



# Isolation and Identification of Endophytic Fungi from *Justicia adhatoda* L. in Tendukheda Region of Damoh District (M.P.)

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## Abstract–

Endophytic fungi live inside the plants without causing any harm to the hosts and produce the secondary metabolites which are known to possess antimicrobial activity. *Justicia adhatoda* has been used in Ayurveda, Unani, and Homeopathy medicine. In this study, 40 samples (25 Leaf and 15 Stem) of plants were collected from the Tendukheda region of Damoh district (M.P.) and screened for the endophytic fungi. The plant first rinsed in running tap water to remove the dust and the other debris present in it. Segments of approximately 0.5 cm were cut with sterile lancet blades and surface sterilized by agitating in 70% ethanol (5 s), followed by treatment with 4% Sodium hypochlorite (NaOCl), (90 s) and then rinsed in sterile distilled water (10 s).

40 segments (leaf and stem) from different plants are processed for the isolation of endophytic fungi. A total of 30 endophytic fungi (Fungal classes) were isolated and identified. The leaf segments showed a maximum repository for endophytic fungi than the stem segments. Among the 18 endophytic fungi, the predominant endophytic fungi isolated belonged to the genera of *Alternaria alternata*, *Cladosporium oxysporum*, *Aspergillus fumigatus* and some sterile fungi followed by *Cercospora Oxysporum*. In this study, it is also observed that the majority of the fungi belonged to dematiaceous hyphomycetes. From this study it is concluded that the isolation of endophytic fungi from *Justicia adhatoda* may be a good source for some novel bioactive compound. However, further studies are required to screen these endophytic fungi for production of novel bioactive compounds. Endophytes are potential source of various novel compounds that help to promote plant growth, eliminate plant pathogens, and enable the plant to resist stress – like conditions.

**Keywords–** Medicinal Plant, Endophytic Fungi, Plants stress, *Justicia Adhatoda*

## Introduction–

Endophytes are microorganisms that are present inside the living tissue of various plants (root, fruit, stem, seed, leaf, etc.) which are establishing mutual relationship without any symptom of diseases (Arnold et al. 2003). These endophytes protect their hosts from infectious agents and adverse conditions by secreting bioactive secondary metabolites. The endophytic fungi play important physiological and ecological roles in their host life. Many traditional medicinal plants have played a crucial role in providing primary health care since the dawn of civilization. The knowledge of medicinal plants has been accumulated from different medicinal systems such as Ayurveda, Unani, and Siddha. In India, it is reported that traditional healers use 2500 plant species and 100 species of plants serve as regular sources of medicine. During the last few decades, there has been an increasing interest in the study of these medicinal plants has been witnessed in different parts of the world mainly due to many problems associated with synthetic drugs and with the emergence of multi-drug resistant pathogens (Gritto MJ, et.al., 2015). Out of these, 10,000 species are known for producing medicine. Indian system of medicine uses around 3000 plant species belonging to more than 1000 genera (Vaidyanathan D., et al., 2015).

## Aim of study–

Endophytic fungi live and grow inside the plants, without causing any harm to the hosts and known to produce the secondary metabolites are potential antimicrobial activity. *Justicia adhatoda* has been used in Ayurveda, Homeopathy and Unani medicine. Therefore, present investigation is conducted with traditional known medicinal plants, collected from the Tendukheda region of Damoh district (MP) in these study endophytic fungi to be isolated with a confined technique and identify them.

## Review of literature–

The plant of *Justicia adhatoda* belongs to the family of Acanthaceae, known commonly as Malabar nut tree, is a shrub growing throughout the Indian peninsula. The plant is used in the indigenous system of medicine in India and is a well-known expectorant in both Ayurvedic and Unani Systems of Medicine (Chopra R.N. et al., 1956). The leaves of these plants are used to treat malarial fever, chronic fever, intrinsic haemorrhage, cough, asthma, leprosy, skin diseases, and piles (Sharma P.V., 1996). The plant is reported to show abortifacient (Wakhloo R.L. et al. 1979), antimicrobial (Doshi J.J. and Patel V.K., 1983, Mathew A.S. et al., 1998) and antitussive activities (Dhuley J.N., 1999). Important fungal diseases in medicinal and Aromatic plants and their control (Avan, M. 2021). A friendly relationship between endophytic fungi and medicinal plants: a systematic review (Jia et al. 2016). Plants disease detection and protection technique: A comparative study of the performance (Mahant, M.M. 2025). Fungal endophytes from medicinal plants acting as natural therapeutic reservoir (Chandra et al. 2024)

