Chapter 3

Isolation and Characterisation of the Alkaloid, Vasicine from in vitro Cultures of Justicia adhatoda L.

3.1. ABSTRACT

Justicia adhatoda L, belonging to family Acanthaceae, contains alkaloids like vasicine, vasicinone, deoxyvasicine. These alkaloids give the plant its expectorant activity, antispasmodic, antiseptic and antihelminthic properties. Hence, in the present investigation attempts were made to isolate and characterize alkaloid vasicine from callus and suspension cultures of J. adhatoda. Alkaloids were extracted with methanol, quantified and identified by colour reactions, Thin layer chromatogram (TLC), High performance liquid chromatogram (HPLC) and Fourier transform infrared spectroscopy (FT-IR) using vasicine as standard.

Key words: Justicia adhatoda L, vasicine, expectorant, antispasmodic, High performance liquid chromatogram, Fourier transform infrared spectroscopy
3.2. INTRODUCTION

The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. The use of the medicinal herbs for curing disease has been documented in history of all civilizations. Man in the pre-historic era was probably not aware about the health hazards associated with irrational therapy. With the onset of research in medicine, it was concluded that plants contain active principles, which are responsible, for curative action of the herbs (Ayurveda herbs, 2005).

Before the onset of synthetic era, man was completely dependent on medicinal herbs for prevention and treatment of diseases. With introduction of scientific procedures the researchers, were able to understand about toxic principles present in the green flora. The scientists isolated active constituents of the medicinal herbs and after testing some were found to be therapeutically active. Aconitine, Atisine, Lobeline, Nicotine, Strychnine, Digoxin, Atropine, Morphine etc. are some common examples. Recently research has supported biological activities of some medicinal herbs (Ayurveda herbs, 2005).

Unlike humans and animals, plants are not mobile which makes them very susceptible to attack from pests and predators. During metabolism plants produce enormous number of compounds as part of defense mechanisms which are not essential for primary functions and are called secondary metabolites and are used as pharmaceutical, agrochemicals, aromatics and food additives (Rao and Ravishankar, 2002). Plant derived compounds include many terpenes, polyphenols, steroids, alkaloids and glycosides (Sidhu, 2010).
All plants produce secondary metabolites which are often specific to an individual species or genus during specific environmental conditions making their extraction and purification difficult. Large scale plant tissue culture is an attractive alternative to the traditional methods of plantation, as it offers two advantages: (1) controlled supply of biochemicals independent of plant availability (politics, climate, pests,), and (2) well defined production systems which result in higher yields and more consistent quality of the product. During the last 30 years, plant cell and tissue cultures have been comprehensively studied for the production of secondary metabolites (Rao and Ravishankar, 2002).

Alkaloids are natural compounds found in all plants and are considered to be products of disintegration of proteins. Generally alkaloids are amalgams that do not have any scent and boast of a distinctive outcome on an animals’ body mechanism or function. Among all elements found in the plants, alkaloids are the most powerful as well as very effective.

Vasicine, a bioactive chief principle pyrroloquinazoline alkaloid isolated from the medicinal plant *J. adhatoda* is reported to possess bronchodilatory and expectorant properties. Other than vasicine this plant contains 1-vasicinone, deoxyvasicine, maiontone, vasicol adhatodinine and vasicinol.

During the last 30 years there has been an increasing interest among scientists to produce high value natural plant products by cell culture. The production of a range of chemicals using plant tissue cultures is remarkable. Cell cultures can overcome many of the problems associated with industrial production of these phytochemicals by extraction from field grown plants.
and the production can be carried out throughout the year, unaffected by the season (Narayanaswamy, 1994).

