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Challenges in Biochemical Engineering and Biotechnology for Sustainable Environment

Phytochemical Screening and Bioactivity Studies of *Cassia Fistula* Leaves

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Abstract : The *Cassia fistula* leaves were subjected to solvent extraction of increasing polarity viz methanolic, ethanolic, petroleum ether, chloroform and aqueous and the invitro bioactivity studies like antimicrobial, antioxidant assay and cytotoxic property was analysed. The invitro antimicrobial activity of the Chloroform extract showed 16 mm zone of inhibition against *Pseudomonas aureus***,** and 36 mm against *Aspergillus flavus*. The radical scavenging activity was studied using DPPH and the percentage inhibition values for the ethanolic and petroleum ether extract is 61% and 51% respectively. The HEp2 cell lines were used to study the cytotoxic effect of the extracts and the EC 50 value ranges to 31 ug/ ml for ethanolic extract and 20 ug/ml for petroleum ether extract.

Introduction

Cassia fistula had been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values and this potent herb is used as alternative medicine for centuries. Ayurvedic medicine recognizes the plant as antibilious, aperitif, carminative, and laxative. The plant is also used for adenopathy, burning sensations, leprosy, skin diseases, syphilis, and tubercular glands. Yunani use the leaves for inflammation. There is still not sufficient study about these species and there must be a research focused on achieving the definitive knowledge and its utilization in prophylaxis[2].

Materials and Methods:

Collection of Plant Material:

Healthy, disease free, matured leaves of *Cassia fistula* were collected from Chennai, Tamil Nadu (India).The leaf sample was washed under clean running tap water and was air dried at the laboratory bench for three to four weeks at room temperature. The dried leaf was then homogenized to uniform, fine powder using ball mill. The powdered material was stored in air tight bottles for further analytical purpose[1,11].

Preparation of Solvent Extract:

Ten grams of powdered leaf sample was taken in a conical flask and mixed with hundred ml of solvents viz methanol, ethanol, petroleum ether, chloroform and aqueous. This mixture was kept in dark for forty eight hours for maceration with intermittent shaking.The mixture was then filtered using whatmann filter paper no 42

(125 mm). The filtrate was concentrated using the water bath set at 40^{o}C dried to form a paste and used for further analysis. All the extracts were subjected to phytochemical screening and evaluation of potential bioactivity[3,4].

Phytochemical Analysis:

Phytochemical analysis of all the evaporated solvent extract was conducted following standard procedures[5,12].

Test Pathogens:

The following gram positive and gram negative bacterial and fungal strains were used for anti-microbial studies: gram positive bacteria included *Bacillus subtilisStaphylococcus aureus*and gram negative bacteria *Pseudomonas aeruginosa, E.coli, Salmonella typhi*and two fungal strains namely *Aspergillusflavus* and *Candida albicans.*

Antimicrobial Activity Assay:

Anti-bacterial activity of solvent extracts was determined by cup diffusion method on nutrient agar and potato dextrose agar medium for bacterial and fungal strains respectively. Solvents used for extraction served as control. Plates inoculated with test pathogens were incubated for twenty four hours and zone of inhibition if any around the wells was measured in mm. The procedure was repeated for erythromycin and trimoxazole discs, the synthetic antibiotic as positive control for the bacterial and fungal cultures[10,3].

Antioxidant Activity using Dpph for Free Radical Scavenging

The antioxidant potential of the methanol, ethanol, petroleum ether, chloroform, and aqueous extracts of *Cassia fistula* leaf can be determined on the basis of its scavenging activity of the stable 2,2- diphenyl-1-picrylhydrazyl (DPPH) free radical. The absorption maximum of a stable DPPH radical in methanol was read at 517 nm. Stock solutions of the samples were prepared by dissolving 2 mg of the extracts in 1 ml of methanol to give a concentration of 2mg/ml. It was then prepared to the working concentrations of 10, 20, 30, 40 and 50 ug/ml.

The decrease in absorbance of DPPH radical caused by antioxidants from the different extracts of test sample and standard was noted after 30 minutes using UV-Visible Spectrometer. A blank reading was taken using methanol. IC 50 value (inhibitory concentration to scavenge 50% free radicals) was calculated from % inhibition. Lower values of absorbance indicate intense scavenging of free radicals. The absorbance values were denoted as percentage inhibition.

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of samples})}{(\text{Absorbance of control})} \times 100$$

The effective concentration of sample required to scavenge DPPH radical by 50% (IC 50) was obtained by linear regression analysis of dose response curve plotting between % inhibition and concentration[6,7].

