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INVESTIGATION OF PHYTOCONSTIUENTS AND HPTLC PROFILE OF CHLOROFORM, ETHANOL AND AQUEOUS EXTRACT OF *CASSIA AURICULATA*

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ABSTRACT

Preliminary phytochemical investigation of chloroform, ethanolic and aqueous extracts of cassia auriculata were evaluated and HPTLC studies were carried out using CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 and WIN CATS-4 software were used. Research study showed the presence of terpenoids, bitter, tannins, saponins, glycosides, amino acids and flavonoids. HPTLC finger printing of chloroform, ethanolic and aqueous extracts of Cassia auriculata powder revealed presence of seven components at the wavelength of 254 and 366 nm. Rf 0.65-.7, 0.85 and 0.79 in chloroform and ethanolic extracts confirms the presence of bitter, steroids and terpenoids respectively. The HPTLC profiles of ethanolic and chloroform at Rf value at 0.6-0.8 and 0.1 for the presence of saponins and tannins respectively using anisaldehyde as spraying agent. The aqueous extract of same plant Rf value were found to be at 0.1-0.39 identifies for the presence of amino acids, using ninhydrin as visualizing agent. It can be concluded that HPTLC fingerprint analysis of seed, flower and seed and flower juice powder extract of *Cassia auriculata* can be used as a analytical tool for the accurate identification of the plant and it is useful as a phytochemical indicator.

KEYWORDS: Cassia auriculata, Phytochemical Screening, HPTLC Fingerprinting.

INTRODUCTION

Cassia auriculata usually known as Tanner's Cassia is an important medicinal plant used in traditional systems of medicine.[1] It also growing natural in Central Provinces and Western peninsula and cultivated in other parts of India.[2] The plant has been reported to have antipyretic, hepatoprotective[3] antidiabetic, antiperoxidative and antihyperglyceamic[4] and microbicidal activity.[5]

Cassia auriculata Linn (Family: Caesalpiniaceae) commonly known as Tanners Senna, is distributed throughout hot deciduous forests of India and holds a very prestigious position in Ayurveda and Siddha systems of medicine. The plant is used in the traditional system of medicine for urinary disorders, female antifertility, leprosy, worm infestation, diarrhoea, disease of pittam; bark is used in skin conditions; bark as astringent; leaves, flowers and fruits as anthelmintic; seeds for eye troubles, diabetes.[6,7,8] In ayurveda, its seeds are used to treat various gastrointestinal disorders.[9]

In the present investigation we have reported the isolation of bioactive compounds from chloroform, ethanol and aqueous extracts of Cassia Auriculata seed (BC), seed and flower juice (BBC), flower (PC). We have also investigated their phytochemical analysis through HPTLC method.

MATERIALS AND METHODS

1. Plant materials and preparation of extracts

Cassia auriculata L was collected from natural habitat, Mysore area, Karnataka. And authenticated by an expert and voucher specimen is kept in JSS Pharmacy College (No-Ayu/PhD/11-14) the taxonomic identification was carried out with the help of botanist. Extracts were prepared using exact weighed sample powder in the measured volume of solvents like, chloroform, ethanol and aqueous extract. Vaccum dried extracts are used for the experiment. Solvents used after distillation.

2. HPTLC Profile (High Performance Thin Layer Chromatography)

HPTLC studies were carried out following the method of Harborne[10] and Wagner[11] et al.

2.1 Sample Preparation

The chloroform, ethanol and aqueous extracts of Cassia Auriculata seed (BC), seed and flower juice (BBC), flower (PC)

10 mg of each crude extract were dissolved in 1.5 ml of suitable solvents taken in a vial. Chloroform and ethanol and aqueous extracts obtained were evaporated under reduced pressure using rotovac evaporator. Each extract residue was re-dissolved in 1ml of chromatographic grade chloroform, ethanol and water, which was used for sample application on pre-coated silica gel 60F254 aluminium sheets.

2.2 Development of Chromatogram

After the application of sample, the chromatogram was developed in Twin trough glass chamber 10x 10 cm saturated with selected solvent for 15 minutes.

