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Evaluation of Antibacterial and DPPH Radical Scavenging Activities of the Leaf Extracts of *Cassia fistula* Linn from South India

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Abstract

The antibacterial and DPPH radical scavenging activities of the leaf extracts of *Cassia fistula Linn* were investigated. The antibacterial potential of the petroleum ether, chloroform, ethyl acetate and methanol extracts of the leaves of *Cassia fistula Linn* were studied against human pathogenic bacteria viz. *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens* by 'agar well diffusion' method. Leaf ethyl acetate, chloroform and methanol extracts of *Cassia fistula Linn* exhibited pronounced activity against Gram-positive and Gram-negative bacteria and their activity is quite comparable with the standard antibiotics such as tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin screened under similar conditions.

Among the leaf extracts of *Cassia fistula* studied, methanol extract showed potent scavenging activity on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. The remarkable antibacterial and antioxidant activity exhibited by the plant extract can be attributed to the synergic effect of the active compounds present in it. The results obtained showed that the leaf methanol extract of *Cassia fistula* can be considered as good sources of natural antioxidants and antimicrobial compounds and can be incorporated into the drug formulations.

Keywords: *Cassia fistula*; Antibacterial activity; Agar well diffusion method; DPPH radical scavenging activity; Drug formulations

Introduction

Cassia fistula L., (Leguminosae), a semi-wild Indian Labernum (also known as the Golden Shower), is distributed in various countries including Asia, South Africa, Mexico, China, West Indies, East Africa and Brazil. It is an ornamental tree with beautiful bunches of yellow flowers [1]. *Cassia fistula* plant parts are known to be an important source of secondary metabolites, notably phenolic compounds [2]. The herb contains anthraquinones, flavonoids and flavan-3-ol derivatives [1]. The seeds are rich in glycerides with linoleic, oleic, stearic and palmitic acids as major fatty acids together with traces of caprylic and myristic acids [3]. Besides phenolics and their derivatives, a certain amount of alkaloids have also been reported in the flowers [4], while traces of triterpenes have been observed in both flowers and fruits. Four new compounds, 5-(2-hydroxyphenoxymethyl) furfural, (2'S)-7-hydroxy-5-hydroxymethyl-2-(2'-hydroxypropyl) chromone, benzyl-2-hydroxy-3, 6-dimethoxybenzoate, and benzyl 2β-O-D-glucopyranosyl-3,6-dimethoxy benzoate, together with four known compounds, 5-hydroxymethylfurfural, (2'S)-7-hydroxy-2-(2'-hydroxypropyl)-5-methylchromone, and two oxyanthraquinones, chrysophanol and chrysophanein, were also isolated from the seeds of *Cassia fistula* [5].

The whole plant possesses medicinal properties useful in the treatment inflammatory diseases, rheumatism, anorexia and jaundice [6]. Singh SK and Singh S [7] isolated *Cassia fistula Linn*. seed mucilage and evaluated the potential of the mucilage as a binder for conventional tablet formulations. A new bioactive flavone glycoside 5,3',4'-trihydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)- O- β-D-galactopyranoside with antimicrobial activity was reported [8]. Anti-inflammatory and antioxidant activities of the aqueous and methanolic extracts of the *Cassia fistula Linn*. bark were assayed in albino rats [9]. It has been reported that the stem bark of *Cassia fistula* is also a potential source of lupeol, β-sitosterol and hexacosanol [10].

The plant has a high therapeutic value and it exerts an antipyretic and analgesic effect [11]. Besides its pharmacological uses, the plant extract is also recommended as a pest and disease control agents in India [12-14]. *Cassia fistula* plant organs are known to be an important source of secondary metabolites, notably phenolic compounds. Fistucacidin, an optically inactive leucoanthocyanidin (3,4,7,8,4'-pentahydroxyflavan) was first extracted from the heartwood [15]. Vaishnav and Gupta [16] (1996) showed the presence of rhamnetin 3-O-gentibioside in *Cassia fistula* roots. The compound 1, 8-dihydroxy-3-anthraquinone carboxylic acid was isolated from the pods [17]. A bianthraquinone glycoside, fistulin, together with kaempferol has been isolated from ethanolic extracts of *Cassia fistula* flowers [18]. Misra et al. [19] isolated a new diterpene, 3β-hydroxy-17-norpimar-8(9)-en-15-one from the pods of *Cassia fistula*.

