Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (3):100-104
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

HPTLC fingerprint analysis and antimicrobial activity of leaf extracts of *Cassia fistula* L

Chavan R. T.[a], Kadam A. S.[a*] and Kote J. R.[b]

[a]*Dept. of Botany, Toshniwal College, Sengaon, Dist. Hingoli, (M.S.) India*
[a*]*Dept. of Botany, D.S.M. College, Jintur, Dist. Parbhani, (M.S.) India*
[b]*School of Life Sciences S.R.T.M. University, Nanded*

ABSRTACT

The present research was carried out to investigate the HPTLC fingerprint analysis and antimicrobial activity of various extracts of leaves of Cassia fistula L. Preliminary phytochemical screening was done by the methods of Treas and Evans and sofowora. The HPTLC analysis were carried out as Harbone and Wagnar et. al. described. The phytochemical screening showed the presence of alkaloids, carbohydrates, glycosides, saponins, triterpenes, tannins, flavonoids, photobatalin and anthraqunies in methanol and aqueous extracts of leaf. The analysis of methanolic extract of leaves by HPTLC confirmed the presence of flavonoids(Peak 4) and alkaloids(Peak 3).The in vitro antimicrobial activity was performed by agar well diffusion method of Perez et. al.,. Of the different extracts methanol, ethanol and aqueous extracts showed significant antimicrobial activity against gram negative bacteria P. aeruginosa and S. aureus also.This study concluded that leaf of C.fistula L. possess significant flavonoids and alkaloids which might be a responsible active principle for the antimicrobial activity.

Key words: HPTLC Finger Print, phytochemistry, antimicrobial activity, *Cassia fistula* L.

INTRODUCTION

Plants are considered not only as dietary supplement to living organism but also traditionally used for treating many health problems. Medicinal values of many plants still remain unexplored for its enumerable activity of compounds. pharmogonostic investigations of plants are carried out to find novel drugs or templates for the development of new therapeutic agents [1]. Many plant extracts have been shown to inhibit the growth of microorganisms;these extracts consist of chemicals and are usually considered to play a role in defense reactions of plants, towards infections by pathogenic microorganisms [2].

Cassiafistula L.(Caesalpiniaceae) a very common plant known for its medicinal properties, commonly known as Amaltas and in English popularly called Indian Laburnum has been extensively used in Ayurvedic system of medicine for various ailments. It is distributed in various regions including Asia, South Africa, China, West Indies and Brazil [3]. It is a semi-wild deciduous tree with compound leaves, greenish grey bark, beautiful bunches of yellow flowers and also used in traditional medicine for several indications [4-5].In the Indian literature, this plant has been described to be useful against skin diseases, liver troubles, tuberculous glands and in the treatment of rheumatism, hematemesis, pruritus, leucoderma and diabetes [6].

MATERIALS ANDMETHODS

Plant material
Leaves of *Cassia fistula* L. were collected from Parbhani district in Maharashtra. It was rinsed severally with clean tap water to make it dust free and subjected to drying in a dark place at room temperature for few days. The dried leaves were grounded in fine powder form using the grinder and stored in air tight bottles.

Preparation of plant extracts
The leaves powder was subjected to successive extraction with methanol, ethanol, aqueous, petroleum ether and acetone using soxhlet apparatus. The collected extracts were concentrated by heating till the boiling point so as unwanted solvents either evaporated or removed and the negative control of the solvents get reduced. The remaining plant extracts used for phytochemical and antimicrobial assays.

Test Microorganisms
Authentic pure cultures of human pathogenic bacteria like gram positive *Staphylococcus aureus* (SRTCC 1073), *Bacillus subtilis* (SRTCC 1091),and gram negative *Pseudomonas aeruginosa* (SRTCC 708) and *Escherichia coli* (SRTCC 3260) and two species of fungi viz. *Aspergillus niger* (SRTCC1073) and *Candida albicans* (SRTCC 3971). These microorganisms were obtained from the School of Life Sciences, S.R.T. M. University, Nanded (M.S.).

Preparation of Test Organisms Suspension
The test organisms were maintained on slants of medium containing nutrient agar (2.5 gm/10 ml) and sub cultured once a week. The slants were incubated at 37^0C for 24 hrs and stored under refrigeration. The inoculums was 1×10^8cells/ml in each case[7].

