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Extraction, isolation and chromatographic estimation of Aloe-Emodin and Physcion from *Cassia Fistula* root

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Abstract: The large and untapped resources of chemical compounds with significant medical potential found in medicinal plant species make them valuable as sources for biomedicine. Traditional medical systems like Ayurveda and Chinese Traditional Medicine are just two of the many that use the essential medicinal plant *Cassia fistula* L. It is a medium-sized tree that loses its leaves in the autumn. Its name is from its beautiful yellow flowers and long, rod-shaped seeds with pulp inside. The plant's roots were extracted using petroleum ether, chloroform, alcohol, and water in that order, and the products were looked at for preliminary phytochemical screening and antioxidant activity. Analysis of phytochemicals like alkaloids, flavonoids, carbohydrates, glycosides, protein, amino acids, saponins, and triterpenoids showed that most elements were present. Furthermore, this work concentrated on isolating and purifying aloe-emodin and Physcionusing column chromatography and then determining their purity using HPTLC. Different extraction solvents were tested, and ethanolic produced the highest yield of both. Toluene: Ethyl acetate: Methanol at 5:4:1 (v/v) showed superior resolution of Rf values at 0.7 and 0.9 as aloe-emodin and Physcion, respectively. Different solvents with different polarities were tried before using TLC to separate aloe-emodin and Physcion. The Toluene-ethyl acetate-formic acid ratio was increased when the silica gel column chromatography polarity was applied to the alcohol extract.

Introduction

There are traditional therapies that use botanical substances everywhere in the world. Herbal remedies continue to be one of the most prevalent forms of therapy available to the global population, even as the thrilling potential of gene therapy awaits us as we approach the new century. Given Western medicine's advancement, new synthetic pharmaceuticals and herbal treatments must meet worldwide standards for excellence, protection, and effectiveness. Herbal medications benefit from being accessible to patients in the same regions where certain traditional medicines are found. Herbal medications developed for global usage follow a different development process than synthetic pharmaceuticals. The primary way plants are used as medicine will stay the same as a source of medicines and a base for getting semi-synthetic chemical compounds used in the food, cosmetics, and fragrance industries (Chand and Shekhar, 2017). Golden shower is a good choice for a single plant. When the leaves fall off, they can look rough and untidy for a short time, but the bright flowers more than make up for this. Some places have placed this as a street tree, which has done well there. Golden-Shower needs full sun and dirt that drains well. The trees can handle some salt and some dryness. Even though conditions just below freezing hurt Golden-Shower, it will grow back when it gets warmer. When trees are young, they need to be pruned sometimes to shape them and give them a regular crown (Kirtikar and Basu, 2003).

The herb has excellent medicinal value and contains analgesic and antipyretic properties. Additionally, it has been discovered to have hypoglycemic and anti-

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inflammatory properties. This plant has been mentioned in Indian literature as helping treat skin conditions, liver problems, and tuberculous glands. It has also been proposed as a remedy for diabetes, haematemesis, pruritus, and leucoderm. There are no reports on the plant's pharmacognostic investigations, however. Therefore, this work is an attempt in this direction, and it consists of a preliminary phytochemical screening of numerous extracts of *Cassia fistula* Linn, as well as an evaluation of their morphology and physical properties, the determination of their physicochemical constants, etc. *Cassia fistula* root has tonic, febrifuge, astringent, and potent purgative properties. They are used for chest discomfort, blood dysentery, migraine, and joint pain.

Additionally helpful are fever, heart conditions, retained excretions, and biliousness, Amaltas root. It also treats different skin conditions, biliousness, rheumatoid arthritis, haemorrhages, wounds, ulcers, and boils. *Cassia fistula* is extensively cultivated as an ornamental plant in the tropics due to its abundant yellow blossoms. Botanically speaking, *Cassia fistula* is a tree that can reach heights of 6-9 metres, has a straight trunk, and has pale grey, smooth bark when young but brown, rough bark when fully old. The branches are pretty slender and spread out far. The plant has compound, pinnate, deciduous leaves with anything from three to eight leaflet pairs. Pendulous racemes of pubescent, glabrous flowers (about 4-7 cm in diameter) are carried by the plant. Yellow corollas and antheriferous stamens accompany a tall, hairy calyx with obtuse and rectangular segments. The fruit of this plant is a legume, and it contains many smelly seeds. After the flowers fade away, the long green pods of immaturity become black. The dark brown pulp of this species is sticky, mucilaginous, sweetened, and has a mildly off-putting aroma. The bark is found in thick, curved, or flat shards; it has an inner surface that is rough and red, with parallel striations, and an outside surface that is smooth to rough with warty areas. lamination, fracture; distinctively sweet taste and aroma as well as astringent (Kokate et al., 2008).

Materials and methods

Plant Materials

Cassia fistula Linn Plant material was collected from Malegaon region of Nashik district (Maharashtra). Plants were authenticated by Prof. Arvind S. Dhabe, Head and Professor on 16/10/2021 and the herbarium was deposited in the Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (India). *Cassia fistula* Linn. is frequently planted as an attractive plant in tropical and subtropical regions. In late April, it blooms. Trees are covered in abundant golden blossoms, often with hardly any leaves to be seen. It will thrive in arid environments. The conditions for this tree's growth include full light and well-drained soil. The tree produces solid, long-lasting wood (Yücedağ et al., 2019).

