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A Comparative Pharmacognostic Study and Phytochemical Analysis of Root, Stem, and Leaf of Five Antidiabetic Medicinal Plants (*Cassia fistula* Linn., *Tinospora cordifolia* Willd., *Gymnema sylvestre* Retz., *Acacia catechu* Willd. and *Holarrhena antidysenterica* Linn.) for Promoting Conservation of Endangered Plant Species

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder affecting a significant proportion of the world's population. Plants are the reservoir of natural anti-diabetic compounds which stimulate the pancreatic beta cells for the production of insulin. In recent times, herbal medicines are gaining popularity over commercial synthetic drugs as they are less costly, easily available, and have fewer side effects than oral hypoglycemic drugs. *Cassia fistula* Linn. (Amaltas), *Tinospora cordifolia* Willd. (Guduchi), *Gymnema sylvestre* Retz. (Gurmar), *Acacia catechu* Willd. (Khadira) and *Holarrhena antidysenterica* Linn. (Kurchi) have been used to prepare herbal medicines to treat diabetes for ages. Generally, a specific plant part is used extensively in the preparation of herbal medicines which eventually causes the plants to get endangered/extinct. These plants can be protected if a specific plant part can be replaced by another part (s) of the same plant having the same active component(s). The comparative analysis included macroscopic observations, powder microscopy study, physicochemical and phytochemical studies of root, stem, and leaf of five plants having potential anti-diabetic properties. Powdered microscopy of roots showed pitted vessels in *Cassia*, perforated vessels in *Tinospora*, broad and pitted vessels in *Holarrhena*. Reticulate thickening was observed in tracheids of *Tinospora* and *Gymnema*, vasicentric tracheids were seen in *Holarrhena*. Single-celled trichomes were present in stems of *Cassia*, *Tinospora, Gymnema,* and *Holarrhena*. Phytochemical analysis showed the presence of flavonoids, alkaloids, saponin, and carbohydrates in root, stem, and leaf samples. Saponin is considered one of the most potent anti-diabetic compounds. Except for *Acacia catechu*, saponin was present in all other plant extracts.

INTRODUCTION:

Diabetes mellitus is a metabolic disorder that is chronic and resulting from insulin deficiency. Along with hyperglycemia, diabetes is associated with macrovascular and microvascular complications, which are the major causes of morbidity and death in diabetes.[1] Conventional antidiabetic drugs are effective, however, possess unavoidable side effects.[2,4] Medicinal plants may act as an alternative source of antidiabetic drugs. It is estimated that 25% of the world population is affected by diabetes.[3,5]

Natural products, particularly of plant origin, are the main quarry for discovering promising lead candidates and play an imperative role in the upcoming drug development programs[6,8,9,10]. Ease of availability, low cost, and least side effects make plant-based preparations the main key player of all available therapies, especially in rural areas[11]. Moreover, many plants provide a rich source of bioactive chemicals, which are free from undesirable side effects and possess powerful pharmacological actions[12,13,14,15,16]. Plants have always been an exemplary source of drugs with many of the currently available drugs being obtained directly or indirectly from them[1,17,18].

In developing countries like India, medicinal plants have been used to treat diabetes to overcome the burden of the cost of conventional medicines to the population. Nowadays, treatments of diseases including diabetes using medicinal plants are recommended[19] because these plants contain various phytoconstituents such as flavonoids, terpenoids, saponins, carotenoids, alkaloids, and glycosides, which are considered to possess antidiabetic properties[20].

Ayurveda, one of the Indian traditional medicinal systems, has a rich history of its effectiveness against diseases. Archaeo-botanical excavations show pieces of evidence on the use of medicinal plants in the Middle Gangetic region since the 2nd millennium BCE and these plant-based formulations are still used in Ayurvedic folk medicines.[6]

India occupies a special position in the area of herbal medicines as it is one of the very few countries which are capable of cultivating plants that are useful for the production of plant-derived medicines[(23)]. The lesser or negligible side effects of herbal medicine result in increasing its demand by 12%-15% per year[(24)]. The side effect of antidiabetic drugs is the driving force for discovering alternative sources of natural antidiabetic agents[7].

There are various antidiabetic drugs used in the treatment of diabetes that can be used either in monotherapy or in combination form. Metformin, Glimepiride, and Pioglitazone are commonly used as oral hypoglycemic drugs.[30] Phytochemical experiments showed that both modern synthetic antidiabetic drugs and specific plant extracts have similarities in their structures. This result could be helpful in establishing the fact that both oral antidiabetic drugs and specific plant extracts have similar compounds present in them.

For the production of herbal medicines, a particular plant part(s) is used extensively which eventually pushes the plant to get endangered or extinct. The other parts of the plants remain unused despite having potent active components present in them.

The current research work included the pharmacognostic as well as phytochemical studies of five different medicinal plants which are well known for their anti-diabetic property. The plants analyzed in this research were *Cassia fistula* (Aragvadha/ Amaltas), *Tinospora cordifolia (Guduchi/ Gulancha*), *Gymnema sylvestre* (Gurmar), *Acacia catechu* (Khadira), and *Holarrhena antidysenterica* (Kurchi).

The objective of the research was to find out the presence of active chemical constituents in the root, stem, and leaf of these five medicinal plants and provide definite information on whether commercially used plant parts could be replaced by other plant parts (root, stem, or leaf) of the same plant. This result may be helpful in the conservation of the plant species from getting endangered and extinct. The study also aims at providing comparative accounts on the pharmacognostic properties of these plant samples as the results would be helpful in the identification of those plants from their powdered forms. This comparative study was not documented previously. Hence, the present study would be helpful for further research work related to antidiabetic herbal medicines.

MATERIAL AND METHODS:

Collection and Identification of Plant Materials:

The recent research work was performed at the Institute of Post Graduate Ayurved Education & Research(IPGAER), Kolkata, West Bengal, India. Root, stem, and leaves of *Cassia fistula* (Aragvadha/ Amaltas)[Family-Caesalpiniaceae], *Tinospora cordifolia(Guduchi/Gulancha*) [Family- Menispermaceae], *Gymnema sylvestre* (Gurmar) [Family- Asclepiadaceae], *Acacia catechu* (Khadira) [Family- Mimosaceae] and *Holarrhena antidysenterica* (Kurchi) [Family- Apocyanaceae] were collected from the medicinal plants garden, IPGAER, Kolkata and identified by Plant Scientist, Botanical Survey of India, Shibpur, India(Ref. No. BSI/CNH/SD/Tech./2018, BSI/CNH/SD/Tech./2019, BSI/CNH/SD/TECH.005-2019).

Preparation and Extaction of Plant Samples:

The root, stem, and leaf samples were air dried at room temperature for seven days. Dried root, stem, and leaf materials were then cut into small pieces. Next, the dried materials were powdered using a grinder (Hammer mill). The powdered plant samples were then passed through No. 40 and No. 120 mesh sieve for phytochemical analysis and pharmacognostic study respectively. Next, the powdered plant samples were packed in air-tight containers and sealed for storage prior to extraction. 5 grams of each air-dried powdered stem sample were macerated (cold extraction) separately with 100 ml of solvents (methanol and water) for 72 hrs.

I) Macroscopic and Organoleptic Study

Macroscopic and organoleptic characters of root, stem, and leaf of the abovementioned five antidiabetic medicinal plants were examined thoroughly.

II) Pharmacognostic Studies

The powders of root, stem, and leaf of these five plants were passed through sieve # 120 and mounted on clean grease-free glass slides for microscopic observations.

