formulations can be interpreted. The alcoholic extract of *lavangadi vati* and *lavangadi vati* suspension has been extracted and used for this technique (Figure 5-8).



(a) *K. pneumonia* (b) *S. aureus* (c) *S. mutan*

Figure 1: Agar Well Diffusion of *Lavangadi Vati*



(a) *K. pneumonia* (b) *S. aureus* (c) *S. mutan*

Figure 2: MIC of *Lavangadi Vati*



(a) *K. PNEUMONIA* (b) *S. AUREUS* (c) *S. MUTAN*

Figure 3: Agar Well Diffusion of *Lavangadi Vati* Suspension



(a) *K. pneumonia* (b) *S. aureus* (c) *S. mutans*

Figure 4: MIC of Lavangadi Vati Suspension

Table 1: Result of Disc Diffusion Test of lavangadi vati and lavangadi vati suspension

S. No.	Samples	75 µl/ml	50 µl/ml	25 µl/ml	10 µl/ml	5 µl/ml	Ciprofloxacin
	<b>Klebsiella</b>						
1	Lavangadi Vati	15mm	13mm	10mm	R	R	25mm
2	Lavangadi Vati Suspension	10mm	08mm	R	R	R	23mm
	<b>Staph aureus</b>						
1	Lavangadi Vati	16mm	14mm	12mm	R	R	28mm
2	Lavangadi Vati Suspension	12mm	10mm	R	R	R	30mm
	<b>S. mutans</b>						
1	Lavangadi Vati	20mm	18mm	12mm	10mm	R	35mm
2	Lavangadi Vati Suspension	12mm	R	R	R	R	38mm

Note: S-Sensitive; R-Resistant

Table 2: Result of MIC Method of lavangadi vati and Lavangadi vati suspension

S. No.	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
	<b>Klebsiella</b>										
1	Lavangadi Vati	S	S	S	S	R	R	R	R	R	R
2	Lavangadi Vati Suspension	S	S	S	R	R	R	R	R	R	R
3	Ciprofloxacin	S	S	S	S	S	S	S	S	S	R
	<b>Staph aureus</b>										
1	Lavangadi Vati	S	S	S	R	R	R	R	R	R	R
2	Lavangadi Vati Suspension	S	S	R	R	R	R	R	R	R	R
3	Ciprofloxacin	S	S	S	S	S	S	S	S	R	R
	<b>S. mutans</b>										
1	Lavangadi Vati	S	S	S	S	R	R	R	R	R	R
2	Lavangadi Vati Suspension	S	S	S	S	S	S	R	R	R	R
3	Ciprofloxacin	S	S	S	S	S	S	S	R	R	R

Note: S-Sensitive; R-Resistant

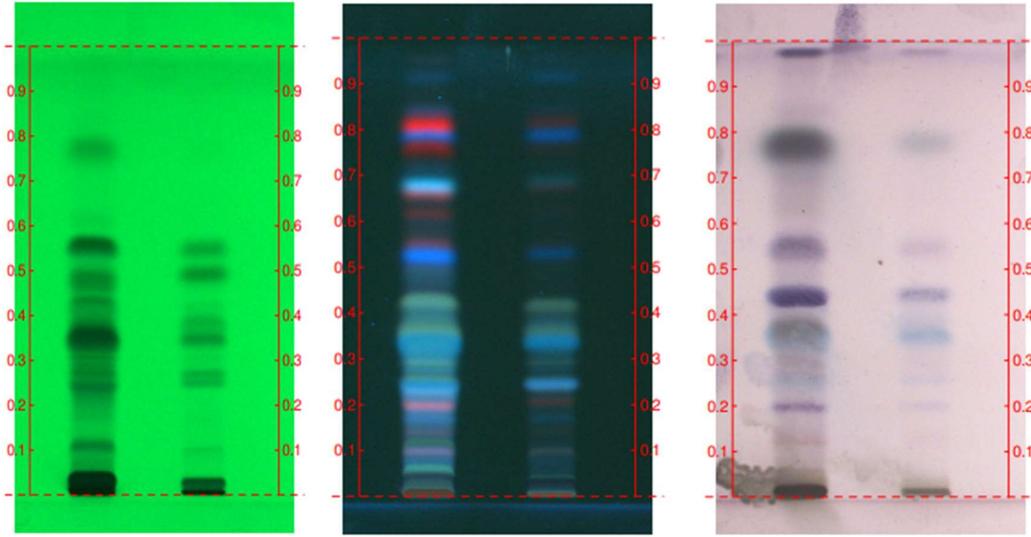


Figure 5: HPTLC of *Lavangadi vati* (left) & *Lavangadi vati* Suspension (right) at 254nm, 366nm and visible light

