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*RESEARCH ARTICLE*

# IN-VITROSTUDIES ON ANTIFUNGALACTIVITY OF SOMEMISTLETOE SPECIES

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## Abstract

In vitro evaluation of antifungal activity by disc diffusion method was carried out on leaf extracts of *D.falcata, D. falcata var pubescens, V. monoicum and V. orientale and* stem extracts of *D. falcata, D. falcata var pubescens, V. articulatum* and *V.orientale* using three different solvents viz., methanol, n-hexane and ethyl acetate. The antifungal activity was tested on three fungal strains include *Fusarium oxysporum, Phytophthora infestans, Sclerotium rolfsii.* Overall leaf extracts exerted better inhibitory activity than stem counterparts. Among all, only *V. orientale* was effective against the three fungal species.Compared to stem extracts, leaf extracts of *D. falcata* var *pubescens* have shown higher antifungal activity.



## Introduction:-

About 80% of the world's population depend on traditional medicine for primary health care (Ekhaise and Okoruwa, 2001).Traditional medicine involves the plant extract–derived medicines for about 85% (Si-Yuan Pan et al., 2013).About 250,000 to 300,000 plant species which exist on Earth, around 5000 plant species only were investigated for chemical compounds with pharmacological and biological activities and more than 25% of pharmaceutical molecules are plant based. The 200,000 known secondary metabolites are grouped in to phenolics, terpenoids, steroids and alkaloids which have significant functions in plants and the bioactive secondary metabolites identified with a broad range of pharmacological and therapeutic potentials (Pandita and Pandita 2021).

The plant derived antifungal compounds will create promising antimycotics for human mycotic diseases.Fungal infections can lead to death, particularly for immune compromised patients and with opportunistic infections (Rathi SanjeshkumarGotam 2013).The number of patients suffering from invasive fungal infection is increasing among organ transplant recipients, haematological patients undergoing hematopoietic stem cell trans-plantation, AIDS, cancer, immunosuppressive therapy, chronic pulmonary diseases, major surgery, etc.(Ibanez-Martinez et.al,2017) Due to its high mortality rate, there is a need for discovery of new and potential antifungal drugs.

Pathogenic fungi are the infectious agents in plants and humans, causing alterations during developmental stages, including post-harvest. In fruit and vegetables, there is a wide variety of fungal strains, causing certain aspects such as decline in quality, nutritional value, organoleptic characteristics, and limited shelf life(Agrios, 2004).Phytopathogenic fungi are controlled by synthetic chemicals on usual practice; however, synthetic chemicals are not ecofriendly and use of such synthetic chemicals is restricted due to the harmful effects on human health and the environment (Harris et al., 2001).The spread of multidrug-resistant strains of fungus and the poor number of

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drugs available make it necessary to discover novel antifungal compounds from natural products including medicinal plants. There is a need for investigation of diverseplants to identify their antifungal potential.

Mistletoes are semiparasitic flowering plants in the sandalwood order Santalales. Mistletoes grow on different host trees; have been widely used in traditional and folk medicine; Mistletoes might be a potential source of new antibacterial and antifungal drugs and complementary therapies to treat diabetes, hypertension, liver diseases, epilepsy and Alzheimer's disease. Multi therapeutic activity is due to the presence of many biologically active phyto compounds. The chemicalcomposition of mistletoe depends on part of theplant and host species as well as the place and time of harvest. (Szurpnicka et al, 2020).

## Material and Methods:-

In the present study, one species and one variety of genus *Dendrophthoe* and three species of genus *Viscum* were collected from different forest regions (Srisailam&Talakona) of Andhra Pradesh. All the plant species selected for the present study are parasitic flowering plants belong to the Loranthaceae and Santalaceae, families. The tested species are collected from different regions (Srisailam&Talakona) are duly authenticated by Botanical Survey of India (BSI), Deccan regional center, Hyderabad. Herbarium specimens of each of thespecies have been maintained separately in the lab. The list of the species tested is presented in Table.1&Fig-I.

