**Title-The antitubercular activity of *lavangadi vati* and its modified suspension form -An invitro study with FTIR characterization.**

**Short Running Title-** Antitubercular activity and FTIR characterization of *Lavangadi Vati* and its suspension.

**Keywords-** FTIR, Herbal suspension, Lavangadi vati, Tuberculosis.

**Authors-** Dr. Meenu Swaminath, Dr. R.S Hiremath* & Dr. V.S. Mannur

**Addresses of Authors**

3rd *year PG Scholar,* Department of *Rasa shastra & Bhaishajya Kalpana, B M Kankanwadi Ayurveda Mahavidyalaya, Shahpur, Belagavi-590005*

*drmeenuswami@gmail.com*

**Professor, HOD, Dept of Rasa Shastra & Bhaishajya Kalpana, B M Kankanwadi Ayurveda Mahavidyalaya, Shahpur, Belagavi-590005*

*rasamruta70@gmail.com*

*Professor & H.O.D, Dept. of Quality Assurance, KLE College of Pharmacy, Nehru Nagar, Belagavi-590010*

*vvinodmannur@klepharm.edu*

**Abstract**

Tuberculosis, a global public crisis, ranked seventh position in the global morbidity and mortality. The current scenario, the medical scientist has been in search of novel Tb drugs and regimens that will reduce the current treatment duration, counter effect drug resistance and cop up the side effects of the drug and disease. This study deals with *Lavangadi vati* is a polyherbal formulation, which is effectively using in case of cough even associated with rhinitis and asthma which has been modified into suspension. For the anti-bacterial assessment, H37 RV strain of *Myco bacterium tuberculosis* was used as the reference strain. Fingerprinting of *lavangadi vati* and its modified suspension form was performed using Fourier Transform infrared spectroscopy. *Lavangadi vati* and suspension reveals a positive result in the management of *Myco bacterium tuberculosis* in the in vitro study in the concentration of 25 µg/ml,50 µg/ml,100µg/ml comparing with the standard drugs. FTIR spectrum confirmed the presence of alcohol, carboxylic acid, aromatic and amine groups in *lavangadi vati* and *lavangadi vati* suspension which are responsible for the medicinal properties of the formulation. This study helps to bring a conclusion that *lavangadi vati*, a polyherbal formulation and its modified suspension form can be used as an additional therapy for tuberculosis. FTIR characterisation could be used as a standardisation profile for the developed modified liquid dosage form of lavangadi vati suspension.

**Introduction**

Tuberculosis caused by mycobacterium tuberculosis, is the 13$^{th}$ leading cause of death and the second leading infectious killer after Covid-19. Developing countries like India still on the trouble to manage tuberculosisThere is urgency of finding newer anti-mycobacterial agents without side - effects to combat due to increase in Multidrug-resistant and Extensive Drug Resistance Strain of *Myco bacterium tuberculosis*. The current first line treatment of tuberculosis in modern medicine requires minimum of 6 months of therapy, which is also affected with drug resistance. We are in a need of searching new drug or established formulation showing proven activity in the management of tuberculosis. In the field of ayurveda wide range of dosage forms mentioned to manage common to chronic ailments, in which each herbal formulation showing enormous activity in the management of several infections caused by various organisms.

Infection is a kind of invasion of the body by pathogenic microorganisms leading to various diseases. *Janapadodhamsa* is a state of widespread outbreak of infectious disease and *Aupasargika*

*rogas* are those transmitted from person to person.[1]The first and foremost outbreak of infectious disease are common cold associated with rhinitis and dry/wet cough. One of the causative factors of cough is due to various infections in the upper and lower respiratory tract which is considered as *krimija* type, due to *Haemophilus influenzae* can be easily remedied by *Lavangadi vati* formulation.[2]*Lavangadi vati* is a unique formulation practising from decades in the management of *pratisyaya* (running nose)associated with *kasa* (cough), which is specifically act on respiratory tract disease and effectively proved to be useful in covid -19 infected cases.[3,4] *Lavangadi vati's* dose form may lose its acceptability in extreme age groups, necessitating a modified form for wider use. So, an attempt has been made to change soild dosage form into liquid dosage form and standardisation of the phytoconstituents with Fourier transformed infrared (FTIR) spectroscopic analysis.

