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Research Article

ANTIFUNGAL PROPERTY OF *NARAVELIA ZEYLANICA (*L.) DC AGAINST STORAGE PATHOGENS OF GINGER (*ZINGIBER OFFICINALIS*)

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ABSTRACT

Presently there has been an increased interest in using herbal extracts and their phytocomponents as an alternative to control plant pathogenic microorganisms. Using agar cup diffusion method, different extracts of *Naravelia zeylanica* were evaluated for their potential antifungal activity against five phytopathogenic fungi, commonly involved in the post harvest diseases of Ginger. Among all the tested extracts, chloroform extract exhibited promising antifungal activity with maximum inhibition zones of 39.67mm followed by methanol, acetone and petroleum ether extract. Water extract was inactive against all the fungi except *Aspergillus niger*. Minimum inhibitory concentration (MIC) values for the most active extracts determined by broth macro dilution technique found ranging between 1.56-3.12 mg/ml. In an approach towards development of eco-friendly antifungal control strategy, the obtained results hints on an existing potential of *N. zeylanica* extracts in the control and management of post harvest fungal pathogens of Ginger.

Key words: Ginger, *Naravelia*, *Aspergillus*, MIC.

INTRODUCTION

Ginger (*Zingiber officinale Rosc*) (Family: *Zingiberaceae*) is an herbaceous perennial, the rhizomes of which are used as a spice. It has also been traditionally used as antimicrobial, antioxidant, anti-inflammatory agents. India is the leading producer of ginger in the world. Over the last few years, rhizome diseases have affected the crop in many states of India resulting in decline of rhizome yield from 1:8 ratios (seed rhizome to harvested rhizome) to 1:4. Fungi are significant destroyers of foodstuffs and grains during storage, rendering them unfit for human consumption by retarding their nutritive value and often by producing mycotoxins.[1,2] A huge amount of ginger is affected by fungi in storage condition despite of its own antifungal property[3]. It is reported that more than fifteen fungi are responsible for rhizome rot of ginger all over the world. *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Monilia* sp, *Penicillium* sp. etc. are among the fungi, which can be controlled by using different types of chemical fungicides. As these synthetic chemicals have adverse effects on environment as well as on human health due to non judicious and inappropriate application, the search for some alternatives from natural sources like medicinal plants to protect the crop (ginger) from fungal agents is the urgent need of the day[4, 5].

In India there are more than 2600 plant species of which not less than 700 are noted for their uses as medicinal herbs[6] and the Northeast India is considered as the store house of medicinal plants and often referred to as biodiversity hotspot[7, 8]. Some plants and plant parts having medicinal value are used to reduce the activity of fungi to a great extent[9]. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not been adequately evaluated[10], it has been proved that ethanol and methanol extracts of *Tecoma stans* were highly effective against many pathogenic bacteria *and* pathogenic fungi belongs to all species of *Aspergillus* and *Alternaria.*

Naravelia zeylanica DC. belongs to the family Ranunculaceae is a woody climber distributed in Indo-Malaysian region[12] and is largely used as medicine from the ancient period. In Ayurveda, the plant has been used as an astringent, bitter antipyretic and anti-inflammatory agent. It is also useful in pitta, dermatopathy, leprosy, rheumatic problem, and wound healing and ulcer protection[13]. The traditional medicine practitioners use the leaf and stem juices for treating the internal worms, psoriasis and dermatitis[14].

The present study has been undertaken to screen the *in vitro* efficacy of the leaves of *N. zeylanica* grown in Assam, a North Eastern state of India, against some common fungal pathogens of Ginger and to determine the minimum inhibitory concentrations (MIC) by using methanol, chloroform, acetone, petroleum ether and water extracts of the plant.

MATERIALS AND METHODS

Sample collection

Healthy and infected rhizome of Ginger samples were collected from different markets of Lakhimpur, Kamrup and Goalpara districts of Assam, India in the months of May and June, 2011.

Isolation and Identification

Isolation of fungi has been done according to different prescribed methods[15,16]. Identification has been confirmed later by observing their morphological characteristics under the microscope and also with the help of available literature[17-19].

Pathogenicity test

All the isolated pathogens have been subjected to pathogenecity test as described by Koch (1882).

