



INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES
(Int. J. of Pharm. Life Sci.)

Phytochemical Screening and TLC Fingerprinting of *Cassia fistula* Linn. Fruit pulp

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Abstract

Traditional herbal medicines are moving from fringe to mainstream use with a large number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. The *Cassia fistula* is used to cure haematemesis, constipation, chlorosis, urinary disorders, biliousness, rheumatic condition, wounds, ulcers, skin diseases, diabetes. The present study was aimed to investigate the preliminary phytochemical screening of *Cassia fistula* fruit pulp. The fruit pulp extract of *Cassia fistula* were prepared using different solvents like Petroleum ether, Benzene, Chloroform, Acetone, Alcohol, Methanol, and Water. The phytochemical screening of the fruit pulp extracts was performed. The thin layer chromatography (TLC) of Chloroform extract was studied. The extracts were analyzed for the presence and absence of alkaloids, steroids, volatile oils, tannins, carbohydrates, flavonoids and saponins. After derivatization seven bands were found in TLC plate of chloroform fraction. Rf value of bands observed for this fraction lies between 0.12 to 0.75. These studies provided referential information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario of lack of regulatory laws to control quality of herbal drugs.

Key-Words: *Cassia fistula*, fruit pulp, phytochemical screening, TLC, Secondary metabolites

Introduction

India is considered to be a country having rich emporia of medicinal plants and where ancient systems of medicine such as Ayurveda, Siddha and Unani medicines have been in practice for many years. Ayurveda (4000-600 B.C.), Rigveda (4500-1600 B.C.) and Atharvaveda (1200 B.C.) are traditional indigenous systems of medicines (Mahesh B, Satish S., 2008). Ayurveda literally means "Science of life". According to Ayurveda, health is an indication of normal biological processes, which help to maintain mental and physical alertness and happiness of human being (Sukh dev 1997). Charak Samhita is the first recorded treatise on Ayurveda which was followed by Sushruta Samhita around 900 B.C. charak samhita dealt primarily with medicine while Sushruta Samhita was concerned with the advanced state of knowledge on the general principles and details of treatment (Solecki, 1975; Kirtikar and Basu, 1984; Sharma *et al.*, 2008).

Each plant species in this universe has its own specific set of secondary metabolites and these secondary metabolites are widely present in medicinal herbs and plants (Harborne, 1984). Sandhu and Arora (2000) have reported that these secondary metabolites are responsible for antimicrobial activity of plant extracts.

These secondary metabolites such as phenols, flavonoids, quinones, essential oils, alkaloids, sterols, thymol, coumarines and triterpenoids are untapped reservoirs of various valuable chemicals (Lefevre *et al.*, 2008). Natural plant-based remedies are used for both acute and chronic health problems, from treating common colds to controlling blood pressure and cholesterol. Herbal plant formulations also have preventive effect against plant pathogenic microbes.

Cassia fistula (Linn.) belongs to family Fabaceae and Sub-family Caesalpinioideae is a very common plant known for its medicinal properties are a semi-wild in nature. It is distributed in various regions including Asia, South Africa, China, West Indies and Brazil. It is 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 deciduous and mixed-monsoon forests throughout greater parts of India, ascending to 1300 m in outer Himalaya, is widely used in traditional medicinal system of India (Prashanth *et al.*, 2006; Gupta, 2010). It is a deciduous tree with greenish grey bark, compound leaves, leaf lets are each 5-12 cm long pairs. It is a semi-wild tree known for its beautiful bunches of yellow flowers and also used in traditional medicine for several indications. A fruit is cylindrical pod and seeds many in black, sweet pulp separated by

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transverse partitions. The long pods which are green, when unripe, turn black on ripening after flowers shed. Pulp is dark brown in colour, sticky, sweet and mucilaginous. (Danish *et al.*, 2011; Bhalerao and Kelkar, 2012).

C. fistula is widely used in traditional medicines for various medicinal properties. The pulp of the ripe pods possesses a mild, pleasant purgative action (Bahorun *et al.*, 2005). Various biological activities of the pod pulp such as antibacterial, antifungal, antioxidant, antileishmanial, and hypolipidemic activity were reported (Duraipandiyan and Ignacimuthu, 2007; Siddhuraju *et al.*, 2002; Satorelli *et al.*, 2007; Gupta and Jain, 2009). In Ayurvedic medicinal system, *C. fistula* was used against various disorders such as haematemesis, pruritus, constipation, chlorosis, urinary disorders, biliousness, rheumatic condition, wounds, ulcers, skin diseases, diabetes, and other ailments (Alam *et al.*, 1990; Asolkar *et al.*, 1992).

