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### ***In-vitro* antifungal activity of *Naravelia zeylenica* DC (Linn).**

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#### **ABSTRACT**

*The Present Investigation was focused on the antifungal activity of Naravelia zeylenica DC (Linn)., Plant Extract with different organic solvent for aqueous, ethanol, ethyl acetate, chloroform, petroleum ether, this solvent against pathogenic fungus namely Acmeilaclavatus, Fusarium oxysporum, alternaria solani, aspergillus niger, aspergillus flavus. The agar disc – diffusion methods was used to determine the inhibitory effect of test plant. The plant showed inhibitory effect on test organisms. The extract of naraveliazeylenica, zones of inhibition against on fungal organisms. The ethyl acetate, extract was found to be more effective against Fusarium oxysporum, alternaria solani, aspergillus niger, other organic solvent plant extract showed moderate effect against the test organisms.*

**Key words:** Antifungal activity, *Alternaria solani* and ethyl acetate.

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#### **INTRODUCTION**

India has about 45,000 plant species and among them, several thousands have been claimed to possess medicinal properties against human diseases. Crude extracts of some well-known medicinal plants are used to control some of the plants pathogens [1]. Medicinal plants represent a rich source of antimicrobial agents [2] and natural antioxidants. Many of the plant materials used in traditional medicines are readily available in rural areas at relatively cheaper than modern medicines. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs [3]. *Naravelia zeylanica* DC (Ranunculaceae) is a woody climber with tuberous roots; opposite, ovate, cordate leaflets; small flowers arranged in panicles and red coloured achenes along with long feathery styles [4]. The plant occurring wild in the warm regions of Eastern Himalayas, Assam, Bengal, Bihar and greater parts of Deccan Peninsula in India [5] *Naravelia Zeylanica* has been extensively used in Ayurveda as an astringent, bitter, antipruritic and anti-inflammatory. It is also used in pitta helminthiasis, dermatopathy, leprosy, rheumatism, odontalgia, cephalalgia, colic inflammation wound healing & ulcer protection. The root and stem have a strong penetrating smell and is used to relieve malarial fever and headache. Root and stem paste is applied externally for psoriasis, itches and skin allergies [6]. *N. zeylanica* is used as a source of drug for intestinal worms, skin disease and toothache particularly in Kerala. The traditional medicinal practitioners residing in the vicinity of Bhadra wild life sanctuary, Karnataka are using the leaf and stem juices for treating psoriasis & dermatitis [7].

The present work focused on evaluating the anti-fungal activity of aerial part of *Naravelia zeylanica* to revivalist folk claiming leaf.

## MATERIALS AND METHODS

### Plant collection and preparation:

*Naravelia zeylanica* was collected from Kolli hills of Namakkal district, Tamilnadu, India. The plant was authenticated by [4]. The aerial part of leaf collected, dried and shade. These dried plant mechanically powdered and stored in air tight container these powdered used for further anti-fungal activity. Antimicrobial Activity (Disc Diffusion Method) [8] Microorganisms are collected from (NCBT) National College, (Autonomous) Department of Biotechnology at Tiruchirappalli

### PREPARATION OF PLANT EXTRACT

#### Ethanol extract:

5g of plant powder is soaked with 50ml of ethanol and kept for room temperature after 5 days the extract are filtered with the help of Whattman No.1 filter paper.

#### Aqueous extract:

5g of plant powder 50ml of distill water and heated at 40°C for 1 hour, after that is filtered with the help of Whattman No. 1 filter paper. This extract is kept in the refrigerator.

#### Ethyl Acetate extract:

5g of plant powder soaked 50ml of Ethyl acetate, kept for room temperature and after 3 days the extract are filtered with the help of Whattman No. 1 filter paper.

#### Chloroform extract:

5g of plant powder is soaked with 50ml of Chloroform, kept for room temperature and after 48 hours filtered with the help of Whattman No. 1 filter paper. These extracts are kept in the refrigerator.

#### Petroleum ether extract:

5g of plant powder is soaked with 50ml of Petroleum ether, kept for room temperature and after 48 hours filtered with the help of Whattman No. 1 filter paper. These extracts are kept in the refrigerator.