Recent investigations have been intensified by the potentialities of endophytic fungus strains in production of bioactive metabolites such as taxol, pestalocide, torreyanic acid and enzymes, xylanase, isoflavonoids, and asparagines (Dar R.A. et al., 2015). Isolation and identification of endophytic fungi from *Ricinus communis* Linn.

and their antibacterial activity (Sandhu S.S. et al., 2014). The crude extract of *Adhatoda vasica* leaves was found to have conspicuous antifeedant and toxic effects on the larvae of *S. littoralis* (Sadek M. M., 2003). The plant contains alkaloids such as Vasicine, vasicinone, deoxyvasicine, vasicol, adhatodine and vasicinol (Claeson U.P. et al. 2000). Vasicine is reported to have bronchodilatory, respiratory stimulant and uterine stimulant effects (Gupta O. P. et al. 1977). Vasicine acetate showed antimycobacterial activity (Ignacimuthu S. & Shanmugam N. 2010).

Various researchers have been able to isolate number of endophytic fungi from some medicinal plant and also reported that they may role growth promoter in plant as well as health care in human beings (Ibrahim et al., 2021; Ahmed, MAK et al., 2021; Eram D. et al. 2018; Selim K. et al., 2018) worked on Biodiversity and antimicrobial activity of endophytic associated with Egyptian the medicinal plant for endophytic fungi.

### Methods and Materials–

Collection of plant material: Healthy parts of plant like leaves and stem of *Justicia adhatoda* L. were collected from the Tendukheda region of Damoh district (M.P.). The plant materials were brought to the laboratory in sterile polythene bags and keep it in cold temperature (10°C) in the refrigerator. Isolation of endophytic fungi the plant materials were taken out from the refrigerator and placed under room temperature. They were washed with running tap water; leaf segments were equally cut by sterilized scalpel from the mid portions of healthy leaves to include the midribs. The cut segments were surface sterilized by immersing into the following series of solutions: sterile dish. H<sub>2</sub>O for 60 s, 70% ethanol for 60 s, 2.5% sodium hypochlorite for 4 min, 70% ethanol for 30 s, and a final rinsing in a sterile dish. H<sub>2</sub>O three times. About 100 µL of the final rinse water was inoculated on Malt Extract Agar (MEA) medium to succeed the surface sterilization. The sterilized plant leaves were cut into five segments (5 mm), and 20 leaf segments per individual plant were placed on the surface of MEA plate (five segments for each plate), supplemented with 0.05 gram of streptomycin sulphate per 100 mL of medium to inhibit bacterial growth and incubated at 28 °C ± 2 °C.

**Table (1) – Endophytic fungi isolated from plant *Justicia Adhatoda***

Site of location	No. of samples	No. of fungi isolated	CF (%)
Leaf	25	20	50
Stem	15	10	37.5
Total	40	30	75%

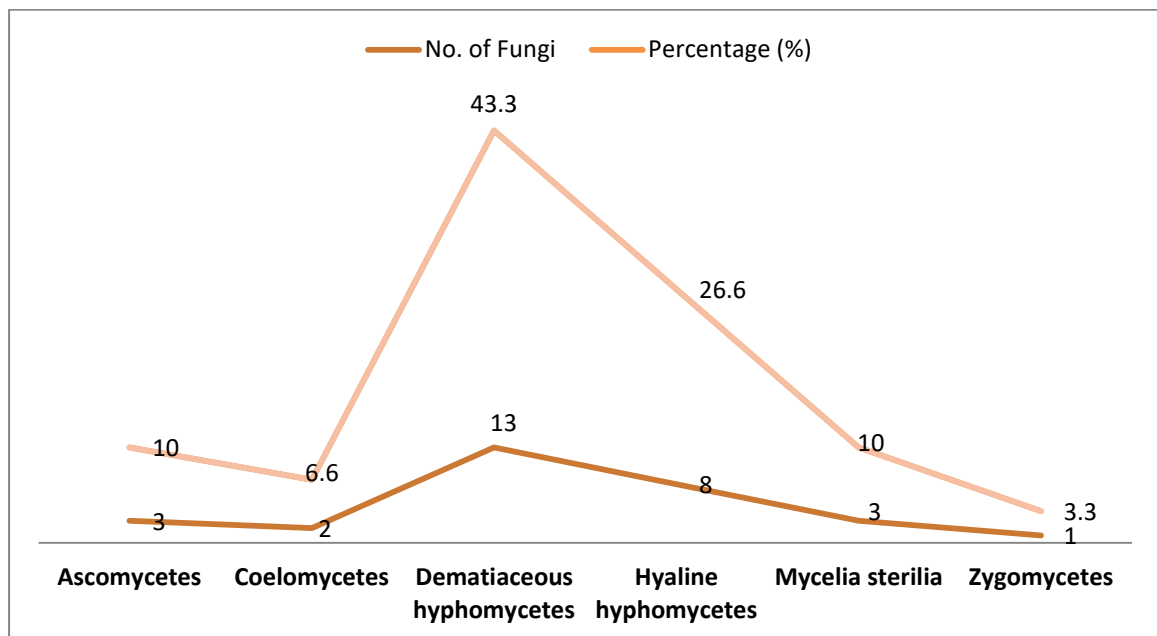
**Table (2) – Taxonomic position of endophytic fungi**

