IDENTIFICATION, ISOLATION AND CHARACTERIZATION OF *JASTICIA ADHATODA* PLANT BY UV, IR AND RP-HPLC

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ABSTRACT

Natural products have a wide range of chemical ingredients. The plant *Jasticia adhatoda* was chosen from the Acanthaceae family for this investigation. Bronchodilator, expectorant, and antibacterial properties.Extraction of herb was done using suitable traditional methods and evaluated by various physicochemical methods for alkaloids, tannins, saponins, flavanoids, and phenolicsetc. Herbal extract was applied for the suitable dosage form development, particularly for pediatric patient followed by optimization studies. The primary alkaloid vasicine found in plant extract was identified and validated in the current investigation through early qualitative phytochemical screening. The color of the zone was compared to the shade of the reference standards for the quantitative measurement and confirmation of vasicine. Short-wave UV 254 nm dots were seen, as well as five spots in long-wave UV 366 nm.With a corresponding correlation coefficient, the linearity standard curve was determined between 2 and 6 g/spot. All tracks are shown in a 3D graph of uniformity at 254nm and 366nm. HPTLC examination revealed a vivid orange colored zone that corresponded to the presence of alkaloids discovered in this study.

Keywords: *Jasticia adhatoda*, UV Spectroscopy, FTIR, RP-HPTLC

INTRODUCTION

The Malabar nut tree, *Jasticia adhatoda* (AV), belongs to the family *Jasticiaceae* *(Acantaceae)*. Plant Leaves, roots and young plants of AV contain the quinazoline

alkaloids such (vasicine, 7-hydroxyvasicine, vasicinolone, 3deoxyvasicine, vasicolinone, triterpenes, anisotine) betaine, steroids carbohydrate and alkanes. Triterpenes (a-amirine) and flavonoids (apigenin, astragalin, kaempferol, quercetin, vitexin) have been discovered in the flowers. The Malabar nut tree, *Jasticia adhatoda*, belongs to the *Acanthaceae* family [1-2].

Plant is a small evergreen, sub-herbaceous plant bush found throughout India, particularly in the lower Himalayas (up to 1300 metres above sea level). It is grown in India, Sri Lanka, Burma, and Malaysia. It is generally known as vasica in Ayurveda, Indian medicine, and is a plant in the traditional system of medicine.Plant grows to about 1.5-2.0m in height, with leaves about 1015cm long and 5.0cm wide, white or purple flowers, and 4-seeded fruits. The leaves are dark green on top and pale yellow on the bottom. Flowers are white and arranged in a pedunculated spike. *Jasticia adhatoda* leaves have been used for over 2000 years in Ayurvedic medicine to treat respiratory disorders [3-4].

Medicinal plants have long played an important role in global health care systems. The active principles of the plant are the roots, leaves, and flowers, which have a variety of pharmacological properties and are used to treat cough, chronic bronchitis, rheumatism, asthma, and bronchial asthma. Plant leaves and roots contain several alkaloids (mostly quinazoline alkaloid, vasicine and vasicinone, vasicinolone and vasicol), which have bronchodilator properties. Sedative, expectorant, anti epileptic, anthelmintic, bronchial antiseptic, and bronchodilator activity on the bronchi. For centuries, leaf extract formulations have been used in the diagnosis of asthma and bronchitis in various countries. It alleviates respiratory cough and shortness of breath. Individual plant extracts were subjected to qualitative phytochemical screening to determine the presence of certain chemical constituents [5-7].

The herb adhatoda is a well-known medicinal plant. Plant leaves, flowers, and roots are all used in various medication compositions. *Jasticia adhatoda* is a tall, dense shrub with broad, many-branched, evergreen leaves that are lance-shaped. The blooms of the AV plant are dense and small, with the stems of the tips being shorter than the leaves. Husks are the leaf structures of plants.present on the points, which are veined in a pointed manner [8-10].

MATERIALS AND METHODS

Selection and authentication of plant

The plant was selected *Jasticia adhatoda L.* belonging to *Acanthaceae*family. The plant was collected from and certified by Botanical Survey of India, Western

Regional Centre, 7-Koregoan Road, Pune-411001, India.

Materials and Methods

Leaves was collected from the botanical garden,The leaves were dried in the shade for 10-14 days at room temperature. After crushing the dried leaves in a commercial electrical stainlesssteel blender, the material was converted into powder form and saved for further analysis **[11]**.

