

# APPLIED RESEARCH IN BOTANY VOLUME-1

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## 9. EFFECT OF MEDICINAL PLANTS ON PATHOGENIC FUNGI

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<b>ABSTRACT</b>	<p>To investigate the antifungal potentials of leaves of <i>Ficus elastica</i>, <i>Alternanthera sessilis</i>, <i>Cassia fistula</i>, <i>Datura metel</i> to assess the antimicrobial activity and determine the zone of inhibition of extracts on fungal. The present study of four plants, i.e., <i>Alternanthera sessilis</i>, <i>Cassia fistula</i>, <i>Datura metel</i>, and <i>Ficus elastica</i>, showed activity against five fungi strains, i.e., <i>Aspergillus flavus</i>, <i>Aspergillus niger</i>, <i>Fusarium oxysporum</i>, <i>Pythium sps.</i> and <i>Rhizopus sps.</i> The Soxhlet method was used for the collection of extract. Effect of plant extracts against <i>Aspergillus flavus</i> out of the four plants. <i>Cassia fistula</i> (leaves) shows the maximum inhibition zone, whereas <i>Alternanthera sessilis</i> shows the minimum inhibition area. In the pathogenic fungi <i>Aspergillus niger</i>, <i>Alternanthera sessilis</i> (leaves) offers the ultimate, and <i>Cassia fistula</i> and <i>Ficus elastica</i> show the minimum zone of inhibition. The effect of plant extracts was observed in <i>Fusarium oxysporum</i>, in which the <i>Datura metel</i> shows the maximum area of inhibition and minimum observed in <i>Ficus elastica</i>. Potato's Root rot disease is caused by <i>Phythium sps.</i> <i>Datura metel</i> and <i>Alternanthera sessilis</i> show the complete inhibition zone, whereas a minimum area of inhibition was observed in the <i>Ficus elastica</i>. <i>Datura metel</i> is highly effective on <i>Rhizopus sps.</i> Indicates the maximum zone of inhibition and minimum was observed in <i>Alternanthera sessilis</i>.</p>
<b>KEYWORDS</b>	<i>Ficus elastica</i> , <i>Alternanthera sessilis</i> , <i>Cassia fistula</i> , <i>Datura metel</i> , Sanjay Gandhi National park, pathogenic fungi , Soxhlet method.

### INTRODUCTION:

In recent years, interest in novel, safe and effective antifungal agents has grown with increased incidences of fungal infections in immunocompromised patients. In natural products with antimycotic activity, higher plants remain largely unexpected compared to microorganisms and marine invertebrates. Some plants' biological

activities were reported, and in the present study, the antifungal activity of some native plants is evaluated. Plants have limitless ability to synthesize aromatic compounds, mostly phenols or their oxygen-substituted derivatives. Most of the secondary metabolites, of which at least 10,000 have been isolated, the number are estimated to be less than 10% of the total. These substances serve as plant defense mechanisms against predation by bacteria, fungi, viruses, insects, and herbivores. A compound like terpenoids gives plants their odors; others (quinones and tannins) are responsible for plant pigment. Many combinations are accountable for plant flavor (e.g., the terpenoid capsaicin from chili, peppers)—spices used by humans to season valuable food yield as medicinal compounds.

Fungi cause important human and plant diseases, especially in tropical regions. Despite the existence of potent antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for an endless search and development of antifungal compounds. To discover new lead compounds, screening plant extracts to detect secondary metabolites with relevant biological activities was done.

Five species from the genus *Alternanthera* occur in Tamil Nadu [*Alternanthera sessilis*, *Alternanthera tenella*, *Alternanthera philoxeroides*, *Alternanthera paronychioides*, and *Alternanthera pungens*.] Out of which except *Alternanthera sessilis*, others are non-native and tropical America. Two of these non-native species, such as *Alternanthera tenella* and *Alternanthera philoxeroides*, become invasive weeds in India. *Alternanthera sessilis* (sessile joy weed), a member of the Amaranthaceae family, is a weed and grows in both wetlands condition and uplands and can grow on various soil types. The plant spreads by seeds, wind, and water-dispersed (Scher, 2004). It acts as a weed of rice throughout tropical regions and other cash crops like sugarcane and bananas. Shoots and leaves are consumed as a vegetable in Southeast Asia (Vimala, N., E.K. and Krishnan, V. (2016). *Alternanthera sessilis* is used internally against intestinal inflammation, externally to treat wounds, treat hepatitis, tight chest, bronchitis, asthma, lung troubles, stop bleeding, and hair tonic. *Alternanthera philoxeroides* the whole plant is used as medicine and the system of medicine is folk and traditional medicine for the treatment of wound, fever and milk secretion (Theiengburanathaum W Dictionary of Thai herbs).