3.3. REVIEW OF LITERATURE

Plant cells are factories of chemical compounds produced for carrying out biochemical pathways of survival and propagation. Plants have been the source for many important drugs because they are able to produce various chemical entities and bioactive molecules through the process known as metabolism. Plant cells carry out both primary and secondary metabolism. Secondary metabolites are plant pigments (such as alkaloids, isoprenoids etc.) responsible for the colours, flavours and smell in plants which also acts as a source of drugs, fine chemicals, insecticides, dyes, flavours and fragrances. Plant derived secondary metabolites have played an essential role as medicine for thousands of years. Currently, secondary metabolites with bioactivity are being isolated and used either directly or after chemical modification. Their pharmacological value is increasing due to the constant discoveries of their potential roles in healthcare and as lead compounds for new drug development (Shilpa et al., 2010).

Secondary metabolism of plants is activated only during particular stages of growth and development or during periods of stress, limitation of nutrients or attack by microorganisms (Shilpa et al., 2010). These are organic compounds that are not directly involved in the normal growth, development or reproduction of organisms. Unlike primary metabolites, absence of secondary metabolites results not in immediate death, but in long-term impairment of the organism’s survivability/ fecundity or aesthetics or perhaps in no significant change at all.
Biotechnological approaches, specifically plant tissue culture plays a vital role in search for alternatives to production of desirable medicinal compounds from plants (Rao and Ravishankar, 2002). On a global scale, medicinal plants are mainly used as crude drugs and extracts.

Higher plants are inexhaustible sources of a wide range of biochemicals such as flavours, fragrances, natural pigments, pesticides and pharmaceuticals (Chattopadhyay et al., 2002). Recently, the ethyl acetate extract of *Alpinia officinarum* has been reported interestingly of possessing dual property of anti-microbial and anti-inflammatory (Subramanian et al., 2008) and methanol extract of *Ocimum basilicum* has been reported to have anti-inflammatory activity (Selvakumar et al., 2007a).

The plant based drug discoveries gained interest with the development of anti-cancer and anti-infectious agents and are being isolated for the treatment of various other disease including metabolic disorders like Diabetes and Obesity such as Metformin, a commercially available anti-diabetic drug derived from *Galega officianalis*. The Synthetic aspirin used by the modern world today is a derivative of a plant-based drug (Shilpa et al., 2010).

Currently many of these secondary metabolites are isolated by solvent extraction from the naturally grown whole plants. The continued destruction of plants has posed a major threat to the plant species getting extinct over the years (Chattopadhyay et al., 2002). Moreover, the natural habitats of many plants are disappearing due to environmental and geopolitical instabilities, therefore making it very difficult to acquire important secondary metabolites and in the process leaving many potentially useful compounds left undiscovered. Plant cell culture is considered as a
promising alternative for producing bioactive compounds that are difficult to be obtained by chemical synthesis or plant extraction. Plant cell culture studies have been carried out based on the fact that each plant cell in the culture exhibits totipotency, wherein the cell has full set of genes necessary for all the functions of a plant, including secondary metabolism (Shilpa et al., 2010). Cell culture systems are useful in large-scale culturing of plant cells, which form a continuous and reliable source of secondary metabolites and can be purified easily due to the absence of significant amounts of pigments. This method removes all seasonal constraints and eliminates the geographic barriers for production of secondary metabolites (Karuppusamy, 2009).

Growth phase, nitrogen source, carbon source, phosphates, inorganic supply of other nutrients and plant growth regulator concentration are played a critical role in secondary metabolite accumulation (Shilpa et al., 2010).

A suspension culture is developed by transferring the relatively friable portion of a callus into liquid medium and is maintained under suitable conditions of aeration, agitation, light, temperature and other physical parameters. However, various strategies may have to be adopted to obtain a fairly homogeneous suspension culture. Inclusion of pectinase, reduced the clumping of cells and polyvinyl pyrrolidone, stopped browning of the culture medium. It was observed that the development of an active and segregated population of plant cells in suspension cultures was dependent on the zone of collection of the plant species, the genetic make-up, the callus texture and the medium/hormone combinations (Chattopadhyay et al., 2002).

