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RESEARCH ARTICLE

# Comparative Pharmacognostical and Phytochemical Study of *Cassia auriculata* and *Cassia fistula*

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## Abstract

*Cassia auriculata L.* (CAL) popularly identified as Tanner's Cassia and *Cassia fistula L.* (CFL) is generally known as Golden Shower. Both plants belong to the Family, Fabaceae. These plants are used in skin disease, as hepatoprotective, as anticancer agent and as antioxidant agent. The intention of current article is to put forward the comparative pharmacognostical analysis of *Cassia auriculata* and *Cassia fistula* roots in terms of macroscopic evaluation, microscopic evaluation, physicochemical evaluation, extractive values and phytochemical analysis. Thin Layer Chromatography study was carried out for CAL and CFL and data pertaining to the above cited evaluations were recorded for both, CAL and CFL roots. The present study may help in differentiating among these species and these pharmacognostic parameters may serve as a tool for identification, authentication and standardization of CAL and CFL.

**Keywords:** *Cassia auriculata*, *Cassia fistula*, Microscopy, Phytochemical evaluation

## 1. Introduction

CAL is usually well-known as Tanner's Cassia, which fit in to the Family Fabaceae. The said plant is spread in Indian county and subcontinents. In Indian traditional system of medicine, the leaf and flower along with Triphala are utilized in the management of diabetic problems. The root of *cassia* is alexeteric and reported to be useful in thirst and respiratory problems. The leaves showed anthelmintic potential and they are supportive in the management of ulcers. The flowers are also reported to be useful in the treatment of throat complications[1,2]. Preclinical and clinical research have showed that roots have ephroprotective potential, leaves also showed liver protective action along with other health benefits[3–11].

CFL normally identified as Golden Shower belonging to Family Fabaceae, also well-known as Amaltas. The herb is found throughout the country. It is scattered in numerous countries including Asia, Mexico, East Africa, South Africa and West Indies along with Brazil. The root is generally consumed as a stimulant and febrifuge. It also shows potential as a strong laxative. In ayurvedic literature, root is used in skin problems like leprosy. The flowers are useful in treating cough and related problems, even flatulence. In Unani system of medicine, the leaf of CFL diminishes the edema. The flowers are used as a purgative. The seeds are used as an emetic. The described uses of CFL are as antibacterial, liver protective, wound healing, anti feedant, larvicidal, antifungal, protease inhibitor, anticancer and antifertility, antioxidant action[12–20].

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# 2. Materials and Methods

## 2.1 Procurement and Authentication of Plants

The roots of the plant CAL and CFL had been gathered from regions of Walgaon Road, Amravati, Maharashtra and some area of Bhopal, Madhya Pradesh respectively. Both the herbs have been validated by Safia College of Science, Bhopal, (MP), and the voucher specimen records were 159/Bot/Safia/2010 (CAL) and 160/Bot/Safia/2010 (CFL).

## 2.2 Preparation of Flavonoid Rich Fraction

The extraction of selected part of the herb for getting flavonoids rich fraction had been carried out. Roots of CAL and CFL (2 kg each) were dried under shade, and ground to get the coarse powdered material. Powdered roots of the plant were extracted with water by decoction method. While preparing the decoction, some precaution was taken regarding temperature, it was ensured that temperature did not exceed 40°C ±5°C as it would lead to precipitation or crystallization of various phytoconstituents which will not be soluble in any solvent for further process. The extracted aqueous sample was filtered and alcohol (ethanol) was poured to precipitate out polysaccharides which are present in the roots of the individual plant. The precipitated solution was filtered and the filtrate was evaporated to $1/4^{th}$ of the total volume. This was consecutively extracted with equivalent quantity of ethyl acetate with the aid of separating funnel to get separate fraction of root constituents. Then the ethyl acetate extract was acidified to raise the yield of the extract. The ethyl acetate fraction was evaporated to obtain a precipitate which was then dissolved in methanol and evaporated gradually to obtain crystalline powder.

