Original Research Article

IN VITRO ANTIFUNGAL ACTIVITY OF *CASSIA FISTULA* L. EXTRACTS AGAINST *MACROPHOMINAPHASEOLINA* (TASSI.) GOID.

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ABSTRACT

Aims: To evaluate the anti fungal activity of *Cassia fistula* alcoholic and aqueous extracts against *Macrophominaphaseolina*.

Methods: Ethanolic, methanolic and aqueous extracts were prepared and qualitative and quantitative estimation 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%). While screening for secondary metabolites, cardiac glycosides were found to be lacking in all three solvent- extracts, while sterols and terpenoids are absent in the aqueous extract. Methanolic extract contained 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.). 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 furtherbe explored to formulate antifungal agents.

Keywords:*Antioxidant activity, antifungal activity, Cassia fistula, Macrophominaphaseolina*

1. INTRODUCTION

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

There is a high demandof chickpea seeds for itsnutritional 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 chickpea.The most destructive soil-borne disease is Dry Root Rot, which is spread on by *Macrophominaphaseolina* (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 from blooming to podding [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 fungicide, weedicide, pesticide, 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 neighbours. 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 take the stimulatory effects of growth into consideration [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, which 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 work, phytochemical, and antioxidant activity of *Cassia fistula* L., were determined and antifungal activity checked against *Macrophominaphaseolina*.

2. Material 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 obvious physical or microbiological problems. The leaves were cleaned with distilled water before being 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 (ethanol, methanol, and water) for 24–42 hours 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, labelled, 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 using standard methods [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 using standard procedures.

2.4 Determination of Antimicrobial activity

Macrophominaphaseolina (Tassi.) Goid, culture strains (NFCCI No. 4832) was 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 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 anioxidant 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 measured with a spectrophotometer. Ascorbic acid was utilized as a reference.

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 ANOVA at $p<0.05$. Appropriate post-hoc tests were performed, including the "Duncan Multi-Distance Test" (DMRT) and the "Least Significant Difference" (LSD).

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 significant.

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

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

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	+	+	+
Cardic 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 (-) indicate absence

3.3 Quantitative analysis:

The results for the quantitative estimation of leaf extract of *C. fistula* are tabulated in Table 3.Maximum amount of phenolic, flavonoid, tannin and alkaloid content wasobserved in themethanolic extract while least found in aqueous extract.

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)

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 pathogen, i.e., 68.07% while aqueous extract appears less significant (60.71%). Results were compared with standard Bavistin are presented.

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

Concentration	**Percent Inhibition**(%)			
(µg/ml)	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)

3.5 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 (Figure 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.

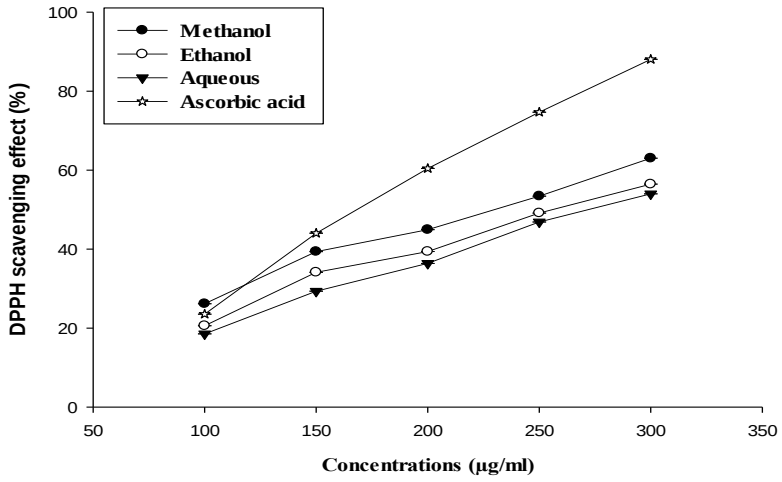

Figure 1. Total antioxidant activity of *C. fistula* leaf extracts in different solvents and Ascorbic acid as standard.

4. DISCUSSION:

In the present study, the presence of high TPC values in methanolic extract of *C.fistula* were 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 effects [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 botanical extracts are a source of cost effective and non-hazardous fungicides, as well as they have no human and environmental, health hazard or implications, so *C. fistula* could be a good antifungal efficacy, which may be used for formulating new, safer, and ecofriendly fungicides.

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