RESEARCH ARTICLE

ANTIFUNGAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS FROM STEM AND LEAF OF *Cassia fistula*

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ABSTRACT

Cassia fistula has been recognized as a multipurpose medicinal plant which has been used to treat blood disorders, cardiac problems, diabetic mellitus, and skin diseases in Ayurvedic medicine. This study aimed to investigate the antifungal activity of different extracts of stem and leaf of *C. fistula* against *Aspergillus niger* and *Candida albicans* as in-*vitro.* The plant parts of *C. fistula* were collected in Kurunegala, Sri Lanka. It was cleaned, shade- dried and powered. The powdered parts were macerated with petroleum ether, ethyl acetate and methanol separately. The antifungal activity was determined using agar well diffusion method against *tested* fungi by employing Itraconazole as the standard. The extracts were screened for phytochemicals separately. The obtained diameter of zones of inhibition (mm) of the extracts was evaluated via analysis of variance (ANOVA) ($P<0.05$) by using SPSS (version 25). The results indicated that all the extracts of *C. fistula* showed antifungal activity against *A. niger and C. albicans* except petroleum ether extract of the stem, which showed no activity against *A. niger*. Phytochemical analysis revealed that the extracts of *C. fistula* contain different phytochemicals including alkaloids, terpenoids, tannins and phenolic compounds which were responsible for the antifungal activity. The inhibitory effects showed by standard, and extract of *C. fistula* differed significantly ($P<0.05$). Further identification of antifungal active phytochemicals present in the extracts facilitates the formulation of new pharmaceuticals.

Keywords: *Cassia fistula*, *Candida albicans*, *Aspergillus niger*, Phytochemicals, antifungal activity,

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1. INTRODUCTION

Antifungals are substances that are used to prevent the growth of fungi. Severe fungal infections have been more common since the mid-twentieth century [1]. The rise in fungal infections is attributable to an increase in the number of individuals with impaired immune systems, such as those suffering from acquired immunodeficiency syndrome (AIDS), and those who have undergone organ transplantation and chemotherapy [2].

Only a few pharmacological classes are available as antifungal agents compared to the medication classes available for antibacterial agents. Polyenes, Triazoles and Echinocandins are antifungal medications that are commonly utilized in clinical practice. Triazoles, for example, have decreased efficiency because they are resistant to some fungal strains [3]. Therefore, there is a need to discover new antifungal agents.

Plants are one of the important sources of antifungal agents. Traditional use of plants as remedies indicate that plants are a source of pharmaceuticals. Earlier the crude form of drug obtained from plants was used to treat a variety of diseases since they were readily available and inexpensive [4]. At present plants are selected as sources of drugs due to the structural diversity of the substances which serve as templates for modern drug synthesis, the number of diseases that can be potentially prevented or treated by these medications and their frequency of usage in disease treatment [5]. In this regard, plant secondary metabolites are currently exploited in medicine development. Only around 15% of the 300,000 species of plants on the globe have been examined to identify their therapeutic effects which encourages to find out the new bioactive compounds from natural origin [6].

There are several plant species that have antifungal activity which has long been utilized in traditional medicine. *Cassia fistula*, *Albizzia procera*, *Costus speciosus* and *Toddalia asiatica* are some of the most well-known plants with antifungal properties [7].

Cassia fistula is a valued medicinal plant due to its wide range of medicinal characteristics and therapeutic applications. *C. fistula* belongs to the Fabaceae family of plants. Golden shower tree is the popular name for this plant. The tree is called as ‘Konrai’ in Tamil and as ‘Ehela’ in Sinhala. East Africa, Sri Lanka, China, India,

Mauritius, Amazon, Brazil and Mexico are some regions that are among the tree's native habitats [8].

The plant has many pharmacological activities such as antitussive, central nervous system activity, leukotriene inhibition, antipyretic, antioxidant, laxative, anti-inflammatory, wound healing activity, hepatoprotective effect, antifungal, larvicidal and ovicidal activity, hypocholesterolemic, hypoglycemic, hypolipidemic, antibacterial, antitumor, anti-parasitic and anti-leishmaniatic activities [9].