There is a still not sufficient study about *Cassia fistula* and there must be a research focused on achieving the definitive knowledge on this plant and utilization as antioxidant and antibacterial agent. Hence the present investigation focuses on the evaluation of antioxidant and antibacterial activities of the leaf extracts of *Cassia fistula Linn*.

Materials and Methods

Plant material

The leaves of *Cassia fistula* were collected from Thrissur district of Kerala, South India and authenticated by Dr. Kochuthressia M.V., HOD, Department of Botany, Vimala College, Thrissur. Voucher

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specimen is deposited in the specially maintained herbarium, Department of Botany, Vimala College, Thrissur.

Preparation of plant extracts

Fifty grams of the powered plant material were extracted successively with 150 mL of petroleum ether, chloroform, ethyl acetate and methanol as solvents for 24 hours by Soxhlet equipment.

Test microorganisms

The microorganisms used for antibacterial activity evaluation were obtained from Microbial Type Culture Collection and gene bank (IMTECH, Chandigarh, India), which were maintained on Nutrient broth media. They were Gram-positive bacteria such as *Bacillus cereus* (MTCC-1305), *Staphylococcus aureus* (MTCC-96) and *Enterobacter faecalis* (MTCC-5112) and Gram-negative bacteria such as *Salmonella paratyphi* (MTCC-735), *Escherichia coli* (MTCC-729), *Klebsiella pneumoniae* (MTCC-109), *Pseudomonas aeruginosa* (MTCC-647), *Proteus vulgaris* (MTCC-426) and *Serratia marcescens* (MTCC-86).

Culture medium and inoculums

The stock cultures of microorganisms used in this study were maintained on Plate Count Agar slants at 4°C. Inoculum was prepared by suspending a loop full of bacterial cultures into 10 mL of nutrient broth and was incubated at 37°C for 24 hours. On the next day Muller-Hinton agar (MHA) (Merck) sterilized in a flask and cooled to 45-50°C was distributed by pipette (20 mL) into each sterile Petri dish and swirled to distribute the medium homogeneously. About 0.1 mL of bacterial suspension was taken and poured into Petri plates containing 20 mL nutrient agar medium. Using the L-shaped sterile glass spreader bacterial suspensions were spread to get a uniform lawn culture.

Antibacterial activity assay

The agar well diffusion method is used for the antimicrobial evaluations. Wells of 8mm (0.8cm) diameter were dug on the inoculated nutrient agar medium with sterile cork borer and 50 µL of the petroleum ether, chloroform, ethyl acetate and methanol extracts of the leaves of *Cassia fistula* were added in each well. Wells introduced with 50 µL of pure petroleum ether, chloroform, ethyl acetate and methanol served as negative controls. The plates were incubated at 37°C over night and examined for the zone of inhibition. The diameter of the inhibition zone was measured in mm. The standard antibiotic drugs such as tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin were also screened under similar conditions for comparison. An extract was classified as active when the diameter of the inhibition was equal to or larger than 8mm [20]. All the assays were performed in triplicate and expressed as average values.

DPPH free radical scavenging assay

The DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating free radical-scavenging activities of antioxidants [21]. Hydrogen or electron donation abilities of the compounds were measured from the bleaching of the purple-colored methanol solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). This spectrophotometer assay uses the stable radical DPPH as a reagent. The sample solution of material (50 µL) at four concentrations (1.0, 0.5, 0.25 and 0.125 mg/mL) was mixed with freshly prepared methanolic solution of DPPH (634 µM) and allowed to stand for 30 min at room temperature. The absorbance was then measured at 515 nm using a spectrophotometer and the inhibition of free radical DPPH in percent (%) was calculated using the formula below:

The percent of inhibition of DPPH reduction (decolourization)

$$\% \text{ of inhibition} = \frac{A_0 - A_{sample}}{A_0} \times 100$$

where (A_0) is the absorbance of the control (blank) and (A_{sample}) is the absorbance of the test compound. The compound concentration demonstrating 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage against sample concentration. Tests were carried out in triplicate. Samples and DPPH were dissolved in methanol. L-ascorbic acid was used as positive control.

