

Original Research Article

ANTIFUNGAL ACTIVITY OF *CASSIA FISTULA* L. EXTRACTS AGAINST *MACROPHOMINA PHASEOLINA* (TASSI.) GOID.: **IN VITRO STUDY**

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.

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. 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 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 (%) = $W_2/W_1 \times 100$, where W_2 =weight of the extract after evaporating solvent, W_1 =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 28-30°C 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.

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	+	+	+
	Keller kellani test	-	-	-
Cardiac glycosides				
Flavonoids	Alkaline test	+	+	+
Phenolics	Ferric chloride test	+	+	+
Saponins	Foam test	+	+	+
Sterols	Liebermann-Burchard test	+	+	-
Tannins	Ferric chloride test	+	+	+
Terpenoids	Salkowski's test	+	+	+

(+) indicates presence (-) indicates absence

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 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 pathogens, 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 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)

3.5 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.

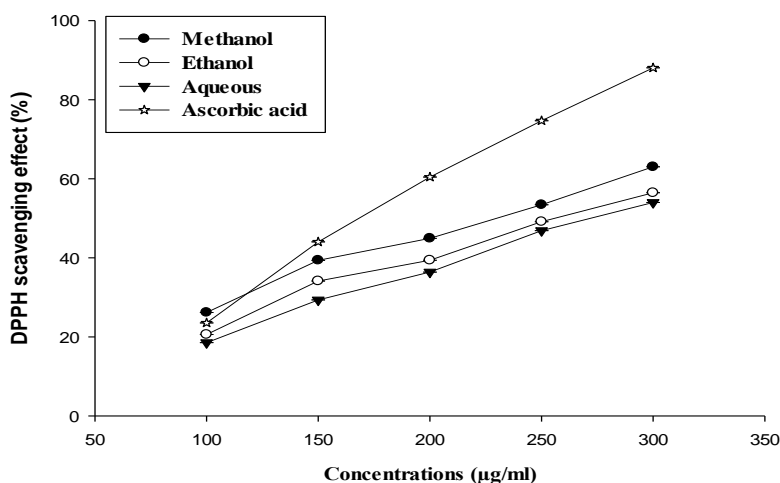


Figure 1. Total antioxidant activity of *C. fistula* leaf extracts in different solvents and Ascorbic acid as standard.

4. DISCUSSION:

In the present study, the presence of high TPC values in 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.

REFERENCES

1. Lewis GP, Schrire BD, Mackinder BA, Rico L, Clark R. A linear sequence of legume genera set in a phylogenetic context—a tool for collections management and taxon sampling. *South African Journal of Botany*. 2013 Nov 1;89: 76-84.
2. Wallace TC, Murray R, Zelman KM. The nutritional value and health benefits of chickpeas and hummus. *Nutrients*. 2016 Nov 29;8(12):766.
3. Anonymous. Technical Manual - Chapter: 2 General Properties of Dry Peas, Lentils & Chickpeas. DPLC/Dry Pea and Lentil Council, USA. 2015. <http://www.usapulses.com/chapter-2-generalproperties.htm>.
4. Dubey S, Raghav RS, Singh P. Enhancement of productivity for chickpea (*Cicerarietinum* L.) through front line demonstration in farmer's fields. *Legume Research*. 2017;40(2):335-7.
5. Rai A, Irulappan V, Senthil-Kumar M. Dry root rot of chickpea: a disease favored by drought. *Plant Disease*. 2022 Feb 28;106(2):346-56.
6. Bhandari S, Poudel DK, Marahatha R, Dawadi S, Khadayat K, Phuyal S, Shrestha S, Gaire S, Basnet K, Khadka U, Parajuli N. Microbial enzymes used in bioremediation. *Journal of Chemistry*. 2021 Feb 8; 2021:1-7.
7. Jabran K. Allelopathy: Introduction and concepts. In: (Jabran K. Eds.) *Manipulation of Allelopathic Crops for Weed Control*. Springer Briefs in Plant Science. Springer, Cham. 2017:1-12.
8. Rice EL. *Allelopathy*. Academic Press Inc. The University of Okalahoma. Norman Oklahoma. 1984.