Extraction

Soxhlet extraction was used to extract plant material (20 g) with methanol. The crude extracts were evaporated to dryness at 50ºC and stored in a screwed cap bottle at -18ºC.The yield of the extract obtained was reported as percentage and was calculated as follows equation:

$$\text{Yield } \% = \frac{Wa - \quad (g)}{Dry\ Plant\ Used(g)} \times 100 \quad \text{------}$$

-------------- **[1]**

Where, Wa = weight of bottle + crude extract (g),

Wb = weight of bottle (g). **[12]**

Pre-formulation Studies

Pre-formulation study is the process of optimizing the delivery of drug through determination of physicochemical properties of the new compound that could affect drug performance and the development of an efficacious, stable and safe dosage form. It gives the information needed to define the nature of the drug substance and provide a framework for the drug combination with pharmaceutical excipients in the dosage form. Hence, pre-formulation studies were performed on the obtained sample of drug for identification including colour, tests, solubility analysis and melting point determination and compatibility studies **[13-14]**.

Preliminary Study of Herbal Drugs

The methanol extracts of *Jasticia adhatoda* were analyzed for color, odour, and taste, loss on drying, total ash. To determine purity of the sample, melting points of *Jasticia adhatoda* extract were determined by capillary method. The capillary filled with extract powder was placed in Thiel's tube filled with liquid paraffin. The tube was heated and the melting point of extract powder was noted when last particle melted **[15-16]**.

Solubility Analysis:

Solubility analysis was done which include the selection of suitable solvent, to dissolve the respective drug as well as various excipients. About 10 mg quantities of the extract sample were added into 5 mL of individual solvents and the solubility was noted after proper shaking **[17-18]**.

Identification of Drug:

The obtained extract was examined by using FTIR spectrophotometer analysis by diffuse reflectance method (Shimadzu Corp., Japan). The infrared spectrum of *Jasticia adhatoda e*xtract, Gelatin, Propylene glycol, Stevia and physical

mixture of *Jasticia adhatoda* (*Jasticia adhatoda L.)* extract, and Gelatin, Propylene glycol, Stevia was recorded. Sample was mixed along with IR grade KBr in 1:100 proportions and IR spectrum was recorded and compared with the reference standard infrared spectra [19].

UV- spectrum: (Determination of λ max):

The standard solutions of *Jasticia adhatoda* powder were prepared in phosphate buffer (pH 6.8) and water. The prepared solutions were scanned between 800-200 nm by UV-visible spectrophotometer [5].

High Performance Thin Layer Chromatography Method

HPTLC chromatograms create a quick, efficient, and repeatable method of analysing vasicine. The combination of a working standard of vasicine produced a peak in the HPTLC chromatogram, as well as the corresponding UV spectra. The presence of marker compounds such as vasicine was detected in the HPTLC chromatogram of methanolic extract [20-21].

Optimum situation:

Dioxane-ammonia (9:1) was used as the solvent system in Silica gel 60 F254 precoated plates (10 x 10 cm).Precoated HPTLC plates were used to spot test samples.The plate's bandwidth was set to 6 mm, and the ascending mode was used for thin layer chromatography development.The saturation time was 25 minutes.TLC plates were developed up to a length of 8 cm. For quantification, the TLC plates were scanned at 254 nm. *Jasticia adhatoda* extract as a drug, sample Detection: Dragendroff reagent [5].

RESULT AND DISCUSSION

Physicohemical characterization

Sr. No.	TEST	OBSERVATION	RESULT
1.	**Test for Carbohydrates** **a) Molisch's test** **b) Benedict's test**	**Violet ring at junction.** **Green color appears.**	**Carbohydrates were present.** **Carbohydrates were present.**
2.	**A)Test for anthraquinone glycosides** **B) Test for Saponin glycosides**	**Ammonical layer do not show pink color.** **Persistent foam observed.**	**Anthraquinone glycosides were absent.** **Saponin glycosides were present.**
3.	**Test for alkaloids** **a) Mayer's test** **b) Dragendroff's test** **c) Wagner's test** **d) Hager's test**	**Precipitate observed.** **Orange brown ppt observed.** **Reddish brown pptobserved.** **Yellow ppt observed.**	**Alkaloids were present.** **Alkaloids were present.** **Alkaloids were present.** **Alkaloids were present.**
4.	**Test for tannins compounds** **a) Lead acetate solution** **b) Bromine water**	**White precipitate observed.** **Decoloration of bromine water.**	**Tannins were present.** **Tannins were present.**