The finally obtained powder was investigated for the occurrence of active phytoconstituents. The powder tested positive for Shinoda test for the Flavonoids. The positivity for flavonoids was also confirmed by TLC in appropriate solvent system. Similarly, the roots of both plants were used to obtain saponin rich fraction. Crushed plant material was macerated with ethanol: water (70:30) for seven days after defatting with petroleum ether (40:60). The extract so obtained was filtered through a muslin cloth followed by filter paper. Later this was concentrated using rotary vacuum evaporator (40˚C) with precaution that extract does not get powdered. N-butanol was used to treat concentrated extract to obtain n-butanol soluble fraction. N-butanol fraction was further reacted with chilled diethyl ether to form a precipitate. This combination with precipitate was reserved at -20˚C for 24 hrs. Precipitates were further alienated by centrifugation. These precipitates were dissolved in methanol and methanol was evaporated, to get a crystalline powder[21].

Powder was analysed for the presence/absence of different phytoconstituents. Positive results for froth test and hemolysis test was observed in powders, which confirms the occurrence of saponin in powder. Further purity of powder was confirmed on the basis of TLC. It was observed that few extra spots were present in solvent system (BAW). Obtained powder was further purified using column chromatography by solvent on the basis of their polarity. Purity was again confirmed on the basis of TLC.

## 2.3 Macroscopical Characters

External features, dimensions and organoleptic properties of roots were studied.

## 2.4 Microscopical Characters

### *2.4.1 Collection of Specimens*

The plant specimen was gathered. Precaution was taken to choose healthy sample. The root was dipped in FAA (Formalin + acetic acid + 70% ethanol). After 24 hours of fixing, the samples were dehydrated with graded series of tertiary butyl alcohol (TBA). Infiltration of the specimens was conceded by slow addition of paraffin wax until TBA solution attained saturation. Then the roots were moved into paraffin blocks.

### *2.4.2 Sectioning*

With the help of Rotary Microtone, the paraffin entrenched specimens were sectioned. The width of the sections was 10 – 12 µm. Dewaxing was conceded by customary method. Toluidine blue, a polychromatic stain was used to stain the sections. Satisfactory results were obtained by staining and some cytochemical reactions were also obtained. Cellulose walls were stained pink color with the dye, lignified cells stained blue, dark green to suberin, dark violet to mucilage, light blue to the protein bodies where essential sections were also stained with safranin and fast green and potassium iodide (for starch). Glycerine mounted temporary preparations were cleared with sodium hydroxide (NaOH) and after staining they were mounted in glycerine medium and the variant cell components were studied and measured.

## 2.5 Microphotography

Microscopic characterization of tissues was done with micrographs. Nikon lab photo 2 microscopic units were

used for taking photographs with different magnifications. Intense field was used for standard observation of the components. Polarizer was used to study the crystals, starch, grains and lignified cells. The results were vivid against dark background under the polarizer. Scale bars were used to indicate magnifications of the figures[23].

## 2.6 Physicochemical Evaluation of Crude Powdered Material[22,23]

### 2.6.1 Ash Value

Ash values for the roots such as total ash value, acid insoluble and water soluble ash value were investigated.

### 2.6.2 Extractive Value

Extractive values for the roots such as water soluble and alcohol soluble extractive values were investigated.

## 2.7 Thin Layer Chromatography

### 2.7.1 Detection of Flavonoids and their Glycosides

Detection: At UV 365nm and visible light the developed TLC plate was observed. Flavonoids and their glycosides emerged as yellow, dark blue, orange spots. The color gels intensified on exposure of the plates to ammonia vapors. Following spraying agents were used for finding the flavonoids and their glycosides,

a) Vanillin-Sulphuric acid
b) Anisaldehyde-sulphuric acid

### 2.7.2 Detection of Saponins

Detection: The developed TLC plate was experiential in visible light and in UV at 365 nm. Following spraying agents were used for detection of saponins,

a) Vanillin-Sulphuric acid
b) Anisaldehyde-sulphuric acid

## 2.8 High performance thin layer chromatography

### 2.8.1 Sample Application

Commercially available pre–coated plates of silica gel GF254 were used. Flavonoid rich fractions were spotted on plates with bandwidth of 6mm. Application rate was 10µl/ min, using Linomat V applicator (Automatic TLC plate applicator, Camag, Switzerland). A sample volume of 10 and 20µl was applied.