## Materials and methods

In ayurveda, pulmonary tuberculosis is compared to *rajayaksma (Tuberculosis)*. The fundamental causes of all ayurvedic symptoms are kasa (cough), swasa (asthma), and pratisyaya (running nose), which are the manifestations of *dhathu kshaya* (tissue depletion) primarily in the reverse order of *saptha dhatus* (seven tissues) with the depletion of *ojas* (essence of seven tissues).[5]

### Preparation of *lavangadi vati*

Raw materials required for *lavangadi vati* were procured from GMP Certified KLE Ayurveda Pharmacy, Khasbhag, Belagavi and Dept of Rasa Shastra and Bhaishajya Kalpana, KAHER's Shri B. M. Kankanwadi Ayurveda Mahavidyalaya Belagavi. The additives/excipients required to be acquired from KLE College of Pharmacy, Belagavi. Drugs were authenticated in AYUSH approved Drug Testing laboratory of KAHER's Shri B.M.K Ayurveda Mahavidyalaya, Shahapur, Belagavi, Karnataka. After proper identification and assessment of the quality,20gms each of *lavanga (Syzygium aromaticum), maricha (Piper nigrum), vibhitaka (Terminalia bellarica)* and 120 gms of *khadira* (*Acacia catechu*) were made into fine powder separately and sieved through cloth (80-100 mesh) and mixed homogenously to prepare *Lavangadi churna*. For the preparation of *vati*, *babbula kashaya* (*Acacia arabica* in decoction form) prepared by the general procedure of decoction preparation. The *churna* (powder) is homogeneously triturated with *babbula Kashaya* in *khalva yantra* and *vati* is prepared of 1gm size. [6,7,8]

**Preparation of *lavangadi vati suspension***

For the easiness in the preparation of suspension, *lavangadi vati* was powdered. 50 ml of water warmed in water bath and add 4gms of Sodium CMC and made a homogenous solution and kept for 12 hrs.*Lavangadi vati* in powder form and mannitol were added one after other in the mortar and pestle and triturated well.To this, add sodium CMC solution till a homogenous mixture in colloidal form.Appropriate quantity of methyl paraben, propyl paraben and sodium benzoate were added and mixed properly after dissolving in 10ml of water.The preparation as per the [Table 1].Finally, 100 ml volume of suspension was prepared .It was stored in air tight container and kept in cool and dry place.[9]

**Phytochemical screening[10]**

The water soluble and alcohol soluble extract of *lavangadi vati* is prepared. Phytochemical screening done as per standards.

**Anti-tubercular screening**

**Mtb strain and culture medium**

For the anti-bacterial assessment, *Mycobacterium tuberculosis*, H37 RV strain (ATCC No- 27294) was used as the reference strain. Isoniazid – 1.6 µg/ml Ethambutol – 1.6 µg/ml Pyrazinamide-3.125µg/ml Rifampicin – 0.8 µg/ml Streptomycin- 0.8µg/ml as the controls.

**Antitubercular Test protocol**

In the case of *lavangadi vati* and *lavangadi vati* suspension, the anti-Mycobacterial activity of substances was evaluated against *M. tuberculosis* using a microplate Alamar Blue test (MABA). This method has good agreement with the proportional and BACTEC radiometric methods, is non-toxic, and makes use of a thermally stable reagent. In order to reduce the amount of medium that dries out in the test wells during incubation, 200 l of sterile deionized water was supplied to all of the sterile 96-well plate's outside perimeter wells. 100 l of the Middlebrook 7H9 broth were added to the 96-well plate, and compounds were serially diluted right there on the plate. The final drug levels examined ranged from 100 to 0.2 g/ml. Parafilm was used to cover and seal the plates, which were then incubated for five days at 37°C. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. [11]