The demand for novel antifungal agents from natural resources has been increased worldwide for preservation of various species of plants and habitat (Velu S. et al., 2014). Certain fungi cause various infections on plants and animals, mostly shows adverse effects in nature. So considering all of them as adverse effects there in need of new cheaper and harmless antifungal agents to get minimized on them.

The exploration of novel antimicrobial agents from natural resources such as plant or plant products and others has been used mainly for treating diseases, food safety and food preservation purpose (Hammer et al., 1999). Thus, plants are the good resource to get remedy on them. In the present investigation aqueous extracts were subjected for antifungal activity against strains *Aspergillus niger* and *Trichoderma viride*.

More than 100 species of genus *Ficus* are found throughout the world. *Ficus elastica* is native to southeastern Asia and a substantial tropical deciduous, evergreen tree with more than 800 species. The Bark, root, leaves, fruit, and latex of this plant produce a unique fruit which is actually an inverted flower. The Moraceae plant family includes *Ficus* as one of the main plants with biological activities already described such as antiplasmodial (Muregi et al., 2003), antioxidant (Phan et al., 2012), anticancer (Mbosso et al., 2012, Mbosso et al., 2015, Mbosso et al., 2016a), antimicrobial, antiulcer (Galati et al., 2001) and anti-pyretic. Note that the latex of some species of *Ficus* is exploited in traditional folk medicine for its antihelmintic activity in South and Central America (De-Amorin et al., 1999).

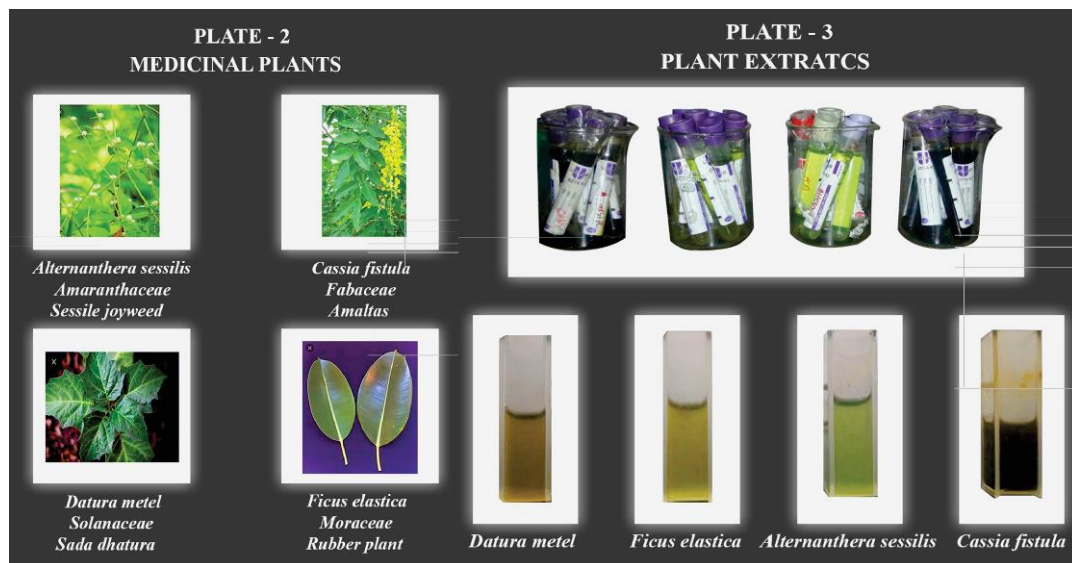
*Datura* is a genus of nine species of poisonous vespertine flowering plants belonging to the family Solanaceae. The toxic herb *D. metel* is invasive to many tropical regions, including Cuba the Galapagos Islands, Micronesia, Fiji, French Polynesia, Kiribati, Hawaii, New Caledonia, Seychelles, Niue, China, and Taiwan as well as parts of Kenya, Tanzania, and Uganda as an agricultural weed, casual alien, cultivation escape, garden thug, naturalized, noxious weed, sleeper weed. *Datura metel* is a plant which grows throughout the year and is known as Devil's Trumpet (Duke, J.A. and E.S. Ayensu, 1985). The plant was firstly described by the scientist Linnaeus in the year 1753 (Dabur, R., M. Ali, H. Singh, J. Gupta and G.L. Sharma, 2004). A novel antifungal pyrrole derivative from *Datura metel* leaves. Die Pharmazie: Int. J. Pharmaceut. Sci., 59: 568-570. *Datura* has wide applications in ayurvedic medicines too. Many constituents of *Datura* are used in ayurvedic preparations, which help in the treatment of hair fall and many other skin disorders (Soni, P., A.A. Siddiqui, J. Dwivedi and V. Soni, 2012). The seeds of *Datura metel* have the potential of treating bleeding disorders (Murthy, B.K., S. Nammi, M.K. Kota, R.V.K. Rao, N.K. Rao and A. Annapurna, 2004) The plant is also known to possess antimicrobial and anti-inflammatory activity (Alabri, T.H.A., A.H.S. Al Musalami, M.A. Hossain, A.M. Weli and Q. Al-Riyami, 2014). Atropine, one of the important constituent of the *Datura metel*, dilates the pupil and also helps in the eye surgery (Satyavati, G.V. and M.K. Raina, 1977) *Datura metel* plant extract is also known to have herbicidal activity as methanolic extracts of the plant made from the dried leaves can remove unwanted weed.

