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Antifungal activity of different parts of *Cassia angustifolia* Vahl. collected from Rajasthan, Gujrat and Tamil Nadu states of India

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Abstract
The goal of this study was to look into the antifungal properties of the leaves, stem, and roots of *Cassia angustifolia* Vahl. and effect of different climatic conditions on the activity. The root, stem, and leaves of the ethnomedicinal plant *Cassia angustifolia* Vahl. collected from Rajasthan, Gujrat, and Tamilnadu districts of India were extracted using chloroform, acetone, and petroleum ether and these extracts were evaluated for their antifungal potential against four fungi *Aletrnaria alternata, Sclerotinia sclerotiorum, Fusarium oxysporum*, and *Penicillium notatum* by agar disc diffusion method. The antifungal potentials of extracts were compared to that of regular ketoconazole in terms of zone of inhibition. The results demonstrated a notable suppression of fungal growth of the selected fungi by the plant extracts. In terms of Minimal Inhibitory Concentration (MIC) (g/ml), the antifungal activity of various plant components ranged from 6.5 to 400 g/ml. The plants' phytochemical compositions were examined. The presence of different secondary metabolites contributed to the *Cassia angustifolia's* microbial activity. Pet ether extract of the stem from Rajasthan showed the lowest MIC value against *Alternaria alternata* (15 mg/L) and Pet ether extract of the stem from Tamilnadu showed the lowest MIC value against *Sclerotinia sclerotiorum* (25 mg/L). Acetonep extract of the root from Gujrat showed the lowest MIC value (6.25 mg/L) against *Penicillum notatum* while chloroform extract of the leaves from Tamilnadu showed the lowest MIC value (25 mg/L) against *Fusarium oxysporum*. Results revealed that antifungal activity of the plant was also affected by climatic conditions.

Keywords: *Cassia angustifolia*, antifungal activity, climatic conditions, MIC value etc

Introduction

The three indigenous medical systems are Ayurveda, Unani, and Siddha. Siddha medications can be made either as a single substance or in combination using the raw form of medicinal herbs. According to Ramchander *et al.* (2017) [24], *Cassia angustifolia*, also known as Swarnapatri in Sanskrit, is a medicinal plant belonging to Caesalpinaceae family. It is a tiny shrub with long, spreading branches, alternate leaves with pinnate leaflets arranged in pairs of 5-8, and vivid yellow flowers. Common names for the plants are Senehe (Sinhala), Indian senna (English), and Nilavagai (Tamil) (Arambewella and Silva, 1999; Thayalini *et al.*, 2019) [2,27]. A commercially grown shrub with a variety of branches that goes by the name of "tinnevelly senna" is found in southern India. Almost all parts of the plant including the leaves, pods, and seeds, are utilised widely for their therapeutic characteristics. Ayurveda uses formulas as Punch Sakaara Churna, Shtshakaar Churna, and Yashtyaadi Churna, while Unani uses Safoof-e-Mulaiyia and Majoon-e-Senaai, which are widely renowned for their ability to improve overall health. There are a few more products on the market, such as the Sidhha medication Nilaavaari Churna, which is used to treat biliousness, stomach distention, and constipation. Other preparations made from *Cassia angustifolia* Vahl include Sarivadya sava, Ayulax, Kultab tablet, Pylend tablet, and Raktansoo syrup (Ramchander *et al.*, 2017) [24]. In both the Indian and global markets *Cassia angustifolia* Vahl. is in high demand (Nilofer and Singh, 2018) [22]. The leaves are believed to have astringent, cathartic, depurative, anthelmintic, cholagogue, expectorant, and febrifuge properties. These properties make the leaves helpful for treating tumors, leprosy, leukoderma, jaundice, typhoid fever, and vitiated conditions of "pitta" and "vata," as well as constipation. Senna's primary active ingredients, which are anthraquinone derivatives, are what give it its laxative effects. Sennosides A, B, C, and D, kaemferol, phytosterols, glycosides of rhein, and chrysophanic acid were all chemical components of them. Additionally, *C. angustifolia* was found to contain tinnevelin glycoside, separating it from *C. auriculata*, the species most frequently used to make the medicine senna. Their seeds also have water-soluble polysaccharides in addition to leaves (Chaubey & Kapoor, 2001) [10]. Peroxidase activity was also identified by Arrieta *et al.* (2002) [3], and they came to the conclusion that it might contribute to anthraquinone biosynthesis. The extracts also have virucidal effects and topical anti-inflammatory properties (Cuellar *et al.*, 2001). Wang *et al.* (2002) [28] also identified a protein which regulates senna-induced increase of gastrointestinal motility in mucous colon. According to Kojima *et al.* (2001) [17], the leaves containing sennosides are effective sources for health teas and can be purchased separately or in a variety of herbal formulations at drug stores (such as Herbolax, Periderm granules, Senolax, and Verechni tablets) (Shrivastav *et al.*, 2006).

