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EVALUATION OF PHENOLIC CONTENT, PHYSICO-CHEMICAL AND HPTLC ANALYSIS OF SOME HERBAL DRUGS USED IN SIDDHA SYSTEM FOR DIABETES

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ABSTRACT

Many herbs identified in Siddha system of medicine have potent anti-diabetic and anti-hyperglycemic activity for the management of diabetes. The phenolic compounds present in them act as antioxidants and show reduction in blood glucose levels. In the present study, *Cassia auriculata* Linn. (Flower), *Coccinia indica* Wight & Arn. (Whole plant), *Gymnema sylvestre* (Retz.) R. Br. ex Schult. (Leaf) and *Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms) (Whole plant) were selected to evaluate the phenolic content and also to fix the pharmacopoeial standards. The physico-chemical, preliminary phytochemical, HPTLC analyses and powder microscopy were carried out to ascertain the authenticity. All the results obtained helps in the identification of the selected plants and thereby ensures the quality of the plants from adulteration and substitution. The phenol content evaluated using UV spectrophotometer confirmed the presence of polyphenols in different concentrations which might be one of the reasons for the anti- diabetic activity of the selected plant materials.

KEYWORDS: Diabetes, Siddha system, identification, secondary metabolites, phenol content, UV spectrophotometer.

INTRODUCTION

Diabetes is a chronic, metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves. According to WHO, about 422 million people have diabetes, the majority living in low-and middle-income countries. 1.6 million deaths reported each year are directly attributed to diabetes. The number of cases and the prevalence of diabetes have been steadily increasing over the past few decades. Diabetes is therefore a growing public health concern all over the world. There is a globally agreed target to halt the rise in diabetes by 2025. Siddha system of medicine is one of the earliest traditional medicine systems in the world which works efficiently to manage diabetics. In this system of medicine, diabetes mellitus is described as neerizivu noi or madhumegam.

There are many herbs in the Siddha system of medicine which have potent anti-diabetic and anti-hyperglycemic activity for the management of diabetes. Secondary metabolites or phytoconstituents present in these herbs are responsible for the therapeutic activity. Phenolics, alkaloids, saponins, terpenes, lipids etc. are the main classes of plant secondary metabolites. The phenolic compounds in herbs act as antioxidants due to their redox properties, allowing them to act as reducing agents, hydrogen donors, free radicals, quenchers and metal chelators.[1] They show reduction in blood glucose levels.[2] Plant phenolics include phenolics acids, flavonoids, tannin and the less common stilbenes and lignins.

In the present study, four plants traditionally used for the treatment of diabetes in the Siddha system of medicine are selected to evaluate the phenolic content. They are *Cassia auriculata* Linn. (Flower), *Coccinia indica* Wight & Arn. (Whole plant), *Gymnema sylvestre* (Retz.) R. Br. ex Schult. (Leaf) and *Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms) (Whole plant). The physico-chemical, preliminary phytochemical, HPTLC analyses and powder microscopy are also carried out to ensure the authenticity of these plants.

1. *Cassia auriculata* Linn

Cassia auriculata belonging to the family Cesalpinaceae is a legume shrub with large bright yellow flowers. It is

commonly known as Avaram or Tanner's cassia. The vernacular names of the plant are Hindi & Bengali - Tarwar; Gujarati - Awal; Marathi –Taravada; Telungu - Tangedu; Tamil - Avarai, Avirae; Malayalam – Aveeram, Avara; Kannada – Taravada-gida, Avarike Chakusina-gida; English – Tanner's cassia.[3]

It prefers drought and dry habitats, therefore are easily found in the tropical climates in India, Sri Lanka and Myanmar.[4] Besides its medicinal value, it is valuable as a tanning material and as a green manure crop. The parts of the plant used in medicine are seeds, leaves, flowers, roots and barks.[5] *C. auriculata* is used in Indian traditional medicine as a tonic, astringent, anthelminthic and as a remedy for diabetes, chylous urine, conjunctivitis and skin diseases. It removes bad odour of body.[6] It is also used for the treatment of ulcers, leprosy and liver disease. Hypolipidemic, antioxidant, antipyretic and hepatoprotective effect of *C. auriculata* have been reported.[7] It is one of the major components of beverage called kalpa herbal tea which has been widely consumed by people suffering from diabetes mellitus, constipation and urinary tract diseases.[8] One herbal formulation 'Avaaraipanchaga chooranam' which is prepared by using *C. auriculata*, is also very effective in treating diabetic population for controlling the blood sugar.[9]

2. *Coccinia indica* Wight & Arn.

Coccinia indica is a herb belonging to the family *Cucurbitaceae*. It is a slender scandent climber also known as Ivy gourd. The vernacular names of the plant are Sanskrit – Bimba; Hindi –Kanduri; Bengali: Telakucha; Kannada – Kaagethonde; Malayalam – Koval; Tamil – Kovai; Telugu – Dondakaya.[10]

It grows upto 8 m and has cosmopolitan distribution. It is indigenous to Bengal and other parts of India. *C. indica* also grows abundantly in Tropical Africa, Australia, Fiji and throughout the oriental countries. It is famous for its hypoglycemic and antidiabetic properties in traditional systems.[11] Different parts of this plant namely the roots, leaves and fruits are used in folklore medicine for several conditions such as jaundice, wounds, ulcers, stomach ache, skin disease, fever, asthma and cough. It has been reported to possess hypoglycaemic, hypolipidemic and antioxidant properties.[12] Anti-inflammatory, analgesic and antipyretic activity of fruit and leaves were also found to be significant.[13]

3. *Gymnema sylvestre* (Retz.) R.Br. ex Schult.

G. sylvestre is a perennial, woody climber belonging to the family Asclepiadaceae or the "milk weed" family.[14] The vernacular names of the plant are Sanskrit: Meshashringi; Hindi: Gurmar, Merasingi; Bengali: Meshashringi, Malayalam: Chakkarakkolli Tamil: Shirukurinja and Telgu: Podapatri.[15]

The plant is found in tropical and subtropical regions, well distributed in parts of central and southern India, southern part of China, tropical Africa, Malaysia, and Sri Lanka.[16] *G. sylvestre* is a potent antidiabetic plant and is used in Folk, Ayurvedic, Siddha and Homeopathic systems of medicine. It is also used in the treatment of asthma, eye complaints, snakebite, urinary complaints, stomach problems, piles, chronic cough, breathing troubles, colic pain, cardiopathy, constipation, dyspepsia and hemorrhoids, hepatosplenomegally etc. It also possesses antimicrobial, antihypercholesterolemic, anti-inflammatory and sweet suppressing activities.[17]

4. *Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms)

Tinospora cordifolia belongs to the family Menispermaceae. The vernacular names of the plant are Sanskrit: Guduchi; Hindi & Bengali: Giloe; Punjabi: Gilo; Malayalam: Chittamritu; Tamil: Sindal and Telugu: Somida.[18]

It is a genetically diverse, large, deciduous climbing shrub with greenish yellow typical flowers, found at higher altitude. It is indigenous to the tropical areas of India, Malaysia, and China. It is a well reported plant possessing numerous medicinal values including anti-diabetic property. The anti-diabetic activity of *T. cordifolia* has been reported experimentally and clinically in numerous scientific journals. In addition, it is recommended for burning sensation, fever, edema etc. It has been scientifically validated in various animal models for hypoglycemic, immunomodulatory, anti-inflammatory, antioxidant and other pharmacological activities.[19]

MATERIALS AND METHODS

The plant materials were collected and authenticated by the botany experts of Pharmacognosy Department, SRRI, Thiruvananthapuram. The selected samples were dried in shade, cut, crushed, and kept in airtight bottles for all analysis.

Physico-chemical analysis: The physico-chemical parameters were determined by standard methods.[20]

Preliminary Phytochemical analysis: The phytochemical constituents of the alcohol extracts of the selected plant materials were screened as per standard methods to identify the major natural chemical groups such as alkaloids, flavonoids, steroids, phenols, tannin, saponin, carbohydrates and terpenoids. General reactions in these analyses revealed the presence or absence of these compounds in the plant extracts.[21,22]

High Performance Thin Layer Chromatographic (HPTLC) analysis: HPTLC analysis is one of the sophisticated instrumental techniques based on thin layer chromatography.[23] The advantages of automation, scanning, full optimization, selective detection principle and minimum sample preparation enable it to be a powerful analytical tool for chromatographic information of natural products which is unique to individual plant.

Application of sample: Alcohol extracts of plant materials were used for chromatographic studies. For preparing the extracts, 1g of each drug was refluxed with 10 ml of alcohol and filtered. The extract is concentrated on a water bath to 1 ml. Each plant extract was applied in the form of band in different tracks with Camag microlitre syringe of Automatic TLC Sampler 4 (ATS4) on a precoated silica gel 60 F254 (Merck) plate.

Pre- conditioning (Chamber saturation): A 10x10 twin trough glass chamber was lined with filter paper before adding the mobile phase. The chamber was saturated with the mobile phase, Toluene: Ethyl acetate (5: 1) for 30 minutes prior to the development to ensure the uniform distribution of solvent vapours.

Chromatographic development, Detection and Visualization: Linear ascending developments of the extracts were done in twin trough glass chamber saturated with the selected mobile phase. The developed plate was air dried and visualized under UV short and UV long. The chromatograms obtained for the alcohol extracts of the plant materials were documented.

Scanning densitometry: After visualization, the plate was scanned at 254 nm and 366 nm using TLC Scanner 4 with winCATS software for interpretation of data and the results were documented.

