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Antifungal Activity of *Cassia fistula* L. Extracts against *Macrophomina phaseolina* (TASSI.) Goid.: *In vitro* Study

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To evaluate the anti-fungal properties of *Cassia fistula* with alcohol soluble and aqueous extracts against *Macrophomina phaseolina*.
Methods: Ethanolic, methanolic and aqueous extracts were prepared and qualitative and quantitative analysis of phytoconstituents was done. The antioxidant and antifungal activity of the extracts were determined.
Results: It was observed that the methanolic extract had the highest (15.65%) extraction yield, whereas ethanolic extract had the lowest (12.45%) extraction yield. While screening for secondary metabolites, cardiac glycosides were found to be lacking in all three solvent extracts, while sterols was absent in the aqueous extract. The methanolic extract contained the highest amounts of phenolic compounds (13.38±0.060 mg GAE/g dw.), flavonoid (10.58±0.074 mg QUE/g dw.), tannin (11.43±0.052 mg TAE/g dw.), and alkaloid (16.18±0.062 mg AE/g dw.). The highest antioxidant activity was observed in the methanolic extract at 300 µg/ml (% inhibition, 63.02%). Antifungal activity against *M. phaseolina* was also highest (68.07%) for the methanolic fraction.
Conclusion: *C. fistula* extracts may be further be explored to formulate antifungal agents.

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Keywords: Antioxidant activity; antifungal activity; Cassia fistula; Macrophomina phaseolina.

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.), commonly known as Bengal gram, is a significant annual plant of the pea family (Fabaceae) that is widely known for its nutritional seed value in many countries. Chickpeas are widely produced in India, Africa, Central, and South America [1]. It provides a substantial number of vital proteins and vitamins that are beneficial to human health [2]. Furthermore, they are also used as raw material for food processing and value-added industrial products [3].

There is a high demand for chickpea seeds for their nutritional value mainly in the vegetarian diet in developing nations such as India, necessitating increased attention to its sustainable production [4].

About 172 pathogens are responsible for diseases in chickpeas. The most destructive soil-borne disease is Dry Root Rot, which is infected by *Macrophomina phaseolina* (Tassi) Goid (*Rhizoctonia bataticola* Taub.). The disease's severity varies according to the temperature and moisture content of the chickpea plant and causes significant losses on the plant from blooming to pod formation [5].

Although synthetic fungicides have long been used to control phytopathogenic fungi, their excessive use can be detrimental to human beings, the environment, and non-target species, negatively impacting biodiversity. Phytochemicals generated from plants exhibit antifungal capabilities [6]. Today, the global endeavor in contemporary agriculture is to limit the use of toxic chemicals such as fungicides, weedicides, pesticides, and so on, by adopting new biological and ecological ways. Utilizing the chemical interactions between plants is one of these strategies [7]. Allelopathy and competition are two examples of the impacts that individual plants in a shared ecosystem have on their nearby neighbors. Competition entails the active absorption of limited resources by one organism, resulting in a decrease in supply and hence growth inhibition of other organisms; nevertheless, allelopathy occurs when one species stops developing due to chemicals emitted by another species [8,9]. However, while defining allelopathy, some researchers also consider the stimulatory effects of growth [10].

The Indian laburnum, sometimes known as the "golden shower tree," or *Cassia fistula* L. (Fabaceae), is a tree with therapeutic benefits [11]. Secondary metabolites including tannins, terpenoids, alkaloids, flavonoids, glycosides, and others are abundant in plant tissues, and have been shown to exhibit antibacterial [12,13,14]; anti-inflammatory [15]; antioxidant [16]; wound healing [17]; antifungal [18] and anticancer activity [19], characteristics when tested in vitro. *C. fistula* includes important bio-natural components that are highly beneficial for providing crucial medicinal benefits.

In the present studies, the phytochemical, and antioxidant activity of *Cassia fistula* L., were determined and antifungal activity was checked against *Macrophomina phaseolina*.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh *Cassia fistula* leaves were randomly selected from the trees growing campus of Banasthali Vidyapith in Tonk, Rajasthan, with no visible disease symptoms. The leaves were cleaned with distilled water before being air dried for two to three days in the shade. The dried samples were then processed into a fine powder using a grinder and kept in a container until extraction was needed.

2.2 Extraction of Leaf Samples

20g of leaf sample was dissolved in 100 ml of solvent (80% ethanol, 80% methanol, and water) for 24–42h at room temperature. Then, the extracts were filtered through sterile muslin cloth. The filtrates from each solvent were evaporated in a desiccator. The dried extracts were packaged in airtight containers, labeled, and kept in a refrigerator (2-4°C) for further use.

