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**Research Article**

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# Phytochemical Analysis of the Methanol Extract from Leaves of *Cassia fistula* L

**Laxman V Gavali, Vitthal S Shivankar, Yashwant A Gaikwad, Amit Surve Tejas Patil, Vaibhav Mali and Gurumeet C Wadhawa***

*Post Graduate Department of Chemistry, Karmaveer Bhaurao Patil College Vashi, Navi Mumbai, Maharashtra, India*

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**ABSTRACT**

*Our aim of study is to investigate the presence of various plant chemicals like salts steroids, triterpenes, alkaloids, saponins, tannins, anthraquinones, flavonoids and iso flavonoid compounds or their derivatives with their biomedical applications .Plant used for study are traditionally known we carry out phytochemical study of the dried leaf of the plant, to extract and fractionate the leaf and thin layer chromatography of the different fractions to study different components. In the phytochemical investigation of the plant, the powdered leaves was gives tests positive for steroids, triterpenes, alkaloids, sap, tannins, anthraquinones and flavonoids. In Navi Mumbai, Panvel region theses leaves are used on wound healing activity like tridex procubace and gulmohar tree.*

**Keywords:** Flavonoids; Alkaloids; Materials; Tests; Phytochemical; Chromatography; Tannins; Extraction; Colour

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## MATERIALS AND METHODS

### Collection and preparation of plant material

Plant leaves are used for the study of plant material collected from panvel region of raigad district indentified by Dr.B.K.Auti of RKMM College Ahmednagar and herbarium is preserved in Rayat shikshan Sansthsa Karmaveer Bhaurao Patil college Vashi as voucher no (001) The leaves were dried at room temperature for 13 days and when properly dried the leaves were powdered using clean pestle and mortar, and the powdered plant was size reduced with a sieve. The fine powder was then packed in airtight container to avoid the effect of humidity and then stored at room temperature.

Cassia fistula is a medium sized deciduous tree, 10 m tall with a straight trunk to 5 m, 1 m diameter and spreading branches. Stem bark pale grey, smooth and slender when young and dark brown and rough when old. Leaves alternate, pinnate, 30-40 cm long, with 4-8 pairs of ovate leaflets, 7.5-15 cm long, 2-5 cm broad, entire, the petiolules 2-6 mm long. Flowers bright yellow in terminal, drooping racemes, 30-60 cm long; calyx oblong, obtuse, pubescent; corolla with five subequal, obovate, shortly clawed petals, to 3.5 cm across; stamens 10, upper three with erect filaments to 0.7 cm long and with basifixed anthers; lower three curved and filaments with dorsifixed anthers and the median four stamens with erect filaments, to 1 cm long and with versatile, curved anthers; pistil sessile or stalked, ovary pubescent, style to 0.5 cm long and with terminal stigma.Fruit an indehiscent pod, 40-60 cm long by 1-2 cm diameter, cylindrical, pendulous and terete, containing 25-100 seeds. The pod develops numerous transverse septa between the seeds. When fresh the pods contain a black pulp which on drying adheres to the septThe drug "C. fistula", a mild laxative is obtained from the sweetish pulp around the seed. Reported to be laxative, aperient, astringent, purgative, and vermifuge, Indian laburnum is a folk remedy for burns, cancer, constipation, convulsions, delirium, diarrhoea, dysuria, epilepsy, gravel, hematuria, pimples, and glandular tumors. Ayurvedic medicine recognizes the seed as antibilious, aperitif, carminative, and laxative, the root is used for adenopathy, burning sensations, leprosy, skin diseases, syphilis, and tubercular glands, the leaves for erysipelas, malaria, rheumatism, and ulcers, the buds for biliousness, constipation, fever, leprosy, and skin disease, the fruit for abdominal pain, constipation, fever, heart disease, and leprosy. Yunani use the leaves for inflammation, the flowers for a purgative, the fruit as anti-inflammatory,

antipyretic, abortifacient, demulcent, purgative, refrigerant, good for chest complaints, eye ailments, flu, heart and liver ailments, and rheumatism, though suspected of inducing asthma. Seeds are considered emetic. Zimbabweans use the pulp for anthrax, blood poisoning, blackwater fever, dysentery, and malaria. Ghana natives use the pulp from around the seed as a safe and useful purgative. Konkanese use the juice to alleviate ringworm and blisters caused by the marking nut, a relative of poison ivy. Leaf poultices are applied to the chilblains so common in the upper Sind; also used in facial massage for brain afflictions, and applied externally for paralysis and rheumatism, also for gout. In the West Indies, the pulp and/or leaves are poulticed onto inflamed viscera, e.g. the liver. The bark and leaves are used for skin diseases, flowers used for fever, root as a diuretic, febrifuge, for gout and rheumat. Throughout the Far East, the uncooked pulp of the pods is a popular remedy for constipation. A decoction of the root bark is recommended for cleaning wounds.