Plant Material and Extract Preparation

Leaves of *N. zeylanica* was collected from Kamrup district, Assam in the month of March, 2011, authenticated by a Taxonomist, Dr. G.C. Sharma from Gauhati University and herbarium was prepared and deposited as voucher specimen (IASST/MEP/H No. 36/07) in the Medicinal Plant and Biochemistry Laboratory of IASST for future reference.

Freshly collected plant material was dried under shade and 100 gm of the fine dried powdered material was then extracted separately with 1000ml each of methanol, chloroform, acetone and petroleum ether in an extractor at room temperature. The extracts were concentrated in a rotary evaporator (Buchi R 124, German) at $\leq 40^{o}$ C, the yield of extracts were 4.50, 3.75, 4.15 and 2.50 gm respectively. For

the aqueous extract, 100 gm of powdered plant materials were heated in 1000ml water for an hour in a water bath at 35°C, filtered and finally lyophilized to dryness; it yielded 6.0 gm of extract. The plant extracts were dissolved in Dimethyle Sulfoxide (DMSO) accordingly to reach the concentrations of 20mg/ml, 10 mg/ml, and 5mg/ml. All the extracts were kept in refrigerator at 4°C for future use.

Preparation of fungal inoculums

The organisms isolated from ginger were sub cultured and maintained in Potato dextrose agar slants at 4°C. For the experiment, freshly cultured slants were used for preparing spore suspension in 0.9% saline water. The fungal spore suspension was adjusted to give a final concentration of 1-5x10^5 cfu/ml.

Antifungal activity

The *in-vitro* antifungal screening was done using agar cup diffusion method[20]. 200µl of fungal spore suspension was uniformly spread over solidified Potato Dextrose Agar (PDA) (Himedia) plates with a sterilized spreader. With the help of sterilized cork borer wells of 6mm diameter were made in the centre of the agar plates. The wells were filled with 200µl of the respective plant extracts and allowed to diffuse at room temperature for an hour. The plates were then incubated at 28 ± 2°C for 48 -72 hours depending upon the growth rate of the fungal pathogen. The antifungal activities of the extracts were determined by measuring the diameter of the inhibition zone around the wells which were filled by the extracts. Carbendazim (1mg/ml) and DMSO (2%) were used as positive and negative control respectively.

Minimum Inhibitory concentration (MIC) of all the extracts was determined by the visual broth macro dilution method[21] using Potato Dextrose broth medium. The minimum concentration of the extract that exhibited no visual growth of the fungus is considered as the MIC value.

RESULTS

The results of the *in-vitro* antifungal activity of methanol, chloroform, acetone, petroleum ether and water extracts of leaves of *N. zeylanica* against *Fusarium oxysporum* (Fo) *Aspergillus niger* (An), *Aspergillus flavus* (Af), *Monilia* sp (Ms) *and Penicillium* sp (Ps) are presented in Table 1. At 20mg/ml concentration, the chloroform extracts showed maximum antifungal activity with inhibition zones of 42, 40, 31, 26 and 26mm respectively against *A. niger*, *Penicillium* sp, *A. flavus*, *F.oxysporum* and *Monilia* sp. Methanol extract was most active *against A. niger* (32mm), followed by *Penicillium* sp. (27mm), *A. flavus* (24mm), *Monilia* sp. (20mm) and *F.oxysporum* (17mm). Acetone extract produced inhibition zones of 21, 18, 17, 13 and 8mm respectively against *A. niger*, *Penicillum* sp., *A. flavus*, Monillia sp and *F.oxysporum*. For the petroleum ether extract, *A. niger* (16mm), was most susceptible followed by *Penicillum* sp.(12mm), *A. flavus* (11mm), *Monilia* sp (11mm) and *F.oxysporum* (6mm). The water extract produced weak inhibition only against *A .niger* whereas it was completely inactive against the rest tested fungi. Minimum inhibitory concentration (MIC) of the extracts against all the phytopathogenic fungi was found in between 1.56-12.5 mg/ml (Table 2).

Table 1: Antifungal activity of *N. zeylanica* extracts.