**Table 1:-**The list of tested plant species (semi-parasites).

| S.NO | Scientific name* | Family | Location | Evaluated part of the plant | Host species |
|---|---|---|---|---|---|
| **1** | *Dendrophthoe falcata var pubescens* (Hook.f.) V.Chandras | Loranthaceae | Srisailam | Stem, Leaves | *Samanea saman* |
| **2** | *Dendrophthoe falcata* (L.f.)Ettingsh | Loranthaceae | Talakona | Stem, Leaves | *Azadirachta indica* |
| **3** | *Viscum articulatum Burm.f.* | Santalaceae | Talakona | Stem | *Dalberjia paniculata* |
| **4** | *Viscum monoicum* Roxb.ex DC. | Santalaceae | Srisailam | Leaves | *Ficus racemosa* |
| **5** | *Viscum orientale* Willd. | Santalaceae | Srisailam | Stem, Leaves | *Strychnosnux vomica* |

*Authentication by BSI, Deccan regional center Hyderabad

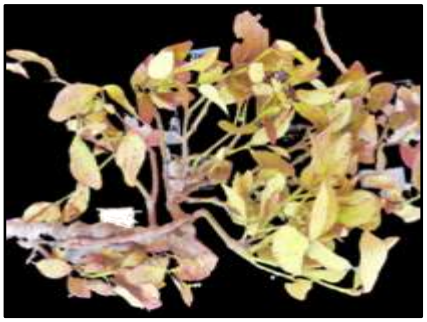
**Fig 1:- Plant species and variety.**

***Dendrophthoe falcata var pubescens***

***Dendrophthoe falcata***

*Viscum articulatum*

*Viscum monoicum*



*Viscum orientale*

**Preparationof plant extracts: -**
The leaves and stems were separated, and surface sterilized with 0.1% Hg Cl 2 for 5 minutes washed thrice with sterilized distilled water 5 minute each time. They were shade dried and powdered. Powders of the test material were dissolved in three different solvents viz methanol, ethyl acetate, and n-hexane for in vitro antimicrobial studies.

**Selection of micro organisms:**
Inoculums of three fungal strains were selected in the present study viz., *Fusarium oxysporum*, *Phytophthora infestans, Sclerotium rolfsii* were obtained from Department of biotechnology, Mahatma Gandhi University, Nalgonda, Telangana, India. Antifungal activity was tested employing a disc diffusion method.

**Disc diffusion method:**
Dissolve 24 gm of PDB in 1000 ml water to obtain PDB-Potato Dextrose Broth for fungal growth. The broth was sterilized by autoclaving at 121$^{o}$C and 15 lb. pressure for fifteen minutes. The sterilized medium (20 ml) was poured in sterilized Petri dishes under aseptic conditions, allowing them to solidify on a plane table.

Inoculation of fungal strains in autoclaved PDB media and incubate 3-4 days at 30º C in a shaker for fungal growth. From that 20 µl of fungal culture was taken and inoculated by inoculation loop on freshly prepared autoclaved agar plates. Filter paper discs (Whatman N0.1 filter paper) of about 6 mm in diameter impregnated with the test compound at the desired concentration are placed on the agar surface on the fungal plate. The incubation of the plates was done for 2 to 4 days at 30ºC in the BOD incubator. Inhibition diameteraround each disc was measured by measuring scale and recorded. Negative control was prepared with only methanol extract used for extraction.

The inhibition percentage (I %) was calculated using the formula $I\%=(C-T)*100/C$

Where I = Inhibition % ofmycelialgrowth (growthreductionovercontrol), C = radial growthoffungus in thecontrolplate (mm), T = radial growthoffungus on theinoculatedplate.

## Results:-

Leaf and stem extracts of the five test species were assessed for antifungal activity against three selected fungal strains viz *Fusarium oxysporum, Sclerotium rolfsii* and *Phytophthora infestans* by disc diffusion method using three different solvents viz., methanol, n- hexane and ethyl acetate.