Preparation and Storage

plant material was first washed with water, followed by 95 % ethanol to prevent deterioration of plant material during storage and drying. Plant material was dried under shade until complete removal of water from it. Such dried material was powered by using pulverized and passed through sieve number 80. We cleaned the soil off the chosen plants' roots in running water, then chopped them into small pieces and dried them in the shade. These were disintegrated into a coarse powder (using sieve no. 10/44) and stored in a sealed, marked container (Siddhuraju et al., 2002).

Pharmacognostic Investigation

Morphology Features

The outward appearance was analysed using the techniques described in the literature. A basic microscope was used for the macro research. Khandelwal., 2008 was used to establish the flower and root colours, aromas, flavours, sizes, and shapes. External traits such as shape, size, texture, surface properties, and cracked surface were used to identify *Cassia fistula* roots using the given methodology and compared to the reported monograph. Sensing organs were used to examine the crude medication for characteristics such as colour, aroma, flavour, and fracture.

Transverse section & powder characteristics

Root free-hand sections were cut from live plants and stained with various chemicals by established protocols. Plant material was disaggregated using the method described. The scales were de-aggregated by boiling them in a NaOH (5% w/v) solution for 5 minutes. Pieces were treated with a 25% v/v chromic acid aqueous solution for 30 minutes at room temperature after chilling and washing with water. The sections were cleaned using chloral hydrate solution after being stained with phloroglucinol-hydrochloric acid (1:1) and toluidine blue. The powdered form of the medications allowed for closer examination under the microscope. Powdered medication was tested for the presence of lignified fibres, Calcium oxalate crystals, and starch grains using phloroglucinol-hydrochloric acid (1:1) solution, acetic acid, and iodine solution, in that order. Motic Images 2000 (version 1.3) analysis software was used to collect the series of digital images from a Motic Digital microscope equipped with a 1/3" CCD camera imaging accessories. The micrometric data were calculated as the mean and standard deviation

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of at least 30 individual measurements for each sample (Kokate, 1994).

Fluorescence analysis

Standard technique 28 was used to analyse the fluorescence of powdered medicine extracted from the root of *Cassia fistula*. The powdered root was subjected to different acidic and basic solutions and then studied in a UV-visible chamber at short and long wavelengths. Different chemical components can be identified because they glow in the visible spectrum when exposed to light. Many naturally occurring substances, including alkaloids, become fluorescent when exposed to ultraviolet radiation but not when exposed to daylight. If the active ingredients are not fluorescent, reagents can turn them into fluorescent byproducts. Therefore, it is a crucial pharmacognostical metric in assessing crude medicines (Hafez et al., 2019).

Physiochemical Investigation

Total ash, acid insoluble ash, water soluble ash, sulphated ash, moisture content, and other physicochemical parameters of the root of *Cassia fistula* Linn. were calculated.

Extraction and isolation of Cassia fistula

Roots taken from *Cassia fistula* Linn. for this investigation were washed thoroughly to remove debris or dirt before the extraction process began. Then, as night fell, it wilted and died. Leaves were spread in a tray and air-dried for a period of 7 days. The parched Root was then pulverized using a laboratory pulverized and the powder (particle size approximately 0.4mm) was used for extraction. Extraction is the basic step in herbal drug preparation, and it helps the plant metabolites to get solubilized in solvents. The critical factors that affect the efficiency of the extraction process are solubility of metabolites in the menstruum, temperature of extraction, particle size of the plant materials, etc. A molecule may be water soluble or water insoluble based on its chemical makeup. The powdered dried Root of *Cassia fistula Linn.* (500grams) were successively extracted by using Alcohol (Sigma Aldrich), in a Soxhlet extracting apparatus. Continue the extraction process for a day or until the solvent gets decolorized. When the extraction was complete, the liquid was collected, strained through Whatmann No. 1 filter paper, and then concentrated using a rotary vacuum evaporator at low pressure. Weight of *Cassia fistula Linn.* Alcoholic dried extract was determined, and further purification was performed using column chromatography (Sayed et al., 2023).

Qualitative phytochemical investigation of Cassia fistula Linn Extract

Qualitative Phytochemical investigation of *Cassia fistula Linn* extracts was done to determine the various phytoconstituents present in the plant and were subjected to routine qualitative chemical analysis to identify the nature of phytochemical constituents present in it. The existence and nonexistence of various phytochemical constituents, like alkaloids, carbohydrates, glycosides, saponins, amino acids and proteins, flavones and flavanones, tannins and phenolics, steroids, fixed oils were determined using standard established techniques (Hajare et al., 2023).

Quantitative phytochemical investigation of Cassia fistula Linn Extract

Calculating the total phenolic, flavonoid, and terpenoid content of alcoholic extracts of *Cassia fistula* root is part of this task.

Estimation of Total Phenolic Content

As McCune and Johns (2002) indicated, total phenolic content was calculated using the Folin-ciocalteu colorimetric method. Catechin served as the standard, while ethanol served as the blank for the standard-curve-making process. All measurements were taken in triplicate, and the data is presented as means standard errors. Catechin equivalents (CEQ), measured in milligrammes per milligramme of dry extract, were used to quantify the total phenolic content.

Estimation of total flavonoid content

Alcoholic extracts of the leaves and roots of a few species of Cassia were analysed for their total flavonoid content using the aluminium chloride colorimetric method published by Kim et al., 2004. To create a standard curve, Rutin was used as the gold standard. All calculations were performed three times, and the final results were presented as the mean standard error of the mean (REQ) in milligrammes per milligramme of dry extract (Mondal, 2014).

