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Bioefficacy of *Psoralea corylifolia* Seed Extracts Against *Colletotrichum capsici* Causing Anthracnose of Chilli

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ABSTRACT

Laboratory bioassays were carried out to evaluate the antifungal potential of, acetonic, ethanolic methanolic and chloroform extract of Psoralea corylifolia. Seed against Colletotrichum capsici causing Anthracnose of Chilli. Different concentrations of solvent extracts (250, 500, 750 and 1000 µl) were prepared and their effect against the tested fungal pathogen was tested using poison food technique on potato dextrose agar (PDA). All the tested solvent extracts at various concentrations differ in mycelial growth inhibition of Colletotrichum capsici by 31.24 to 96.99%. Maximum mycelial growth inhibition of fungal pathogen was observed in methanolic extract at 1000 µl concentration (96.99%). Methanolic extract was fractioned on thin layer chromatography (TLC) plates using different eluting solvent systems. From all solvent systems, Toluene: Ethyl acetate:Methanol (24:05:1.5 v/v) separated more number of secondary metabolites from methanolic extract of Psoralea corylifolia Seed. The present study concludes that methanolic extract of Psoralea corylifolia Seed possess antifungal activity and can be used for management of Colletotrichum capsici.

Key words: *Chilli, Anthracnose, Psoralea corylifolia and Thin layer chromatography.*

INTRODUCTION

Chilli is an important commercial crop grown in india. Although production is high in India. Among all the diseases, anthracnose disease is major constraint to chilli production worldwide resulting in high yield losses. This fungal disease caused by *Colletotrichum* species drastically reduces the quality and yield of fruit resulting in low returns to farmers. In india, in severe cases, pre harvest and post harvest losses comprise up to more than 50%. Significant yield losses[13].

The *Psoralea corylifolia* is commonly known as babji, bakuchi and bavanchi. Is belongs to Fabaceae family. Bakuchi grows throughout india, *Psoralea corylifolia* Linn, has multifarious uses as it is an important component of Ayurveda. It is remedy as acts antifungal. In the present investigation it was found that phenols, alkaloids, tannins, flavonoids and saponin are present in seeds of plant. TLC and HPLC also confirmed these results[15].

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MATERIAL AND METHODS

Extraction yield and antifungal activity of solvent extracts of *Psoralea corylifolia* Seed

The experiment was carried out at department of Plant Pathology, Dr. PDKV, Akola, Maharashtra (India). The pathogen *Colletotrichum capsici* was isolated from infected chilli fruits from field. *Psoralea corylifolia* Seeds were procured from Akola city of Maharashtra, India. These seeds were dried under shade, powdered with grinder and 40 g of this powder was soaked separately in 200 ml acetone, ethanol, methanol and chloroform for four days at 150 rpm on rotary shaker. After filtration, solvents were evaporated at room temperature. The percentage of extraction yield was calculated by using following formula[10].

$$\text{Extraction yield \%} = \frac{\text{Weight of extract}}{\text{Weight of ground seed material}} \times 100$$

Resultant crude extract was dissolved as 1g in 10 ml dimethyl sulphoxide (DMSO) to prepare stock solution. To study antifungal mechanism of *Psoralea corylifolia* Seed extracts against *Colletotrichum capsici* by poison food technique was followed as suggested by Nene and Thapliyal[14], The experiment was conducted in Completely Randomized Design (CRD) with three replications, 1.25, 2.50, 3.75 and 5.00 ml of stock solution was separately mixed with 100 ml of sterilized molten potato dextrose agar medium respectively. The medium was thoroughly shaken for uniform mixing of the extract. Twenty ml of medium amended with extracts was poured separately into each of the 90 mm sterilized Petri plates to maintain desired concentrations of 250, 500, 750 and 1000 µl/20ml medium. The control was maintained similarly with DMSO. After solidification, each plate was inoculated with 5 mm mycelial disc taken from the periphery of seven day old fungal cultures and incubated at 28±2°C. The observations were taken on the day when the growth of colony touched the periphery in the control. The per cent inhibition of mycelial growth over control was calculated by using the formula given by Vincent[19], and the data analyzed statistically.

$$\text{Percent inhibition (I)} = \frac{\text{C-T}}{\text{C}} \times 100$$

Where,

C = Growth of fungus in control in mm

T = Growth of fungus in treatment in mm

Preliminary phytochemical screening

Preliminary phytochemical analysis of *Psoralea corylifolia* seed methanolic extract was performed by following the method of Thenmozhi *et al*[18].

