*Mycopath* (2012) 10(2)**:** 77-82 **Research Article**

# Antifungal potential of methanolic leaf and bark extracts of *Cassia fistula* L. against *Ascochyta rabiei*

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## Abstract

*Cassia fistula* L. was examined for its *in vitro* antifungal potential against *Ascochyta rabiei* (Pass.) Lab., the causal agent of chickpea blight (*Cicer arietinum* L.). Screening bioassay with methanolic leaf and bark extracts were performed *in vitro* against *A. rabiei*. All the concentrations of methanolic leaf and bark extracts significantly reduced fungal growth. Leaf extract exhibited greater antifungal activity than the bark extract. Leaf extract was selected for bioassays guided fractionation. Methanolic leaf extract was partitioned among *n*-hexane, chloroform, ethyl acetate and *n*-butanol. Minimum inhibitory concentration (MIC) of the four separated fractions and a commercial synthetic fungicide (metalaxyl + mancozeb 72 WP) was investigated against *A. rabiei*. Chloroform fraction was found highly effective against *A. rabiei* with MIC value of 0.97 mg $mL^{-1}$ followed by ethyl acetate and *n*-hexane fractions.
**Key words:** Antifungal activity, *Ascochyta rabiei*, *Cassia fistula*, methanolic extracts.

## Introduction

Chickpea (*Cicer aretinum* L.) is the third important pulse crop in the world and one of the major pulse crop grown in Mediterranean basin and rainfed areas of Pakistan (FAO, 2004). It is a rich source of vegetable protein for human nutrition (Hulse, 1991). Ascochyta blight caused by fungus *Ascochyta rabiei*, is the major disease limiting chickpea productivity worldwide (Chongo *et al*., 2003). It causes 20-25% yield loss in chickpea annually and may cause total crop failure under epidemic conditions (Cho *et al.,* 2008). The fungus can infect all above ground parts of the plant and is most prevalent in areas where cool, cloudy and humid weather occurs during the crop season and pathogen attacks the crop at both vegetative and podding stages (Ali *et al.,* 2011). Infections arise from seed borne inoculum or from wind borne spores (ascospores) results in elongated, sunken, dark lesions on aboveground part. Lesions often girdle stems, weaken and break branches and petioles (leaf stems), and kill all plant parts above the lesion. Within the lesions, the fungus produces fruiting bodies (pycnidia) that become visible as tiny, black, raised spots, often arranged in concentric rings (Wiese *et al.,* 1995).

Several management strategies are intended to prevent the occurrence of blight disease, utilization of resistant varieties is considered as best option (Iqbal *et al*., 2005). However, the chickpea cultivars lack stability in tolerance and new pathotypes of the pathogen appeared (Singh and Reddy, 1991). Use of foliar sprays and seed dressing fungicides is another way to control chick pea blight disease effectively but pesticides pollute the environment and cause health hazards. Increasing public concern on environmental issues requires alternative disease management systems, which are less pesticide dependant or based on naturally occurring compounds (Cuthbertson and Murchie, 2005). Botanical derivatives are more environmentally safe than synthetic chemicals (Hashim and Devi, 2003). Several antifungal compounds present in certain plant species have been used to control fungal pathogens (Masoko *et al.*, 2007; Romero *et al*., 2008).

*Cassia fistula* known as the golden shower tree is a flowering plant in the family Fabaceae, native to southern Asia, from southern Pakistan east through India to Mayanmar and south to Sri Lanka. This is a medium-sized tree, growing to 10–20 m tall with fast growth. In Ayurvedic medicine, this golden shower tree is known as *aragvadha*, meaning "disease killer" its root is considered a very strong purgative, and self-medication or any use without medical supervision is strongly advised against in Ayurvedic texts (Karanth *et al*., 2006). *C. fistula* has well-known for antifungal and antimicrobial activities (Duraipandiyan and Ignacimuthu, 2010; Nayan *et al.,* 2011). The present study was carried out to evaluate the antifungal activity of *C. fistula* against *A. rabiei*, the causal agent of chick pea blight.

