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Antimicrobial activity of some rare endangered and threatened medicinal plants against phytopathogens

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Abstract
Increase of environmental pollution due to use of pesticides is alarming. One of the alternatives is to reduce the pesticide use and use of botanicals as biocontrol agents. The present investigation was undertaken to test antifungal activity of four RET medicinal plants *viz., Oroxylum indicum, Embelia ribes, Celastrus paniculatus, and Saraca asoca* against four phytopathogens *viz., Sclerotium rolfsii, Fusarium oxysporum, Alternaria solani*, and *Phytophthora capsici* was screened *in-vitro*. Aqueous leaf extract of *E. ribes* and *O. indicum*at 15% concentration provided a significant inhibition of mycelial growth of fungi. *E. ribes* leaf extract inhibited the mycelial growth of *A. solani* and *F. oxysporum* upto 86.52% and 43.26% respectively, whereas leaf extract of *O. indicum* inhibited the fungal growth of *S. rolfsii* up to 80.39%. Thus the present study revealed that these two plant extracts have proved to be effective as bio control agents for suppressing the growth of phyto pathogens.

Keywords: Botanical, Phytopathogens, Plant extract, Antifungal, Mycelial growth inhibition, Antimicrobial activity.

Introduction
The success of crop production depends on the ability of the farmers to overcome challenges to maintain functional and profitable farms. One of the most important challenges is, preventing and controlling pest and diseases, which can ruin agricultural crops. The diseases caused by bacteria, fungi and viruses can cause a significant loss of many economic crops worldwide (Dewa, 2016) [1]. Approximately direct yield losses caused by pathogens, animals, and weeds, are altogether responsible for losses ranging between 20% and 40% of global agricultural productivity (Savary *et al.*, 2012) [2] and Crop losses from fungal diseases are estimated to be about 14 per cent (Jantasorn *et al.*, 2016) [3]. Phytopathogenic fungi are controlled by synthetic fungicides; however, the use of these is increasingly restricted due to the harmful effects of pesticides on human health and the environment (Harris *et al.*, 2001) [4]. The increasing demand of production and regulations on the use of agrochemicals and the emergence of pathogens resistant to the products employed, justifies the search for novel active molecules and new control strategies. Plants are used as promising alternative or complementary to cure many human as well as plant diseases because of their anti-microbial activity, non phytotoxicity, systemicity and biodegradability (Ngegba *et al.*, 2018) [5]. Plants produce a number of secondary metabolites which includes flavonoids, phenols, phenolic glycosides, unsaturated lactones, sulphur compounds, saponins, cyanogenic glycosides and glucosinolates (Das *et al.*, 2010) [6] due to which plant extract possess fungicidal activity.
Considering the vast potentiality of plants as sources for antimicrobial agents. RET plants also reported to possess potent antimicrobial activity due to the presence of number of secondary metabolites and able to suppress pathogens. This investigation is undertaken to screen the antimicrobial activity of some RET Medicinal plants against selected pathogens.

Materials and Methods
The experiments on antimicrobial activity of RET plants were carried out at Division of Plant Pathology ICAR-IIHR, Hesaraghatta, Bangalore. Leaves of selected RET plants were collected from the Field Gene Bank of RET medicinal plants, Division of Plant Genetic

Resources (PGR), ICAR-IIHR, Bengaluru. Plants included under this study are shown in Table 1.

Table 1: List of plant species tested for antifungal activity.

Sl.no.	Plant extract	Common names	Family	Plant part used
1.	*Oroxylum indicum* Vent.	Indian trumplet flower	Bignoniaceae	Leaf
2.	*Embelia ribes* Burm F.	Vidang	Myrsinaceae	Leaf
3.	*Celastrus paniculatus* Willd.	Jyotishmathi	Celastraceae	Leaf
4.	*Saraca asoca* (Roxb) Willd.	Ashoka	Caesalpinaceae	Leaf

Preparation of Plant Extracts

Fresh leaves after collecting from field, 100grams were weighed and washed repeatedly with tap water to remove adhering dust followed that were washed with distilled water to avoid contamination. Leaves were cut into small pieces and they were dried for 2 hours to remove moisture. 100 ml of distilled water was added and crushed using a grinder, then filtrate was collected using double layered muslin cloth.