Thin layer chromatography (TLC)

The TLC plates were prepared as described by Harborne[6], Briefly, 25 g of silica gel-G (Hi media, Manufactured, India) was mixed with 50 ml of distilled water and the slurry formed was uniformly spread over TLC plates with a thickness of 0.25 mm using the spreader. The plates were allowed to dry at room temperature and heated in an oven at 110 ^{0}C for 1 h. Crude extract of *Psoralea corylifolia* Seeds was diluted in distilled water and 10 µl sample was applied on TLC plates at equal distance with the help of capillary tubes. TLC plate was kept in chromatography chamber, containing tested solvent system and allowed to run until it reached as 3/4th position. The developed chromatogram on TLC plates was allowed to air dry and observed under UV light. The bands were noted and the Rf value (Relative front) of separated bands were calculated by measuring the distance travelled by solute and the solvent.

RESULT AND DISCUSSION

Extraction yield of extracts

Extraction yield of *Psoralea corylifolia* Seed in each solvent were determined as described in "Materials and Methods" and presented in Table 1. The extraction yield was affected significantly by the solvent used for extraction. This is principally related to the polarity and capability to extract substances that can be dissolved in the used solvent. Methanol exhibited (15.55%) maximum extraction yield whereas minimum extraction yield was observed in chloroform (10.27%). The methanol was found extensively useful for extraction yield, and was the most capable to extract more substances or the plants used contain more substances that preferably dissolve in Methanol. Boulenouar *et al*.[4], reported 21.91, 2.90, 2.05 and 2.11% extraction yield of *Calotropis procera* leaves in Methanol, Ethyl acetate, Dichloromethane and Hexane, respectively. Wavare *et al*.[21], obtained extraction yield of *Gaillardia* sp. flower in methanol (10.88%). Methanol was found extensively useful for get higher extraction yield, and was the most capable to extract more substances or the plants used contain more substances that preferably dissolve in Methanol. However Priyanka pandey *et al.*[15], obsereved best extraction efficacy of *Psoralea corylifolia* seed in ethanol.

Table 1. Effect of different solvents on per cent extraction yield from dry weight of *Psoralea corylifolia* Seeds

Sr. No.	Solvent	Extraction yield(%)
1	Acetone	12.47
2	Ethanol	13.77
3	Methanol	15.55
4	Chloroform	10.27

Antifungal activity of different solvent extracts of *Psoralea corylifolia* Seeds

In the present work attempts were made to discover potential antifungal activity of four solvent extracts of *Psoralea corylifolia* Seeds against *Colletotrichum capsici* at various concentrations i.e. 250, 500, 750 and 1000 µl, respectively. The result from Fig. 1 showed that moderate effect of all extracts against *Colletotrichum capsici* at 250 µl (31.24 to 47.92%) and 500 µl (37.52 to 79.52%) concentration, but at higher concentration i.e. 750 µl (67.20 to 91.87%) and 1000 µl (77.56 to 96.99%) it effectively inhibits the mycelia growth as compared with control. Earlier study of Johnny *et al*.[8], suggested that Crude extract of *P. betle* in methanol inhibited 85.25% of radial growth of *C. capsici.* Whereas, petroleum ether extract of seeds of *Psoralea corylifolia* was recorded a maximum antifungal activity (93.5%) in *Aspergillus flavus oryzae*[12,2]. The results are also in lined with the results of Kamber *et al*.[9], showed highest inhibition (74.19%) of *C. capsici* in methanolic extract of *M. indica*. Rajput and Palakshappa[17] also reported neem based formulations were effective against *C. capsici.* Prasad and Anamika[16] found that ethanol extract of *Lantana camera* possess significant fungicidal effect on growth of *C. gloeosporioides.* Begum and Nath[1] tested efficacy of four botanical oils *viz.*, Garlic, Neem, Polyalthia and Citronella at different concentrations and inhibited growth of *C. capsici*. Marigold and *gaillardia* extracts effectively suppressed growth (81.59%) of *F oxysporum* f. sp. *Ciceri*[20].