S. No.	Isolated endophytes	Fungal class	Leaf	Stem	Total	(%)
1	<i>Alternaria</i> sp.	Dematiaceous hyphomycetes (deuteromycetes)	2	1	3	10
2	<i>Aspergillus fumigatus</i>	Hyaline hyphomycetes	2	1	3	10
3	<i>Aspergillus niger</i>	Hyaline hyphomycetes	1	1	2	6.6
4	<i>Aspergillus terreus</i>	Hyaline hyphomycetes	0	1	1	3.3

5	Bipolaris spp.	Dematiaceous hyphomycetes (deuteromycetes)	0	1	1	3.3
6	Cladosporium oxysporium	Dematiaceous hyphomycetes (deuteromycetes)	2	1	3	10
7	Colletotrichum sp	Dematiaceous hyphomycetes (deuteromycetes)	1	0	1	3.3
8	Cercospora oxysporum	Dematiaceous hyphomycetes (deuteromycetes)	2	1	3	10
9	Curvularia spp.)	Dematiaceous hyphomycetes (deuteromycetes)	1	0	1	3.3
10	Dreschelrasp	Dematiaceous hyphomycetes (deuteromycetes)	1	0	1	3.3
11	Emericellandulans	Ascomycetes	1	1	2	6.6
12	Fusarium oxysporum	Hyaline hyphomycetes	1	0	1	3.3
13	Mucor	Zygomycetes	0	1	1	3.3
14	Penicillium brevicompactum	Hyaline hyphomycetes	1	0	1	3.3
15	Phomopsis sp	Coelomycetes	1	0	1	3.3
16	Phoma spp.	Coelomycetes	1	0	1	3.3
17	Sterile forms	Mycelia sterilia	2	1	3	10
18	Verticillium sp	Ascomycetes	1	0	1	3.3
<b>Total</b>			<b>20</b>	<b>10</b>	<b>30</b>	<b>100%</b>

Table (3) – Percentage of isolated fungal class

S. No.	Fungal class	No. of Fungi	Percentage (%)
1	Ascomycetes	03	10
2	Coelomycetes	02	6.6
3	Dematiaceous hyphomycetes	13	43.3
4	Hyaline hyphomycetes	08	26.6
5	Mycelia sterilia	03	10
6	Zygomycetes	1	3.3
<b>Total</b>		<b>30</b>	<b>100%</b>



**Figure- Graph between isolated fungi and their percentage (%)**

The plates were checked daily for any fungal growth; single isolates grown out from the tissues were re-inoculated on fresh MEA plates and maintained at 4°C in MEA slants (Kahtani, et al. 2020). Identification of endophytic isolates, the endophytic fungal isolates were identified up to genus level based on the basis of micro and macro morphological characters such as colony morphology, pigmentation, growth pattern, spore structures, and other hyphal characteristics with the help of relevant literature and keys. The microscopic examination was also done to study their reproductive spores. Cultures which failed to produce spores were grown on different minimal media and incubated for several weeks to months.