*Cassia fistula*, commonly known as golden shower purging cassia,<sup>[3]</sup> Indian laburnum,<sup>[4]</sup> or pudding-pipe tree,<sup>[5]</sup> is a flowering plant in the subfamily,

Caesalpinioideae of the legume family, Fabaceae. The species is native to the Indian subcontinent and adjacent regions of Southeast Asia. It ranges from eastward throughout India to Myanmar and Thailand and south to Sri Lanka and southern Pakistan. It is a popular ornamental plant and is also used in herbal medicine. It is both the national tree and national flower of Thailand. It is the state flower of Kerala in India.

The genus *Cassia*, comprising 600 species widely distributed worldwide and is well known for its diverse biological and pharmacological properties. (Panda, S. K. Brahma, S. and Dutta, S. K. 2010) *Cassia fistula* L. belongs to Caesalpinioideae family is widely used for its medicinal properties. In the Indian literature, this plant has been described to be useful against skin diseases, liver troubles, tuberculosis and its use in the treatment of rheumatism, haematemesis, pruritus, leucoderm and diabetes. (Alam et al., 1990; Asolkar et al., 1992). Some studies have also been done on antimicrobial activity of *C. fistula* flower and seed along with some other Indian medicinal plants (Misra et al., 1996; 1997; Valasraj et al., 1997; Perumal Samy et al., 1998; Phongpaichit et al., 2004; Kumar et al., 2006; Duraipandiyan and Ignacimuthu, 2007; Sangetha et al., 2008). These works give little information on antifungal property of this plant. Hence in the present experiment an attempt has been made to evaluate the antifungal activity of different leaf extracts (petroleum ether, chloroform, ethanol, methanol and aqueous) against human pathogenic fungi and the biological activities of the extracts in terms of MIC and MFC were also determined.

#### MATERIAL AND METHOD:





- A: Collection of Plant Materials:** The different Plant species were collected from Sanjay Gandhi National Park free from the disease
- B: Plant Extracts:** Solvents like ethanol extracted the selected plant to isolate active factors that can be the potential antifungal agent. Fresh plant materials leave (each one was 8 g) were extracted with ethanol (150ml) by Soxhlet extractor.
- C: Antifungal activity test:** The agar diffusion method has performed screening of antifungal activity (Perez et al., 1990). The methanol and aqueous extracts of five different plants tested against plant pathogen *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, *Pythium sps.* and *Rhizopus sps.* The Czapek Dox Agar medium was poured into the sterile Petri plate and allowed to solidify. Cotton swabs spread pathogenic fungal culture evenly distributed over the media by using sterile cotton swabs uniformly. Sterile cork borer to make a Wells of (6 mm) in the medium. 200µl of each extract into the separate wells. The plates at 27°C -30°C for 48-72 hrs. After the incubation, the inhibition of the zone at regular intervals. The area of inhibition is the diameter of the inhibition of the site.
- D: Media preparation:** Czapek Dox Agar medium was prepared by using sterilized distilled water with standard pH. Table 1: Composition of Czapek Dox Agar media for one liter was as follows:

