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EVALUATION OF ANTIFUNGAL ACTIVITY OF SOLVENT EXTRACTS OF *PICRORHIZA KURROA* RHIZOME ON SEED BORNE FUNGI OF MAIZE

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ABSTRACT

Antifungal activity three solvent extracts viz., methanol, petroleum ether and chloroform extract of *P. kurroa* rhizome was tested at 100, 250, 500, 750, 1000, 1250, 1500, 1750 and 2000 ppm concentration respectively against ten species of fungi viz., *Fusarium graminearum*, *F. oxysporum*, *Aspergillus flavus*, *A niger*, *A. terreus*, *Penicillium* sp, *Curvularia lunata*, *Drechslera halodes*, *Alternaria alternata* and *Cladosporium cladosporides* isolated from maize seeds. Maximum activity was observed in methanol extract and six fungi were completely inhibited at 1500 to 2000 ppm concentration. Methanol extract was followed by petroleum ether extract and three fungi were completely inhibited at 2000 ppm concentration. Least inhibition was observed in chloroform extract and none of the test fungi were inhibited completed at different concentration of rhizome extract. All the result were compared with two synthetic fungicides Dithane M-45 and Thiram and all the test fungi were completely inhibited at 2000 ppm concentration.

KEYWORDS: *Picrorhiza kurroa*, Fungi, Solvent extract, Antifungal activity, Synthetic fungicides.

INTRODUCTION

Plants have been an essential part of human society since the civilization started, as a source of food, shelter, medicine, etc. and now a day's becoming a source of natural poisons .[1] It has been established that up to 25% of the drugs prescribed in conventional medicines are allied directly or indirectly to natural substances mostly of plant origin,[2] The search for naturally occurring materials with biological activity and use of naturally occurring antifungal substances in plant chemotherapy is gaining more importance. Secondary products which are considered as final products of plant metabolism or metabolite refuses have important ecological functions for the plant which synthesize them. One of these functions is to protect the plants against infections/invasions by pathogens ,[3] Each year, pests reap a tremendous harvest of the food intended for animal production and human sustenance. Numerous attempts have been made to quantify these losses on a worldwide basis. The use of chemical sprays, dust or seed treatment for protecting plants from the ravages of the pathogens is not an innovation of the 20th century. Synthetic fungicides have effectively controlled plant diseases for a number of years, but increasing concern over environmental effects of the currently used fungicides has highlighted the need for the development of alternative types of selective control or of methods of crop protection with or without reduced use of conventional fungicides,[4] It has been estimated that hardly 0.1% of the agrochemicals used in crop protection reach the target pest leaving the remaining 99.9% to enter the environment to cause hazards to non-target organisms including humans,[5] Plant kingdom represents an extra-ordinary reservoir of novel molecules. Among them higher plants is a treasure house of phytochemicals which serves as valuable drugs that have helped combat several fatal diseases world over. The reliance on the use of indigenous medicine plants has a long history and a number of plants used in traditional medicine have been reported to process antimicrobial activity,[4,6] Hence in the present study, different solvent extracts viz., methanol, petroleum ether and chloroform extract of *Picrorhiza kurroa* rhizome belongs to family Scrofulariaceae was investigated for the antimicrobial activity against ten different species of fungi isolated from maize seeds.

MATERIALS AND METHODS

Test Plant

Healthy rhizome of *P. kurroa* were collected from the market, Mysore. The rhizome was washed two to three times thoroughly with running tap water and once with sterile distilled water and air dried. The dried samples were preserved for further use.

Solvent extraction

Completely dried rhizome of *P. kurroa* were powdered. 25 grams of fine powder of seeds of *P. kurroa* was filled in the thimble and extracted successively with petroleum ether, chloroform and methanol for 48 hours. The obtained solvent extracts were concentrated in rotary flash evaporator. The concentrated extracts were preserved in airtight brown bottle until further use,[7]

Isolation of seed borne mycoflora from maize

Standard blotter method was employed for isolation of seed borne biodeterioration causing fungi. Three layers of blotters equivalent to the size of the petridish were soaked in distilled water, the surplus water is drained from the blotters and placed in the lower lid of the petridish. Four hundred seeds of each of the samples were placed on the blotters at the rate of ten seeds per plate. These plates were incubated for seven days at 22 ± 2^{o} C under alternating cycles of 12/12 hours of NUV light and darkness. After the period of incubation the seeds were observed under stereobinocular microscope and the fungi associated with these seeds were identified based on their growth habit, mycelial structure and spore morphology using standard manuals,[8] All the fungi associated with the seeds were isolated and their pure cultures maintained on specific media The fungi were subcultured periodically.