**Fourier transformed infrared (FTIR) spectroscopic analysis**

*Lavangadi vati* and its modified suspension form was checked utilizing Fourier Transform Infra-Red Spectroscopy. Sample was kept in IR cell and runed out 48 scans over sample with IR beam. Sample undergoing molecular vibration on the absorbance of IR beam of light. Vibrational frequencies will be measured in the term of wavenumbers (Cm-1) versus percentage transmittance. Wavenumbers in the term cm-1 is indication of functional group at different positions. Peaks will be interpreted and given in the form of graph peaks. [12,13] The peaks obtained from the analysis were then interpreted by using the standard ranges of absorption of different functional groups.

**RESULTS**

The phytochemical screening of *lavangadi vati* reveals the presence of carbohydrate, reducing sugar, protein, tannin, alkaloid, glycoside, saponin, steroid, flavonoid, monosaccharide, aminoacid given .[14,15]

**Anti-tubercular study**

In the current study Alamar Blue assay by minimum inhibitory concentration has been assessed for the interpretation of anti-tubercular activity of both *lavangadi vati* and *lavangadi vati* suspension. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink. Both *lavangadi vati and lavangadi vati* suspension reveals a positive result in the management of *mycobacterium tuberculosis* in the in vitro study in the concentration of 25 µg/ml,50 µg/ml,100µg/ml comparing with the standard drugs, whereas *lavangadi vati* suspension at 12.5 µg/ml,25 µg/ml,50 µg/ml and 100 µg/ml. [ Figure 1-2 and Table -2]

**Fourier transformed infrared (FTIR) spectroscopic analysis**

FTIR is a non-invasive analytical tool allowing a fast and simultaneous qualitative as well as quantitative determination of natural products and their constituents. As a result of the absorption of electromagnetic radiation at different frequencies, infrared spectrum is formed and it is corelate to the vibration of specific sets of chemical bonds formed within a molecule.[16] So this spectrum helps to identify the functional group of the active components based on the peak value in the region of infra-red radiation. In the case of *lavangadi vati* 17 peaks were obtained and 7 similar peaks were noticed in the lavangadi vati suspension. The interpretation given in [Table 3-4& Figure 3-4]

## DISCUSSION

Drug resistance tuberculosis have become a major global crisis facing by the medical field. The scientific research fraternity constantly working for a solution for this along with in search of less side effect medicine. As per the current study *lavangadi vati* and its suspension effectively showing its antitubercular activity in minimum dosage. Carbohydrate having anti -tussive activity.[17]Monosaccharides having anti-inflammatory, anti-microbial, anti-diabetic activity.[18] Tannins having anti-oxidant, antimicrobial, antiviral, cardio protective and anti ulcerant activity.[19] Alkaloids having anti-cancer, anti-inflammatory, Anti-malarial, Anti-microbial, Anti-hypertensive, Anti-diabetic, Anti-oxidant. Alkaloids directly act on the central nervous system in the human body and also affect nucleic acid, DNA (Deoxy Ribonucleic acid), RNA (Ribonucleic acid), membrane permeability and proteins.[20] Anthraquinone glycoside possess antibacterial, antiparasitic, insecticidal, fungicidal, and antiviral properties. They are also used as anticancer agents.[21] Saponins having antibacterial, anti-inflammatory, antifungal and antiviral activities.[22] Steroids having anti-tussive,anti-inflammatory,anti-bacterial,hepatoprotective,cardiotonic,anti-tumour activity.[23] Flavonoids possess antioxidative activity, free radical scavenging capacity, coronary heart disease prevention and antiatherosclerotic, hepatoprotective, anti-inflammatory, and anti-microbial activity .[24] Monosaccharides which are the building blocks of disaccharides like sucrose(common sugar) and polysaccharides (such as cellulose and starch), having antioxidant and antimicrobial activity.[25] Amino acids having potent antibacterial, antifungal, antiparasitic, and antiviral properties.[26]