**Extraction of the powdered leaf of the plant**

**Maceration:**

Collected plant leaves around 100 gm are soked in 350 ml of methanol in conical flask was cork with Teflon and shake on shaker for 30 min. and kept at room temperature for 48 years then refluxed for 2 hours ,then cool ,and evaporate all methanol to dryness, in evaporating dish.

**Fractionation of the methanol extract:**

The methanol extract (2 g) was placed at the top of a silica gel (28 g) wet packed in a chromatographic column and eluted with gradient of hexane and ethyl acetate at ratio of 8:2. 20 ml of the elute were collected in small bottles and labeled 1, 2, 3….19, successively. The fractions collected were spotted on thin layered chromatography (TLC) plate and developed. The plate was allowed to dry and sprayed with 20% sulphuric acid then heated in an oven at 105°C for 15 min.

**Fractionation of the methanol extract:**

2 gm of powered leaves placed on column around 30 gm of silica gel is taken and eluted with solvent hexane and etylacetate (8:2) and eluent are collected in conical flask labelled as 1-20 depend on TLC observed during column.

**Phytochemical analysis of *Paullinia pinnata***

**Identification of sterols and triterpenes:**

Three grams of the powdered leaves was placed in a test tube and 10 ml of 50% alcohol was added, the tube was then placed on a water bath and heated for 3 min. It was then allowed to cool to room temperature and filtered. The filtrate was then evaporated in an evaporating dish to dryness and 5 ml of petroleum ether was added to the dish and stirred for 5 min, the petroleum ether portion was then decanted and discarded. 10 ml of chloroform was then added and stirred for about 5 min, it was then transferred into test tube and 0.5 mg of anhydrous sodium sulphate was added and shaken gently and filtered, the filtrate was then divided into two test tubes and used for the following tests.

**Lieberman-Burchard's reaction:** To test tube I, equal volume of acetic anhydride was added and gently mixed. Then 1 ml of concentrated $H_2S0_4$ was added down the side of the tube. The appearance of a brownish-red ring at the contact zone of the two liquids and a greenish colour in the separation layer indicates the presence of sterols and triterpenes.

**Salwoski's test:** To test tube II, 2 to 3 drops of concentrated sulphuric acid was added to form a lower layer. Reddish-brown colour at the inter phase indicates the presence of steroidal ring.

**Identification of alkaloids:**

The powdered leaves (2 g) were boiled in a water bath with 20 ml of 5% sulphuric acid in 50% ethanol. The mixture was cooled and filtered. A portion was reserved. Another portion of the filtrate was put in 100 ml of separating funnel and the solution was made alkaline by adding two drops of concentrated ammonia solution. Equal volume of chloroform was added and shaken gently to allow the layer to separate. The lower chloroform layer was run off into a second separating funnel. The ammoniacal layer was reserved. The chloroform layer was extracted with two quantities each of 5 ml of dilute sulphuric acid. The various extracts were then used for the following test:

**Wagner's test:** To the filtrate in tube III, 1 ml of wagner's reagent was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids (Evans, 2002).

**Mayer's test:** To the filtrate in test tube I, 1 ml of mayer's reagent was added drop by drop. Formation of a greenish coloured or cream precipitate indicates the presence of alkaloids (Evans, 2002).
**Dragendoff's test:** To the filtrate in test tube II, 1 ml of dragendoff's reagent was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids (Evans, 2002).

**Identification of tannins:**
Two grams of the leaves was extracted with 10 ml of 50% alcohol, it was then filtered and the filtrate was divided into three portions for the following tests.

**Ferric chloride test:** Three drops of diluted solution of FeCl3 was added to the test tube I, production of a blue or greenish-black colour that changes to olive green as more ferric chloride is added indicates the presence of tannins (Evans, 2002).