All the extracts obtained were finally centrifuged at 10, 000 rpm for 10 minutes at 4 ^{0}C (Ambika pathy *et al*., 2011) [7]. Centrifugation is carried out till the solid pellets disappears in centrifuge tubes this depends on plant species; herbaceous leaves take more time for settling pellets whereas tree species requires centrifugation to the maximum of 5-6times. The supernatant was collected in separate tubes and then the supernatant was sterilized by filtration through 0.22 µm sterile syringe filters (PVDF hydrophilic HIMEDIA Syringe-driven Filters) and stored at -4 °C and stored in centrifugation tubes as stock solution until further use. The resulting aqueous extract was used for the fungal growth inhibition assay.

Plant pathogens

Phytopathogenic fungal cultures of *Sclerotium rolfsii, Fusarium oxysporum, Alteraria solani* and *Phytophthora capsici* were collected from Division of Plant Pathology, IIHR, Bangalore. The cultures were preserved in slants containing potato dextrose agar (PDA) except *P. capsici* which was preserved using Carrot Agar (CA) till used.

Evaluation of Antifungal Activity of Plant extracts

The antifungal activity of the RET plant extracts against the fungal pathogens was assessed by employing Poisoned Food Technique (Vinesh and Devendra., 2013) [8]. Requisite amount of the plant extract was dissolved into sterile Potato Dextrose Agar (PDA) medium and to the Carrot Agar (CA) medium to obtain the desired concentrations. The media was then poured into sterile Petri dishes and allowed to solidify.

Discs of 5mm diameter of the actively growing fungal culture were transferred aseptically to the center of the media poured petri dishes of the treatment and control sets. Treatment and control sets were incubated at 25±2 ˚C for seven days. Radial growth in treatments and control were measured at 3rd day, 5th day and 7th day, except for *S. rolfsii* where this fungus grows fast and completes within three days so radial growth was taken on 1st day, 2nd day and on 3rd day after inoculation. This was expressed as the mean growth along two axes on two predraw perpendicular lines on the reverse side of each plate and compared with the control sets and the percentage of mycelia inhibition was calculated using the following formula (Gupta *et al*. 2014) [9]:

$$I = \frac{[C - T]}{C} \times 100$$

Where, I = Percent Inhibition
C = Colony Diameter in Control
T = Colony Diameter in Treatment

Result and Discussion

The data related to antimicrobial activity of RET medicinal plant botanicals against phytopathogens has been illustrated in table 1, 2, 3, 4, 5.

All four extract showed positive response towards all pathogens except for *Phytophtora capsici*.

Effect of plant extract against *A. solani.*

It was confirmed from the results (Table.2) aqueous leaf extract of 4 plant species showed significant inhibition in the mycelial growth of *A. solani*. Among four plant extracts used, *E. ribes* at 15% concentration was found to be the most effective against *A. solani* and caused highest inhibition in the mycelial growth (86.52%), followed by *O. indicum* (61.74%), *S.asoca* (59.57%), *C. paniculatus* (25.05%). This was confirmed with the earlier work of Deepti and Nidhi, (2015) [10] that plant extract of *Eucalyptus obliqua* (15%) showed good inhibitory effect against *A. soalni*. This antimicrobial activity of *E. ribes* may be due to presence of phytochemicals (Mohammad *et al*., 2010) [11] which acts as fungicidal agent.

Table 2: Effect of plant extracts on growth of *A. solani*

Plant species	Mean Diameter of Mycelial Growth of (mm)			Percentage of Mycelial Growth Inhibition (%)		
	DAY 3	DAY 5	DAY 7	DAY 3	DAY 5	DAY 7
Oroxylum indicum	11.90	15.20	19.40	50.62	61.42	61.74
Embelia ribes Burm F.	2.40	5.00	6.80	89.78	87.31	86.59
Celastrus paniculatus Willd.	14.80	30.10	38.00	38.59	23.60	25.05
Saraca asoca (Roxb) Willd.	10.40	21.60	20.50	56.97	45.18	59.57

Effect of plant extract against *S. rolfsii.*

It was also observed from the results (Table.3) aqueous plant extract of 4 plant species showed significant inhibition in the mycelial growth of *S. rolfsii*. Among the plant extract used *O. indicum* at 15% concentration, was found to be the most effective against *S. rolfsii*.and caused highest inhibition in the mycelial growth (80.35%), followed by *E.ribes* (13.29%), *C. paniculatus* (2.82%). And *S.asoca* showed no effect this may work out higher concentration. Similar results were observed from the aqueous extracts of *Azadiracta indica* (Farooq *et al*., 2010) [12]. Antimicrobial activity of *O.indicum* is due to presence bioactive compounds which acts as antifungal agents.

