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Antifungal and growth promoting potentiality of seeds of *Psoralea corylifolia* L.

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ABSTRACT

Antifungal potentiality of aqueous and solvent extracts of seeds of *Psoralea corylifolia* L. were tested against five *Aspegillus* species *in vitro* employing poisioned food technique. In aqueous extract, among five *Aspergillus* species tested *A. ochraceous* , *A. flavipes* , *A. columnaris* and *A. fumigatus* were completely inhibited at 45% and 50% concentration. *A. candidus* recorded 90.0% inhibition at 50% concentration. More than 60% to 91% inhibition of Mycelial growth was observed in all the species of *Aspergillus* tested in Petroleum ether extract at 0.5, 1.0 and 2.0% respectively. Significant inhibition of mycelial growth was also observed in Benzene extract followed by Chloroform, Methanol and least inhibition was observed in Ethanol extract. Aqueous extract of seeds of *P. corylifolia* showed significant increase in seed germination and seedling vigour of maize treated at 20% concentration for 12 hours duration compared to 10,30,40 and 50% concentration soaked for 3,6 and 24 hours duration. The Minimum Inhibitory concentration was determined for all the test fungi.

Keywords: *Psoralea corylifolia*, *Aspergillus*, Antifungal activity,Seed germination, Seedling Vigour.

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INTRODUCTION

Agriculture plays a vital role in the economy of every nation that exists. Despite the significant achievements in food grain production, Indian agriculture continues to face serious challenges from ever increasing population. Modern agriculture has been supplying the required food for the world's ever increasing population by managing both field and storage fungi. About thirty percent of the food was lost by storage fungi which is playing a dominant role in biodeterioration. To manage biodeterioration causing fungi and the regular practice of farmers is to use a large quantities of chemical fertilizers, chemical growth regulators and chemical pesticides. The ill effects associated with the use of chemical fungicides like carcinogenicity and teratogenicity which cause a serious health problems. There is a urgent need to search for alternative strategies for the management of pre and post harvest crop diseases. Medicinal plants represents a rich source of antimicrobial agents [1]. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country [2]. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world [3]. 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 [4]. In the recent years, research on medicinal plants has attracted a lot of attentions globally. Large body of evidence has accumulated to demonstrate the promising potential of Medicinal Plants used in various traditional, complementary and alternate systems of treatment of human diseases.

Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc, which have been found *in vitro* to have antimicrobial properties[5]. Plant derived drugs remains important resource especially in developing countries to combat serious disease. *In vitro* evaluation for antifungal potency of plants against phytopathogenic fungi in general and biodeterioration causing fungi in particular is the first step towards developing plant based fungicides. This is an important first step towards developing ecofriendly approach for the prevention of biodeterioration of grains during storage. Hence in the present investigation *Psoralea corylifolia* L. (Seed) belongs to family Fabaceae were subjected to aqueous and successive solvent extraction and further evaluation of these extracts against important biodeterioration causing fungi in maize *in vitro* and to test the potentiality in seed germination and seedling vigour in maize seeds.

MATERIALS AND METHODS

Test Plant

Shade dried, healthy seeds of *P. corylifolia* were collected from seed market, Mysore. The seeds were washed thoroughly 2-3 times with running tap water and once with sterile

distilled water, air dried at room temperature on a sterile blotter, and used for preparation of extracts[6].

Extraction

Aqueous Extraction

One hundred grams of the thoroughly washed and air dried healthy seeds of *P. corylifolia* were macerated with 100 ml sterile distilled water in a waring blender (Waring international, new hart-ford, CT, USA) for 5 minutes. The macerate was filtered through double-layered muslin cloth, and then centrifuged at 4000g for 30 minutes. The supernatant was filtered through Whatman No.1 filter paper and sterilized at 120^0 C for 10 minutes, which served as 100% aqueous mother extract. The extract was preserved aseptically in a sterile brown bottle at 5^0 C until further use [7].

