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Physicochemical Profiling of Lasunadi Vati – A Polyherbal Ayurvedic Formulation

P. Rajalakshmi[1], N. Ravichandran[2], V. Sudha[1], R. Lavanya1, S. Sriram*[1], P. Brindha[1]

[1]Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA Deemed University, Thanjavur-613401, Tamil Nadu, India.

[2]Srinivasa Ramanujan Centre, SASTRA Deemed University, Kumbakonam - 612001, Tamil Nadu, India.

***Corresponding author- Dr. S. Sriram**

School of Chemical and Biotechnology,

Centre for Advanced Research in Indian System of Medicine (CARISM),

SASTRA Deemed to be University, Thanjavur, Tamil Nadu, 613401, India.

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KEYWORDS

Lasunadi vati, *Ayurveda*, Physicochemical characteristics, Instrumental analysis

ABSTRACT:

Background: *Lasunadi vati* (LV) is a polyherbal *Ayurvedic* preparation, traditionally used for treating dyspepsia, diarrhea and gastroenteritis. Even though LV is a commonly used formulation, no proper has yet been done on the chemical characterization of LV.

Aim: The present study intends to carry out a physicochemical fingerprinting of LV involving Organoleptic and microscopic characterization, Estimation of ash content, Surface characterization studies using modern analytical techniques such as Scanning Electron Microscope (SEM), Thermogravimetric analysis (TG-DTA), X-ray fluorescence spectroscopy, Fourier Transform Infra-Red (FT-IR) spectroscopy and X-ray diffraction.

Materials and Methods: All the analyses were carried out in the State-of-the-art laboratory facility available in SASTRA University, Thanjavur.

Results and Discussion: The microscopic characterization confirmed the presence of all the herbal ingredients present in LV as mentioned in *Ayurvedic* literature. The surface morphology showed a fused network. All the physicochemical characteristics (Total ash, Acid insoluble ash, Soluble ash and Moisture content) were all found to be within the permissible limits mentioned in *Ayurvedic* pharmacopeia. The XRF results on elemental composition of LV was in accordance with the details mentioned in *Ayurvedic* Formulary of India. The results of TG-DTA and FT-IR indicated the presence of organic moieties in the formulation. The XRD pattern of LV indicated the presence of sulphur which is one of the main ingredients of the formulation.

Conclusion: The present study has provided a thorough physicochemical fingerprinting of the formulation *LV*

INTRODUCTION

Ayurveda – the knowledge or science (*Veda*) for longevity (*Ayus*) aims at maintaining and promoting positive health through a holistic approach [1] that provides cure and remedy for various human ailments, with drugs obtained from indigenous plants, animal products and minerals [2]. In the field of *Ayurveda*, even though various types of *kalpanas* (formulations) are being used, *Vati kalpana* are preferred more due to its easy administration, palatability, convenient form of dispensing and transportation. *Vati kalpana* is a Ayurvedic procedure in which the powder of raw drugs (Herbal or Herbo-minerals) is triturated together with certain Kasayam/Juice/honey and the medicines are

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prepared in the form of pills or tablets [3]. *Lasunadi vati* (LV) is a polyherbal *Ayurvedic* medicine used for treating dyspepsia, diarrhea and gastroenteritis and has carminative, laxative, stomachic and stimulant properties [4]. These medicinal properties of *LV* are mainly due to its ingredients that include *Alium sativum* (Garlic), *Cuminum cyminum* (Cumin), rock salt, herbal purified sulphur, *Zingiber officinalis* (Ginger Rhizome), *Piper longum* (Long pepper fruit), *Piper nigrum* (Black pepper), *Asafoetida* and lemon juice each having a rich array of well documented therapeutic activities. Garlic is useful in treating infections, cancer, heart diseases, hemorrhoids, rheumatism, dermatitis, abdominal pain, cough, loss of appetite and loss of weight [5]. It also acts as a gastric stimulant and carminative [6]. Cumin has been used as a carminative and eupeptic for treating mild digestive problems, as an astringent in broncopulmonary disorders, as an analgesic and has also been used for treating cough [7-8]. Rock salt has long been used as a Laxative, Carminative, Stomachic and Diuretic in *Siddha* system of medicine [9]. Sulphur is used to treat flatulence, gastric ulcer and diarrhoea. It is an excellent laxative and also enhances bile juice secretion [10]. *Zingiber officinalis* has been traditionally known for its medicinal activity against constipation and indigestion [11]. The roots of *Piper longum* has been used as a stomachic, laxative and carminative in *Ayurveda* [12]. The hot water extract of *Ferula asafoetida* finds use as an oral carminative in the Indian traditional medicinal system [13-14]. Lemon juice has been widely used in traditional medicine as a diuretic and laxative [15]. Even though the medicinal properties of LV have been well documented over the years, there have not been any study on the chemical characterization of the poly herbal preparation. Hence in the present study, a systemic chemical profiling of commercially available LV was carried out as per AYUSH parameters and with the help of modern analytical techniques to obtain more insight about the morphological, structural, elemental and chemical characterization of the *Ayurvedic* preparation.