Derivatization: The plate was derivatised using vanillin-sulphuric acid reagent, heated at 105°C by placing on CAMAG TLC plate heater till the colour of the bands appeared. Then the plate was visualized under white light and scanned at 575 nm. All the data obtained were documented.

Powder microscopy: The powdered plant materials were subjected to powder microscopy studies as per standard protocol.[24] The powdered tissues of drug was mounted in glycerin at room temperature for 24 h and observed under 10X and 40X objective of the microscope for diagnostic powder features.

Estimation of total phenol content: 10 g each of the plant material was extracted with 100 ml of ethanol for 3–4 h. The filtrate of each one was concentrated. The total phenolic content of the extract was determined by the Folin–Ciocalteu method by using UV spectrophotometer (Model: UV3120).[25] The total phenolic content of each plant material was calculated from the calibration curve, and the results were expressed as mg of pyragallol equivalent per g dry weight.

RESULTS AND DISCUSSION

The physico-chemical parameters of the plant materials are given in Table 1 which are important diagnostic features of the plants.

Table 1: Physico-chemical parameters of the selected plants.

Sl.No.	Parameters	Results (%)			
		C. auriculata **(flower)**	***C. indica*** **(whole plant)**	***G. sylvestre*** **(leaf)**	***T. cordifolia*** **(whole plant)**
1	Foreign matter	Nil	Nil	Nil	Nil
2	Loss on drying at 105°C	12.93	18.67	5.96	14.46
3	Total ash content	4.31	9.40	10.48	8.79
4	Acid insoluble ash	0.20	2.35	0.04	0.20
5	Water soluble extractive	17.52	10.07	15.97	6.74
6	Alcohol soluble extractive	20.50	2.42	11.67	4.02
7	Volatile oil	Nil	Nil	Nil	Nil

The total ash value is particularly important in the evaluation of purity of drug. It is the presence or absence of foreign matter such as metallic salts or silica. The water soluble extractive values indicated the presence of sugar, acids and inorganic compounds in the drugs. The alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids etc.

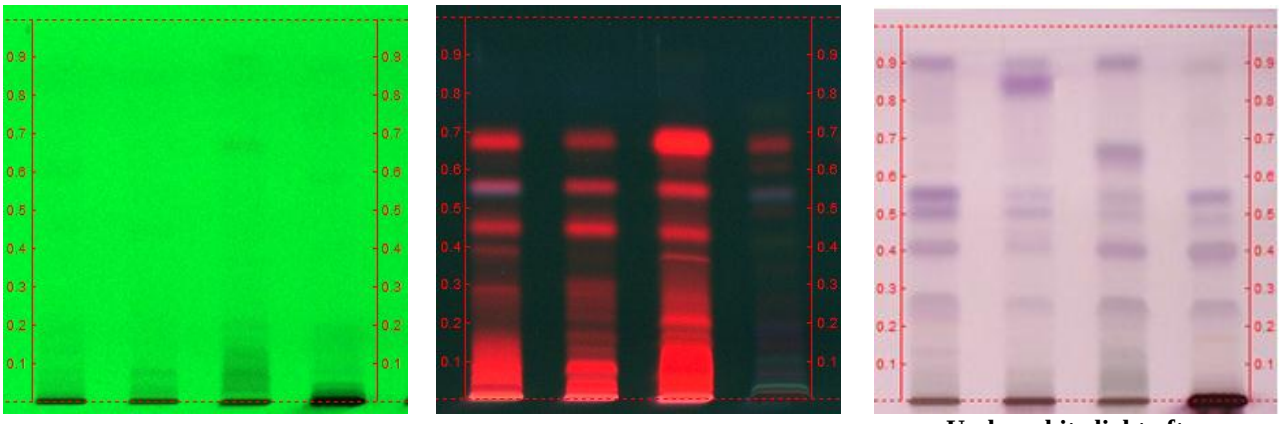
The preliminary phytochemical analyses of the alcohol extracts of the plant materials were investigated and the results of the screening were recorded in Table 2.

Table 2: Preliminary phytochemical analysis of the selected plants.

Sl.No.	Parameters	Results			
		C. auriculata (flower)	*C. indica* (whole plant)	*G.* sylvestre (leaf)	*T. cordifolia* (whole plant)
1	Saponins	-	+	+	+
2	Tannins	+	-	+	-
3	Terpenoids	+	+	+	+
4	Phenols	+	+	+	+
5	Steroids	-	-	+	-
6	Glycosides	-	+	+	+
7	Carbohydrates	+	+	+	+
8	Alkaloids	+	+	+	+
9	Lignin	-	-	-	-
10	Flavones	-	+	+	+
11	Proteins	+	-	+	+
12	Quinones	+	-	-	-
13	Coumarins	-	-	-	-

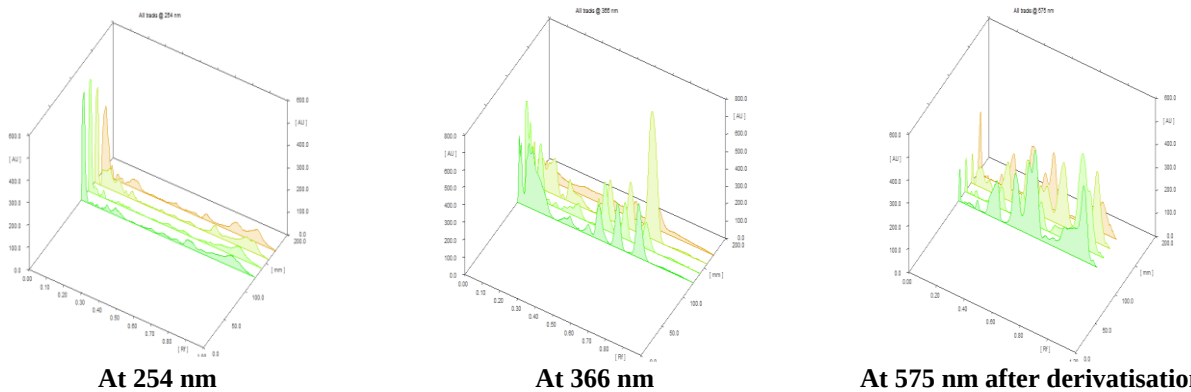
The results revealed the presence of almost all major secondary metabolites in the extracts of the plant materials which are responsible for their potent therapeutic activities.

HPTLC is one of the sophisticated instrumental techniques which takes the quality control of a drug one step ahead. HPTLC fingerprint enables a particular plant to be identified and distinguished from adulterants and substitutes. HPTLC photodocumentation profiles and densitogram profiles of the alcohol extracts of the plant materials at UV short (254 nm), UV long (366 nm) and white light (575 nm) after derivatisation are shown in Figures 1 – 5.



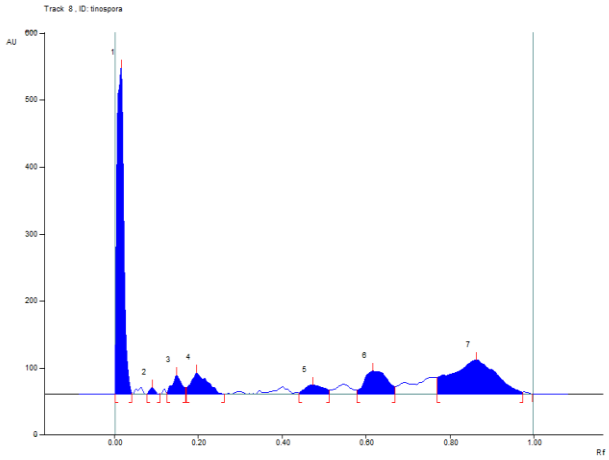
Under UV short **Under UV long** **Under white light after derivatisation**

Figure 1: TLC chromatogram of alcohol extracts of the selected plants viewed under different wavelengths; Solvent system: Toluene: Ethyl acetate (5: 1); Tracks: 1. *T. cordifolia* (whole plant) 2. *G. sylvestre* (leaf); 3. *C. indica* (whole plant); 4. *C. auriculata* (flower).

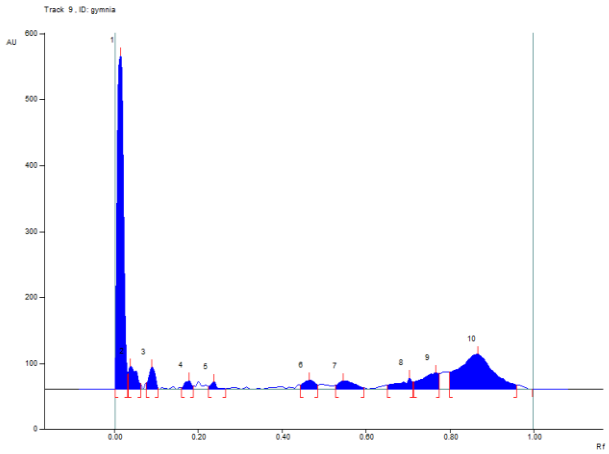


At 254 nm **At 366 nm** **At 575 nm after derivatisation**

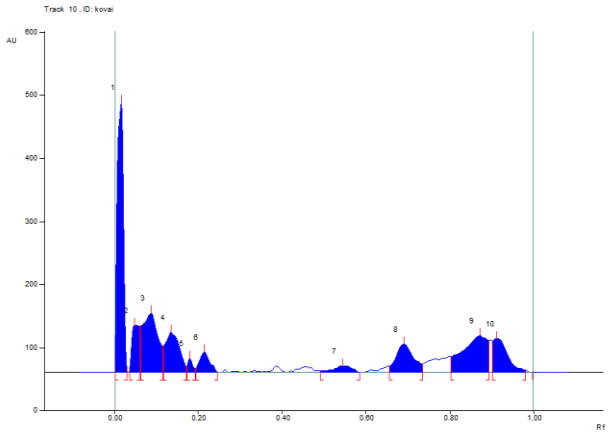
Figure 2: 3D densitometric chromatograms of alcohol extracts of the selected plants at different wavelengths.