## Materials and Methods

### Collection of plant material

Leaves and bark of the *C. fistula* were collected from the Lahore College for Women University, Lahore, Pakistan. The plant material

was dried under sunlight and stored in polythene bags till for use.

**Isolation of pathogenic fungus**

*A. rabiei* was isolated from diseased stem of chick pea. Fungus was purified, pure culture was maintained on 2% PDA (Potato dextrose agar) and stored in refrigerator at 4 $^{o}$C.

**Preparation of organic solvent extracts**

Twenty grams of each plant dried materials were soaked in 100 mL of organic solvent (methanol) and left for three days at room temperature (30 $^{o}$C). After three days, materials were filtered through an autoclaved muslin cloth. Methanolic solvent extracts were evaporated to concentrate it to volume up to 2 mL under dried oven at 35 $^{o}$C and then diluted by adding appropriate amount of distilled water to make a final volume of 100 mL. These stock organic solvents were stored in refrigerator at 4 $^{o}$C for further utilization.

**Antifungal bioassays**

Different concentration of extract i.e. 1, 2, 3, 4 and 5% were prepared by adding 5, 10, 15, 20 and 25 mL of the stock solutions, respectively to prepare 100 mL of PDA to prepare 100 mL of PDA supplemented with chloromycetin capsule to avoid bacterial contamination. Five replicates of each concentration were prepared by adding 20 mL from total of 100 mL in pre-sterilized 9 cm Petri plates. After solidification of medium, mycelial discs (2 mm) of target fungus was placed in the center of each Petri plate. Control treatments were without any plant extracts. Plates were incubated for 7 days at 25±2 $^{o}$C. After incubation period fungal growth diameter was measured by taking average of the three diameters taken at right angles for each colony. Percentage growth inhibition of the fungal colonies was measured by using the following formula:

$$\text{Inhiition (\%)} = \left[ \frac{\text{Growth in control - Growth in treatment}}{\text{Growth in control}} \right] \times 100$$

**Partitioning of plant material**

Methanolic extract of 350 g *C. fistula* leaves were portioned with n-hexane, chloroform ($CHCl_3$) followed by ethyl acetate (EtOAc) and n-butanol at room temperature. The extract was evaporated under vacuum on rotary evaporator (Buchi Switzerland R-210) at 40 $^{o}$C to yield 41 g gummy mass. This methanolic extract (41 g) was partitioned between *n*-hexane and water. The aqueous fraction was successively partitioned with chloroform, ethyl acetate and *n*-butanol (Jabeen *et al.,* 2013) according to increasing polarity order. This partitioning was yielded as gummy mass of *n*-hexane (2 g), chloroform (12 g), ethyl acetate (2 g), *n*- butanol (0.5 g) and remaining water fraction. This partitioning was made by use of separating funnel.

**Assessment of minimum inhibitory concentration (MIC) of the isolated fractions**

The MIC values of the separated fractions and a synthetic fungicide (Metalaxyl + Mancozeb 72 WP) were tested in test tubes by serial dilution micro dilution assay (Jabeen *et al.,* 2011) with few modifications. The four separated fractions were dissolved in dimethyl sulfoxide (DMSO) and were serially diluted with water in test tubes. Maximum concentration1 mg $mL^{-1}$ was prepared by adding 1 mL of DMSO and 1 mL of distilled water, this concentration was further serially diluted and the minimum applied concentration was 0.976 mg $mL^{-1}$. Freshly prepared PDA medium was added to seven days old fungal culture of *A. rabiei* to reach a final conidial concentration $1\times10^{5}$, 100 µL of this was added to test tubes having a diameter of 1.6 and 15 cm long. Test tubes containing DMSO and distilled water were used as control. These test tubes were incubated at 25-30 $^{o}$C. The MIC of the fractions was observed visually after 24, 48 and 72 hours by using inverted microscope to study the fungal mycelia growth.

**Statistical analysis**

Data were analyzed statistically by applying ANOVA followed by Duncan's Multiple Range Test (Steel *et al.,* 1997).