2.3 Detection of Spots

The air-dried plates were viewed in ultraviolet radiation to mid-day light (Figure 1). The chromatograms were scanned by densitometer at 254 and 366 nm after spraying with anisaldehyde as spraying reagent for the detection of bitter, steroid, saponins and tannins in chloroform and alcoholic extract. The aqueous extracts were scanned at same wavelength for the detection of flavonoids and amino acids. The Rf values and finger print data were recorded by WIN CATS software.

2.4. Developing Solvent System

A number of solvent systems were tried, for extract, but the satisfactory resolution was obtained in the solvent dichloro methane: methanol with the ratio of 9.7:3 for chloroform and alcohol extract for the separation of bitter, steroids and terpenoids. Similarly aqueous extract separation solvents like, butanol, acetic acid water used in the ratio of 4:1:1.

2.5. Sample Application

Application of bands of each extract was carried out (4mm in length and 1ul in concentration for leaf) using spray technique. Sample were applied in duplicate on pre-coated silica gel 60F254 aluminium sheets (5 x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

2.6 Development of Chromatogram

After the application of sample, the chromatogram was developed in Twin trough glass chamber 10x 10 cm saturated with solvent dichloro methane: methanol (9.7:3) & butanol, acetic acid water (4:1:1) extract for 15 minutes.

3. Phytochemical screening: Qualitative assay, for the presence of plant phyto constituents such as carbohydrates, alkaloids, glycosides, flavonoids, tannins and saponins were carried out on following standard procedure.[12]

3.1 Preparation of extracts: The plant BC,BBC,PC flowers around (500 gm) were dried at room temperature and reduced to fine powder to particle size (#) 40 then subjected to continuous hot extraction with 90% ethanol in a soxhlet extractor for 48 h. The total ethanol extract was filtered and evaporated to dryness at 40°C under reduced pressure in a rotary evaporator.

3.2 Preliminary phytochemical studies: The preliminary phytochemical screening of ethanol and aqueous extracts were performed to identify the presence of sterols, triterpenoids, flavonoids and tannins.

Test for alkaloids: Small quantities of various extracts were dissolved in dilute sulphuric acid and filtered. The filtrate was treated with Dragendorff's reagent. Appearance of orange brown precipitate in response to the above reagent indicates the presence of alkaloids.

Test for amino acids: Small quantities of various extracts were dissolved in a few ml of water and treated with Ninhydrin reagent. Appearance of purple color indicates the presence of amino acids.

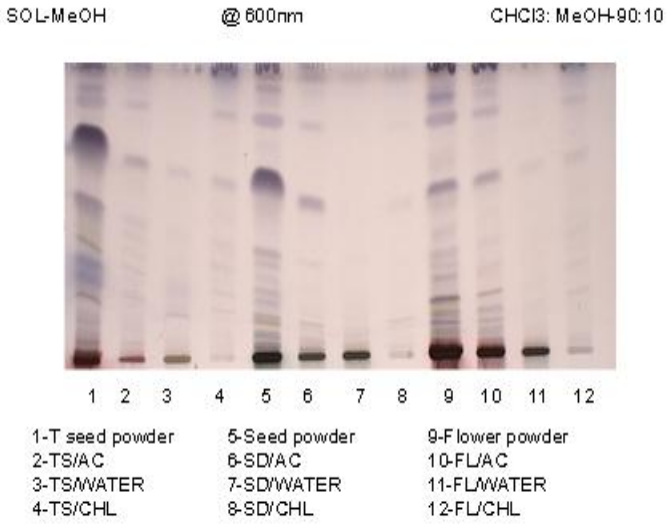
Test for saponins: The extracts were diluted with 20 ml of distilled water and it was agitated on a graduated cylinder for 15 minutes. The presence of saponins was indicated by formation of 1 cm layer of foam.

