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Antifungal activity of ethanolic and petroleum ether extracts of some medicinal plants against the plant pathogenic fungus *Sclerotium rolfsii* sacc.

Bapat U.C.[1], **Smrity Prabha**[2*] **and Jyoti Kumar**[2]
[1]Department of Botany, St. Xavier's College, University of Mumbai, Maharashtra, India.
[2]Department of Botany, Ranchi College, Ranchi, Jharkhand, India.

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Abstract: The anti-fungal activity of ethanolic and petroleum ether extracts of eight medicinal plants, *viz.*, *Acorus calamus Linn, Justicia adhatoda* L. *Lawsonia Inermis L., Lantanacamara* Linn., *Pongamiapinnata* (L.) Pierre, *Solanum nigrum* Linn., *Vitex negundo* L. and *Wedelia chinensis* (Osbeck) Merr. were tested *in vitro* against phytopathogenic fungus *Sclerotium rolfsii* Sacc. was evaluated using poison food method. The results obtained showed that the Petroleum ether extracts of leaves and flowers of *L. camara*, leaves of *Lawsonia inermis* and *S. nigrum* did not inhibit the growth of *S. rolfsii*. However, it was observed that the ethanolic extracts of the rhizome of *A. calamus* and the leaves of *L. Camara* showed 61% and 50% inhibition of the growth of *S. Rolfsii* respectively. The most promising results were obtained with the petroleum ether extract of *A. calamus*, which exhibited 100% inhibition of *S. Rolfsii with MIC of* 10.4 mg/ ml.

Key words: Anti-fungal Activity; *Acorus calamus* rhizome; ethanolic extract; *Lantana camara*; petroleum ether extract; *Sclerotium rolfsii* Sacc.

Introduction

Sclerotium rolfsii Sacc. is a soil-borne fungal pathogen characterised by whitish mat of mycelium and the brownish sclerotia. This pathogen infects a number of vegetables (pumpkin, peanut, sweet potato, *etc.*), crops (wheat, corn, *etc.*) and also some medicinal plants (*e.g. Stevia*). Some of the medicinal plants have been reported to be effective in controlling the growth of this pathogen[1,2].

In this study, nine widely available medicinal plants, *viz.*, *Acorus calamus Linn, Justicia adhatoda* L., *Lawsonia Inermis L., Lantanacamara* Linn., *Pongamiapinnata* (L.) Pierre, *Solanum nigrum* Linn., *Vitex negundo* L. and *Wedelia chinensis*(Osbeck) Merr. have been considered based on their therapeutic uses. *A. calamus* has wide applications as a herbal medicine as its roots and leaves exhibit anti-microbial and insecticidal activities[3,4,5]. *J. adhatoda* is found to be highly effective against various bacterial and fungal infections[6] and the treatment of scabies and other skin diseases[7]. *Lawsonia. inermis* is reported to display antimicrobial activity deriving from asarone[9] present in its leaf, roots and rhizomes tissues. *L. camara* has several therapeutic uses, mainly as herbal medicine[10,11,12]. *Lantana* oil is used externally for leprosy and scabies[13]. *L. camara* leaf ethanolic fraction (EF) and essential oil (EO) demonstrated antibacterial activity[14,15,16,17]. Jain (2004) reported that *Lantana* leaf extracts displayed excellent anti-dermatophytic properties because of free and bound flavanoid fraction[18]. Bajpai (2009) reported that the leaf extracts of *Pongamiapinnata* could have significant applications in food and pharmaceutical industries as it inhibits the growth of some representative food spoilage and food-borne pathogens[19]. It is also very effective against two *human pathogens* (*viz. Epidermophyton floccosum and Candida albicans) and two plant pathogens*[20] (*viz. Alternaria solani and Helminthosporium turcicum). Solanum nigrum* Linn. (Solanaceae), commonly known as 'Black nightshade', has been extensively used in traditional medicine in India and other parts of world to cure liver disorders, chronic skin ailments (psoriasis and ringworm), inflammatory conditions, diarrhoea, eye diseases, etc.[21,22]. *Vitex negundo* L. has been used traditionally in treatment of rheumatism and has anti-microbial, tranquillizer and diuretic properties[23]. Srinivas (2010) observed significant anti-bacterial activity of *V. negundo* against *Bacillus cereus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Pseudomonas putida*[24]. *W. chinensis*has wider applications in treating some skin ailments (such as eczema, acne, and dermatitis), cholagouge, jaundice, diarrhoea, couch, cephalahgia, diphtheria and pertussis, *etc.* Ethanolic leaf extract of *W. chinensis* has been reported to show strong activity against gram-positive bacteria, gram-negative bacteria and some fungi as *Aspergillus niger*, *A. flavus*, *Candida albicans* and *Alternaria alternate*[25].