#### **Analysis–**

Statistical analysis to know the endophyte richness, the frequency of fungal endophytes harboured in plant species were calculated by the number of segments colonized by endophyte species divided by a total number of segments examined  $\times 100$  (Divya C.R. et al. 2016).

$$\text{Colonization frequency (CF \%)} = \frac{\text{No. of segment colonized by fungi}}{\text{Total number of segment observed}} \times 100$$

#### **Formulae –**

Calculation of colonizing frequency Colonization Frequency (CF %) was calculated as described by Suryanarayanan et al. (2003) and Photita (2001). Briefly, proper time of incubation was given for colonizing frequency counting. Colonization frequency (%) of an endophyte species was equal to the number of segments colonized by a single endophyte divided by the total number of segments observed  $\times 100$ . (Table 2 and figure 1) Colonizing frequency % = Morphological Identification of Endophytic fungi the fungi were identified on the basis of morphological characteristics according to Domsch (1980) and Agrawal and Hasija (1980). The colonies appearing on petri plates were sub-cultured into the tube containing potato dextrose agar medium for identification. Fungi were again

cultured from slant to petri plates containing potato dextrose agar medium without antibiotic (Tetracycline) for 7 days.

Morphological identification was done according to the standard taxonomic key including colony diameter, texture, colour and the dimensions and morphology of hyphae and conidia. Mass Production of Antibacterial Metabolites 150 ml of Potato Dextrose Broth was prepared in 250 ml flasks and autoclaved at 15 lbs. psi for 20 min. The medium was inoculated with various fungi culture and incubated at  $28\pm 1^\circ\text{C}$  in the incubator. After 7, 14 and 21 days of incubation the crude cultures broth was filtered and tested for antibacterial activity against the six pathogenic bacteria by using agar well diffusion methods. Antibacterial Activity Test organisms there were six strains of pathogenic bacteria such as *Bacillus subtilis*, *Enterococcus* sp., *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus* were used to evaluate the antibacterial activity of fungi. Antibacterial Activity the agar well diffusion assay method was used to assess the antibacterial activity of the fungi. In this method wells were aseptically made in the seeded media using sterile cork borer and appropriate amount of the bioactive metabolite, Number of segments colonized by fungi, Total number of segment observed.

### Results and discussion –

About 40 segments (25 segments of Leaf and 15 of Stem respectively) of the plant were screened for the isolation of the endophytic fungi. A total of 30 endophytic fungi (Fungal Class) were isolated and identified viz... The leaf segments showed a maximum repository for endophytic fungi than the stem segments. Among the 30 number of endophytic fungi, 16 genera belongs to 18 species viz of *Alternaria*, sp. were isolated from table (2). Tables (1) and (2) showed the CF value and taxonomic position of endophytic fungi. The predominant endophytic fungi isolated belonged to the genera of *Alternaria alternata*, *Aspergillus fumigatus*, *Aspergillus niger*, *A. terreus* and *Cladosporium oxysporium* followed by *Cercospora oxysporum*, *Bipolaris*, *Colletotrichum* sp, *Curvularia* sp, *Dreschelra* sp, *Emericilla nidulans* *Fusarium oxysporum*, *Penicillium brevicompactum*, *Phoma* sp, *Phomopsis* sp, *Mucor* sp, *Verticillium* sp. Some fungi did not produce any reproductive structure as they produced sterile mycelia (no sporulation). These fungi did not sporulate in spite of repeated subculturing on to sporulating media (PDA, Potato dextrose agar, and tap water agar) and hence are grouped on mycelia sterilia. In this study, the majority of the fungi (Table 3) belonged to dematiaceous hyphomycetes or group of fungi imperfection or deuteromycetes (43.3%), followed by hyaline hyphomycetes (26.6%), sterile mycelium fungi (10.0%), Ascomycetes (10.0%), Coelomycetes (6.6%) and zygomycetes (3.3%). The colonization frequency was found to be 75.0% from table (1).

This study was carried out to isolate and identify endophytic fungi from Medicinal Plants *Justacia adhatoda* from the Tendukheda region of Damoh District (M.P.). In the study, a total of 27 fungal colonies were isolated from 40 segments (15 from stem and 25 from leaf). Among the 30 endophytic fungi, the predominant endophytic fungi isolated belonged to the genera of *Alternaria alternata*, *Aspergillus fumigatus*, *Aspergillus niger*, *A. terrious* and *Cladosporium oxysporium* followed by *Cercospora oxysporum*, *Bipolaris*, *Colletotrichum* sp , *Curvularia* sp, *Dreschelra* sp,

*Emericilla nidulans*, *Fusarium oxysporum*, *Penicillium brevicompactum*, *Phoma* sp, *Phomopsis* sp, *Mucor* sp, *Verticillium* sp.