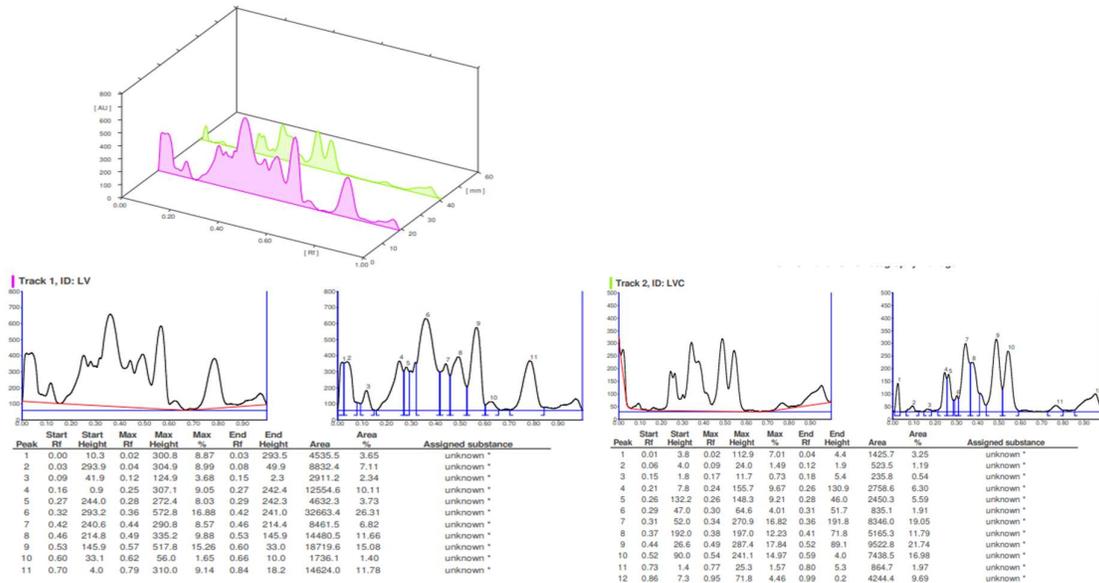


Figure 6: Densitogram of *lavangadi vati* and *lavangadi vati* suspension at 254nm