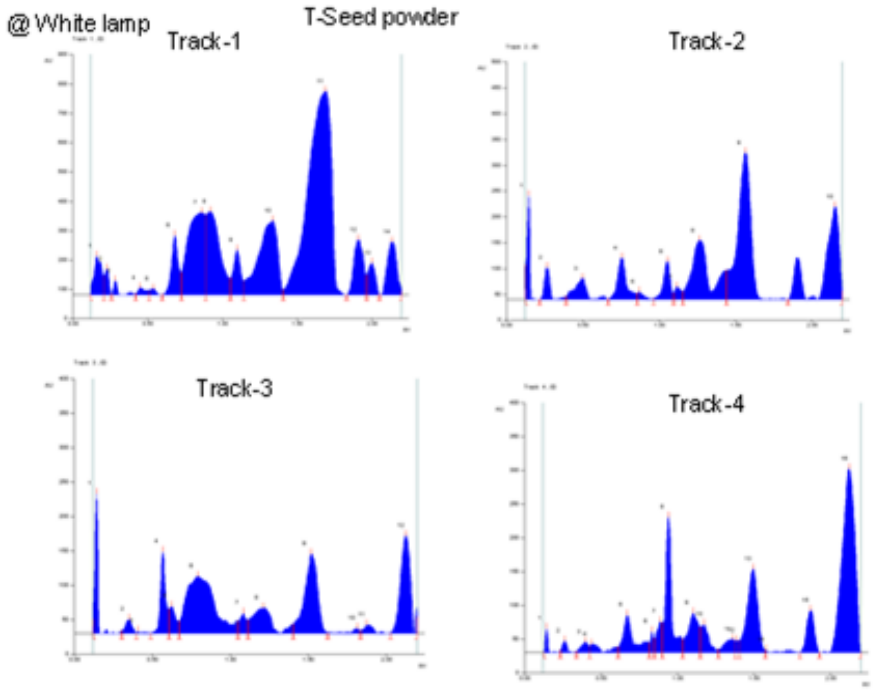
Test for carbohydrates and glycosides: The minimum amount of extracts were dissolved in 5ml of distilled water and filtered. The filtrate was subjected to test for carbohydrates and glycosides.

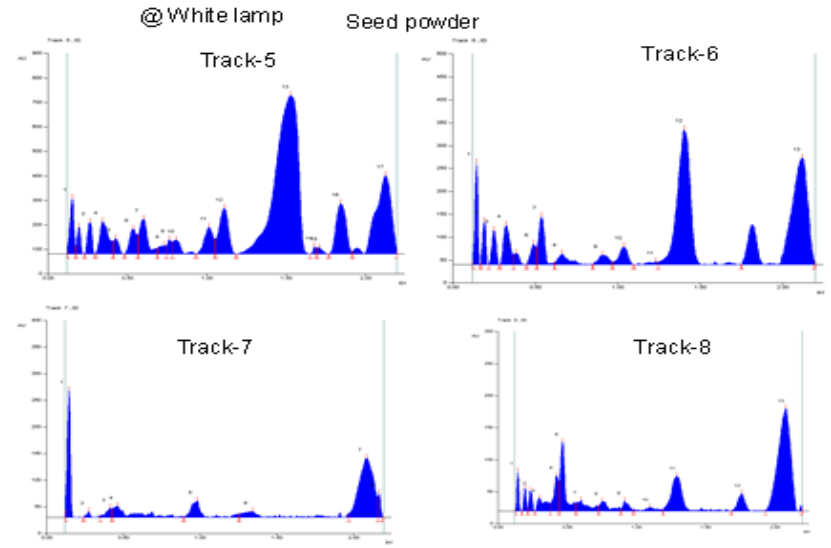
Molisch's test: The filtrate was treated with 2-3 drops of 1% alcoholic α-naphthol and 2ml of conc. sulphuric acid was added along the sides of the test tube. The formation of deep violet ring at the junction of two liquids and the spreading of color on standing shows carbohydrates.

Fehling's test

The filtrate was treated with 1ml of Fehling's solution and heated. Orange precipitate was obtained showing the presence of carbohydrates.



