

**ANTIFUNGAL ACTIVITY OF ETHANOLIC AND PETROLEUM ETHER EXTRACTS
OF SOME MEDICINAL PLANTS AGAINST THE PLANT PATHOGENIC FUNGUS
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ABSTRACT

Anti-fungal activity of ethanolic and petroleum ether extracts of eight medicinal plants (*viz.*, *Acorus calamus* Linn., *Justicia adhatoda* L., *Lawsonia Inermis* L., *Lantana camara* Linn., *Pongamia pinnata* (L.) Pierre, *Solanum nigrum* Linn., *Vitex negundo* L. and *Wedelia chinensis* (Osbeck) Merr.) was tested *in-vitro* (using poison food method) against phytopathogenic fungus *Nigrospora oryzae* (Berk. & Broome) Petch. Results showed that the petroleum ether extracts of leaves of *J. adhatoda*, *L. inermis* and *Solanum nigrum* did not inhibit the mycelia growth of *N. oryzae*. Nevertheless, ethanolic extracts of *A. calamus* rhizome, *P. pinnata* and *L. inermis* showed 87.7%, 50.96% and 49.81% inhibition, respectively against the mycelia growth of *N. oryzae*. The most promising results were obtained with the petroleum ether extract of *A. calamus*, which exhibited 100% inhibition of *N. oryzae* with MIC of 8.41 mg/ml. Anti-fungal activity of different plants' extracts prepared in two different solvents were found to be significantly different from one another in the two-way ANOVA test.

KEYWORDS: Antifungal activity, *Nigrospora oryzae*, *Acorus calamus*, poison food technique.**I. INTRODUCTION**

Nigrospora oryzae (Berk. & Broome) Petch. is a saprophytic fungus, which grows on the debris of various living and dead plants species. It is found widespread across all parts of the world, especially in the tropical areas. Depending upon the environmental conditions and the host, it acts both as an entophyte and a parasite. *N. Orzaye* causes various diseases in plants; *e.g.* grain spots of rice, sorghum, and corn^[1]; tip blight and leaf spot of *A. calamus* in India^[2]; leaf spot disease in date palm.^[3] It is also reported to reduce active chemical components in plants parts having medicinal value.^[2]

Based on their therapeutic uses, eight widely available medicinal plants, *viz.*, *Acorus calamus* Linn., *Justicia adhatoda* L., *Lawsonia Inermis* L., *Lantana camara* Linn., *Pongamia pinnata* (L.) Pierre, *Solanum nigrum* Linn., *Vitex negundo* L. and *Wedelia chinensis* (Osbeck) Merr. have been considered in this study. *A. calamus*, also known as sweet flag (family – Acoraceae), is a perennial medicinal plant and its roots, leaves and rhizome exhibit anti-microbial and insecticidal activities^[4,5,6]. *J. adhatoda* is reported to be effective against various bacterial, and fungal infections^[7] and other skin diseases.^[8] Leaves, roots and rhizomes of *Lawsonia inermis* are reported to display antimicrobial

activity.^[9] Owing to its therapeutic potential, *L. camara* is mainly used as a herbal medicine.^[10,11,12] Ethanolic fraction (EF) and essential oil (EO) of *L. camara* leaves are reported to demonstrate antibacterial activity.^[13,14,15] *Pongamia pinnata* is effective against two human pathogens (*viz.* *Epidermophyton floccosum* and *Candida albicans*) and two plant pathogens^[16] (*viz.* *Alternaria solani* and *Helminthosporium turcicum*). *Solanum nigrum* Linn. (Solanaceae) is used widely in traditional medicine in India and other parts of world to cure liver disorders, diarrhoea, eye diseases, chronic skin ailments, inflammatory conditions, *etc.*^[17] *Vitex negundo* L. has anti-microbial, tranquillizing and diuretic properties^[18] and has traditionally been used in treatment of rheumatism. *W. Chinensis* has wider applications^[19] in treating jaundice, diarrhoea, cough, cephalalgia, diphtheria and pertussis, skin ailments, cholagogue, *etc.*

Although there are several reports on the antimicrobial activity of above-mentioned plants, there are possibly very few references on the antifungal activity of the said plants against *N. orzaye*. Present study aims to evaluate the anti-fungal activity of these plants against the aforesaid fungus.

MATERIALS AND METHODS

A. Collection Of Plant Pathogen

A culture of *Nigrospora oryzae* (Berk. & Broome) Petch. (NFCCI – 2710 isolated from *Brassica sp. root*) was procured from Agharkar Research Institute, Pune.

B. Collection of plant materials

Plant materials were collected from the wild. They were identified and authenticated in the Blatter Herbarium (St. Xavier's College, Mumbai) using 'Flora of Bihar and Orissa'.^[20] Details of plants parts used and the locality from where they were collected are given in Table 1.