Secondary products in plant cell culture can be generated on a continuous year-round basis; there are no seasonal constraints. Plant cell culture eliminates potential political boundaries or geographic barriers to the
production of a crop, such as the restriction of natural rubber production to the tropics or anthocyanin pigment production to climates with high light intensity. Extraction from the *in vitro* tissues is much simpler than extraction from organized, complex tissues of a plant. Plant tissue culture techniques offer the rare opportunity to tailor the chemical profile of a phytochemical product, by manipulation of the chemical or physical microenvironment, to produce a compound of potentially more value for human use. A tremendous research and development efforts have advanced a number of other *in vitro* - derived secondary products to semi commercial status, including vanillin and taxol production in cell cultures. In a myriad of other cases, the *in vitro* processes for secondary metabolite production have fallen far short of expectations and have never approached commercial status. Still, the arena of secondary product formation in cell cultures remains as an industrial pursuit. Engineers and biologists are currently joining forces on a global scale to develop new strategies for streamlining the critical bioprocesses (Karuppusamy, 2009).

Plant cells in suspension cultures often undergo spontaneous genetic variation in terms of accumulation of secondary metabolites, which leads to heterogeneous population of cells in a suspension culture. This variation, known as somaclonal variation, has posed a commercial hurdle to the large scale production of secondary metabolites.

Gaines (2004) stated that secondary metabolites are chemical compounds in organisms that are not directly involved in the normal growth, development or reproduction of organisms.

*In vitro* culture allows the production of secondary metabolites under controlled conditions. Two types of *in vitro* cultures are currently used for
secondary metabolite production; dedifferentiated cultures (callus and suspension cultures) and differentiated cultures (transformed roots and shoots). The former tends to accumulate secondary metabolites only in small amount compared to the whole plant. The approaches used for increasing the production of secondary metabolites include optimization of growth and production of culture media, as well as elicitation and metabolic engineering.

Alkaloids act as a form of nitrogen storage in plants (Harborne, 1998). Various phenolic compounds have attracted the attention of food and medical scientists because of their fragrant aroma, antioxidant and anti-inflammatory properties (Mabinya et al., 2006).

The fresh and dried leaves of *Justicia adhatoda* possess several alkaloids such as vasicine, vasicinone, vasicol, adhatonine, vasicinol etc. Presence of bioactive compounds and their investigation has been reported in *J. adhatoda* L. (Gulfraz et al., 2004). Soni et al. (2008) reported the validation of different methods of preparation of *J. adhatoda* leaf juice by quantification of total alkaloids and vasicine using spectrophotometric method, TLC and HPLC techniques.

Vasicine/ Peganine is a quinazoline type alkaloid. Few of the main chemical constituents of *J. adhatoda* are vasicine (derived from leaves), 2'-hydroxy-4-glucosyloxychalcone, vasicol (from leaves), vasicinone (from leaves, stem and roots), vasicinol (contained in stem and roots), and deoxyvasicinone (from leaves) etc. Vasicine is the major, as well as, the most important active principle of this medicinal plant. It is an optically active molecule in its natural condition but, gets racemized when extracted.
Table 3.1. Medicinal properties of Vasicine