III) Physico-chemical Study

Ash values and extractive values of plant samples were determined by procedures described in Ayurvedic Pharmacopoeia of India (API).

a) Total Ash Value

3 grams of each airdried plant sample were weighted separately. Then they were incinerated in a muffle furnace at 450°C for six hours. The ashes obtained from this method were cooled and weighed on ash less filter paper. The percentages of ashes with reference to the airdried samples were calculated separately.

b) Acid Insoluble Ash Value

The total ash obtained from the above study was boiled with 25 ml of dilute hydrochloric acid for 5 min. The insoluble residues were collected separately on ashless filter paper and washed with hot water. Then the residues were ignited, cooled, and kept for desiccation. The residues thus obtained were weighted and percentages of acid-insoluble ash with reference to air-dried stem samples were calculated.

c) Water Soluble Ash Value

The ash obtained was boiled with 25 ml of distilled water for 5 mins. The soluble matter was collected and washed with hot water, ignited, and weighed. The percentage of water-soluble ash with reference to air-dried sample was calculated.

d) Extractive value

The filtrate was taken and kept for evaporation to dryness and weighed. The percentages of different soluble extractive values were calculated with reference to the air-dried powder. The determination of water and alcohol soluble extractive value was used to evaluate the purity of the constituents.

IV) Phytochemical screening:

Each 5 g of dried and powdered form of root, stem, and leaf samples were mixed separately in 25 ml of different solvents viz. methanol and water. The different extracts were used for standard phytochemical studies. The methanolic and aqueous extracts of different plant parts were used to evaluate the presence of phytoconstituents such as alkaloids, flavonoids, phenols, saponins, tannins, etc. This study was carried out by using standard procedures.

RESULT AND DISCUSSION:

RESULTS:

I) MACROSCOPIC AND ORGANOLEPTIC STUDY

Table No. 1: Macroscopic and Organoleptic Characteristics of Root, Stem & Leaf of *Cassia fistula* (Aragvadha/ Amaltas) [Family-Caesalpiniaceae]

CHARACTERS	OBSERVATIONS					
	Root		Stem		Leaf	
	Fresh	Powder form	Fresh	Powder form	Fresh	Powder form
Colour	Brown	Light brown	Dark brown	Light brown	Dark green	Light green
Texture	Hard	_	Hard	_	Smooth	_
Odour	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless
Taste	Bitter	Bitter	Slightly acidic or bitter	Bitter	Bitter	Bitter
Type	_	_	_	_	Alternate, pinnately compound	_
Shape	Cylindrical	_	Cylindrical	_	Ovate	_
Apex	_	_	_	_	Acute	_
Surface	smooth	_	Densely hairy	_	Upper smooth, lower hairy	_
Venation	_	_	_	_	Pinnately reticulate	_
Length	10-14 cm	_	11-15 cm	_	7-15 cm (each leaflet)	_
Width	0.8-1 cm	_	1-2 cm	_	2-7 cm (each leaflet)	_

The macroscopic and organoleptic study revealed the fresh root, stem, and leaf of *C. fistula* were brown, dark brown, and dark green respectively. The powder form of root and stem were light brown whereas that of the leaf was light green. The shape of the root and stem was cylindrical whereas that of the leaf was ovate. The lengh and width of root were 10-14 cm and 0.8-1 cm respectively. The lengh and width of stem were 11-15 cm and 1-2 cm respectively. Leaves were arranged oppositely. They were pinnately compound and reticulate venation was observed. Each leaflet was 7-15 cm in length and 2-7 cm in width.

Table No. 2: Macroscopic and Organoleptic Characteristics of Root, Stem & Leaf of *Tinospora cordifolia* [Family- Menispermaceae]

CHARACTERS	OBSERVATIONS					
	Root		Stem		Leaf	
	Fresh	Powder form	Fresh	Powder form	Fresh	Powder form
Colour	Brown	Light brown	Light brown	Light brown	Light green	Light green
Texture	Hard	_	Hard	_	Leathery	_
Odour	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless
Taste	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter
Type	_	_	_	_	simple, alternate, exstipulate	_
Shape	Cylindrical	_	Cylindrical	_	Deeply cordate	_
Apex	_	_	_	_	roundish, pulvinate	_
Surface	Smooth	_	Hairy	_	Pubescent above, whitish tomentose below	_
Venation	_	_	_	_	Reticulate	_
Length	12-14 cm	_	15-17 cm	_	10-20 cm	_
Width	10-30 mm	_	0.8- 2 cm	_	8-15 cm	_

The macroscopic and organoleptic study revealed the colors of the fresh root, stem, and leaf of *T. cordifolia* were brown, light brown, and light green respectively. The powdered form of root, stem, and leaf was light brown, light brown, and light green respectively. The shape of the root and stem was cylindrical whereas that of the leaf was deeply cordate at the base. The length and width of root were 12-14 cm and 10-30 mm respectively. The length and width of stem were 15-17 cm and 0.8-2 cm respectively. Reticulate venation was observed in the leaf. The surface of the leaf was pubescent above and whitish tomantose found below the leaf. Each leaflet was 10-20 cm in length and 8-15 cm in width.

Table No. 3: Macroscopic and Organoleptic Characteristics of Root, Stem & Leaf of *Gymnema sylvestre* [Family- Apocynaceae]

CHARACTERS	OBSERVATIONS					
	Root		Stem		Leaf	
	Fresh	Powder form	Fresh	Powder form	Fresh	Powder form
Colour	Brown	Light brown	Grey	Light grey	Light green	Light green
Texture	Hard	_	Hard	_	Smooth, leathery	_
Odour	Odourless	Odourless	Odourless	Odourless	Present	Present
Taste	Bitter	Bitter	Bitter	Bitter	Extremely Bitter	Bitter
Type	_	_	_	_	Simple	_
Shape	Cylindrical	_	Cylindrical	_	Ovate	_
Apex	_	_	_	_	Acute	_
Surface	Smooth	_	Glabrous	_	Hairy	_
Venation	_	_	_	_	Reticulate	_
Length	7-9 cm	_	15-18 cm	_	5-8 cm	_
Width	6-8 mm	_	1-2 cm	_	3-5 cm	_

The macroscopic and organoleptic study revealed the colors of fresh root, stem, and leaf of *Gymnema sylvestre* were brown, grey, and green respectively. The leaf was extremely bitter in taste. The powdered form of root, stem, and leaf was light brown, light grey, and light green respectively. The shape of the root and stem was cylindrical whereas that of the leaf was ovate. The length and width of root were 7-9 cm and 6-8 mm respectively. The length and width of stem were 15-18 cm and 1-2 cm respectively. The upper leaf surface was hairy, the apex was acute. Reticulate venation was observed in the leaf. Each leaflet is 5-8 cm in length and 3-5 cm in width.