Antimicrobial Activity Assay
The *In vitro* antimicrobial activity of leaves extracts of *Cassia fistula* L. was determined by the agar well diffusion method [8]. The plant extracts were dissolved in distilled water at concentration 2 mg/ml. The standard antimicrobial solution containing 50µl streptomycin was inoculated with 20µl microbial suspension having concentration 1×10^8cells/ml. 0.1 ml extract was added to each well. The plates containing bacteria were incubated at 37^0C for 24 hrs and those containing fungi were incubated 25^0C for 7 days. Positive antimicrobial activity was based on growth inhibition zone and compared with standard drug [9].The diameter of zone of inhibition surroundings each of the well was recorded.

Preliminary Phytochemical Analysis
All the plant extracts were subjected to systematic phytochemical screening for the presence of chemical constituents[10-12].

High Performance Thin Layer Chromatography (HPTLC)
A. *HPTLC Profile*
HPTLC studies were carried out by following the methods of [13]

B. Sample Preparation
The methanolic bark extracts was dissolved in HPTLC grade methanol which were used for sample application on precoated silica gel 60 GF254 aluminium sheets.

C. *Developing Solvent System*
A number of solvent system were tried, for extract but the satisfactory resolution was obtained in the solvent toluene-ethyl acetate- formic acid (7:3:0.1).

D. Sample Application
The samples 5µl were spotted in the form of bands of width 6 mm with 100µL sample using a Hamilton syringe on silica gel which was precoated on aluminum plate GF-254 plates (20×10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

E. Development of chromatogram

The mobile phase consisted of toluene-ethyl acetate- formic acid (7:3:0.1) and 15µl of mobile phase was used per chromatography run. The linear ascending development was carried out in a (20×10 cm) twin through glass chamber saturated with the mobile phase.

F. *Detection of spots*

The developed plate was dried by hot air to evaporate solvents from the plate. The developed plate was sprayed with anisaldehydesulphuric acid reagent as spray reagent and dried at 100 ^{0}C in hot air oven for 3 minutes. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images under UV light at 254 and 366 nm, respectively. The R_f values and finger print data were recorded by WIN CATS software.

RESULTS AND DISCUSSION

The results of the phytochemical analysis of leaves are presented in Table 1. The results revealed the presence of alkaloids, carbohydrates, glycosides, saponins, triterpenes, fats and oils, tannins, , flavonoids, photobatalin and anthraqunonines. Among the different extracts methanol and aqueous extracts of leaves showed the presence of alkaloids, carbohydrates, glycosides, saponins, triterpenes, fat and oils, tannins, flavonoids, photobatalin and anthraqunonines. Panda *etal*.(2011) reported that *Cassia fistula* leaves showed the presence of alkaloids, flavonoids, carbohydrates, glycosides, protein and amino acids, saponins, and triterpenoids in polar extracts (ethanol, methanol, and aqueous) compared with nonpolar extracts (petroleum ether and chloroform).

The results of HPTLC of *C. fistula*L. leaves shown in Fig 1 . Under the UV 366 nm showed five peaks, the highest Rf values are 0.72(43.51%) 0.63(28.13%) and 0.86(20.14%) respectively. The peaks 4, 3 and 5 are the major groups of all chemicals in *C. fistula* L. leaves. From the results it was confirmed that flavonoids and alkaloids are the major group of chemicals in methanol extract of leaves (peak 4&3).HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials and it allows for the analysis of a broad number of compounds both efficiently and cost effectively. HPTLC studies have shown that it is more versatile than ordinary TLC methods, as the spots were well resolved. Though further work to characterize the other chemical constituents and perform quantitative estimation with marker compounds is also necessary, this data can also be considered along with the other values for fixing standards to this plant.

Table 1.Phytochemical analysis of leaves of *Cassia fistula* L.