Column chromatography

They used a mobile phase of 50 ml Toluene, 40 ml ethyl acetate, and 10 ml Methanol to dissolve 10 gm of dried and partially filtered alcohol extract. Therefore, column chromatography was performed on the produced solution. Column chromatography used a glass column (Merck: 120-240 mm) packed with 650 g of silica gel. Colour bands produced in column helped researchers gather extract fractions that contained certain secondary metabolites. Toluene: ethyl acetate:(5:4:1) was used as the developing solvent system for the TLC silica gel 60 F254 plate on which all the collected fractions were spotted. After pooling fractions with identical Rf values,

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the organic solvent was extracted using a rotary evaporator. UV spectrophotometry at 434 nm and 286 nm were used to examine the Aloe-emodin and Physcion concentrations, respectively.

Purification of Aloe-emodin and Physcion

After separation by column chromatography, we heated methanolic solutions of Aloe-emodin and Physcion. After the mixture was completely dissolved, toluene was added to bring the ethyl acetate to methanol ratio to 5:4:1, and the mixture was stored at 5°C for 12 hours. Filtration was used to separate the crystals once they were acquired. Petroleum ether was used to precipitate the crystals. HPTLC analysis was used to determine the purity of individual crystals.

Characterization of isolated compounds chromatographic techniques

Characterizing isolated compounds using chromatographic techniques is crucial in modern analytical chemistry and natural product research. Chromatography is a versatile and powerful separation technique that allows scientists to separate, identify, and quantify individual components in a mixture. Here are some standard chromatographic techniques used for the characterization of isolated compounds.

High Pressure Thin Layer Chromatography (HPTLC) profile

Validating the identity of a crude medication requires standardising it. This research may therefore provide a foundation for correct plant identification. The HPTLC chromatographic fingerprint profile of *Cassia fistula* root extracts column fraction was investigated.

Sample preparation and application

Extracts at a concentration of 5 mg/ml were produced in chromatographic-grade solvents and subsequently filtered with Whatman filter paper No. 1. Prepared samples of different fractions were applied on TLC aluminium sheets silica gel 60 F 254 (Merck) 07 µl each with band length of 5 mm using Linomat 5 sample applicator set at a speed of 150 nl/sec.

Developing solvent system

For each extract, multiple solvent solutions were tested to see which would provide the best resolution and the most significant number of spots, however, Toluene proved to be the clear winner. Acetate of ethyl Methanol (5:4:1)

Development of chromatogram

For 20 minutes, at a distance of 80 mm, the chromatograms were produced in a twin trough glass chamber saturated with a solvent mixture of toluene, ethyl acetate, and methanol (5:4:1).

Scanning and detection of spots

TLC plates were sprayed with a detection reagent (5% methanolic potassium hydroxide and heated at 110°C for 5 minutes) and then viewed in the visible light range of 400-600nm. Spots were visible without derivatization at 434 and 286 nm wavelengths. The extracts were scanned at 434 and 286 nm in absorbance mode on a CAMAG HPTLC Densitometer (Scanner 3), as well as at 350 and 600 nm with a deuterium and tungsten lamp and a slit size of 6.0 x 0.45 macro. Colour and Rf values of the resolved bands were recorded (Mane et al., 2023).

Results and Discussion

The following Pharmacognostic evaluations exposed the dried roots of *Cassia fistula* Linn. (Amaltas). Studies at a macroscopic level were conducted to create pharmacognostic standards for plant authentication and to guarantee the appropriate quality of roots, as well as for field identification of plants (Rahim et al., 2023). Comparisons and observations of *Cassia fistula* L. Tabulated below are the findings. The morphology of the Amaltas genus *Cassia fistula* Linn. It has a light brown colour, a fragrant smell, and no flavour.Cork, cortex, sclerenchyma, medullary rays, phloem, xylem arteries, xylem fibres, and xylem parenchyma are all present in the root's transverse slice, which has a round contour.Cork has four to six layers and is a dark brown, thick-walled, angular rectangle. The cortex has four to five layers of tightly packed, polygonal parenchymatous cells with intracellular space, followed by a continuous ring of lignified sclerenchymatous cells. The periderm is continuous and of constant thickness all the way around the root. The radial thickness is 300 micrometres. Collateral vascular bundles include xylem on the inside and phloem on the outside, while medullary rays range from uniseriate to biseriate and reach up into the phloem. The middle cortical zone is rich in calcium oxalate crystals. Only prismatic crystals can be found here. Collapsed phloem cells generate dark, thick, tangential lines in the secondary phloem. The non-collapsed phloem is located within the collapsed portion. The cylinder of secondary xylem is solid and round. Elements of broad, round vessels are found dispersed throughout. The vessel element's diameter might be between 40 and 150 m. An amorphous gum-like substance fills several of the vessel parts. Both xylem fibres and xylem parenchyma are part of the secondary xylem. The fibres are of the libriform type; they are thick-walled and have a small lumen. The parenchyma of the xylem tissue forms a substantial sheath around the vessels. Figure 1 depicts the obtained outcomes. Cork cells, lignified fibres, pitted veins, stone

