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Original Article

ASSESSMENT OF ANTIFUNGAL ACTIVITY OF *CASSIA FISTULA* L. FRUIT PULP AGAINST *ALTERNARIA SOLANI*

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ABSTRACT

In the present study antifungal activity of crude and partially purified extracts of *Cassia fistula* L. fruit pulp has been assayed against *Alternaria solani* which is responsible for early blight of potato. Cold and hot extracts of fruit pulp was prepared in different organic solvents, which were subsequently recycled by rotary vacuum evaporator. Antifungal activity of different fractions was determined by poison food technique. Maximum percent extractive value was obtained with alcoholic extract. Maximum inhibitory activity was observed with 100% alcohol crude extract and partially purified chloroform extract against *Alternaria solani*. Mancozeb and bavistin were used as standards. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of chloroform fraction of *Cassia fistula* fruit pulp was investigated against *Alternaria solani*. Results suggest that *Cassia fistula* L. fruit pulp extract can be used to develop a biocontrol agent against *Alternaria solani*. The antifungal activity of the *Cassia fistula* was due to the presence of various secondary metabolites. Hence, this plant can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities.

Keywords: Antifungal activity, Cold extraction, Hot extraction, Poison food technique, MIC, MFC.

INTRODUCTION

Drugs derived from natural sources play a significant role in the prevention and treatment of diseases. In many countries, traditional medicine is one of the primary health care systems [1]. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented. Natural products of higher plants, may give a new source of antimicrobial agents with possibly novel mechanisms of action [2].

In the recent years, researches on medicinal plants have attracted a lot of attention globally. Evidences have been accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary, and alternative systems of treatment of human diseases. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, and glycosides etc., which have been found *in vitro* to have antimicrobial properties [3]. Herbal medicines have been known to man since centuries. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. Traditional medicine continues to be a valuable source of remedies that have been used by millions of people around the world to secure their health [4].

Alternaria solani is the causal agent of early blight of potato that leads to major damages to potato crop. It is a major foliar disease of potato crop and Losses due to early blight typically are around 20-25%; however, there have been cases of 70-80% losses [5]. It produces irregular to circular dark brown spots on the lower (older) leaves, excessive defoliation may lead to death of the plant and consequent yield loss [6]. *A. solani* overwinters as mycelium or conidia in plant debris, soil, infected tubers or on other host plants of the same family. The disease is controlled primarily through the use of cultural practices such as resistant cultivars and foliar fungicides, crop rotation, removal and burning of infected plant debris, and eradication of weed hosts helps reduce the inoculums level for subsequent plantings [7]. The most common and effective method for the control of early blight is through the application of foliar fungicides. Protectant fungicides recommended for early blight control are maneb, mancozeb, chlorothalonil, and triphenyl tin hydroxide [8]. But negative side of the use of synthetic fungicides is that they are harmful for human, soil as well as wildlife health, and enter the food chain and cause several deleterious effects on biosphere, contributing to significant declines in populations of beneficial soil organisms, soil acidification, and diminished resistance to diseases [9]. Natural plant products are important sources of new agrochemicals for the control of plant diseases. A search for an environmentally safe and economically viable strategy for the control of diseases has led to an increased use of plant based products in agriculture [10].

Cassia fistula (Linn.) belongs to family Fabaceae and Sub–family Caesalpinioideae is a very common plant known for its medicinal properties are a semi-wild in nature. It is distributed in various regions including Asia, South Africa, China, West Indies and Brazil. It is commonly known as Amaltas and in English popularly called "Indian Laburnum" has been extensively used in Ayurvedic system of medicine for various ailments. It is deciduous and mixed-monsoon forests throughout greater parts of India, ascending to 1300 m in outer Himalaya, is widely used in traditional medicinal system of India [11]. There are several reports of antimicrobial activities of *Cassia fistula* [12, 13, 14]. The present paper reports the antifungal activity of crude and partially purified extracts of *Cassia fistula* L. against *Alternaria solani*.

MATERIALS AND METHODS

Collection of plant material

The healthy, infection free, mature pods were collected from the campus of University College of Science, Mohanlal Sukhadia University, Udaipur in May-June 2013, and were dried in shade. The pods were broken with the help of a pestle to extract out the pulp. The pulp was grounded in an electrical grinder after removal of the seeds from the pulp. The ground material was passed through sieve of mesh size 60 to obtain a fine powder which was used to prepare the extract.