	c) Dilute iodine solution	Transient red color observed.	Tannins were present.
5.	**Test for Phenolic compounds** **a) Ferric Chloride test** **b) Dilute Potassium permanganate solution**	Deep blue-black color observed. Decoloration observed.	Phenolic compounds were present. Phenolic compounds were present.
6.	**Test for flavonoids** **a) Shinoda test** **b) Sulfuric acid test**	Pink color observed. Orange color observed.	Flavonoids were present. Flavonoids were present.
7.	**Test for amino acid**	Purple or bluish color observed.	Amino acid were present.
8.	**Test for Organic acid** **a) Oxalic acid test** **b) Tartaric acid** **c) Citric acid** **d) Malic acid**	White precipitate observed. Precipitate observed. Precipitate observed on boiling and then cooling. Precipitate not observed on adding absolute alcohol.	Oxalic acid were present. Tartaric acid were present. Citric acid were present. Malic acid wereabsent.
9.	**Test for In-Organic elements** **a) Test for Calcium** **b) Test for Magnesium** **c) Test for Sodium** **d) Test for Potassium** **e) Test for Iron** **f) Test for Sulphate.** **g) Test for Phosphate**	White precipitate observed. White crystalline precipitate observed. Yellow precipitate observed. Yellow precipitate observed. Dark blue coloration observed. White crystalline precipitate not observed. Yellow crystalline precipitate not observed.	Calcium were present. Magnesium were present. Sodium were present. Potassium were present. Iron were present. Sulphate were absent Phosphate were absent.

Preformulation study

Physical properties of *Justicia adhatoda L.* extract powder was found to be grey brown colour, brownish crystalline appearance, characteristic odour, bitter taste, pH is 6.460.The softening purpose of concentrate was discovered to be 210^0C, which agrees with the announced dissolving point (209-211^0C).

Determination of λ_{max}:

The UV spectrum obtained after the proper dilution of the extract. The extract solution was scanned at 800-200 nm and wavelength (λ_{max}) was found to be 298 nm in phosphate buffer pH 6.8 using a double beam UV spectrophotometer (Shimadzu 1800, Japan).

FTIR Spectroscopy

To investigate the drug's compatibility with excipients, the IR spectra of the drug in combination with excipients in a 1:1 ratio were studied and compared with standard peaks found in individual monographs in the compendial literature. The characteristics IR absorption region of important bands necessary in the elucidation of drug and excipients presented below. Result of the

Preformulation study suggested that all the studied excipients were compatible with *Jasticia adhatoda* extract powder.
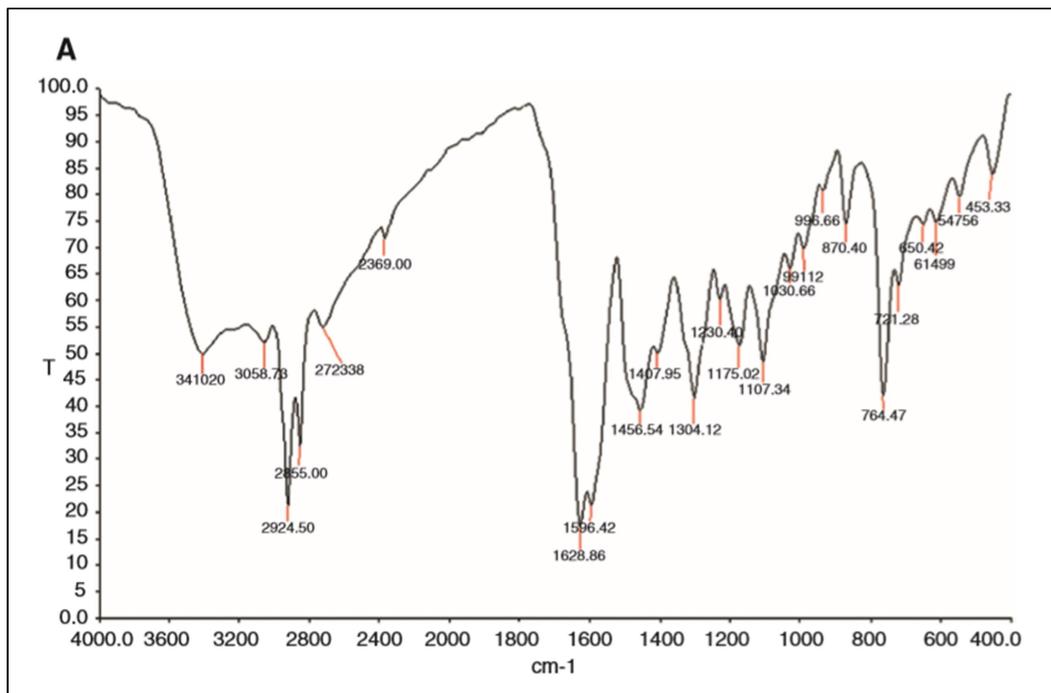