<table>
<thead>
<tr>
<th>Medicinal properties of vasicine</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Antioxidant and anti-inflammatory activity</td>
<td>Chakraborty and Brantner, 2001; Srinivasrao <em>et al</em>., 2006</td>
</tr>
<tr>
<td>Radioprotective/genoprotective activity</td>
<td>Jahangir <em>et al</em>., 2006</td>
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<tr>
<td>Radiation protective ability</td>
<td>Sharma <em>et al</em>., 2009</td>
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<tr>
<td>Antioxidant property</td>
<td>Pandit <em>et al</em>., 2004</td>
</tr>
<tr>
<td>Mucus liquefying/expectorant activity</td>
<td>Rachana <em>et al</em>., 2011; Sharafkhaneh <em>et al</em>., 2007</td>
</tr>
<tr>
<td>Potentiated the action of oxytocin, smooth muscle stimulant activity, effect on uterus, mammary gland, guinea pig ileum and guinea pig tracheal muscle.</td>
<td>Madappa <em>et al</em>., 1989</td>
</tr>
<tr>
<td>Uterotonic effect similar to that of oxytocics</td>
<td>Rachana <em>et al</em>., 2011</td>
</tr>
<tr>
<td>Control the capillary hemorrhages and correct the drug induced bone marrow depression.</td>
<td>Rachana <em>et al</em>., 2011</td>
</tr>
<tr>
<td>Inhibit IgE-dependent mediator secretion</td>
<td>Gibbs, 2009</td>
</tr>
<tr>
<td>Anticestodal activity</td>
<td>Yadav and Tangpu, 2008</td>
</tr>
<tr>
<td>Antileishmanial activity</td>
<td>Khaliq <em>et al</em>., 2009</td>
</tr>
<tr>
<td>Anti-helminthic activity</td>
<td>Al-Shaibani <em>et al</em>., 2008</td>
</tr>
<tr>
<td>Anti-bacterial activity</td>
<td>Karthikeyan <em>et al</em>., 2009</td>
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</table>
One of the derivatives of vasicine is Bisolvon/ bromhexine (N-cyclo-N-methyl-(2-amino-3, 5-dibromo-benzyl) amine hydrochloride). It has been reported to possess mucus liquefying/expectorant activity by Rachana et al. (2011) and Sharafkhaneh et al. (2007).

Ambroxol, a widely used semisynthetic, secretolytic agent developed from vasicine, is found to inhibit IgE-dependent mediator secretion from human mast cells and basophils, which are the main effector cells of allergic inflammation (Gibbs, 2009). Ambroxol hydrochloride is a metabolic product of bromhexine.

There are studies describing various methods for extraction of vasicine from J. adhatoda. Soni et al. (2008) compared different methods of extraction of vasicine including the classical method in which, the crushed leaves are subjected to wet heat followed by squeezing the extract as well as modernization of the same by exposing the leaves to steam at 15 lbs. The total alkaloid content varied from 0.3 mg ml\(^{-1}\) to 5.93 mg ml\(^{-1}\) and vasicine content varied from 0.2 mg ml\(^{-1}\) to 5.64 mg ml\(^{-1}\) for different methods.

Although J. adhatoda is the main source of obtaining vasicine, other plants were also worked upon to isolate this molecule. Vasicine was isolated from a different plant namely Sida cordifolia by Ghosal et al. (1975). Susag et al. (2003) isolated vasicine from two species of Afrogalega: Galega battiscombei (Bak.f) Gillett and Galega lindblomi (Harms) Gillett. Bagchi et al. (2003) reported its isolation from A. beddomei and the content of vasicine was compared to Adhatoda zeylanica / vasica. They also reported that Adhatoda zeylanica/ vasica showed wide seasonal variation throughout the year. The variation of vasicine was found from 1.22 % to 2.57 % in a
year. Peganine/vasicine hydrochloride was isolated from *Peganum harmala* seeds in dehydrated form by Khaliq *et al.* (2009).

Chromatography is used to separate components of a mixture. The technique relies on the fact that the different molecules in the mixture move through the matrix at different rates and so can be separated and then identified. The substance chosen for the chromatography is known as the stationary phase through which the mixture moves. The choice of stationary phase will vary with the mixture being examined. The special advantages of thin layer chromatography include versatility, speed and sensitivity. Silica gel is the most widely used adsorbent for TLC. Versatility is due to the fact that a number of different adsorbents besides cellulose may be spread on to the glass plate. The greater speed of TLC is due to the more compact nature of adsorbent when spread on plate. The sensitivity of TLC is that separation on less than µg amounts of materials can be achieved if necessary. Preparative TLC is carried out using thick (up to 1 mm) instead of thin layers of adsorbent. Separated constituents are removed by scrapping off the adsorbent at the appropriated places on the developed plate, eluting the powder with a solvent and finally centrifuged to remove the adsorbent (Harborne, 1998; Radoslaw and Tadeusz, 2003; Gocan and Cimpan, 2004; Mabinya *et al.*, 2006).