Test fungi

Ten species of fungi viz., *Fusarium graminearum, F. oxysporum, Aspergillus flavus, A niger, A. terreus, Penicillium* sp, *Curvularia lunata, Drechslera halodes, Alternaria alternata* and *Cladosporium cladosporides* isolated from maize seeds were used as test fungi for antifungal activity assay.

Antifungal activity assay of solvent extract by poisoned food technique

One gram of all the three solvent extract viz., petroleum ether extract, chloroform extract and methanol extract was dissolved in 1ml of Dimethyl sulfoxide (DMSO) solvents, Czapek Dox Agar (CDA) medium with different concentration of each of the solvent extracts viz., 100, 250, 500, 750, 1000, 1250, 1500, 1750 and 2000 ppm were prepared. CDA medium amended with the same concentrations of these respective solvents served as control. Five mm mycelial discs from the margins of seven day old cultures of all the test fungi were placed at the center of medium. All the plates were incubated at 36 ±1°C for seven days and ten replicates were maintained for each treatment. The percent inhibition of mycelial growth was determined by the formulae PI = C-T/C×100, where C=Diameter of control colony,

T=Diameter of treated colony,[3] The same procedure was followed for two synthetic fungicides Dithane M-45 and Thiram at 2000 ppm standard recommended concentration. All the data obtained were subjected for statistical analysis.

STATISTICAL ANALYSIS

The data were subjected to Tukey's HSD analysis. Data on percentages were transformed to arcsine and analysis of variance (Anova) was carried out with transformed values. The means were compared for significance using Tukey's HSD (P=0.05).

RESULT

The antifungal activity different solvent extracts of *P. kurroa* rhizome tested against ten species of fungi showed a promising result in methanol extract. Complete inhibition was observed in six fungi. *F. oxysporum* and *A.flavus* were completely inhibited at 1500 ppm concentration. *C. cladosporides* and *F. graminearum* was inhibited at 1750 ppm and showed 60 to 88% inhibition from 1000 to1500 ppm concentration. *A. alternata, A niger , A. terreus, C. lunata, Penicillium* sp. and *D.halodes* were inhibited at 2000 ppm concentration (Table 1). Methanol extract was followed by Petroleum ether extract and recorded maximum inhibition in all the test fungi. Compete inhibition was observed in *C. cladosporides, A. flavus* and *C. lunata* at 2000 ppm concentration. *F. graminearum* recorded 87.0%, *F. oxysporum* recorded 55.0%, *A niger* recorded 89.0%, *A. terreus* recorded 53.0%, *Penicillium* sp. recorded 92.0%, *D. halodes* recorded 80.0% and *A. alternata* recorded 75.0% in 2000 ppm concentration of rhizome extract. Significant activity was observed in all the different concentrations tested (Table 2). In chloroform extract, moderate activity was observed in all the test fungi. At 2000 ppm concentration tested, *F.graminearum* showed 37.0%, *F. oxysporum* (35.0%), *A. flavus* (49.0%), *A niger* (38.0%), *A. terreus* (29.0%), *Penicillium* sp.(34.0%), *C. lunata* (39.0%), *D. halodes* (11.0%), *A. alternata* (41.0%) and *C. cladosporides* (88.0%) inhibition respectively. No complete inhibition was observed in any test fungi tested at different concentration of rhizome extract (Table 3). Compared to synthetic fungicides Dithane M-45 and Thiram, all the fungi were completely inhibited at 2000 ppm concentration.

Table 1: Antifungal activity of Methanol extract of *Picrorhiza kurroa* (rhizomes) against seed borne fungi of maize