Figure 2: (A) FTIR of Standard Vasicine

Interpretation of IR Spectrum (Standard. Vasicine)

At that frequency, the primary absorption peak of the drug showed O-H stretching hydroxyl group (alcohol) group at 3410 and C-H stretching (Alkane) group at 2924, C-O stretching (Ketone) group at 1628, and C-N stretching (Amine) group at 1304.
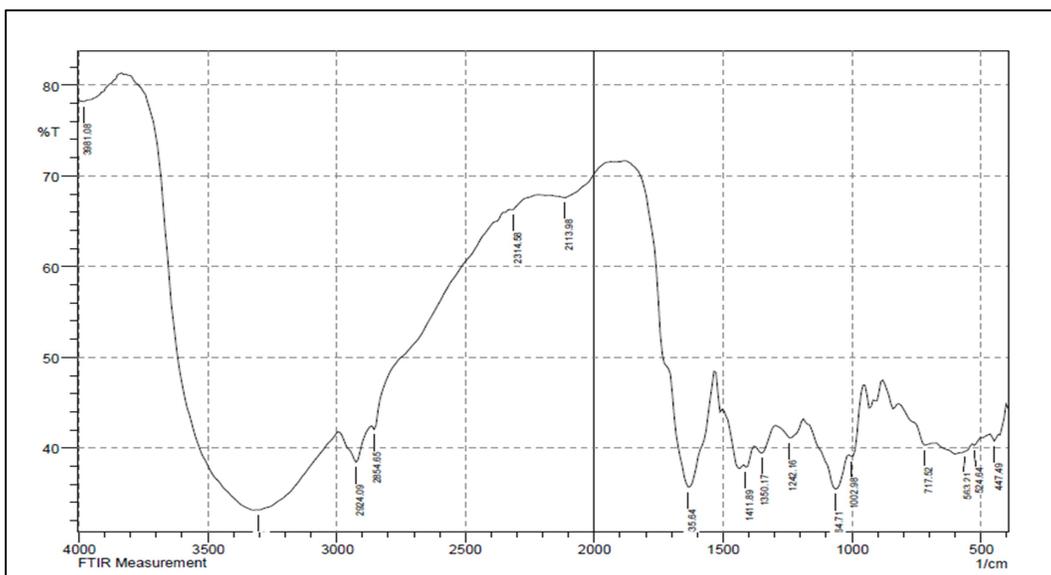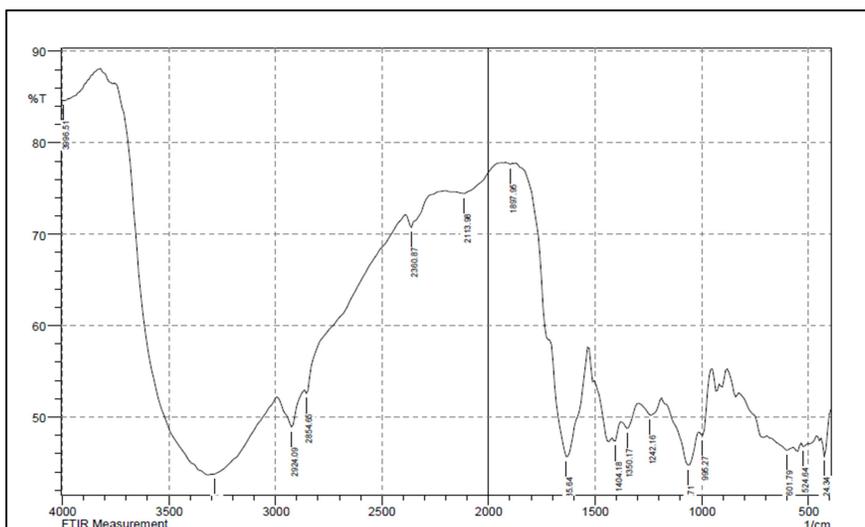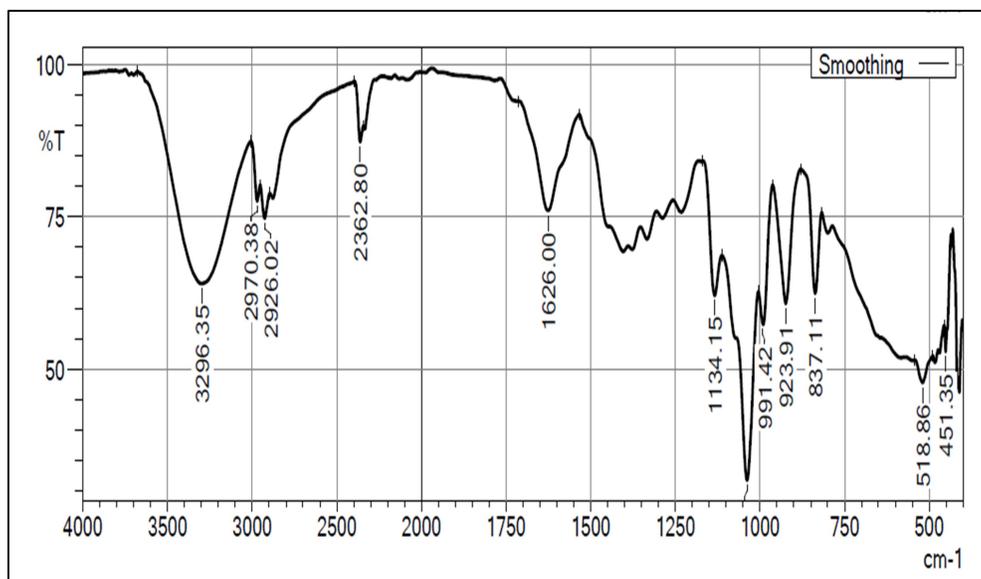