On more than 380 host species of plants, *Aletrnaria alternate*, a fungus, has been observed to cause leaf spot and other diseases. It is an opportunistic pathogen that affects a variety of hosts and causes rots, blights, and leaf spots on different plant sections. In addition, it can lead to upper respiratory tract infections and asthma in people with weakened immune systems (Wiest *et al.*, 1987) [29]. It requires a warm, moist atmosphere to survive. It frequently grows where there is a humid climate or where there has been a lot of rain. The fungus is propagated through spores as well as living in seeds and seedlings. When plants are left

in gardens over the winter dead, this disease thrives. Among plant pathogens, *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most dangerous and widespread. More than 60 names, including cottony rot, watery soft rot, stem rot, drop, crown rot, blossom blight, and, perhaps most frequently, white mould, have been used to describe diseases brought on by this fungus. The fungus affects more than 400 plant species globally, including significant crops and a large number of weeds (Boland and Hall, 1994; Kim *et al*., 2011) [7, 15]. *S. sclerotiorum* is a hazard to monocotyledonous plants like onions and tulips as well as dicotyledonous plants like sunflower, soybean, oilseed rape, edible dry beans, chickpea, peanut, dry pea, and different vegetables (Bolton *et al*., 2006) [8]. In every type of soil on earth, the species *Fusarium oxysporum* is well represented among the communities of soilborne fungi (Gordon & Martyn, 1997) [13]. All *F. oxysporum* strains are saprophytic, meaning they can grow and survive for a long time on organic matter found in soil and in the rhizosphere of many different plant species. Additionally, some strains of *F. oxysporum* can cause tracheomycosis or root rots in different plant species by penetrating the roots and invading the vascular system (Olivain & Alabouvette, 1997) [23]. Numerous economically significant plant species suffer serious harm at the hands of wilt-inducing strains of *F. oxysporum* (Fravel *et al*., 2003) [12]. The environmental occurrence of *Penicillium notatum*, also known as *Penicillium chrysogenum*, is widespread (Hu *et al*., 2013) [14]. It grows on wood, decomposing vegetables, and the dirt. *P. notatum* is a blue-green mould that is typically found in temperate and subtropical regions (Barcus *et al*., 2005) [5]. In immunocompetent hosts, it has only infrequently been implicated in the development of human disease; nevertheless, infection with this microbe seems to be more widespread and manifests in immunocompromised patients with a more severe clinical picture. On media for fungi culture, it frequently grows quickly. Because *P. notatum* infection is uncommon and has non-specific clinical and imaging characteristics, it is frequently challenging to be identified (Shokouhi *et al*., 2016) [25].

There is a paucity of literature on these pathogens. Additionally, the management of infectious disorders is limited since many medications are no longer effective against bacteria. It's crucial to test new plant-based antibacterial agents. Therefore, in the current study, an effort has been made to assess the antifungal activity of chloroform, acetone, and petroleum ether extracts of *C. angustifolia's* roots, stems, and leaves collected from Rajasthan, Gujrat, and Tamil Nadu against the aforementioned fungi, *Aletrnaria alternate, Sclerotinia sclerotiorum, Fusarium oxysporum,* and *Penicillium notatum.*

Materials and methods

Plant materials

The mature plants of *Cassia angustifolia* Vahl. were collected from Rajasthan, Gujarat, and Tamilnadu, India to observe the effects of climatic conditions on bioactivity of plants. Samples from India were collected from Jaipur, whereas other samples were collected from Salem, Tamil Nādu, and Surat, Gujarat. During summers, the temperature rises in Rajasthan, in Tamilnadu, the weather is humid while in Gujarat, the temperature remains constant. Leaves, stem and roots were separated and washed. After air drying, the plant material was grinded to make fine powder and stored at cool place.