Combined knowledge of biological activity and chemical constituents of the plant is desirable for discovery of new class of compounds. Thin layer chromatography (TLC) is routinely used as valuable tool for qualitative determination of small amounts of impurities. Molecular markers generally refer to biochemical constituents, including primary and secondary metabolites and other macromolecules such as nucleic acids (Kalpana, *et al.*, 2004). TLC has been used as a broad spectrum screen for detection of drug abuse. TLC results are only qualitative and cannot be quantified (Andrew *et al.*, 1988; Jones and Gierasch 1994).

In the present study partially purified extracts of *Cassia fistula* fruit pulp were subjected to rapid qualitative phytochemical tests for confirming the presence of primary and secondary metabolites. TLC was used to isolate the active compound from most effective fraction.

Material and Methods

Collection of plant material

The healthy, infection free, mature pods were collected from the campus of University College of Science, Mohanlal Sukhadia University, Udaipur, Rajasthan, India. The herbarium specimen plant was identified from Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India. Where a voucher number RUBL 211505 specimen was deposited. The pods were shade dried at room temperature and broken with the help of a pestle to extract out the pulp. The pulp was grounded in an electrical grinder after removal of the seeds from the pulp. The ground material was passed through sieve of mesh size 60 to obtain a fine powder which was used to prepare the extract.

Preparation of extracts

Reflux method of solvent extraction was used for successive separation of different partially purified organic constituents present in dried plant material (Harborne, 1984). Solvent series used for successive separation was as follows:

Petroleum ether → Benzene → Chloroform → Acetone → Alcohol → Methanol → Water

40 gm dry fruit pulp powder was kept in Soxhlet extraction unit and extracted with 280 ml petroleum ether till all petroleum ether soluble fractions was extracted. Residue was dried in an oven below 50°C and used for extraction with next solvent in series. Fractions obtained at every step including aqueous fraction were vacuum dried in a rotary evaporator and the dried residue was used as extract.

Phytochemical study of fruit pulp extracts

Qualitative methods were used for the identification of different secondary metabolites or phytochemicals present in the plant extracts. Various fractions of fruit pulp obtained by successive extraction were then subjected to qualitative test suggested by Kokate *et al.*, 1990. The extracts were analyzed for the presence and absence of alkaloids, steroids, volatile oils, tannins, carbohydrates, flavonoids and saponins.

Tests for Detection of Secondary Metabolites

Alkaloids

Alkaloids are compounds having one or more nitrogen containing heterocyclic ring. Presence of alkaloids in the partially purified fractions was tested by performing Mayer's test or Wagner's test or Hager's test. Reaction with Mayer's reagent produces a cream coloured precipitate; Hager's reagent gives yellow precipitate while Wagner's reagent results in formation of reddish brown precipitate.

Small amount of extract was stirred with few drops of dilute HCl and filtered. The filtrate was tested with various alkaloid reagents and observed for development of coloured precipitate.

Volatile Oils

The odorous volatile chemical constituents of plants are known as volatile or essential oils. Sudan III test was used to detect presence of volatile oils. Development of red colour on mixing with Sudan III indicates the presence of volatile oils.

Small amount of extract was mixed with Sudan III dye and observed for development of red colour.

Tannins

Chemically, tannins contain the mixture of complex organic substances in which polyphenols are present. Development of green colour indicates the presence of condensed tannins whereas blue colour indicates the presence of hydrolysable tannins.

Small amount of extract was taken and treated with alcohol FeCl_3 solution and observed for colour development.

Saponin

Saponins are complex glycoside compounds in which the a glycone is triterpenoid or steroidal in nature. Foam test was used to detect presence of saponins.

Small amount of extract was diluted with 20 ml of distilled water; then shaken in graduated cylinder for 15 minutes. Formation of a layer of foam at surface indicates the presence of saponin.

Carbohydrates

Carbohydrates are widely distributed in plants and can be detected by Molish's test and Fehling's test. Small amount of extract was dissolved in 5 ml distilled water and filtered.

Development of purple colour on addition of few drops of α -naphthol and conc. H_2SO_4 to the filtrate indicates presence of sugars.

Similarly small quantity of filtrate was heated with equal amount of Fehling A and Fehling B solution. Development of brick red colour indicates the presence of carbohydrates.

Flavonoids

Flavonoids usually occur in plants as glycosides in which one or more of phenolic hydroxyl groups are combined with sugar residues. Alkaline reagent test was used to detect flavonoids.

Small amount of extract was mixed with aqueous NaOH. Development of reddish brown colour shows presence of flavonoids.

Sterols

Sterols are triterpenes which are based on cyclopentane perhydroxy phenanthrene ring system. They are also called as phytosterols; Liebermann's Burchard test was used for detection of phytosterols.

Small amount of extract was mixed with 2 ml CHCl_3 and 1 ml of acetic anhydride. Subsequently concentrated H_2SO_4 was added gradually through the side of the test tube. Formation of brown coloured ring at junction of two layers indicates the presence of sterols.