Solvent Extraction

The dried seeds of *P. corylifolia* were powdered with the help of waring blender. 25 grams of fine powder of *P. corylifolia* was filled in the thimble and extracted successively with petroleum ether, benzene, chloroform, methanol and ethanol for 48 hours. All the solvent extracts were concentrated using rotary flash evaporator under reduced pressure. The extracts were preserved in airtight brown bottle until further use [8].

Test Fungi

Five species of *Aspergillus* viz., *A. fumigatus*, *A. candidus, A. ochraceous, A. flavipes* and *A. columnaris* isolated from maize seeds were used as test fungi for antifungal activity assay.

Antifungal Activity Assay by Poisoned Food Technique

Aqueous Extract

Different concentrations of the Malt Extract Salt Agar(MESA) medium with the aqueous extracts of seeds of *P. corylifolia* viz., 10,15, 20,25, 30,35, 40,45 and 50% were prepared and poured into sterile petriplates and allowed to cool and solidify. Five mm mycelium disc of seven day old cultures of species of *Aspergillus* were placed at the centre of the petriplates and incubated at 25 ± 1° C. The MESA medium without the aqueous extract but with the same concentration of sterile distilled water served as control. The colony diameter was measured in millimeter. For each treatment three replicates were maintained. The percent inhibition of mycelial growth if any was determined by the formulae PI = C-T/C x 100 Where C= Diameter of control colony, T=Diameter of treated colony. Minimal inhibitory concentration (MIC) for each of the test fungi was determined [9,10]. The data were subjected to statistical analysis by ANOVA and Tukey's HSD.

Solvent Extract

The dried seeds of *P. corylifolia* were aseptically powdered and subjected to solvent extraction successively with Petroleum ether, Benzene, Chloroform, Methanol and Ethanol employing Soxhlet apparatus[11]. Each of the solvent extracts were collected separately and stored in small brown bottles and stored at 5^0 C until further use. One gram of each of the solvent extract was dissolved in 10 ml of respective solvents, which served as the mother solvent extracts. MESA medium with different concentration of each of the solvent extracts viz., 0.5%, 1.0% and 2.0% were prepared and used for *Aspergillus* species. MESA medium amended with the same concentrations of these respective solvents served as control. Five mm mycelial discs from the margins of 7day old culture of *Aspergillus* were placed on MESA medium. The plates were incubated at $25{\pm}1^o$ C for seven days and three replicates were maintained for each treatment. The colony diameter was measured in mm and percent inhibition if any was determined as described earlier [9].

Effect of the Aqueous Extract Treatment on Seed Germination and Seedling Vigour of Maize

Seed Treatment

Maize seeds were soaked in 10, 20, 30, 40 and 50% concentration of the aqueous seed extract for 3, 6, 12 and 24 hours duration. Seeds treated with sterile distilled water and soaked for 3, 6, 12 and 24 hours served as control.

Seed Germination and Seedling Vigour

Effect of aqueous seed extract of *P. corylifolia* on seed germination and seedling vigour of maize under laboratory condition was studied by treating the seeds and subjecting them to germination test and vigour index analysis. Seeds treated with extract and untreated seeds were subjected to germination test following the procedure of paper towel method [12]. Seedling vigour was determined at the end of 14 days of incubation following the method of [13]. The experiment was carried out with four replicates of 100 seeds each and repeated three times.

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).

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RESULTS

Antifungal Activity Assay by Poisoned Food Technique

Aqueous Extract

Table 1: Effect of aqueous extract of seeds of *P. corylifolia* L. on mycelial growth of *Aspergillus* species