cells, and medullary rays are all visible under a powder microscope, as depicted in Figure 2.Light emitted by Fluorescence analysis was performed on powdered roots of *Cassia fistula* L after they were treated with various chemicals. Short- and long-wavelength UV light were used for the observations, respectively. Table 2 displays the outcomes. The results presented above provide credence to the traditional preparation of *Cassia fistula* root powder for its beneficial therapeutic effects. The ash values of dried leaves of *Cassia fistula* Linn. was ascertained to confirm the identity and cleanliness of the crude drugs. Ash values indicate the existence of inorganic substances that naturally exist in drug and those which adhere to the drugs like inorganic adulterating substances. Total ash value is useful for detecting excess of sandy and earthy matter associate with the crude drug. Water soluble ash values signify the existence of cellulosic substances. A high acid insoluble ash value for leaves indicates the presence of silicacious substances. Sulphated ash value is used for determining the content of inorganic impurities. Ash values, including total ash, water soluble ash, acid insoluble ash and sulphated ash of *Cassia fistula* Linn. was investigated. The total ash value was found to be (8.13±0.26%w/w). The ash that is soluble in water was (5.10±0.30%w/w) which denotes that cellulosic substances are less present in *Cassia fistula* Linn. root. The ash that is not soluble in acid was present more in *Cassia fistula* Linn. root (1.22±0.18%w/w) indicating presence of silicacious substances. Sulphated Ash was found to be in *Cassia fistula* Linn. (0.49±0.10%w/w) denoting the content of inorganic impurities associated with the crude drug. However, the leaves of *Cassia fistula* Linn included a significant amount of ash. Table 3 displays the findings. The moisture content of a crude medication can be roughly estimated by doing a loss on drying experiment. Contamination of crude pharmaceuticals is possible due to their low moisture content. Roots of the *Cassia fistula* Linn plant experienced a loss of (4.57±0.12%w/w) upon drying. In order to get the most out of the root of *Cassia fistula* Linn, its aerial portions were dried and ground into a powder before being extracted several times with alcohol. Table 4 displays the % yields of *Cassia fistula* Linn extracts. Extractive values aid in assessing the solvent solubility of particular ingredients, which can be used to detect counterfeit or depleted pharmaceuticals. When the extraction was complete, the liquid was collected, strained through Whatmann No. 1 filter paper, and then concentrated using a rotary vacuum evaporator at low pressure. Table 5 displays the range of hues and textures offered by the several *Cassia fistula* Linn root extracts.In order to determine the numerous phytoconstituents present in the plant, phytochemical tests were conducted before completing pharmacological examination of *Cassia fistula* Linn extract. Alkaloids, carbohydrates, glycosides, saponins, amino acids and proteins, flavones and flavanones, tannins and phenolics, steroids and fixed oils, and so on, all had their presence or absence determined using the accepted procedures. Table 6 displays the collected data. Alcoholic root extracts of *Cassia fistula* Linn. have been analysed for their total phenolic content. Catechin equivalent weights of 45.27 and 40.23 mg/g dry were found. Tables 7, 8 display the results of this investigation showing that the overall phenolic and flavonoid content was highest when compared to catechin (Sun and Shahrajabian, 2023). Figures 3, 4 depicted the data and graph respectively. The spots were identified using a TLC plate coated with silica gel 60 and a developing solvent solution of methanol. The organic solvent was extracted using a rotary evaporator after pooling fractions with identical Rf values. Figure 5 depicts the assembled parts. TLC and HPTLC Chromatography were used to characterise the total Aloe emodin and Physcion content obtained at 434 nm and 286 nm, respectively. Multiple bands were observed on TLC for each fraction. UV light at 434 and 286 nm revealed bands on TLC plates both before and after derivatization. Spraying TLC plates with 5% potassium hydroxide in methanol caused the band colours to shift (Kour et al., 2023). Both aloe-emodin and Physcion could be found in isolated fractions of root extract. Both aloe-emodin and Physcion concentrations in the sample were found to be adequate when compared to the standards. HPTLC densitometric analysis substantiates the presence of Aloe-emodin. The findings are shown in Tables 9–11 and Figures 6–8.

Table 1. Morphology of roots

Parameter	*Cassia fistula Linn*
Part	Bark
Type	Tap
Condition	Sliced
Color	Reddish brown externally & Light Pink internally
Odour	Mild characteristic
Taste	Slightly bitter
Size	
Length (cm.) Breadth (cm.)	18-35 5-12
Shape	Cylindrical
Fracture	Tough & fibrous

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Table 2. Fluorescence behaviour of powdered roots of *Cassia fistula* Linn.

Sr. No.	Reagent used	*Cassia fistula* Linn		
		Day Light	Short U.V. light (254 nm)	Long U. V. light (360 nm)
1	Dried Powder as such	Buff	Black	Light brown
2	1N HCL	Brown	Black	Light green
3	50% HCL	Light brown	Black	Light green
4	1N H_2SO_4	Light brown	Black	Brown
5	50% H_2SO_4	Light brown	Black	Greenish black
6	1N HNO_3	Brown	Black	Yellowish brown
7	50% HNO_3	Dark brown	Black	Yellowish brown
8	25%NH3	Brown	Black	Dark brown
9	5%Fecl3 (Aqueous)	Greenish black	Black	Greenish black
10	5% Fecl3 (Alcoholic)	Greenish black	Black	Greenish black
11	Picric acid	Yellowish Brown	Greenish Black	Light Brown
12	Acetic acid	Light brown	Black	Dark brown
13	1N NaOH (Aqueous)	Reddish brown	Black	Dark brown
14	1N NaOH (alcoholic)	Dark reddish brown	Black	Dark brown
15	Iodin water	Brownish Black	Black	Brownish Black
16	Distilled Water	Light brown	Blackish brown	Brown