MATERIALS AND METHODS

Materials

LV was purchased from the local market of Thanjavur, Tamil Nadu, India. Hydrochloric acid, nitric acid and sodium chloride were procured from Merck, Mumbai. Phosphate buffered saline solution was purchased from Gibco, USA.

Methods

Organoleptic characterization

For organoleptic characterization, the colour, taste, odour and consistency were tested according to the methods described in *Ayurvedic* pharmacopeia.

Microscopic characterization

Small amount of sample was treated with chloral hydrate, phloroglucinol, iodine potassium iodide (IKI), Sudan Black B and oil red. The type and shape of crystals, fibres, xylem vessels elements, starch grains, stone cells, starch grains, trichomes, parenchyma cells and presence of oil globules, aleurone grains were detected by using Carl Zeiss microscope and the photomicrographs were taken with the help of ProgRes software and digital camera [16].

Estimation of Total Ash, Acid Insoluble Ash and Water-Soluble Ash

One gram of the LV was weighed accurately and taken in a silica crucible. The sample was spread uniformly on the bottom of the crucible and incinerated at 450^{o}C for 3 hours and allowed to cool naturally to room temperature. The residue was weighed and total ash was estimated [17]. The residue from total ash estimation was boiled with 25 mL of dilute hydrochloric acid for 5 minutes. The insoluble matter was washed with hot water, transferred to a crucible and dried. The mass of the residue was determined to estimate the acid insoluble ash [17]. The residue from total ash estimation was boiled with 25 mL of distilled water for 5 minutes. The insoluble matter was washed with hot water, transferred to a crucible, dried and weighed. The water-soluble ash was determined by subtracting the mass of insoluble matter from the total ash [17].

Determination of Loss on Drying (LOD)

One gram of LV was taken in a crucible and dried in an oven at 105°C for about 5 hours. The sample was allowed to cool and the dry mass was determined. The difference in mass was used to determine the loss on drying and expressed in percentage.

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Electron Microscopy

The surface morphology of LV was qualitatively assessed using a scanning electron microscope (Vega 3 SBH, TESCAN, Czech Republic). The sample was mounted on a brass stub and sputter coated with platinum and introduced into the specimen chamber. Imaging was carried out at acceleration voltages between 3 kV and 5kV.

Thermogravimetric Analysis

The thermal analysis of the sample was carried out using thermogravimetry (SDT Q600, TA Instruments, USA). 5 mg of the sample was introduced in an alumina cup and heated gradually in a nitrogen atmosphere at the rate of 10°C/minute.

Spectroscopic Analysis

LV was pelletized using a 25-ton hydraulic press to prepare thin discs of 34 mm diameter. The elemental composition of *LV* was determined using an X-ray fluorescence spectrometer (S8 Tiger, Bruker AXS, Germany) using a 4 kW Rhodium anode X-m ray tube.

LV was mixed with KBr and pelletized using a hydraulic press. The Fourier Transform Infra-Red (FTIR) spectra were recorded between 4000–400 cm–1 in a FTIR spectrometer (Spectrum 100, Perkin Elmer, USA).

XRD Analysis

The crystallinity of LV was analyzed using an X–ray diffractometer (D8 Focus, Bruker, Germany), by irradiating with Cu–Kα radiation. The analysis was performed from 10° to 60° (2q) with a step size of 0.01°.

RESULTS AND DISCUSSION

Organoleptic characterization

The organoleptic characteristics of LV was studied and the results are tabulated in Table 1.