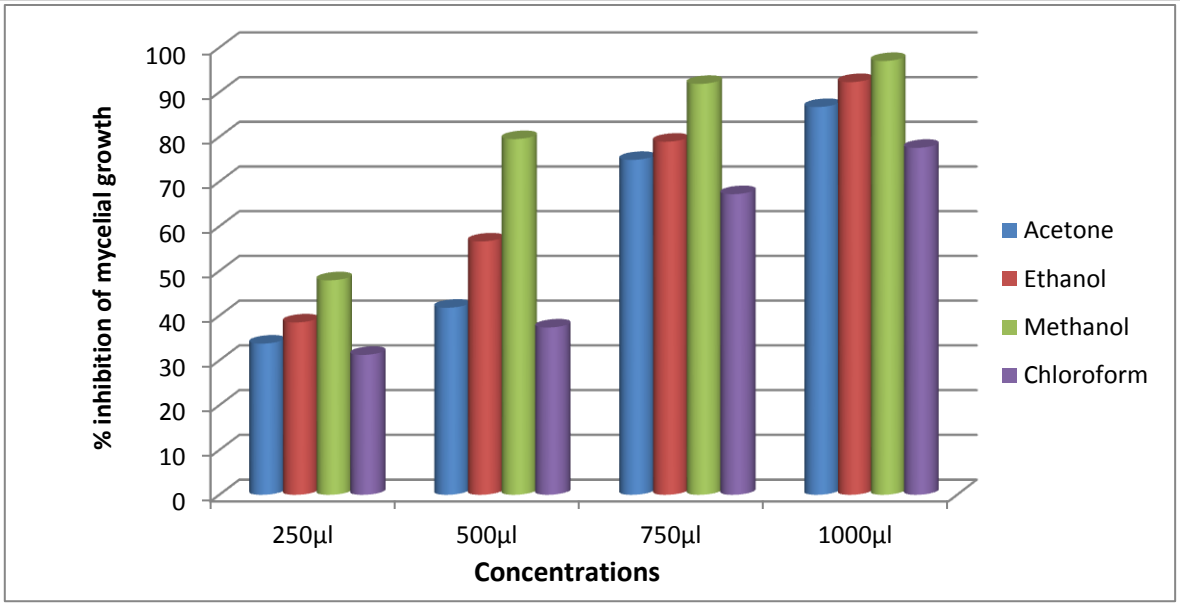


Fig. 1. Effect of different concentrations of solvent extracts of *Psoralea corylifolia* Seeds on mycelial growth of *Colletotrichum capsici.*

The efficacy of these botanicals may be due to the presence of antifungal constituents in the form of phenolics, resins and gummy and non-volatile substances of unknown nature. In present study among all the solvent extracts, methanolic extracts of *Psoralea corylifolia* Seeds were found to be effective against *Colletotrichum capsici*.

Phytochemical analysis of *Psoralea corylifolia* Seed methanolic extract

Table 2. Preliminary phytochemical analysis of methanolic extracts of *Psoralea corylifolia* Seeds

Test	Presence or absence of Phytochemicals
Alkaloids	+
Saponins	+
Tannins and phenolics	+
Fixed oils and fats	+
Steroids and sterols	-
Cardio glycosoides	+
Flavonoids	+

+ presence, - absence

It was revealed from Table 2 that, Alkaloids, Saponins, Tannins and phenolics, Fixed oils and fats, Cardio glycosoides and Flavonoids were present in methanolic extract of *Psoralea corylifolia* Seed whereas, Steroids and sterols were found absent in extract. Similar findings also observed by Borate *et al*.[3], who showed that the *Psoralea corylifolia* seeds contain of alkaloids, carbohydrates, flavonoids, glycosides and saponin. However the steroids and terpanoids were absent. Idris and Adam[7] found alkaloids, flavonoid, reducing compound, glycosides, saponins, and triterpens in *D. stramonium* leaves. The presence of tannins, alkaloids, terpenoids, cardiac glycosides, saponins, phlobatanins and flavonoids in ethanolic and methanolic extract of Lantana was reported in leaves part[5].