Micro-organisms tested

Root, stem, and leaves of the plants were extracted using chloroform, acetone, and pet ether for anti-fungal activity against the selected fungi (*Aletrnaria alternata, Sclerotinia sclerotiorum, Fusarium oxysporum,* and *Penicillium notatum*). Four strains of fungi were used for anti-fungal screening. Clinical laboratory isolates were procured from the Microbiology Laboratory, SMS Medical College, Jaipur.

Culture and maintenance of micro-organisms

Pure cultures of mentioned fungal strains were used as indicator organisms. The fungi were grown in potato dextrose agar (PDA) medium. Fungal cultures were maintained on the medium for 72 h of sub-culturing, usually incubated at 37°C and stored at 4°C for future experiments. A fresh suspension of test organism in saline solution was prepared from a freshly grown agar slant before every anti-fungal assay.

Preparation of extracts

From all three locations, *in vivo* parts i.e. root, stem and leaves of *Cassia angustifolia* Vahl. were washed under tap water followed by distilled water, then shade dried. Dried plant parts were then coarsely powdered. Selected extracts were obtained by macerating 100 g of dried powder of different parts in selected solvents and separately kept on a rotary shaker for 24 hr. Each extract was filtered and centrifuged for 15 min at 5000 rpm, dried under reduced pressure and stored in airtight sterile bottles at 4°C.

Microbiological screening

The antimicrobial activity was performed with the agar well diffusion method (Andrews, 2001) [1] Inhibition zone and minimum inhibitory concentration (MIC) were calculated (Barsi and Fan, 2005).

Determination of antifungal assay

The anti-fungal activity of the experimental plant was investigated by agar well diffusion method (Andrews, 2001) [1]. The fungal strains were subcultured on potato Dextrose Agar (PDA: Merck, Germany) medium and respectively incubated at 37°C for 24 h and 25°C for 2-5 days. Suspensions of fungal spored were prepared in sterile PBS (Phosphate buffered saline) and adjusted to a concentration of 106 cells mL^{-1}. Dipping a sterile swab into the fungal suspension was rolled on the surface of the agar medium. The plates were dried at room temperature for 15 min. Wells of 6 mm in diameter and about 10 mm apart were punctured in the culture media using sterile cork borer. The test compound (40 µL) was introduced in the well. Plates were incubated at 37°C. After incubation of 72 hrs, bioactivities were determined by measuring the diameter of inhibition zone (mm). The diameters of zone of inhibition produced were with those of standard ketoconazole used as standard anti-fungal agent. All the experiments were performed in triplicates and mean values were taken.

Determination of minimum inhibitory concentration

The least concentration, which can inhibit the fungal growth on the media plates, is known as minimum inhibitory concentration (MIC) (Barsi and Fan, 2005). The most commonly employed methods are the tube dilution method and agar dilution method (Andrews, 2001) [1]. Less drug required for inhibiting the growth of the organism, lesser is

its MIC value. Consequently, drugs that have low MIC values are more effective antimicrobial agents. The resistivity of microbes to antibiotics is seen by its MIC score and can monitor their activity.

The serial dilution technique of extracts were followed when performed MIC representing different concentrations. 100, 50, 25 and 12.5 µg/ml of chloroform, acetone, and pet ether extracts were used. The same agar well diffusion method was used with serial dilutions of extracts for inhibiting fungal culture with their respective inoculum and incubated for 72 hrs. at 37°C. Now MIC was calculated based on the least visible growth of the lowest concentration, killing 99.5% of the used inoculum.

The visible zone of inhibition was quantitatively assessed based on inhibitory activity on strains along with MIC. Their Activity Index was also calculated with the help of the given formula.

IZ (Inhibition zone) = in mm (Includes diameter of disc-6mm)

AI (Activity Index) = IZ of test sample/IZ of standard (as in Table 1)

***(**Values are mean of triplicate readings)

Standard: Ketoconazole (60µl/disc)