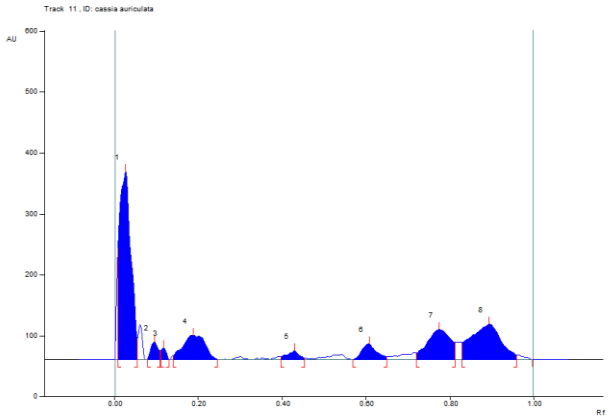
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.02 Rf	487.0 AU	74.15 %	0.04 Rf	1.1 AU	5492.8 AU	44.61 %
2	0.08 Rf	0.1 AU	0.09 Rf	10.1 AU	1.54 %	0.11 Rf	0.7 AU	85.1 AU	0.69 %
3	0.13 Rf	2.2 AU	0.15 Rf	28.0 AU	4.27 %	0.17 Rf	9.5 AU	428.6 AU	3.48 %
4	0.17 Rf	9.7 AU	0.20 Rf	32.0 AU	4.87 %	0.26 Rf	0.1 AU	857.5 AU	6.96 %
5	0.44 Rf	1.8 AU	0.47 Rf	14.2 AU	2.17 %	0.51 Rf	5.8 AU	433.5 AU	3.52 %
6	0.58 Rf	5.6 AU	0.62 Rf	34.5 AU	5.25 %	0.67 Rf	11.0 AU	1300.2 AU	10.56 %
7	0.77 Rf	25.1 AU	0.87 Rf	51.0 AU	7.76 %	0.98 Rf	2.9 AU	3714.0 AU	30.17 %



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	505.8 AU	70.09 %	0.03 Rf	22.9 AU	5318.1 AU	48.17 %
2	0.03 Rf	24.0 AU	0.04 Rf	35.3 AU	4.89 %	0.06 Rf	8.9 AU	466.2 AU	4.22 %
3	0.08 Rf	9.6 AU	0.09 Rf	34.0 AU	4.71 %	0.10 Rf	1.2 AU	358.2 AU	3.24 %
4	0.16 Rf	2.3 AU	0.18 Rf	13.3 AU	1.84 %	0.19 Rf	4.8 AU	148.5 AU	1.35 %
5	0.22 Rf	4.3 AU	0.24 Rf	11.4 AU	1.59 %	0.27 Rf	0.5 AU	114.1 AU	1.03 %
6	0.44 Rf	6.1 AU	0.47 Rf	13.5 AU	1.88 %	0.49 Rf	6.5 AU	271.5 AU	2.46 %
7	0.53 Rf	6.1 AU	0.55 Rf	12.3 AU	1.70 %	0.60 Rf	2.2 AU	345.5 AU	3.13 %
8	0.65 Rf	5.9 AU	0.70 Rf	17.4 AU	2.41 %	0.71 Rf	11.3 AU	364.8 AU	3.30 %
9	0.71 Rf	11.4 AU	0.77 Rf	25.2 AU	3.49 %	0.77 Rf	24.2 AU	697.3 AU	6.32 %
10	0.80 Rf	25.7 AU	0.87 Rf	53.4 AU	7.39 %	0.96 Rf	6.5 AU	2956.1 AU	26.78 %

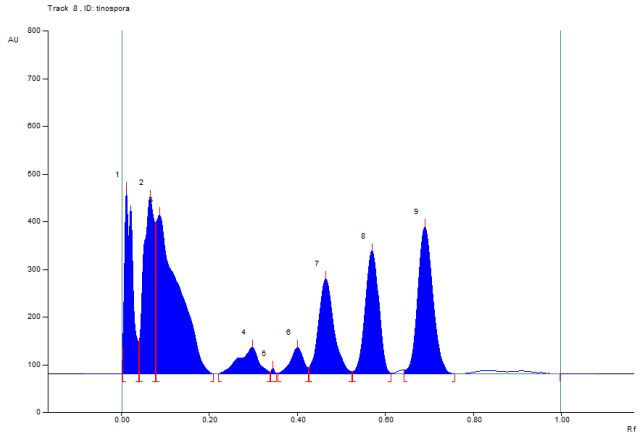


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.02 Rf	427.2 AU	48.50 %	0.03 Rf	4.3 AU	4429.5 AU	29.15 %
2	0.04 Rf	3.4 AU	0.05 Rf	74.1 AU	8.42 %	0.06 Rf	72.9 AU	964.2 AU	6.34 %
3	0.06 Rf	73.3 AU	0.09 Rf	94.0 AU	10.67 %	0.12 Rf	41.5 AU	2398.2 AU	15.78 %
4	0.12 Rf	42.1 AU	0.14 Rf	63.6 AU	7.22 %	0.17 Rf	8.5 AU	1416.8 AU	9.32 %
5	0.17 Rf	9.6 AU	0.18 Rf	22.6 AU	2.57 %	0.19 Rf	6.1 AU	178.4 AU	1.17 %
6	0.19 Rf	6.5 AU	0.22 Rf	32.2 AU	3.66 %	0.25 Rf	0.0 AU	540.7 AU	3.56 %
7	0.49 Rf	2.1 AU	0.55 Rf	10.4 AU	1.18 %	0.58 Rf	0.2 AU	314.1 AU	2.07 %
8	0.66 Rf	9.9 AU	0.69 Rf	44.6 AU	5.06 %	0.74 Rf	12.8 AU	1287.9 AU	8.47 %
9	0.80 Rf	24.2 AU	0.87 Rf	58.3 AU	6.62 %	0.89 Rf	50.4 AU	2334.4 AU	15.36 %
10	0.90 Rf	49.7 AU	0.91 Rf	53.8 AU	6.11 %	0.98 Rf	3.2 AU	1332.7 AU	8.77 %

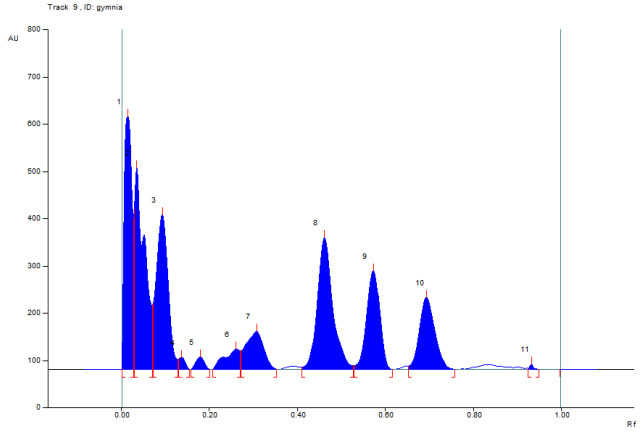


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	182.7 AU	0.03 Rf	308.3 AU	56.60 %	0.05 Rf	34.1 AU	5900.4 AU	43.42 %
2	0.08 Rf	1.5 AU	0.10 Rf	28.8 AU	5.28 %	0.11 Rf	14.7 AU	354.7 AU	2.61 %
3	0.11 Rf	15.2 AU	0.12 Rf	19.2 AU	3.53 %	0.13 Rf	0.4 AU	167.8 AU	1.24 %
4	0.14 Rf	6.2 AU	0.19 Rf	40.2 AU	7.38 %	0.25 Rf	0.2 AU	1502.2 AU	11.05 %
5	0.40 Rf	4.7 AU	0.43 Rf	15.0 AU	2.76 %	0.45 Rf	2.9 AU	283.5 AU	2.09 %
6	0.57 Rf	0.1 AU	0.61 Rf	25.7 AU	4.72 %	0.65 Rf	5.4 AU	624.6 AU	4.60 %
7	0.72 Rf	10.6 AU	0.77 Rf	49.5 AU	9.09 %	0.81 Rf	28.3 AU	1900.1 AU	13.98 %
8	0.83 Rf	28.7 AU	0.89 Rf	57.9 AU	10.63 %	0.96 Rf	7.5 AU	2855.1 AU	21.01 %

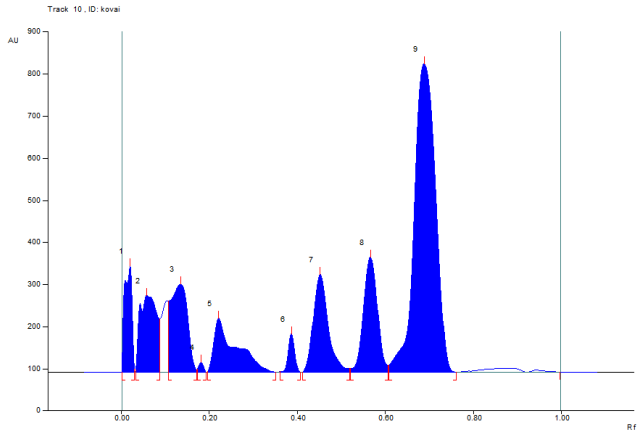
Figure 3: HPTLC fingerprinting profiles and R_f tables of alcohol extracts of the selected plants at 254 nm