*Lavangadi vati* has components that are antipyretic, antiallergic, immunomodulatory, antioxidative, anti-inflammatory, and analgesic. The mixture, which has *katu vipaka* (pungent after taste), *ushna virya* (hot potency), and *katu-tikta-kashaya rasa* (pungent-bitter-astringent taste), clears the bronchial constriction and functions as a *rasayana*(rejuvenator) at the cellular level of the tissues. Both *lavangadi vati* and *lavangadi vati* suspension shown the anti-bacterial activity. The *lavangadi vati* suspension shown its activity at lower concentration of 12.5µg/ml whereas the lower concentration of *lavangadi vati* was 25 µg/ml. This can be interpreted as when compared to soild dosage form, liquid dosage form suspension is better in bio availability and action. The anti-tubercular drugs act on three ways i.e., it inhibits protein synthesis, inhibit cell wall synthesis and hydrolyses is nicotinic acid, ethionamide directly inhibit mycolic acid synthesis. Eventually the damage cell membranes, activate enzymes and denaturate proteins so that cell walls are damaged by decreased permeability. It further disrupts the transport of important organic ions into the cells resulting in inhibition of growth even to cell lysis. The lipophilic nature of the compounds like *lavanga*, *maricha* etc., were an important property of the bioactive constituents in these plant partitions as they could penetrate the hydrophobic outer membrane of *M. tuberculosis* to exert

inhibitory effects. The identification of these compounds supported the higher anti-TB activity of the non-polar solvent partitions as discussed above. Ideally, the specific bioactive constituents should be isolated and elucidated. Flavonoid, sterol, phenol, diterpene, sesquiterpene, triterpenoid, anthraquinone, and alkaloid showed the activity of anti-tuberculosis. The phenol compounds damage the cell membrane, activate enzymes and denaturate protiens thereby cell wall is damaged by decreased permeability. This will further disrupt the transport of main organic ions into the cell resulting in inhibition of growth leading to cell lysis.[27] Flavonoids mainly act through inhibiting cytoplasmic membrane function as well as by inhibition of DNA gyrase and β-hydroxyacyl-acyl carrier protein dehydratase activities.[28] Alkaloid are already proven as potent anti-tubercular activity.[29] Alkaloid act by disrupting the peptidoglycan component of the bacterial cells thereby the cell wall layer is not formed intact and act on amino acid of cell wall and bacterial DNA.[30] Similarly anthraquinone activate the enzyme and denature the bacterial protein and cell wall is damaged.[31,32]