Results

Antifungal potential of crude (Chloroform, Acetone and Pet ether) extracts of stem, root and leaf parts of *Cassia angustifolia* Vahl. Collected from Rajasthn, Gujrat, and Tamilnadu were assessed by IZ, AI (Table-1), and MIC (Table-2). In the present investigation, total 27 extracts were tested. The anti-fungal activity was determined by using agar well diffusion method and micro-dilution method. Chloroform extract of stem part collected from Tamilnadu was found to be the most active against *Penicillium notatum* (IZ=11mm, MIC=12.5 µg/ml, AI=0.73) followed by collected from Gujarat against *Penicillium notatum* (IZ=10 mm, MIC=12.5 µg/ml, AI=0.66) and from Rajasthan against *Sclerotinia sclerotiorum* (IZ=10 mm, MIC=25 µg/ml, AI=0.454). Leaves from Tamilnadu were found again to be the most active against *Penicillium notatum* (IZ=10 mm, MIC=12.5 µg/ml, AI=0.66) followed by leaves from Tamilnadu against *Fusarium oxysporum* (IZ=9 mm, MIC=25 µg/ml, AI=0.225). In case of root, no significant results were observed in any of the extracts. Acetone extract of root part collected from Gujarat was found to be the most active against *Penicillium notatum* (IZ=11mm, MIC=6.25µg/ml, AI=0.73) followed by collected from Rajasthan (IZ=10mm, MIC=12.5µg/ml, AI=0.66) and Tamilnadu (IZ=10mm, MIC=12.5 µg/ml, AI=0.66). Roots from Tamilnadu were also showed significant activity against *Alternaria alternata* (IZ=10mm, MIC=25 µg/ml, AI=0.416). Stem and leaf extracts were not found that much active against any of the tested fungus. Roots collected from all the three locations were found inactive against *Fusarium oxysporum.* Petroleum ether extract of root part collected from all the three locations were found to have very less activity against *Alternaria alternate, Sclerotinia sclerotiorum* and *Penicillium notatum* while no activity against *Fusarium oxysporum.* Stem extracts from Rajasthan showed good activity against *Penicillium notatum* (IZ=10mm, MIC=12.5 µg/ml, AI=0.66) and *Alternaria alternata* (IZ=10mm, MIC=15 µg/ml, AI=0.416) while from Tamilnadu against only *Alternaria alternata* (IZ=10mm, MIC=100 µg/ml, AI=0.416). In case of leaves, extracts from Rajasthan (IZ=11mm, MIC=12.5 µg/ml, AI=0.73) and Gujarat (IZ=10mm, MIC=12.5 µg/ml, AI=0.66) showed very good activity against *Penicillium notatum.* No other extract exhibited significant antifungal activity. Almost all the extracts found inactive against *Fusarium oxysporum.* The most susceptible organism in the present study was *Fusarium oxysporum* followed by *Sclerotinia sclerotiorum.* Among all the crude extracts, chloroform extracts found to be most bioactive followed by acetone extracts. MIC values (Table-3) were evaluated for plant crude extracts which had shown activity in diffusion assay. The range of MIC of extract recorded was 6.25-400 µg/ml. In present investigation, lowest MIC value 6.25µg/ml was recorded against *P. notatum*, showing significant antimicrobial potential of test extract and higher susceptibility of *P. notatum*. Significant differences were recorded in the antifungal activity of the various extracts collected from different locations.

Discussion

Inter-specific variation is known to emerge from phenotypic changes caused by environmental factors and genotype. Geographically diverse species tend to be more diverse (Wilikie *et al.*, 1993) [30]. Climate change is strongly correlated with plant architecture, flowering, fruiting, phytochemistry, and in-situ competition with other species. The life cycle, distribution, and phytochemical makeup of the world's flora, including medicinal and aromatic plants, are all being significantly impacted by climate change (Kumar *et al*., 2017) [19]. India is known for its extreme seasonal variations in temperature and rainfall as well as other environmental and climatic factors (Krishnarajua *et al*., 2005) [18]. Agro-climatic conditions have a significant impact on the phytochemical makeup of plants. In order to understand how variations in these geo-climatic elements would affect the plants' phenology, nutritional, antioxidant, and secondary metabolite levels, it is necessary to grow the plants under a variety of temperature, precipitation, soil moisture, and fertility circumstances. Numerous secondary metabolites are present in plants and contribute to important biological processes (Xiong, 2002) [31]. The phyto-constituents in various extracts are affected by various geographic and climatic conditions. The climatic component of temperature has a major impact on the biological activities of plants gathered from various parts of India. The study showed that plants taken from Tamilnadu had the highest antifungal activity, followed by those from Gujarat and Rajasthan. Selecting locations for a plant's mass production to increase its medicinal and commercial values might be aided by screening *Cassia angustifolia* plants for their antifungal capability in connection to their activity from different climatic zones (Kumar *et al*., 2017) [19]. By