### 2.8.2 Development of Chromatogram

The plate was allowed to run in twin trough chamber (20×10) using the solvent system. After developing, the plates were air dried and observed under Camag UV chamber 4.

### 2.8.3 Densitometric Scanning

The developed plates were scanned using densitometer at 254 nm (Camag TLC scanner – 4, combined with integration software, Win CATS 4.06, Switzerland).

# 3. Results

## 3.1 Microscopy

### 3.1.1 Microscopic Characters of CAL Roots

The root measuring 2.3mm diameter was studied. The surface of the root was deeply and irregularly fissured. The periderm outer part exfoliates into small, irregular fragments. The root consists of thick periderm, narrow cortex and wide and continuous secondary phloem. The secondary xylem was thick and dense (Figure 1).

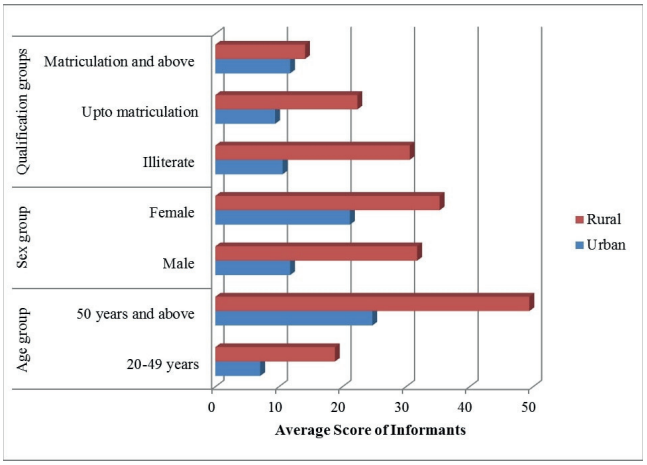
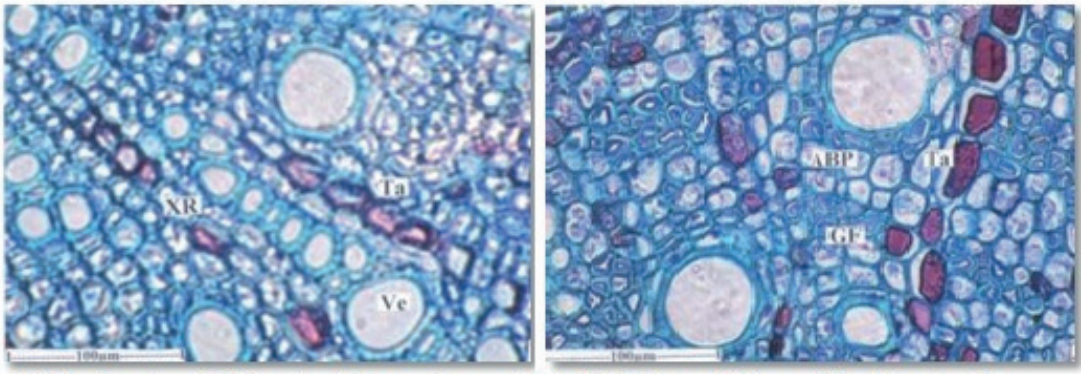



**Figure 1.** Transverse section of root - entire view.

The periderm consists of wide, darkly stained and thick walled phloem which was 150 cm thick. The cells contain darkly stained amorphous inclusions. The cells were tabular in shape and supervised. The inner part of the periderm had phelloderm which was 30 cm thick. It consists of thin walled radial files of tabular cells. The cortical zone was narrow comprising large, polygonal, compact thick walled cells.

Cortex was followed by secondary phloem which was 80cm thick. It includes sieve elements, phloem rays and phloem parenchyma. The inner part of the phloem consists of fairly wide, rectangular cells arranged in regular, radial files. The outer part of the phloem includes a narrow zone of collapsed phloem where the delicate cells were crushed into dark, thick tangential line. The inner part includes circular, clusters of sieve elements where the elements were narrow, thick walled cells.