Infrared spectroscopy is widely used in both research and industry as a simple and reliable technique for measurement, quality control and dynamic measurement. IR spectra are usually contributed to the structural elucidation, when new compounds are encountered in plants. It is used to identify unknown materials present in a specimen. The technique works on the fact that bonds and groups of bonds vibrate at characteristic frequencies. The range of measurement is from 4000 to 667 cm\(^{-1}\). Many of the functional
groups can be identified by their characteristic vibration frequencies makes
the IR spectrum the simplest and often the most reliable method of assigning
a compound to its class (Harborne, 1998; Walton and Brown, 1999).

High performance liquid chromatography is a form of column
chromatography used frequently in biochemistry and analytical chemistry.
HPLC is mainly used for those classes of compounds, which are non-
volatile, e.g. higher terpenoids, phenolics of all types, alkaloids, lipids and
sugars. It works best for compounds, which can be detected in the ultraviolet
and visible spectrum. HPLC procedures normally operate at ambient
temperature, so that the compounds are not subjected to the possibility of
thermal rearrangement during separation (Harborne, 1998; T¨Uzen and
¨Ozdem_Ir, 2003).

3.4. SPECIFIC OBJECTIVES

The study was designed for extraction, isolation, characterization and
quantification of vasicine from in vitro cultures of J. adhatoda L.

1a) To develop a protocol for suspension culture of J. adhatoda L.

1b) To extract, isolate and characterize vasicine from callus and
suspension cultures of J. adhatoda L.

1 c) To quantify vasicine production from callus and suspension
cultures of J. adhatoda L.
3.5. MATERIALS AND METHODS

3.5.1. SUSPENSION CULTURE

In order to initiate suspension culture of *J. adhatoda*, 50gms of friable calli from culture tubes were transferred to 250 ml Erlenmeyer flask containing 50 ml of MS medium. The flasks were incubated on a rotary shaker at 120 rpm using the same culture conditions used for callus culture. The suspension culture medium was analyzed every 5 days for secondary metabolite production using thin layer chromatography. Both suspension and callus extracts were taken for TLC.

3.5.2. EXTRACTION OF SECONDARY METABOLITE

3.5.2.1. Extraction from suspension culture

From the suspension culture of friable calli, the supernatant was collected and extracted with methanol for 30 minutes by using a separating funnel. The extract collected (VAS II) and concentrated to half the volume.

3.5.2.2. Extraction from callus

500 mgs of callus from culture was ground well with a mortar and pestle in 10ml volume of methanol. Remove the supernatant and repeat the procedure for three times. The supernatant collected was evaporated to half the volume (VAS I).

3.5.2.3. Thin layer chromatographic separation

The extracts collected from callus culture (VAS I) and suspension culture (VAS II) were chromatographed over a silica gel plate.TLC was carried out on ‘Merck silica gel 60’ plates. The methanol extracts of samples were spotted on the plates using capillary tubes about 2 cm from the lower end of the plates. The plates were developed with Ethyl acetate: Methanol:
Water (100: 13.5: 10) in a pre-saturated chromatographic chamber. The solvent was allowed to run up to 2/3rd of the plate. Developed plates were then dried in a hot air oven at 100°C. The methanol extracts of VAS I and VASII were used for HPLC and FT-IR analysis after separation with TLC.

3.5.2.4. Analysis of samples

Detection of the samples in the TLC plate was done under UV light of 245 and 365 nm. The colour of the spots was noted with Dragendorff’s and the Rf values were calculated. The TLC analysis suggested the possibility of an alkaloid as one secondary metabolite. The conformation of the presence of alkaloid was done by HPLC and FT-IR. The orange coloured spots were separately scrapped off from the plates, and the individual fraction isolated was quantified using spectrophotometric analysis (Soni et al., 2008) and identified using FT-IR and HPLC analysis with standard vasicine (SPIC India Ltd, Chennai). Quantification of total alkaloids was done by spectrophotometric method with tropaeolin ‘OO’ (Sigma Aldrich, India). Coloured complex developed was measured at 545 nm against blank. The amount of total alkaloids in the samples was calculated using standard curve of vasicine. The content of the total alkaloids was expressed as vasicine.