Results

Antibacterial screening

The leaf extracts of *Cassia fistula* showing the zone of inhibition in millimeters, for Gram positive and Gram negative bacteria are summarized in Table 1. In addition, the inhibition zones formed by standard antibiotics and those of negative controls are listed in Table 2.

Antioxidant activity

The antioxidant activity of *Cassia fistula* leaf extracts in solvents of varying polarity were measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH. The method is based on the reduction of alcoholic DPPH• solutions in the presence of a hydrogen donating antioxidant. DPPH• solutions show a strong absorption band at 515 nm appearing as a deep violet color. The absorption vanishes and the resulting decolourization is stoichiometric with respect to degree of reduction. The remaining DPPH•, measured

	Diameter of inhibition zones (mm/50 µL)			
	Cassia fistula Leaf extracts			
Microorganisms	A	B	C	D
1. Bacillus cereus	32	22	13	12
2. Enterobacter faecalis	26	16	13	12
3. Salmonella paratyphi	22	18	16	12
4. Staphylococcus aureus	28	16	14	12
5. Escherichia coli	18	14	13	12
6. Proteus vulgaris	24	20	16	14
7. Klebsiella pneumonia	26	18	13	12
8. Pseudomonas aeruginosa	24	20	15	14
9. Serratia marcescens	28	22	14	12

A: methanol; B: ethyl acetate; C: chloroform; D: petroleum ether
Used concentrations: 50 µL of 10 mg/mL of plant extracts.

Table 1: Inhibition zones formed by *Cassia fistula* leaf extracts.

	Diameter of inhibition zones (mm/50 µL)				
Microorganisms	**Tob**	**Gen**	**Oflo**	**Cip**	**Control**
	10 µg	**10 µg**	**10 µg**	**10 µg**	**A, B, C, D**
1. *Bacillus cereus*	28	32	34	30	--
2. *Enterobacter faecalis*	26	32	32	26	--
3. *Salmonella paratyphi*	25	30	28	30	--
4. *Staphylococcus aureus*	26	28	24	24	--
5. *Escherichia coli*	30	36	32	34	--
6. *Proteus vulgaris*	26	30	24	32	--
7. *Klebsiella pneumoniae*	26	32	32	36	--
8. *Pseudomonas aeruginosa*	26	24	32	28	--
9.*Serratia marcescens*	24	32	30	30	--

Controls- A: methanol; B: ethyl acetate; C: chloroform; D: petroleum ether
Tob: tobramycin, Gen: gentamicin sulphate, Oflo: ofloxacin, Cip:ciprofloxacin

Table 2: Inhibition zones formed by the standard antibiotics and negative controls.

after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant. The results of the free radical scavenging activity of the leaf extracts *Cassia fistula* assessed by DPPH assay and amount of the sample needed for 50% inhibition of free radical activity, IC_{50} values were summarized in Table 3.

Discussion

Antibacterial screening of leaf extracts

As can be seen from Table 1, the leaf extracts of *Cassia fistula* showed pronounced antibacterial activity against all the microorganisms tested. Among the leaf extracts, methanol extract exhibited higher activity than the other extracts and petroleum ether extract showed least activity. Methanol (18-32 mm/50 µl inhibition zone), ethyl acetate (14-22 mm/50 µl inhibition zone), chloroform (13-16 mm/50 µl inhibition zone) and petroleum ether (12-14 mm/50 µl inhibition zone) extracts of the leaf exhibited marked activity against all the tested organisms such as *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens*.