# Results and Discussion

**Antifungal activity of methanolic leaf extract of *C. fistula***

The methanolic leaves and bark extract of *C. fistula* was significantly inhibited the *in vitro* growth of *A. rabiei* at all concentrations (Fig. 1 & 2). Growth of the fungus was significantly inhibited by 1, 2, 3, 4 and 5% of methanolic leaves extract resulted in decline of fungal growth by 55, 34, 50, 33 and 56 %, respectively. On the other hand, methanolic bark extract significantly suppressed the diameter of *A. rabiei* by 49, 45, 57, 48 and 44% with concentration 1, 2, 3, 4 and 5%, respectively. Numerous antifungal constituents present in certain plant species have been used for controlling fungal pathogens (Romero *et al*., 2008; Javaid and Amin, 2009). Earlier Priya *et al.* (2010) studied the antifungal activity of *C. fistula* leaves, fruits, seeds and stem bark extracted in water,

aqueous methanol (40%) and methanol against *Candida albicans*, *Aspergillus niger*, *Epidermophyton floccosum* and *Trychophyton mentagrophytes*. All the extracts of the showed good antifungal active antifungal activity is due to the presence of anthraquinone derivative, 6-9-dimethoxy-3-methyl,1,4,5-trihydroxy-anthraquinone-2-caroboxylic acid.

**Minimum inhibitory concentration (MIC) bioassays**

MIC of the four separated fractions viz. *n*-hexane, chloroform, ethyl acetate and *n*-butanol along with a reference synthetic fungicide (metalaxyl + mancozeb, 72 WP) was observed against *A. rabiei* obtained after 24, 48 and 72 hours incubation period (Table 1). Synthetic fungicide and chloroform fraction was found to be most effective antifungal as its highest and lowest (0.97-500 mg $mL^{-1}$) concentration totally inhibits the mycelial germination of *A. rabiei* even after 72 hrs incubation period (Ahmed *et al.*, 2007). Other fractions were comparatively less antifungal and the n-butanol fraction was least effectual. Both control treatments (distilled $H_2O$ and DMSO) were promoting the spore germination and mycelium growth of test fungus as mycelium of *A. rabiei* was visually observed even after 24 hrs incubation period. Similar results were observed by Timothy *et al.* (2012) with methanolic leaf extract of *Cassia alata* against different plant pathogenic. The MIC of four compounds isolated from chloroform fraction of methanolic leaf extract of *M. azedarach* against *A. rabiei* was checked by Jabeen *et al.* (2011) and found promising results.

# Conclusion

Different concentrations of methanolic leaf extracts (1-5%) significantly reduced the fungal growth. Chloroform fraction of *C. fistula* leaves has potential antifungal activity against *A. rabiei*.

**Table 1:** MIC values of different organic fractions of methanolic leaf extract of *Cassia fistula* and synthetic fungicide (Metalaxyl + Mancozeb) against *Ascochyta rabiei* after 24, 48 and 72 hours incubation periods.

| Fractions | Concentration ( mg $mL^{-1}$) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **500** | **250** | **125** | **62.5** | **31.25** | **15.62** | **7.81** | **3.90** | **1.95** | **0.97** |
| 24 hours after incubation | | | | | | | | | | |
| Control ($H_2O$) | + | + | + | + | + | + | + | + | + | + |
| Control (DMSO) | + | + | + | + | + | + | + | + | + | + |
| *n*-Hexane | - | + | + | + | + | + | + | + | + | + |
| Chloroform | - | - | - | - | - | - | - | - | - | - |
| Ethyl acetate | - | - | - | - | + | + | + | + | + | + |
| *n*-Butanol | - | + | + | + | + | + | + | + | + | + |
| Metalayl+mancozeb | - | - | - | - | - | - | - | - | - | - |
| 48 hours after incubation | | | | | | | | | | |
| Control ($H_2O$) | + | + | + | + | + | + | + | + | + | + |
| Control (DMSO) | + | + | + | + | + | + | + | + | + | + |
| *n*-Hexane | - | - | - | + | + | + | + | + | + | + |
| Chloroform | - | - | - | - | - | - | - | - | - | - |
| Ethyl acetate | - | - | - | + | + | + | + | + | + | + |
| *n*-Butanol | - | + | + | + | + | + | + | + | + | + |
| Metalayl+mancozeb | - | - | - | - | - | - | - | - | - | - |
| 72 hours after incubation | | | | | | | | | | |
| Control ($H_2O$) | + | + | + | + | + | + | + | + | + | + |
| Control (DMSO) | + | + | + | + | + | + | + | + | + | + |
| *n*-Hexane | - | - | - | + | + | + | + | + | + | + |
| Chloroform | - | - | - | - | - | - | - | - | - | - |
| Ethyl acetate | - | - | - | + | + | + | + | + | + | + |
| *n*-Butanol | - | + | + | + | + | + | + | + | + | + |
| Metalayl+mancozeb | - | - | - | - | - | - | - | - | - | - |