Fungi	Methanol Extract									Dithane M 45	Thiram
	Concentration										
	100 ppm	250 ppm	500 ppm	750 ppm	1000 ppm	1250 ppm	1500 ppm	1750 ppm	2000 ppm	2000 ppm	2000 ppm
	Percent Inhibition (%)										
Fusarium graminearum	8.0^{a} ±0.0	19.0^{b} ±0.0	29.0^{c} ±0.0	40.0^{d} ±0.0	61.0^{e} ±0.0	77.0^{f} ±0.0	88.0^{g} ±0.0	100.0^{h} ±0.1	100.0^{h} ±0.0	100.0^{h} ±0.0	100.0^{h} ±0.0
F. oxysporum	10.0^{a} ±0.1	29.0^{b} ±0.0	41.0^{c} ±0.1	60.0^{d} ±0.0	78.0^{e} ±0.1	92.0^{f} ±0.1	100.0^{g} ±0.0	100.0^{g} ±0.1	100.0^{g} ±0.1	100.0^{g} ±0.0	100.0^{g} ±0.0
Aspergillus flavus	14.0^{a} ±0.0	30.0^{b} ±0.0	47.0^{c} ±0.0	63.0^{d} ±0.1	79.0^{e} ±0.0	91.0^{f} ±0.1	100.0^{g} ±0.1	100.0^{g} ±0.0	100.0^{g} ±0.0	100.0^{g} ±0.1	100.0^{g} ±0.1
A niger	5.0^{a} ±0.0	16.0^{b} ±0.0	24.0^{c} ±0.0	32.0^{d} ±0.0	45.0^{e} ±0.1	60.0^{f} ±0.0	71.0^{g} ±0.1	79.0^{h} ±0.0	85.0^{i} ±0.0	100.0^{j} ±0.0	100.0^{j} ±0.2
A. terreus	5.0^{a} ±0.1	10.0^{b} ±0.0	19.0^{c} ±0.1	32.0^{d} ±0.0	40.0^{e} ±0.0	54.0^{f} ±0.1	62.0^{g} ±0.0	71.0^{h} ±0.0	80.0^{i} ±0.0	100.0^{j} ±0.2	100.0^{j} ±0.1
Penicillium sp.	8.0^{a} ±0.0	18.0^{b} ±0.1	33.0^{c} ±0.0	48.0^{d} ±0.0	62.0^{e} ±0.1	75.0^{f} ±0.0	83.0^{g} ±0.1	91.0^{h} ±0.1	100.0^{h} ±0.1	100.0^{i} ±0.0	100.0^{i} ±0.0
Curvularia lunata	4.0^{a} ±0.0	12.0^{b} ±0.0	20.0^{c} ±0.1	31.0^{d} ±0.0	47.0^{e} ±0.0	58.0^{f} ±0.0	69.0^{g} ±0.1	80.0^{h} ±0.1	85.5^{i} ±0.0	100.0^{j} ±0.0	100.0^{j} ±0.0
Drechslera halodes	6.0^{a} ±0.1	16.0^{b} ±0.0	27.0^{c} ±0.1	35.0^{d} ±0.1	51.0^{e} ±0.1	64.0^{f} ±0.0	78.0^{g} ±0.0	87.0^{h} ±0.0	94.0^{i} ±0.0	100.0^{j} ±0.1	100.0^{j} ±0.1
Alternaria alternata	7.0^{a} ±0.1	18.0^{b} ±0.1	34.0^{c} ±0.0	49.0^{d} ±0.0	62.0^{e} ±0.0	76.0^{f} ±0.1	88.0^{g} ±0.1	95.0^{h} ±0.0	100.0^{i} ±0.0	100.0^{j} ±0.0	100.0^{j} ±0.1
Cladosporium cladosporides	10.0^{a} ±0.0	26.0^{b} ±0.1	39.0^{c} ±0.0	53.0^{d} ±0.1	69.0^{e} ±0.0	80.0^{f} ±0.0	92.0^{g} ±0.0	100.0^{h} ±0.0	100.0^{i} ±0.1	100.0^{j} ±0.1	100.0^{j} ±0.0

- Values are the mean of five replicates, ± Standard error.
- The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey's HSD.
- Pattern of percentage inhibition increase is not uniform for all the microorganisms

Table 2: Antifungal activity of Petroleum ether extract of *Picrorhiza kurroa* (rhizomes) against seed borne fungi of maize