Development of solvent system for TLC (Thin layer chromatography)

TLC is used for separation of mixtures and identification of constituents from extract using different solvents. A number of solvent and solvent mixtures were tried for separation of constituents from chloroform extract of *Cassia fistula* fruit pulp.

TLC Fingerprinting of chloroform extract

TLC fingerprinting of chloroform fraction of fruit pulp performed using precoated silica gel 60 F254 TLC plates (E-Merck) of uniform thickness (20mm x

20mm). A 10 cm length of TLC plate was cut and marked carefully. 10 μ l of plant extract was spotted onto the marked plate with the help of a capillary tube or pipette. Chloroform: ethyl acetate: ethanol: acetic acid (5 ml: 5 ml: 3 ml: 10 μ l) was used as mobile phase. The TLC plate was kept in a chromatographic chamber containing the solvent system and the chamber was covered with glass plate to prevent evaporation of solvent. The plate was allowed to remain in the chamber till the solvent reached up to 8 cm distance. The plate was then observed at short and long wavelengths under UV-florescence analysis cabinet.

Visualization of TLC Plate

The TLC finger printing was derivatized with anisaldehyde sulphuric acid reagent followed by heating at 100°C till coloured bands of various secondary metabolites appeared. The observations were taken before and after derivatization, in visible as well as ultraviolet light. Rf values were calculated as follows:

$$R_f = \frac{\text{Distance traveled by substance}}{\text{Distance traveled by solvent}}$$

Results and Discussion

Observation of Phytochemical screening of partially purified fractions of *Cassia fistula* fruit pulp showed in table 1. Volatile oils were present in benzene, chloroform, acetone and alcohol fractions and flavonoids in benzene, chloroform, acetone and methanol fractions. Saponins were found only in petroleum ether and benzene fractions. Carbohydrates were present in benzene, chloroform and aqueous fractions. Alkaloids were absent in all fractions while steroids were present in chloroform and aqueous fractions. Only acetone fraction showed presence of tannins. Presence of anthraquinone glycosides, sennosides A & B, rhein and its glucoside, barbaloin, aloin, formic acid, butyric acid and their ethyl esters and oxalic acid, pectin and tannin in *Cassia fistula* fruit pulp has been reported by Agarwal and Paridhavi, (2005); Khare, (2007).

Ellof (1998) reported that tannins, saponins polypeptides and reducing sugars are soluble in water whereas terpenoids, flavonoids, alkaloids, and fatty acids are soluble in organic solvents. Similar findings have been reported by several workers (Scalbert, 1991; Zhang and Lewis, 1997). Tannins and reducing sugars are soluble in both water as well as organic solvents but their solubility is more in organic solvents as compared to water. Harborne (1984), Kokate *et al.*, (1990) suggested that extraction of secondary metabolites from plant material by hot extraction with petroleum ether separates sterols, waxes and fatty acids

leaving behind residue containing the defatted plant materials. Subsequently extraction of this residue with benzene separates out sterols and flavonoids. Terpenoids and flavonoids get extracted with chloroform. The last solvents i.e. alcohol removes alkaloids, flavonoids, polyphenols, tannins and reducing sugar from residue. Finally extraction with water yields remaining water-soluble metabolites such as anthocyanin, starch, tannins, saponins, reducing sugar and polypeptides (Scalbert, 1991; Zhang and Lewis, 1997). All the active principles present in plants are saturated organic compound so they get extracted in ethanol or methanol (Cowan, 1999).

The extraction of any plant material with solvent will yield a mixture of compounds. The extract may contain a wide variety of compounds like alkaloids, phenols, tannins, flavonoids, Volatile oils, Saponins and Carbohydrates etc. for the separation and identification of mixtures of constituents from partially purified extract, TLC is commonly performed using different combination of solvents. Higher the retention speed or the low retention time on TLC, better the solvent would be and vice versa. Among the different solvent system (table-2) for TLC of fruit pulp of *Cassia fistula*, Chloroform: ethyl acetate: ethanol: acetic acid in ratio of 5 ml: 5 ml: 3 ml: 10 μ l appeared as ideal solvent for resolution of maximum number of constituents. After derivatization of TLC, seven bands were observed for chloroform fraction of *Cassia fistula* fruit pulp. Changes in the colour of bands suggest that there is presence of different secondary metabolites in extract. Rf value of bands observed for this fraction lies between 0.12 to 0.75. The Rf value and colour of bands of chloroform fraction has been summarized in table-3, figure-1 and 2.

TLC profiling of chloroform extracts gives an impressive result that directing towards the presence of number of phytochemical. The TLC method is best choice for the identification of secondary metabolite present in plants. Here the different Rf values indicate the presence of different nature of phytoconstituents in single extracts. Different Rf values of the compound also reflects an idea about their polarity. This information will help in selection of appropriate solvent system for further separation of compound from these plant extracts.