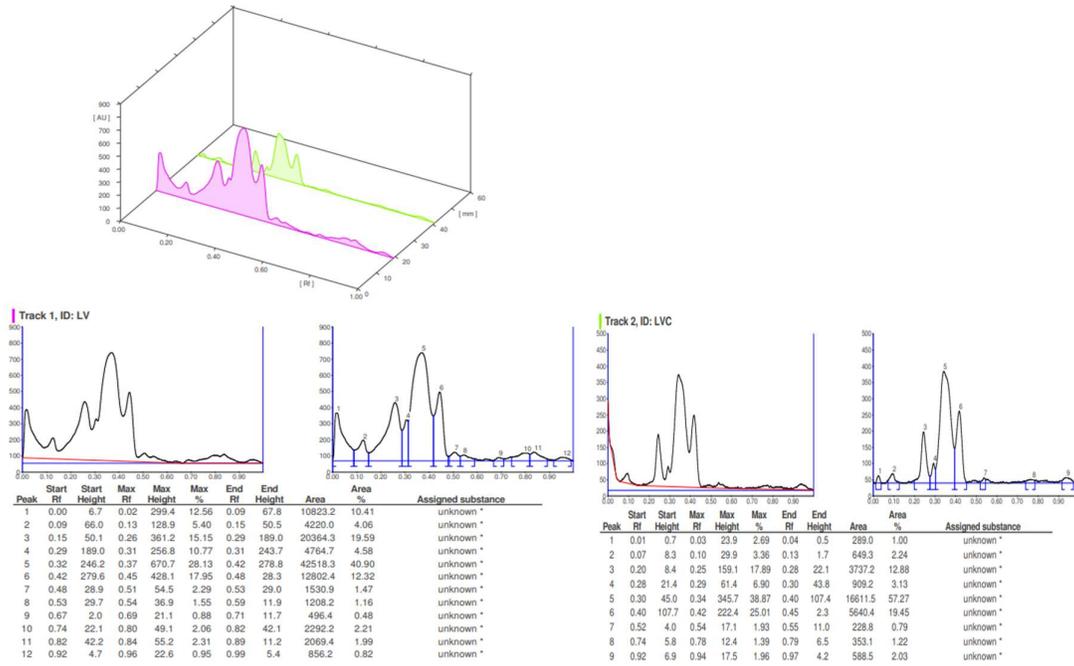


Figure 7: Densitogram of lavangadi vati and lavangadi vati suspension at 366nm

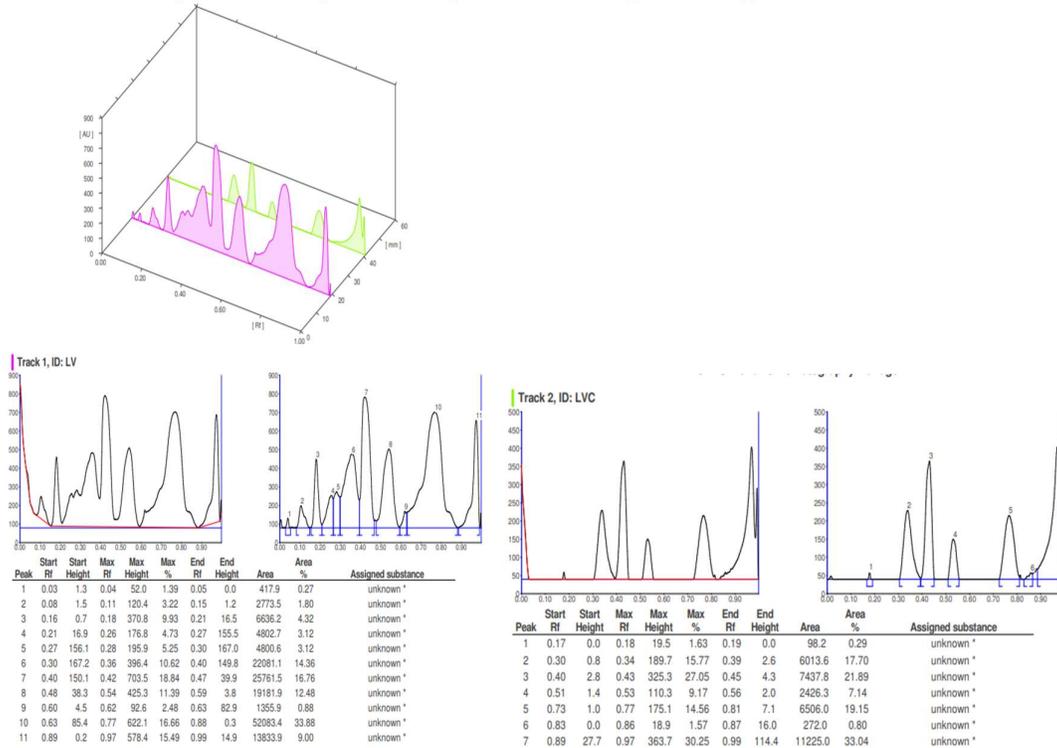


Figure 8: Densitogram of lavangadi vati and lavangadi vati suspension at visible light

**DISCUSSION**

The phyto chemical screening shows the presence of carbohydrate, reducing sugar, pentose sugar, hexose sugar, protein, tannin,

alkaloid, anthraquinone glycoside, saponin, steroid, flavonoid, monosaccharide and amino acid in *lavangadi vati*.

**Anti-bacterial activity**

Both *lavangadi vati* and *lavangadi vati* suspension shown inhibition for *Klebsiella pneumonia* (gram -ve), *Staphylococcus aureus* (gram +ve) and *Streptococcus mutans* (gram +ve). As compared to standard drug Ciprofloxacin, the results revealed that both shown activity in all the three microorganisms. In case of *lavangadi vati*, the growth of inhibition ranges from 10 to 20mm for all the sensitive bacteria

whereas that of *lavangadi vati* suspension ranges from 8mm to 12mm. *Lavangadi vati* shown its minimum inhibitory concentration of 12.5 µg/ml against *K.pneumonia*, 25 µg/ml against *S.aureus*, 12.5 µg/ml against *S.mutans*; whereas *lavangadi vati* suspension shown its minimum inhibitory concentration of 25 µg/ml against *K.pneumonia*, 50 µg/ml against *S aureus* and 3.12 µg/ml against *S mutans* (Figure 9-14).

