INTRODUCTION

Over three-quarters of the world population relies mainly on plants and plant extracts for health care. India is a major exporter of raw medicinal and aromatic plants and processed plant based drugs. Thus, the economic importance of medicinal plants is much more to countries such as India than to the rest of the world. Of these, about 15000-20000 plants have good medicinal value. However, only 7000-7500 species are used for their medicinal values by traditional communities. Nearly 25% of modern therapeutics is of plant origin. Plants have been used in the preparation of traditional medicine for a long time and most of these folk medicines were prepared from locally grown wild plants. Knowledge about the uses of plants was compiled by trial and error and passed down from one generation to another orally. Consumers are also more preferred to use plants as producers’ of secondary metabolites (Holm and Hiltunen, 2002).

Plant secondary metabolites were found to be the sources of various phytochemicals that could be used directly or as intermediates for the production of pharmaceuticals, as additives in cosmetics, food or drink supplements. In recent years, there has been a resurgence of interest in the discovery of new compounds from plants with the aim of finding novel treatment against a variety of illnesses. Many medicinal plants that reported to have the potential for medicinal purpose were investigated for useful active compounds. Many secondary products produced from medicinal plants have been commercialized. The key issues for commercialization of herbal-based products are standardization and consistency of material. Adulteration or microbial and heavy metal contamination is a potential risk. However recent successes of plant-derived products, increasing in production
of the Chinese and Indian herbal medicines and favorable regulation for commercialization have created a fast growing market for herbal based products and neutraceuticals. The increasing demand for medicinal plants will definitely reduce the sustainable supply of medicinal plants in the future. Moreover, plant secondary products are often produced only in small quantities in most of the plant species. It is not always feasible to isolate secondary compounds from intact plants. Besides, plants are endangered by a combination of factors such as over-collecting, unsustainable agriculture practices, urbanization, pollution and climate change, improper regulation on management and conservation. Efforts are therefore inevitable to evolve strategies for mass propagation of several valuable medicinal plants and also to bring about desired improvement for higher yield. Therefore, plant cell and tissue culture techniques can be an alternative approach to maintain sustainable supply of plant materials for producing bioactive compounds continuously under artificially controlled conditions (Thorpe, 2006). It is successfully applied in the propagation of plants with poor and uncertain response to conventional propagation and for plants on the verge of being extinct.

Plant tissue culture has made tremendous progress in plant biotechnology for crop improvement by manipulating genetic material at cellular level and molecular level. Micropropagation is the art and science of plant multiplication *in vitro*. Rapid development of plant tissue culture and growing fear that the earth is losing plant species at an alarming rate has focused attention on alternatives to whole plants for production of natural products. Plant tissue culture is potentially valuable for studying the biosynthesis of secondary metabolites and may also eventually provide an efficient means of producing plant products. Plant tissue culture has been
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Successfully used for the commercial production of pathogen-free plants and to conserve the germplasm of rare and endangered species. Principle advantage of this technology is that it provides year-round production of natural products in response. Shoot organogenesis via callus can be used as an effective method for multiplication of medicinal plants. The widespread use of plant in vitro culture techniques has many advantages when classical methods of in vivo vegetative propagation prove inadequate. In vitro cloning has been proven to be an important tool in speeding up propagation. In vitro propagated plants are often healthier than those cloned in vivo. This is mainly due to rejuvenation and they are often disease-free plants.

Theoretically all living cells are capable of giving rise to full plants and this phenomenon is called cellular totipotency. In cultures, isolated plant cells/tissues may be induced to form an actively growing mass of cells called callus which can be multiplied for an indefinite period by routine subculturing. It is an actively dividing and more or less undifferentiated tissue. It can be obtained from isolating tissues, organs and embryos in vitro; which generally first undergo dedifferentiation before all division starts. A portion of the callus tissue when transferred to the differentiation medium could result in shoot or bud regeneration or the formation of somatic embryos.

The most usual groups of plant growth regulators (PGR) used in tissue culture research are the auxins and cytokinins. The amount of PGR in the culture medium was critical in controlling the growth and morphogenesis of the plant tissues (Skoog and Miller, 1957). The age and physiological state of the mother plant could affect the formation of callus. Matkowski (2004) reported that callus derived from stem and petiole segments collected from the adult plants grew very slowly, became necrotic and eventually died, while satisfactory growth took place in root, stem and leaf callus derived
from *in vitro* germinated young plants. The season of the year to collect the explants could also affect callus initiation of explants derived especially from a field grown plant (George and Sherrington, 1984).