Although there are several reports on the antimicrobial activity of above mentioned plants, there are very few references on the antifungal activity of these plants against phytopathogenic fungi. Present study aims to evaluate the anti-

***Corresponding Author:**
Mrs. Smrity Prabha,
Assistant Professor, Department of Botany,
Ranchi College, Ranchi, Jharkhand, India.
E-mail: smrity80@gmail.com

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fungal activity of these plants against the phytopathogenic fungus *S. rolfsii.*

Materials and Methods

Collection of plant pathogen

A culture of *Sclerotium rolfsii* Sacc. (**NFCCI - 2465**) was obtained from Agharkar Research Institute, Pune.

Collection of plant material

The plant materials were collected from the wild and identified and authenticated using 'Flora of Bihar and Orissa'[26] and Blatter Herbarium. The locality of plants collected from and the parts used are given in table 1.

Table 1: List of plants tested for Antifungal Activity, along with the plants used and locality of collection

S. No.	Plant species	Area of collection	Plant part
1.	*Acorus calamus* Linn.	Kanke Road, Ranchi.	Rhizome
2.	*Lantanacamara L.*	Behind Ranchi College Premises, Ranchi	Leaves, Flower
3.	Lawsonia innermis L.	Bariatu Road, Ranchi	Leaves
4.	Justicia adhatoda L.	Behind Ranchi College Premises, Ranchi	Leaves
5.	*Millettia pinnata*(L.) Panigrahi *(Pongamia pinnata* (L.) Pierre)	Tagore Hill, Morabadi, Ranchi	Leaves
6.	Solanum nigrum L.	Behind Ranchi College Premises	Leaves
7.	Vitex negundo L.	Bariatu Road, Ranchi	Leaves
8.	*Sphagneticola calendulacea* (L.) Pruski (Wedalia chinensis (Osbeck) Merr.)	Medicinal Garden, Behind Botany Department, Ranchi College Premises, Ranchi.	Aerial parts

The collected plants were surface sterilised with 0.1% mercuric chloride and then washed with distilled water 2-3 times and shade dried. The dried plant materials were then ground separately to fine powder using grinder. Powders, thus, obtained were transferred to air tight jars and stored in the dark for future use.

Preparation of extract

10 g each of powdered plant material was soaked overnight in 50 ml of ethanol (EtOH) and petroleum ether (40-60˚c) separately with intermittent shaking. The extracts were then filtered through Whattman no. 1 filter paper and the filtrates were collected separately. To the residue 50 ml of solvent (in which the powder was soaked) was added, stirred well and left for 4 hours at room temperature with intermittent shaking. The extracts were filtered again and the filtrates were collected. This procedure was repeated once again and the filtrates of each plant were pooled together separately. The filtrates, thus obtained, were transferred separately to pre-weighed evaporating dishes and the solvent was evaporated. The residues, thus, obtained were weighed and dissolved in respective solvents to make the final volume of 10 ml for each plant extract. These were used for further anti-fungal studies.

Antifungal Activity Assay

In-vitro anti-fungal activity of the plant extracts was tested following poison food technique[27,28,29]. 1 ml of petroleum ether extract and 0.1 ml ethanolic extract (as at this amount of solvent the fungal growth is not inhibited) of each plant extract were pipetted out separately under aseptic condition and mixed with 19 ml and 19.9 ml of cool molten PDA medium in the Petri dishes respectively to make up the final volume to 20 ml per plate. Each plate was gently swirled on the laboratory bench to ensure even dispersion of extracts and the medium plates were allowed to solidify at room temperature. Mycelial disc (5 mm in diameter) of *S. rolfsi* obtained from 7 day-old culture of the fungus was transferred aseptically to the centre of each Petri dish. The plates were then incubated at 28ºC ± 2ºC and observations were made every day to check the fungal growth. The diameter of the fungal colony (if any) was measured on 3rd, 5th and 7th day. Colony diameter was taken as the mean along three preset diametric lines on the reverse side of the plates. Solvent control plates were kept for comparison. Only PDA culture medium and PDA plus petroleum ether or ethanol served as negative controls whereas PDA plus Bavistin (5 µg/ml) served as positive control. The anti-fungal activity of the extracts was expressed as percent inhibition of mycelial growth. This is calculated using the following formula[30].

$$\% \text{ inhibition of Mycelial Growth} = \frac{DC_{Control} - DC_{Treatment}}{DC_{Control}} \times 100 \qquad (i)$$

where, DC stands for average diameter of fungal colony.