Sr.No.	Phyto Constituents	Chemical test	*C. Fistula leaves extract*				
			P.E.	Met	Eth	Aq	Ac
1	Alkaloids	1. Mayer's test	-	+	-	+	-
		2. Dragendroff's test	-	+	-	+	-
		3. Wagner's test	-	+	-	+	-
		4. Hagers test	-	+	-	+	-
2.	Carbohydrates	1.Molisch's test	-	+	+	+	-
		2. Benedicts test	-	+	+	+	-
		3. Fehlings's test	-	+	+	+	-
3.	Glycosides	1. Modified Borntragers	-	+	-	+	-
		2.legal test	-	+	-	+	-
4.	Saponins	1. Foam test	-	+	-	+	-
		2. froth test	-	+	-	+	+
5.	Triterpences	1. Salkowski test	-	+	-	+	+
		2. LibermannBurchard	-	+	-	+	-
6.	Fats & Oil	1. Stain test	-	+	-	+	-
7.	Tannins	1. Gelatin test	-	+	-	+	-
8.	Flavonoids	1. Alkaline Reagent	-	+	-	+	-
		2. lead acetate test	-	+	-	+	-
		3. Shinoda test	-	+	-	+	-
9.	Photobatalin		-	+	-	-	-
11.	Anthraqunonies		-	+	-	+	-

(- : absent, + : present; P.E.- Petrolem ether, Meth.- Methanol, Eth.- Ethanol, Aq.- Aqueous, Ac- Acetone.)

Table 2.Rf values of the peak of *Cassia fistula* leaf extract

Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %	Assigned substances
1	0.10	0.2	0.13	19.8	5.80	0.16	0.0	447.1	4.28	unknown*
2	0.32	0.1	0.34	21.1	6.17	0.38	6.5	411.7	3.94	unknown*
3	0.50	6.0	0.55	73.9	21.63	0.63	4.3	2937.0	28.13	alkaloid*
4	0.63	4.3	0.68	181.5	53.14	0.72	12.2	4541.7	43.51	flavonoid*
5	0.73	12.3	0.78	45.3	13.26	0.86	8.8	2102.0	20.14	unknown*

Fig.1. Track1 .HPTLC analysis of *Cassia fistula* L. leaves

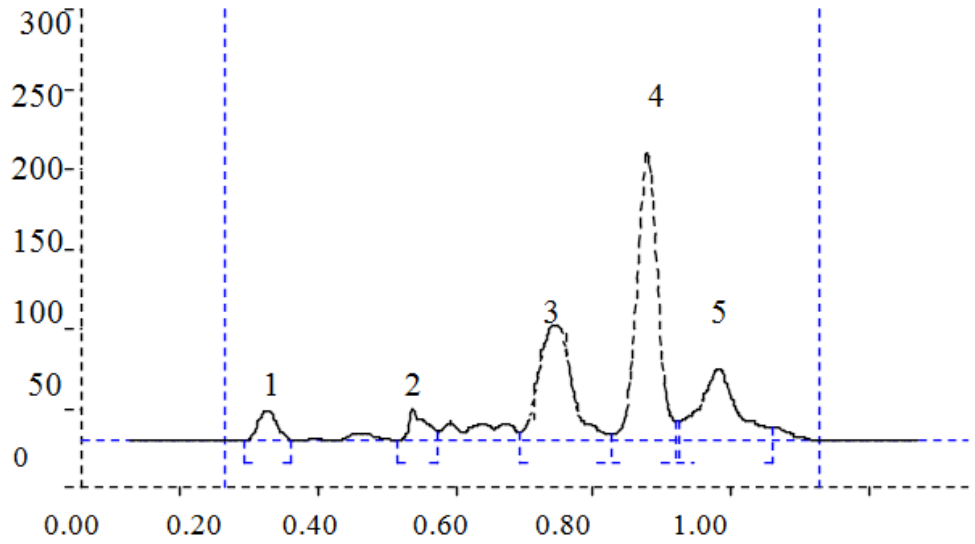

Table 3 : *In vitro* antimicrobial activity of leaves of *Cassia fistula* L

Microorganisms	Diameter of Zone of inhibition in mm of different extracts of leaves(2mg/ml) Mean± S.D.					Stand. reference antibiotic (Streptomycine)
Bacteria	Methanol	Ethanol	Aqueous	Pet. ether	Acetone	
Escherichia coli	11±0.15	19±0.15	**21±0.12**	17±0.10	12±0.12	20±0.12
Staphylococcus aureus	**24±0.12**	20±0.35	21±0.17	16±0.12	21±0.15	21±0.23
Bacillius subtilis	**23±0.37**	20±0.15	20±0.15	17±0.12	22±0.12	22±0.11
P.aeruginosa	**24±0.17**	**21±0.17**	20±0.12	15±0.15	**25±0.12**	20±0.12
Fungi						
Aspergillus niger	**21±0.17**	**20±0.11**	**20±0.12**	17±0.12	19±0.12	20±0.12
Candida albicans	**20±0.15**	**19±0.19**	**19±0.12**	15±0.17	18±0.15	18±0.08