Secondary xylem was about 1 mm in diameter. It consists of narrow, circular, solitary, thick walled diffusely distributed vessels (Figure 2). The vessels were 15-50 µm wide. There were tangential bands of apotrachial parenchyma cells. The parenchyma cells were wide angular and thick walled. The xylem rays were one or two cells thick, straight and they were filled with brown colored tannin. The xylem fibers were mostly gelatinous type. They had inner gelatinous or mucilaginous substance.

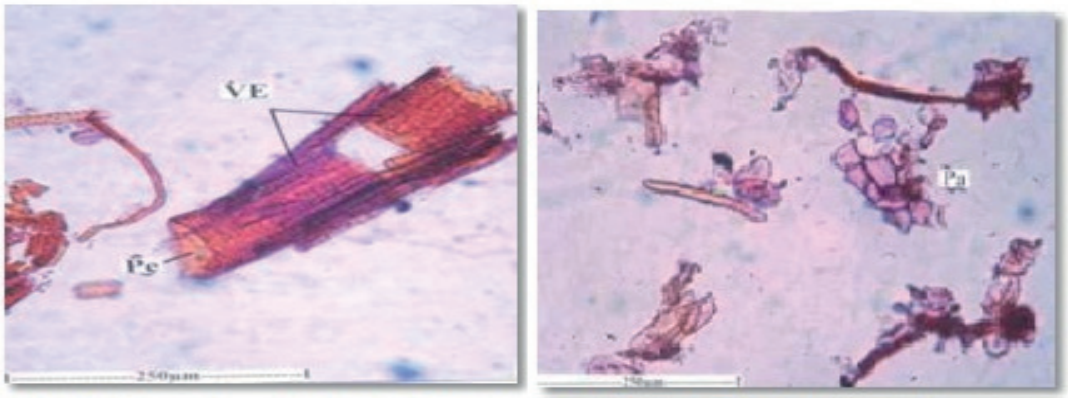



ABP- Apotrachial band of parenchyma, GF- Gelatinous Fibers, Ta- Tannin, Ve- Vessel, XR- Xylem Rays

**Figure 2.** T.S. of secondary xylem showing solitary vessels, apotrachial band of parenchyma and tannin filled xylem rays.

### *3.1.2 Powder Microscopy*

The powder of the root consists of vessels elements, fibers, Sclereids and parenchyma cells. The vessel elements were cylindrical, long or short and wide. The vessel elements range from 100-200 µm long. They had dense, multiseriate, elliptical bordered pits on the lateral walls and wide circular, oblique or horizontal perforation on the end walls. The fibers were narrow and wide. The narrow fibers were thick walled with narrow lumen. They were 710 µm long. The wide fibers were short, thin walled with wide lumen. They were 470 µm long. The Sclereids were long, narrow and fiber like. They have thick lignified walls with dense and prominent canal-like simple pits. Some of the sclereids were wider in the middle and tapering at the ends. Small polygonal thick walled parenchyma cells were seen scattered in the powder. The cells do not have any cell inclusions (Figure 3).




Pe- Perforation, Pi- Pits, VE- Vessel Elements, Pa- Parenchyma

**Figure 3.** Vessel elements and parenchyma cells.

### 3.1.3 Microscopic Characters of CFL Roots

Root sample having minimum 2.2 mm thickness was studied. It was circular in outline with more or less smooth outline. The root consists of periderm, cortex, secondary phloem and secondary xylem cylinder.

Periderm was continuous all around the root and more or less uniform in thickness. It was 300cm thick in radial plane. The periderm includes several layers of dark brown narrowly tabular radial files of phellem cells and 2 layers of wide, square in phelloderm cells.

The cortical zone includes polygonal, compact parenchyma cells and small scattered clusters of gelatinous fibers. Some of the cortical cells also had tannin content. Secondary xylem cylinder was solid with circular outline. It also showed wide, circular, diffusely distributed vessel elements. The vessel element ranges from 40-150µm in diameter. Some of the vessel elements were filled with amorphous gummy substance. The secondary xylem also consists of xylem fibers and xylem parenchyma.

The fibers were libriform type, thick walled with narrow lumen (Figure 4). Xylem parenchyma occurs in the form of thick sheath around the vessels. They are called paratrachial parenchyma. The secondary phloem consists of outer part of collapsed phloem cells forming dark, thick, tangential lines. Intact non-collapsed phloem was found inner to the collapsed part. In this region the phloem elements were intact with small clusters of sieve elements and polygonal wide parenchyma cells. Calcium oxalate crystals were abundant in the middle cortical zone. Prismatic types of crystals were exclusively observed.

