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PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF *ZINGIBER OFFICINALE*.L

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ABSTRACT:

The objectives of the present study was to highlight the use of medicinal plants treating numerous diseases of plants with different etiologies throughout the history. The main aim of this research paper is to determine the in vitro antifungal activity of zingiber rhizome extract against some isolated fungi viz., *F. oxysporum, F. solani, F. moniliforme, H. sativum, C. lindemuthianum, C. lunata, R. solani, A. solani* from different vegetables. Both the aqueous and alcoholic extracts of zingiber were screened for the phytochemical constituents and were tested against fungal pathogens. Result of phytochemical screening, revealed the presence of tannins, alkaloids, saponins, flavonoids, steroids and cardiac glycosides. The aqueous extract exhibited less fungitoxic activity as compared to methanolic extracts. The methanol extract of *Z. offinale* was also effective against all the pathogens with highest inhibition of mycelial was recorded in *A. solani* with 86.41% and lowest by *C. lunata* with 73.99% inhibition of mycelial growth.

KEYWORDS: Medicinal Plants, Phytochemical Screening, Fungal Pathogens, Aqueous extract, Methanolic Extract.

INTRODUCTION:

Agricultural practices have been great concern by using chemicals as management of plant diseases. These chemicals in addition kill various beneficial organisms and their toxity can persist in the soil (Onuegbu, 2002). Excessive use of synthetic pesticides has been implicated in their negative effects on the environment such as soil and water pollutions, long periods of degradation, residual accumulation in the food chain, and less control efficacy against pathogenic microbes with long-term usage (Nega, 2014; Bhavaniramya et al., 2019). The increasing resistance by these microorganisms against these chemicals has been a great concern. Among the various alternatives, natural plant products are used having no side effect and are been used by scientists (Okigbo, 2009). Extracts obtained from these valuable plants have gained attention as scientific interest for having antifungal activity (Santas et al., 2010). Plant secondary metabolites are of low-molecular weight compounds that are not important for sustaining life, but are essential for the survival of the producing organism (Hadacek, 2002). Ginger *(Zingiber officinale*) is a medicinal plant that has been widely used all over the world for various purposes. *Zingiber officinale* commonly known as Ginger, belongs to Zingiberaceae family. Ginger has been valued for its antibacterial properties for thousands of years in Asian cultures. Plant extracts of many higher plants have been reported to exhibit antifungal properties under laboratory trials (Okigbo and Ogbonnaya, 2006). Volatile phytochemical composition of rhizome of ginger were reported

(Prakash et al., 1993; Meepagala et al., 2002; Bhuiyan et al., 2008), and their main composition is Zingiberene and its derivatives, and compounds of pharmacological activity of ginger are gingerols and its derivatives.

The aim of this study was to summarize the actions and application of ginger and its active constituents against fungal pathogen of vegetables.

MATERIAL AND METHODS:

The plant extracts were evaluated *in vitro* through Poison food technique (Nene and Thapliyal, 2000). The supernatant was taken as standard plant extract solution (100%). Further, the extract was diluted by adding sterilized water to get different per cent concentrations. The plant extracts were subjected to boiling temperature of 50°C in water bath to avoid contamination and then incorporated into PDA media by transferring 2ml of each type of plant extract in to a Petridish containing 20 ml melted warm PDA medium and gently shaken for thorough mixing of the extract. The PDA plates containing the plant extracts were inoculated aseptically with different pathogens by transferring 6mm diameter agar disc of 07 days old culture of the pathogen to the centre of PDA medium in Petridish. Three replications were maintained for each treatment. The basal medium (PDA) without any phytoextract served as control. All the inoculated Petridishes were incubated at 25±1°C.The radial growth of the test fungus in the treated plates was measured in all treatments when the pathogen growth touched the periphery in the control Petridishes. The per cent inhibition of fungal growth was estimated by using the formula given by Vincent (1927).

Phytochemical analysis of crude plant extracts:

Chemical test were carried out using aqueous and methanol extract to identify various constituent using standard method (Trease and Evans.,1989).

Test for Tannins:

About 2ml of the extract was stirred with 2 ml of distilled water and few drops of $FeCl_3$ solution were added. Formation of green precipitate was indication of presence of tannins.