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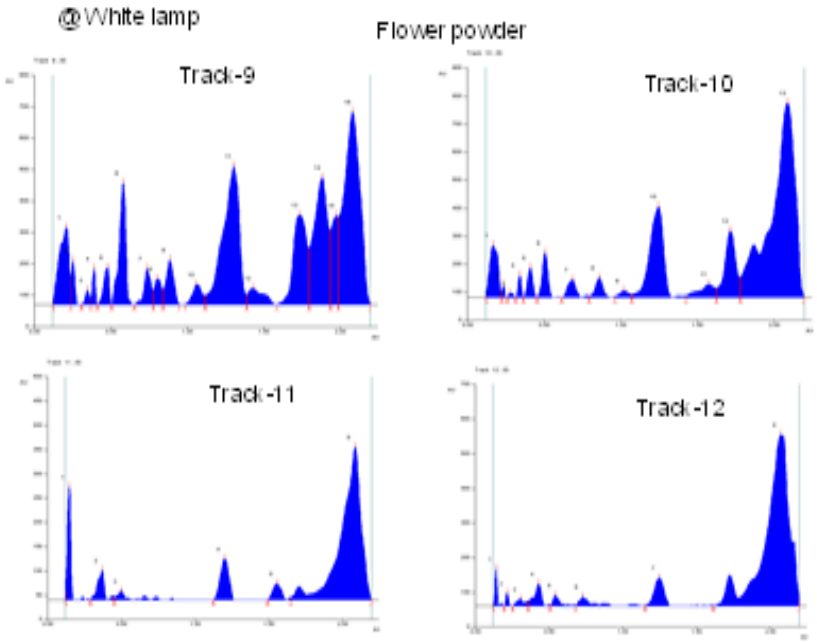
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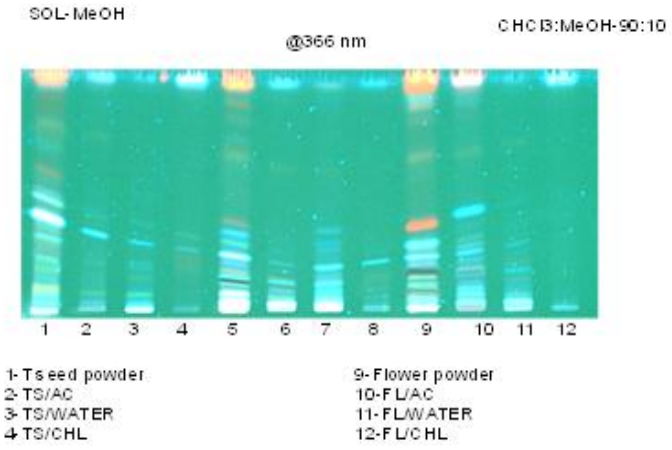
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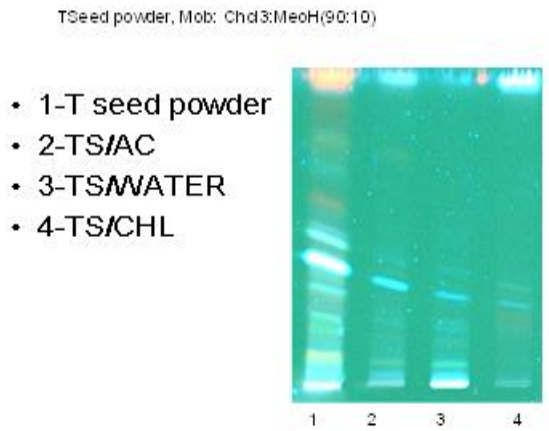

RESULTS AND DISCUSSION

The phytochemical test on chloroform, ethanol and aqueous extracts of *Cassia Auriculata* seed (BC), seed and flower juice (BBC), flower (PC) showed the presence of various phytoconstituents like, glycoside, steroid, protein, tannin, terpenoid, flavonoids and amino acid are present (Table 1).