Table No. 4: Macroscopic and Organoleptic Characteristics of Root, Stem & Leaf of *Acacia catechu* [Family- Leguminosae]

CHARACTERS	OBSERVATIONS					
	Root		Stem		Leaf	
	Fresh	Powder form	Fresh	Powder form	Fresh	Powder form
Colour	Brown	Light brown	dark greyish brown to dark brown	Dark brown	Dark green	Light green
Texture	Hard	_	Hard	_	Smooth	_
Odour	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless
Taste	Bitter	Bitter	Very astringent	Astringent	Astringent	Astringent
Type	_	_	_	_	Alternate, bipinnate, stipulate	_
Shape	Cylindrical	_	Cylindrical	_	Linear to oblong	_
Apex	_	_	_	_	Round, obtuse	_
Surface	Smooth	_	Smooth	_	Smooth	_
Venation	_	_	_	_	Reticulate	_
Length	8-12 cm	_	20-27 cm	_	Leaflet 2.5-8 mm	_
Width	0.5-1 cm	_	1.5-2 cm	_	0.5-1.5 mm	_

The macroscopic and organoleptic study revealed the fresh root, stem, and leaf of *A. catechu* were brown, dark greyish brown to dark brown, and dark green respectively. The powdered form of root and stem was brown whereas that of the leaf was light green. The taste of stem was astringent. The shapes of root and stem were cylindrical whereas that of the leaf was linear to oblong. The length and width of root were 8-12 cm and 0.5-1 cm respectively. The length and width of stem were 20-27 cm and 1.5-2 cm respectively. Reticulate venation was observed in the leaf. Each leaflet was 2.5-8mm in length and 0.5-1.5mm in width.

Table No. 5: Macroscopic and Organoleptic Characteristics of Root, Stem & Leaf of *Holarrhena antidysenterica* [Family- Apocyanaceae]

CHARACTERS	OBSERVATIONS					
	Root		Stem		Leaf	
	Fresh	Powder form	Fresh	Powder form	Fresh	Powder form
Colour	Brown	Light brown	Yellow to grey	Light grey	Light green	Light green
Texture	Hard	_	Hard	_	Smooth, membranous	_
Odour	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless
Taste	Bitter	Bitter	Acrid/ Extremely bitter	Bitter	Bitter	Bitter
Type	_	_	_	_	Simple	_
Shape	Cylindrical	_	Cylindrical	_	Oval	_
Apex	_	_	_	_	Acute	_
Surface	Smooth	_	Glabrous	_	Smooth and glossy	_
Venation	_	_	_	_	Pinnate	_
Length	7-9 cm	_	11-14 cm	_	10-12 cm	_
Width	0.6-0.8 cm	_	0.7-1.5 cm	_	4-10 cm	_

The macroscopic and organoleptic study revealed the colors of the fresh root, stem, and leaf of *H. antidysenterica* were brown, yellow to grey, and light green respectively. The powdered form of root, stem, and leaf was light brown, light grey, and light green respectively. The taste of root and stem was bitter and that of leaf was astringent. The shapes of root and stem were cylindrical whereas that of the leaf was oval. The length and width of root were 7-9 cm and 0.6-0.8 cm respectively. The length and width of stem were 11-14 cm and 0.7-1.5 cm respectively. Pinnate venation was observed in the leaf. Each leaf was 10-12 cm in length and 4-10 cm in width.

II) PHARMACOGNOSTIC STUDY

Powder microscopy study of the root, stem, and leaves of these plants revealed the presence of lignified cork cells, single-celled trichome, simple and compound starch grains, lignified fiber, tracheids with the narrow lumen and tapering ends, xylem vessels with scalariform, reticulate, and spiral thickenings, calcium oxalate crystals and stomata. The findings have been described in Table 6. [Figure 1,2,3,4,5]

Table No. 6: Powder Characters of Roots, Stems, and Leaves of Different Antidiabetic Medicinal Plants

NAME OF PLANTS	PLANT PARTS	POWDER CHARACTERS								
		Cork cells	Trichome	Starch grains	Fiber	Tracheid	Vessel element	Sclereids	Stomata	Crystal
***Cassia fistula* (Aragvadha/ Amaltas)**	Root	Present	_	Simple and compound	Libriform, tapering towards ends	short	pitted	_	_	_
	Stem	Lignified	Unicellular	Simple and compound	Short, pits present, round at tips	short	Short with scalariform thickening	Thick-walled, small, rectangular	_	_
	Leaf	_	Unicellular	Simple and compound	Short, round at the tips	Short	Scalariform thickening	_	Paracytic	_
Tinospora cordifolia (Guduchi/ Gulancha)	Root	_	_	simple	tapering end	reticulate thickening	Simple, pitted, broad, perforated	_	_	_
	Stem	Cells lignified	Single-celled	simple	Libriform, tapering end	reticulate thickening	Broad, annular thickening	_	_	_
	Leaf	_	Single-celled	simple	round or tapering end	Long	Broad, reticulate thickening	_	Anisocytic	_
***Gymnema sylvestre* (Gurmar)**	Root	_	_	simple	Broad lumen	Reticulate thickening	Simple, pitted	_	_	_
	Stem	Cells lignified	Single-celled	simple	Libriform, tapering ends	Long, reticulate thickening	Broad, annular thickening	_	_	_
	Leaf	_	Single-celled	simple	Tapering ends	Long	pitted	_	Paracytic	_
***Acacia catechu* (Khadira)**	Root	_	_	Simple	Tapering towards ends	Short	pitted	_	_	Present
	Stem	Lignified with brownish pigments	Single-celled, non-glandular	Simple	Libriform, pits present	Short	long with reticulate thickening	Thick-walled	_	_
	Leaf	_	_	Simple	Short, round	Short	Scalariform	_	Paracytic	Pres

					at tips		thickening			ent
***Holarrhena antidysenterica* (Kurchi)**	Root	–	–	simple	Tapering ends	Vasicentric	pitted, broad	–	–	–
	Stem	Cells lignified	Single -celled	simple	Libriform, tapering end	Reticulate thickening	Broad, reticulate thickening	Thick-walled	–	–
	Leaf	–	Single -celled	simple	Round or tapering end	Long	Broad, reticulate thickening	–	Anisocytic	–

POWDER MICROSCOPY

i) Powder Microscopy of Root, Stem & Leaf of *Cassia fistula (Aragvadha/Amaltas)*