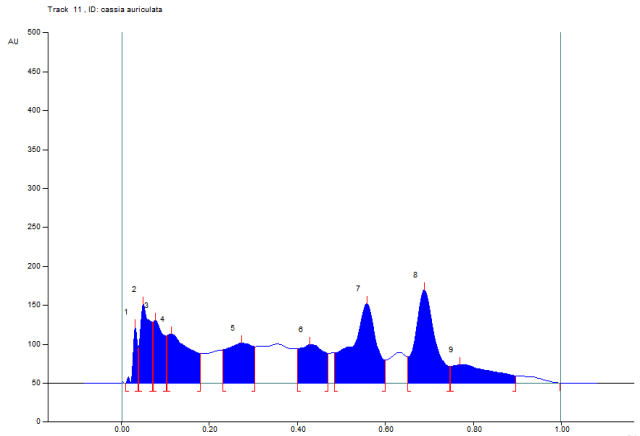
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	55.8 AU	0.01 Rf	387.0 AU	19.54 %	0.04 Rf	67.3 AU	4830.1 AU	11.17 %
2	0.04 Rf	69.7 AU	0.07 Rf	369.5 AU	18.66 %	0.08 Rf	17.1 AU	6332.7 AU	14.64 %
3	0.08 Rf	317.6 AU	0.09 Rf	333.1 AU	16.82 %	0.21 Rf	0.1 AU	11640.3 AU	26.91 %
4	0.22 Rf	0.1 AU	0.30 Rf	56.3 AU	2.84 %	0.34 Rf	4.1 AU	1706.5 AU	3.95 %
5	0.34 Rf	4.9 AU	0.34 Rf	12.1 AU	0.61 %	0.35 Rf	1.0 AU	58.9 AU	0.14 %
6	0.36 Rf	1.6 AU	0.40 Rf	55.7 AU	2.81 %	0.43 Rf	13.4 AU	1115.2 AU	2.58 %
7	0.43 Rf	13.9 AU	0.47 Rf	199.9 AU	10.09 %	0.52 Rf	4.6 AU	4804.1 AU	11.11 %
8	0.53 Rf	4.6 AU	0.57 Rf	258.6 AU	13.05 %	0.61 Rf	1.7 AU	5408.6 AU	12.50 %
9	0.64 Rf	7.5 AU	0.69 Rf	308.6 AU	15.58 %	0.76 Rf	0.1 AU	7357.6 AU	17.01 %



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	535.3 AU	25.29 %	0.03 Rf	19.5 AU	6716.4 AU	17.37 %
2	0.03 Rf	339.4 AU	0.04 Rf	427.1 AU	20.17 %	0.07 Rf	35.2 AU	6953.8 AU	17.99 %
3	0.07 Rf	138.4 AU	0.09 Rf	328.0 AU	15.49 %	0.13 Rf	21.2 AU	6220.4 AU	16.09 %
4	0.13 Rf	21.6 AU	0.14 Rf	25.5 AU	1.20 %	0.16 Rf	0.4 AU	239.8 AU	0.62 %
5	0.16 Rf	0.4 AU	0.18 Rf	26.7 AU	1.26 %	0.20 Rf	0.3 AU	361.4 AU	0.93 %
6	0.21 Rf	0.6 AU	0.26 Rf	42.9 AU	2.03 %	0.27 Rf	39.9 AU	1050.4 AU	2.72 %
7	0.27 Rf	40.1 AU	0.31 Rf	80.3 AU	3.80 %	0.35 Rf	0.3 AU	2218.7 AU	5.74 %
8	0.41 Rf	4.5 AU	0.46 Rf	278.6 AU	13.16 %	0.53 Rf	7.5 AU	6807.7 AU	17.61 %
9	0.53 Rf	7.8 AU	0.57 Rf	208.7 AU	9.86 %	0.62 Rf	0.1 AU	4277.1 AU	11.06 %
10	0.65 Rf	7.2 AU	0.69 Rf	152.6 AU	7.21 %	0.76 Rf	0.1 AU	3746.4 AU	9.69 %
11	0.93 Rf	2.4 AU	0.93 Rf	11.3 AU	0.53 %	0.95 Rf	0.5 AU	67.1 AU	0.17 %

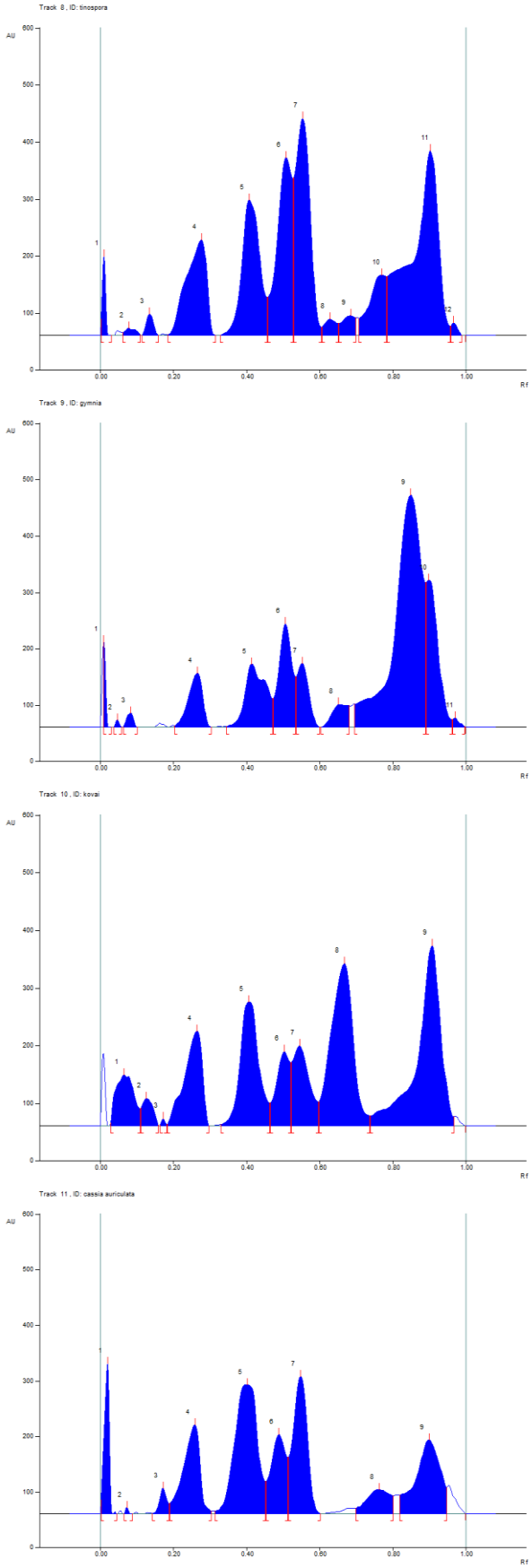


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.02 Rf	253.1 AU	11.90 %	0.03 Rf	7.6 AU	2999.3 AU	5.43 %
2	0.03 Rf	6.3 AU	0.06 Rf	182.8 AU	8.60 %	0.09 Rf	27.0 AU	4976.7 AU	9.02 %
3	0.11 Rf	170.0 AU	0.14 Rf	209.5 AU	9.85 %	0.17 Rf	6.1 AU	5476.0 AU	9.92 %
4	0.17 Rf	7.4 AU	0.18 Rf	23.5 AU	1.10 %	0.19 Rf	0.7 AU	180.4 AU	0.33 %
5	0.20 Rf	1.0 AU	0.22 Rf	129.2 AU	6.08 %	0.35 Rf	0.1 AU	4403.6 AU	7.98 %
6	0.36 Rf	1.4 AU	0.39 Rf	90.9 AU	4.27 %	0.41 Rf	0.5 AU	917.2 AU	1.66 %
7	0.41 Rf	0.7 AU	0.45 Rf	231.8 AU	10.90 %	0.52 Rf	8.7 AU	5423.6 AU	9.83 %
8	0.52 Rf	8.7 AU	0.57 Rf	273.3 AU	12.85 %	0.61 Rf	16.5 AU	5966.8 AU	10.81 %
9	0.61 Rf	17.1 AU	0.69 Rf	732.6 AU	34.45 %	0.76 Rf	0.1 AU	24854.2 AU	45.03 %



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.0 AU	0.03 Rf	72.6 AU	10.90 %	0.04 Rf	45.6 AU	490.8 AU	2.57 %
2	0.04 Rf	45.7 AU	0.05 Rf	101.7 AU	15.28 %	0.07 Rf	78.4 AU	1622.4 AU	8.50 %
3	0.07 Rf	78.9 AU	0.08 Rf	80.7 AU	12.12 %	0.10 Rf	61.1 AU	1341.5 AU	7.03 %
4	0.10 Rf	61.3 AU	0.12 Rf	63.2 AU	9.49 %	0.18 Rf	38.3 AU	2314.8 AU	12.12 %
5	0.23 Rf	43.0 AU	0.28 Rf	51.8 AU	7.79 %	0.30 Rf	47.0 AU	2119.6 AU	11.10 %
6	0.40 Rf	44.5 AU	0.43 Rf	49.6 AU	7.45 %	0.47 Rf	38.2 AU	1901.5 AU	9.96 %
7	0.49 Rf	39.2 AU	0.56 Rf	102.2 AU	15.35 %	0.60 Rf	30.1 AU	4039.7 AU	21.16 %
8	0.65 Rf	33.7 AU	0.69 Rf	119.9 AU	18.01 %	0.75 Rf	21.6 AU	3675.2 AU	19.25 %
9	0.75 Rf	21.5 AU	0.77 Rf	24.1 AU	3.62 %	0.90 Rf	9.7 AU	1589.1 AU	8.32 %

Figure 4: HPTLC fingerprinting profiles and R_f tables of alcohol extracts of the selected plants at 366 nm.