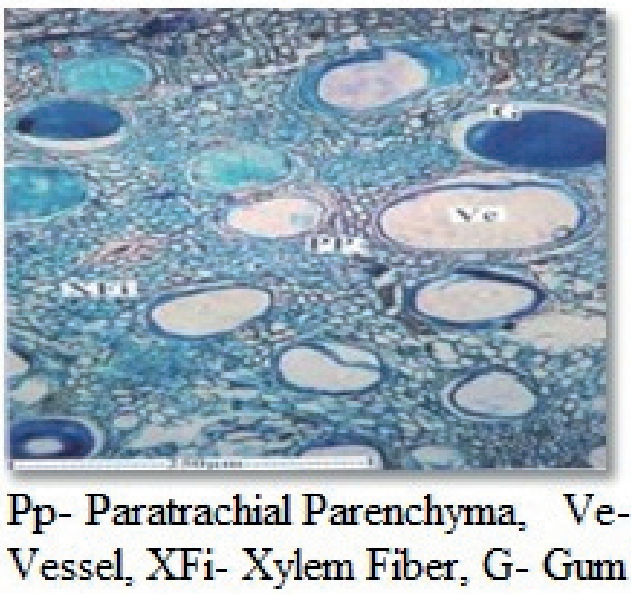



**Figure 4.** Secondary xylem showing gum inclusions in the vessels.

The crystals also occur in the xylem parenchyma which encloses the vessel (Figure 5).

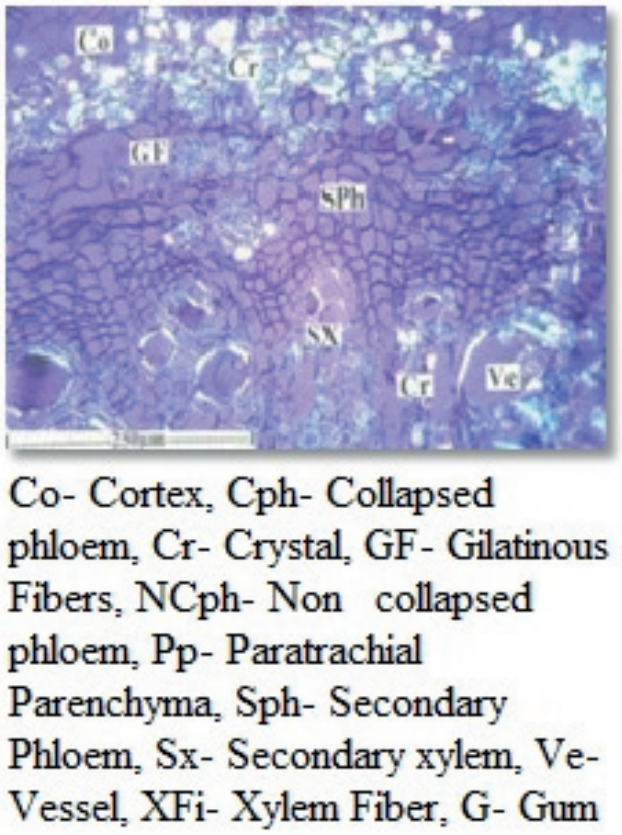



**Figure 5.** Crystal distribution in the bark.

### 3.1.4 Powder Microscopy

The root powder included fiber, vessel elements and periderm fragments. Parenchyma cells were also occasionally seen. The fibers were of two types. Many of the fibers were wide, thin walled and short. They were 1 mm long and 40 µm wide. Some crystalline bodies of unknown chemical nature were often seen inside the wide fibers. Narrow fibers were less common they were 550µm long 10 µm thick. The walls were thick and lignified. The lumen was narrow and no inclusions were seen in the cell lumen. The vessel elements were also of two types. Some were narrow, long and resemble the wide fibers in size and shape. The narrow vessel elements had short tails at both ends. The vessel elements had minute, circular, multiseriate pits on the lateral walls. The perforation of the wide vessels was circular and horizontal in orientation. The narrow vessels elements were 280 µm long 20 µm wide. The wide vessel elements were120 µm long and 40 µm wide.