Figure 2: (B) FTIR of *Justicia adhatoda* Extract

Interpretation of IR spectrum (*Justicia adhatoda* extract)

At that frequency, the primary absorption peak of the drug showed O-H stretching hydroxyl group (alcohol) group at 3302 and C-H stretching (Alkane) group at 2924, C-O stretching (Ketone) group at 1635, and C-N stretching (Amine) group at 1350.
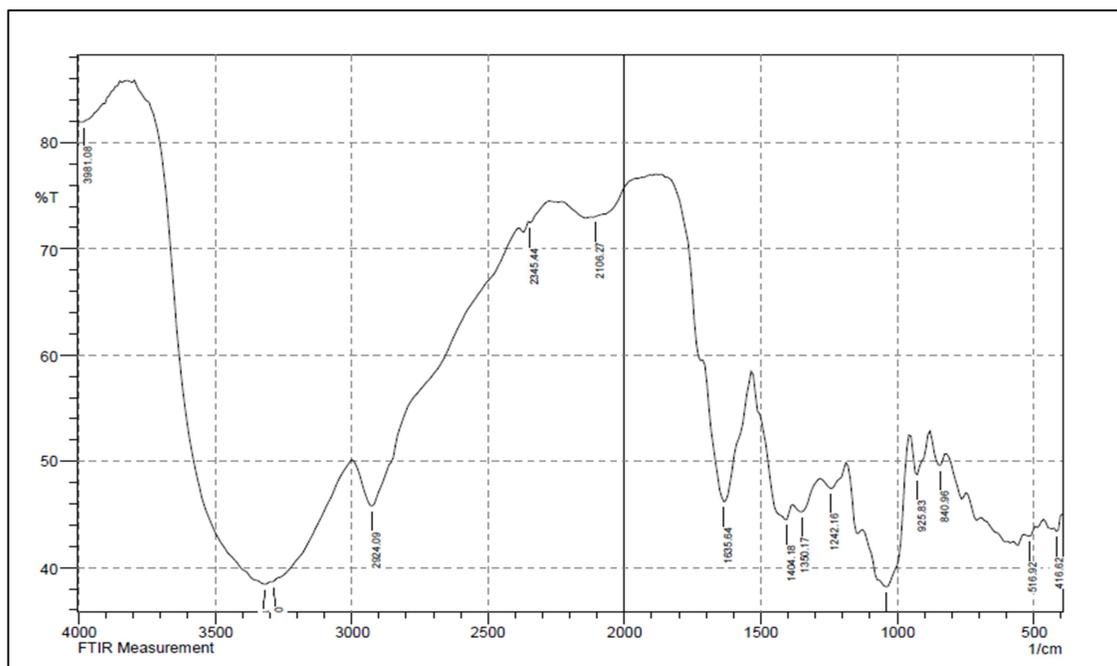

Figure 2: (C) FTIR of Extract+Gelatin

Interpretation of IR spectrum (Extract+Gelatin)

At that frequency, the primary absorption peak of the drug showed O-H stretching hydroxyl group (alcohol) group at 3294 and C-H stretching (Alkane) group at 2924, C-O stretching (Ketone) group at 1635, and C-N stretching (Amine) group at 1350.

Figure 2: (D) FTIR of Extract+Propylene Glycol

Interpretation of IR spectrum (Extract + Propylene Glycol)

At that frequency, the primary absorption peak of the drug showed O-H stretching hydroxyl group (alcohol) group at 3396 and C-H stretching (Alkane) group at 2924, C-O stretching (Ketone) group at 1626, and C-N stretching (Amine) group at 1134.