Test for Alkaloids:

3 ml extract was stirred with 3 ml of 1% HCl on steam bath. Mayer and Wagner's reagent was then added to mixture. Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloid.

Test for Saponins:

5 ml of extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

Test for Cardiac glycosides:

Keller-Kiliani test:

Plant extract treated with 2 ml glacial acetic acid containing a drop of FeCl3. A brown colour ring indicates the presence of positive test.

Test for Steroids:

1 ml extract was dissolved in 10 ml of chloroform and equal volume of concentrated H_2SO_4 was added from the side of test tube. The upper layer turns red and H_2SO_4 layer showed yellow with green fluorescence. This indicates the presence of steroids.

Test for Flavonoid:

To 1 ml of extract, 1 ml of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for flavonoids.

RESULTS:

Table 1. Phytochemical analysis of Zingiber officinale. L

Phytochemical constituents	Methanol extract	Aqueous extract
Carbohydrates	P	P
Glycosides	P	P
Anthraquinone	P	P
Cardiac glycosides	P	P
Saponins	P	P
Steroids and Triterpenes	P	P
Flavanoids	P	P
Tannins	P	A
Alkaloids	A	P

Key: P = Present A = Absent

Table 2: Inhibitory effect of plant extracts of different concentrations on mycelial growth of fungal pathogens of vegetables.

Pathogens/Medicinal Plant	Mycelial growth and percent inhibition	*Zingiber officinale*.L	
		H_2O Extract	MeOH Extract
A. solani	Mycelial Growth (mm)	30.6	10.6
	% Inhibition over control	51.06±1.95	86.41±1.43
C. lindemuthianum	Mycelial Growth (mm)	33.3	13.3
	% Inhibition over control	62.70±0.71	83.07±0.71
C. lunata	Mycelial Growth (mm)	48.3	22
	% Inhibition over control	45.57±0.71	73.99±0.94
F. moniliforme	Mycelial Growth (mm)	47.3	21.3
	% Inhibition over control	47.20±0.97	76.22±1.43
F. oxysporum	Mycelial Growth (mm)	63.3	20.3
	% Inhibition over control	29.35±1.65	76.33±1.08
F. solani	Mycelial Growth (mm)	37.3	21.3
	% Inhibition over control	58.37±0.97	83.90±0.97
R. solani	Mycelial Growth (mm)	40.6	12.6
	% Inhibition over control	54.53±1.78	85.89±1.18
H. sativum	Mycelial Growth (mm)	47.3	21.3
	% Inhibition over control	85.19±0.97	76.22±1.18

Value expressed in mean ± S. E. M of triplicates

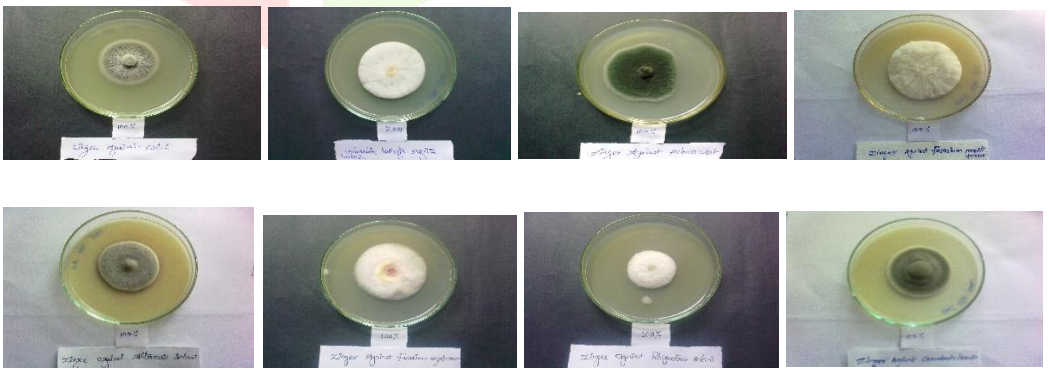

Fig: Antifungal activity of aqueous extract against eight targeted fungal pathogens