Microscopic characterization

Microscopic characterization studies of LV revealed the presence of square and rectangular shaped prismatic calcium oxalate crystals, elongated epidermal cells with stomata (*Lasuna-Allium sativum*). Brick shaped parenchyma cells and amorphous resinous matter, oil containing cells, vessels with spiral thickening and large starch grains (*Sunthi - Zingiber officinale* Rosc.). Bristle hairs and tabular cells, polygonal cells containing oil globules and aleuorone grains. (*Sveta jiraka - Cuminum cyminum* L.). Isodiametric and slightly elongated stone cells, yellow colored sclerenchymatous cells with thick wall, parenchyma cells with oil globules, oval to round, simple and compound starch grains (*Marica - Piper nigrum* L.). Group of oval elongated stone cells, reddish brown content, larger, polygonal irregular cells, endocarp cells with round to oval starch grains and oil globules (*Pippali - Piper longum* L.). The results of the microscopic characterization (Figure 1 & 2) confirm the presence of all herbal ingredients of *Lasunati vati* listed in literature.

Surface morphology

Figure 3 shows the results of surface morphology of LV done through a scanning electron microscope which reveals the formation of a layered fused network.

Physico-Chemical Characterization Studies:

The physicochemical characteristics (Total ash, Acid insoluble ash, Soluble ash and Moisture content) of LV were determined and the results are tabulated in Table 2. The total ash content of the LV sample was determined to be about 16% of which less than 1% was acid insoluble. The moisture content of the sample determined from the weight loss at 105ºC was about 12%. The sample also contains a significant amount of polar molecules as evident from the high water-soluble extractive values (50%).

Thermogravimetric Analysis

The thermogravimetric profile of LV is given in Figure 4. The moisture content in the sample was found to be about 9% which is in close agreement with the loss on drying values reported in Table 2. The residue at 650ºC was about 25%. Further, there is a gradual weight loss between the temperature range of 186ºC-440ºC indicating the presence of organic moieties in the sample.

Spectroscopic Analysis

Table 3 shows the elemental composition of LV analyzed using x-ray fluorescence spectroscopy. The major elements in LV are Sulphur, Chlorine, Sodium, Potassium, Calcium, Silicon, Aluminum, Iron and Magnesium, which is in accordance to the *Ayurvedic*

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formulary of India, that has documented the major inorganic ingredients in LV as Sulphur (*Suddha Gandhaka*) and Rock salt (*Saindhava Lavana*). The XRF data in the present study confirms the presence of these ingredients in the LV sample. The FT-IR spectrum of the LV sample is given in Figure 5. The presence of the strong vibration band at 3412 cm^{-1} may be attributed to the –OH stretch. The bands at 2925 and 2855 cm^{-1} arise due to the asymmetric and symmetric stretching vibrations of the –C-H bond respectively. The appearance of an intense vibration band at 1743 cm^{-1} may be attributed to the carbonyl stretching frequency of ester moieties in the sample. Further, -N-H bending vibrations at 1637 $^{cm-1}$ and –C-H bending vibrations at 1384 cm^{-1} are observed in the sample indicating the presence of organic moieties in *LV*.

XRD Analysis

The x-ray diffraction pattern of LV obtained between 2theta values of 20-60o is presented in Figure 6. The appearance of crystalline peaks at 2 theta values of 23, 26, 27, 32, 36, 44 and 56 correspond to the planes (222), (133), (311), (044), (244), (319) and (551) respectively for sulphur (JCPDS No 88-2300). The XRD results of the present study also confirms the presence of sulphur as a major ingredient in LV.

CONCLUSION

The present study is a comprehensive chemical fingerprint of the commercially available Ayurvedic polyherbal preparation LV based on AYUSH parameters and modern analytical techniques. The results of the study serve as proper scientific evidence about the presence of individual ingredients of *LV* documented in *Ayurvedic* literature.

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Color	Black
Taste	Sour
Odor	No characteristic odor
Texture	Black spherical balls

Table 1: Macroscopic characteristics of *LV*

S No	**Parameters**	**Results**
1	Appearance	Black coloured ball shaped tablet
2	Total Ash	15.91%w/w
3	Acid Insoluble Ash	0.9442%w/w
4	Loss on Drying at 105°C	11.90%w/w
5	Water Soluble Extractive(WSE)	49.52%w/w
6	Alcohol Soluble Extractive(ASE)	18.38%w/w
7	Disintegration time	More than 30min
8	Uniformity of weight	84.12% to 111.30%

Table 2: Physico-Chemical Characteristics of *LV*

Table 3: X-ray fluorescence analysis of *LV*

Element in oxide form

Formula	**Concentration (%)**
SO_3	44.11
Cl	20.62
Na_2O	12.15
K_2O	7.98
CaO	3.74
SiO_2	3.65
Al_2O_3	2.42
MgO	1.9
Fe_2O_3	1.72
P_2O_5	1.33
PbO	0.13
CuO	0.13
MnO	0.06
Cr_2O_3	0.05
SrO	80PPM