The primary and secondary metabolites of *C. fistula* plant have been studied in numerous research. As the primary metabolites, it comprises significant amounts of amino acids, carbohydrates, fatty acids, different minerals and proteins. As secondary metabolites, *C.fistula* contains flavonoids, glycosides, proanthocyanidins, essential oils, terpenoids and polyphenols [10].

C. fistula extracts of leaves, bark and seeds were effective against the fluconazole resistant candida. The potent antifungal agent is gallic acid. Leaves extracts of *C. fistula* are inhibitory towards pathogenic fungi [11]. The flower extract of *C.fistula* has antifungal activity due to the presence of Rhein [12]. Seed extract of *C. fistula* has roseanone as a chemical constituent which is responsible for anti-yeast activity [13]. The methanolic extracts of the bark, root and leaves of *C. fistula* possess antifungal activity [14].

Many studies have been done related to the anti-fungal activities of *C. fistula* in different countries and also, in the different regions of particular country, as the phytochemical composition of a plant varies within region. The presence of type and composition of phytochemicals in the plant is influenced by geographical and ecological factors and the polarity of the solvents effects the type of the phytochemical need to be extract and it could affect the anti-fungal activity [15]. Further, there have been studies conducted on the antifungal activity of leaves of *C. fistula* in different region of Sri Lanka, however, there have been no investigation done on the antifungal activity of leaves of *C. fistula* collected from Kurunegala. Moreover, antifungal activity of stem of *C. fistula* has not been investigated so far. Therefore, this study was carried out to screen the antifungal activity of *C. fistula* plants collected in Kurunegala by using parts such as the stem and

leaf and employing polarity-differentiated solvents such as petroleum ether, ethyl acetate and methanol against pathogenic fungi that cause infections in human.

2. MATERIAL AND METHODS

2.1 Collection and identification of plant material

Fresh and healthy leaves and stem of *Cassia fistula* were collected from home gardens in Kurunegala, Sri Lanka and these plants were authenticated taxonomically by Professor Priyangani Senanayake, Department of Plant and Molecular Biology at University of Kelaniya. The collected materials were washed under running tap water and allowed to dry in sunshade. They were ground into fine powder using an electric grinder. The powders were kept in air sealed brown bottles for further studies.

2.2 Preparation of plant extracts

Three different solvents namely methanol, ethyl acetate and petroleum ether were used for the extraction. For that homogenized dry powder of the leaves with weight approximately 50 g were soaked separately in Duran bottles each containing 500 mL of petroleum ether, ethyl acetate and methanol. The macerated flasks were allowed to stand for 48 hours with intermittent shaking. The supernatant was decanted and were filtered through Whatman No 1 filter paper with pore size 11 µm using suction pump. The solvents were removed using rotary evaporator under reduced pressure below 45 ºC. The concentrated extracts were placed in a vacuum desiccator for further dryness. The yield of each extract was weighed and stored at 4 °C until used. The same procedure was repeated for the dry powder of stem [16].

2.3 *In vitro* screening of antifungal activity

2.3.1 Collection of fungal strains

Two fungal strains were used in this study. They were *Candida albicans* and *Aspergillus niger*, obtained from the culture collections of Department of Botany, University of Jaffna. Fungal strains were subcultured on Sabouraud Dextrose Agar (SDA) medium and incubated at ambient temperature. All fungal suspensions were prepared by suspending fungal culture in sterile saline solution for 72 hrs. The concentration of the suspensions was adjusted 0.5 McFarland standard (Equivalent to 1.5×10^8 CFU/mL).

2.3.2 Antifungal assay

In vitro antifungal activity of leaf and stem crude extracts of *C.fistula* against test organisms were determined by agar well diffusion method in triplicates [11] . Autoclaved Sabouraud dextrose agar (SDA) medium was cooled down to 40 °C, and then each sterile Petri plates was filled with approximately 20 mL of SDA medium and allowed to solidify. Initially the test extracts were prepared by dissolving 50 mg of each extract in 10 mL of methanol, ethyl acetate and petroleum ether separately. Then the concentrations of 2.5 mg/mL and 1.25 mg/mL were prepared from the stock solution by the serial dilution method. The standard, Itraconazole was prepared by dissolving 100 mg in 5 mL distilled water, from that the concentration of 200 µg/mL was obtained. A sterile cotton swab was dipped into the adjusted fungal suspension and the entire surface of the sterile SDA plate was swabbed. A sterile cork borer of 6 mm diameter was used to make wells on agar plate and 100 μL of test plant extracts were added to each well using micropipettes. Whereas Itraconazole (200 µg/ml) and 100 µl of methanol, ethyl acetate and petroleum ether were used as standard and control respectively.