Microorganisms	Inhibition(%)									
	Concentration of the aqueous extract									
	10%	15%	20%	25%	30%	35%	40%	45%	50%	MIC (%)
Aspergillus ochraceous	56.21[a] ±0.23	57.83[b] ±0.22	59.45[c] ±0.21	59.99[d] ±0.67	62.69[e] ±0.20	67.56[f] ±0.1	75.66[g] ±0.0	89.20[h] ±0.3	100.0[i] ±0.0	50
A. flavipes	66.5[a] ±0.32	69.45[b] ±0.34	70.93[c] ±0.35	72.41[d] ±0.35	73.39[e] ±0.12	73.39[f] ±0.12	89.60[g] ±0.2	100.0[h] ±0.2	100.0[h] ±0.7	45
A. columnaris	69.07[a] ±0.15	72.67[b] ±0.43	74.74[c] ±0.46	75.25[d] ±0.12	76.80[e] ±0.11	76.80[f] ±0.11	86.30[g] ±0.5	91.0[h] ±0.8	100.0[i] ±0.5	50
A. fumigatus	56.69[a] ±0.21	60.30[b] ±0.63	65.97[c] ±0.17	68.03[d] ±0.57	67.52[e] ±0.16	71.12[f] ±0.60	87.30[g] ±0.3	100.0[h] ±0.6	100.0[h] ±0.5	45
A. candidus	36.60[a] ±0.0	43.30[b] ±0.6	49.90[c] ±0.6	55.20[d] ±0.9	60.00[e] ±0.0	74.50[f] ±0.6	80.10[g] ±0.5	86.60[h] ±0.9	90.00[i] ±0.3	--

- Values are the mean of three 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 percent Inhibition increase is not uniform for all the microorganisms.

Among the five *Aspergillus* species tested, at 10-15% concentration, complete inhibition was observed in *A. flavipes* and *A.fumigatus* at 45% concentration. *A. ochraceous* and *A. columnaris* was completely inhibited at 50% concentration. *A. candidus* recorded 90.0% inhibition at 50% concentration. The Minimum Inhibitory Concentration (MIC) of *A.ochraceous* (50%), *A.flavipes* (45%), *A.columnaris* (50%) and *A.fumigatus* (45%) was recorded. The percentage of inhibition goes on increasing with increasing the concentration (Table 1).

Solvent Extract

Among the five solvent extract tested at 0.5%, 1.0% and 2.0% concentration, petroleum ether extract recorded a maximum inhibition of all the test fungi. *A.fumigatus* recorded 91.30% inhibition at 2.0% concentration, and 76.36% inhibition at 0.5% concentration. Significant activity was observed in *A.columnaris* (86.60%), *A.candidus* (83.60%), *A.ochraceous* (80.20%) and *A.flavipes* (79.66%)(Table 2).

Table 2: Effect of Petroleum ether, Benzene, Chloroform, Methanol and Ethanol extract of seeds of *P. corylifolia* L. on mycelial growth of *Aspergillus* species