Figure 2: (E) FTIR of Extract + Stevia

Interpretation of IR spectrum (Extract + Stevia)

At that frequency, the primary absorption peak of the drug showed O-H stretching hydroxyl group (alcohol) group at 3317 and C-H stretching (Alkane) group at 2900, C-O stretching (Ketone) group at 1635, and C-N stretching (Amine) group at 1350.

High Performance Thin Layer Chromatography Method

The HPTLC chromatogram allows for a quick, efficient, and repeatable method of testing for vasicine. A mixture of vasicine working standards appeared in the HPTLC chromatogram, along with their corresponding UV spectra. The HPTLC chromatogram of methanolic concentrate demonstrated the closeness of marker mixtures, such as vasicine. HPTLC chromatogram of methanolic extract demonstrating the presence of two marker compounds, vasicine and vasicinone, as well as their UV spectra. HPTLC chromatogram shows standard Vasicine. Spotting standard Vasicine on an HPTLC plate yielded the alignment bend of Vasicine.
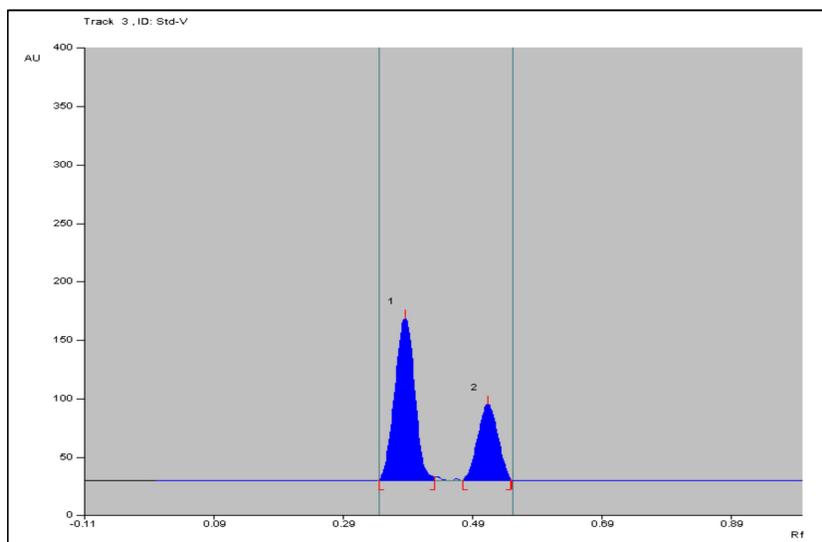
Figure 3: The HPTLC chromatogram of the standard vasicine mixture was shown (Resolution peak in fingerprinting)

Following improvement, the plate was investigated at 254 nm, as shown in **Figure 7**. Vasicine, a biomarker, was found in *Jasticia adhatoda* extract using quantitative estimation. Vasicine and vasicinone have been reported to have bronchodialatory and respiratory stimulation effects and are thus regarded as biological markers for *Jasticia adhatoda* extract standardisation. Few chromatographic methods, such as HPTLC, are available and reported.
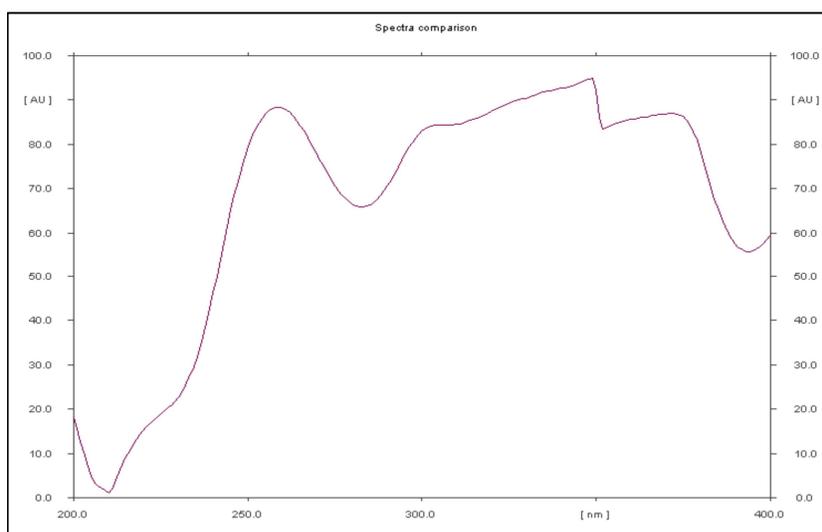