Table 3: Effect of plant extracts on growth of *S. rolfsii*

Plant Species	Mean Diameter of Mycelial Growth of (mm)			Percentage of Mycelial Growth Inhibition (%)		
	DAY 3	DAY 5	DAY 7	DAY 3	DAY 5	DAY 7
Oroxylum indicum	4.90	16.70	16.70	79.73	71.16	80.35
Embelia ribes Burm F.	13.90	47.10	73.70	42.49	18.65	13.29
Celastrus paniculatus Willd.	18.60	44.90	82.60	23.05	22.45	2.82
Saraca asoca (Roxb) Willd.	18.30	44.00	85.00	24.29	24.01	0.00

Effect of plant extracts on growth of *F. oxysporum*

The results also indicate (Table.4) that aqueous plant extract of 4 plant species showed significant inhibition in the mycelial growth of *F. oxysporum*. Among the four plant extract used *E. ribes* at 15% concentration, was found to be the most effective and caused highest inhibition in the mycelial growth (43.26%), followed by *S. asoca* (42.96%), *C. paniculatus* (38.27%). and a minimum growth inhibition was observed in *O.indicum* (18.77%). These findings are in concurrence with those reported by Keerio *et al*., 2017 [13], results of this study reported that neem leaf aqueous extract was found to be more effective in reducing the mycelial growth of *F. oxysporum*. *S. asoca* and found to have good inhibitory effect against *F. oxysporum.*

S. asoca found to have antifungal activity this was confirmed with the work of Divya *et al*., 2014, [14] where aqueous extract with 100% concetration reduced the mycelial growth of *R. solani* and from this it has been concluded from the study that *S. asoca* extract may inhibit mycelial growth of *F. oxysporum* completely at higher concentrations. Antimicrobial activity of *saraca indica* is due to the synergistic action of different chemical constituents in the leaves. (Chandekar, 2011) [15].

Table 4: Effect of plant extracts on growth of *F. oxysporum*

Plant Species	Mean Diameter of Mycelial Growth of (mm)			Percentage of Mycelial Growth Inhibition (%)		
	DAY 3	DAY 5	DAY 7	DAY 3	DAY 5	DAY 7
Oroxylum indicum	23.70	38.30	55.40	17.71	15.08	18.77
Embelia ribes Burm F.	14.50	32.30	38.70	49.65	28.38	43.26
Celastrus paniculatus Willd.	21.60	41.70	42.10	25.00	7.54	38.27
Saraca asoca (Roxb) Willd.	20.00	31.00	38.90	30.56	31.26	42.96

Effect of plant extracts on growth of *P. capsici*

It has been concluded from the results (Table.4) that no plant extract is effective against *P. capsici* and it may have appeared to be more resistant to the extracts. This may be due to fast sporulation of fungus, differences in chemical composition and structure of cell wall of micro-organisms, and it may work out at higher concentration than the concentration used in the study to suppress the growth of fungus.

Table 5: Effect of plant extracts on growth of *P. capsici*

Plant Species	Mean Diameter of Mycelial Growth of (mm)			Percentage of Mycelial Growth Inhibition (%)		
	DAY 3	DAY 5	DAY 7	DAY 3	DAY 5	DAY 7
Oroxylum indicum	43.30	79.20	85.00	15.10	3.06	0.00
Embelia ribes Burm F.	43.20	78.60	85.00	15.29	3.79	0.00
Celastrus paniculatus Willd.	43.30	60.88	85.00	15.10	78.60	0.00
Saraca asoca (Roxb) Willd.	47.10	73.80	85.00	7.65	5.99	0.00

Investigations on the mechanisms of disease suppression by plant products have suggested that the active principles present in plant extracts may either act on the pathogen directly (Amadioha, 2000) [16] or induce systemic resistance in host plants resulting in a reduction of the disease development (Kagale *et al*., 2004) [17]. The four plant extracts *E. ribes*, *O. indicum*, *C. paniculats*, and *S. asoca* shortlisted for pathogen inhibition have potential to be developed as potent fungicides.

Conclusion

As most of the medicinal plants are in the verge of extinction even though they have potent use in the field of medicine and also in plant disease management, the findings of the present investigation could be an important step towards the possibilities of using natural plant products as bio pesticides and it also helps to reduce the residual effect in crop plants. This also helps in creating awareness about use of these RET medicinal plants and their conservation perspective.

The study concluded that *E. ribes* and *O. indicum* found to have highest activity against *A. solani* and *S. rolfsii* respectively. And also shown that all the botanicals can be used as antifungal agents against *A. solani*, *S. rolfsii*, and *F. oxysporum* pathogens.

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