Extraction yield (%) was calculated as follows:

> Extraction yield (%) =W2/W1 *100, where W2=weight of the extract after evaporating solvent, W1=initial dry weight of the sample

2.3 Qualitative and Quantitative Phytochemical Analysis

Phytochemical evaluation for saponins, flavonoids, cardiac glycosides, terpenoids,

steroids, tannins, phenol, anthraquinone, alkaloids, and tannins was done [20,21]. The total phenolic [22,23], flavonoid [24,25], tannin [24,26], and alkaloid [20] content of various fractions of extracts (methanol, ethanol, and aqueous) were determined.

2.4 Determination of Antimicrobial Activity

Macrophomina phaseolina (Tassi.) Goid, culture strains (NFCCI No. 4832) were procured from NFCCI (National Fungal Culture Collection of India), Agharkar Research Centre, Pune, India and maintained at the Department of Bioscience and Biotechnology, Banasthali Vidyapith, Rajasthan. The antifungal assay was performed on a Potato dextrose broth medium [27]. The growth of fungal mycelia at 28-30ºC was determined by observing the dry weight on 7th day.

2.5 Determination of Total Antioxidant Activity by DPPH assay

To determine the total Anti-oxidant activity, DPPH (1, 1 dihydroxy 2- picrylhydrazyl, Sigma Aldrich) free radical scavenging assay was conducted; 1.0ml of DPPH solution (0.135mM DPPH in methanol) was added to (100-300µg/ml) plant extract [28]. The reaction mixture was kept in the dark for 30 minutes and its absorbance at 517nm was observed on a spectrophotometer. Ascorbic acid was utilized as a control.

2.6 Statistical Analysis

The experiments were repeated three times, and the findings were provided as Mean± S.E. All tests were designed using a random block design and Analysis of Variance (ANOVA) at p=0.05. Appropriate post-hoc tests were performed, including the "Duncan Multi-Distance Test" (DMRT) and the "Least Significant Difference" (LSD) using SPSS software.

3. RESULTS

3.1 Determination of Extraction Yield

The % yield for the different solvent extracts of *C. fistula* leaf shown in Table 1. When yields are compared, the Methanolic extract has the most significance.

Table 1. Percentage yield of various solvent extracts of leaf of *C. fistula*

Solvent	**Yield percent** (%)
Methanol	15.65^{c}
Ethanol	12.15^{a}
Aqueous	13.55^{b}

3.2 Qualitative Phytochemical Analysis

The phytochemical analysis of three solvent extracts of *C. fistula* is listed in Table 2. All the three different solvent extracts gave a variety of compounds.

3.3 Quantitative Analysis

The results for the quantitative estimation of leaf extract of *C. fistula* are tabulated in Table 3. A maximum amount of phenolic, flavonoid, tannin and alkaloid content was observed in the methanolic extract while the least was found in the aqueous extract.

Table 2. Phytochemical screening of various solvent extracts of *C. fistula*

Phytochemical compound	**Screening test**	**Solvents** Ethanol	Methanol	Aqueous
Alkaloids	Wagner's test	+	+	+
	Mayer's test	+	+	+
Cardiac glycosides	Keller kellani test	-	-	-
Flavonoids	Alkaline test	+	+	+
Phenolics	Ferric chloride test	+	+	+
Saponins	Foam test	+	+	+
Sterols	Liebermann-Burchard test	+	+	-
Tannins	Ferric chloride test	+	+	+
Terpenoids	Salkowaski's test	+	+	+
(+) indicates presence (-) indicates absence				

Table 3. Quantitative analysis of *C. fistula*leaf extracts

Solvents	Total Phenolic Content (mg GAE/g) DW.	Total Flavonoid Content (mg QUE/g) DW	Total Tannin Content (mg TAE/g) DW	Total Alkaloid Content (mg AE/g) DW
Methanol	13.38±0.060[c]	10.58±0.074[c]	16.18±0.062[c]	11.43±0.052[c]
Ethanol	10.58±0.063[b]	8.68±0.106[b]	8.91±0.065[b]	9.85±0.050[b]
Aqueous	5.88±0.153[a]	6.56±0.059[a]	7.5±0.157[a]	7.91±0.063[a]
(Results are mean ± standard deviation ; *p*=0.05 following ANOVA and LSD)				

Table 4. Antifungal activity of *C. fistula* leaf extracts with different solvents at various concentrations