HPLC analysis of the above sample was carried out in Sree Chitra Institute of Research Centre, Thiruvananthapuram. HPLC is a form of column chromatography used frequently in analytical chemistry to separate, identify and quantify compounds. It utilizes a column that holds chromatographic packing material, (Stationary phase), a pump that moves the mobile phase (s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the
interactions between the stationary phase, the molecules being analyzed and the solvent(s) used.

HPLC equipment used was Varian with a manual injector, a programmable wavelength photodiode array UV detector (280 nm), and column packing with modified silicagel (C 18 column). The linear gradient elution was carried out with solvent A (acetic acid/water (2:98 v/v)) and solvent B (methanol/water/acetonitrile 40:40:20 v/v)) as mobile phase.

For the conformation of spectral characteristics FT-IR spectroscopy was done. It is a measurement technique that provides information about the chemical bonding or molecular structure of materials, whether organic or inorganic molecule that is exposed to infrared rays absorbs infrared energy at frequencies which are characteristic to that molecule. In the FT-IR spectrum, the samples examined along with standard vasicine. FT-IR analysis (Shimadzu) of the test samples was done at Sophisticated Test and Instrumentation Centre (STIC), Cochin University P.O. Kochi. The solvent used for dissolving the sample was methanol.

3.6. RESULT

3.6.1. SUSPENSION CULTURE

The callus of leaf inoculated in the suspension culture medium was analyzed every 5 days for secondary metabolite production using thin layer chromatography. Both suspension and callus extracts were taken for TLC.

3.6.2. CHROMATOGRAPHIC SEPARATION BY TLC

The chromatography plates developed were detected under UV light (Plate 3.1). The plates were sprayed with Dragendorff’s reagent. Orange coloured spots were developed (Wagner and Bladt, 2009) after drying the
plates in oven at 100°C for 15 minutes (Plate 3.2). The Rf value obtained for callus extract and suspension extract were 0.4. The Rf value was similar to that of vasicine (Plate 3.1). The suspension extract of 15th day of inoculation showed maximum diameter of spots in the TLC plate (Plate 3.2).

![TLC plates under UV light](image)

**Plate 3.1** TLC plates under UV light

![TLC chromatography plates](image)

**Plate 3.2** The chromatography plates showing Orange coloured spots developed after sprayed with Dragendorff’s reagent. a) VAS I (10th Day), b) VAS II (10th Day), c) VAS I & VAS II (15th Day)
Figure 3.1: Graphical representation of separation of extract from callus and suspension culture of *Justicia adhatoda* by TLC

Figure 3.1: The diameter of the spots on the TLC plate with VAS I and VAS II on 15\textsuperscript{th} day of inoculation is 4cm, which is greater than the diameter obtained on 10\textsuperscript{th} day (2cm for VAS I and 1cm for VAS II). No spots obtained on 1\textsuperscript{st} and 5\textsuperscript{th} day of inoculation from both (Figure 3.1). On 20\textsuperscript{th} and 25\textsuperscript{th} day the diameter of the spots decreased.

3.6.3. QUANTIFICATION OF VASICINE

Vasicine content was found to be 5.15 mg ml\textsuperscript{-1} and 4.09 mg ml\textsuperscript{-1} in callus and suspension cultures of leaf respectively which were on par with field grown plants (5.07 mg ml\textsuperscript{-1}). Callus and suspension cultures of axillary bud and root tip yielded 5.08 mg ml\textsuperscript{-1} and 3.95 mg ml\textsuperscript{-1} of vasicine and 5.05 mg ml\textsuperscript{-1} and 4.03 mg ml\textsuperscript{-1} of vasicine respectively. All the observations strongly support the presence of vasicine in the extract of callus and suspension cultures of various explants. The results obtained in the present work were promising and suggest a viable, alternative, simple, rapid and
inexpensive methodology for isolation and characterization of vasicine from *J. adhatoda* callus cultures.