using the Agar well diffusion method on nutritional agar medium, the ethanolic and aqueous leaf extracts of *Cassia angustifolia* Vahl. were shown to have antifungal activity against two different fungal strains (*Aspergillus niger*, *Candida albicans*) (Sood *et al*., 2012). When *Cassia angustifolia* Vahl. isolated saponins were examined for their antifungal action, *Colletotrichium dematium* showed the greatest inhibition (Khan and Srivastava, 2009). Many fungi, including *Aspergillus terrus, Aspergillus flavus, Aspergillus niger, Aspergillus junii, S. mercescens,* and *P. aerogenosa,* have been shown to be resistant to *Cassia angustifolia* extracts (Nilofer and Singh, 2018) [22]. Small amounts of antifungal activity of *Cassia angustifolia* against *Aletraria alternata, Sclerotinia sclerotiorum, Fusarium oxysporum,* and *Penicillium notatum* have been reported. At 200 ppm, *C. angustifolia* essential oils reduced the percentage of *Alternaria alternata* that became infected (Mahdavi *et al.*, 2013) [21].

In addition, in the present study, the test extracts' antifungal efficacy was compared to that of ketoconazole. In terms of MIC (g/ml), the antifungal activity of various plant components varied from 12.5 mg/L to 400 mg/L in the case of leaves to 12.5 mg/L to 200 mg/L and 6.25 mg/L to 400 mg/L in the case of stem and root extracts, respectively. While stem and leaf extracts showed encouraging antifungal activity, root extracts were found to be the least effective among all the extracts. The most resistant organism, *Fusarium oxysporum*, with the highest MIC values, whereas the most sensitive one was *Penicillium notatum* had the lowest MIC values. When compared to the standard, numerous extracts showed to be just as excellent as or better than the medication ketoconazole. The potential of antimicrobial compounds found in the extracts and their method of action on various test organisms may be the cause of variations (Barbour *et al*., 2004) [4]. It should be noted that all of the tests were performed using unpurified samples; further purification could even produce more encouraging results (Davarya and Vala, 2011).

Table 1: Antifungal activity of different extracts of root, stem, and leaves of *Cassia angustifolia* Vahl. Collected from different locations.

	Chloroform						Acetone						Petroleum ether						S
	Root		Stem		Leaf		Root		Stem		Leaf		Root		Stem		Leaf		
	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI	
Alternaria alternate																			
R	NA	NA	5	0.208	6	0.25	4	0.16	4	0.166	NA	NA	5	0.208	10	0.416	7	0.291	24
G	2	0.08	3	0.125	3	0.125	8	0.33	6	0.250	3	0.125	NA	NA	9	0.375	6	0.250	
T	3	0.125	6	0.25	6	0.25	10	0.416	7	0.291	5	0.208	NA	NA	10	0.416	6	0.250	
Sclerotinia sclerotiorum																			
R	5	0.227	10	0.454	7	0.318	4	0.181	NA	NA	6	0.272	NA	NA	5	0.227	NA	NA	22
G	4	0.181	8	0.363	5	0.227	2	0.090	3	0.136	4	0.181	5	0.227	7	0.318	NA	NA	
T	NA	NA	5	0.227	3	0.136	4	0.181	4	0.181	5	0.227	6	0.272	8	0.363	4	0.181	
Penicillium notatum																			
R	NA	NA	5	0.33	3	0.20	10	0.66	8	0.53	8	0.53	6	0.40	10	0.666	11	0.733	15
G	6	0.40	10	0.66	8	0.53	11	0.73	8	0.53	9	0.60	5	0.33	8	0.533	10	0.666	
T	5	0.33	11	0.73	10	0.66	10	0.66	7	0.46	8	0.53	NA	NA	7	0.466	6	0.400	
Fusarium oxysporum																			
R	2	0.05	4	0.10	8	0.20	NA	NA	NA	NA	6	0.15	NA	NA	NA	NA	NA	NA	40
G	NA	NA	4	0.10	8	0.20	NA	NA	6	0.15	6	0.15	NA	NA	NA	NA	NA	NA	
T	2	0.05	6	0.15	9	0.225	NA	NA	4	0.10	8	0.20	NA	NA	NA	NA	2	0.05	

Note: IZ- Inhibition zone (mm), AI- Activity Index, S- standard, NA- No Activity, R- Rajasthan, G- Gujrat, T-Tamilnadu

Table 2: Minimum inhibitory concentrations of different extracts of root, stem, and leaves of *Cassia angustifolia* Vahl. collected from different locations.