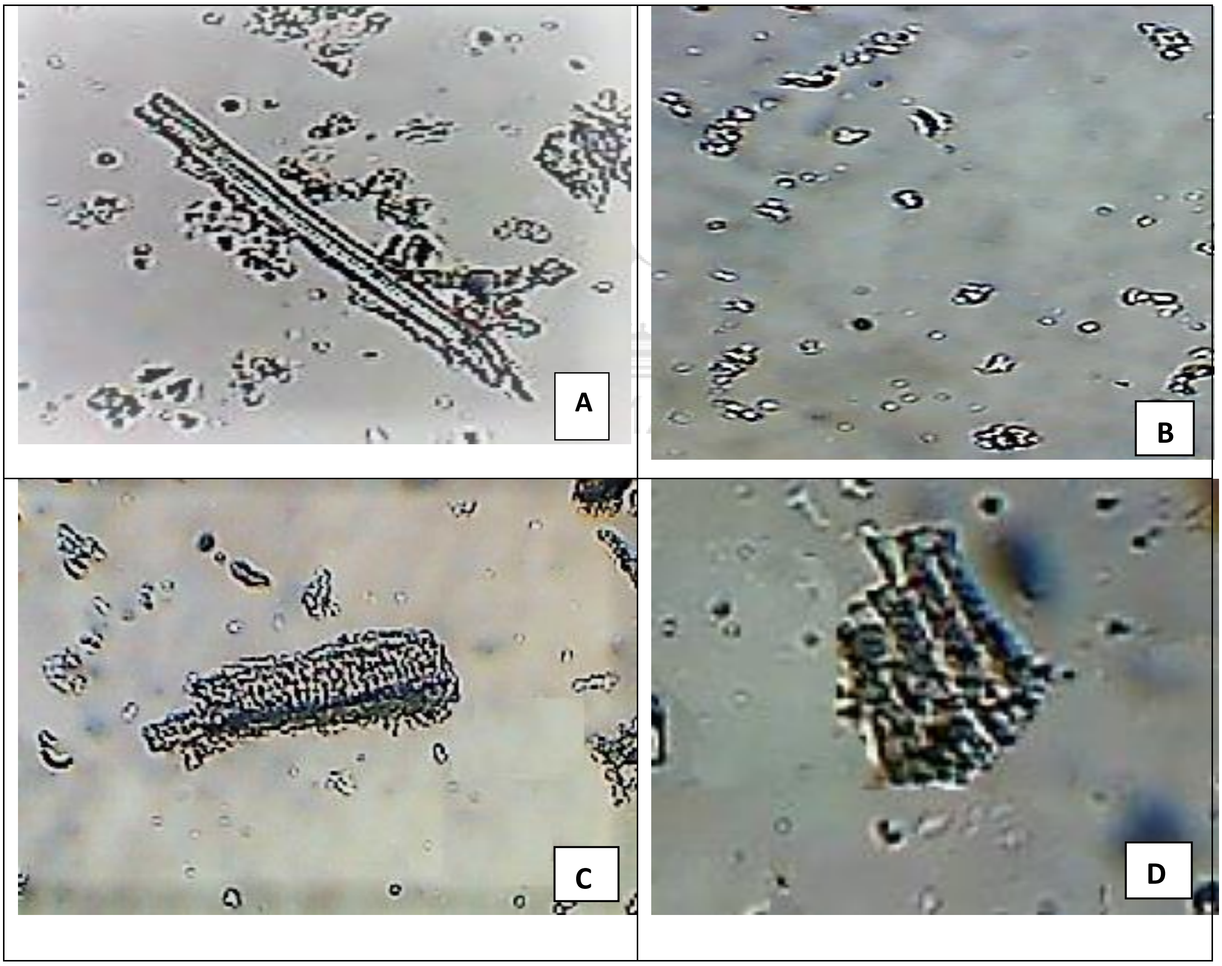

Figure No. 1: Powder microscopy of *Cassia fistula* (Aragvadha): A) Fibres with a lumen in the root, B) Simple and compound starch grains in the stem, C) Vessel with scalariform thickening in the stem, D) Epidermal tissues in the leaf

ii) Powder Microscopy of Root, Stem, and Leaf of *Tinospora cordifolia (Guduchi/ Gulancha)*

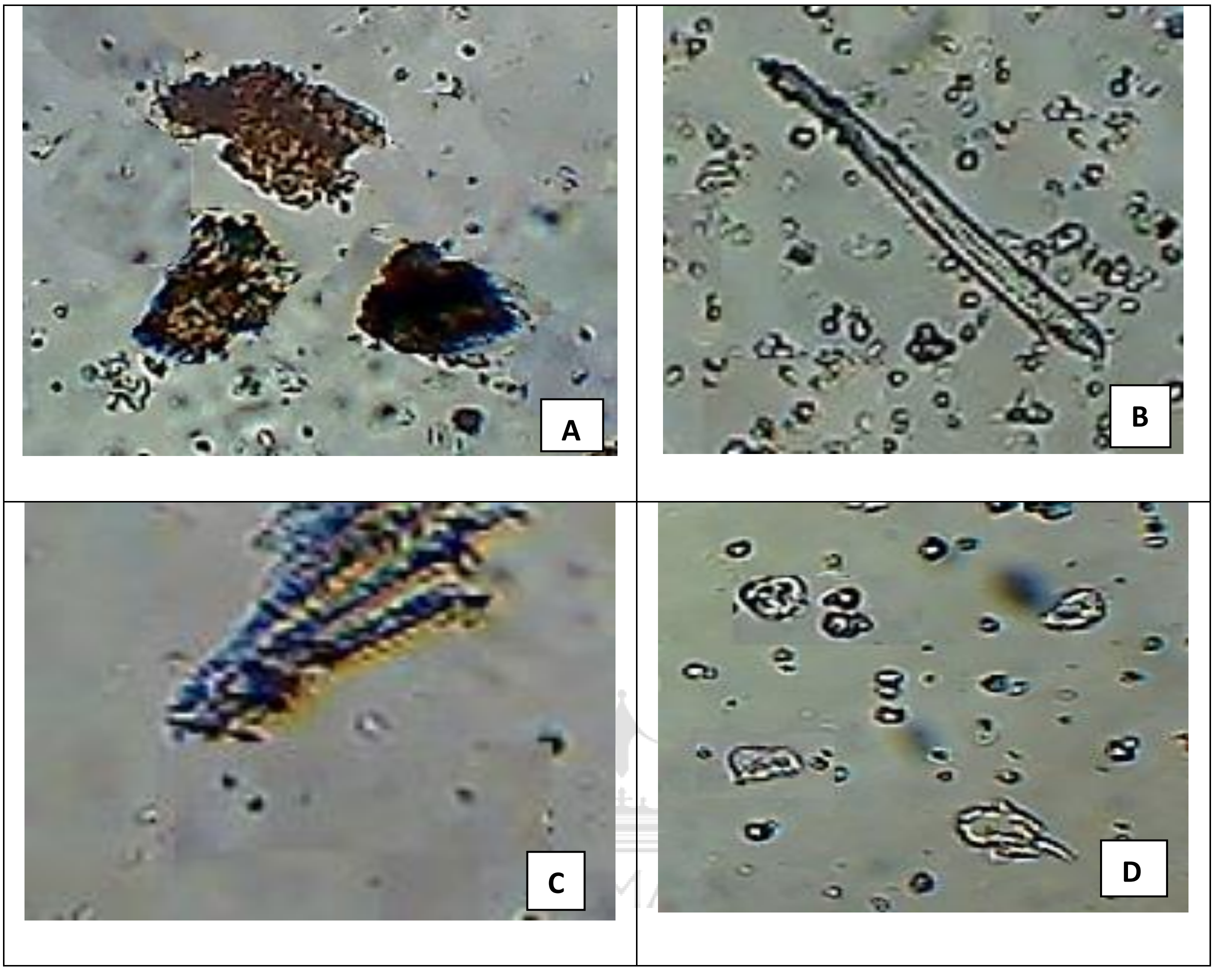

Figure No. 2: Powder microscopy of *Tinospora cordifolia*: A) Cells containing brown pigments in the root, B) Fibre with lumen and tapering ends in the stem, C) Vessel with annular thickening in the stem, D) Anisocytic stomata in leaf

iii) Powder Microscopy of Root, Stem & Leaf of *Gymnema sylvestre* (Gurmar)