Figure 4: Spectrum of standard vasicine

The peak of the UV spectra of vasicine is shown in **Figure 5** of the standard vasicine spectrum. As a mobile phase, the dioxane-ammonia (9:1) solvent system produced R f 0.4–0.5 for vasicine.
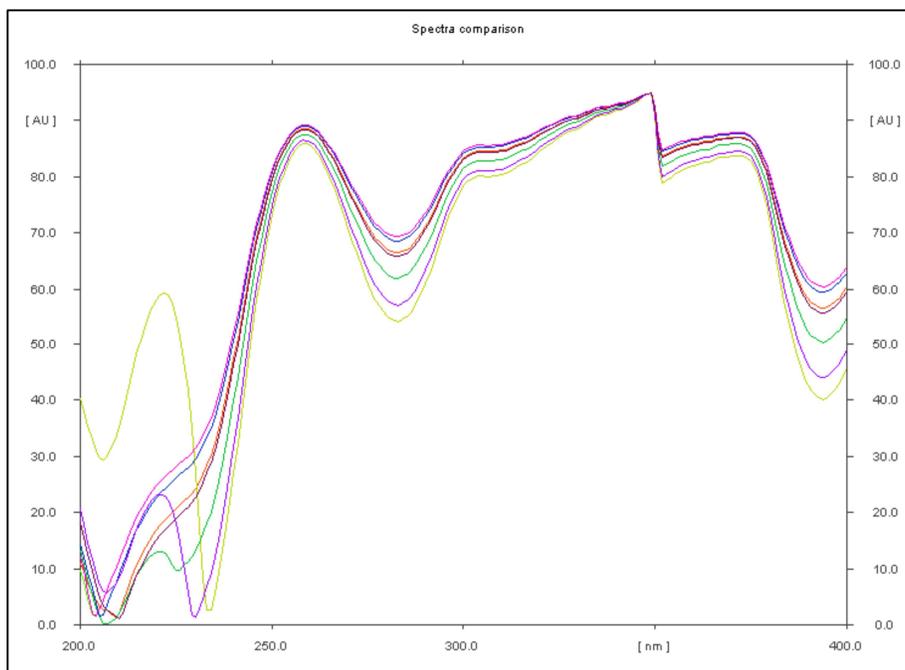
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Figure 5: Overlay spectra of standard vasicine

The spots were observed at 254 nm, and the three-dimensional densitogram configurations of the test samples and standard vasicine revealed that the peaks for all samples corresponding to R f 0.5 were overlaying. The features of this peak's corresponding spectrum were found to precisely match each other, revealing the compounds analogous to R f of the standards.

Me-OH   Me-OHVas.stdVas.std

Figure 6: Fingerprinting derivetization

The reported HPTLC methods have limitations such as the use of ammonia in the solvent system, which is hazardous, a higher limit of detection and quantification and linear range, which demonstrates lower sensitivity and precision, and the use of water in the mobile phase, which has a lower linear range. HPTLC was attempted as a fast, precise, sensitive, and reproducible method with good recoveries for standardisation of *Jasticia adhatoda* extracts.
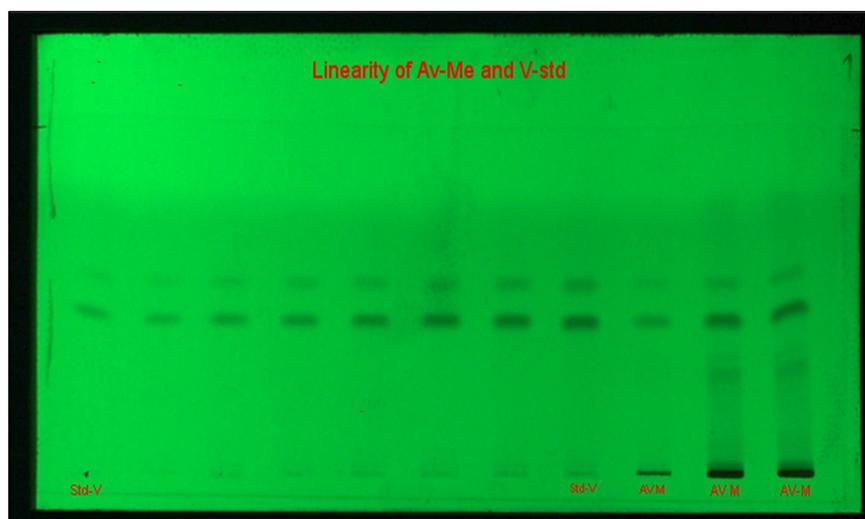