Table 1: Plant Species and their area of collection.

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

C. Preparation of extract

10 gms. each of powdered plant material was soaked overnight separately in 50 ml of ethanol (EtOH) and petroleum ether (40 - 60°C), respectively with intermittent shaking. The extracts were then filtered through Whatman no. 1 filter paper and the filtrates were collected separately. The residues, thus, obtained were dissolved in respective solvents to make the final volume of 10 ml for each plant extract. These were used for further anti-fungal studies.

D. Antifungal Activity Assay

In-vitro anti-fungal activity of the plant extracts was tested following poison food technique.^[21] 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 *N.oryzae* obtained from 3-7 days' old culture of the fungus was transferred aseptically to the centre of each Petri dish. The plates were then incubated at 28°C ± 2°C 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. 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.^[22]

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

Where, DC stands for average diameter of fungal colony.

E. Minimum Inhibitory Concentration (MIC)

The MIC was determined by poison food technique. Those petroleum ether plant extracts, which showed 100% inhibition at highest concentration were selected for the determination of MIC and compared with solvent control plate. The concentration of plant extracts was decreased to find the minimum level of concentration at which 100% inhibition was observed.

The ethanol plant extracts, which showed less than 100% inhibition. The extracts and the samples were prepared in triplicates and all the experiments were done in triplicates for the confirmation of result.

F. Combination of different plants extract

Having seen the efficacy of individual plant extracts in two different solvents, the effectiveness of combination of plant extracts in the afore-said (two) solvents was attempted. This analysis would be useful to find if the formulation prepared from the combined plant extracts is more effective (in lesser dosage) against the said pathogens as compared to individual plant extracts.

For the said analysis, while preparing the petroleum ether solvent formulation, plant extract of *A. calamus* was mixed separately with *V. negundo*, *L. camara leaves* and *W. chinensis* in various proportions, viz., 1:1, 1:2, and 2:1

Similarly, in the ethanolic solvent, *A. calamus* extract was diluted separately with plant extracts of *L. innermis*, *L. camara leaves*, and *P. pinnata* in the said proportions as mentioned above and investigated for their antifungal activity.

II. RESULTS AND DISCUSSION

Findings in respect of the antifungal extracts indicate that negative control plate (solvent) does not inhibit the

growth of *N. Orzyae*, whereas positive control plate (Bavistin - antibiotic) shows 100% inhibition at a concentration of 5 µg/ml (**Figure 1**). The ethanolic extract of *A. vasica* and *S. nigrum* showed negative result (-3.45 % inhibition each) whereas their petroleum ether extract showed zero percent inhibition (**Chart 1**).

Petroleum ether extracts of *W. chinesis*, *L. camara* leaves and *V. nigundo* showed 56.8%, 60.9% and 71.2% inhibition, respectively. Ethanolic extract of *Acorus calamus* showed 87.74% inhibition, while petroleum ether extract of *A. calamus* showed 100% inhibition.

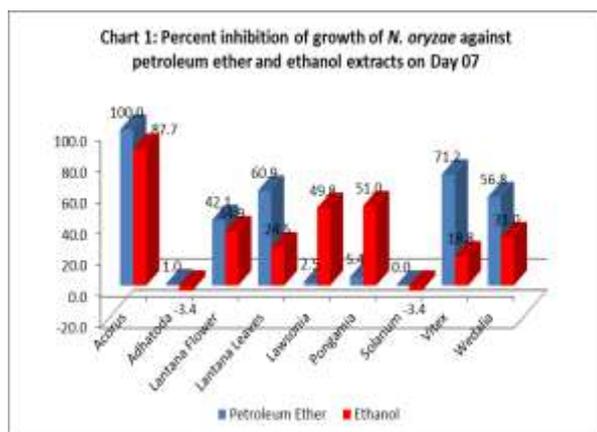


Figure 1A



Figure 1B

Figure 1: Percent Inhibition of *N. orzyae* (Day 07) against eight different Ethanolic and petroleum ether plant extracts.



It was observed that with the enhancement in MIC range of ethanolic extract of *A. calamus* rhizome from 50µl (4.5mg/ml) to 100µl (9mg/ml) % inhibition of mycelial growth increased from 47.2% to 89.6% on day 7 (**Figure 2A and Chart 2**).