Fungi	Inhibition (%)					
	Petroleum ether extract			Petroleum ether control		
	0.5%	1.0%	2.0%	0.5%	1.0%	2.0%
A. ochraceous	66.33^{a}±0.3	67.67^{b}±0.0	80.20^{c}±0.3	0.60^{a} ±0.1	1.31^{b} ±0.0	1.31^{b} ±0.0
A. flavipes	61.53^{a}±0.5	68.90^{b}±0.0	79.66^{c}±0.3	0.00^{a} ±0.3	0.00^{a} ±0.0	0.00^{a} ±0.0
A. columnaris	77.90^{a}±0.4	81.00^{b}±0.5	86.60^{c}±0.0	0.00^{a} ±0.0	0.00^{a} ±0.0	0.00^{a} ±0.2
A. fumigatus	76.36^{a}±0.6	82.31^{b}±0.5	91.30^{c}±0.6	0.00^{a} ±0.0	0.00^{a} ±0.0	0.00^{a} ±0.2
A. candidus	69.43^{a}±0.2	76.00^{b}±0.4	83.60^{c}±0.0	0.00^{a} ±0.0	0.87^{b} ±0.0	0.87^{b} ±0.0
	Benzene extract			Benzene control		
	0.5%	1.0%	2.0%	0.5%	1.0%	2.0%
A.ochraceous	57.70^{a} ±0.1	70.70^{b} ±0.5	76.60^{c}±0.5	0.58^{a}±0.1	0.58^{a}±0.6	0.58^{a} ±0.0
A. flavipes	55.30^{a} ±0.1	63.60^{b} ±0.2	69.90^{c}±0.5	1.23^{a}±0.2	1.91^{b}±0.2	2.60^{c} ±0.0
A. columnaris	70.20^{a} ±0.5	77.20^{b} ±0.0	83.10^{c}±0.5	0.00^{a}±0.3	0.44^{b}±0.0	0.44^{b} ±0.0
A. fumigatus	81.40^{a} ±0.0	83.00^{b} ±0.9	84.70^{c}±0.2	0.48^{a}±0.1	5.92^{b}±0.5	7.55^{c} ±0.2
A. candidus	62.10^{a} ±0.5	67.10^{b} ±0.0	73.80^{c}±0.5	0.00^{a}±0.1	0.66^{b}±0.0	1.50^{b} ±0.0
	Chloroform extract			Chloroform control		
	0.5%	1.0%	2.0 %	0.5%	1.0%	2.0%
A. ochraceous	38.46^{a}±0.1	42.93^{b}±0.2	54.76^{c}±0.0	0.00^{a}±0.1	0.00^{a}±1.5	0.00^{a}±0.1
A. flavipes	34.03^{a}±0.1	43.90^{b}±0.0	64.85^{c}±0.0	0.62^{a}±0.0	6.43^{b}±1.5	10.3^{c}±0.1
A. columnaris	65.20^{a}±0.0	70.90^{b}±0.0	78.50^{c}±0.0	1.42^{a}±0.1	1.88^{b}±1.0	2.34^{c}±0.1
A. fumigatus	63.30^{a}±0.2	68.70^{b}±0.5	75.03^{c}±0.0	0.00^{a}±0.1	0.00^{a}±1.0	0.00^{a}±0.3
A. candidus	21.03^{a}±0.0	32.80^{b}±0.0	42.23^{c}±0.0	0.00^{a}±0.1	1.06^{b}±1.2	1.06^{b}±0.1
	Methanol extract			Methanol control		
	0.5%	1.0%	2.0 %	0.5%	1.0%	2.0%
A. ochraceous	43.63^{a}±0.5	50.20^{b}±0.0	57.50^{c}±0.3	0.58^{a}±1.0	1.31^{b}±0.0	1.31^{b}±0.0
A. flavipes	38.76^{a}±0.5	49.50^{b}±0.0	54.86^{c}±0.5	1.37^{a}±0.0	2.13^{b}±0.0	2.89^{c}±0.0
A. columnaris	62.73^{a}±0.0	65.03^{b}±0.5	73.20^{c}±0.3	1.10^{a}±0.0	4.01^{b}±0.9	4.01^{b}±0.1
A. fumigatus	68.13^{a}±0.5	76.30^{b}±0.2	76.90^{c}±0.4	0.51^{a}±0.0	1.53^{b}±0.3	1.53^{b}±0.0
A. candidus	66.86^{a}±0.0	72.63^{b}±0.5	77.66^{c}±0.3	0.00^{a} ±0.1	0.64^{b}±0.9	0.64^{b}±0.1
	Ethanol extract			Ethanol extract		
	0.5%	1.0%	2.0 %	0.5%	1.0%	2.0%
A. ochraceous	34.56^{a}±0.5	41.60^{b}±1.0	39.40^{c}±0.5	0.00^{a}±0.0	0.00^{a}±0.0	0.00^{a}±0.0
A. flavipes	21.00^{a}±0.0	38.50^{b}±0.9	45.10^{c}±0.0	0.00^{a}±0.0	0.00^{a}±0.0	0.00^{a}±0.0
A. columnaris	60.23^{a}±0.5	64.90^{b}±0.3	68.63^{c}±0.0	0.00^{a}±0.0	0.00^{a}±0.0	0.00^{a}±0.0
A. fumigatus	60.00^{a}±0.3	67.06^{b}±1.0	74.60^{c}±0.5	0.00^{a}±0.0	0.00^{a}±0.0	0.00^{a}±0.0
A. candidus	13.53^{a}±0.5	20.80^{b}±0.6	44.06^{c}±0.0	0.00^{a}±0.0	0.00^{a}±0.0	0.00^{a}±0.0

- Values are the mean of three 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 percent Inhibition increase is not uniform for all the microorganisms.