In FTIR spectrometric analysis, *lavangadi vati* the intense peak occurring at 3385 $cm^{-1}$ indicates -O-H bond of alcohol group;1608 $cm^{-1}$ and 629 $cm^{-1}$indicates C-C and C-H bonds of aromatic group;1290 $cm^{-1}$ and 1232 $cm^{-1}$ indicates C-O bonds of carboxylic acid, ester and ether groups;1035 $cm^{-1}$ indicates amine group. In case of *lavangadi vati* suspension intense peak occurrence are in little lower peak frequencies such as 3318.51 $cm^{-1}$ indicates -O-H bond alcohol group ;1636.99 $cm^{-1}$ indicates C-C bond aromatic group.1078.05 $cm^{-1}$ and 1030.39 $cm^{-1}$ indicates C-N bond amine groups. There was no peaks ranging between 2220-2260 $cm^{-1}$ indicates that no cyanide groups present in both samples which can be interpreted as absence of toxic substance.[33]*Lavangadi vati* shown weak peaks in the frequencies in 3790 $cm^{-1}$ indicates N-H bond of amine group;2890 $cm^{-1}$,1447 $cm^{-1}$,1377 $cm^{-1}$ and 821 $cm^{-1}$ indicates N-H ,C=C and two C-H bonds of aromatic group;2825 $cm^{-1}$,2347 $cm^{-1}$ and 2277 $cm^{-1}$ indicates 0-H and OH bonds of carboxylic group;1774 $cm^{-1}$ indicates C-O bond of amide group;1500 $cm^{-1}$ indicates N-O bond of $NO_2$ and 774 $cm^{-1}$ indicates the C-Cl bond of halide group. The weak peak assigned in the lavangadi vati suspension at 2121.47 $cm^{-1}$,1419.29 $cm^{-1}$ and 882.23 $cm^{-1}$ indicates -O-H- bond carboxylic group, C-C and C-H bonds of aromatic group resply. Although both samples had substantial overlap of each absorption spectrum of various components, each band represent an overall overlap of some characteristic absorption peak of functional groups in the spectra.[34] O-H bend of carboxylic acid reveals the presence of flavonoids, tannins, saponins and glycosides.[35]Eugenol is a phenolic aromatic compound present in lavanga having antibacterial, antiviral, antifungal, anticancer, anti-inflammatory and antioxidant properties.[36]Piperine present in maricha is a type of amide alkaloid that exhibits pleiotropic properties like antioxidant, anticancer, anti-inflammatory, antihypertensive, hepatoprotective, neuroprotective and enhancing bioavailability and fertility-related activities.[37] Kaempferol present

in *khadira* and *babbula* belongs to the flavonoid group has antioxidant, anti-inflammatory, antimicrobial, cardiovascular, and neuroprotective properties.[38]Even quercetin and iso-quercetin present in khadira and babbula also belongs to polyphenolic flavonoid having anticancer, antiviral, antiprotozoal, and antimicrobial effects, treatment of allergic, metabolic, and inflammatory disorders, eye and cardiovascular diseases, and arthritis.[39]

## CONCLUSION

Finally, the results of this study clearly elucidate the antibacterial potential especially anti-tubercular activity of *lavangadi vati* and its modified form suspension. It provides evidence to support their use as an additional therapy for tuberculosis and also to reduce the side effects of allopathic drugs. So, the FTIR characterisation helped to reveal the presence of functional groups present in the *lavangadi vati* and its suspension form, resulting in its chemical standardisation. It is important to keep in mind that a single compound may not be responsible for the observed activity but rather a combination of compounds interacting in an additive or synergistic manner. More definite studies invivo were required to define the mechanism of action of these compounds and elucidate compounds responsible for antitubercular activity of this poly herbal formulation in future.

### Conflict of Interest

No any conflict of interest

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### Author's contribution

Dr. Meenu Swaminath wrote the manuscript, concept designing, preparation of study suspension, definition of intellectual content and done investigation related to study. Dr.R. S Hiremath helped in concept designing, guided in definition of intellectual content, preparation of study suspension and chronological arrangement of matters and corrections of the matters.Dr. Mannur V.S helped in concept designing, Preparation of the study suspension.

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**Tables**

**Table -1 Preparation of *lavangadi vati suspension***

| Sl. No | Ingredient | Quantity |
|---|---|---|
| **1** | *Lavangadi vati churna* | 20 gm |
| **2** | Mannitol | 20 gm |
| **3** | Sodium CMC (4%) | 4 gm |
| **4** | Methyl paraben | 0.05 gm |
| **5** | Propyl paraben | 0.01gm |
| **6** | Sodium benzoate | 0.5gm |
| **7** | Water | 50ml |

**Table -2 Antitubercular activity of *lavangadi vati* and suspension**

| Sl. No. | Sample | 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 |
|---|---|---|---|---|---|---|---|---|---|
| **1** | ***Lavangadi suspension*** | S | S | S | S | S | R | R | R |
| **2** | ***Lavangadi Vati*** | S | S | S | R | R | R | R | R |