Methanol leaf extracts of *V. orientale* has shown considerable inhibitory activity against *S.rolfisii* and *F. oxysporum.*Whereas *D. falcata var pubescens* exerted inhibitory effect against *F. oxysporium* and *P. infestans* while *V. monoicum* exerted highest zone of inhibition against *P. infestans.*

Among all the test species, n- hexane leaf extracts of *D.falcata,* induced inhibition against *F. oxysporum* and *P.* Infestans. Whereas *D.falcata var pubescens* exerted highest zone of inhibition against *F. oxysporium.*

Ethyl acetate leaf extracts of *V.monoicum* exerted inhibition against *F. oxysporum* where as *D.falcata var pubescens* exhibited inhibition against *P. infestans.*

Methanol stem extracts of *V.orientale* exhibited growth retardation against *F.oxysporum* and *P. infestans* whereas *D. falcata* shown inhibitory activity against *F. oxysporum.*None of the N- Hexane stem extracts has shown inhibitory activity against any of the fungal species tested. However, *V.articulatum* exerted high zone of growth inhibition against *P. infestans*. Ethyl acetate stem extracts of *D. falcata* has shown considerable inhibitory activity against *F. oxysporum* and *P. infestans.*

Among all, only *V. orientale* was effective against the three fungal species .Compared to stem extracts, leaf extracts of *D. falcata var pubescens* have shown higher antifungal activity.

**Table 2:- Zone of Inhibition in mm of different leaf extracts.**

| Fungal Species | Plant Species | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Dendrophthoe falcata* | | | *Dendrophthoe falcata var pubescens* | | | *Viscum monoicum* | | | *Viscum orientale* | | |
| | ME | NH | EA | ME | NH | EA | ME | NH | EA | ME | NH | EA |
| *Sclerotium rolfisii* | ** | ** | ** | ** | ** | ** | ** | ** | ** | 21 | ** | ** |
| *Fusarium oxysporum* | ** | 22 | 2 | 20 | 21 | ** | ** | 23 | 22 | 23 | ** | ** |
| *Phytophthora infestans* | ** | 17 | ** | 18 | ** | 16 | 20 | ** | ** | ** | ** | ** |

Key: ME- Methanol extract, NH- N-hexane extract, EA-Ethyl acetate extract; ** - No activity