Element form

Formula	**Concentration (%)**
S	54.84
Cl	20.62
Na	9.01
K	6.63
Ca	2.68
Si	1.71
Al	1.28
Fe	1.2
Mg	1.15
P	0.58
Pb	0.12
Cu	0.1
Mn	0.05
Cr	0.03
Sr	68PPM

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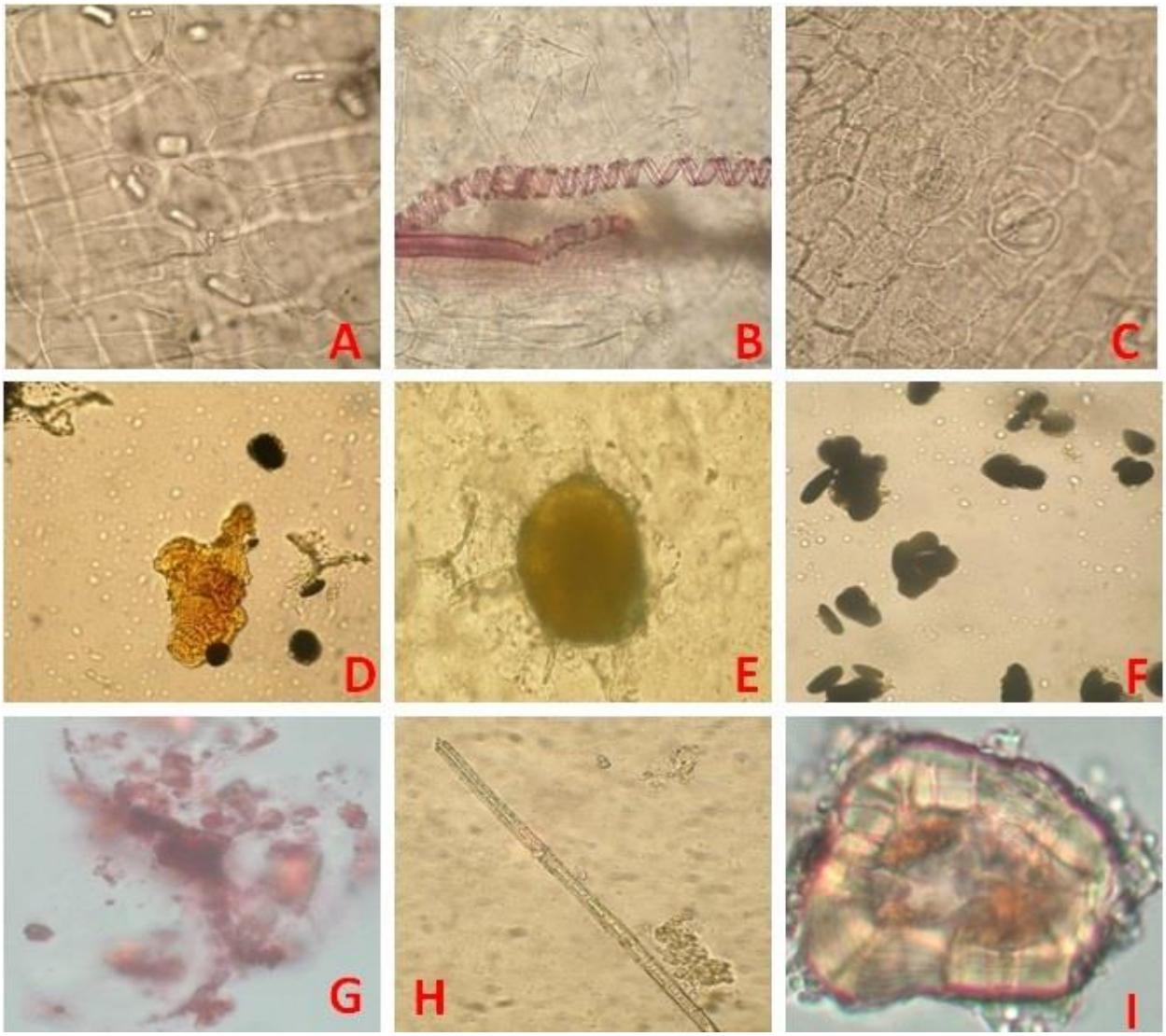