**Bromine water test:** Three drops of bromine water was added to the second portion of the filtrate. A buff coloured precipitate indicates condensed tannins while hydrolysable tannins gave none (Evans, 2002).

**Lead sub-acetate test:** Three drops of lead sub acetate solution was added to the third portion. Occurrence of a coloured precipitate indicates the presence of tannins (Evans, 2002).

**Identification of anthraquinones:**
**Borntrager's test (for free anthracene derivatives):** The powdered leaves (0.5 g) was taken in a test tube and 5 ml of chloroform was added and shaken for 5 min. The mixture was filtered and the filtrate shaken with equal volume of 10% ammonia solution. A pink, red or violet colour in the aqueous layer after shaken indicates the presence of free anthraquinone (Evans, 2002).

**Modified Borntrager's test (for combined anthracene derivatives):** One gram of the powdered leaves was boiled with 5 ml of 10% hydrochloric acid for 3 min. The hot solution was filtered in a test tube, cooled and extracted gently with 5 ml of benzene. The upper benzene layer was pipetted off and shaken gently in a test tube with half of its volume of 10% ammonium hydroxide solution. A rose pink to cherry red colour in the ammonia layer indicates the presence of anthraquinone (Evans, 2002).

**Identification of saponins:**
**Frothing test:** The powdered leaves (0.5 g) was placed in a test tube and 10 ml of distilled water was added and shaken vigorously for 30 s. It was then allowed to stand for 30 min and observed. Formation of honey comb froth indicates the presence of saponins (Safowora, 1993).

**Haemolysis test:** One gram of the leaves was extracted with distilled water and 2 ml of aqueous NaCl solution was placed in a test tube and 2 ml of the filtrate was added to the test tube. Then 3 drops of an animal blood was added to the tube by means of a syringe and mixed gently by inverting the tube (no shaking) and allowed to stand for 15 min. The settling down of the red blood cells denotes the presence of saponins.

**Identification of flavonoids**
Two gram of the powdered leaves sample was completely detanned with acetone. The residue was extracted with warm water after evaporating the acetone on a water bath. The mixture was then filtered while hot, the filtrate was allowed to cool and used for the following test:

**$FeCl_3$ test:** To test tube III, 3 drops of FeCl3 solution was added, production of greenish-black colour indicates the presence of phenolic nucleus (Sofowora, 1993).

**Sodium hydroxide test:** To test tube II, 2 mls of 10% NaOH solution was added, yellow solution indicates the presence of flavonoids which on adding dilute hydrochloric acid becomes colourless (Evans, 2002).

**Shinoda's test:** Few magnesium chips were added to 3 ml of the aqueous solution and 2 drops of dilute hydrochloric acid was added and warmed. A pink or red colour indicates the presence of flavonoids (Evans, 2002).

**Thin layer chromatography**
**Parameters used:**
Absorbent (silica gel) Merck, Germany 120 mesh size, eluting solvent- n-hexane: ethyl acetate (8:2); technique: ascending; visualization aids: day light, methanol-sulphuric acid and heated at 105°C for 15 min.

**Development of thin layer chromatoghraphy for the extract**
The extract was applied onto the plate about 1.5 cm above the edge and 0.5 cm away from the margin, when the spot was dried, the plate was observed and then sprayed with methanol-sulphuric acid and then heated in oven at 105°C for 15 min. The solvent used for the mobile phase was n-hexane and ethyl acetate (8:2).

**Column chromatography of the extract**
The methanolic extract of the powdered leaf was added into a column pre-packed with silica gel. It was then run using n-hexane/ethyl acetate (8:2) and the separated fractions were collected separately in bottles.

**TLC of the fractions**
Using capillary tubes, the various fractions collected from column chromatography were spotted on a silica gel pre-coated plate 1.5 cm from the base and 0.5 cm away from the edge. Each plate was allowed to dry before putting it in a chromatographic tank containing specific solvent system. The developed plate was sprayed using methanol sulphuric acid.

## RESULTS

The followings are the results of analysis of phyto-chemical constituents in *P. pinnata* leaf.
**Identification of sterols and triterpenes**
**Lieberman-burchard's test:** A violet ring was formed at the contact zone of the two liquids; the upper layer becomes green which indicates the presence of sterols.