Table 3. Ash values of *Cassia fistula* Linn. (%w/w)

Sr. No.	Type of Ash	*Cassia fistula* Linn
01	Total ash value	8.13±0.26
02	Water soluble ash value	5.10±0.30
03	Acid insoluble ash value	1.22±0.18
04	Sulphated Ash	0.49±0.10

Table 4. Loss on drying values

Sr. No.	Crude Drug	LOD
01	***Cassia fistula* Linn**	6.89±0.12

Table 5. The percentage yield

Sr. No.	Solvent	*Cassia fistula* Linn
		Percentage Yield (%W/W)
01	Alcohol	13.23
02	Petroleum. Ether	8.09
03	Chloroform	2.56
04	Aqueous	11.50

Table 6. Colour and consistency of different extracts roots of *Cassia fistula* Linn.

Sr. No.	Solvent	*Cassia fistula* Linn	
		Color	Consistency
01	Alcohol	Chocolate brown	Semisolid
02	Petroleum. Ether	Yellowish brown	Semisolid
03	Chloroform	Brown	Solid
04	Aqueous	Dull brown	Solid

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Table 7. Standard curve of Catechin for Phenolic content

Concentration (µg/ml)	Absorbance at 765 nm #
0	0
25	0.111
50	0.161
100	0.212
200	0.422
400	0.781
600	1.235
800	1.652
1000	1.992

Table 8. Standard curve of Rutin for Flavonoid content

Concentration (µg/ml)	Absorbance at 510nm #
0	0
25	0.036
50	0.068
100	0.102
200	0.225
400	0.421
600	0.711
800	0.958
1000	0.994

Table 9. Rf Values of TLC Fingerprinting of alcohol fraction of *Cassia fistula* Root

Sr. No.	Sample	Color of bands		Rf Value (cm)
		Before Derivatization	After Derivatization	
01	Aloe emodin Fr1	Light brown	Dark brown	0.11
02	Aloe emodin Fr2	Light brown	Dark brown	0.19
03	Physcion Fr1	Light brown	Dark brown	0.10
04	Physcion Fr1	Light brown	Dark brown	0.15

Table 10. Data interpretation of Aloe-emodin

Component 1: Aloe emodin					
Lane type	**y-Pos [mm]**	**Area**	**Height**	**CV [%]**	**Amt. [µl]**
Standard 1: Aloe Emodin	78.9	1259.73	301.16	n/a	5
Sample 1: CFR-Fraction -1 [5µl]	76.6	1005.95	100.166	n/a	2.2
Sample 2: CFR-Fraction-1 [3µl]	77.2	80.95	70.123	n/a	1.2
Sample 3: CFR-Fraction-2 [5µl]	76.6	75.36	20.39	n/a	0.8
Sample 4: CFR-Fraction-2 [3µl]	78.7	60.04	12.204	n/a	0.4
Sample 5: CFR-Fraction-3 [5µl]	77.4	65.99	9.586	n/a	0.6
Sample 6: CFR-Fraction-3 [3µl]	75.3	62.22	7.203	n/a	0.63

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Table 11. Data interpretation of Physcion

Component 2: Physcion					
Lane type	**y-Pos [mm]**	**Area**	**Height**	**CV [%]**	**Amt. [µl]**
Standard 1: Physcion	101.5	111.742	112.26	n/a	5
Sample 1: CFR-Fraction -1 [5µl]	102.4	80.584	28.341	n/a	4.8
Sample 2: CFR-Fraction- 1 [3µl]	101.6	27.991	15.104	n/a	2.6
Sample 3: CFR-Fraction- 2 [5µl]	102.6	140.256	35.752	n/a	6
Sample 4: CFR-Fraction- 2 [3µl]	102.6	216.817	48.549	n/a	8.2
Sample 5: CFR-Fraction- 3 [5µl]	102.9	283.082	70.88	n/a	12
Sample 6: CFR-Fraction -3 [3µl]	102.4	202.168	60.20	n/a	10