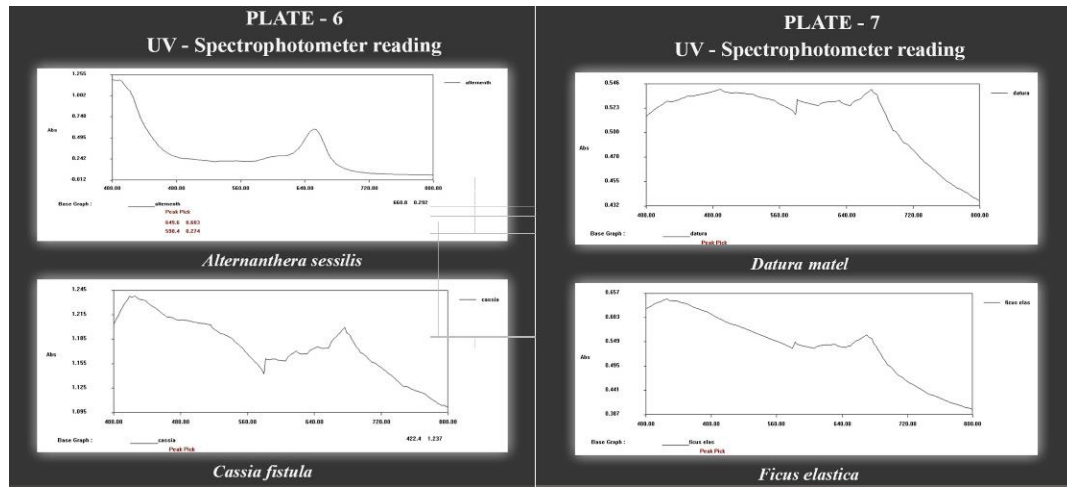
Sr.No.	CHEMICALS	FORMULA	WEIGHT(gm)
1	Sodium Nitrite	NaNO <sub>3</sub>	2.0
2	Diphosphate Phosphate	K <sub>2</sub> HPO <sub>4</sub>	1.0
3	Magnesium Sulphate	MgSO <sub>4</sub> .7H <sub>2</sub> O	0.5
4	Potassium chloride	KCL	0.5
5	Ferrous Sulphate	FeSO <sub>4</sub> .7H <sub>2</sub> O	0.01
6	Sucrose	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	30.0
7	Agar		20

- E: UV-Spectrophotometer Analysis:** UV-visible Spectrophotometric analysis was conducted on the *Alternanthera sessilis*, *Cassia fistula*, *Datura metel*, *Ficus elastica*, extracts using a UV-visible spectrophotometer (Perkin Elmer, USA Model: Lambda 950) with a slit width of 2nm, using a 10-mm cell at room temperature. The extract was examined under visible and UV light in the wavelength ranging from 300-800nm for proximate analysis.
- F: In vitro Antifungal Activity:** Plate inhibition assay & spore germination assay will be used for testing antifungal properties of various extract / fractions.
- G: The pathogenic fungi are collected from ICAR New Delhi:**

Table 2:

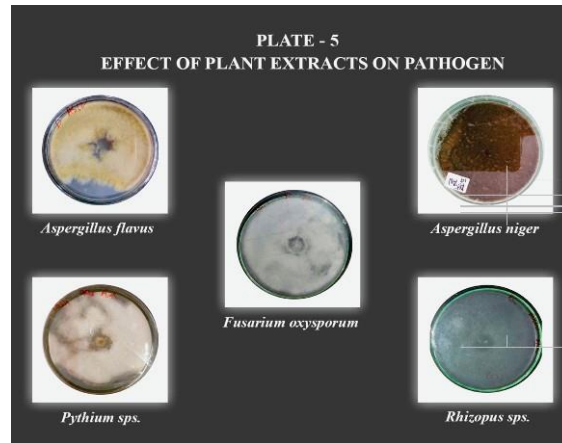
Sr. No.	Name of culture	ITCC No.
1	<i>Pythium aphanidermatum</i>	502