### TEST STRAINS AND FUNGAL SUB-CULTURED

Strain fungi were obtained from NCBT, anti-fungal activity of *Naravelia zeylanica* fungal strain for *Acmellaclavatus*, *Fusariumoxysporum*, *Alterneria solani*, *Aspergillus niger*, *Aspergillus flavus* all are sub cultured by Potato Dextrose Agar (PDA) medium having the following combination in

Peeled potato	-	20g
Dextrose	-	20g
Agar	-	15g
Distilled water	-	1000ml.

Peeled and sliced potato was boiled in 500ml of distilled water for 30 minutes and the extracts was obtained by filtering through, a muslin cloth. To extract, 20g of dextrose was added. At the same time 15g of agar was melted in other half of the distilled water. These two 500ml were mixed well and autoclave.

Inoculated plates were incubated at 27.2°C for 24 hours. Isolation and identification of the bacteria was subsequently done to study the antibacterial activity of *N. zeylanica*.

### SCREENING FOR ANTI FUNGAL ASSAY

#### Antifungal activity test

Antifungal activity was screened by disc diffusion methods (DDM) [8] The Ethanol , Aqueous , Ethyl Acetate , Chloroform , Petroleum ether extracts of *Naravelia Zeylanica* plant leaf were tested plants pathogen fungus. The PDA medium was poured in to the sterile petriplate and allowed to solidify. The test fungal culture was evenly spreader over the media by sterile cotton swabs. Then wells (6 mm) were made in the medium using sterile cork borer. 200µl of each extracts were transferred in to the separate wells. The plates were incubated at 27°C for 48-72 hrs. After the incubation the plates were observed for formation of clear incubation zone around the well indicated the presence of antifungal activity. The zone of inhibition was calculated.

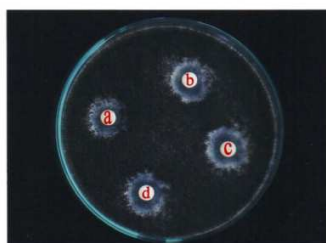
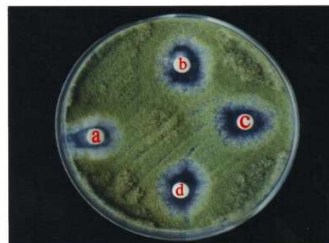
## RESULTS AND DISCUSSION

Effect of antifungal activity of medicinal plant against *Naravelia zeylanica* the results of antifungal property of *Naravelia zeylanica* were given in Table: 1 and Plate: 1

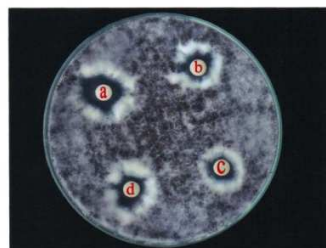
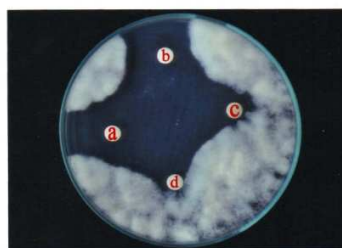
The ethyl acetate extract showed higher antifungal activity with *Alternaria solani*, *Aspergillus niger* and *Fusarium oxysporum* and moderate effect against *Aspergillus flavus* and no activity against *Acmella clavatus*. Ethanol extract showed moderate inhibitory effect against *Alternaria solani*, *Aspergillus niger* and *Fusarium oxysporum* and no activity against *Aspergillus flavus* and *Acmella clavatus*.

## Plate - 1

## CHLOROFORM EXTRACT

*Acmella clavatus**Aspergillus Flavus*

## ETHYL ACETATE EXTRACT

*Fusarium oxysporum**Alternaria solani**Aspergillus niger*

## Plate - 1

a - 25%

b - 50%

c - 75%

d - 100%

**Chloroform extract** showed medium effect against *Acmella clavatus*, *Aspergillus flavus* and *Alternaria solani* and no activity against *Fusarium oxysporum* and *Aspergillus niger*.