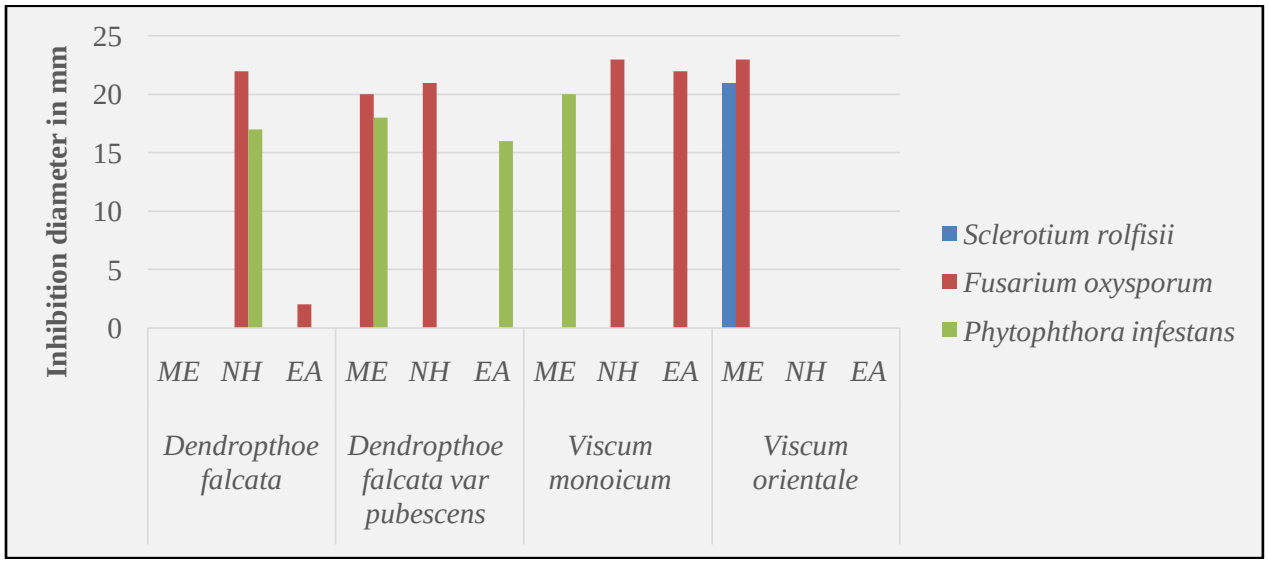



**Fig 2:-** Zone of Inhibition in mm of different leaf extracts.

**Table 3**:- Zone of Inhibition in (mm) of different stem extracts.

| Fungal Species | Plant Species | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Dendrophthoe falcata* | | | *Dendrophthoe falcata var pubescens* | | | *Viscum articulatum* | | | *Viscum orientale* | | |
| | **ME** | **NH** | **EA** | **ME** | **NH** | **EA** | **ME** | **NH** | **EA** | **ME** | **NH** | **EA** |
| *.Sclerotium rolfisii* | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| *Fusarium oxysporum* | 20 | ** | 22 | ** | ** | ** | ** | ** | ** | 22 | ** | ** |
| *Phytophthora infestans* | ** | ** | 22 | ** | ** | ** | ** | 17 | ** | 23 | ** | ** |

Key: ME- Methanol extract, NH- N-hexane extract, EA-Ethyl acetate extract; ** - No activity