Concentration (µg/ml)	Percent Inhibition(%)			
	Methanol	Ethanol	Aqueous	Bavistin
100	27.23±0.014[a]	25.49±0.014[a]	21.78±0.024[a]	25.08±0.038[a]
150	35.53±0.014[b]	30.81±0.014[b]	28.49±0.024[b]	55.60±0.038[b]
200	47.84±0.024[c]	43.54±0.043[c]	38.49±0.037[c]	71.06±0.014[c]
250	59.63±0.14[d]	55.75±0.029[d]	50.38±0.014[d]	86.70±0.029[d]
300	68.07±0.024[e]	65.31±0.025[e]	60.71±0.024[e]	94.67±0.025[e]
(Results are mean ± standard deviation ;*p*=0.05 following ANOVA and LSD)				

3.4 Antifungal Activity:

The percentage of mycelial growth inhibition of *M. phaseolina* was studied using plant extracts in various solvents. The antifungal effects of *C. fistula* plant extract were tested at five different concentrations (100, 150, 200, 250 and 300µg/ml) and represented in Table 4. At 300µg/ml, methanol extract effectively reduced the emergence of selected pathogens, i.e., 68.07% while aqueous extract appears less significant (60.71%). Results were compared with standard Bavistin are presented.

3.5 Total Antioxidant Activity

The efficacy of three distinct solvent extracts to scavenge DPPH free radicals was tested and compared to the standard, ascorbic acid. The methanolic extract of *C. fistula* was observed to be more active than the aqueous and ethanolic extracts (Fig. 1). However, the extracts' DPPH radical scavenging capacities were lower than those of ascorbic acid (82.29%) at 300µg/ml. This result clearly shows that the extracts have proton-donating potential and might be used as free radical inhibitors or scavengers, possibly functioning as primary anti-oxidants.

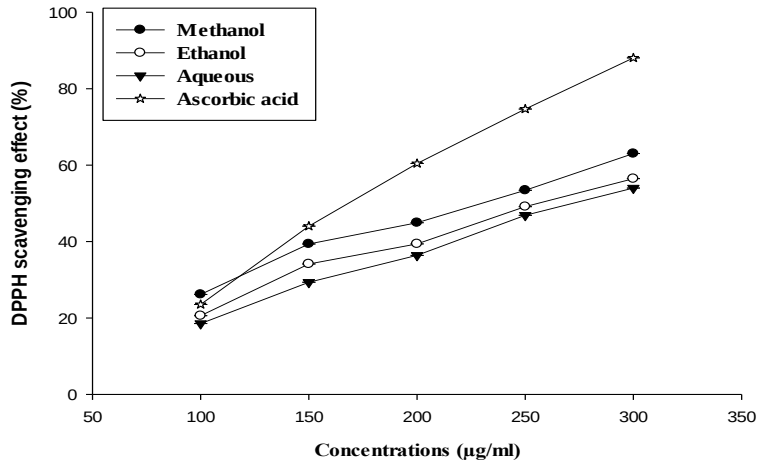

Fig. 1. Total antioxidant activity of *C. fistula* leaf extracts in different solvents and Ascorbic acid as standard

4. DISCUSSION

In the present study, the presence of high TPC values in the methanolic extract of *C.fistula* was observed. Plant phenolic compounds are powerful antioxidants [29,30] as well as antimicrobials [31,32]. Various studies have demonstrated flavonoids in plant extracts to have antioxidant and antifungal properties [33,34]. The presence of tannins in all extracts may explain their strong bioactivities, as tannins are known to have significant antioxidant and antimicrobial activities [30]. Alkaloids also have antimicrobial and antioxidant properties [35].

These findings give scientific evidence to support traditional therapeutic usage and highlight the possibility of developing an antimicrobial and antioxidant agent from the *C. fistula* plant. Sharma [36] revealed that methanolic extracts of *C. fistula* flowers, leaves, stem bark, and pulp exhibit strong antioxidant activities. Rajput et al. [37] evaluated the antifungal effects of *C. fistula* leaf extracts produced in acetone, methanol, and diethyl ether against *C. albicans* and found substantial results. Variable activities of plant components such as flowers, leaves, pods, seeds, and stem bark have been recorded in various solvents [38,39,40]. Previously, methanol extracts of *C. fistula* showed strong antibacterial and antifungal activity on microorganisms [41]. According to in vitro data, this medicinal plant looks to be fascinating and promising as a potential source for emerging antimicrobial and antioxidant pharmaceuticals.

5. CONCLUSION

To design realistic management methods, it is necessary to understand the compatibility of bio-control agents with other components of the production system. According to current research, certain plant extracts are a source of cost-effective and non-hazardous fungicides, towards human and environment, thus, *C. fistula* could be a good antifungal efficacy, which may be used for formulating new, safer, and ecofriendly fungicides.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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