Determination of Cellular Toxicity by MTT Assay:

HEp-2 cell line was obtained from National Centre for cell sciences (NCCS), Pune, India. The cells were grown at 37^{o} C under a humidified, 5% CO_2 atmosphere in DMEM medium supplemented with 10% fetal calf serum (FCS), 2Mm glutamine, 100 units/ml of penicillin and 50 ug/ml of streptomycin. A sub confluent monolayer culture was trypsinized and 200 ul of the cells were transferred into each well of the flat bottomed 96 well plate starting with column 2 and ending with column 11 and placing approximately 0.5-10 x 10 ^{3} cells. 200 ul of growth medium was added to the eight wells in column 1 and 12 where column 1 will be used as blank and column 12 helps to minimize the edge effect. The plates were incubated until the cells are in their exponential phase of growth during which a serial dilution of the samples of the different extracts of *Cassia fistula* were added and incubated. At the end of the drug exposure fresh medium was added and 15ul of MTT was added to all of the wells. The plates were then wrapped in aluminium foil and incubated for 4 hrs. The cells were then examined by immediately recording the Absorbance at 570nm[8,9].

Results:

Phytochemical Analysis:

Phytochemical analysis of all the solvent extracts revealed the presence of tannin only in ethanolic and methanolic extracts but was absent in aqueous, petroleum ether and chloroform extracts (Table 1). Presence of terpenoids was observed in aqueous, methanolic and petroleum ether extracts only and found absent in ethanol and chloroform extracts. Cardiac glycosides were absent in all the five extracts of the plant. Flavonoids were present in aqueous, methanolic, ethanolic and chloroform extracts but not in petroleum ether. The component phytosterol was found to be present in all the five extracts. The phytochemical anthraquinone was absent in all the five extracts.

Table-1: Phytochemical Screening of Aqueous, Ethanol, Methanol, Petroleum Ether and Chloroform Extracts of *Cassia Fistula* Leaves

S.No.	Phytochemicals	Leaf Extracts				
		Aqueous	Ethanol	Methanol	Petroleum Ether	Chloroform
1	Tannin	-	+	+	-	-
2	Saponins	+	+	+	+	+
3	Terpenoids	+	-	+	+	-
4	Cardiacglycocides	-	-	-	-	-
5	Antraquinone	-	-	-	-	-
6	Flavanoids	+	+	+	-	+
7	Phytosterol	+	+	+	+	+

Antimicrobial Activity of *Cassia Fistula* Leaf Extracts:

Antibacterial activity assay of all the five solvent extracts revealed that chloroform extracts showed significant activity against all the organisms while ethanol and methanol extracts showed good activity (Table 2). The petroleum ether extract had activity against *Salmonella typhi* and *Staphyllococcus aureus* but not for the other organisms. The aqueous extract had activity against *Salmonella typhi* and not against any other organism. The anti-bacterial activity of different solvents extracts of *Cassia fistula* (zone of inhibition measured in mm)

Table-2: Antimicrobial Activity of *Cassia Fistula* Leaf Extracts

S.No.	Bacterial Organisms	Aqueous	Ethanol	Methanol	P.Ether	Chloroform
		Zone Of Inhibition (mm)				
1.	*S. t*	12	12	14	14	13
2.	*P. a*	ND	ND	12	ND	16
3.	*B. s*	ND	ND	11	ND	15
4.	*E. c*	ND	12	15	ND	11
5.	*S. a*	ND	12	ND	13	10
S.No.	**Fungal Organisms**	**Aqueous**	**Ethanol**	**Methanol**	**P.Ether**	**Chloroform**
		Zone Of Inhibition (mm)				
6.	*C. a*	ND	16	18	ND	16
7.	*A. f*	ND	13	ND	ND	35

S.t = Salmonella typhi, P.a = Pseudomonas aureus, B.s = Bacillus subtilis, E.c = E.coli, S.a = Staphyllococusaureus, C.a = Candida albicans, A.f = aspergillusflavus
The zone of inhibition expressed in mm. (ND) indicates No Dimension.

Determination of In Vitro Antioxidant Activity Free Radical Scavenging Activity (Dpph)

The in vitro antioxidant free radical scavenging activity for the aqueous, ethanol, methanol, petroleum ether and chloroform extracts of *Cassia fistula* leaves were done with DPPH assay. The result of percentage inhibition was represented in table 3. The % inhibition values for the ethanolic and petroleum ether extract is 61% and 51% respectively. The EC 50 value ranges to 31 ug/ ml for ethanolic extract and 20 ug/ml for petroleum ether extract.