Petroleum ether extract was followed by Benzene extract and at 2% concentration, maximum inhibition was observed in *A.fumigatus*(84.70%), *A.columnaris* (83.10%), *A.ochraceous* (76.60%), *A.candidus* (73.80%) and *A.flavipes* (69.90%) respectively. Benzene extract was followed by chloroform extract and recorded 78.50% inhibition in *A.columnaris*,

75.03% in *A. fumigatus*, 64.85% in *A.flavipes*, 54.76% in *A.ochraceous* and 42.23% in *A.candidus* at 2.0% respectively(Table 2).

In methanol extract, significant activity was observed in *A.fumigatus* and recorded 76.90% inhibition, *A.candidus* recorded 77.66% inhibition, 73.20% inhibition in *A.columnaris* at 2.0% concentration respectively. Moderate activity was observed in *A.ochraceous* and *A.flavipes* at 2.0% concentration(Table 2).

In ethanol extract, significant activity was observed in *A.fumigatus* and recorded 74.60% inhibition and *A.columnaris* recorded 68.63% inhibition at 2.0% concentration. Moderate activity was observed in *A.flavipes*(45.10%), *A.candidus* (44.06%) and *A.ochraceous*(39.40%) at 2.0% concentration(Table 2).

Effect of The Aqueous Extract Treatment on Seed Mycoflora, Seed Germination And Seedling Vigour of Maize

Seed Germination And Seedling Vigour

Table 3: Effect of aqueous seed extract of *P. corylifolia* L. on seed germination and seedling vigour of maize

Duration of seed Treatment (hours)	Concentration (%)	Germination (%)	Vigour index (MRL + MSL) x germination %
3 hours	10	74.00^{e} ±0.0	1017.5^{g}±0.0
	20	76.00^{g} ±.5	1029.8^{i}±0.3
	30	73.00^{d} ±0.1	1010.7^{d}±0.0
	40	71.00^{b} ±0.3	1004.6^{b}±0.1
	50	71.00^{b} ±0.3	1004.6^{b}±0.1
	Control	71.00^{b} ±0.2	1015.2^{f}±0.1
6 hours	10	74.00^{e} ±0.0	1036.0^{k}±0.0
	20	77.00^{h} ±0.5	1031.9^{j}±0.5
	30	72.00^{c} ±0.1	1029.6^{i}±0.1
	40	72.00^{c} ±0.5	1017.5^{g}±0.0
	50	0.0^{a} ±0.0	0.0^{a}±0.0
	Control	72.00^{c} ±0.2	1036.0^{k}±0.1
12 hours	10	75.00^{f} ±0.1	1008.5^{c}±0.2
	20	88.00^{i} ±0.2	1398.5^{l}±0.3
	30	74.00^{e}±1.0	1021.2^{h}±0.1
	40	73.00^{d}±0.5	1011.6^{e}±0.0
	50	0.0^{a}±0.0	0.0^{a}±0.0
	Control	72.00^{c}±0.2	1010.1^{d}±0.1
24 hours	10	75.00^{f}±0.1	1008.5^{c} ±0.2
	20	88.00^{i}±0.2	1398.5^{l}±0.3
	30	0.0^{a}± 1.0	0.0^{a}±1.0
	40	0.0^{a}±0.0	0.0^{a} ±0.0
	50	0.0^{a}±0.0	0.0^{a}±0.0
	Control	72.00^{c}±0.2	1010.1^{d}±0.1

- Values are the mean of three 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.