Fungi	Petroleum Ether Extract									Dithane M 45	Thiram
	Concentration										
	100 ppm	250 ppm	500 ppm	750 ppm	1000 ppm	1250 ppm	1500 ppm	1750 ppm	2000 ppm	2000 ppm	2000 ppm
	Percent Inhibition (%)										
Fusarium graminearum	4.0^{a} ±0.0	11.0^{b} ±0.0	20.0^{c} ±0.1	32.0^{d} ±0.0	45.0^{e} ±0.0	60.0^{f} ±0.0	71.0^{g} ±0.0	82.0^{h} ±0.1	87.0^{i} ±0.0	100.0^{j} ±0.0	100.0^{j} ±0.0
F. oxysporum	2.0^{a} ±0.1	8.0^{b} ±0.0	17.0^{c} ±0.0	26.0^{d} ±0.0	33.0^{e} ±0.1	40.0^{f} ±0.1	49.0^{g} ±0.0	51.0^{h} ±0.0	55.0^{i} ±0.1	100.0^{j} ±0.0	100.0^{j} ±0.0
Aspergillus flavus	7.0^{a} ±0.0	18.0^{b} ±0.1	34.0^{c} ±0.1	50.0^{d} ±0.1	68.0^{e} ±0.0	78.0^{f} ±0.0	89.0^{g} ±0.0	94.0^{h} ±0.0	100.0^{i} ±0.1	100.0^{j} ±0.1	100.0^{j} ±0.1
A niger	5.0^{a} ±0.0	15.0^{b} ±0.0	28.0^{c} ±0.0	37.0^{d} ±0.0	51.0^{e} ±0.0	64.0^{f} ±0.0	73.0^{g} ±0.1	82.0^{h} ±0.1	89.0^{i} ±0.0	100.0^{j} ±0.0	100.0^{j} ±0.2
A. terreus	2.0^{a} ±0.1	8.0^{b} ±0.0	13.0^{c} ±0.0	17.0^{d} ±0.0	23.0^{e} ±0.0	29.0^{f} ±0.1	35.0^{g} ±0.0	42.0^{h} ±0.0	53.0^{i} ±0.0	100.0^{j} ±0.2	100.0^{j} ±0.1

Penicillium sp.	2.0^{a} ±0.1	13.0^{b} ±0.1	22.0^{c} ±0.1	31.0^{d} ±0.1	42.0^{e} ±0.1	57.0^{f} ±0.0	70.0^{g} ±0.1	82.0^{h} ±0.0	92.0^{i} ±0.0	100.0^{j} ±0.0	100.0^{j} ±0.0
Curvularia lunata	3.0^{a} ±0.0	15.0^{b} ±0.0	31.0^{c} ±0.0	46.0^{d} ±0.1	63.0^{e} ±0.0	77.0^{f} ±0.1	85.0^{g} ±0.0	94.0^{h} ±0.0	100.0^{i} ±0.0	100.0^{j} ±0.0	100.0^{j} ±0.0
Drechslera halodes	4.0^{a} ±0.0	9.0^{b} ±0.1	18.0^{c} ±0.0	27.0^{d} ±0.0	40.0^{e} ±0.0	53.0^{f} ±0.0	63.0^{g} ±0.0	72.0^{h} ±0.0	80.0^{i} ±0.1	100.0^{j} ±0.1	100.0^{j} ±0.1
Alternaria alternata	2.0^{a} ±0.1	14.0^{b} ±0.1	25.0^{c} ±0.0	35.0^{d} ±0.1	44.0^{e} ±0.0	51.0^{f} ±0.0	60.0^{g} ±0.0	68.0^{h} ±0.0	75.0^{i} ±0.0	100.0^{j} ±0.0	100.0^{j} ±0.1
Cladosporium cladosporides	6.0^{a} ±0.0	18.0^{b} ±0.0	28.0^{c} ±0.1	41.0^{d} ±0.0	55.0^{e} ±0.1	68.0^{f} ±0.0	81.0^{g} ±0.0	92.0^{h} ±0.1	100.0^{i} ±0.0	100.0^{j} ±0.1	100.0^{j} ±0.0

- Values are the mean of five replicates, ± Standard error.
- The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey's HSD.
- Pattern of percentage inhibition increase is not uniform for all the microorganisms

Table 3: Antifungal activity of Chloroform extract of *Picrorhiza kurroa* (rhizomes) against seed borne fungi of maize