Fungi		Chloroform			Acetone			Petroleum ether		
		Rot	Stem	Leaves	Root	Stem	Leaves	Root	Stem	Leaves
Alternaria alternate	R	200	100	100	100	100	200	100	15	50
	G	100	100	100	50	100	100	200	100	100
	T	100	100	50	25	100	100	200	100	100
Sclerotinia sclerotiorum	R	100	25	50	100	200	100	200	100	200
	G	100	50	100	100	100	100	100	50	200
	T	200	100	100	100	100	100	50	25	100
Penicillium notatum	R	200	50	100	12.5	25	25	50	12.5	12.5
	G	50	12.5	25	6.25	12.5	12.5	100	50	12.5
	T	50	12.5	12.5	12.5	25	12.5	200	50	25
Fusarium oxysporum	R	100	100	50	200	200	100	200	200	400
	G	200	100	50	100	100	100	200	200	400
	T	100	100	25	100	100	100	400	200	100

Note: MIC- Minimum Inhibitory Concentration (mg/L), R- Rajasthan, G- Gujrat, T- Tamilnadu.

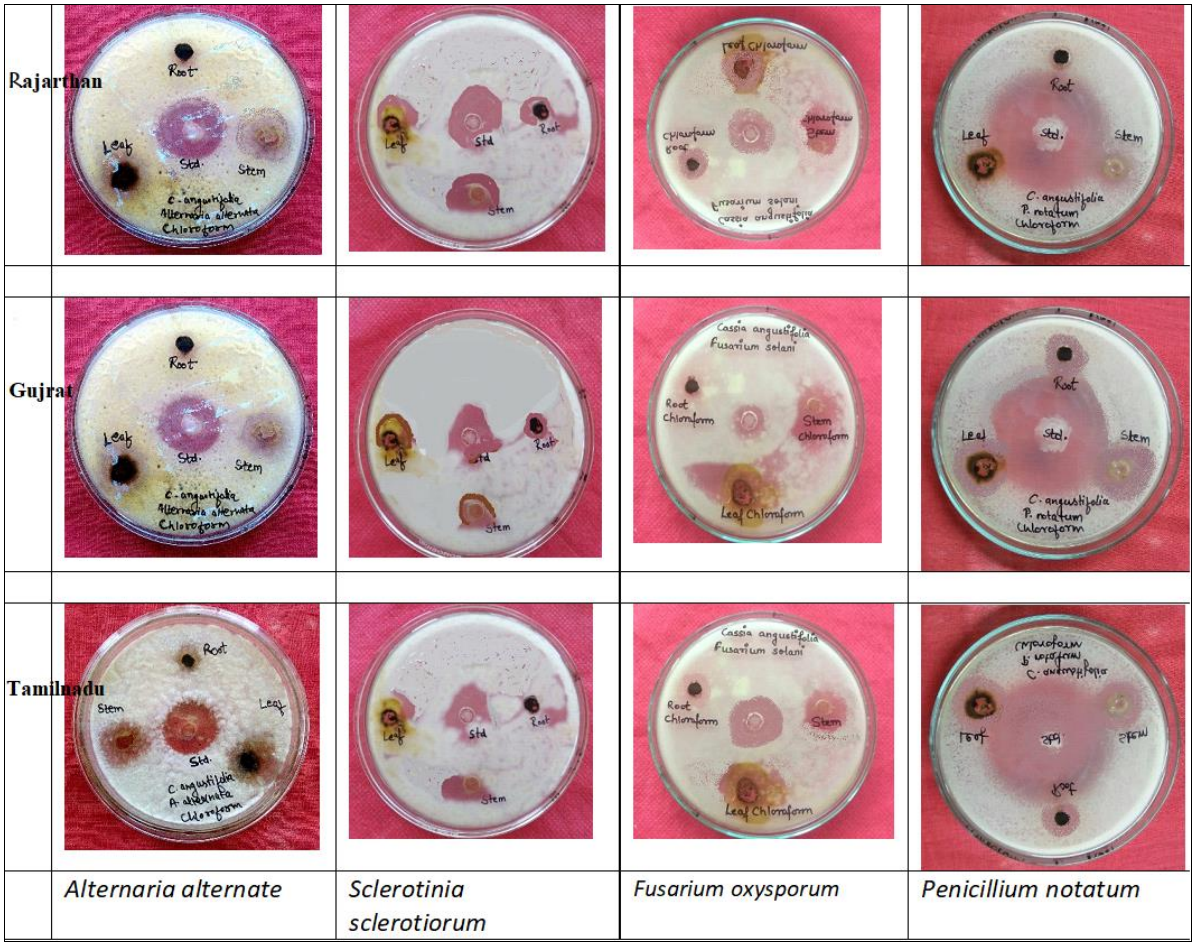

Fig. 1: Photographs of anti-fungal activity of chloroform extract of Cassia *angustifolia* Vahl. collected from Rajasthan, Gujrat, and Tamilnadu locations against selected fungal strains.

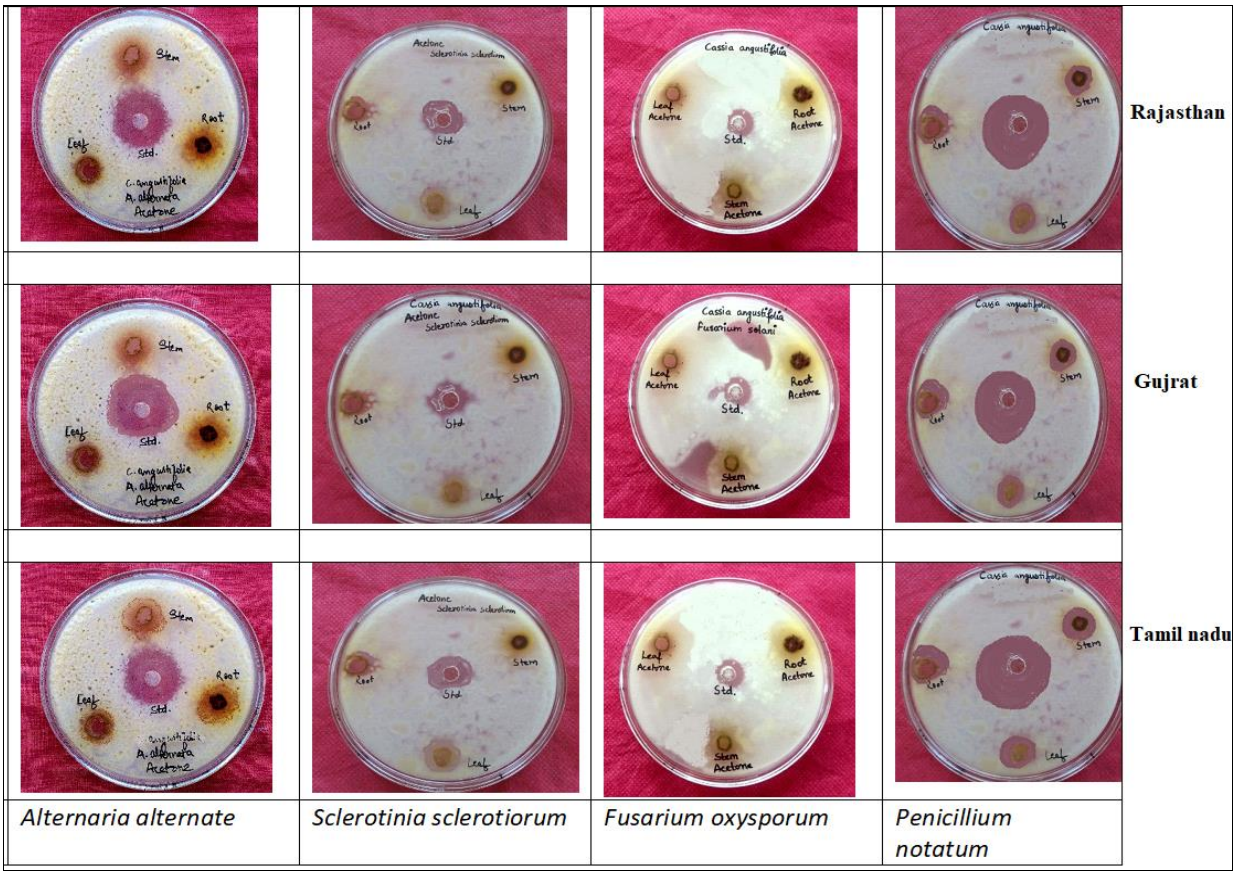

Fig. 2: Photographs of anti-fungal activity of acetone extract of Cassia *angustifolia* Vahl. collected from Rajasthan, Gujrat, and Tamilnadu locations against selected fungal strains.