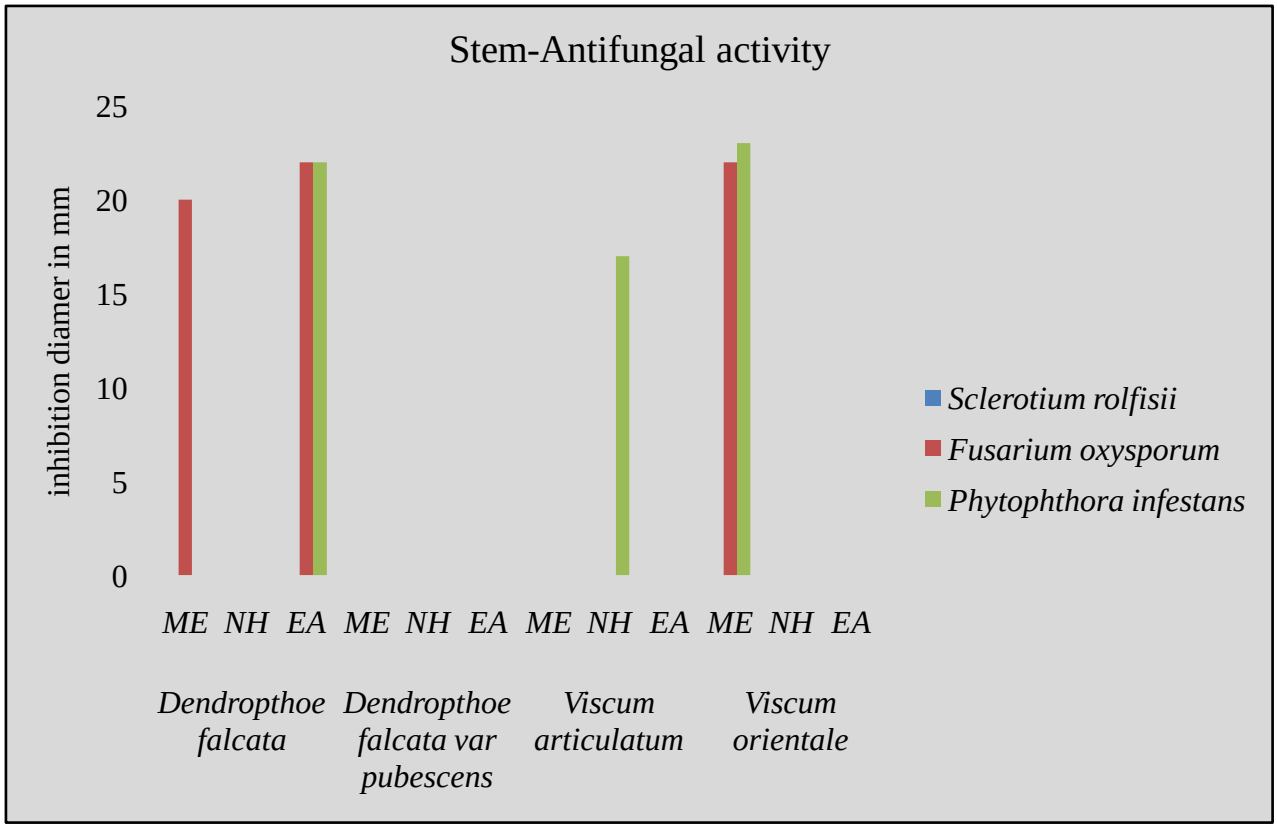
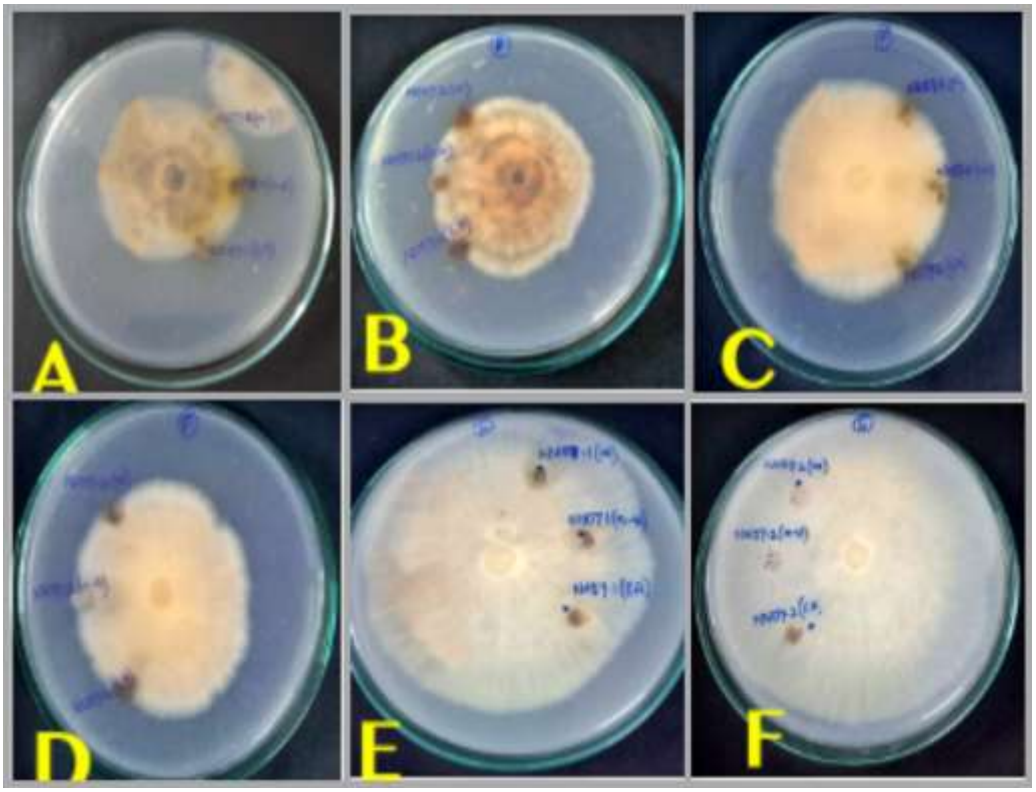