Plant cell cultures are initiated by transferring friable soft callus to liquid nutrient medium of the same composition as used for callus culture. Cell culture has to be agitated on an orbital shaker of between 90 and 150 rpm which serves both to aerate the culture and to disperse the cells. Kirsi and Wolfgang (2002) have stated that cell culture technique is an important system for genetic and breeding studies. Suspension cell cultures can also be used as the material of choice for biochemical and molecular investigation of plant secondary metabolite (Dicosmo and Misawa, 1995). The production of useful compounds by plant cell cultures has become increasingly significant for the last few decades, especially the production of pharmaceutically important plant metabolites. Several compounds e.g., shikonin, berberine, and ginseng saponins have commercially produced from *in vitro* cell cultures.

There are several factors affecting secondary metabolite production using plant cell cultures such as plant growth regulators, medium nutrients, physical factors and biological factors. Auxin and cytokinin affecting the production of secondary metabolite in plant cell cultures have been extensively investigated. It is well known that auxin is essential and cytokinin is preferable to induce cell differentiation and to maintain cell proliferation *in vitro* (George and Sherrington, 1984).

Hairy root induction and elicitors were found to be effective in increasing the production of secondary metabolites. Physical factors such as light, temperature, medium pH, aeration rate, can also affect secondary
metabolite synthesis in a cultured plant cells. Optimization of medium nutrients is also important to increase the productivity of particular secondary metabolites.

**About the plant**

*Justicia adhatoda* L. is one of the important medicinal herbs reported in the medicinal flora. The present study mainly targeted on the *in vitro* cultivation and secondary metabolite production in *J. adhatoda*.

Plate.1 *Justicia adhatoda* L.

Plate.2 *J. adhatoda* plant with buds and flowers

**CLASSIFICATION**

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<tr>
<th>Kingdom</th>
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<tr>
<td>Division</td>
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<td>Botanical Name</td>
<td><em>Justicia adhatoda</em> Linn.</td>
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<td>Syn</td>
<td><em>Adhatoda vasica</em> Nees.</td>
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*Justicia adhatoda*, Syn. *Adhatoda vasica* is one of the most important medicinal shrubs of family Acanthaceae, and subclass Asteridae growing in India and Pakistan. *Justicia* is a medicinal plant of common occurrence in Kerala. This shrub grows on the plains of India and in the lower Himalayans, up to a range of 1000 meters above sea level. This plant is also cultivated in other tropical areas. It will grow well in low moisture areas and dry soils. Botanically, the plant is a profusely branching shrub growing up to a height of 1.5 metres. Two major species are medicinally important viz., *Justicia adhatoda* (Valiyaadalodakam) and *Adhatoda beddomei* (Chittadalodakam or cheriyaadalodakam).

*J. adhatoda* is a small evergreen plant with broad, lanceolate leaves measuring 10 to 16 centimeters in length and 5 centimeters wide. They become greenish-brown when dried and have a bitter taste. They have a smell similar to strong tea. The wood of the stem is soft, and makes charcoal for gunpowder. Flowers of this plant are in the shape of a lion’s head; hence these plants are also called as Simhamukhi. The flower has large, attractive, white petals, streaked with purple on the lower lip. The fruit is a small capsule with 4 seeds.

This plant is like mother to doctors and is known as Vaidyamata (Anshutz, 1996) in Sanskrit. These are able to balance Kapha in the body and got the name Kaphahari. Other common names of this plant are Malabar Nut, Adulsa, Arusha, Vasaka, Arusa, Adathodai, Bakash, Adathoda, Adalodakam, Adusoge, Addasaramu, Lion’s Muzzle, Stallion’s Tooth etc.

The leaves, roots, flowers and stem bark of this plant are used in medicinal applications. *J. adhatoda* has been using in the traditional Indian medicine for thousands of years to treat respiratory disorders. The plant is
useful in treating bronchitis, tuberculosis and other lung and bronchiole disorders. A decoction of the leaves may be used to help with cough and other symptoms of colds. The soothing action helps to alleviate irritation in the throat and the expectorant will help loosen phlegm deposits in the airway. A poultice of the leaves may be applied to wounds for their antibacterial and anti-inflammatory properties. The poultice is also helpful in relieving rheumatic symptoms when applied to joints. It has been used to control both internal and external bleeding such as peptic ulcers, piles and bleeding gums.