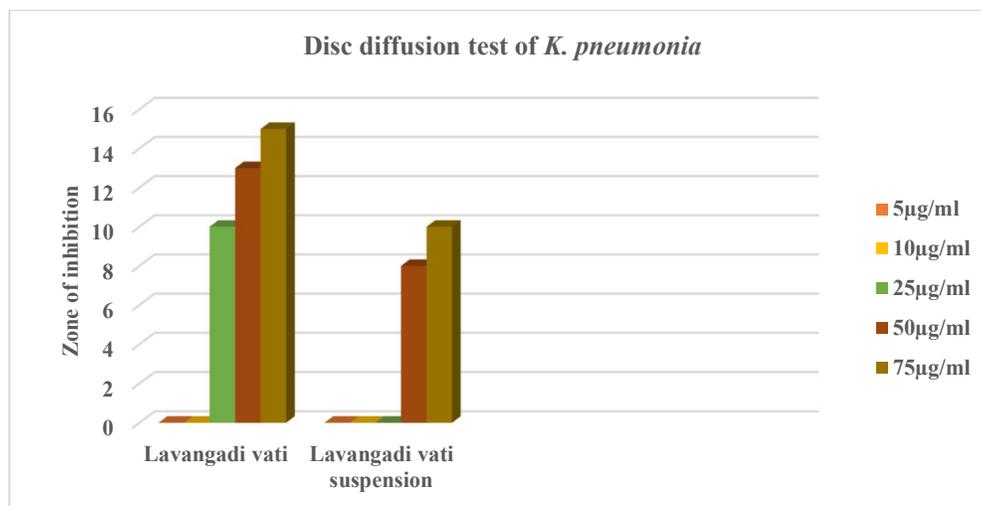


Figure 9: Disc diffusion Test of *K. Pneumonia*

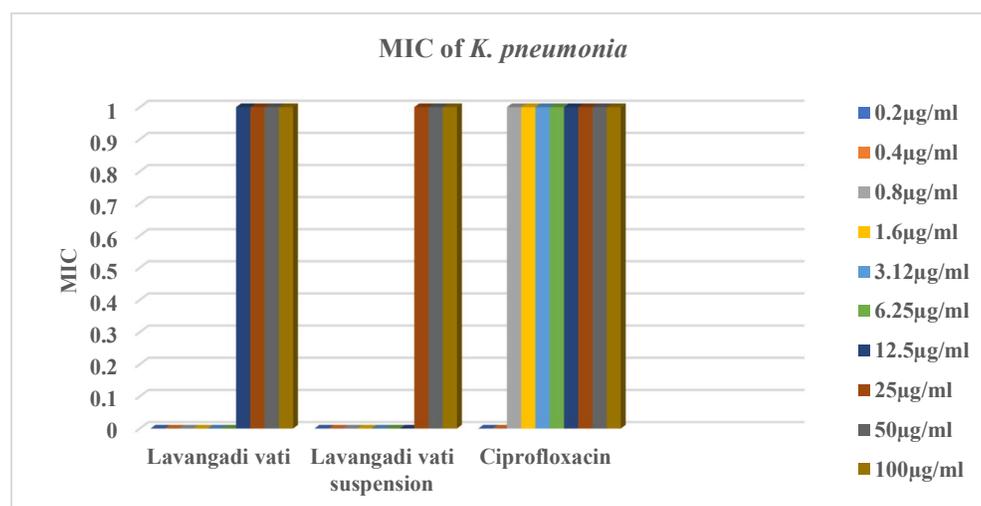