![Standard graph of vasicine](image)

**Figure 3.2. Standard graph of vasicine**

![The concentration of vasicine in suspension cultures of *J. adhatoda* in different growth periods.](image)

**Figure 3.3. The concentration of vasicine in suspension cultures of *J. adhatoda* in different growth periods.**
3.6.4. IDENTIFICATION OF THE SECONDARY METABOLITES BY HPLC AND FT-IR:

The TLC analysis suggested the possibility of an alkaloid as one of the component of the extract. Conformation of the presence of alkaloid was done by HPLC and FT-IR. The orange colour spot obtained in TLC were scrapped off from the plates and was subjected to HPLC (figure 3.4 B and C) and FT-IR (figure 3.5 A and B) analysis along with vasicine standard (Figure 3.4 A and 3.5 C).

![Figure 3.4 A. HPLC Chromatogram of Vasicine Standard](image)
Figure 3.4 B. HPLC Chromatogram of VAS II

Figure 3.4 C. HPLC Chromatogram of VAS I
For the conformation of spectral characteristics FT-IR spectroscopy was done using extracts and vasicine standard. In the FT-IR spectrum of the extracts examined, a characteristic broad band of range 3000-3600 cm$^{-1}$ was observed which was indicative of aromatic hydrogen and aromatic hydroxyl groups. The band of 1660 cm$^{-1}$ might be indicating the presence of saturated cyclic C-O or keto-enol conformation. Also bands at wave numbers 1020 cm$^{-1}$ might be indicative of C-N, 1114 cm$^{-1}$ might be of C-H and 1448 cm$^{-1}$ might be of aromatic hydrogen present in the suspected molecule. FT-IR spectra of the extracts were comparable with that of standard vasicine.

![FT-IR Spectrum of Vasicine](image)

**Figure 3.5. A.** FT-IR Spectrum of VAS 1
Figure 3.5. B. FT-IR Spectrum of VAS-II

Figure 3.5. C. FT-IR Spectrum of standard vasicine
3.7. DISCUSSION

In the present study an efficient protocol has been developed for the production of vasicine as a secondary metabolite through callus and suspension cultures of *J. adhatoda* L. The calli of leaf, axillary bud and root tip inoculated into the suspension culture showed cell separation and multiplication of callus. Suspension culture was often hindered by bacterial and fungal contamination. Separation, purification and characterization of vasicine have also been done by TLC, HPLC and FT-IR spectroscopy.

The secondary metabolite present in *J. adhatoda* was first confirmed as alkaloid by preparative TLC. The thickness of the plate, polarity and choice of solvent are important factors in the separation of alkaloids. There was a significant variation in the values of diameter of the spots. The spots were found to be more intense in 15th day of inoculation into suspension culture. In the present work the retention time detected under HPLC analysis is almost matching to the retention time of standard vasicine. The observations of FT-IR spectrum also strongly support the presence of vasicine in the extracts used.

VASICINE:

![Structure of Vasicine](image)

- **Molecular Formula**: C₁₁H₁₂N₂O
- **Formula Weight**: 188.226

From the findings upon quantification of vasicine production during different time periods, it was observed that initially maximum vasicine content was found in root tip callus suspension but maximum vasicine
production observed on 15th day of suspension culture was not significantly different in the case of suspension cultures of root tip, axillary bud and leaf calli.

The results obtained in the present work were promising and suggest a viable methodology for isolation and characterization of secondary metabolites. Once appropriate technology is developed callus suspensions can be used for the commercial production of vasicine from *J. adhatoda*. 