Isolation of pathogenic fungus

Alternaria solani was isolated from infected leaves of potato by single spore inoculation method. Fungus was purified; pure culture was maintained on PDA (Potato dextrose agar) and stored in refrigerator at 4 ^{o}C.

Inoculum disc

Seven day old culture of the test fungus was used for the preparation of inoculum disc of 6 mm in diameter.

Preparation of Plant extract

Cold Extraction

Crude extract was prepared by according to the modified cold extraction method suggested by Shadomy and Ingraff [15]. 100% alcohol, 50% alcohol as well as 100% aqueous extract of fruit pulp was prepared by dissolving 20 gm dried and powdered plant material in 100 ml of solvent (alcohol/ water) for 24 h. The suspension was filtered through Whatman filter paper no.1 then vacuum dried with the help of rotary vacuum evaporator. The dried residue was used as extract and solvent was recycled.

Hot Extraction

Reflux method of solvent extraction was used for successive separation of different partially purified organic constituents present in dried plant material [16]**.** Solvent series used for successive separation was as follows:

Pet. ether → Benzene → Chloroform → Acetone → Alcohol →Methanol →Water

This method involves continuous extraction of powdered dried plant material in soxhlet apparatus with a series of organic solvents. Each time before extracting with next solvent the plant material was dried in an oven below 50°C. 40 gm dry plant powder was kept in Soxhlet extraction unit and extracted with 280 ml solvent.

Percent Extractive Value

Crude extract and fractions obtained at every step including aqueous fraction were vacuum dried in a rotary evaporator. The dried extract and fractions were weighed and their percentage in terms of the dry weight of the plant material was estimated by the following formula given below.

$$\text{Percent extractive} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100$$

Antifungal activity of Plant extracts

Antifungal activity of crude 100% alcohol, 50% alcohol as well as 100% aqueous extract and partially purified fractions of fruit pulp of *Cassia fistula* against *Alternaria solani* was done by Poison food technique [17]. 100 mg of extract was dissolved in 10 ml solvent to prepare stock solution of 10mg/ml concentration. 9 ml of molten PDA medium was poured into test tubes and then autoclaved. The molten sterilized medium along with 1 ml of stock solution was placed into Petri plates and **i**n the control set no extract was used. After the solidification of the media, 6 mm inoculum disc of 7 days old culture of the fungus was aseptically inoculated upside down in the centre of the Petriplate and incubated at 25±2ºC.

The average diameter of the fungal colonies was measured on the 7th day of incubation and percentage of mycelial growth inhibition was calculated by the following formula given below.

Mycelial growth inhibition = gc-gt / gc× 100

Where,

gc = Growth of mycelial colony after incubation period in control set subtracting the diameter of inoculums disc;

gt = Growth of mycelial colony after incubation period in treatment set subtracting the diameter of inoculum disc.

Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

Minimum inhibitory concentration (MIC) was determined by broth dilution method [18]. Potato dextrose broth (PDB) was used for determining inhibitory activity. 200 mg of the extract was dissolved in 10 ml of acetone to prepare stock solution of 20 mg/ml. Two fold serial dilution method was used for the preparation of 10 mg/ml to 0.0195 mg/ml concentration from the stock solution. Thus prepared concentration was serially diluted with sterile broth medium to attain final concentration of 1000 µg/ml to 1.95 µg/ml. All these tubes were than respectively inoculated with 100 µl of spore suspension (1×10^6 spores/ml) and incubated at 25 ± 2°C for 72 h. One tube containing extract free autoclaved medium was used as control. Three replicates of each concentration were maintained and experiment was repeated thrice. A loopful of fungal biomass from each tube containing 9 ml broth medium and MIC as well as all concentrations were streaked onto the surface of sterile PDA slants and incubated at 25 ± 2°C for 72 h. Presence or absence of growth was observed after respective incubation time. Appearance of growth indicates that the extract concentration is just fungistatic and absence of growth indicates that extract concentration is fungicidal.

RESULTS AND DISCUSSION

Plants have variety of secondary as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils. These metabolites play very important role in defense against insects, herbivores and microorganisms [19, 20]. The antifungal activity of different organic solvent extracts of *Cassia fistula* was reported by Hajra *et al*., [13].

In the present study *C. fistula* fruit pulp extracts prepared in water, alcohol and various organic solvents were screened for antifungal activity. Results of percent extractive value of various fractions of crude and hot extracts are depicted in table 1 and 2. Maximum percent extractive value was obtained with 50% alcoholic crude extract *i. e.* 56.05% and alcohol fraction of partially purified extract *i. e*. 28.5%. Results of antifungal activity of crude as well as partially purified extracts of fruit pulp against *Alternaria solani* are presented in table 3 and 4. The inhibitory activity of the extracts was compared with standard fungicides like mancozeb and bavistin are presented in table 5.