Figure 10: MIC test of *K. Pneumonia*

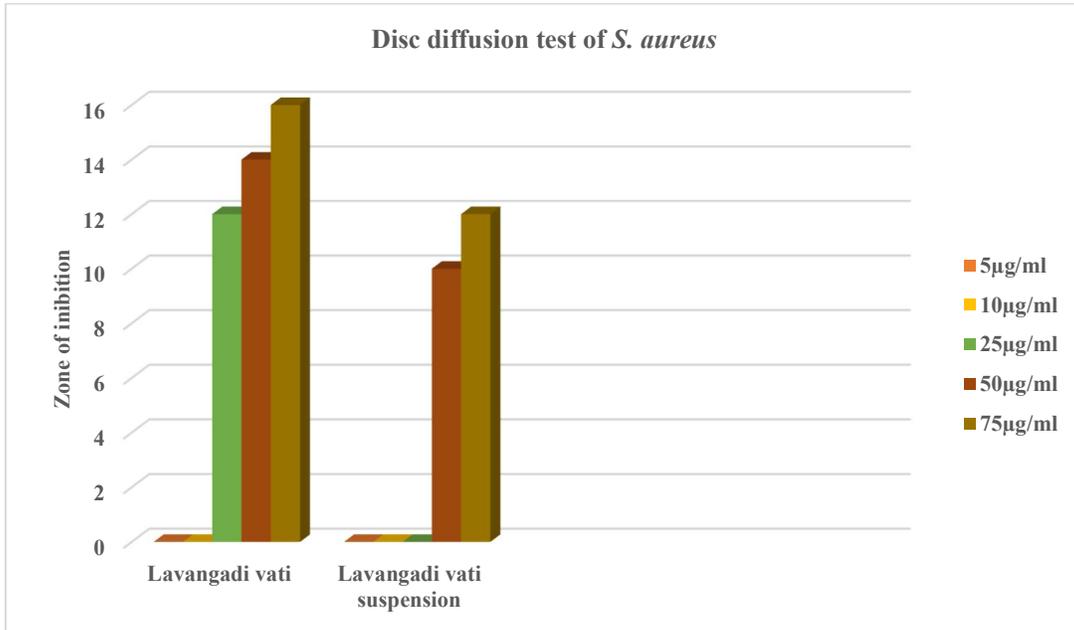


Figure 11 Disc diffusion test of *S. aureus*