**Salwoski's test:** A reddish brown colour was observed at the interphase which indicates the presence steroid ring.

**Identification of tannins**
**Ferric chloride test:** A greenish precipitate was formed which indicates the presence of condensed tannins.

**Bromine water test:** A buff colour precipitate was observed which indicates the presence of tannins.

**Lead sub-acetate test:** A coloured precipitate was observed indicating the presence of tannins.

**Identification of alkaloids**
**Wagner's test:** A reddish-brown precipitate was formed which indicates the presence of alkaloids.

**Mayer's test:** A cream (buff) coloured precipitate was formed which indicates the presence of alkaloids.

**Dragendoff's test:** A reddish-brown precipitate was formed which indicates the presence of alkaloids.

**Identification of flavonoids**
**Ferric chloride test:** A greenish-black colour was observed which indicates the presence of flavonoids.

**NaOH test:** A yellow coloured solution was formed which indicate the presence of flavonoids.

**Shinoda's test:** A pinkish coloured solution was observed which indicates the presence of flavonoids.

**Identification of saponins**
**Frothing test:** Honey comb froth was formed which persisted for about 10 minutes indicating the presence of saponins.

**Haemolysis test:** The red blood cell settled down in the test tube which indicates the presence of saponins.

**Identification of anthraquinones**
**Borntrager's test:** A pink colour solution was formed showing the presence of free anthracene derivative.

**Modified Borntrager's test:** A pinkish colour was formed in the ammonia layer which indicates the presence of anthraquinone.

**Layer chromatography:** Technique used: Ascending; eluting solvent: n-hexane:ethyl acetate (8:2); visualization aids: Day light, methanol-sulphuric acid and heated at 105°C for 15 min.

(a) Before spray: Number of spot = 3; Colour: Spot 1: Light yellow, Spot 2: Yellow, Spot 3: Green.
(b) After spray: Number of spot = 6; Colour: Spot 1: Light yellow, Spot 2: Yellow, Spot 3: Pink, Spot 4: Green, Spot 5: Violet, Spot 6: Purple.
Retention factor (Rf)= Distance moved by the
Component/Distance moved by the solvent

| Extraction Fraction | Colour After Spray | Number of spots | Retention Factor |
|---|---|---|---|
| 1 | Light yellow | 7 | 0.6 |
| | yellow | | 0.9 |
| | pink | | 0.79 |
| | Green | | 0.58 |
| | Purple | | 0.68 |
| 2 | Light yellow | 3 | 0.57 |
| | pink | | 0.8 |
| | Green | | 0.6 |
| 3 | Pale Purple | 3 | 0.78 |
| | Green | | 0.9 |
| | Violet | | 0.45 |
| 4 | Violet | 1 | 0.58 |

| | | |
|---|---|---|
| Spot 1 Rf value = 0.72/7.2 = 0.10 | Spot 2 Rf value = 2.3/7.2 = 0.32 | Spot 3 Rf value = 2.9/7.2 = 0.40 |
| Spot 4 Rf value = 3.0/7.2 = 0.42 | Spot 5 Rf value = 3.2/7.2 = 0.44 | Spot 6 Rf value = 3.5/7.2 = 0.49 |

## DISCUSSION

Phytochemical analysis of the leaves of *P. pinnata* was successfully carried out, hexane/ethyl acetate at ratio (8:2) was found to be a good solvent system for the separation of the active constituents of the plant and using TLC, the separation of these constituents on the chromatogram was carried out. The powdered leaf was tested positive for steroids, triterpenes, alkaloids, saponins, tannins, anthraquinones and flavonoids. These results agreed with the literature review on the plant which showed these chemical constituents to be present.The TLC chromatograms of elutes collected showed different spots and colours ranging from fairly coloured to distinctively visible colours after spraying with 20% sulphuric acid indicating the presence of such chemical constituents (Plates 1 to 4 and Table 1).

## CONCLUSION

The phytochemial constituents of the leaf of *Cassia fistula* L was investigated. The leaf was found to constitute steroids, triterpenes, alkaloids, saponins, tannins, anthraquinones and flavonoids. The leaf is an African woody vine widely used in traditional medicine for the treatment of malaria and as a remedy against different forms of pains and as a natural cure (Jimoh et al., 2007). The presence of the constituents was also found to be similar to those reported for most medicinal plants.

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