The plates were incubated at room temperature for 2-5 days. Antifungal activity was determined by measuring the diameter of inhibition zone around the well. The average values were taken after taking readings in two different directions.

2.3.3 Activity Index

Activity index (AI) used to measure the antifungal activity in comparison with standard, which is calculated by the following formula;[17]

$$AI = \frac{Zone\ of\ inhibition\ shown\ by\ extracts}{Zone\ of\ inhibition\ shown\ by\ standard}$$

2.4 Phytochemical screening

Preliminary phytochemical tests were done to identify the primary and secondary metabolites in the extracts. The extracts were tested for the identification of carbohydrate, protein, lipids, Cardiac glycoside, Terpenoids, Flavanoids, Alkaloids, Tannins, Sterol, Phenolic compound and Anthraquinone as described in the literature [18][19]. The results of preliminary phytochemical tests were indicated by the presence of phytochemicals as “+” and the absence of phytochemicals as “-”.

2.5 Data analysis

All analysis were undertaken in triplicate and quantitative values were presented as means ± standard deviation. The statistical significance was evaluated by the analysis of variance (ANOVA) followed by Tukey's test using SPSS version 25. Differences between means were considered significant if P-values lower than 0.05 ($p<0.05$)[20][21].

3 RESULTS AND DISCUSSION

3.1 Yield percentages of different extracts of *C. fistula*

The yield in percentage of methanol, ethyl acetate and petroleum ether extracts of *C. fistula* are shown in Table 1.

Table 1: Yield percentages of different extracts

Parts of plant	Type of solvent	Weight of crude extract (g)	Yield percentage (% w/w)
Leaf	Methanol	3.250	6.5
	Ethyl acetate	1.883	3.7
	Petroleum ether	1.481	3.0
Stem	Methanol	0.769	1.5
	Ethyl acetate	0.870	1.7
	Petroleum ether	0.212	0.4

The yield in percentages of the methanol, ethyl acetate and petroleum ether extracts of the leaf of *C. fistula* were 6.5%, 3.7% and 3.0% respectively. The yield percentages of the methanol, ethyl acetate and petroleum ether extracts of the stem of *C. fistula* were 1.5%, 1.7% and 0.4 % respectively.

A study was conducted in Jaffna by Jeyaseelan *et.al*, 2012, revealed that the yield percentage of the petroleum ether extract of the leaf (2.2%) was slightly lower than this study (3.0%). Meanwhile, the yield percentage of the ethyl acetate extract of leaf was higher (4.16%) compared to this study (3.7%)[22]. A study was conducted by Panda *et.al*, 2010, exhibited the yield of the methanol extract of the leaf was lower (4.34%) compared to the yield in this study (6.5%) [23]. The yield percentage of the stem was reported here for the first time.

The reason for the variation in the yield percentages can be due to the difference in the ecological and geographical variation of plant material which can affect the composition of the phytoconstituents present in the different parts of the plant [24][25]. Meanwhile, the method that was used to extract the chemical constituents, type of solvent used, and the time taken for soaking and shaking of the extracts can influence the amount and type of phytoconstituent extracted from the plant parts [26].

3.2 Antifungal activity

3.2.1 Mean zones of inhibition by agar well diffusion method

The *in vitro* screening of antifungal activity of *C. fistula* leaf and stem extracts were done against two pathogenic fungal species *A.niger* and *C.albicans*. The test was done in triplicate for each concentration of each extract and the standard. The mean and standard deviations of the zones of inhibition against *A.niger, C.albicans* are shown in Table 2 and Table 3 respectively. The zone of inhibition was increased with the increasing concentration of the extract. Meanwhile, there was no zone of inhibition observed for the negative controls. All the tested extracts exhibited antifungal activity against *A. niger* and *C. albicans* except petroleum ether extracts of the stem. When observing the zones of inhibition, in general, the growth of both the fungal strains was inhibited by the different extracts of *C. fistula*.