Figure 1: Microscopic characteristics of *LV*

A. Square and rectangular calcium oxalate crystals; B. spiral thickened xylem elements, C. Elongated epidermis with stomata, D. Resinous matter, E. Resin containing cells; F. Starch grains; G. oil containing cells; H. Trichome; I. Striated sclerenchyma cell

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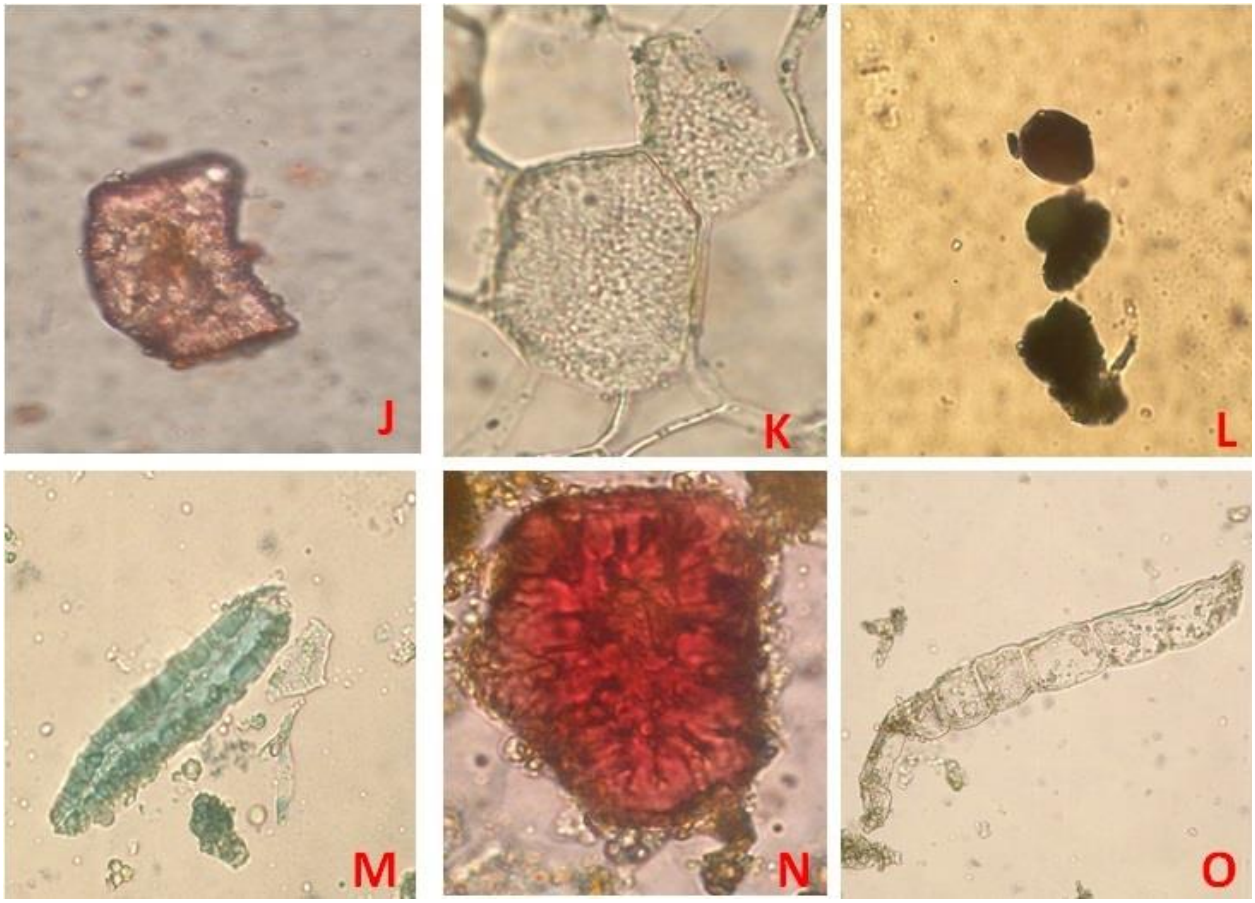

Figure 2: Microscopic characteristics of *LV*

J. Stone cell; K. Aleuorone grains; L. Simple large starch grains; M. Elongated Sclereids; N. Stone cell and O. Multicellular trichome