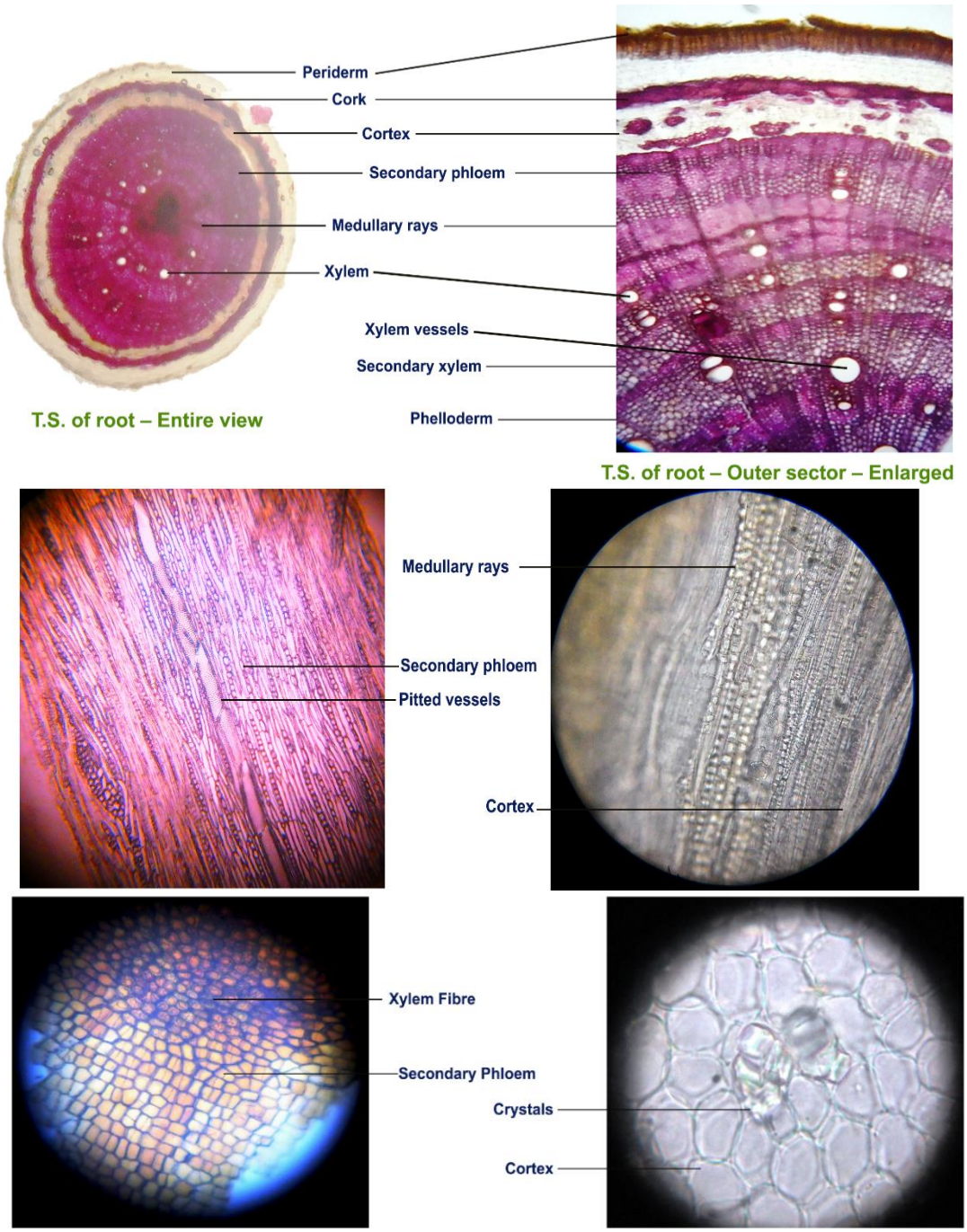

Figure 1. T.S. of Root of Cassia fistula Linn

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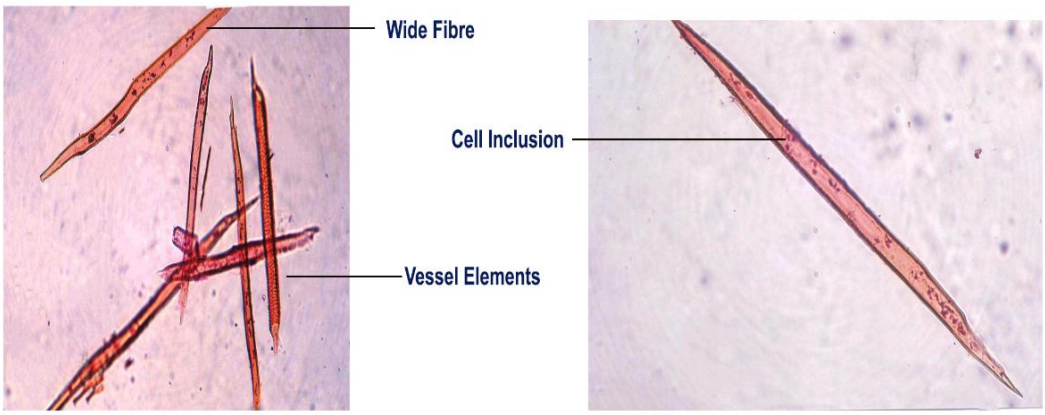

Figure 2. Powder microscopy of root of Cassia fistula Linn.