Extracts	Conc. (mg/ml)	Inhibition Zone (mm)				
		A. flavus	*A. niger.*	*F.oxysporum*	*Monilia* sp.	*Penicillum* sp.
AE	20	16.67±0.41	21.00±0.71	08.33±1.08	13.33±1.08	18.33±0.41
	10	10.00±1.41	10.67±0.82	06.33±0.41	07.00±0.71	11.00±0.71
	5	07.00±0.71	06.33±0.41			09.00±0.71
CE	20	31.00±0.71	41.67±1.08	26.00±0.58	25.67±0.41	39.67±1.08
	10	21.00±0.71	25.67±1.08	16.67±0.41	14.67±1.08	24.00±1.41
	5	12.00±0.71	18.33±0.41	11.33±0.82	09.67±1.08	16.00±0.71
ME	20	24.00±0.71	32.00±0.71	17.00±0.71	20.00±0.71	27.33±0.82
	10	15.67±0.82	20.00±0.71	10.67±1.08	12.33±0.41	16.67±0.41
	5	09.00±0.71	10.33±0.41	08.00±0.71	08.00±0.71	09.00±0.71
PE	20	11.00±0.71	15.67±1.08	06.33±0.41	11.00±0.71	12.33±0.41
	10	06.33±0.41	10.33±0.41	_	06.33±0.41	07.67±0.41
	5	_	06.33±0.41	_	_	_
WE	20	_	04.00±0.71	_	_	_
	10	_	_	_	_	_
	5	_	_	_	_	_
Carbendazim	1mg/ml	19.67±1.08	20.00±0.71	21.33±0.41	16.33±0.82	36.00±0.71
DMSO		_	_	_	_	_

CE-Chloroform Extract, ME-Methanol Extract, AE-Acetone Extract, PEE-Petroleum Ether Extract, WE-Water Extract, (-)- no inhibition zone.

Table 2: Minimum inhibitory concentration of the plant extracts (mg/ml).

Fungal strains	Minimum inhibitory concentration (mg/ml)				
	CE	ME	AE	PEE	WE
Penicillum sp	1.56	3.12	6.25	6.25	-
Fusarium. oxysporum	3.12	6.25	12.5	12.5	-
Aspergilus niger	1.56	1.56	3.12	6.25	12.5
Aspergilus flavus	1.56	3.12	6.25	6.25	-
Monilia sp	3.12	3.12	6.25	6.25	-

CE-Chloroform Extract, ME-Methanol Extract, AE-Acetone Extract, PEE-Petroleum Ether Extract, WE-Water Extract, (-) - MIC >12.5.

DISCUSSION

Pre and post harvest bio-deterioration and spoilage of grains, vegetables, fruits and agricultural products due to infestation by insects and microorganisms may cause losses of up to 100%, whereas pathogenic fungi alone causing nearly 20% reductions in the yield of major crops[22]. Additionally serious problems in the environment and the toxic effects of synthetic chemicals on non-target organisms have led to the investigations of exploiting pesticides of plant origin [23]. The use of plant products as bio-control agents for the management of plant diseases have achieved greater significant in recent times due to its readily available nature, antimicrobial activity, easy bio-degradability, non-phytotoxicity and are the sources of thousands of useful

phytochemical of great diversity, which have inhibitory effect on all types of microorganisms besides inducing resistance in host.

In the present investigation, antifungal activity of *Naravelia zeylanica* extracts was recorded against all the isolated fungal pathogens of ginger. The antifungal activity was recorded higher in chloroform and methanol extracts compared to acetone and petroleum ether extracts. Water extract however exhibited weak activity only against *A. niger*. Based on the *in vitro* observations, the activity of the extracts can be arranged as - Chloroform > methanol > acetone > petroleum ether > water. Plants contain alkaloids, tannins, quinones, coumarins, phenolic compounds, phytoalexins and others, which are known for antifungal activity. N. *zeylanica* also contain some of the important compounds having antifungal activity[24].

Previously, many extracts and essential oils from plants have been reported to have broad spectrum antifungal activity against plant pathogenic fungi. It was reported that the growth of *Fusarium oxysporum* mycelium can be inhibited by using cold extract of *Nicotiana tabacum*[25] and the growth and sporulation of fungal pathogens on sweet potato and yam could be able to reduced by using garlic (*Allium sativum*)[26]. Biologically active plant derived pesticides are expected to play an increasingly significant role in crop protection. Exploitation of naturally available chemicals from plants, which retards the reproduction of undesirable microorganisms, would be a more realistic and ecologically sound method for plant protection and will have a prominent role in the development of future commercial pesticides for crop protection strategies, with special reference to the management of plant diseases[27, 28].

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