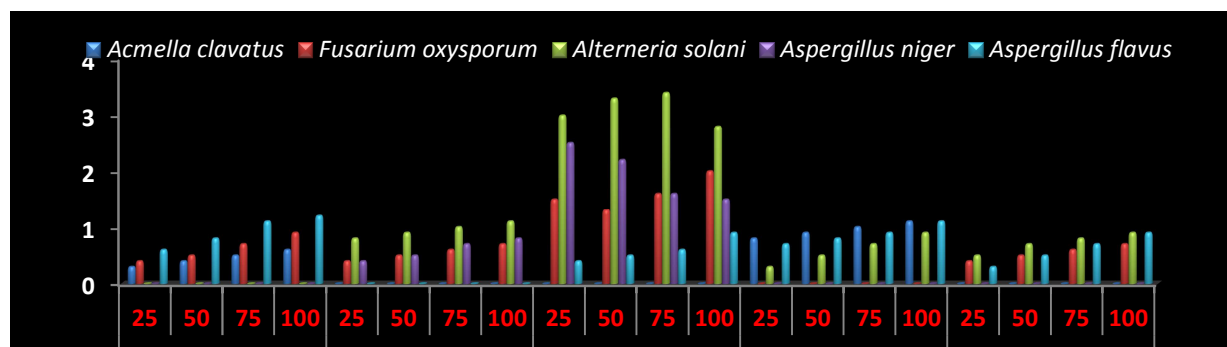
**Petroleum ether extract** showed moderate activity against *Fusarium oxysporum*, *Alternaria solani* and *Aspergillus flavus* and no activity against *Acmella clavatus* and *Aspergillus niger*.

**Aqueous extract** showed high rate of inhibitory activity against *Aspergillus flavus* and moderate activity against *Acmella clavatus* and *Fusarium oxysporum* and no activity in *Alternaria solani* and *Aspergillus niger*.

The results of the inhibition of fungal radial mycelial growth and MIC value are summarized. It appeared that the crude extract of *N. zeylanica* inhibited radial mycelial growth of the entire test fungal at a concentration of 100 µg/ml medium to varying degrees from 25 to 100 these indicators that ethyl acetate extract of *N. zeylanica* could be used as an eco-friendly antifungal against in the control of fungal diseases. Antifungal activities of other plants have also been reported by [9 - 13]. Natural products from plants are known to control some infectious disease; the use of plant secondary metabolites for treatment of fungal disease has received less attention. The finding of active principles may be interesting in the search for new efficacious and safe antimicrobial agent against a variety of pathogenic fungal our result could stimulate further pharmacological studies seeking new antimicrobial agent from the plant resources. The present investigation conform that there are antifungal properties in the crude extract of *N. zeylanica*.

**Table; 1 Zone of Inhibition Spectrum of the Medicinal Plant against *Naravelia zeylanica***

S. No	Organisms	Diameter of the zone inhibition extract concentration (µg/ml) cm values																			
		Aqueous				Ethanol				Ethyl acetate				Chloroform				Petroleum ether			
		25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
1	<i>Acmella clavatus</i>	0.3	0.4	0.5	0.6	-	-	-	-	-	-	-	-	0.8	0.9	1.0	1.1	-	-	-	-
2	<i>Fusarium oxysporum</i>	0.4	0.5	0.7	0.9	0.4	0.5	0.6	0.7	1.5	1.3	1.6	2.0	-	-	-	-	0.4	0.5	0.6	0.7
3	<i>Alternaria solani</i>	-	-	-	-	0.8	0.9	1.0	1.1	3.0	3.3	3.4	2.8	0.3	0.5	0.7	0.9	0.5	0.7	0.8	0.9
4	<i>Aspergillus niger</i>	-	-	-	-	0.4	0.5	0.7	0.8	2.5	2.2	1.6	1.5	-	-	-	-	-	-	-	-
5	<i>Aspergillus flavus</i>	0.6	0.8	1.1	1.2	-	-	-	-	0.4	0.5	0.6	0.9	0.7	0.8	0.9	1.1	0.3	0.5	0.7	0.9



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