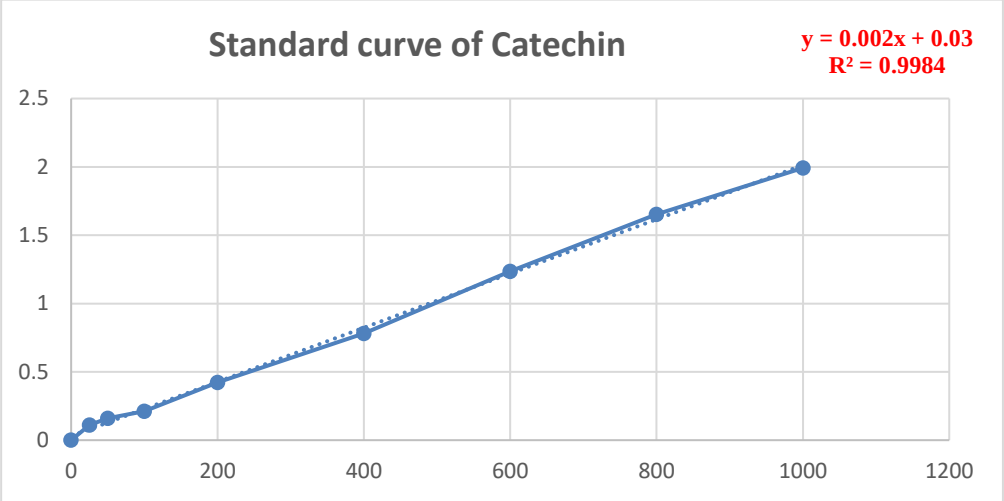

Figure 3. Standard curve of Catechin for phenolic content

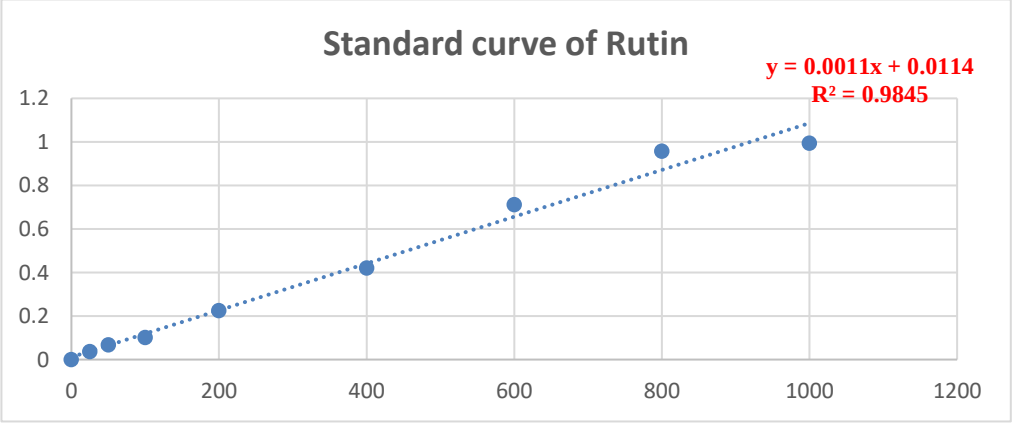

Figure 4. Standard curve of Rutin

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Figure 5. Column Chromatography of Cassia fistula root extract