The results of the antimicrobial activities of the different extracts of leaves are presented in Table 3. The results revealed that all the solvent extracts were found to be effective against the tested microbes. Among the different extracts methanol extracts have shown significant antimicrobial activity compared with standard reference antibiotic streptomycin except *E. coli*. The acetone, methanol and ethanol extracts of leaves found effective against gram negative bacteria *P. aeruginosa*. Aqueous extract were found effective against gram negative bacteria *E.coli*.

The highest antifungal activity was exhibited by methanol, ethanol and aqueous extracts of leaves against *Aspergillus niger* and *Candida albicans*. Perumalsamy and Ignacimuthu [14] reported that *Cassia fistula* leaf extracts showed antibacterial activity against Escherichia *coli, Klebsiellaaerogenes, Pseudomonas aeruginosa* and *Proteus vulgaris.* Vasudevan [15] reported that alcoholic extract of leaves of *Cassia fistula* showed antimicrobial activity against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherechia coli.* Abu [16] also reported that extracts from the leaves and pods of *C. fistula* showed significant antimicrobial activity. This study was supported by previous studies of Wins [5] that the Gram positive bacteria are strongly inhibited by all the extracts of *Cassia fistula* than Gram negative bacteria and it shows minimum activity against *Pseudomonas aeruginosa.*

Acknowledgement
The authors are thankful to the Director School of Life Sciences Swami Ramanand Teerth Marathwada University Nanded-431606 (M.S.) for Laboratory facility and also thankful to Anchrom Enterprises Pvt. Ltd. Mumbai (M.S.), India for HPTLC analysis.

REFERENCES

[1] C Anushia, P Sampathkumar and L Ramkumar,*Global Journal of Pharmacology*,**2009**, 3,127-130.
[2] CH Fawcett and D M Spencer,*Phytopathol*, **1976**, 8, 403-418.
[3] PV Kumar, NS Chavan, H Padh and M Rajani, **2006**,*J. Ethnopharmacol*, 107,182-188.
[4] RK Gupta,**2010**,*CBS Publishers & Distributers*, 1st edition, 116-117.
[5] WTMurugan andM Murugan, **2013**,*IJREB*, 1, 32-41.
[6] MM Alam, MB Siddiquiand W Hussian, **1990**,*Fitoterapia*, 61,240-242.
[7] M Chessbrough, Linacre House, Jordon Hill, Oxford, **2000**
[8] C Perez, M Paul and P Bazerque, **1990**,*Acta. Bio. Med. Exp*, 15**,** 113-115.
[9] GRM Perez, JG Avila, MA Zavala, GS Perez, and GC Perez, **1996**, *Phytomedicine*, 3,186.
[10] CK Kokate, Practical Pharmacognosy (4th Ed.)VallabhPrakashan, **1993**, pp.107-111.
[11] GE Trease and WC Evans,Pharmacology. 15thEdn,saunders publishers, London,**2002**, pp.42-44.
[12] A Sofowora, Screening plants for Bioactive Agents. In medicinal plants and Traditional medicine in Africa. 2ndEdn., Spectrum Books Ltd. Sunshine House, Ibadan, Nigeria, **1993**, pp.134-156.
[13] H Wagnerand SBladt, Drug analysis, A Thin Layer Chromatography Atlas, 2nd Springer, New York, **1996**, pp.3-335.
[14]RPerumalsamy and S Ignacimuthu, **1998**,*Journal of Ethanopharmcology*, 69, 63-71.
[15] DT Vasudevan, KR Dinesh, SG Krishana, SKSreekanth and S Shekar, **2009**,*Journal Chem. Sci*, 7, 2363-2367.
[16]KA Abu, SW Lasaki, and AA Adeyemi,**1999**, *NigerianjournalofNaturalProductsandMedicine*, 3,123-12