The leaf methanol extract exhibited significant activity against *Bacillus cereus* (32 mm/50 µl inhibition zone), *Enterobacter faecalis* (26 mm/50 µl inhibition zone), *Staphylococcus aureus* (28 mm/50 µl inhibition zone), *Proteus vulgaris* (24 mm/50 µl inhibition zone), Klebsiella pneumonia (26 mm/50 µl inhibition zone), *Pseudomonas aeruginosa* (24 mm/50 µl inhibition zone) and *Serratia marcescens* (28 mm/50 µl inhibition zone) and are comparable with the standard antibiotic tobramycin (10 µg). The activity of leaf methanol extract against *Bacillus cereus* (32 mm/50 µl inhibition zone), *Staphylococcus aureus* (28 mm/50 µl inhibition zone) and *Pseudomonas aeruginosa* (24 mm/50 µl inhibition zone) are comparable with the standard antibiotic gentamicin (10 µg) screened under similar conditions.

DPPH free radical scavenging activity assay

The DPPH free radical scavenging activity of the leaf extracts of *Cassia fistula* are sorted in descending order: Leaf methanol extract > Leaf ethyl acetate extract > Leaf chloroform extract > Leaf petroleum ether extract. Out of the four samples tested *Cassia fistula* leaf methanol extract showed the highest scavenging activity (% inhibition 84.99 82.05, 76.02 and 71.11 at 1.0, 0.5, 0.25 and 0.125 mg/ml respectively), followed by *Cassia fistula* leaf ethyl acetate extract. Leaf petroleum ether extract exhibited least DPPH radical scavenging ability with % inhibition 72.37 69.57 46.14 and 42.64 at 1.0, 0.5, 0.25 and 0.125 mg/ml respectively.

The dose dependent variation in the DPPH radical scavenging activity of essential oil and leaf extracts of *Cassia fistula* are given in Figures 1 and 2. *Cassia fistula* leaf methanol extract possess potent free radical-scavenging activity. The amount of the sample needed for 50% inhibition of free radical activity is expressed by IC_{50}. Lower IC_{50} value indicates higher antioxidant activity (Figure 3).

By comparing the IC_{50} value of the leaf extracts of *Cassia fistula* with that of the authentic antioxidant L-ascorbic acid (Figure 3), it was found that the antioxidant activity of *Cassia fistula* leaf methanol extract (IC_{50}: 88.23 µg/ml) was quite comparable with that of L-ascorbic acid (IC_{50}: 70.40 µg/ml). IC_{50} value of *Cassia fistula* leaf ethyl acetate extract (IC_{50}: 101.56 µg/ml) is not significantly different from that of L-ascorbic acid (IC_{50}: 70.40 µg/ml).

Concentration (mg/ml)	IC_{50} (µg/ml)			
Samples	1.0	0.5	0.25	0.125
	Radical scavenging effect (%)			
C. fistula leaf methanol extract	84.99	82.05	76.02	71.11 88.23 ± 0.08
C. fistula leaf ethyl acetate extract	83.03	77.98	72.93	62.97 101.56 ± 0.06
C. fistula leaf chloroform extract	79.38	72.65	70.27	49.51 126.0 ± 0.01
C. fistula petroleum ether extract	72.37	69.57	46.14	42.64 257.35 ± 0.03
L-ascorbic acid	96.03	93.83	91.18	86.34 70.40 ± 0.04

Table 3: DPPH free radical scavenging activity of the leaf extracts of *Cassia fistula*.

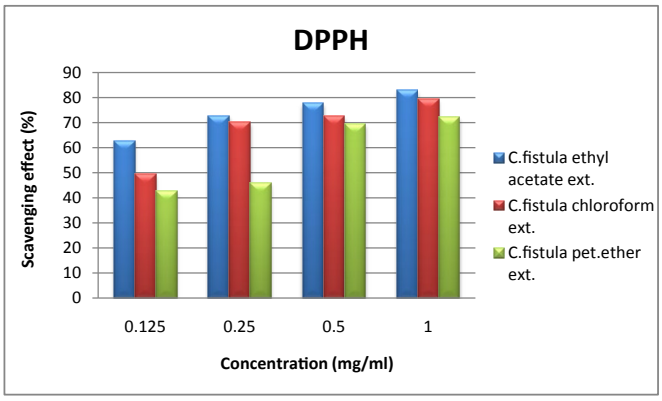

Figure 1: DPPH free radical scavenging activity of leaf ethyl acetate, chloroform and petroleum ether extracts of *Cassia fistula*.