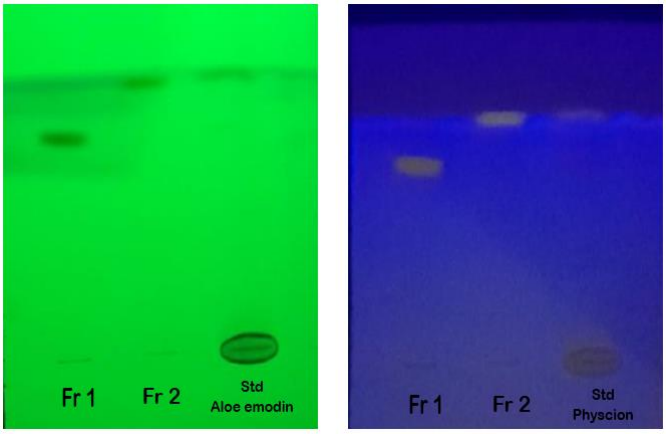

Figure 6. TLC Estimation of Aloe-emodin and Physcion under UV 434nm and 286 nm

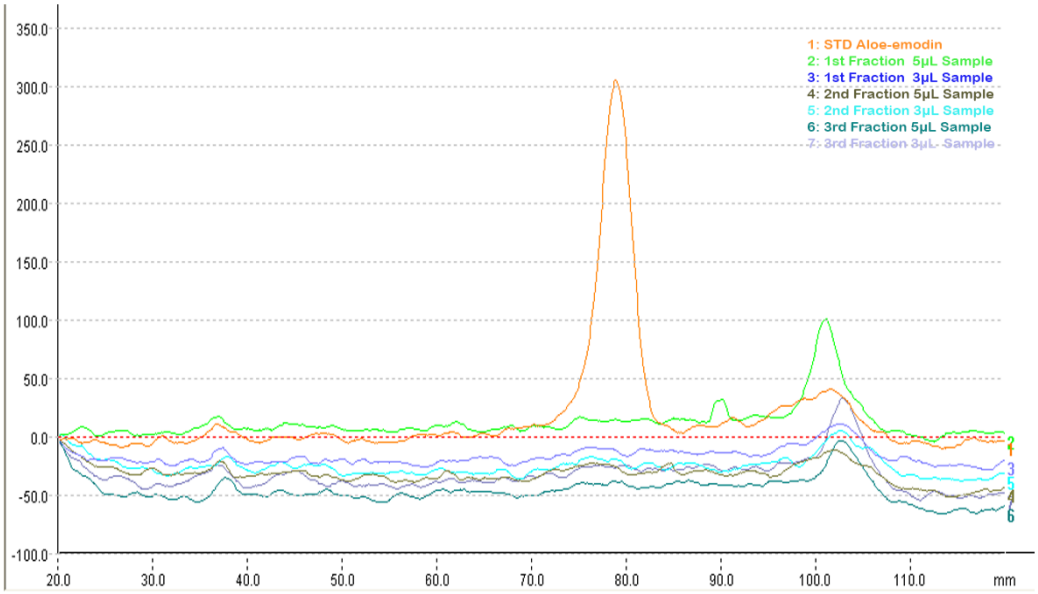

Figure 7. HPTLC Estimation of Aloe-emodin

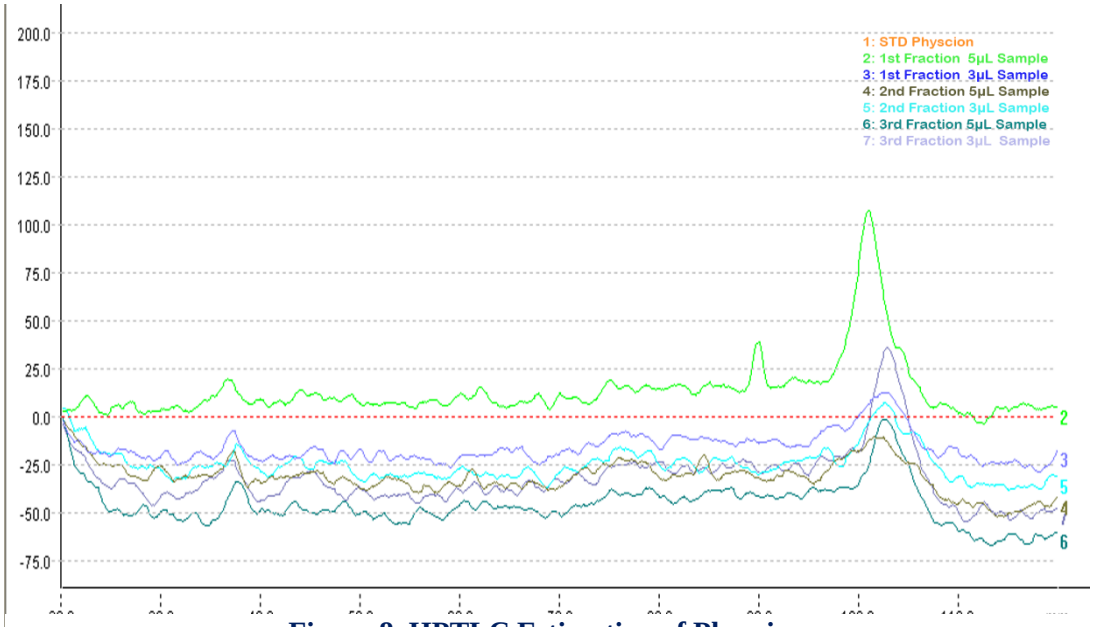

Figure 8. HPTLC Estimation of Physcion

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Conclusion

Family Fabaceae, subfamily Caesalpinioideae: that's where you'll find *Cassia fistula* (Linn.). It's a semi-wild plant that's often cultivated for its useful therapeutic characteristics. Asia, Africa, China, the Caribbean, and South and Central America, all have it in their own markets. Amaltas, often known as "Indian Laburnum" in English, is widely utilised in the Ayurvedic medical system to treat a wide range of conditions. The mature pods of *Cassia fistula*, which are both disease-free and in good health.In pharmacognostical screening the macroscopy, microscopy, powder studies, histochemical studies and physico-chemical analysis were carried out. Pharmacognostical standards acquired during the observation are valued apparatuses for the identification of the plant material. The chemical profile of the crude medicine was established through preliminary phytochemical screening. Most plants' physiologic or therapeutic activities can be traced back to a wide range of phyto-constituents. Thus, pharmacological examination of *Cassia fistula* was preceded by phytochemical research. Linn extract was performed to identify the plant's unique phytoconstituents. Alkaloids, carbohydrates, glycosides, saponins, amino acids, proteins, flavonoids, tannins, phenolic acids, steroids, and fixed oils were all tested and found to be present or absent according to established protocols. The percentage yield indicates how many soluble constituents may be extracted from plant material using various solvents such alcohol, petroleum ether, chloroform, and water. The standard curve's phenolic content shows that it was generated by measuring the absorbance of catechin standard solutions at 765 nm. Regression was performed on a graph depicting concentration against absorbance. With a correlation coefficient of 0.997, we get the following straight-line regression equation: y = 0.002x + 0.054. Alcoholic extract of *Cassia fistula* roots was used to discover Aloe emodin and Physcion.

Flavonoids absorbance at 510 nm of Rutin standard solutions of varying concentrations was used to generate the standard curve. Regression analysis and data plotting were performed. A straight line with a correlation coefficient of 0.996 was found to have a regression equation of y = 0.001x + 0.001. Crude alcoholic extracts were prepared by cold Successive extraction in a Soxhlet assembly by hot extraction method was followed to procure different partially purified fractions of the crude extract. Rapid qualitative photochemical studies were performed on Physcion and aloe emodin fractions to establish the presence of primary and secondary metabolites. The aim of these experiments was to identify the active component and isolate it.

We used precoated silica gel 60 F254 TLC plates (E-Merck) of uniform thickness (20mm x 20mm) to conduct TLC and HPTLC fingerprinting of the alcoholic fraction of the root, which was derivatized with 5% methanolic Potassium hydroxide and then heated at 100°C until coloured bands of various secondary metabolites appeared. Both visible light and ultraviolet light at 254 nm and 336 nm were used to make the measurements. They used a glass column (Merck: 120-240 mm) loaded with 650 g of silica gel for column chromatography after dissolving the dried, partially purified alcoholic fraction of *Cassia fistula* root in their mobile phase, which consisted of toluene, ethyl acetate, and methanol at a ratio of 5:4:1.

Conflict of interest

The authors state that no conflicting interests is found in this work.

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