Table 3: In Vitro Antioxidant Activity (Dpph Assay) for Aqueous, Ethanol, Methanol, P.Ether and Chloroform Extracts of *Cassia Fistula*

S.NO	Concentration (µg)	% Inhibition				
		Aqueous	Ethanol	Methanol	P.Ether	Chloroform
1	10	1.13	51.31	40.17	22.65	4.20
2	20	1.53	13.26	41.30	51.18	16.39
3	30	28.79	60.70	43.42	25.03	15.14
4	40	25.03	20.15	44.30	11.90	35.66
5	50	8.39	25.03	31.16	35.16	10.63

% Inhibition of DPPH free radical scavenging activity for different extracts of *Cassia fistula* leaves

Determination of Cellular Toxicity by Mtt Assay

The cellular cytotoxic effect by MTT assay of the ethanol, methanol and chloroform extracts of *Cassia fistula* leaves was performed in HEp2 cell lines and the results are represented in table 4. The IC_{50} values were found by plotting a graph between concentration of the extract added and % cytotoxicity. The IC_{50} values for ethanolic extracts are 55µg, chloroform extract is 135µg and that of methanolic extract is 143µg. Of all the extracts the ethanolic extract has the highest activity at the lowest concentration.

Table 4: Cellular Toxicity by Mtt Assay for the Ethanol, Methanol, Chloroform Extracts of *Cassia Fistula* Leaves

S.No	Concentration (Ug)	% Cytotoxicity		
		Ethanol	Methanol	Chloroform
1	20	30.40	15.20	12.60
2	40	37.14	20.29	12.66
3	60	54.26	22.49	26.66
4	80	55.74	29.23	28.67
5	100	58.40	36.95	31.17
6	120	58.64	39.15	40.82
7	140	58.77	48.39	53.21
8	160	61.86	61.92	61.24

Discussion:

Diseases due to Infection were considered as the worst nightmare for the early human civilisation whose occurrence was accompaigned with the consumption of enormous lives. The prevention of the spread of these diseases through biomolecules was sought earnestly worldwide. However the profound use of synthetic chemicals as therapeutics has altered the mode of survival of the pathogenic organisms conferring them resistance to antibiotics. Henceforth the search for a molecule of varied chemical diversity was on the lookout and plants have drawn our attention because of their rich phytochemicals that potentially evades infections. The present study focuses on the bioactivity study of the *Cassia fistula* leaf. The phytochemical analysis of the methanolic, ethanolic, petroleum ether, chloroform and aqueous leaf extracts were analysed for the invitro bioactivity studies like antimicrobial, antioxidant assay and cytotoxic property. Preliminary phytochemical analysis on aqueous, ethanol, methanol, petroleum ether and chloroform extract of *Cassia fistula* leaves revealed the presence of secondary metabolite like tannin, saponins, tarpenoids, flavonoids and phytosterol (table 1) some of which chemical compounds have been associated to anti-bacterial activities and thus have curative properties against pathogens. The in-vitro microbial activities are shown in table 2. This showed a wide spectrum activity against most bacterial and fungal strain studies. The standard antibiotic co trimaxazole used as positive control for fungal strains inhibited the growth of the test organisms under study at the smallest concentration four microgram per milli litre. The inhibition zones produced significantly higher for the chloroform extract *A.flavus* and high for the methanol extract for *C. albicans* as compared to standard drug used. It is also observed from these results that the chloroform extract have wide anti-bacterial activity against both gram positive, gram negative and fungal strain under study with highest activity observed in *A. flavus and*

C.albicans. The ethanol and methanol extract also has activity against most of the organisms. The results of the investigation are indicative of possible pure active principle of natural origin from the extract with possible high potency which could serve as a lead to the isolation of chemotherapeutic agents. It is not surprising that there are differences in the anti-microbial activity of plant species are due to the phytochemical constituents and the differential occurrence in species[13,14]. The potential for developing anti-microbials from higher plants appear rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant based anti-microbials have enormous therapeutic potential as they can serve the purpose with lesser side effects.

The in vitro antioxidant radical scavenging activity by DPPH assay revealed that the ethanolic and petroleum ether plant extracts of *Cassia fistula* have significant antioxidant activities when compared with those of the other extracts. The degree of reduction in absorbance measurement is indicative of the radical scavenging power of the extracts. However the crude plant extracts needs to be purified by fractionation to isolate potential antioxidant compounds which could find application in food industries to prolong shelf life and to replace the synthetic antioxidants because of their potential health risks and toxicity. Further these plants could be intensively studied and can be formulated to a neutraceutical which help to prevent diseases caused due to oxidative damage. The cytotoxicity profile of the plant extracts on HEp 2 cells revealed that *Cassia fistula* had positive results. These plants should be further studied for their efficacy and toxicity in invivo models and the results validated for its use in therapeutic purposes.

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