## 3.2 Thin Layer Chromatography

TLC characterization of fractions of CAL and CFL has been discussed in Table 1. Flavonoids rich extracts of both the plants were subjected to HPTLC studies using solvents of varying polarity, Rf values of the separated components were found out. The number of components separated in the extracts, their Rf values and their percentage area were represented in respective tables.

**Table 1.** TLC characterization of fractions of *Cassia auriculata* and *Cassia fistula*

| Sr. No. | Mobile Phase | Spraying Reagent | No. of Spots | | Rf Value | | Inference |
|---|---|---|---|---|---|---|---|
| | | | CAL | CFL | CAL | CFL | |
| 1 | Chloroform: Methanol | Ammonia vapour/ VS reagent | 2 | 1 | 0.24, 0.83 | 0.62 | Flavonoids |
| 2 | Ethyl acetate: Formic acid: Glacial acetic acid: Water | Ammonia vapour/ VS reagent | 2 | 3 | 0.35, 0.53 | 0.27, 0.42, 0.70 | Flavonoids |
| 3 | Chloroform: Gallic acid: Methanol: Water | Anisaldehyde sulphuric Acid reagent | 1 | 2 | 0.22 | 0.47, 0.71 | Saponins |
| 4 | Chloroform: Methanol: Water | Vanillin phosphoric acid reagent | 2 | 2 | 0.14, 0.20 | 0.57, 0.61 | Saponins |

HPTLC was carried out by using following solvent systems, ethyl acetate: acetic acid: formic acid: water [100:11:11:26] (EAFW). HPTLC Fingerprinting of CAL at 254 has been discussed in Figures 6 and 7. HPTLC Fingerprinting of CFL at 254 shown in Figures 8 and 9. Correlation between reported and observed Rf values of various constituents has been observed in Table 2.