According to results, best antifungal activity was observed with 100% alcoholic crude extract and partially purified chloroform fraction against *A. solani* and % mycelial growth inhibition is 53.27% and 93.88% respectively given in table 3, 4. The second highest inhibition showed by 100% aqueous crude extract and benzene, petroleum ether, acetone fractions of partially purified extracts. 50% alcoholic crude extract and alcohol, methanol and water fractions of partially purified extracts showed less inhibition against *A. solani*. All data were compared with water as control in which no plant extract and fungicides used.

Table 1: Percent Extractive value of Crude Extract of *C. fistula* Fruit pulp

S. No.	Type of Extract	Weight of dried extract (gm)	% Extractive value
1	100% alcohol	4.58	22.9
2	100% aqueous	9.81	49.05
3	50% alcohol	11.21	56.05

Table 2: Percent Extractive value of Different Partially Purified Fractions of *C. fistula* Fruit pulp Extract

S. No.	Type of Extract	Weight of dried extract (gm)	% Extractive value
1	Petroleum ether fraction	0.05	0.12
2	Benzene fraction	0.1	0.25
3	Chloroform fraction	0.06	0.15
4	Acetone fraction	0.43	1.07
5	Alcohol fraction	11.4	28.5
6	Methanol fraction	3.33	8.32
7	Water fraction	4.02	10.05

Table 3: Antifungal Activity of Crude Extract of *C. fistula* Fruit pulp against *A. solani*

S. No.	Type of Extract	Growth Diameter after 7 days (mm) ± SD	% Mycelial growth inhibition
1	100% alcohol	35.67 ± 1.52	53.27
2	100% aqueous	43.33 ± 1.15	43.23
3	50% alcohol	47 ± 1.73	38.42

Table 4: Antifungal Activity of Various Partially Purified Fractions of *C. fistula* Fruit pulp Extract against *A. solani*

S. No.	Type of Extract	Growth Diameter after 7 days (mm) ± SD	% Mycelial growth inhibition
1	Petroleum ether	13.67 ± 1.15	82.09
2	Benzene	9 ± 1.00	88.20
3	Chloroform	4.67 ± 0.57	93.88
4	Acetone	17.67 ± 1.15	76.85
5	Alcohol	44.67 ± 1.15	41.48
6	Methanol	46.33 ± 2.08	39.30
7	Water	54.67 ± 1.15	28.37

Table 5: Antifungal Activity of standard fungicides with water control against *A. solani*

S. No.	Standard fungicides and water control	Growth Diameter after 7 days (mm) ± SD
1	Mancozeb	14.67 ± 1.52
2	Bavistin	35.67 ± 1.52
3	Water	76.33 ± 1.15

The results of MIC and MFC of chloroform fraction of Cassia fistula fruit pulp was observed at 2.5 mg/ml and 5 mg/ml respectively.

Harborne, Cowan and Kokate *et al.,* suggested that successive extraction of plant secondary metabolites should be done in petroleum ether followed by benzene, chloroform, acetone, alcohol, methanol and finally with water *i. e.* from non polar to polar solvents [16,19,21]. Extraction of secondary metabolites from plant material by hot extraction with petroleum ether separates sterols, waxes and fatty acids leaving behind residue containing the defatted plant materials. Subsequent extraction of this residue with benzene separates out sterols and flavonoids. Terpenoids and flavonoids get extracted with chloroform. The last solvent *i. e.* alcohol extracts alkaloids, flavonoids, polyphenols, tannins and reducing sugar from the residue. Finally extraction with water yields remaining water soluble metabolites such as anthocyanins, starch, tannins, saponins, reducing sugars and polypeptides[22,23].

CONCLUSION

The present study proved that antimicrobial properties of *C. fistula* fruit pulp on *Alternaria solani*. In the current investigations, 100% alcoholic crude extract and partially purified chloroform extract of *C. fistula* fruit pulp was found to be active on *Alternaria solani* as compared to standard fungicides. Further studies are necessary to isolate and reveal the active compounds of the extract. Further, purifications of these potent partially purified fractions will be used to develop natural fungicides which will do not have any environmental hazards.

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CONFLICT OF INTERESTS

Declared None

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