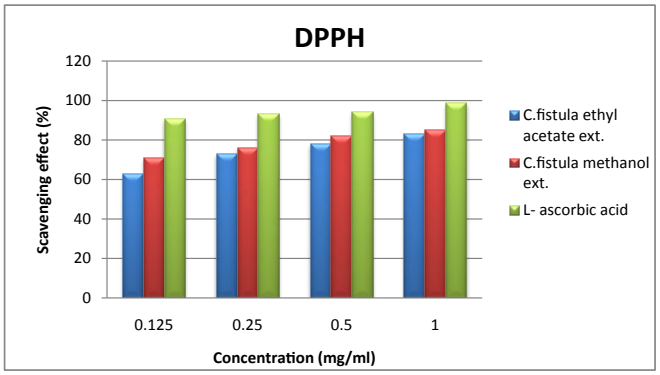

Figure 2: DPPH free radical scavenging activity of leaf ethyl acetate and methanol extracts of *Cassia fistula* and L-ascorbic acid.

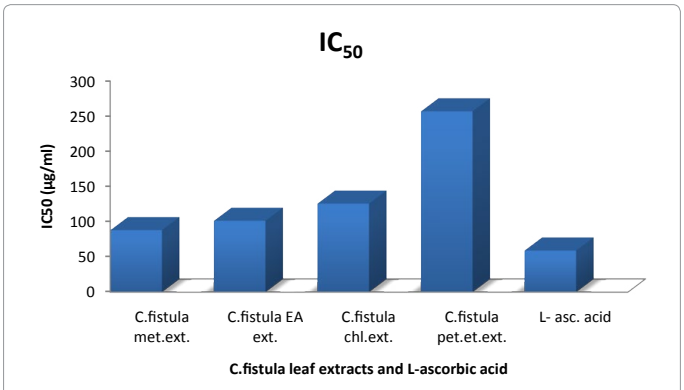

Figure 3: IC_{50} values of *Cassia fistula* leaf methanol extract, leaf ethyl acetate extract, leaf chloroform extract, leaf petroleum ether extract and L-ascorbic acid.

Conclusions

The leaf extracts of *Cassia fistula* showed varying degrees of antibacterial activity on the microorganisms tested. It is interesting to note that even crude extract of this plant showed prominent activity against various pathogenic bacteria where modern therapy has failed. Due to the emergence of the antibiotic resistant pathogens, plants are being looked upon as an excellent alternate to combat the spread of multi drug resistant microorganisms.

From the above experiment it can be inferred that leaf methanol extract of *Cassia fistula* showed significant activity against Gram-positive and Gram-negative bacteria. The activity of leaf methanol extract was found to be quite comparable with the standard antibiotics screened under similar conditions. So they can be used as an external antiseptic in the prevention and treatment of bacterial infections caused by various pathogenic bacteria such as *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens*, which have developed resistance to antibiotics. The incorporation of these samples into the drug formulations is also recommended. This study demonstrated that the methanolic leaf extract of *Cassia fistula* is as effective as modern medicine to combat pathogenic microorganisms.

Among the leaf extracts of *Cassia fistula* studied, leaf methanol extract showed potent scavenging activity on DPPH free radical comparable with the standard antioxidant L-ascorbic acid. Antioxidant activities of the extracts from medicinal plants are mainly attributed to the active compounds present in them. This can be due to the high percentage of main constituents, but also to the presence of other constituents in small quantities or to synergy among them. The methanolic leaf extract of *Cassia fistula* was rich in phenolic compounds, saponins, flavonoids and tannins. These compounds are reported to have antioxidant activity [1].

Free radicals are known to play a definite role in a wide variety of pathological manifestations of pain, inflammation, cancer, diabetes, alzheimer; hepatic damage etc. antioxidants fight free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms. Hence, the present investigation revealed that methanolic leaf extract of *Cassia fistula* can be considered good sources of natural antioxidants.

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