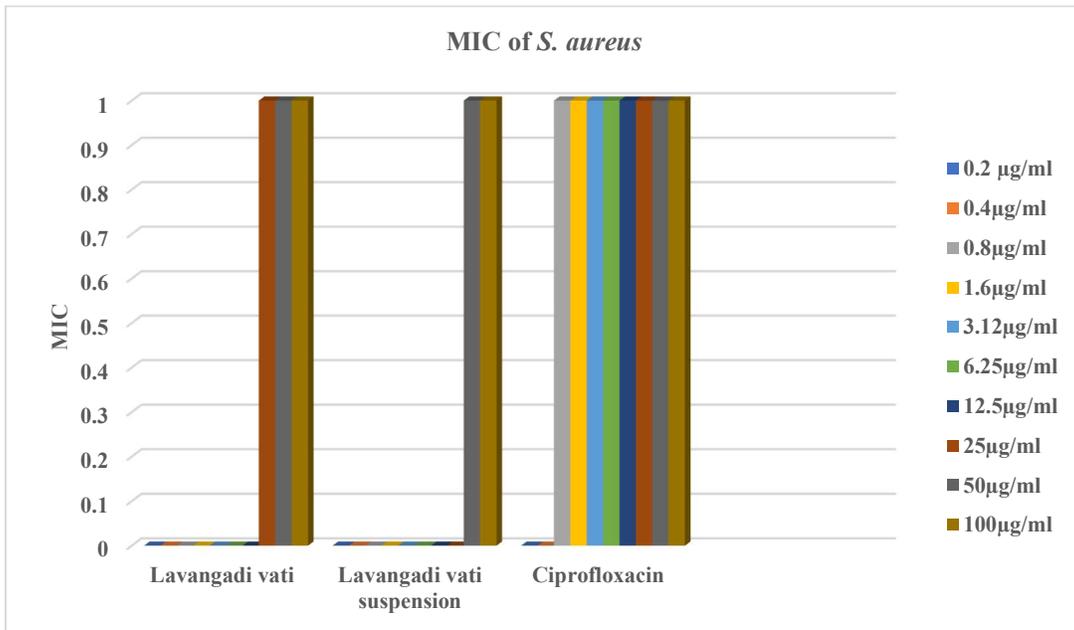
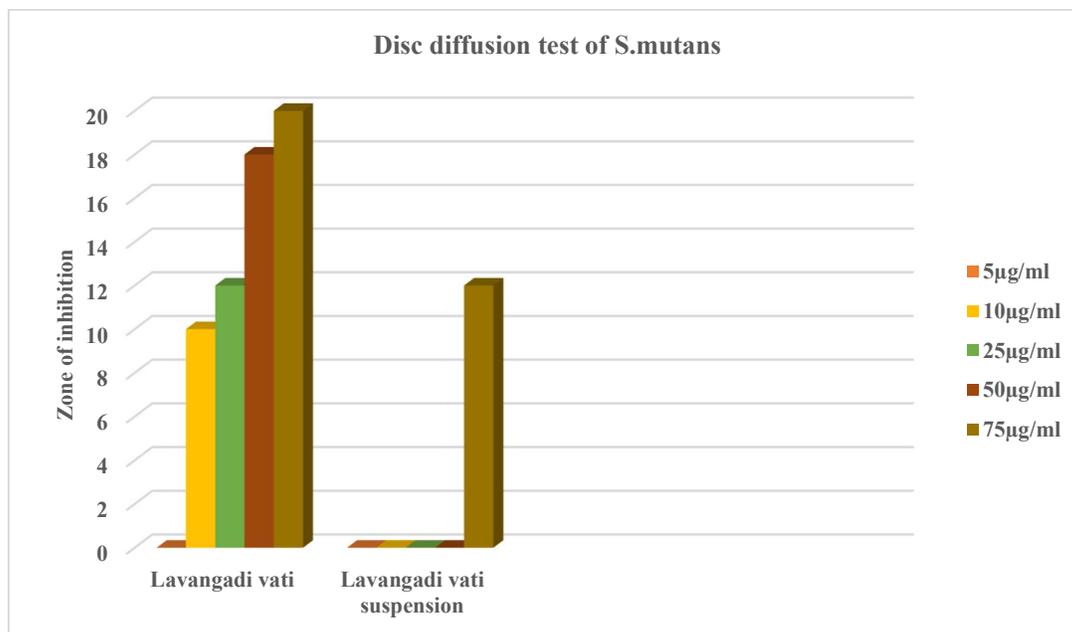
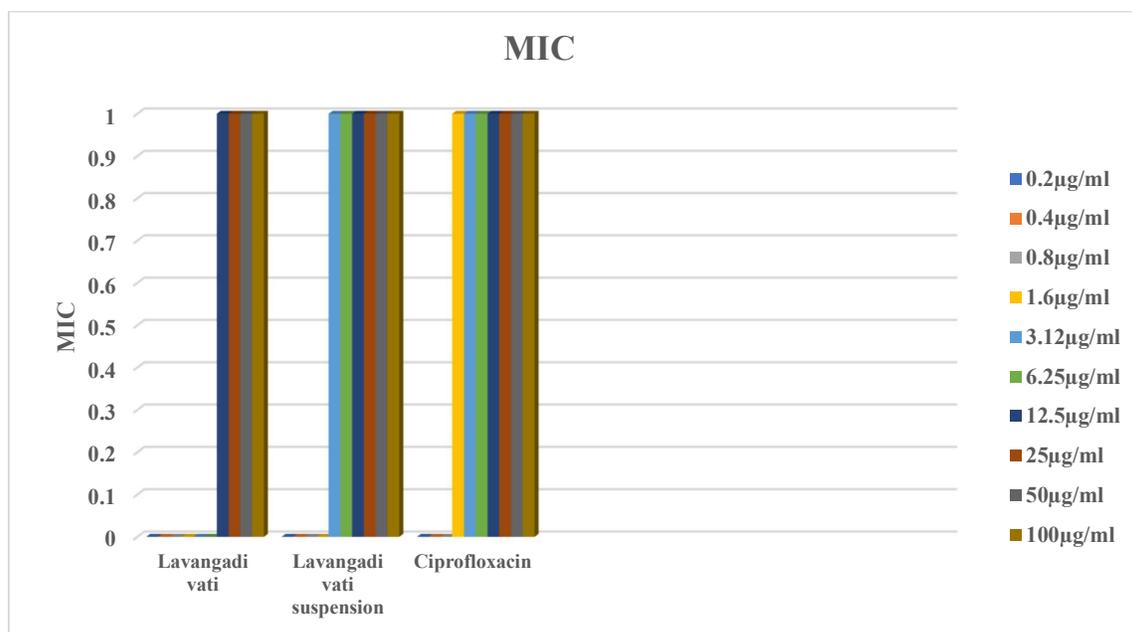