Fungi	Chloroform Extract									Dithane M 45	Thiram
	Concentration										
	100 ppm	250 ppm	500 ppm	750 ppm	1000 ppm	1250 ppm	1500 ppm	1750 ppm	2000 ppm	2000 ppm	2000 ppm
	Percent Inhibition (%)										
Fusarium graminearum	2.0^{a} ±0.0	5.0^{b} ±0.0	9.0^{c} ±0.1	14.0^{d} ±0.0	18.0^{e} ±0.0	21.0^{f} ±0.0	27.0^{g} ±0.0	32.0^{h} ±0.0	37.0^{i} ±0.1	100.0^{j} ±0.0	100.0^{j} ±0.0
F. oxysporum	1.0^{a} ±0.1	4.0^{b} ±0.1	8.0^{c} ±0.0	12.0^{d}±0.1	15.0^{e} ±0.1	20.0^{f} ±0.1	25.0^{g} ±0.1	30.0^{h} ±0.0	35.0^{i} ±0.0	100.0^{j} ±0.0	100.0^{j} ±0.0
Aspergillus flavus	3.0^{a} ±0.0	7.0^{b} ±0.1	13.0^{c}±0.0	19.0^{d} ±0.0	25.0^{e} ±0.0	32.0^{f} ±0.1	38.0^{g} ±0.0	43.0^{h} ±00	49.0^{i} ±0.0	100.0^{j} ±0.1	100.0^{j} ±0.1
A niger	2.0^{a} ±0.1	4.0^{b} ±0.0	7.0^{c} ±0.1	11.0^{d} ±0.1	18.0^{e} ±0.0	23.0^{f} ±0.0	28.0^{g} ±0.1	33.0^{h} ±0.0	38.0^{i} ±0.0	100.0^{j} ±0.0	100.0^{j} ±0.2
A. terreus	-	-	-	4.0^{d} ±0.1	9.0^{e} ±0.1	13.0^{f} ±0.0	17.0^{g} ±0.0	22.0^{h} ±0.0	29.0^{i} ±0.0	100.0^{j} ±0.2	100.0^{j} ±0.1
Penicillium sp.	1.0^{a} ±0.0	3.0^{b} ±0.1	8.0^{c} ±0.0	12.0^{d} ±0.0	17.0^{e} ±0.0	21.0^{f} ±0.1	25.0^{g} ±0.1	29.0^{h} ±0.1	34.0^{i} ±0.0	100.0^{j} ±0.0	100.0^{j} ±0.0
Curvularia lunata	2.0^{a} ±0.1	5.0^{b} ±0.0	10.0^{c}±0.0	16.0^{d} ±0.1	22.0^{e} ±0.1	26.0^{f} ±0.0	31.0^{g} ±0.0	35.0^{h} ±0.0	39.0^{i} ±0.0	100.0^{j} ±0.0	100.0^{j} ±0.0
Drechslera halodes	-	-	-	-	-	1.0^{f} ±0.0	3.0^{g} ±0.0	6.0^{h} ±0.0	11.0^{i} ±0.1	100.0^{j} ±0.1	100.0^{j} ±0.1
Alternaria alternata	3.0^{a} ±0.0	5.0^{b} ±0.1	10.0^{c}±0.0	15.0^{d} ±0.0	21.0^{e} ±0.0	26.0^{f} ±0.0	32.0^{g} ±0.0	37.0^{h} ±0.0	41.0^{i} ±0.0	100.0^{j} ±0.0	100.0^{j} ±0.1
Cladosporium cladosporides	6.0^{a} ±0.0	11.0^{b}±0.1	24.0^{c}±0.1	35.0^{d} ±0.0	48.0^{e} ±0.1	64.0^{f} ±0.1	78.0^{g} ±0.1	88.0^{h} ±0.1	100.0^{i}±0.0	100.0^{j} ±0.1	100.0^{j} ±0.0

- Values are the mean of five replicates, ± Standard error.
- The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey's HSD.
- Pattern of percentage inhibition increase is not uniform for all the microorganisms.

DISCUSSION

Many higher plants produce economically important organic compounds. The emergence and spread of antimicrobial resistance is a growing problem in both developing and developed countries and threatens to become a global crisis,[9] It has been established that up to 25% of the drugs prescribed in conventional medicines are allied directly or indirectly to natural substances mostly of plant origin,[2] Long before the development of modern medicines, India in ancient times was entirely dependent on herbal medicines for health care. Currently, the antimicrobial activities of many plants species have been reported.[10,11] Several higher plants and their constituents have shown success in plant disease control and proved to be harmless and non phytotoxic unlike chemical fungicides,[12] Indiscriminate use of chemical not only hazardous to living beings but adversely affects the microbial population present in the ecosystem .[13] In the present study, different solvent extracts of *P. kurroa* rhizome when tested against ten species of fungi, methanol extract recorded a highest inhibition against all the test fungi followed by petroleum ether extract and chloroform extract. Hence it is very clear indication that the bioactive compounds were present predominately in methanol extract and in petroleum ether extract. Further isolation of the bioactive principle can be proceeded in methanol and petroleum ether extract.

CONCLUSION

Form the above observation, it can be concluded that, maximum inhibition was observed in methanol extract followed by petroleum ether and chloroform extract. Hence, a further research is necessary to isolate and identify the active principles from methanol and petroleum ether extract and tested against different species of fungi of different crops and also against different species of bacteria.

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