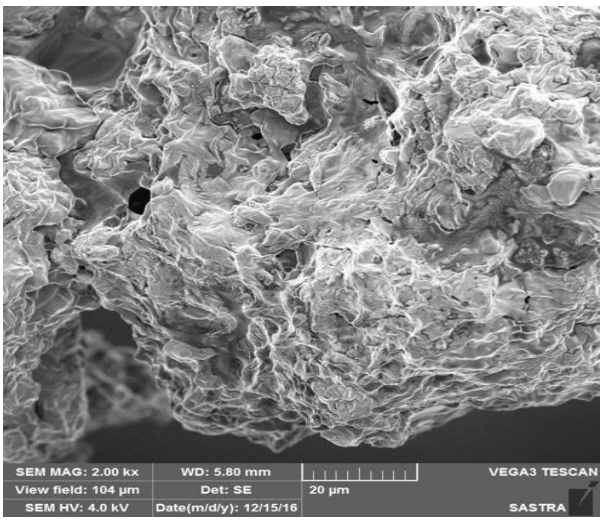

Figure 3: Surface morphology of *LV*

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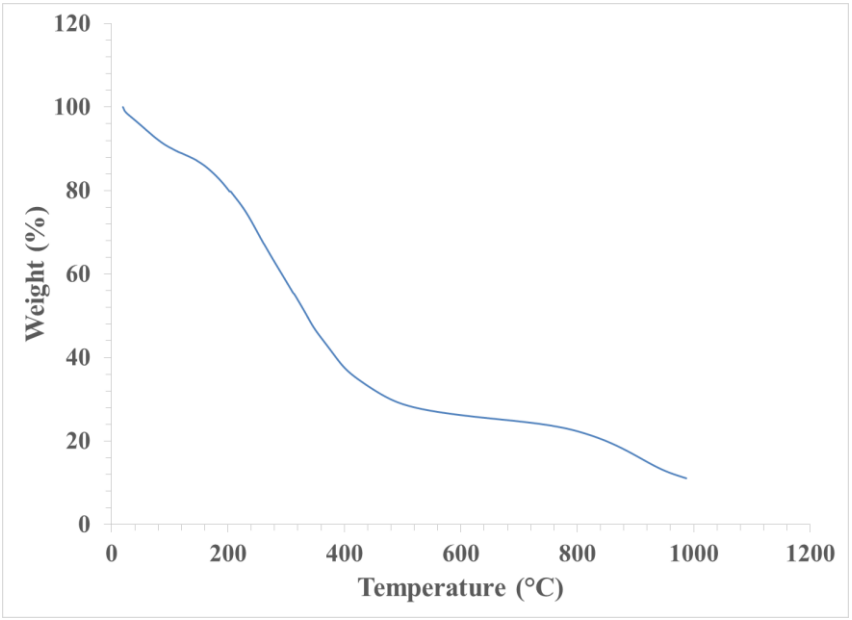

Figure 4: Thermogravimetric profile of *LV*

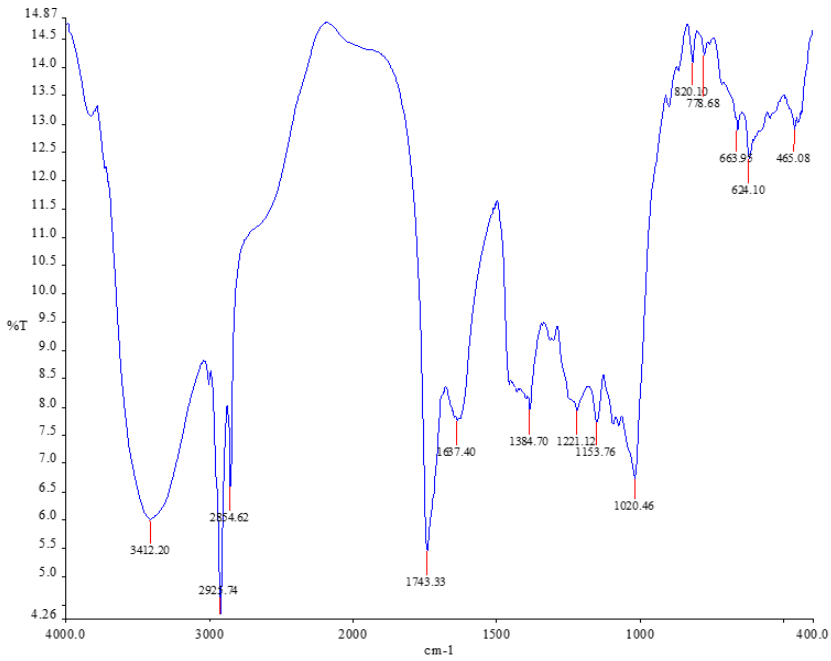

Figure 5: FTIR Spectrum of *LV*