Conclusion

In the present study the qualitative tests of extracts showed significant indication about the presence of metabolites. Preliminary phytochemical investigations tests are useful to isolate the pharmacologically active principles present in the plant. *Cassia fistula* is known as a rich source of tannins, flavonoids and glycosides

present in it, might be medicinally important and/or nutritionally valuable. It is an important medicinal plant with diverse pharmacological spectrum. The plant shows the presence of many chemical constituents which are responsible for varied pharmacological and medicinal property. The evaluation needs to be carried out on *Cassia fistula* in order to uses and formulation of the plant in their practical clinical applications, which can be used for the welfare of the mankind and formation of herbal drugs.

Acknowledgement

One of the authors (Deepa Hada) is thankful to University Grant Commission (UGC), New Delhi, India, for providing financial assistance.

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Table 1: Phytochemical screening of various fractions of *Cassia fistula* fruit pulp extract

Fractions	Alkaloids	Steroids	Volatile oils	Tannins	Carbohydrates	Flavonoids	Saponins
Petroleum ether	-ve	-ve	-ve	-ve	-ve	-ve	+ve
Benzene	-ve	-ve	+ve	-ve	+ve	+ve	+ve
Chloroform	-ve	+ve	+ve	-ve	+ve	+ve	-ve
Acetone	-ve	-ve	+ve	+ve	-ve	+ve	-ve
Alcohol	-ve	-ve	+ve	-ve	-ve	-ve	-ve
Methanol	-ve	-ve	-ve	-ve	-ve	+ve	-ve
Aqueous	-ve	+ve	-ve	-ve	+ve	-ve	-ve

+ve = Presence, -ve = Absence

Table 2: Composition and Resolution of developing solvent system for TLC

S. No.	Solvent system	Separation
1.	Petroleum ether	Satisfactory
2.	Toluene	Not good
3.	Chloroform	Significant
4.	n- butanol	Satisfactory
5.	Ethyl acetate	Significant
6.	Acetone	Not good
7.	2- propanol	Satisfactory
8.	Ethanol	Satisfactory
9.	Methanol	Good
10.	Water	Not good
11.	Acetic acid	Not good
12.	Chloroform: ethyl acetate	Not good
13.	Chloroform: ethyl acetate: ethanol	Satisfactory
14.	Chloroform: ethyl acetate: ethanol: acetic acid	Significant

Table 3: Rf Values of TLC Fingerprinting of chloroform fraction of *Cassia fistula* fruit pulp

S. No.	Total number of Bands	Colour of Bands		Rf Value
		Before Derivatization	After Derivatization	
1.	7 Bands	Light brown	Dark brown	0.12
2.		Light green	Dark green	0.18
3.		Light yellow	Dark yellow	0.31
4.		Light brown	Dark brown	0.55
5.		Light green	Dark green	0.62
6.		Light pink	Dark pink	0.70
7.		Dark green	Dark green	0.75

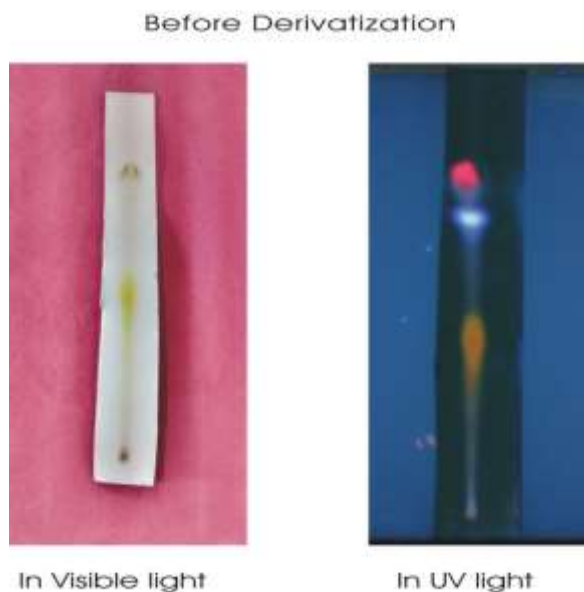


Fig. 1: TLC plate of chloroform extract (Before derivatization)



Fig. 2: TLC plate of chloroform extract (After derivatization)

How to cite this article

Hada D. and Sharma K. (2015). Phytochemical Screening and TLC Fingerprinting of *Cassia fistula* Linn. Fruit pulp. *Int. J. Pharm. Life Sci.*, 6(3):4334-4340.

Source of Support: Nil; Conflict of Interest: None declared

Received: 20.02.15; Revised: 01.03.15; Accepted: 17.03.15