Mycelium present: +; Mycelium absent: -

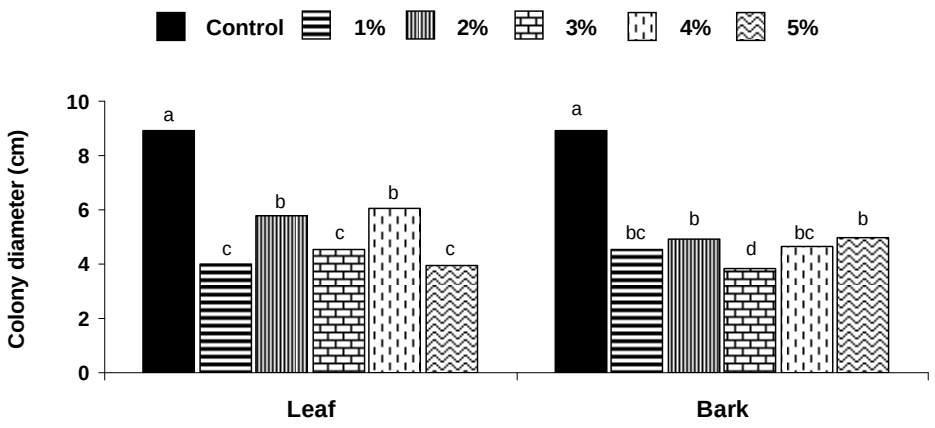


**Fig. 1:** Effect of methanolic leaf and bark extracts of *Cassia fistula*, on radial growth of *Ascochyta rabiei.* Values with different letters (separately for each plant part) show significant difference as determined by DMR Test at 5% level of significance.

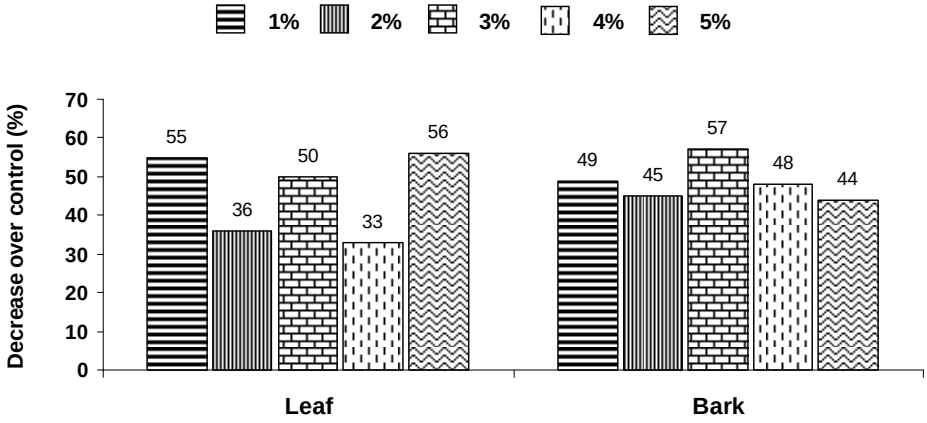


**Fig. 2:** Percentage decrease in diameter of *Ascochyta rabiei* due to different concentrations of methanolic leaf and bark extracts of *Cassia fistula*.

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