Figure 12: MIC test of *S. aureus*

Figure 13: Disc diffusion test of *S. Mutans*Figure 14: MIC test of *S. mutans*

All the ingredients of *lavangadi vati* already proven antibacterial activity, antioxidant, anti-asthmatic and expectorant activity. *Faujdar et al* conducted the antibacterial activity of *lavanga* against gram negative uropathogens and 9-16 mm inhibitory zone shown for *K.pneumoniae*.

[9] *Coralie Pavesi et al* studied the anti-fungal and antibacterial activity of eugenol which is the main phytoconstituent of *lavanga* and proven 3.8 +0.7mm inhibitory zone for *S.aureus*. The hydrophobicity of the volatile oil present in *lavanga* help in the partition of the lipids and disrupt the outer

membrane of gram-positive and gram negative bacteria and also enhance the protein leakage of cell membrane, thereby further growth of the bacteria is stopped.

[10] Pavithra et al conducted the antibacterial activity of black pepper and analysed the minimum inhibitory concentration ranged between 62.5 to 125 ppm for gram positive bacteria and 125 to 250ppm for gram negative bacteria. The mechanism of antibacterial activity also determined by membrane leakage of UV<sub>260</sub> and UV<sub>280</sub> absorbing material spectrophotometrically and concluded that leakage of intracellular material might be the cause of cell death in bacteria due to loss of control over cell membrane permeability.

[11] Dharmaratne et al studied the antibacterial activity of *terminalia bellarica* fruit extract and interpreted the methanol and aqueous extract of the fruit having non-toxic polar antibacterial compounds with high thermal stability, which inhibits the growth of *S.aureus*.

[12] Negi et al conducted the invitro antimicrobial activity of *acacia catechu* and analysed that the maximum zone of inhibition (20mm) was found to be exhibited against *S.aureus*.

[13] Vijayasanthi et al evaluated the antibacterial potential of *acacia arabica* and concluded most susceptible organism was *S.aureus* showed inhibition zone of 15.5mm and MIC value of 0.125 mg/ml. [14] Based on the results and the recent research studies, the

antimicrobial activity of *lavangadi vati* and *lavangadi vati* suspension were proven.

HPTLC fingerprinting by interpreting the bands formed and densitogram of *lavangadi vati* shown 11 bioactive compounds with Rf value 0.03, 0.08, 0.15, 0.27, 0.29, 0.42, 0.46, 0.53, 0.60, 0.66, 0.84 at short wave, 12 bioactive compounds with Rf value 0.09, 0.15, 0.29, 0.31, 0.42, 0.48, 0.53, 0.59, 0.71, 0.82, 0.89, 0.99 at long wave and 11 bioactive compounds with Rf value 0.05, 0.15, 0.21, 0.27, 0.30, 0.40, 0.47, 0.59, 0.63, 0.88, 0.99 at visible light whereas *lavangadi vati* suspension shown 12 bioactive compounds with Rf value 0.04, 0.12, 0.18, 0.26, 0.28, 0.31, 0.36, 0.41, 0.52, 0.59, 0.80, 0.99 at short wave, 9 bioactive compounds with Rf value 0.04, 0.13, 0.28, 0.30, 0.40, 0.45, 0.55, 0.79, 0.97 at long wave and 7 bioactive compounds with Rf value 0.19, 0.39, 0.45, 0.56, 0.81, 0.87, 0.99 at visible light. By analysing the Rf value both *lavangadi vati* and *lavangadi vati* suspension has shown similarity in peaks under UV 254nm, 366nm and visible light, which was shown as band and densitograms in (Figure 3-6). It has been proven that there was no interaction between the *lavangadi vati* and its excipients and a stable suspension can be developed.

## CONCLUSION

As per the results, present study can be concluded as both *lavangadi vati* and its

modified suspension form can be used as an effective antibacterial compound for upper and lower respiratory tract infection, which helps to reduce the cost and the risk of antibiotic consumption. Further in vivo studies and structural analysis of the phytoconstituents quantitatively needed for future studies.

### Conflict of Interest

No any conflict of interest

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