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	28.8 AU	0.01 Rf	139.4 AU	7.74 %	0.03 Rf	0.0 AU	860.3 AU	1.52 %
2	0.06 Rf	5.0 AU	0.08 Rf	13.0 AU	0.72 %	0.11 Rf	0.7 AU	252.3 AU	0.45 %
3	0.12 Rf	0.3 AU	0.14 Rf	37.2 AU	2.07 %	0.16 Rf	0.1 AU	473.0 AU	0.83 %
4	0.19 Rf	1.3 AU	0.28 Rf	167.5 AU	9.31 %	0.31 Rf	0.5 AU	6430.4 AU	11.34 %
5	0.33 Rf	0.1 AU	0.41 Rf	237.3 AU	13.18 %	0.46 Rf	67.0 AU	8060.3 AU	14.22 %
6	0.46 Rf	67.5 AU	0.51 Rf	311.6 AU	17.31 %	0.53 Rf	75.3 AU	8954.0 AU	15.79 %
7	0.53 Rf	276.6 AU	0.56 Rf	380.1 AU	21.11 %	0.61 Rf	14.0 AU	10486.4 AU	18.50 %
8	0.61 Rf	14.5 AU	0.63 Rf	28.6 AU	1.59 %	0.65 Rf	20.6 AU	650.3 AU	1.15 %
9	0.65 Rf	20.7 AU	0.69 Rf	34.3 AU	1.91 %	0.70 Rf	30.5 AU	855.8 AU	1.51 %
10	0.71 Rf	30.1 AU	0.77 Rf	105.7 AU	5.87 %	0.78 Rf	03.2 AU	3344.9 AU	5.90 %
11	0.79 Rf	103.3 AU	0.90 Rf	323.7 AU	17.98 %	0.96 Rf	16.0 AU	16067.1 AU	28.34 %
12	0.96 Rf	16.6 AU	0.97 Rf	21.8 AU	1.21 %	0.99 Rf	0.6 AU	259.7 AU	0.46 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	150.7 AU	0.01 Rf	150.7 AU	10.61 %	0.03 Rf	0.0 AU	653.1 AU	1.50 %
2	0.04 Rf	0.2 AU	0.05 Rf	12.6 AU	0.89 %	0.06 Rf	0.3 AU	86.5 AU	0.20 %
3	0.06 Rf	0.3 AU	0.08 Rf	24.8 AU	1.75 %	0.10 Rf	0.1 AU	319.3 AU	0.73 %
4	0.20 Rf	2.5 AU	0.27 Rf	95.6 AU	6.73 %	0.30 Rf	0.2 AU	2597.3 AU	5.98 %
5	0.34 Rf	0.7 AU	0.41 Rf	111.8 AU	7.87 %	0.47 Rf	50.5 AU	4333.2 AU	9.97 %
6	0.47 Rf	50.7 AU	0.51 Rf	182.9 AU	12.88 %	0.54 Rf	89.3 AU	4654.4 AU	10.71 %
7	0.54 Rf	89.4 AU	0.55 Rf	113.0 AU	7.96 %	0.60 Rf	0.2 AU	2475.5 AU	5.70 %
8	0.60 Rf	0.0 AU	0.65 Rf	40.4 AU	2.84 %	0.68 Rf	37.8 AU	1234.8 AU	2.84 %
9	0.70 Rf	40.6 AU	0.85 Rf	411.1 AU	28.95 %	0.89 Rf	56.1 AU	20991.6 AU	48.32 %
10	0.89 Rf	256.7 AU	0.90 Rf	260.9 AU	18.37 %	0.96 Rf	13.1 AU	5902.8 AU	13.59 %
11	0.96 Rf	13.6 AU	0.97 Rf	16.2 AU	1.14 %	1.00 Rf	0.8 AU	195.6 AU	0.45 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	4.4 AU	0.06 Rf	88.3 AU	6.37 %	0.11 Rf	30.0 AU	3140.0 AU	6.18 %
2	0.11 Rf	30.8 AU	0.13 Rf	47.0 AU	3.39 %	0.16 Rf	1.4 AU	955.5 AU	1.88 %
3	0.16 Rf	1.0 AU	0.17 Rf	12.1 AU	0.87 %	0.18 Rf	2.6 AU	83.5 AU	0.16 %
4	0.18 Rf	2.8 AU	0.26 Rf	164.2 AU	11.85 %	0.30 Rf	0.4 AU	5569.0 AU	10.96 %
5	0.33 Rf	2.3 AU	0.41 Rf	214.6 AU	15.48 %	0.46 Rf	39.9 AU	7638.7 AU	15.03 %
6	0.47 Rf	40.2 AU	0.50 Rf	128.3 AU	9.26 %	0.52 Rf	09.5 AU	3292.1 AU	6.48 %
7	0.52 Rf	109.8 AU	0.55 Rf	138.4 AU	9.99 %	0.60 Rf	41.5 AU	4400.8 AU	8.66 %
8	0.60 Rf	42.1 AU	0.67 Rf	281.3 AU	20.30 %	0.74 Rf	17.5 AU	11903.2 AU	23.42 %
9	0.74 Rf	17.5 AU	0.91 Rf	311.8 AU	22.50 %	0.97 Rf	15.6 AU	13842.6 AU	27.24 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	24.6 AU	0.02 Rf	269.9 AU	21.00 %	0.05 Rf	0.0 AU	2594.5 AU	6.95 %
2	0.06 Rf	0.0 AU	0.07 Rf	11.2 AU	0.88 %	0.09 Rf	0.0 AU	61.9 AU	0.17 %
3	0.14 Rf	0.5 AU	0.17 Rf	45.7 AU	3.55 %	0.19 Rf	18.5 AU	627.8 AU	1.68 %
4	0.19 Rf	18.8 AU	0.26 Rf	160.0 AU	12.45 %	0.30 Rf	5.1 AU	5020.1 AU	13.44 %
5	0.31 Rf	4.9 AU	0.40 Rf	232.3 AU	18.08 %	0.45 Rf	57.8 AU	10180.7 AU	27.26 %
6	0.45 Rf	57.9 AU	0.49 Rf	142.2 AU	11.06 %	0.51 Rf	01.1 AU	4011.8 AU	10.74 %
7	0.51 Rf	101.2 AU	0.55 Rf	247.1 AU	19.23 %	0.60 Rf	0.2 AU	6811.5 AU	18.24 %
8	0.70 Rf	9.6 AU	0.76 Rf	43.7 AU	3.40 %	0.80 Rf	31.8 AU	1952.4 AU	5.23 %
9	0.82 Rf	33.8 AU	0.90 Rf	132.9 AU	10.34 %	0.95 Rf	47.1 AU	6088.9 AU	16.30 %

Figure 5: HPTLC fingerprinting profiles and R_f tables of alcohol extracts of the selected plants at 575 nm after derivatisation.

These peaks can be attributed to the various phytoconstituents present as major constituents in the analysed plants.

Table 3: R_f values and colour of bands obtained at different wavelengths.

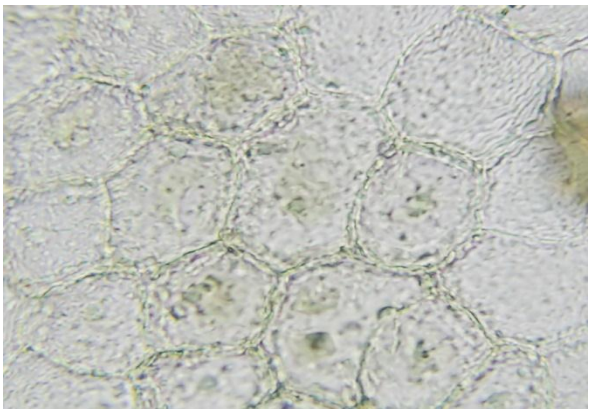
Wave lengths	254 nm		366 nm		575 nmafter derivatisation	
	R_f values	Colour	R_f values	Colour	R_f values	Colour
C. auriculata (Flower)	0.10 0.12 0.19 0.77 0.89	light green light green light green light green light green	0.05 0.08 0.12 0.28 0.43 0.56 0.69 0.77	purple fluorescent green green purple light yellow purple red light green	0.07 0.17 0.26 0.41 0.49 0.55 0.90	light purple light purple purple dark purple light purple dark purple light purple
C.indica (Whole plant),	0.05 0.09 0.14 0.18 0.22 0.69 0.87	light green light green light green light green light green light green light green	0.06 0.14 0.18 0.22 0.39 0.45 0.57 0.69	fluorescent red red red red red red red fluorescent red	0.06 0.13 0.26 0.41 0.51 0.55 0.67 0.90	 light purple light purple purple purple purple purple dark purple dark purple
G.sylvestre (Leaf)	0.04 0.09	light green light green	0.04 0.09 0.14 0.18 0.26 0.31 0.46 0.57 0.69	fluorescent red fluorescent red light red light red light red light red fluorescent red red fluorescent red	0.05 0.08 0.27 0.41 0.51 0.55 0.85 0.90	 light purple light purple purple purple purple purple dark purple purple
T.cordifolia (Whole plant)	0.09 0.20 0.62	light green light green light green	0.07 0.09 0.30 0.40 0.47 0.57 0.69	fluorescent red fluorescent red light red light red red pink red	0.08 0.14 0.28 0.41 0.51 0.56 0.90	light purple light purple purple purple dark purple dark purple dark purple