NB:S-Sensitive; R-Resistant

**Table 3 FTIR of *Lavangadi Vati***

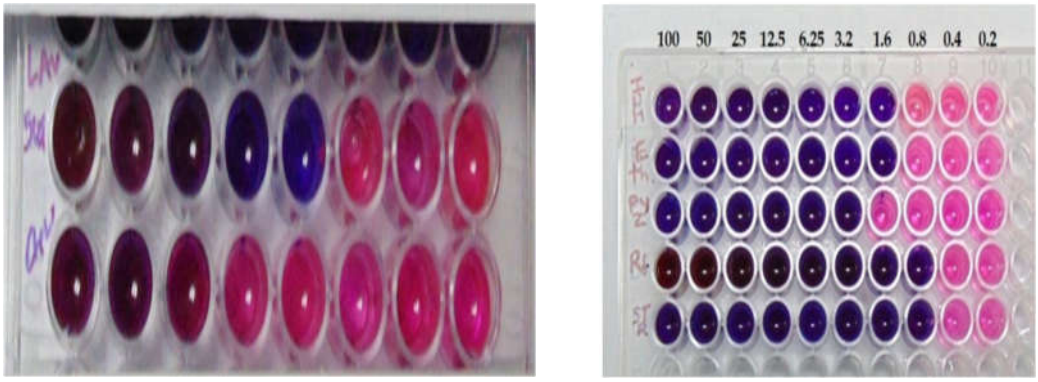
| Sl.no | Peaks | Bond | Functional group |
|---|---|---|---|
| 1 | 3790 | N-H | Amine |
| 2 | 3385 | -O-H | Alcohol |
| 3 | 2890 | C-H | Aromatic/Aliphatic |
| 4 | 2825 | O-H | Carboxylic acid |
| 5 | 2347 | OH | Carboxylic acid |
| 6 | 2277 | OH | Carboxylic acid |
| 7 | 1774 | C-O | Amide |
| 8 | 1608 | C-C | Aromatic |
| 9 | 1500 | N-0 | NO2 |
| 10 | 1447 | C=C | Aromatic |
| 11 | 1377 | C-H | Aromatic |
| 12 | 1290 | C-O | Carboxylic acid, Ester, Ether |
| 13 | 1232 | C-O | Carboxylic acid, Ester, Ether |
| 14 | 1035 | C-N | Amine |
| 15 | 821 | C-H | Aromatic |
| 16 | 774 | C-Cl | Halide |
| 17 | 629 | C-H | Aromatic |

**Table No 4 FTIR of *Lavangadi Vati* Suspension**

| Sl.no | Peak | Bonds | Functional group |
|---|---|---|---|
| 1 | 3318.51 | -O-H | Alcohol |
| 2 | 2121.47 | OH | Carboxylic acid |
| 3 | 1636.99 | C-C | Aromatic |
| 4 | 1419.29 | C=C | Aromatic |
| 5 | 1078.05 | C-N | Amine |
| 6 | 1030.39 | C-N | Amine |
| 7 | 882.23 | C-H | Aromatic |

**Figures**

**Figure -1 Antitubercular activity of (a)*lavangadi vati*, suspension and (b)control drugs**




(a) (b)