In Ayurvedic medicine, *J. adhatoda* has been used for a multitude of disorders including: bronchitis, leprosy, blood disorders, heart troubles, thirst, asthma, fever, vomiting, loss of memory, leucoderma, jaundice, tumors, mouth troubles, sore-eye, fever and gonorrhea.

This herb exhibits antispasmodic, expectorant and blood purifying qualities. *J. adhatoda* has also been used to speed delivery during childbirth. Justicia leaves are rich source of vitamin C. There are different alkaloids present in Justicia and is used in the treatment of cold, cough, bronchitis, asthma, rheumatism, etc. It has sedative properties. Though leaves are the primary source of medicine, flowers, roots and fruits are also used. The leaves of the plant contain two major alkaloids called vasicine and vasicinone 1, 2.

![Structural formula of Vasicine and Vasicinone](image)

The plant is valued for containing bronchodilator alkaloids, mainly vasicine. All parts of the plant are used in herbal medicine and particularly the leaves are credited with insecticidal and parasiticidal properties. Chemical
compounds found in leaves and roots of this plant includes essential oils, fats, resins, sugar, gum, amino acids, proteins, vitamin ‘C’ etc.. The root is useful in strangury, leucorrhoea, bronchitis, asthma, bilious vomiting, sore eyes, fever and gonorrhoea. It is a valuable antiseptic and antihelminthic. The leaves are considered as a very efficacious remedy for all sorts of coughs and asthma, diarrhoea and dysentery. The leaves are also used for rheumatism. The flowers and the fruit are bitter, aromatic and antispasmodic. The fresh flowers are used in ophthalmia, lessen strangury and jaundice (Gulfraz et al., 2006).

The medicinal properties of *J. adhatoda* are well known in India and several other countries for many years. The leaves contained an essential oil and alkaloids quinazoline, vasicine, vasicinone and deoxyvasicine (Shinawie, 2002). The roots contained vasicinolone, vasicol, peganine and 2’-hydroxy-4-glucosyl-oxychalcone. The flowers contained– D-glucoside, kaempferol and its glucosides, as well as the bioflavonoid, namely quercetine (Gulfraz et al., 2004).

The leaves are mostly used in the treatment of respiratory disorders in Ayurveda. The alkaloids, vasicine and vasicinone present in the leaves, possess respiratory stimulant activity; whereas, vasicine, at low concentrations, induced bronchodilation and relaxation of the tracheal muscle. However, at high concentrations, vasicine offered significant protection against histamine-induced bronchospasm in guinea pigs. Vasicinone, the auto-oxidation product of vasicine has been reported to cause bronchodilatory effects both *in vitro* and *in vivo* (Shinawie, 2002).

The plant is conventionally propagated by seeds and by stem cuttings. Chomchalow and Sahavacharin (1981) first attempted regeneration of *J. adhatoda* through tissue culture. Later Jaiswal *et al.*, (1989) reported regeneration of
*J. adhatoda* plantlets *in vitro* by culturing nodal explants on MS medium. However, only limited number of plantlets was produced in both cases.

The present investigation was therefore, undertaken to establish a protocol for *in vitro* propagation of *J. adhatoda* with a view to isolate and characterize the bronchodilatory alkaloid namely vasicine.

**Objectives:**

The study was designed and executed to achieve the following broad objectives:

1. To micropropagate *Justicia adhatoda* L. through direct organogenesis.
2. To induce indirect organogenesis and histological analysis of organogenic and non-organogenic calli obtained from *in vitro* cultures of *J. adhatoda* L.
3. To isolate and characterize the alkaloid vasicine from *in vitro* cultures of *J. adhatoda* L.
4. To study the effect of elicitors on the production of vasicine from *J. adhatoda* L. cell suspension cultures.
5. To develop salinity resistant somaclones of *J. adhatoda* L. from callus and compare vasicine production from somaclones.
6. To study DNA polymorphism among *J. adhatoda* L. somaclones differing in vasicine production.
7. To attempt hairy root transformation of *J. adhatoda* L. for enhanced production of vasicine.
8. To test the antimicrobial activity of callus extracts of *J. adhatoda* L. in comparison with vasicine.