Table 2 The inhibitory effect of C.fistula at different extracts concentrations on A.niger.

Part of plant	Extract	Concentration (mg/mL)	Mean ± Std deviation of zone of inhibition (mm)	AI
Leaf	Petroleum ether	5	13.7 ± 0.577^{de}	0.51
		2.5	12.3 ± 1.12^{de}	0.46
		1.25	11.7 ± 0.577^{e}	0.43
	Ethyl acetate	5	19.3 ± 0.577^{a}	0.72
		2.5	16.3 ± 0.577^{bc}	0.60
		1.25	14.3 ± 0.577^{cd}	0.53
	Methanol	5	21.3 ± 1.16^{a}	0.79
		2.5	16.7 ± 0.577^{b}	0.62
		1.25	12.0 ± 0.000^{e}	0.44
	Petroleum ether	5	$.00 \pm 0.000^{d}$	0.00
		2.5	$.00 \pm 0.000^{d}$	0.00
		1.25	$.00 \pm 0.000^{d}$	0.00

Stem	Ethyl acetate	5	9.33 ± 0.577^{a}	0.35
		2.5	7.33 ± 0.577^{b}	0.27
		1.25	6.33 ± 0.577^{bc}	0.23
	Methanol	5	8.67 ± 0.577^{a}	0.32
		2.5	7.33 ± 0.577^{b}	0.27
		1.25	6.00 ± 0.000^{c}	0.22
	Itraconazole	0.2	27.0 ± 0.000	1.00

Values are represented as mean±standard deviation; Values with different superscripts in the same column differ significantly (P<0.05)

Table 3 The inhibitory effect of C.fistula at different extracts concentrations on C. albicans.

Part of plant	Extract	Concentration mg/mL	Mean ± Std. Deviation of zone of inhibition (mm)	AI
Leaf	Petroleum ether	5	18.67 ± 0.577^{b}	0.79
		2.5	15.67 ± 0.577^{de}	0.66
		1.25	14.67 ± 0.577^{e}	0.62
	Ethyl acetate	5	22.33 ± 0.577^{a}	0.94
		2.5	17.67 ± 0.577^{bc}	0.75
		1.25	15.33 ± 0.577^{de}	0.65
	Methanol	5	18.67 ± 0.577^{b}	0.79
		2.5	16.33 ± 0.577^{cd}	0.69
		1.25	15.00 ± 0.000^{de}	0.63
Stem	Petroleum ether	5	13.33 ± 0.577^{c}	0.56
		2.5	12.33 ± 0.577^{c}	0.52
		1.25	10.33 ± 0.577^{d}	0.44
	Ethyl acetate	5	18.00 ± 0.000^{b}	0.76
		2.5	16.67 ± 0.577^{b}	0.70
		1.25	12.33 ± 0.577^{c}	0.52
	Methanol	5	22.33 ± 0.577^{a}	0.94
		2.5	21.33 ± 0.577^{a}	0.90
		1.25	17.67 ± 0.577^{b}	0.75
	Itraconazole	0.2	23.67 ± 0.577	1.00

Values are represented as mean±standard deviation; Values with different superscripts in the same column differ significantly (P<0.05)

Evaluation using Tukey test revealed that that there was a significant difference ($P > 0.05$) in the zone of inhibition among the different extracts of *C. fistula* against the *A. niger*.

Among the leaf extracts of *C. fistula*, the methanol extract exhibited highest activity at 5 mg/mL (21.33±1.55 mm) against *A. niger*. This result was comparable to the previous study done in India by Hajra *et.al* [27].

The ethyl acetate extract of the leaf against *A. niger* showed higher zone of inhibition at 5 mg/mL (19.33±.577) compared to petroleum ether extract of leaf (13.67±.577 mm). A study conducted in Bangladesh by Ali *et.al* also demonstrated similar results for the inhibition of *A. niger* by *C. fistula* leaf extracts [28].