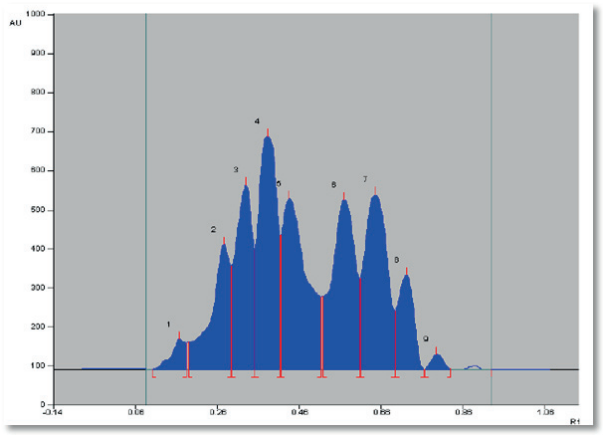


**Figure 6.** HPTLC fingerprinting of CAL at 254 nm.

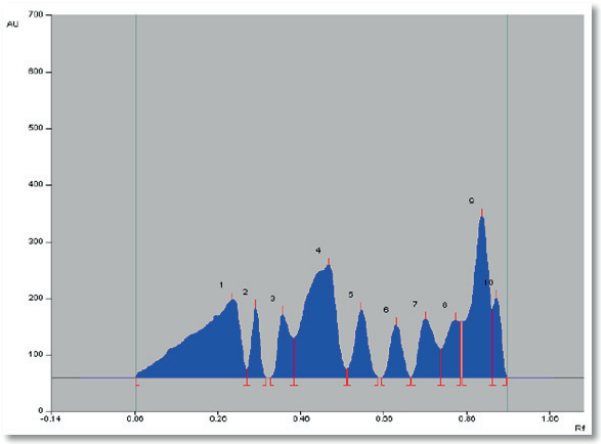


**Figure 8.** HPTLC Absorbance of CFL at 254 nm.

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.10 Rf | 0.1 AU | 0.17 Rf | 76.5 AU | 2.50 % | 0.18 Rf | 37.6 AU | 2162.4 AU | 1.80 % | unknown * |
| 2 | 0.19 Rf | 69.0 AU | 0.27 Rf | 318.9 AU | 10.42 % | 0.29 Rf | 33.2 AU | 12180.9 AU | 10.13 % | unknown * |
| 3 | 0.29 Rf | 264.7 AU | 0.33 Rf | 472.2 AU | 15.43 % | 0.35 Rf | 33.0 AU | 15210.3 AU | 12.65 % | unknown * |
| 4 | 0.35 Rf | 305.3 AU | 0.38 Rf | 596.3 AU | 19.49 % | 0.41 Rf | 41.0 AU | 21576.1 AU | 17.94 % | unknown * |
| 5 | 0.41 Rf | 343.7 AU | 0.43 Rf | 436.5 AU | 14.27 % | 0.51 Rf | 35.8 AU | 21147.3 AU | 17.59 % | unknown * |
| 6 | 0.52 Rf | 186.5 AU | 0.57 Rf | 433.2 AU | 14.16 % | 0.61 Rf | 31.9 AU | 19830.2 AU | 16.49 % | unknown * |
| 7 | 0.61 Rf | 233.5 AU | 0.65 Rf | 446.2 AU | 14.58 % | 0.69 Rf | 49.0 AU | 19911.7 AU | 16.56 % | unknown * |
| 8 | 0.69 Rf | 150.1 AU | 0.72 Rf | 241.0 AU | 7.88 % | 0.76 Rf | 0.2 AU | 7330.7 AU | 6.10 % | unknown * |
| 9 | 0.77 Rf | 0.3 AU | 0.79 Rf | 38.9 AU | 1.27 % | 0.83 Rf | 1.4 AU | 900.9 AU | 0.75 % | unknown * |

**Figure 7.** HPTLC fingerprinting of CAL at 254 nm.

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.06 Rf | 1.3 AU | 0.29 Rf | 137.0 AU | 9.70 % | 0.33 Rf | 13.9 AU | 12595.4 AU | 25.11 % | unknown * |
| 2 | 0.33 Rf | 14.4 AU | 0.35 Rf | 123.4 AU | 8.75 % | 0.38 Rf | 0.0 AU | 1774.9 AU | 3.54 % | unknown * |
| 3 | 0.39 Rf | 0.0 AU | 0.42 Rf | 112.0 AU | 7.94 % | 0.44 Rf | 39.4 AU | 2510.8 AU | 5.01 % | unknown * |
| 4 | 0.44 Rf | 69.5 AU | 0.53 Rf | 197.7 AU | 14.01 % | 0.57 Rf | 14.6 AU | 11540.3 AU | 23.01 % | unknown * |
| 5 | 0.57 Rf | 14.6 AU | 0.61 Rf | 119.6 AU | 8.47 % | 0.65 Rf | 0.3 AU | 2818.5 AU | 5.62 % | unknown * |
| 6 | 0.65 Rf | 0.0 AU | 0.69 Rf | 93.0 AU | 6.59 % | 0.72 Rf | 0.0 AU | 2016.6 AU | 4.02 % | unknown * |
| 7 | 0.73 Rf | 0.1 AU | 0.76 Rf | 102.9 AU | 7.29 % | 0.80 Rf | 50.1 AU | 3141.5 AU | 6.26 % | unknown * |
| 8 | 0.80 Rf | 50.1 AU | 0.83 Rf | 100.6 AU | 7.13 % | 0.84 Rf | 38.0 AU | 2712.4 AU | 5.41 % | unknown * |
| 9 | 0.85 Rf | 97.9 AU | 0.90 Rf | 284.4 AU | 20.15 % | 0.92 Rf | 18.0 AU | 8985.6 AU | 17.91 % | unknown * |
| 10 | 0.92 Rf | 119.4 AU | 0.93 Rf | 140.9 AU | 9.99 % | 0.96 Rf | 0.7 AU | 2067.6 AU | 4.12 % | unknown * |