Highly significant increase in seed germination(76.0%, 77.0%, 88.0% and 88.0%) and seedling vigour(1029.8, 1031.9, 1398.5 and 1398.5) was observed in the seeds treated with 20% concentration of the extract for 3, 6, 12 and 24 hours duration respectively. The seeding vigour increased with increased period of soaking in 20% concentration upto 12 hours. No significant increase in seed germination or seedling vigour was observed at 24 hours treatment in 20% concentration over 12 hours treatment. At 30% concentration treatment, marginal increase in seed germination (73.0%, 72.0% and 74.0%) and seedling vigour (1010.7, 1029.6 and 1021.2) was observed at 3, 6 and 12 hours of treatment over control. However, total germination failure of seeds was observed in this concentration at 24hours treatment. At 40% concentration, significant decrease in seed germination (71.0%, 72.0% and 73.0%) and vigour index(1004.6, 1017.5 and 1011.6) were observed in 3, 6 and 12 hours duration of treatment compared with control. At 24 hours period of soaking, total germination failure was observed in 30 and 40 concentration. At 50% concentration, highly significant reduction in seed germination and vigour index was observed at 3 hours treatment, total germination failure was observed in this concentration at 6, 12 and 24 hours treatment compared with control (Table 3).

DISCUSSION

Plants, as extracts and in various other forms, are being used for centuries in different traditional systems of medicine for the treatment of human ailments, particularly those caused by pathogenic bacteria, fungi as well as viruses. Their use against plant pathogens, though a relatively recent practice, has gained momentum due to the well-known problems associated with the use of synthetic pesticides for the purpose. Use of plant products for the control of human and plant diseases has certain advantages. Plants are a repository of various biomolecules responsible for different biological activities. India is endowed with rich plant biodiversity and Karnataka is one of the hot spots of plants diversity. Many plants have been evaluated for different biological activities world over [14-17]. The results of the present investigations clearly reveal that the aqueous extract and some solvent extracts (Petroleum ether, Benzene and Chloroform) of the seeds of *P. corylifolia* are antifungal active. It has been demonstrated that total inhibition of mycelial growth of majority of *Aspergillus* species tested could be achieved at 40% conc. Among the solvents tested, Petroleum ether extract was highly antifungally active suggesting that the active principle responsible for the antifungal activity is extracted better in Petroleum ether compared with the other solvents. A few reports are available on the antifungal potential of Petroleum ether and Methanol solvent extracts of this plant on human pathogenic fungi [18-21]. However, there is lack of information on the antifungal potential of the solvent extracts on a wide range of species of phytopathogenic *Aspergillus* species. In the present investigation, the antifungal potential of aqueous and solvent extracts of *P. corylifolia* has been demonstrated. Evaluation of different concentration of seed extract on seed germination and seedling vigour by paper towel method revealed that 40 and 50% concentration of treatment and at 12 and 24 hours duration resulted in total germination failure suggesting that higher concentrations with longer duration of treatment is highly phytotoxic to maize seeds. This is evident from the fact that at 24hours periods of treatment with 30 and 40% of aqueous extract and 6, 12 and 24 hours treatment of 50% aqueous extracts

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resulted in 100% loss in seed germination compared with control. Further investigations are necessary to isolate and characterize the active principle responsible for the antifungal activity of this plant.

CONCLUSION

The present study demonstrates that aqueous and solvent extracts of *P. corylifolia* were antifungal active against five *Aspergillus* species tested *in vitro* condition by poisoned technique. It was observed that at 40% concentration of the aqueous extract total inhibition of mycelial growth was observed and in solvent extract, 2% concentration was more significant to inhibit mycelial growth. It was also observed that maize seeds at 20% concentration of the aqueous extract soaked for 12 hours duration soaking, significant increase in seed germination and seedling vigour was observed. Hence from this investigation it can be concluded that seeds of *P. corylifolia* was a potent plant material for future isolating antimicrobial drug for the management of seed borne pathogens which is an ecofriendly approach.

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