**EXPERIMENTAL RESULTS:**



**Table 3: UV- Spectrophotometer readin**

Sr. No	Plant extracts	$\lambda_{max}$ (nm)
1	<i>Alternanthera sessilis</i>	649.6, 0.603 / 598, 0.274
2	<i>Cassia fistula</i>	422.4, 1.237 / 675.2, 1.200
3	<i>Datura metel</i>	488.0, 0.540 / 668.8, 0.540
4	<i>Ficus elastica</i>	425.6, 0.644 / 667.2, 0.562



**Table 4: Effect of plant extracts against *Aspergillus flavus***

Sr.No.	Plant extracts	Family	Parts used	Zone of Inhibition (mm)
1.	<i>Alternanthera sessilis</i>	Amaranthaceae	Leaves	10
2.	<i>Cassia fistula</i>	Fabaceae	Leaves	20
3.	<i>Datura metel</i>	Solanaceae	Leaves	15
4.	<i>Ficus elastica</i>	Moraceae	Leaves	15

**Table 5: Effect of plant extracts against *Aspergillus niger***

Sr.no	Plant extracts	Family	Parts used	Zone of Inhibition (mm)
1.	<i>Alternanthera sessilis</i>	Amaranthaceae	Leaves	12.2
2.	<i>Cassia fistula</i>	Fabaceae	Leaves	5
3.	<i>Datura metel</i>	Solanaceae	Leaves	7
4.	<i>Ficus elastica</i>	Moraceae	Leaves	5

**Table 6: Effect of plant extracts against *Fusarium oxysporum***

Sr. No.	Plant extracts	Family	Parts used	Zone of Inhibition (mm)
1.	<i>Alternanthera sessilis</i>	Amaranthaceae	Leaves	15
2.	<i>Cassia fistula</i>	Fabaceae	Leaves	7
3.	<i>Datura metel</i>	Solanaceae	Leaves	17
4.	<i>Ficus elastica</i>	Moraceae	Leaves	5

**Table 5: Effect of plant extracts against *Pythium sps.***

Sr. No.	Plant extracts	Family	Parts used	Zone of Inhibition(mm)
1.	<i>Alternanthera sessilis</i>	Amaranthaceae	Leaves	10
2.	<i>Cassia fistula</i>	Fabaceae	Leaves	7
3.	<i>Datura metel</i>	Solanaceae	Leaves	10
4.	<i>Ficus elastica</i>	Moraceae	Leaves	5

### RESULTS AND DISCUSSION:

Extract of *Alternanthera sessilis*, *Cassia fistula*, *Datura metel*, and *Ficus elastica* analyzed by UV-Spectrophotometer to identify phytoconstituents present in methanolic. The appearance of one or more peaks in the region indicates unsaturated group and heteroatoms such as Sulphur, Nitrogen, and Oxygen. The pathogenic fungi were isolated from rhizosphere soil using the Czapek Dox Agar medium, which acts as a standard medium for fungi's growth. Plant materials from Sanjay Gandhi National park according to their antimicrobial effect. (Misra and Dixit 1977, Gehlot 1997, Khilare and Gangawane 1997). The present study of four plants, i.e., *Alternanthera sessilis*, *Cassia fistula*, *Datura metel*, and *Ficus elastica*. have antifungal activity against *Aspergillus flavus* *Aspergillus niger*, *Fusarium oxysporum*, *Pythium sps.* and *Rhizopus sps.* The extracts by Soxhlet method (Luque de Castro, M. D., and L. E. Garcia-Ayuso). Effect of plant extracts against *Aspergillus flavus* out of the four plants *Cassia fistula* (leaves) shows the maximum inhibition zone whereas *Alternanthera sessilis* shows the minimum inhibition area. In the pathogenic fungi *Aspergillus niger*, *Alternanthera sessilis* (leaves) deliver the ultimate, and *Cassia fistula* and *Ficus*

*elastica* show the minimum zone of inhibition. The effect of plant extracts on *Fusarium oxysporum* in which the *Datura metel* shows the maximum area of

inhibition and the minimum observed in *Ficus elastica*. Potato's Root rot disease by *Phythium* spp. *Datura metel* and *Alternanthera sessilis* show the ultimate inhibition zone, whereas the minimum zone of inhibition in the *Ficus elastica*. *Datura metel* is highly effective on *Rhizopus* spp. It delivers the maximum area of inhibition and the minimum in *Alternanthera sessilis*.

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