These results were similar to the studies of Rajeshwari et al. (2017) they isolated and identified numbers of endophytic fungi from the medicinal plant *Terminalia chebul* (Dhanalakshmi et al., 2013). Who had isolated *Alternaria* spp., *Aspergillus* spp., *Bipolaris* spp., *Exophiala* spp., *Nigrospora* spp., and *Penicillium* spp. from *Moringa oleifera* as dominant endophytic fungi? (Barnabas et al., 2013) also reported *Aspergillus* spp., as the predominant isolate in the leaves, stem, and roots of *Moringa oleifera*. They have also reported that most of fungal species belonged to deuteromycetes (43.3%), followed by hyaline hyphomycetes (26.6%), sterile mycelium fungi (10.0%), Ascomycetes (10.0%), Coelomycetes (6.6%) and zygomycetes (3.3%), from table no. 3.

The same results are showed the presence of endophytic fungi in *Avicennia officinalis* that isolated the endophytic fungi namely reported *Aspergillus*, *Penicillium*, *Curvularia*, *Cladosporium*, *Phoma*, and *Fusarium* species (Job N. et al., 2015). According to some reports of workers, *Alternaria* sp. as endophytes has a potential to produce many antimicrobial metabolites. The endophytic fungi are able to produce many antimicrobial metabolites, such as colletotric acid (Zou et al., 2000) and griseofulvin, (Park et al., 2005) reported from *Alternaria* species ubiquitous fungal genus including saprobic, endophytic and pathogenic species.

### **Conclusion –**

Medicinal plants are a good source for isolation of endophytic fungi that colonize the tissue without causing apparent symptoms. Endophytic organisms have received considerable attention as they are found to protect their hosts against pests, pathogens and even domestic herbivores. In this study, a total of 18 endophytic fungi and 30 fungal classes were isolated from the *Justicia adhatoda*.

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### **References–**

1. Ahmed Mohamed Aly Khalil, Saad El-Din Hassan, Sultan M. Alsharif, Ahmed M. Eid, Emad El-Din Ewais, Ehab Azab, Adil A. Gobouri, Amr Elkelish and Amr Fouda (2021); Isolation and Characterization of Fungal Endophytes Isolated from Medicinal Plant *Ephedra pachyclada* as Plant Growth-Promoting, *Biomolecules*, 11, 140
2. A.L. Kahtani, Md. Fouda, A.; Attia, K.A.; Al-Otaibi, F.; Eid, A.M.; Ewais, E.E.-D.; Hijri, M.; St-Arnaud, M.; Hassan, S.E.-D.; Khan, N.; (2020); Isolation and characterization of plant