The lowest zones of inhibitions at 5 mg/mL were observed for the ethyl acetate and methanol extracts of the stem among the other extracts for *A.niger* which were 9.33±.577 mm and 8.67± .577 mm respectively.

Evaluating the zone of inhibition against *C. albicans* the using Tukey test revealed that there was a significant difference was noticed in the inhibition zone produced by the different extracts of *C. fistula.*

Ethyl acetate extract of leaf showed better activity compared with other leaf extracts. The zone of inhibition for ethyl acetate was 22.33±.577 mm with 0.94 AI at 5 mg/mL concentration. The methanol and petroleum ether extracts of the leaf showed identical activity with a mean zone of inhibition of 18.67±.577 mm against *C. albicans* at the same concentration. The zone of inhibition exhibited by the standard Itraconazole against *C. albicans* was 23.67±.577 mm. According to previous study, the zones of inhibition obtained for the methanol and petroleum ether extracts of leaf against *C. albicans* were 10.6±.5mm and 12.6±.5mm respectively [21]. These values were lower compared to zones measured in this study.

Among the stem extracts, the highest activity against *C. albicans* was exhibited by the methanol extract and the clear zone was observed as 22.33±0.577 mm with 0.94 AI value at 5mg/mL concentration.

3.2.2 Preliminary phytochemical screening

Table 4 Preliminary phytochemical screening of different extracts of Cassia fistula

No.	Phytochemicals	Leaf			Stem		
		M	**EA**	**PE**	**M**	**EA**	**PE**
1	Carbohydrate	+	-	+	+	+	+
2	Protein	-	-	+	-	+	+
3	Lipid	+	-	+	+	+	+
4	Cardiac glycoside	-	-	+	-	-	-
5	Alkaloids	+	+	+	-	-	+
6	Terpenoids	+	+	+	+	-	-
7	Flavonoids	+	+	+	+	-	-
8	Tannins	+	+	+	-	+	+
9	Phenols	+	+	+	+	-	-
10	Sterol	-	-	-	+	+	+
11	Anthraquinones	-	-	-	-	-	-

+ present; - absent; M- Methanol; EA – Ethyl acetate; PE- Petroleum ether

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Table 4 indicates the presence of different phytochemicals in the different extracts of *C. fistula*. Phytochemical analysis revealed the presence of alkaloid, terpenoids, flavonoids, tannins and phenols in all the leaf extracts. Sterols and anthraquinones were absent from all the leaf extracts. The similar results were obtained for the leaf extracts of methanol and ethyl acetate in a previous study, conducted in India[29].

The presence of sterols, carbohydrate and lipids were observed in all the extracts of the stem. Among the stem extracts, alkaloids were present only in the petroleum ether extract. Terpenoids, flavonoids and phenols were present in the methanolic extract of the stem. Tannins were present only in the ethyl acetate and petroleum ether extracts of the stem. Anthraquinones were absent in all the parts of *C. fistula* in this study.

According to previous study the metabolites that exhibit antifungal activity were flavonoids, tannins, phenols, alkaloids and peptides[30]. The antifungal activity exhibited by the different extracts of *C. fistula* may be due to the presence of the above- mentioned phytochemicals.

The methanol extracts of *C. fistula* showed highest antifungal activity than other two extracts for both strain except methanol extract of stem against *C. albicans*. An earlier study revealed that most of the phytochemicals were present in the polar extracts of the plant[15]. Meanwhile, *Pinto et.al* mentioned that the presence of flavonoids, tannins, coumarins, quinones, lignans, and neolignans in a natural source which showed strong antifungal activity[31]. Similar to the previous study, alkaloid, flavonoid, tannin and phenol were present in the extracts of leaf, this could be contributed to the higher antifungal activity of the plant.

4 CONCLUSION

Among the different parts of *C. fistula*, the leaf extracts showed the highest antifungal activity against the two fungal pathogens, *A.niger* and *C.albicans* and stem showed highest activity against *C.albicans* at 5 mg/ mL concentration. The other extracts exhibited moderate antifungal activity. The results revealed that antifungal activity of leaf of *C.fistula* may be useful to treat the infections caused by tested fungal strains. However, further studies are required to isolate the active phytochemicals which were corresponding to the antifungal activity.

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