**Figure 9.** HPTLC fingerprinting of CFL at 254 nm.

**Table 2.** Correlation between reported and observed Rf value

| Sr.no | Name of the Constituents | Reported Rf value | Observed Rf value |
|---|---|---|---|
| 1 | Chlorgenic Acid | 0.45, 0.50 | 0.44 |
| 2 | Isochlorgenic acid | 0.80 | 0.80 |
| 3 | Caffeic acid | 0.90 | 0.92 |
| 4 | Rutin | 0.40 | 0.41 |
| 5 | Apigenin 7–o–glucoside | 0.75 | 0.76 |
| 6 | Quercetin | 0.35-0.75 | 0.29, 0.35, 0.38 |
| 7 | Quercetin | 0.80, 0.74 | 0.72, 0.84 |
| 8 | Isoquercitrin | 0.80, 0.60 | 0.57, 0.61, 0.79 |
| 9 | Avicularin | 0.85 | 0.83 |
| 10 | Vitexin, 2–rhamnoside | 0.45 | 0.33, 0.42 |
| 11 | Vitexin | 0.70 | 0.65 |
| 12 | Luteolin | 0.40-0.55 | 0.51 |
| 13 | Kampeferol | 0.40 | 0.31, 0.47 |
| 14 | Flavone 6 – glycosides | 0.65 | 0.69, 0.65, 0.67 |
| 15 | Isoorientin | 0.45 | 0.42 |

## 4. Discussion

All plants have their unique nature, structure, appearance, chemical constituents and therapeutic efficacy. Therefore, it is essential to study organoleptic properties of a medicinal plant, not only for quality control and standardization but also to recognize its structure and biology.

CAL and CFL were used by traditional practitioners for management of several ailments. Regularity of herbal products is a multifaceted task due to their diverse composition in all forms of the material. To protect reproducible quality of herbal products, appropriate control of starting material is essential. The primary step towards ensuring quality of preliminary material is verification of the species. Now a days there has been a rapid increase in the standardization of many medicinal herbs of latent healing significance. Although many fresh techniques are available still identification of herbs by pharmacognostic properties is more liable. The macroscopic and microscopic explanation of a medicinal plant is the first step to create an identity and the purity of materials and should be conceded by undertaking any other tests, as described by World Health Organization[24].

Hence, it was felt desirable to pursue study on pharmacognostical and preliminary phytochemical studies of CAL and CFL roots to supplement useful information with regard to the exact identity of this plant

and, as this plant is extensively used in indigenous system of medicine.

Morphological assessment of drugs refer to valuation of drugs by taste, size, color, odor, shape and special features, like texture, touch. It is a method of qualitative evaluation based on the study of morphological and sensory summary of whole drugs. Organoleptic estimation is the conclusions drained from studies resulted due to feeling on organs of senses. All these parameters were recorded for roots of both the plants, CAL and CFL[25].

Microscopical techniques provide thorough information on the crude drug. Microscopical scrutiny of crude drugs from plant source is vital for the recognition of the grounded or crushed materials. Single-handed thorough microscopy could not provide complete assessment profile of a herbal drug, still it can supply underneath evidence, which when combined with other analytical parameters can be used to find the full confirmation for standardization and estimation of herbal drugs. Restricted burning of crude drugs result in an ash residue consisting of inorganic materials (metallic salts and silica). This value varies within fairly wide limits and hence a significant factor for the intention of evaluation of crude drugs. More direct contamination, such as by sand or earth, is immediately detected by the ash value. The quantity of active constituents in a given quantity of plant material when extracted with solvents is determined from the different extractive values obtained. Extractive values were used as revenue of evaluating crude drugs which were not eagerly expected by other means[26]. Presence of bioactive components can be ascertained on the basis of various phytochemical testing. Thus in present investigation, extracts of CAL and CFL were subjected to phytochemical testing, which revealed that both plant extracts of CAL and CFL have shown the presence of various pharmacologically active chemicals such as flavonoids, saponins, glycosides, phenolic compounds, tannins. This confirmed that the targeted active molecules were present in selected extracts.

## 5. Conclusion

The present study reports the comparison between macroscopic, microscopic, physicochemical parameters and phytochemical parameters of CAL and CFL. These parameters may help in differentiating among these species. Thus, pharmacognostic parameters may serve as a tool for identification, authentication and standardization of CAL and CFL.

## 6. Acknowledgements

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## 7. Conflict of Interest Statement

There are no conflicts of interest among all the authors with publication of manuscript.

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