Results and Discussion

The antifungal activity of the extracts in terms of the colony diameter and % inhibition showed following results. Colony diameter increases with the progression of time (days) in the control plates (negative and positive) indicating that solvents alone as well as antibiotic (Bavistin) do not inhibit the growth of *S. rolfsii.* It is also observed that the plates having ethanol and petroleum ether extracts of Lantana flower, *Acorus* rhizome, *Vitex, Wedalia,* and *J.adhatoda leaves* did not show any growth (*i.e.*100% inhibition) on day 3 as compared to the ethanolic extracts and the two controls (PDA medium alone and Medium + ethanol). On day 5, fungal growth was observed in the plates with ethanolic and pet ether extracts of all the plants except the petroleum ether extracts of *A. calamus* rhizome. For the petroleum ether extracts, higher

inhibition was observed with *W. Chinensis* (68%), and *V. negundo* (48%), while for ethanol extract, highest inhibition was observed with *A. calamus* (69.6%), followed by *L. camara* leaves (49.0%). On day 7, fungal growth was observed in all the plants *i.e.* having ethanolic or petroleum ether extracts of plants studied except the pet ether extract of *A. calamus* (Figure 1). The petroleum ether extracts of *A. calamus* exhibited 100% inhibition of growth of *S. Rolfsii.*

Fig. 3: Inhibition Zone of *Sclerotium rolfsii* (Day 07) against *A. calamus* rhizome petroleum ether extract at different concentrations.

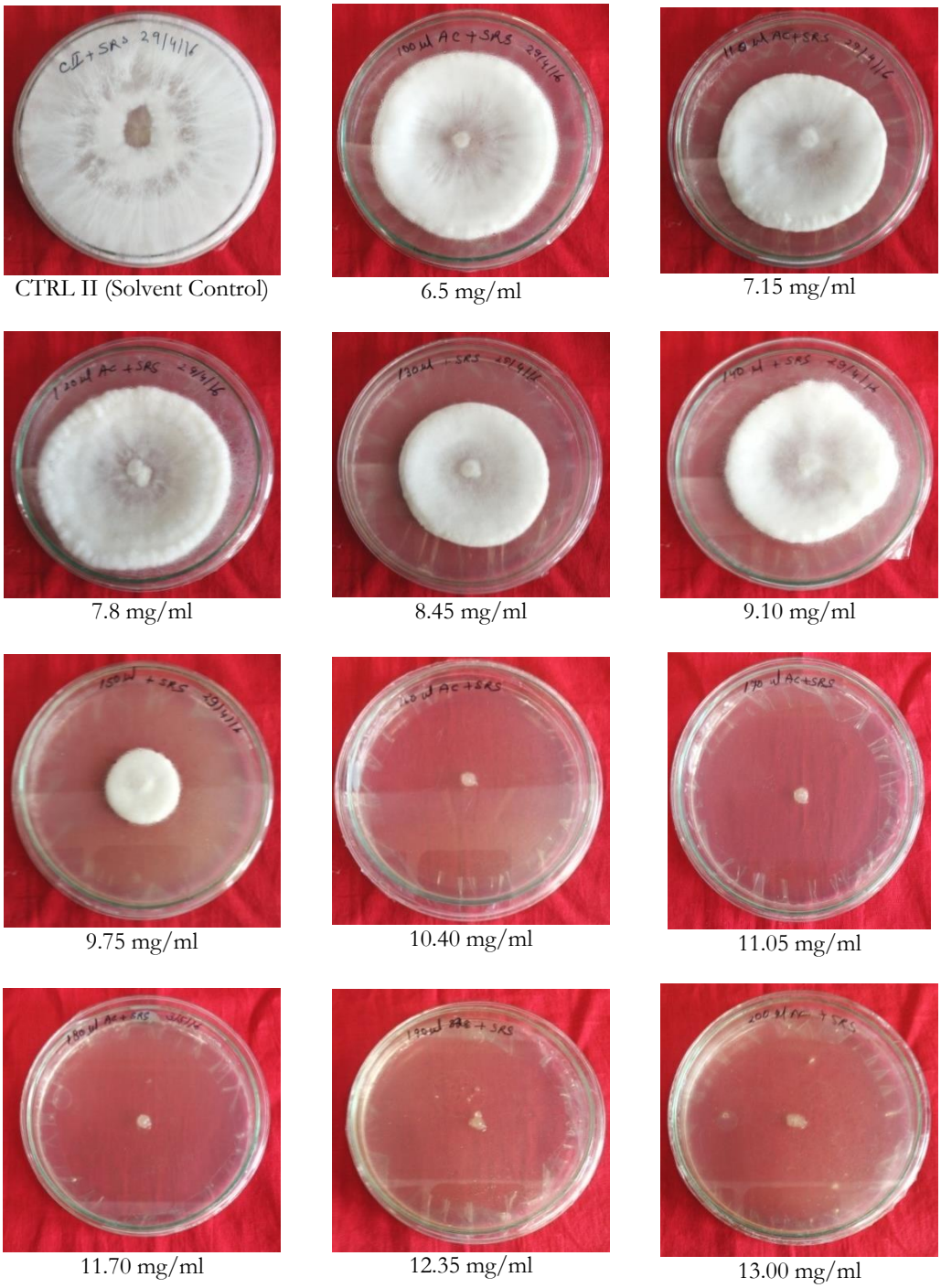
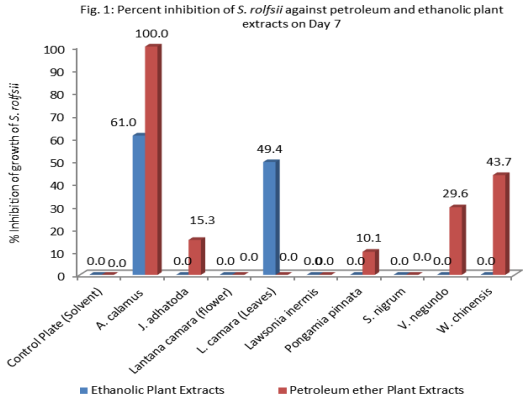