Powder microscopy

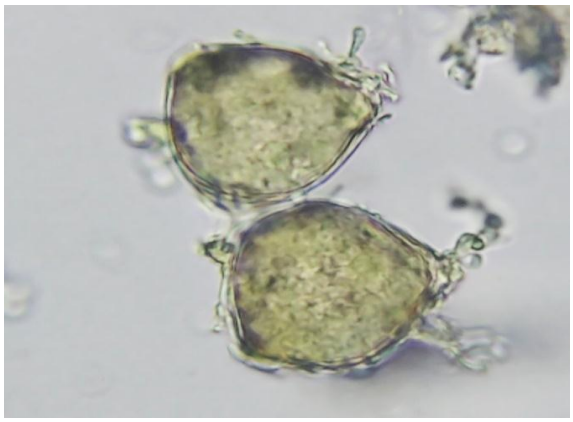
Microscopic investigations of the plant materials supply invaluable supporting evidence when used in association with other analytical methods for the identification of drugs. Unique powder characteristics ensure the botanical authentication of the plant materials. The powder microscopy of the selected plants is given in figures 6, 7, 8 and 9.

1. *C. auriculata* (Flower)

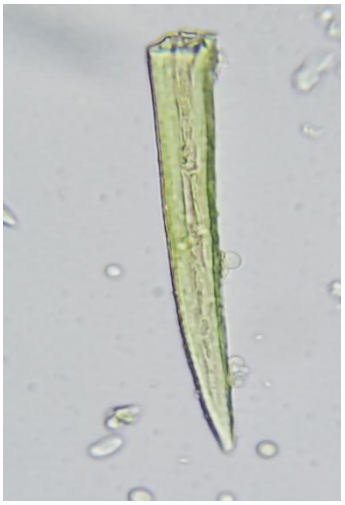
Powder is dark brown in colour without any characteristic smell and taste. Powder characteristics include uniseriate unicellular trichome, tannin cells, pollen grains, spiral vessels, epidermal cells of petal etc.



Epidermal cells of petal

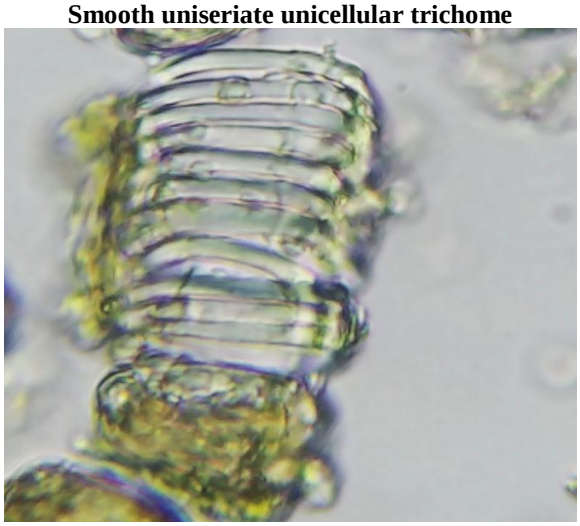


Tannin cells

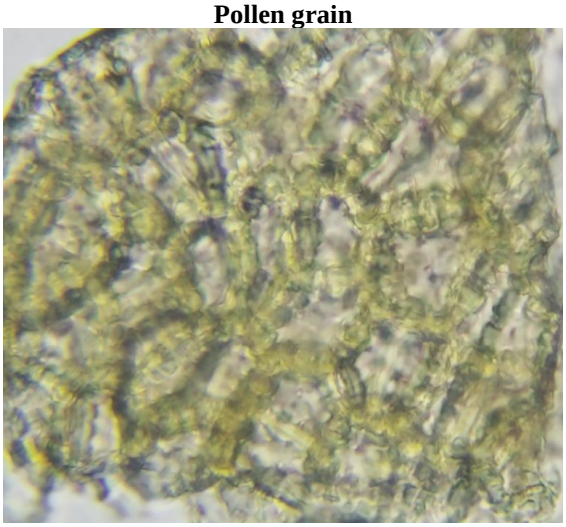


Smooth uniseriate unicellular trichome

Pollen grain



Spiral vessel



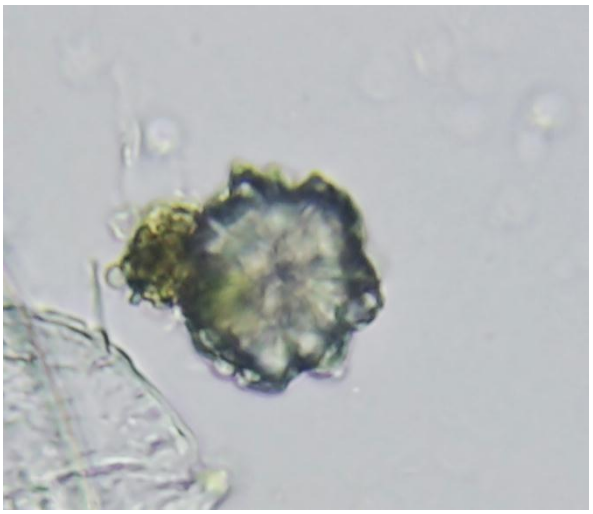
Group of stone cells

Figure 6: Powder microscopy of *Cassia auriculata* (Flower)

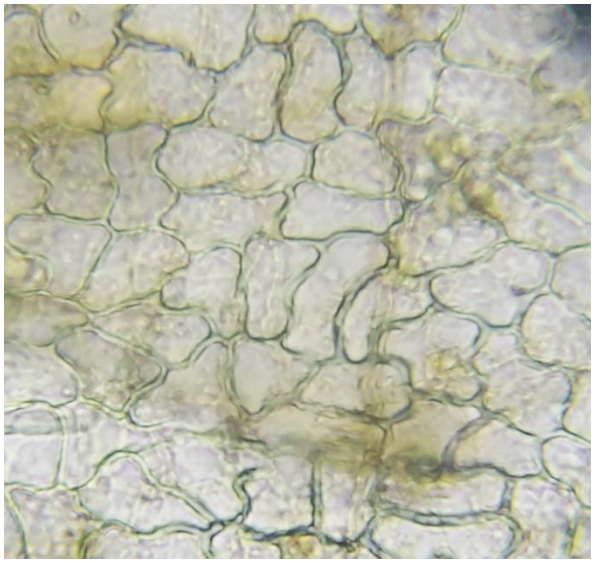
2. *C. indica* (Whole plant)

The powder is dark green in colour without any characteristic smell and taste and the powder characters observed are spiral vessels, prismatic and rosette calcium oxalate crystals, anomocytic stomata, wavy epidermal cells, uniseriate multicellular trichomes (both smooth and warty type), pitted vessel etc.