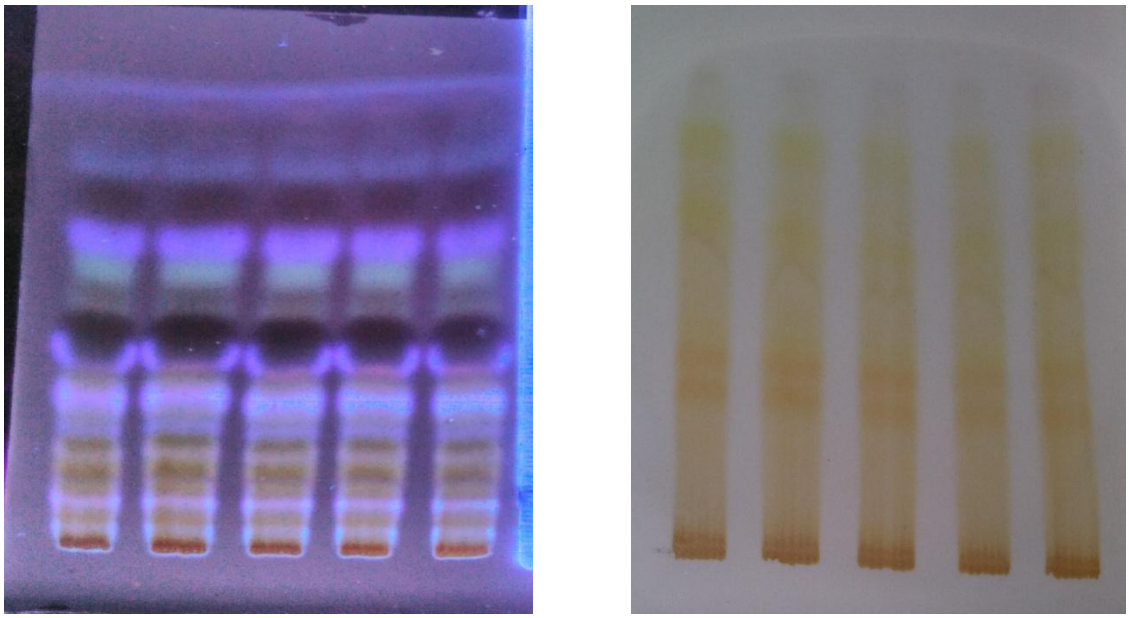
Separation of compounds by TLC

Table 3. Effect of different solvent systems on separation of compounds on TLC from *Psoralea corylifolia* Seed methanolic extract

Sr. No.	Solvent system	Proportion	Methanolic extract of *Psoralea corylifolia*	
			Rf	**Colour**
1	Ethyl acetate : methanol	3:7	0.82	Light green
			0.63	Blue
			0.45	Light brown
2	Ethyl acetate : acetone	4:6	0.72	Dark blue
			0.65	Light blue
3	Toluene : ethyl acetate : methanol	24:5:1.5	0.12	Light blue
			0.14	Light brown
			0.16	Brown
			0.22	Black brown
			0.27	Light blue
			0.37	Light pink
			0.39	Dark blue
			0.50	Light black
			0.58	Light yellow
			0.62	Light blue
			0.70	Black blue
			0.83	Light blue
			0.86	Green
4	Methanol	100%		Smear of compounds, no bands
5	Ethyl Acetate: acetic acid : petroleumether	19:1:5	0.05	Blue
			0.62	Light blue
			0.85	Black
6	Ethyl Acetate: acetic acid : petroleumether	15:6:4		Smear of compounds, no bands
7	Ethyl Acetate: acetic acid : petroleumether	20:6:4		Smear of compounds, no bands
8	Ethyl acetate : methanol : petroleumether : water	19:3:3		Smear of compounds, no bands
9	Ethyl acetate : methanol : benzene	20:6:3		Smear of compounds, no bands
10	Ethyl acetate : methanol : butanol	19:1:6		Smear of compounds, no bands
11	Petroleumether : ethyl acetate	02:01		Smear of compounds, no bands

Total 11 solvent systems were screened for efficient separation of bands according to polarity. The Rf values and colour of separated bands in different solvent systems under UV transilluminator are summarised in Table 3. Most of the compounds were visible when eluted with toluene : ethyl acetate : methanol (24:5:1.5), The Rf values of compounds separated from methanolic extract of *Psoralea corylifolia* Seed on TLC plates were were 0.12, 0.14, 0.16, 0.22, 0.27, 0.37, 0.39, 0.50, 0.58, 0.62, 0.70, 0.83 and 0.86. (Plate 1). Borate *et al*.[3], observed the retention factors (Rf) of ethanol and aqueous extracts *Psoralea* in different solvent systems. The ethanol extracts produces three fraction having Rf

0.16, 0.33, 0.44 and 0.91 under ethyl acetate: methanol (3:7) solvent system. The results of TLC indicate that methanol extracts has number of chemical constituents. Similar finding was reported by Khan and Nasreen[11] who showed 7 Rf values of methanol extracts of *Datura metel*. Thin layer chromatography was prepared in eight solvent systems. Out of which, toluene/ ethyl acetate (1:1) showed higher band separation in almost all extracts. Wavare *et al*.[21], detected the maximum Rf value separation of floral extract of *Gaillardia* sp. in butanol : acetic acid : dichloromethane : distilled water (30:10:15:5 v/v). Idris and Adam[7] identified the active fractions of *Datura stramonium* leaves extract on thin layer chromatography technique in solvent system butanol : acetic acid : water (4:1:5).



UV- Light Day light

Plate 1. TLC of *Psoralea corylifolia* Seed methanolic extract

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