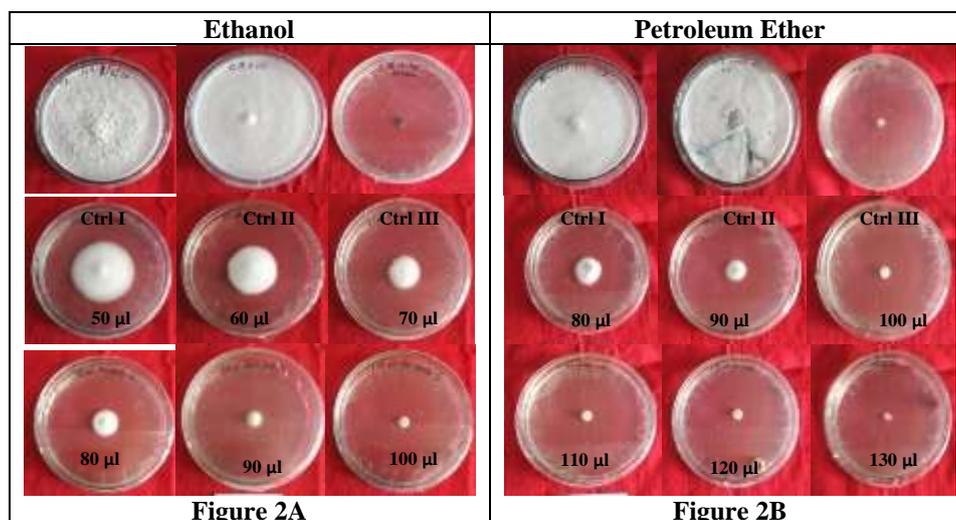
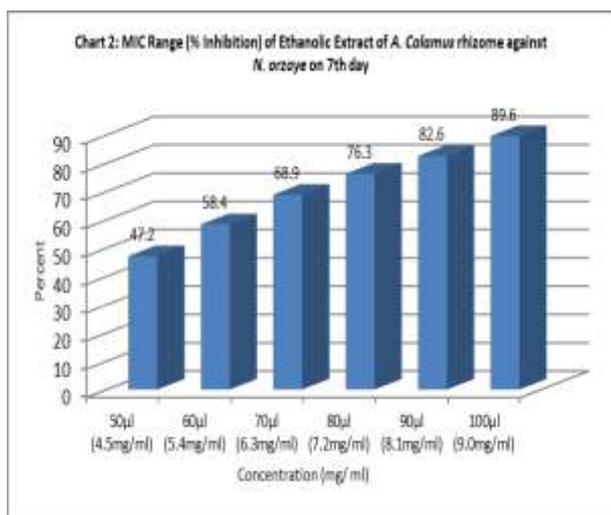


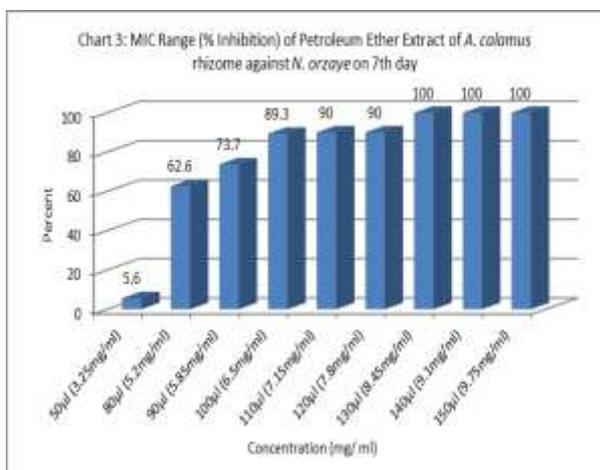
Figure 2A

Figure 2B

Figure 2: Inhibition Zone of *N. orzyae* (Day 7) against Ethanolic and petroleum ether extract of *A. calamus* rhizome at different concentrations.



On the contrary, petroleum ether extract of rhizome of *A. calamus* at the concentration of 8.45 mg/ml (130 µl) and above showed complete inhibition of mycelial growth of *N. oryzae* on the 7th day (Chart 3 and Figure 2B). Bapat, *et al.* (2016)^[23] reported 100% inhibition of mycelial growth of *S. Rolfzii* against petroleum ether extract of *A. calamus* at the concentration of 10.4 mg/ml. whereas Gauria *et al.* (2017)^[24] reported that resin extract of *Gardenia resinifera* was the most effective against *N.oryzae* - showing 77.2% inhibition of mycelial growth at the concentration of 111 mg/ml.



Amongst the combinations tried, the mixture of *A. calamus* and *V. negundo* (2:1) in petroleum ether solvent yielded highest percent inhibition of 91.9% against *N. Oryzae* (Chart 4). However, when the same combination is used in 1:1 proportion, percent inhibition against the said pathogen reduces to 70.37%. This mixture even in the proportion of 1:1 yielded higher percent inhibition as compared to other plant extracts combinations.

On the whole, as compared to individual petroleum ether plant extract of *V. negundo*, which showed a maximum % growth inhibition of 71.2%, the combination, mixed with *A. calamus*, in proportion of 2:1, showed much higher % growth inhibition (91.85%) of *N. oryzae*.