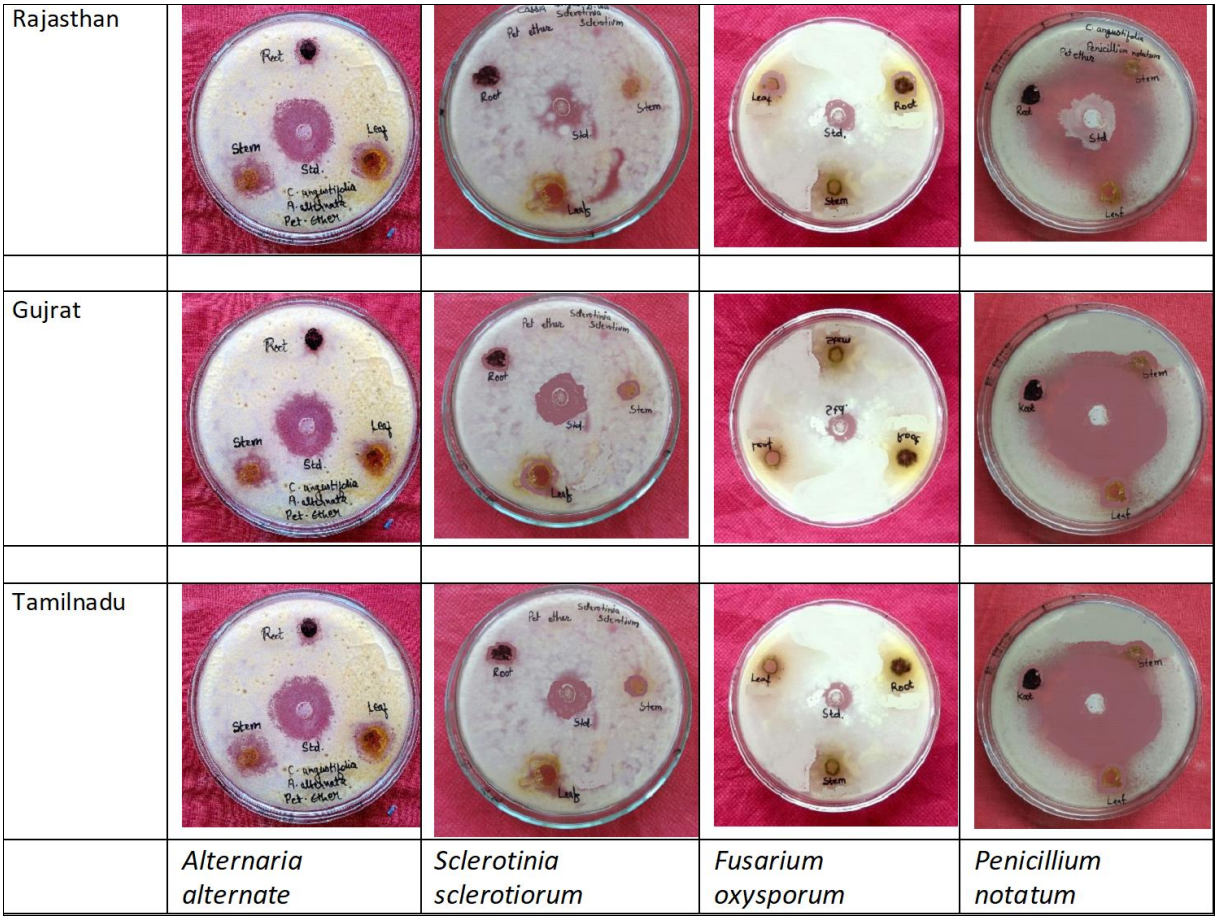

Fig 3: Photographs of anti-fungal activity of pet ether extracts of Cassia *angustifolia* Vahl. collected from rajasthan, Gujrat, and Tamilnadu locations against selected fungal strains (std- standard).

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