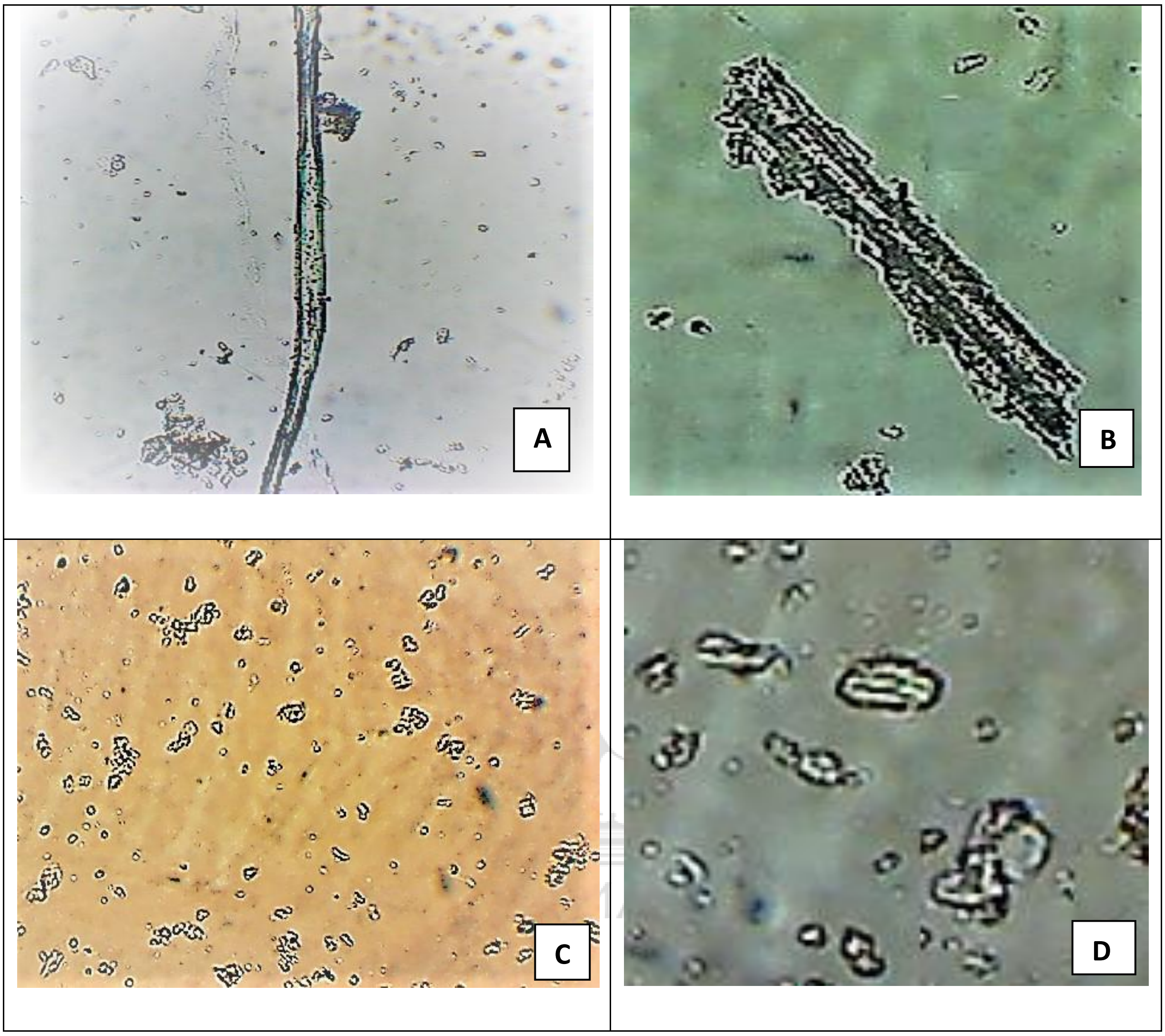

Figure No. 3: Powder microscopy of *Gymnema sylvestre*: A) Fibre with a broad lumen in the root, B) Vessel with scalariform thickening in the stem, C) Starch grains in the stem, D) Paracytic stomata in leaf

iv) Powder microscopy of root, stem & leaf of *Acacia catechu* (Khadira)

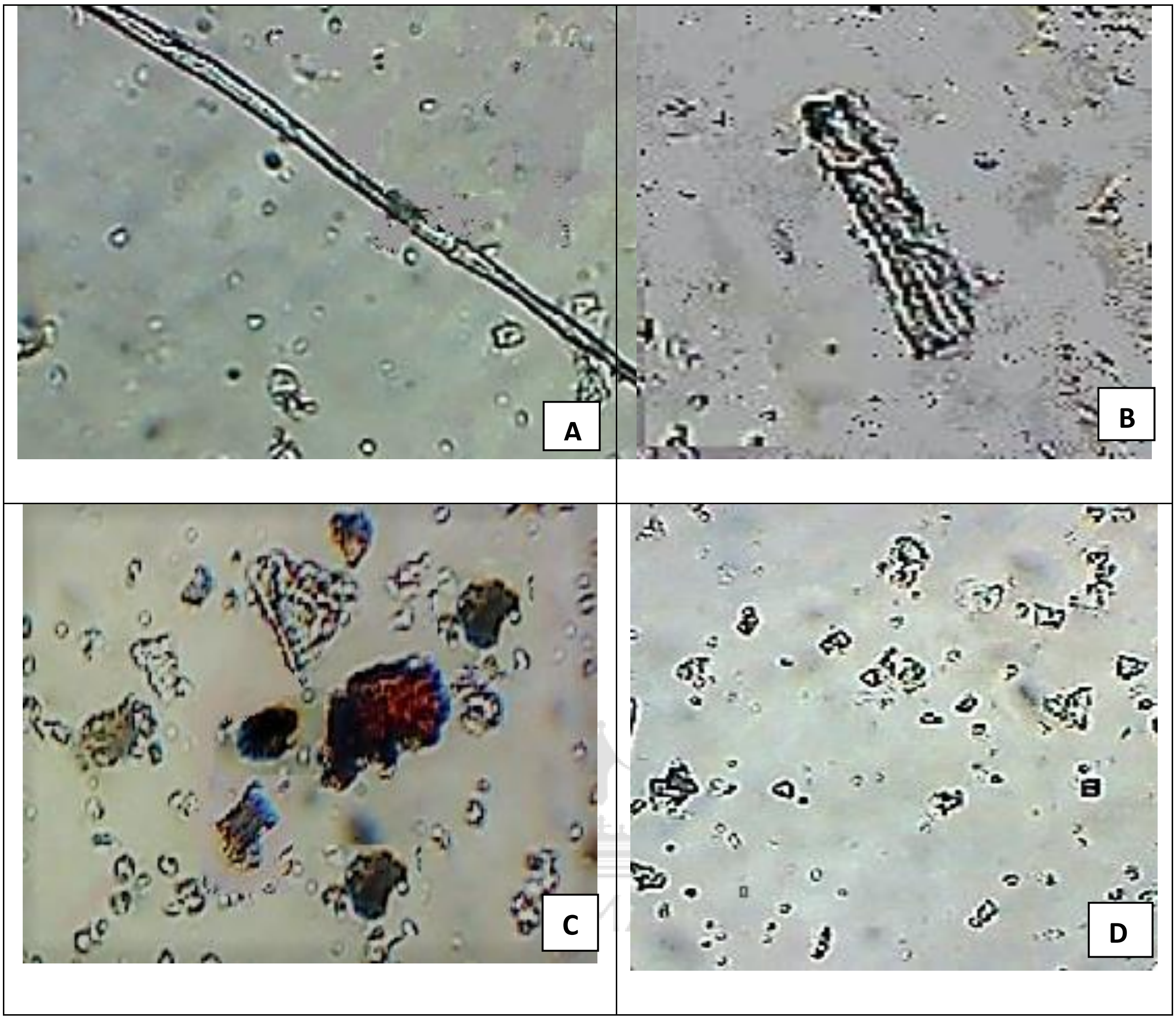

Figure No. 4: Powder microscopy of *Acacia catechu* (khadira): A) Fibre in the root, B) Vessel with annular thickening in the stem, C) Tissue containing brown pigments in the stem, D) Starch grains in leaf

v) Powder Microscopy of Root, Stem & Leaf of *Holarrhena antidysenterica* (Kurchi)