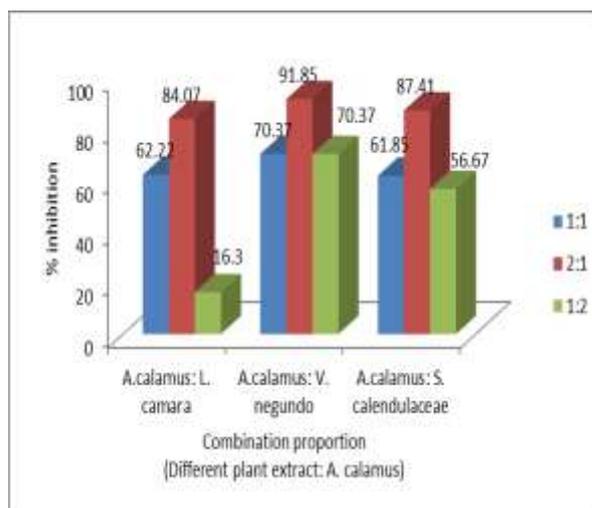


Chart 4: Percent inhibition of combined petroleum ether plant extract of *L. inermis*, *P. pinnata* and *L. camara* with *A. calamus* against leaves against *N. oryzae* on Day 7.

For the ethanolic solvent, the combination of *A. calamus* and *P. pinnata* (2:1) yielded a maximum 64% inhibition against *N. oryzae* (Chart 5). The ethanolic solvent formulation is not that effective as *A. calamus* (in ethanolic solvent) alone in the same amount yielded 68% inhibition against the said pathogen. This suggests that ethanolic extract formulation of *A. calamus* with other plant extracts does not show significant result *vis-à-vis* *A. calamus* alone.

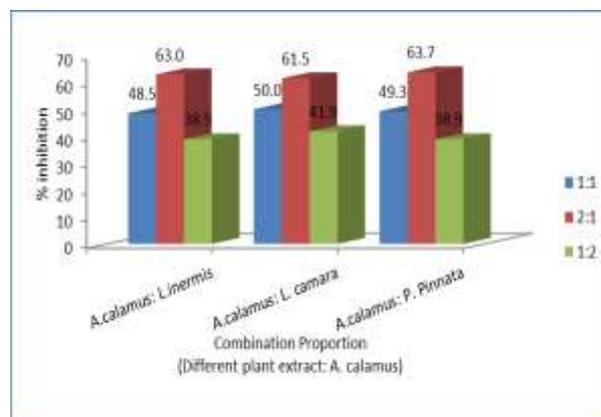


Chart 5: Percent inhibition of combined ethanol plant extract of *A. calamus* with *L. inermis*, *P. pinnata* and *L. camara* leaves against *N. oryzae* on Day 7.

III. STATISTICAL ANALYSIS

Two-way ANOVA test was used to compare if the anti-fungal activity of different plants extracts prepared in two different solvents were significantly different from one another. Interaction between the solvents and plants extracts concentration was also included in the analysis. Two-way ANOVA result rejects the null hypothesis of no significant difference between activities of two different solvents on the mycelial growth of the aforesaid fungus at 1% level of significance (Table 4). Null hypothesis of no significant differences in anti-fungal

activity of the said nine plants' parts was also found to be rejected at 1% level of significance. The interaction effect of the two factors (Solvent*Plant Extract

concentration) was also found to have statistical significant variation in anti-fungal activity of the plants extracts at 1% level of significance.

Table 4: ANOVA (two-way with replication) Table for the effect of two solvents of nine different plants' extracts on the mycelia growth of *Nigrospora oryzae*.

Source of Variance	Degree of freedom	Sum of Square	Means of Square	F-test	p-value
Solvent	1	0.58	0.582424	10.29***	0.0025
Extract	10	560.95	56.09448	991.67***	0.00
Solvent* Extract	10	111.47	11.14709	197.06***	0.0000

Note: ***: Significant at 0.01 level.

IV. CONCLUSION

Of the eight plants extracts tested for their inhibitory activity *in-vitro* against *N. Oryzae*, petroleum ether extract of rhizome of *A. calamus* exhibited complete inhibition (100%) of the growth of the fungus on the 7th day at 8.41 mg/ ml concentration. Ethanolic extracts of *A. calamus* rhizome, *P. pinnata* and *L. inermis* showed 87.7%, 50.96% and 49.81% inhibition, respectively against the mycelia growth of *N. Oryzae* on the 7th day. On the whole, the combined petroleum ether extracts of *L. camara*, *V. negundo*, and *S. calendulacea*, mixed, individually with *A. calamus* in proportion of 2:1 showed a significantly enhanced % growth inhibition of *N. oryzae*. Two-way ANOVA test suggests that anti-fungal activity of different plants extracts prepared in two different solvents are significantly different from one another.

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