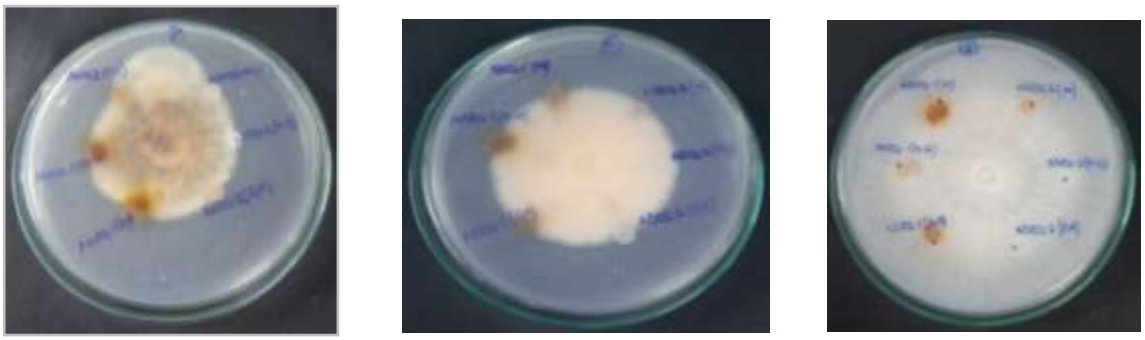

**Fig 3:-** Zone of Inhibition in (mm) of different stem extracts.

**Plate-I:** Antifungal activity of different solvent extracts of *Dendrophthoe falcata.*



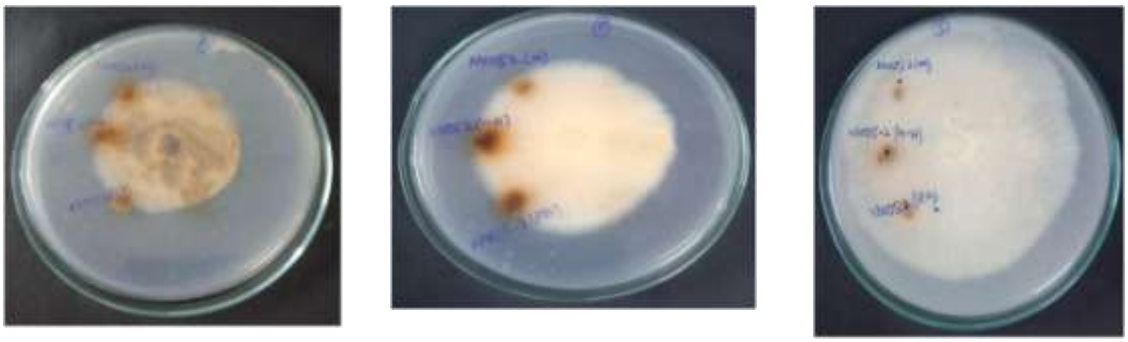

A &B *P.infestans;* C&D *F.oxysporum*; E&F *S.rolfisii*

**Plate-II:** Antifungal activity of different solvent extracts of *Dendrophthoe falcata var. pubescence*



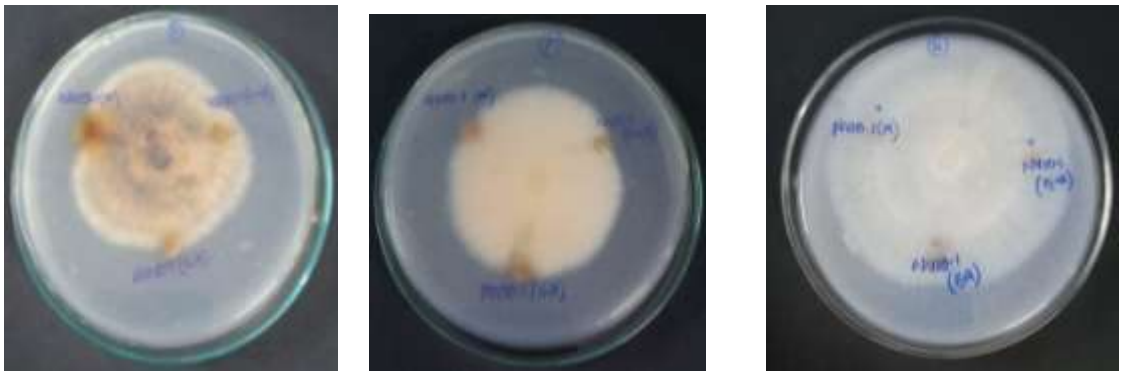
*P.infestans* *F.oxysporum* *S.rolfisii*