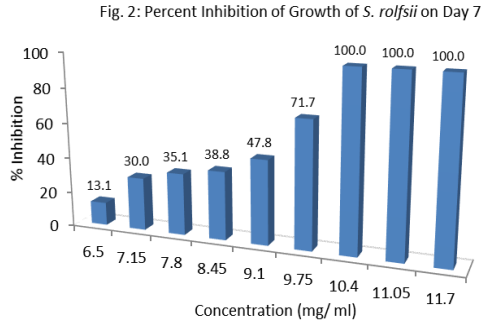
Table 2: ANOVA (two-way with replication) for the effect of two solvents on eight different plants' extracts in % inhibition of mycelia growth of S. Rolfsii

Source of Variance	Degrees of freedom	Sum of Square	Means of Square	F-test	p-value
Solvent	1	8.61	8.61	76.11***	0.00
Extract	10	277.33	27.73	245.25***	0.00
Solvent* Extract	10	77.36	7.74	68.41***	0.00

***: Significant at 0.01 level.



It was observed that the petroleum ether extract of rhizome of *A. calamus* at the concentration of 10.4 mg/ml and above, completely inhibits the mycelial growth of *S. rolfsii* on 3rd, 5th and 7th days (Figure 2 and Figure 3) indicating the minimum inhibitory concentration (MIC) to be 10.4 mg/ ml.



Statistical Analysis

Two-way ANOVA results suggested that among all the plants tested, the petroleum ether extracts of *A. calamus*, *W. chinensis* and *V. negundo* showed statistically significant inhibition at 0.01 level when compared with the -ve control. Among these three extracts, the % inhibition exhibited by the petroleum ether extract of *A. calamus* is highly significant (0.01 level of significance) (Table 2).

Conclusion

Of the eight plants extracts tested for their inhibitory activity *in-vitro* against *S. rolfsii*, petroleum ether extract of rhizome of *A. calamus* exhibited complete inhibition (100%) of the growth of the fungus on the 7th day at 10.40 mg/ ml concentration. The petroleum ether extracts of leaves of *W. chinensis* and *V. negundo* showed 43.7% and 29.6% inhibition, respectively on the 7th day. Ethnolic extract of leaves of *L. camara* showed only 49.4% inhibition of growth of fungus.

ANOVA results confirm that the inhibition showed by the extract of *A. calamus* is statistically significant at 0.01 level.

The results suggest that the extract of *A. calamus* could be used as biological fungicide. Blend of the extract of *W. chinensis, V. negundo* and *L. camara* may be tested for their combined effect on the growth of the fungus. However, further, studies need to be carried out *in vivo* for confirmation. Phyto-chemical studies should be carried out to find out the active principle(s) in the extract of *A. calamus*.

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