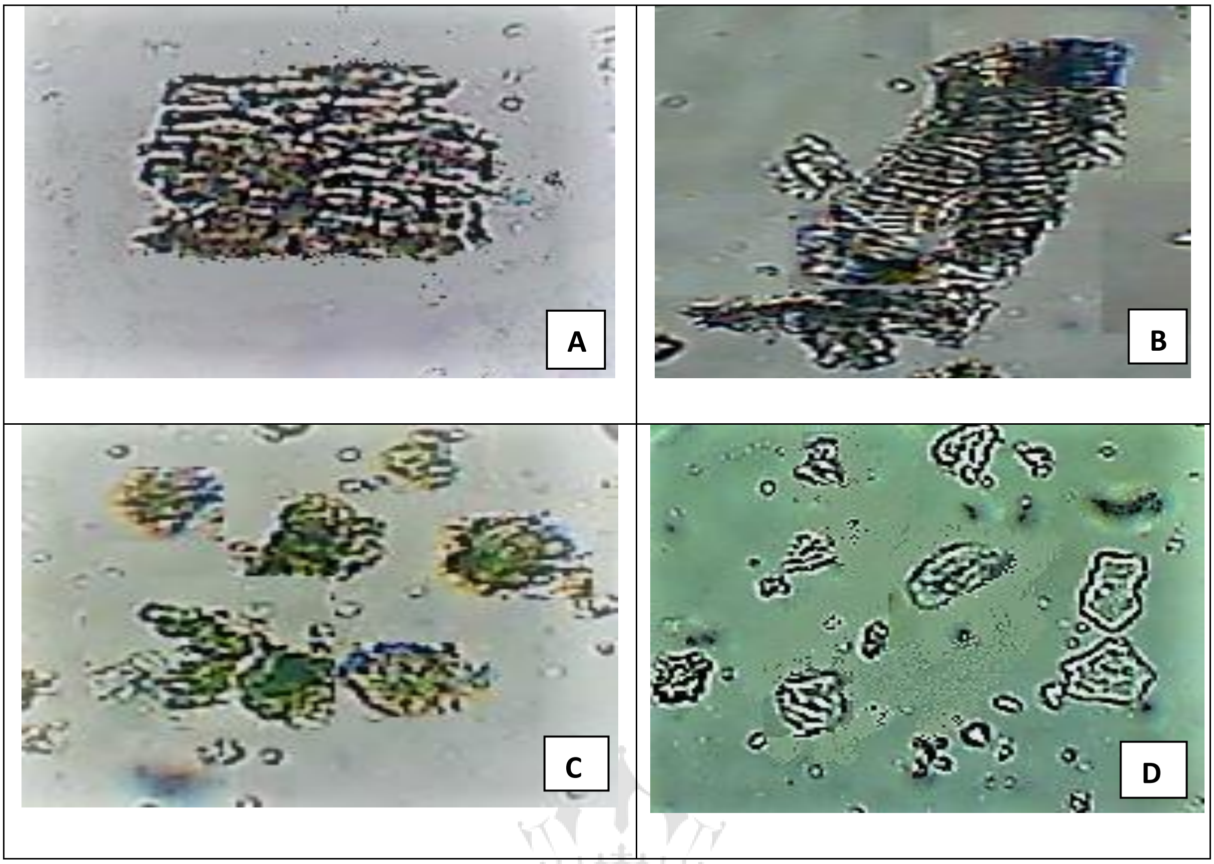

Figure No. 5: Powder microscopy of *Holarrhena antidysenterica*: A) Fibres in a bundle in the root, B) Vessel with reticulate thickening in the stem, C) Epidermal tissue in stem, D) Anisocytic stomata in leaf (upper view)

Comparative Pharmacognostic Characters

Distinctive similarities and dissimilarities between powder characters of root, stem, and leaves of these plants were observed under a microscope. The roots of different plants showed different characteristics. A pitted vessel was seen in *Cassia*, both perforated and pitted vessels were seen in *Tinospora*, broad and pitted vessels were seen in *Holarrhena*. Reticulate thickening was observed in tracheids of *Tinospora* and *Gymnema*, vasicentric tracheids were seen in *Holarrhena*. In *Cassia*, fibre was libriform. *Gymnema* showed fibers with broad lumen, *Acacia* and *Holarrhena* showed fibers with tapering ends. Crystal was seen in the root of *Acacia*. Tracheids were present in stems of *Tinospora.* Vessels in stems of *Gymnema* and Holarrhena had reticulate thickening. Except for Cassia, fibers were present in stems and had tapering ends. Xylem fibers in *Cassia* had round ends. Single-celled trichomes were present in stems of *Cassia*, *Tinospora*, *Gymnema,* and *Holarrhena*. In the case of xylem vessels, various types of thickenings such as reticulate, annular, scalariform thickenings were observed. Thick-walled sclereids were observed in stems of *Cassia, Acacia,* and *Holarrhena.* Crystal was observed in the leaf of *Acacia*. Anisocytic stomata stomata were observed in *Tinospora* and *Holarrhena* leaves. Paracytic stomata was seen in *Cassia, Gynmnema and Acacia* leaves. Starch grains were commonly seen in all of the root, stem, and leaf samples.

III) PHYSICO-CHEMICAL PROPERTIES

Table No. 7: Physico-Chemical Properties of Roots of Different Antidiabetic Medicinal Plants

PARAMETERS	RESULT % W/W				
	***Cassia fistula* (Amaltas)**	***Tinospora cordifolia* (Guduchi)**	***Gymnema sylvestre* (Gurmar)**	***Acacia catechu* (Khadira)**	***Holarrhena antidysenterica* (Kurchi)**
Loss on drying	16.08	11.67	10.87	8.54	11.58
Total ash value	11.90	8.24	8.26	12.82	6.38
Acid insoluble ash	7.81	2.02	2.71	2.61	1.77
Water soluble ash	0.21	0.71	0.65	5.83	8.11
Alcohol soluble extractive value	9.52	43.67	12.52	5.42	30.68
Water soluble extractive value	5.23	38.55	7.71	2.67	32.36

In the case of root samples, loss on drying at 105 degrees C and acid insoluble ash were maximum for *Cassia fistula.* Total ash value was maximum in the case of *Acacia*. Maximum value of water soluble ash was observed in *Horarrhena*. Both alcohol soluble and water soluble extractive values were maximum in the case of *Tinospora*.

Table No. 8: Physico-chemical Properties of Stems of Different Antidiabetic Medicinal Plants

PARAMETERS	RESULT % W/W				
	***Cassia fistula* (Amaltas)**	***Tinospora cordifolia* (Guduchi)**	***Gymnema sylvestre* (Gurmar)**	***Acacia catechu* (khadira)**	***Holarrhena antidysenterica* (Kurchi)**
Loss on drying	11.74	12.55	9.35	7.81	13.44
Total ash value	8.52	7.24	10.61	12.43	4.57
Acid insoluble ash	1.63	1.21	3.23	1.64	0.60
Water soluble ash	8.06	0.55	5.25	7.92	8.60
Alcohol soluble extractive value	24.83	47.88	5.63	4.73	31.22
Water soluble extractive value	17.98	35.68	16.21	2.80	33.53

In the case of stem samples, loss on drying at 105 degrees C was maximum in the case of *Holarrhena antidysenterica*, total ash value was maximum in *Acacia*, acid insoluble ash value and water-soluble ash were seen maximum for *Gymnema* and *Holarrhena* respectively. Both alcohol soluble and water-soluble extractive values were seen maximum in the case of *Gymnema.*