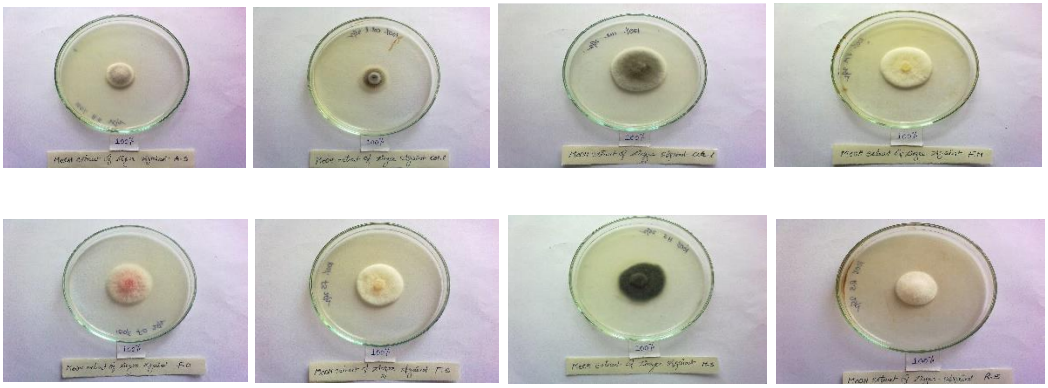

Fig: Antifungal activity of Methanolic extract against targeted fungal pathogens.

Phytochemical screening of Methanolic plant extract showed presence of flavanoids, glycosides, saponins, tannin and terpenoids with the absence of alkaloid.

From the above results, it is clear that the methanol extract of *Z. offinale* was effective against all the pathogens with highest inhibition of mycelial was recorded in *A. solani* with 86.41% and lowest by *C. lunata* with 73.99% inhibition of mycelial growth. Aqueous extract shows averagely 54.25% inhibition against all pathogens, while methanol extract of *Z. officale*, was significantly effective and revealed 80.25% inhibition of mycelial growth of all eight tested pathogens.

DISCUSSION:

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural resources. Natural products perform various functions, and many of them have interesting and useful biological activities. Approximately 25 to 50 % of current Pharmaceuticals are derived from plants (Upadhyay *et al*, 2011). Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries (Kamali and Amir, 2010).The aqueous extract exhibited less fungitoxic activity as compared to methanolic extracts. It could also be due to incomplete extraction of the active principles (El-Mahmood *et al.*, 2008). The present results are in accordance with Pinelo *et al.* (2004), who suggested that the chemical characteristics of the solvent, the method used during the extraction process and diverse structural and compositional aspects of the natural products result in each material-solvent system showing distinct behavior.

Results of this work suggest that fungitoxic compounds are present in Z. officinale extracts since they were able to control the growth of the fungal pathogens tested. This is in agreement with the work of Udo et al. (2001) who worked on the inhibition of growth and sporulation of fungal pathogens in Ipomoea batatas and Dioscorea sp by garlic extracts. The antimicrobial activity of these plants also agrees with the work of Adejumo and Langenkamper (2012), which showed that methanolic extracts of leaves of botanicals possessed antimicrobial properties. Also Okigbo amd Nmeka (2005) used leaf extracts of Xylopia aethiopica and Z. officinale to control yam tuber rot caused by Aspergillus niger, Aspergillus flavus and Fusarium oxysporum. A. melegueta extract was also used by Okigbo and Ogbonnaya (2006) in the control of F. oxysporum and A. niger rot in yam tubers.

From this result, it is essential to investigate the specific constituents which are responsible for this observed activity. The in vivo study is also required to confirm the usefulness of the obtained results.

CONCLUSION:

In conclusion, the in vitro results of this study confirmed the potentiality of *Z. officinale* as one of the best sources for controlling fungal growth. Hence, further work is necessary to evaluate its potentiality in vivo on targeted pathogens. This can provide an alternative means for the control of vegetable diseases by farmers. Investigations are also needed to characterize, formulate and market the active principles of these extracts which may provide avenues for the discovery of novel antifungal compounds. These biofungicidal botanicals are environmentally safe; therefore, they could successfully replace the toxic and hazardous synthetic compounds and be exploited as ideal treatment for future plant disease management programs.

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