Figure 7: HP-TLC plate showing the distinct separationof vasicine after development (Fingerprinting at 254nm)

The major alkaloid vasicine present in plant extract was identified and confirmed using preliminary qualitative phytochemical screening in the current study. After comparing the colour of the zone to the shades of the reference compounds, the quantitative determination and confirmation of vasicine was performed. Short wave UV 254 nm spots were observed, as were five long wave UV 366 nm spots.
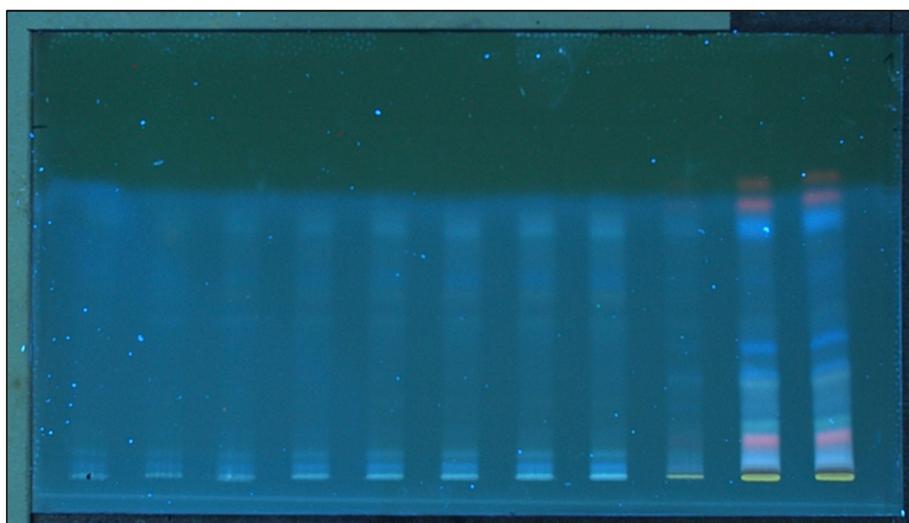

Figure 8: Fingerprinting at 366nm

The spectrum of the standard compound Vasicine and the corresponding spot present in *Jasticia adhatoda* leaves matched exactly, indicating that no interference from other plant constituents or excipients was present. Visual inspection under UV light revealed few spots, but densitometer analysis revealed many more.
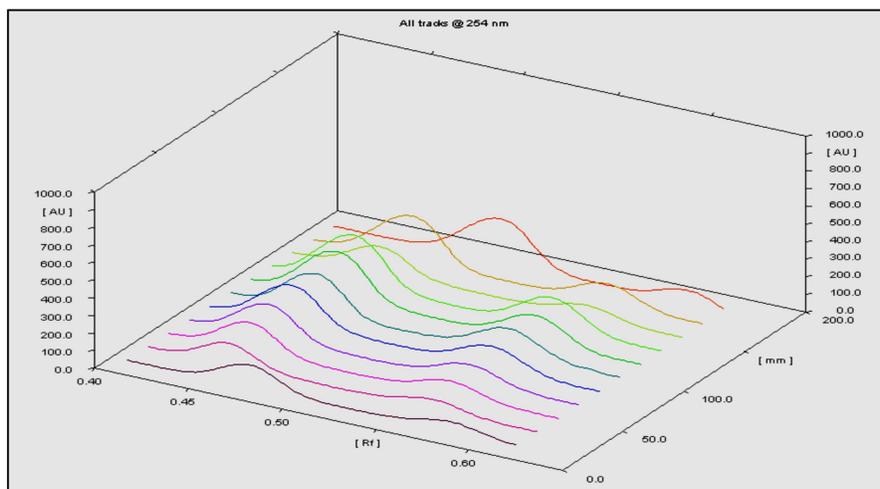

Figure 9: 3D Graph of Linearity

The linearity standard curve was found to be between 2 and 6 g/spot, with a correlation coefficient of 0. All tracks are shown in a 3D linearity graph at 254nm and 366nm. The HPTLC analysis was coloured in a bright orange zone that corresponded to the proximity of the alkaloids identified in the current study.

CONCLUSION

Phytochemical study identified the no. of phytoconstituents in the *Jasticia adhatoda* extract. Having bronchodilation, expectorant and antimicrobial activity. Extraction of herb was done using suitable traditional methods and evaluated by various physicochemical methods for alkaloids, tannins, saponins, flavanoids, and phenolics etc. further proceeded for characterization by UV, FTIR and RP-HPTLC confirmed the presence of flavonoids in the isolated fraction of *Jasticia adhatoda* extract.

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