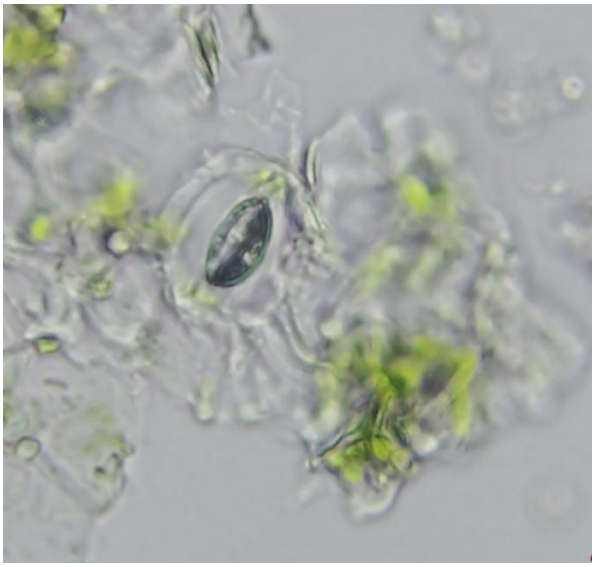
Spiral vessel



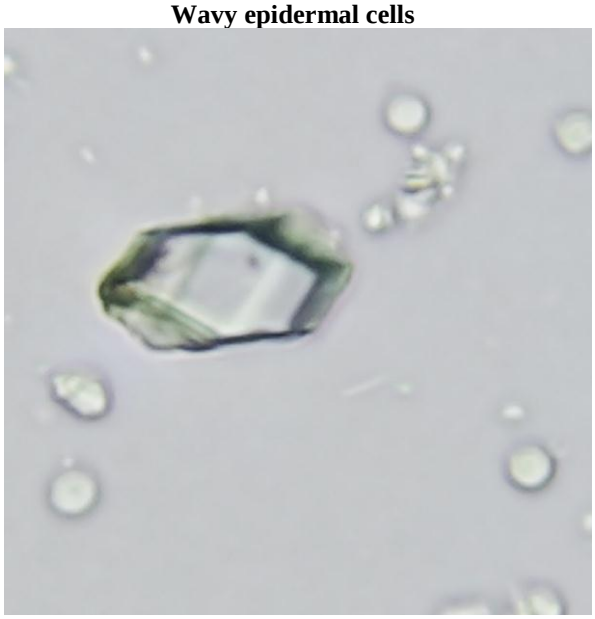
Rosette calcium oxalate crystal



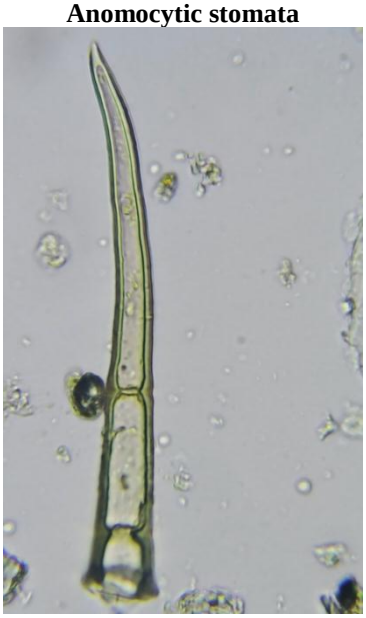
Wavy epidermal cells



Anomocytic stomata



Prismatic calcium oxalate crystal



Smooth uniseriate multicellular trichome

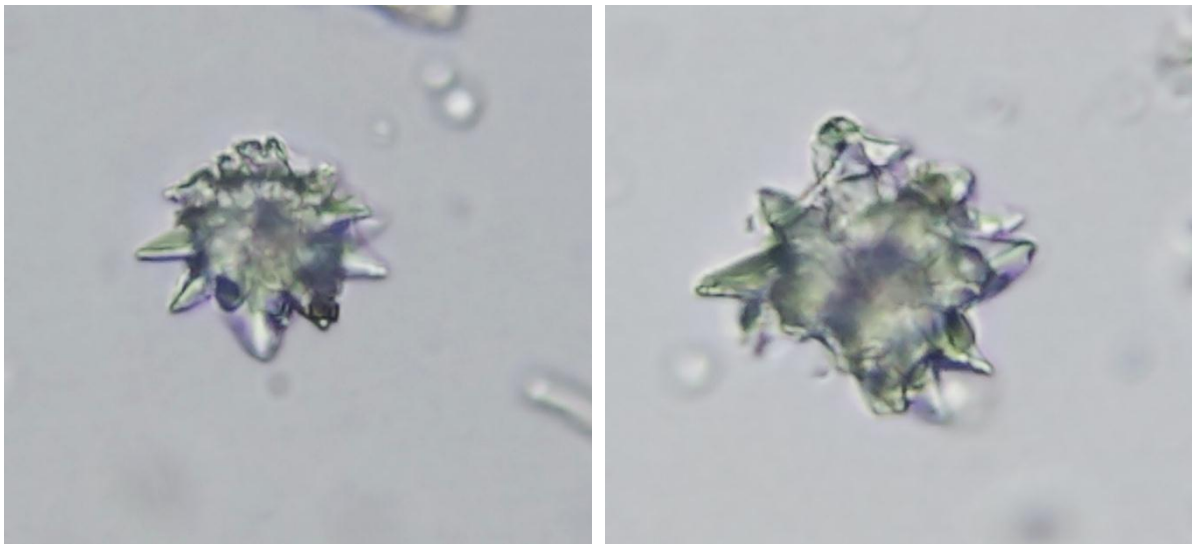
Warty uniseriate multicellular trichome

Pitted vessel

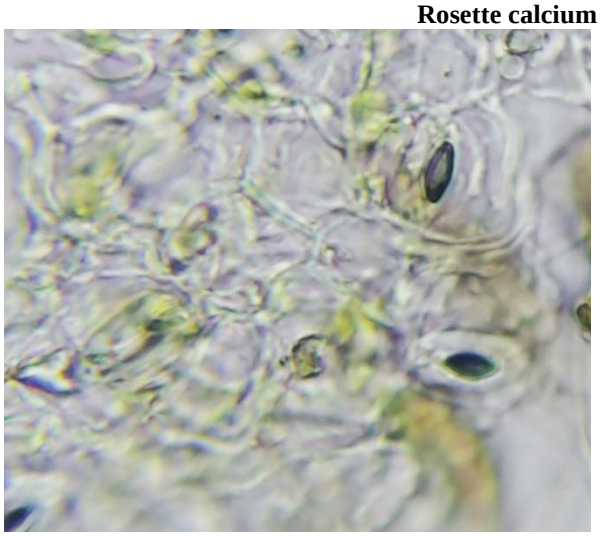
Figure 7: Powder microscopy of *Coccinia indica* (Whole plant).

3. G. sylvestre (Leaves)

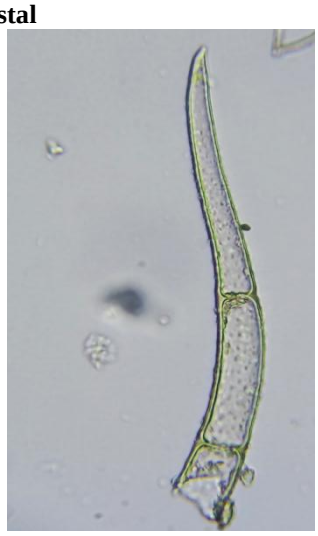
The powder is light green in colour without any characteristic smell and taste and the powder characters include rosette calcium oxalate crystal, uniseriate multicellular trichomes, paracytic stomata, starch grain, spiral vessels etc.



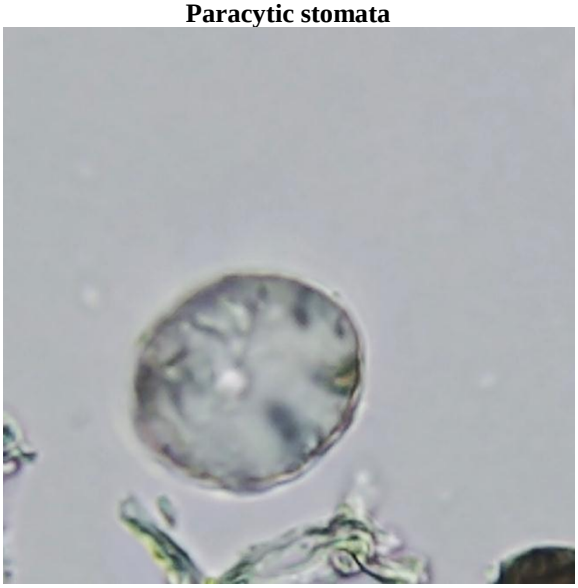
Rosette calcium oxalate crystal



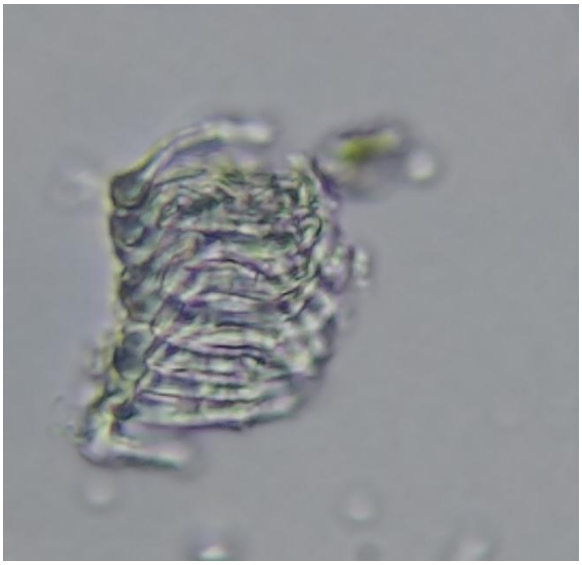
Paracytic stomata



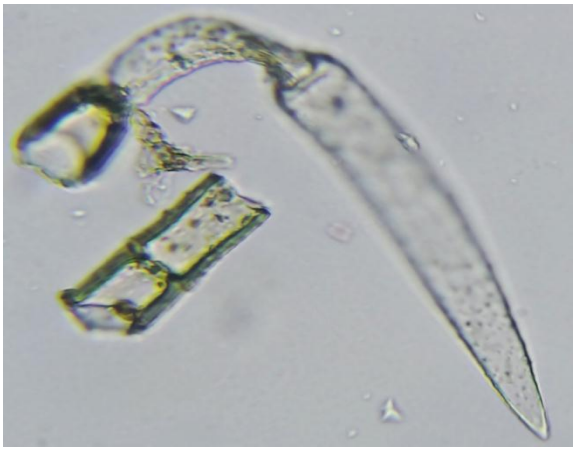
Uniseriate multicellular trichome



Starch grain



Spiral vessel

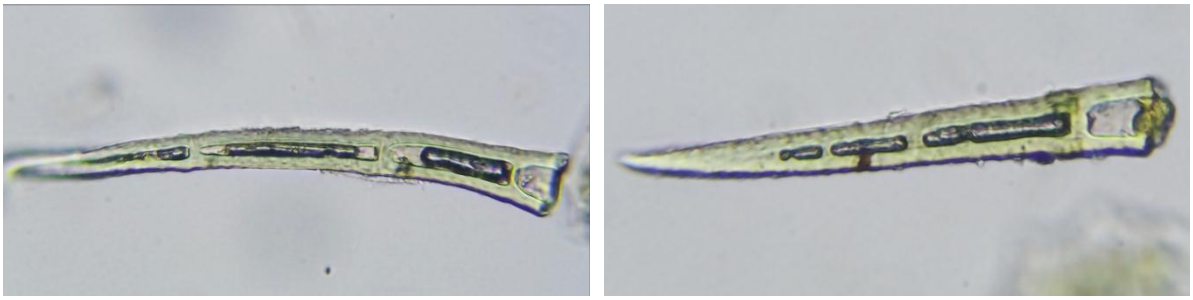


Trichome with basal and stalk cell

Figure 8: Powder microscopy of *Gymnema sylvestre* (Leaves).