The results from HPTLC finger print scanned at wavelength 254 and 366 nm for chloroform and alcoholic extract of *Cassia Auriculata* seed and flower (BC, BBC, PC). The results from HPTLC finger print scanned at wavelength at two different wavelength 254 and 366 nm for chloroform and ethanolic extract of *Cassia Auriculata* extract showed for presence of bitter, terpenoids and steroids phytoconstituents at Rf values ranging from 0.65-0.7, 0.79 and 0.85 respectively. The corresponding HPTLC chromatogram was presented in Figure 1, 2 and 3. The results from HPTLC finger print scanned at wavelength 254 and 366 nm for chloroform extract and ethanolic extract of *Cassia Auriculata* extract. There are two polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.1and 0.6 to 0.8, in which 1.5 -3.1 % and 1.81-1.99 % saponins and tannins in chloroform extract. The chromatograms were shown in Fig 4,5 and 6. The aqueous extract also scanned at similar wavelength and found amino acids in the Rf value ranging from 0.1 -.039 using ninhydrin as spraying reagent. The relevant chromatograms were shown in Fig 7,8 and 9. The comparative experimental values of chloroform, ethanolic and aqueous extract are shown in Table 2.

Table 1: Preliminary phytochemical screening of chloroform, ethanol and aqueous extracts of *Cassia Auriculata* seed (BC), seed and flower juice (BBC), flower (PC).

S.NO	Ext code	Tannins %	Flavanoids %	Bitters %	Saponins %	Terpenoids	Steroids	Alkaloids %
1	BBC-AQ	+	+	+	+++	-	-	-
2	BC-AQ	+	+	+	+++	-	-	-
3	PC-AQ	+	+	+	++	-	-	-
4	G-AQ	+	+	+	++	-	-	-
5	BBC-ET	+	+	+++	++	+	+	+
6	BC-ET	+	+	+++	+	+	+	+
7	PC-ET	+	+	+++	+	+	+	+
8	BBC-CF	+	+	+++	+	+	+	+
9	BC-CF	+	+	+++	+	+	+	+
10	PC-CF	+	+	+++	+	+	+	+

BBC-AQ: Seed and flower juice (Bhavita Beeja Choorna) Aqueous extract; BC-AQ: (Seed) Beeja Choorna Aqueous extract; PC-AQ: Flower (Pushpa churna) Extract; BC: Beeja Churna Aqueous Extract; + indicates presence of active compounds and – describes absence of active compounds.

Table 2: A Comparative Experimental Evaluation of extract of Seed, flower, seed and flower.

Sl.No.	Ext Code	Tannins In % W/W	Flavanoids In % W/W	Bitters In % W/W	Saponins In % W/W	Alkaloids In % W/W
1	BBC-AQ	4.71	0.13	7.3	28.4	0.01
2	BC-AQ	4.60	0.11	8.9	31.3	0.02
3	PC-AQ	4.32	0.14	5.1	23.6	0.011
4	G-AQ	4.51	0.17	8.6	22.32	0.15
5	BBC-ET	5.37	1.21	82.6	10.44	0.33
6	BC-ET	5.20	1.72	88.4	11.89	0.28
7	PC-ET	5.08	1.51	83.1	8.33	0.19
8	BBC-CF	1.81	0.38	93.1	1.5	0.21
9	BC-CF	1.78	0.42	95.1	2.3	3.24
10	PC-CF	1.99	0.21	83.45	3.1	0.94

BBC-AQ: Bhavita Beeja Choorna (Seed and flower) Aqueous extract; BC-AQ: Bhavita Choorna (seed and flower) Aqueous extract; PC-AQ: Pushpa churna Extract (Flower); BC: Beeja Churna (seed) Aqueous Extract;

CONCLUSION

On the basis of preliminary screening of phytochemical, it was concluded that, the bitters are enriched in the chloroform and ethanol extract of seed extract (BC), seed and flower juice

(BBC), seed (PC) and saponins. The amino acids are major in the aqueous extract of seed and flower juice (BBC), seed (BC) and flower (PC). The other phytochemical like steroids, terpenoids, flavonoids, alkaloids also present which are in minor extent.

From the HPTLC studies, it has been found that chloroform, ethanol and aqueous extracts contain not a single compound but a mixture of compounds and work is in progress to identify the possible mechanism of action and to identify the lead molecules.

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