**Plate-III:** Antifungal activity of different solvent extracts of *Viscum articulatum.*



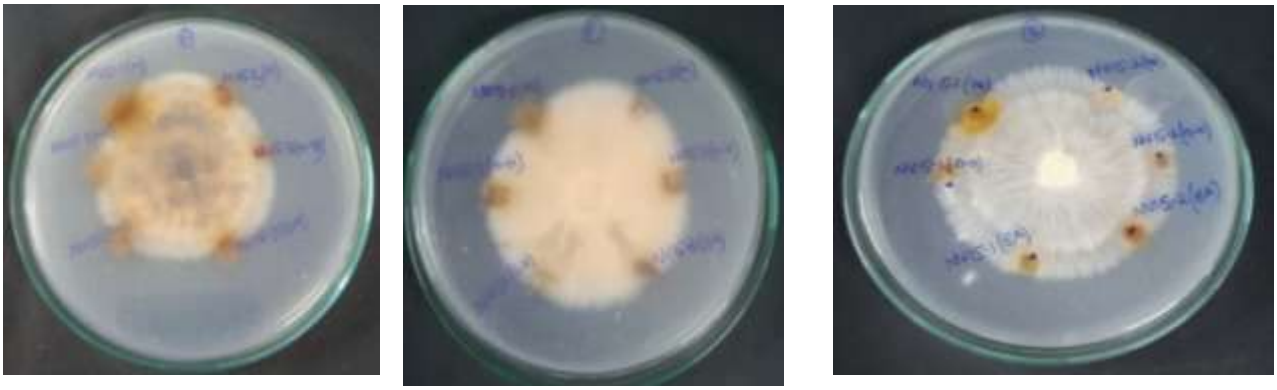
*P.infestans* *F.oxysporum* *S.rolfisii*

**Plate-IV:-** Antifungal activity of different solvent extracts of *Viscum monoicum* .



*P.infestans* *F.oxysporum* *S.rolfisii*

**Plate-V:** Antifungal activity of different solvent extracts of *Viscum orientale*



P.infestans F.oxysporum S.rolfisii

## Discussion:-

The present investigation indicates that antifungal activity was more pronounced with methanol extracts of the test species compared to ethyl acetate extracts. Methanol leaf and stem extracts of *V.orientale* found to be very efficacious against the three fungal isolates. Common to most *Viscum* species, the occurrence of Viscotoxins might have conferred on its antifungal property (Guidiciet al. 2004) (Szurpnickaet al. 2020). Other test species in the present study exhibited moderate to high antifungal activity against *F. oxysporum* and *P.infestans* but failed to exert any activityagainst the fungal isolate *S. rolfsii*. On the whole, *D. falcata var. pubescens* and *V. articulatum* were least performers against the three fungal isolates while stem extracts of *D.falcata* and leaf extracts of *V.monoicum* exerted moderate antifungal activity against *F. oxysporium* and *P. infestans*. (Pattanayaket al 2008)(Priya and Neelamegam 2016)

Relatively, fungi had been known to equip with higher permeability barriersbecause of its intricate and complex cell structure than bacteria, conferring higher resistance to the tested extracts. Earlier reports also support the negligible antifungal activity of several parasitic plants (Osadebe 2008).

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