**Figure -2 MIC study on *Tuberculosis***

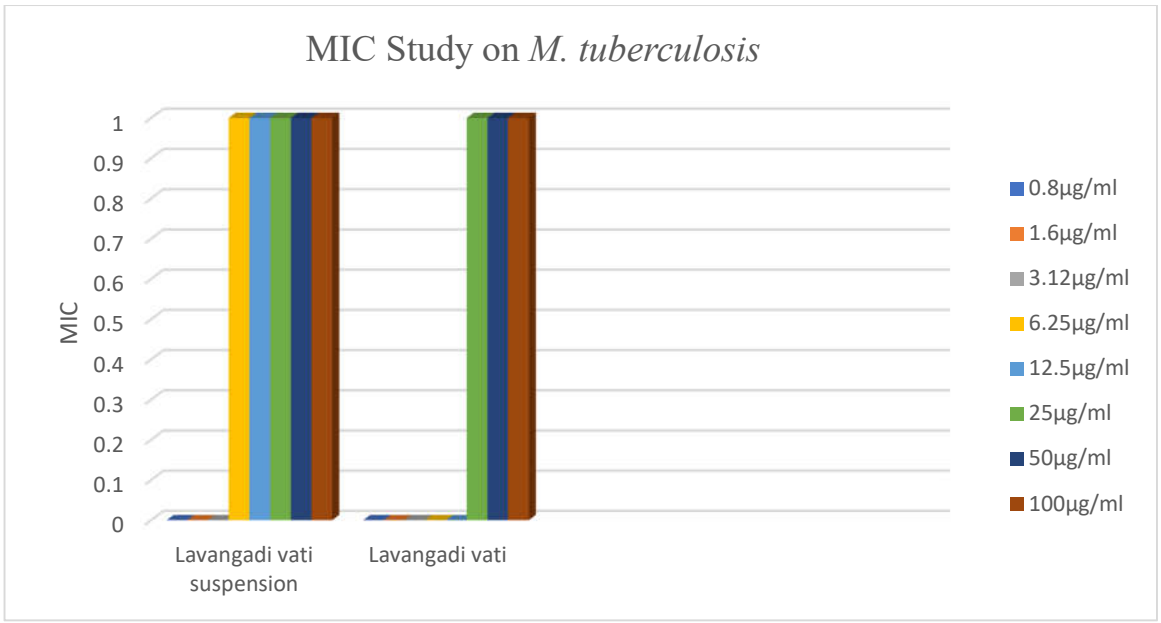


**Figure 3 FTIR Study of *Lavangadi Vati***

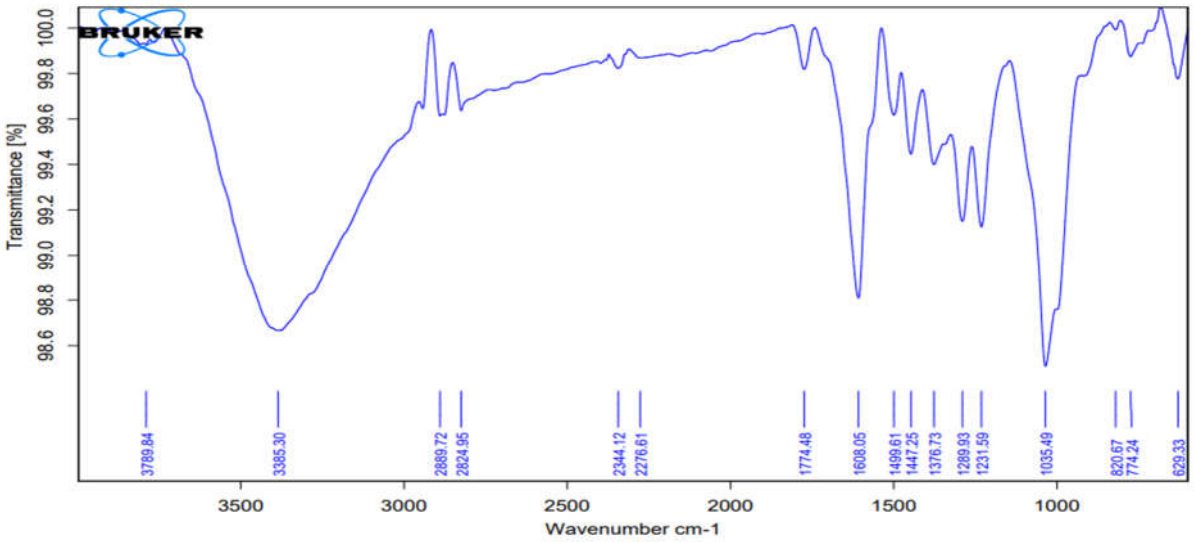



**Figure 4 FTIR Study of *Lavangadi Vati* Suspension**