- growth promoting endophytic bacteria from desert plants and their application as bio inoculants for sustainable agriculture. *Agronomy*: 10-1325.
3. Arnold A.E., Mejia L.C., Kyllö D., Rojas E.I., Maynard Z., Robbins N., Herre E.A. (2003); Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of National Academy of Sciences USA*, 100:15649–15654.
  4. Barnabas J, Murthy S, Jagdeesh S. (2013); Antimicrobial properties of endophytic fungi isolated from *Cynodactylon* and *Moringa oleifera*. *Int J Biol Pharm Res* 4(2):98-104.
  5. Barron, G.L. (1972); “The genera of hyphomycetes from soil.” Robert. E. Krieger Publishing Co. Inc New York, Pp:364
  6. Booth, C. (1971). *The genus Fusarium*. CMI, Kew, Surrey, England pp237.
  7. Chopra R. N., Nayar S. L., Chopra I. C. (1956); *Glossary of Indian Medicinal Plants*. New Delhi, India: Council of Scientific and Industrial Research.
  8. Daniya Eram, Manoj kumar Arthikala, Govindappa Melappa and Gustavo Santoyo (2018); *Alternaria* species: endophytic fungi as alternative sources of bioactive compounds. *Italian Journal of Mycology* vol. 47; 40-54.
  9. Dar R.A., Rather SA, Mushtaq S, Qazi P.H. (2015); Purification and characterization of endophytic fungal strains from four different high value medicinal plants of Kashmir valley. *Int. J Phytopharm*, 5(1):8-11.
  10. Dhanalakshmi R., Umamaheswari S., Sugandhi P., Prasanth D.A. (2013); Biodiversity of the endophytic fungi isolated from *Moringa oleifera* of Yercaud hills. *Int J Pharm Sci Res*, 4(3):1064-8.
  11. Divya C.R., Sharada M.S., Ashwini V. (2016); Isolation and identification of endophytic fungi in *Trigonella foenum-graceum* L. and their antibacterial activity. *Imp J Interdiscip. Res*, 2(9):516-21.
  12. Dhuley J. N. (1999); Antitussive effect of *Adhatoda vasica* extract on mechanical or chemical stimulation-induced coughing in animals. *Journal of Ethnopharmacology*. 67(3):361–365.
  13. Doshi J. J., Patel V. K., Venkatakrishna-Batt H. (1983); Effect of *Adhatoda vasica* massage in pyorrhoea. *International Journal of Crude Drug Research*; 21(4):173–176.
  14. Gilman, J. C. (1957). *A manual of soil fungi*, (2nd edition) IOWA State University press USA, Pp: 450
  15. Gritto M. J., Nandagopalan V., Doss A. (2015); Ethno-botanical study on the traditional healers in Pachamalai hills of Eastern Ghats, Tamilnadu, South India. *J Med Plants Studies*, 3(2):80-5.
  16. Gupta O. P., Sharma M.L., Ghatak B.J.R., Atal C.K. (1977); Potent uterine activity of alkaloid vasicine. *Indian Journal of Medical Research*, 66(5):865–871.
  17. Ignacimuthu S., Shanmugam N. (2010); Anti mycobacterial activity of two natural alkaloids, vasicine acetate and 2-acetyl benzylamine, isolated from Indian shrub *Adhatodavasicina* Ness. Leaves. *Journal of Biosciences*; 35(4):565–570.

18. Job N, Manomi S, Philip R. (2015); Isolation and characterization of endophytic fungi from *Avicennia officinalis*. *Int J Res Biomed Biotechnol*, 5(1):4-8.
19. Kapoor L. D. (2001); "Handbook of Ayurvedic Medicinal Plants." Boca Raton, Fla, USA: CRC Press; (pp. 416–417).
20. Mahant, M. M. (2025); "Plant disease detection and protection technique: A comparative study of the performance." *Int. J. of applied and Universal Research* 12 (3): 10-15
21. Mathew A. S., Patel K. N., Shah B. K. Investigation on antifeedant and anthelmintic potential of *Adhatodavasica nees*. *Indian Journal of Natural Products and Resources*, 1998; 14:11–16.
22. Rajeshwari S., Revaths R. and Arvind Prasanth D. (2017); Isolation and identification of endophytic fungi from *Terminalia chebula* of eastern Ghat, Tamil Nadu. *Assian J. Pharm.Clinical Res.* 10(4)21-23.
23. Sadek M. M. (2003); Antifeedant and toxic activity of *Adhatodavasica* leaf extract against *Spodoptera littoralis* (Lep., Noctuidae) *Journal of Applied Entomology*. 127(7):396–404.
24. Selim K., ElBech A. AbdelEl-Rahman T., El-diwany-A. (2018); Biodiversity and antimicrobial activity of endophytic associated with Egyptian medicinal Plants. *Fermentation* 4:49.
25. Sharma P. V. (1996); *Classical Uses of Medicinal Plants*. 1st. addition Varanasi, India: Chaukhambha Visvabharti.
26. Sutton, B.S (1980); "The Coelomycetes. CMI, Kew, Survey, England. Pp: 696.
27. Vaidyanathan D., Senthilkumar M.S., Sisubalan N, Basha M.G. (2014); Studies on ethnomedicinal plants used by Malayali Gounder Tribes in Pachamalai of Eastern Ghats, Tamil Nadu, India. *Adv Appl Sci Res*, 5(1):244-53. 3.
28. Wakhloo R.L., Wakhloo D., Gupta O.P., Atal C.K. (1979); Vasicine hydrochloride, a new drug for interruption of pregnancy. *Journal of Obstetrics and Gynaecology of India*, 29:939–940.