Table No. 9: Physico-chemical Properties of Leaves of Different Antidiabetic Medicinal Plants

PARAMETERS	RESULT % W/W				
	***Cassia fistula* (Amaltas)**	***Tinospora cordifolia* (Guduchi)**	***Gymnema sylvestre* (Gurmar)**	***Acacia catechu* (Khadira)**	***Holarrhena antidysenterica* (Kurchi)**
Loss on drying	15.07	11.78	8.63	8.18	11.7
Total ash value	7.71	8.21	10.64	13.51	4.41
Acid insoluble ash	5.42	1.52	3.62	2.30	0.40
Water soluble ash	1.51	0.85	4.81	6.42	7.31
Alcohol soluble extractive value	12.70	45.67	4.61	8.67	30.16
Water soluble extractive value	17.06	35.77	18.70	2.34	34.54

In the case of leaf samples, loss on drying at 105 degrees C was maximum in case of *Cassia*, total ash value was seen maximum in *Acacia.* Acid insoluble ash value and water-soluble ash value were maximum in case of *Cassia* and *Holarrhena* respectively. Both alcohol soluble and water-soluble extractive values were maximum in the case of *Acacia.*

Comparative Physico-chemical Properties

In the case of root samples, loss on drying nd acid insoluble ash values were maximum for *Cassia fistula*. Total ash value was maximum in the case of *Acacia*. Maximum value of water soluble ash was observed in *Horarrhena*. In the case of Tinospora, both alcohol soluble and water soluble extractive values were maximum.

In the case of stem samples, loss on drying was maximum in the case of *Holarrhena antidysenterica,* total ash value was maximum in *Acacia*. Acid insoluble ash and water-soluble ash values were seen maximum for *Gymnema* and *Holarrhena* respectively. Both alcohol soluble and water-soluble extractive values were seen maximum in the case of *Gymnema.*

In the case of leaf samples, loss on drying was maximum in case of *Cassia.* Acid insoluble and water-soluble ash values were maximum in case of *Cassia* and *Holarrhena* respectively. In the case of *Acacia*, total ash value as well as both alcohol soluble and water-soluble extractive values were maximum.

IV) PHYTOCHEMICAL PROPERTIES

Alkaloid, flavonoid, carbohydrates, phenol, tannin, saponin, protein, and amino acids were present in methanolic and aqueous plant extracts [Table 10, table 11].

Table No. 10: Phytochemical Screening (Methanolic Extract)

Antidiabetic medicinal plants	Plant parts	Phytochemical constituents						
		Alkaloid	Flavonoid	Carbohydrate	Phenol	Tannin	Saponin	Protein and amino acid
***Cassia fistula* (Aragvadha/ Amaltas)**	Root	+	+	+	+	+	_	_
	Stem	+	+	+	+	+	_	_
	Leaf	+	+	+	+	+	_	_
Tinospora cordifolia (Guduchi/ Gulancha)	Root	+	+	_	+	+	_	+
	Stem	+	+	+	+	+	+	_
	Leaf	+	+	_	+	_	_	_
***Gymnema sylvestre* (Gurmar)**	Root	+	+	+	+	+	_	+
	Stem	+	+	+	+	+	+	_
	Leaf	+	+	_	+	_	+	_
***Acacia catechu* (Khadira)**	Root	_	+	+	+	+	_	+
	Stem	+	+	+	+	+	_	_
	Leaf	+	+	_	+	_	_	_
***Holarrhena antidysenterica* (Kurchi)**	Root	+	+	_	+	+	+	_
	Stem	+	+	_	+	+	_	_
	Leaf	+	+	_	+	+	+	_

[Present +, Absent -]

Table No. 11: Phytochemical Screening (Aqueous Extract)

Antidiabetic medicinal plants	Plant parts	Phytochemical constituents						
		Alkaloid	Flavonoid	Carbohydrate	Phenol	Tannin	Saponin	Protein and amino acid
***Cassia fistula* (Aragvadha/ Amaltas)**	Root	+	–	–	+	+	–	–
	Stem	+	–	–	+	+	+	–
	Leaf	+	+	–	+	+	+	–
Tinospora cordifolia (Guduchi/ Gulancha)	Root	+	+	+	+	+	–	+
	Stem	+	+	–	+	+	–	–
	Leaf	–	+	–	–	–	+	+
***Gymnema sylvestre* (Gurmar)**	Root	+	+	+	+	+	–	+
	Stem	+	+	+	+	+	+	–
	Leaf	–	+	–	–	–	+	+
***Acacia catechu* (Khadira)**	Root	+	–	–	+	+	–	+
	Stem	+	+	–	+	+	+	–
	Leaf	+	+	–	+	–	+	–
***Holarrhena antidysenterica* (Kurchi)**	Root	–	–	+	+	+	–	–
	Stem	+	+	–	+	+	+	+
	Leaf	+	+	+	–	–	+	–

[Present + , Absent -]

Comparative phytochemical properties

In the case of methanolic extracts of plant samples, alkaloid was absent in the root sample of *Acacia catechu*. Carbohydrate was not present in root and leaf samples of *Tinospora* *cordifolia*, leaves of *Gymnema sylvestre* and *Acacia catechu*, and all methanolic extracts of *Holarrhena antidysenterica*. Tannin was absent in leaf of *Tinospora cordifolia* and *Acacia catechu*. Saponin was present in the stem of *Tinospora cordifolia*, the root of *Holarrhena antidysenterica,* stem and leaf of *Gymnema sylvestre*. Protein and amino acid were present in *Tinospora cordifolia*, *Gymnema sylvestre*, and *Acacia catechu*.

Aqueous extracts of plant samples showed the absence of alkaloids in the leaf of *Tinospora cordifolia*, *Gymnema sylvestre*, and root of *Holarrhena antidysenterica*. Flavonoids were absent in root and stem of Cassia, root of *Acacia catechu*, and *Holarrhena antidysenterica*. Carbohydrate was present in the root of *Tinospora cordifolia*, *Gymnema* sylvestre, and *Holarrhena antidysenterica.* Phenol was absent in leaves of *Tinospora cordifolia, Gymnema sylvestre*, and *Holarrhana antidysenterica*. Tannin was absent in leaves of *Tinospora cordifolia, Gymnema sylvestre, Acacia catechu*, and *Holarrhana antidysenterica*. Saponin was absent in all root samples. Protein and amino acids were present in the root and leaf of *Tinospora cordifolia, Gymnema sylvestre, the* root of *Acacia*, root, and stem of *Holarrhena antidysenterica.*

DISCUSSION:

Diabetes mellitus (DM) is a serious and common metabolic disorder affecting a large number of the population in the world. Oral hypoglycemic agents and insulin are mostly used in the treatment of diabetes but they have significant side effects on the human body and lead to many complications. Biguanides, sulfonylureas, thiazolidinediones and alpha glucoside inhibitors are the antidiabetic agents which are widely used to control increased blood sugar level. They have limited use because of undesirable secondary complications. [21]

Medicinal plants have been used by mankind since ancient times for the treatment of various ailments and maintenance of good health. In India, traditional plant remedies have been used to treat diabetes since the time of Charaka and Shusruta[22]. Herbal medicines have been gaining popularity for the treatment of diabetes because of their efficiency in reducing blood sugar levels without having many side effects on the human body.