9. Zimdahl RL. Fundamentals of Weed Science. Academic Press; 2018 Feb 7.
10. Journet ARP. Etherington, J.R. Plant Physiological Ecology. Bryologist; 2006, 82, 508.
11. Rana R, Saklani K, Gaurav N. Phytochemical Analysis and Antimicrobial Activity of Leaf and Seed Extract of *Cassia fistula*. International Refereed Multidisciplinary Journal of Contemporary Research. 2017;5(4):24-30.
12. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews. 1999 Oct 1;12(4):564-82.
13. Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. Indian Journal of Pharmacology. 2000 Jul 1;32(4): S81-118.
14. Irshad M, Eneji AE, Hussain Z, Ashraf M. Chemical characterization of fresh and composted livestock manures. Journal of Soil Science and Plant Nutrition. 2013 Mar;13(1):115-21.
15. Rajeswari K, Sankar G, Rao A, Seshagirirao JV. RP-HPLC method for the simultaneous determination of Atorvastatin and Amlodipine in tablet dosage form. Indian Journal of Pharmaceutical Sciences. 2006 Mar 1;68(2).
16. Irshad M, Zafaryab M, Singh M, Rizvi M. Comparative analysis of the antioxidant activity of *Cassia fistula* extracts. International Journal of Medicinal Chemistry. 2012;2012.
17. Kumar VP, Chauhan NS, Padh H, Rajani M. Search for antibacterial and antifungal agents from selected Indian medicinal plants. Journal of Ethnopharmacology. 2006 Sep 19;107(2):182-8.
18. Tanveer SA, Latif AB, Ashiq KA, Qayyum ME, Bajwa MA. A comprehensive review on pharmacological and phytochemical potential of *Cassia fistula* L: A Magical Herb. International Journal of Biology, Pharmacy and Allied Science. 2019;8(6):1134-57.
19. Irshad M, Mehdi SJ, Al-Fatlawi AA, Zafaryab M, Ali A, Ahmad I, Singh M, Rizvi MM. Phytochemical composition of *Cassia fistula* fruit extracts and its anticancer activity against human cancer cell lines. Journal of Biologically Active Products from Nature. 2014 May 4;4(3):158-70.
20. Lahare RP, Yadav HS, Bisen YK, Dashahre AK. Estimation of total phenol, flavonoid, tannin and alkaloid content in different extracts of *Catharanthus roseus* from Durg district, Chhattisgarh, Indian School Bulletin 2021; 7:1-6.
21. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 2005 Aug 19;4(7):685-8.
22. Chakraborty D, Mandal SM. Fractional changes in phenolic acids composition in root nodules of *Arachishypogaea* L. Plant Growth Regulation. 2008 Jul; 55:159-63.
23. CI KC, Indira G. Quantitative estimation of total phenolic, flavonoids, tannin and chlorophyll content of leaves of *Strobilanthes kunthiana* (Neelakurinji). Journal of Medicinal Plants. 2016; 4:282-6.
24. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complementary and Alternative Medicine. 2012 Dec; 12:1-2.
25. Aliyu AB, Ibrahim MA, Musa AM, Musa AO, Kiplimo JJ, Oyewale AO. Free radical scavenging and total antioxidant capacity of root extracts of *Anchomanes difformis* Engl. (Araceae). Acta Poloniae Pharmaceutica 2013 Jan 1;70(1):115-21.
26. Govindappa M, Naga SS, Poojashri MN, Sadananda TS, Chandrappa CP. Antimicrobial, antioxidant and in vitro anti-inflammatory activity of ethanol extract and active phytochemical screening of *Wedelia trilobata* (L.) Hitchc. Journal of Pharmacognosy and Phytotherapy. 2011 Apr;3(3):43-51.
27. Chakraborty D, Mandal SM, Chakraborty J, Bhattacharyaa PK, Bandyopadhyay A, Mitra A, Gupta K. Antimicrobial activity of leaf extract of *Basilicum polystachyon* (L) Moench. 2007; 45(8): 744-748.doi: <http://nopr.niscpr.res.in/handle/123456789/5316>

28. Alam MN, Bristi NJ, Rafiquzzaman M. Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*. 2013; 21(2): 143-152. doi: <https://doi.org/10.1016/j.jsps.2012.05.002>
29. Adedapo AA, Jimoh FO, Koduru S, Masika PJ, Afolayan AJ. Assessment of the medicinal potentials of the methanol extracts of the leaves and stems of *Buddleja saligna*. *BMC Complementary and Alternative Medicine*. 2009 Dec;9(1):1-8.
30. Adesegun SA, Fajana A, Orabueze CI, Coker HA. Evaluation of antioxidant properties of *Phaulopsis fascisepala* CB Cl. (Acanthaceae). *Evidence-Based Complementary and Alternative Medicine*. 2009 Jun 1; 6:227-31.
31. Lai HY, Lim YY, Kim KH. *Blechnum orientale* Linn-a fern with potential as antioxidant, anticancer and antibacterial agent. *BMC Complementary and Alternative Medicine*. 2010 Dec; 10:1-8.
32. Kaur GJ, Arora DS. Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi*. *BMC complementary and alternative medicine*. 2009 Dec;9(1):1-0.
33. Yoshida T, Konishi M, Horinaka M, Yasuda T, Goda AE, Taniguchi H, Yano K, Wakada M, Sakai T. Kaempferol sensitizes colon cancer cells to TRAIL-induced apoptosis. *Biochemical and Biophysical Research Communications*. 2008 Oct 10;375(1):129-33.
34. Lopez-Lazaro M. Distribution and biological activities of the flavonoid luteolin. *Mini Reviews in Medicinal Chemistry*. 2009 Jan 1;9(1):31-59.
35. Mandal P, Babu SS, Mandal NC. Antimicrobial activity of saponins from *Acacia auriculiformis*. *Fitoterapia*. 2005 Jul 1;76(5):462-5.
36. Saeed M, Naseer S, Hussain S, Iqbal M. Phytochemical composition and pharmacological effects of *Cassia fistula*. *Scientific Inquiry and Review*. 2020 Mar 19;4(1):59-69.
37. Rajput M, Kumar N. Medicinal plants: A potential source of novel bioactive compounds showing antimicrobial efficacy against pathogens infecting hair and scalp. *Gene Reports*. 2020 Dec 1; 21:100879.
38. Duraipandiyar V, Ignacimuthu S. Antibacterial and antifungal activity of *Cassia fistula* L.: An ethnomedicinal plant. *Journal of Ethnopharmacology*. 2007 Jul 25;112(3):590-4.
39. Panda SK, Brahma S, Dutta SK. Selective antifungal action of crude extracts of *Cassia fistula* L.: A preliminary study on *Candida* and *Aspergillus* species. *Malaysian Journal of Microbiology*. 2010 Jun 1;6(1):62-8.
40. Ali MA, Sayeed MA, Bhuiyan MS, Sohel FI, Yeasmin MS. Antimicrobial screening of *Cassia fistula* and *Mesua ferrea*. *Journal of Medicinal Sciences*. 2004;4(1):24-9.
41. Archana H, Bose VG. Evaluation of phytoconstituents from selected medicinal plants and its synergistic antimicrobial activity. *Chemosphere*. 2022 Jan 1; 287:132276.