4. *T. cordifolia* (Whole plant)

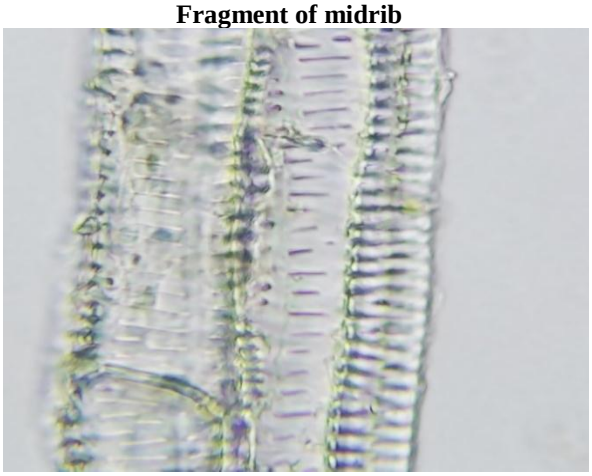
The powder is dark green in colour without any characteristic smell and taste and the powder characters revealed uniseriate trichomes, anomocytic stomata, pitted vessels, spiral vessels, starch grains, calcium oxalate crystal etc.



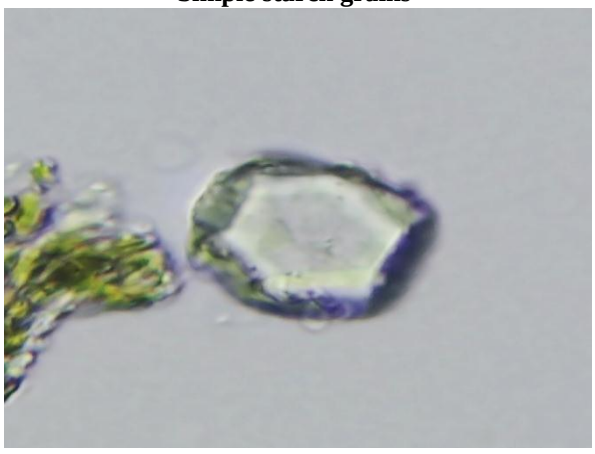
Smooth uniseriate multicellular trichomes

Fragment of midrib

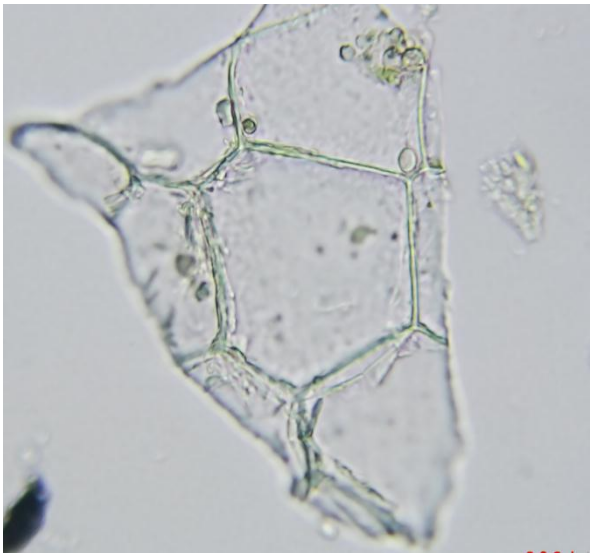
Simple starch grains



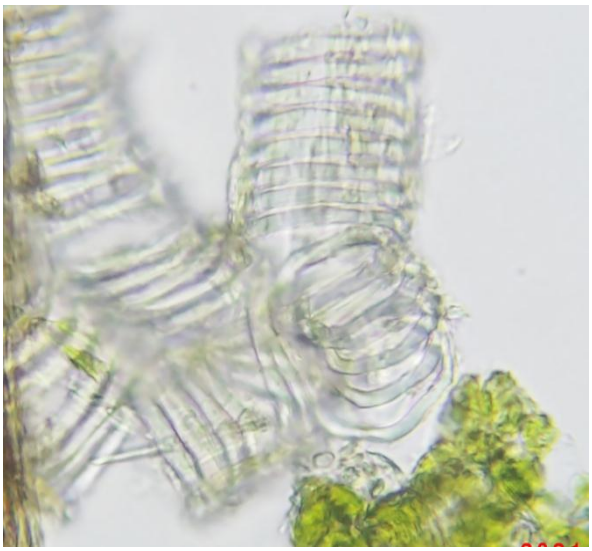
Pitted vessel



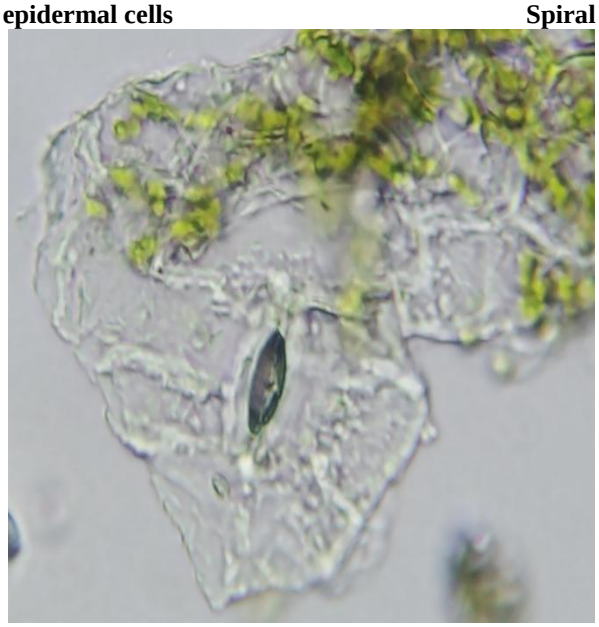
Calcium oxalate crystal



Surface view of epidermal cells



Spiral vessels



Anomocytic Stomata

Figure 9: Powder microscopy of *Tinospora cordifolia* (Whole plant).

Estimation of total phenol content

The total phenolic content in the plant extract was determined by using Folin-Ciocalteu (F-C) colourimetric method with Pyragallol as a standard compound. Alcohol was selected as the extracting solvent as the phenolic compounds are more soluble in polar organic solvents due to the presence of a hydroxyl group. The absorbance obtained at various concentrations of pyragallol was used for constructing the calibration curve (Fig 10). F-C method is based on the transfer of electrons in alkaline medium from phenolic compounds to phosphotungstic/ phosphomolybedic acid complexes to form blue coloured complexes, $(PMoW_{11}O_{40})^{-4}$ that are determined spectrophotometrically at 760 nm. Total phenolic content of each extract was calculated from the regression equation of calibration curve (y = 0.422x - 0.3524, R^2 = 0.992) and expressed as mg pyragallol equivalents per gram of extract (mg/g). The results are tabulated in Table 3.

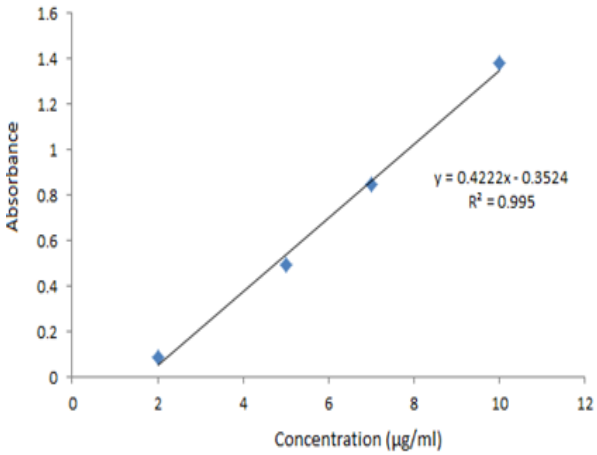


Figure 10: Calibration curve for standard pyrogallol equivalent.

Table 4: Total phenol content of the selected plants.

Parameter	Results			
	C. auriculata **(flower)**	***C. indica*** **(whole plant)**	***G.* sylvestre (leaf)**	***T. cordifolia*** **(whole plant)**
Total Phenol content	178.5mgPGE/g	132.24mg PGE/g	108.52mg PGE/g	111.51mgPGE/g

The content of phenolic compounds in alcohol extracts ranged from 108.52mg PGE/g to 178.5mgPGE/g. *C. auriculata* (flower) had the greatest phenol content while the smallest phenolic contents were found in *G*. sylvestre (leaf). The hydroxyl groups of the phenolic compounds in plant extracts are responsible for facilitating free radical scavenging and thereby reducing the diabetic complications.[26,27]

CONCLUSION

All the results obtained from physio-chemical, preliminary phytochemical, HPTLC and powder microscopy analysis helps in the identification of *C. auriculata* (Flower), *C. indica* (Whole plant*), G. sylvestre* (Leaf) and *T. cordifolia* (Whole plant). The parameters laid down can be considered as standards to ensure the quality of the plants from adulteration and substitution. HPTLC fingerprint showed the presence of various phytoconstituents in the plants which are responsible for the therapeutic activity of the plants. The phenol content evaluated using UV spectrophotometer confirmed the presence of polyphenols in different concentrations which might be one of the reasons for the anti- diabetic activity of the selected plant materials.

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