In India, *Cassia fistula* (Aragvadha/ Amaltas) [Family Caesalpiniaceae], *Tinospora cordifolia (Guduchi/ Gulancha*) [Family- Menispermaceae], *Gymnema sylvestre* (Gurmar) [Family- Asclepiadaceae], *Acacia catechu* (Khadira) [Family- Mimosaceae], and *Holarrhena antidysenterica* (Kurchi) [Family- Apocyanaceae] have been commonly used for the production of herbal medicines in the treatment of diabetes. The stem bark of *Cassia fistula* shows a reduced level of blood sugar level[26]. The aqueous extract of stem of *Tinospora cordifolia*, commonly known as *Guduchi sattwa* in Ayurveda, is recommended for the treatment of diabetes mellitus[27]. *Gymnema sylvestre* leaves contain gymnemic acids(triterpene saponins) which have antidiabetic activity[28]. The aqueous bark extract of *Acacia catechu* is useful for the treatment of diabetes[29]. Methanolic extract of stem of *Holarrhena antidysenterica* shows results in reducing blood sugar level[30].

The current research included i) pharmacognostic similarities and dissimilarities and ii) comparative account on the phytochemical analysis between root, stem and leaves of five medicinal plants [*Cassia fistula* (Aragvadha/ Amaltas), *Tinospora cordifolia (Guduchi/ Gulancha*), *Gymnema sylvestre* (Gurmar), *Acacia catechu* (Khadira) and *Holarrhena antidysenterica* (Kurchi)]. These plants have been used extensively in Ayurveda for the treatment of diabetes. The study would help provide information on whether other plant parts(root/stem/leaf) could be useful in replacing the leaf, bark and stem of these plants for the production of herbal medicines.

The macroscopic and organoleptic study showed different characteristics of powdered plant parts. Physico-chemical properties of plant samples showed various parameters. The powdered microscopic study revealed significant characteristics of powdered plant parts. In case of powdered root samples, different plants showed different characteristics. A pitted vessel was observed in the powdered root of *Cassia*. In case of root of *Tinospora*, both perforated and pitted vessels were seen, broad and pitted vessels were seen in *Holarrhena* root samples. Reticulate thickening was observed in tracheids of *Tinospora* and *Gymnema* roots, vasicentric tracheids were seen in *Holarrhena* root. In *Cassia* root, fibre was libriform. *Gymnema* showed fiber with broad lumen, *Acacia* and *Holarrhena* showed fibers with tapering ends. Crystal was seen in the root of *Acacia*. Tracheids were present in stems of *Tinospora*. *Gymnema* and *Holarrhena* stems showed reticulate thickenings. Except for the stem of *Cassia*, fibers were present in stems and had tapering ends. Xylem fibers in *Cassia* had round ends. Single-celled trichomes were present in stems of *Cassia*, *Tinospora, Gymnema,* and *Holarrhena*. In the case of xylem vessels, various types of thickenings such as reticulate, annular, and scalariform thickenings were observed. Thick-walled sclereids were observed in stems of *Cassia, Acacia*, and *Holarrhena*. Calcium oxalate crystals were observed in the leaf of *Acacia*. Anisocytic stomata stomata were seen in *Tinospora* and *Holarrhena* leaves. Paracytic stomata was observed in *Cassia*, *Gynmnema and Acacia* leaves.

Anisocytic stomata and paracytic stomata were seen in the leaf of *Tinospora* and *Cassia* respectively. Starch grains were commonly seen in all of the root, stem, and leaf samples.

Analysis obtained from various research works proved that different phytochemical components such as flavonoids, alkaloids, tannins, saponins, glycosides, terpenes are responsible for the anti-diabetic activities of the plants. The research work focused on finding whether the same plant has potential phytoconstituent in all its root, stem, and leaf. This way extensive use of a particular plant part could be reduced as another part of the same plant can be used to extract the same active chemical constituent.

The result showed that methanolic extracts of all root, stem, and leaf of *Cassia fistula* contain alkaloids, flavonoids, tannins, carbohydrates, and phenol. So, instead of only stem bark, the root and leaf can also be used for the preparation of anti-diabetic herbal medicine. Methanolic extracts of root and leaf of *Tinospora cordifolia* showed the presence of alkaloids, flavonoids, tannin, and phenol. So, instead of the only stem, the root and leaves can also be used for the preparation of anti-diabetic plant-based medicine. In the case of *Gymnema sylvestre*, both stem and leaf extracts showed the presence of saponin. So, instead of only leaves, stem can also be used for the production of anti-diabetic herbal medicine. Root, stem, and leaf extracts of *Acacia catechu* showed the presence of alkaloids, flavonoids, tannins, carbohydrates, phenol, proteins, and amino acids; saponin was not present. So, all root, stem, and leaf may be used (instead of the only stem) for the treatment of diabetes. In the case of *Holarrhena antidysenterica*, methanolic extract of root, stem, and leaf showed the presence of alkaloids, flavonoids, tannins, phenols, and saponins. So, roots and leaves can be used for the treatment of diabetes instead of the only stem of *Holarrhena*.

A further phytochemical study conducted to compare the similarities in structures between modern antidiabetic drugs and plant extracts showed promising results. Similar compounds were found to be present in both modern antidiabetic drugs and specific plant extracts. Further research and analysis would provide specific data about the similar compounds present in both oral antidiabetic drugs and specific plant extracts.

CONCLUSION:

Diabetes is one of the major health challenges of the 21st century. Apart from allopathic medicines, traditional herbal medicines play a significant role in the treatment of diabetes. Medicinal plants are being considered as alternative sources of antidiabetic agents. Herbal medicine is gaining popularity over conventional allopathic drugs due to its lesser side effects on the human body. The current study revealed comparative pharmacognostic and phytochemical characteristics of five medicinal plants which have antidiabetic properties. *Cassia fistula*, *Tinospora cordifolia*, *Gymnema sylvestre*, *Acacia catechu*, and *Holarrhena antidysenterica* showed the presence of cork cells, trichomes, tracheids, xylem vessels, phloem elements, starch grains, and crystals in their powdered root, stem, and leaf samples. Root of Tinospora showed the presence of perforated as well as pitted vessels.Reticulate thickening was present in tracheids of roots of *Tinospora* and *Gymnema* and stems of *Gymnema* and *Holarrhena*. Single celled trichome is considered as an identifying character of plant species. The presence of single-celled trichomes were seen in powdered stem of *Cassia, Tinospora, Gymnema* and *Holarrhena.* Calcium oxalate crystals were present in root and leaf of *Acacia.* Thick-walled sclereids were present in stems of *Cassia, Acacia,* and *Holarrhena.* Anisocytic stomata stomata were observed in *Tinospora* and *Holarrhena* leaves. Paracytic stomata was found in *Cassia*, *Gynmnema and Acacia* leaves. Starch grains were present in the root, stem, and leaf of all plants. Phytochemicals such as alkaloids, flavonoids, phenols, tannin, saponin, carbohydrates were present or absent depending on the plant extracts. Saponin showed significant results in the treatment of diabetes. Except for *Acacia catechu*, saponin was present in the root, stem, and leaves of all other plants. So, it can be concluded that the root, stem, and leaves of these plants also contain a significant amount of phytochemicals that are responsible for antidiabetic properties. Instead of excessive uses and extracting only a specific plant part for the production of antidiabetic herbal drugs, all root, stem, and leaves can be used. This way these plants can be saved from getting endangered.

Further researches are needed to separate the active constituents from the plants extracts. The intermolecular reactions are also needed to be studied for a better understanding of the curative properties of the active components.

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CONFLICT OF INTEREST:

There is no conflict of interest.

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