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Original Article

Profiling of Iron Weed: An Ethnopharmacological Approach

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ABSTRACT

Background: *Baccharoides anthelmintica* L., known as Iron weed or Kali Jeeri, is a traditional medicinal plant used for treating asthma, sores, inflammation, and various other ailments. It boasts pharmacological activities including antimicrobial, anticancer, antidiabetic, anti-inflammatory, analgesic, antipyretic, and larvicidal properties. Despite its therapeutic potential, comprehensive anatomical information, particularly macroscopic, microscopic, physicochemical parameters, and HPTLC fingerprinting is lacking, which is crucial for its authentication and prevention of adulteration. Objective: This study aims a detailed analysis focusing on its pharmacognostical, physicochemical, phytochemical parameters, and HPTLC fingerprinting to detect alkaloids and diterpenes contributing to its medicinal properties in both whole plant and seed forms. **Materials and Methods:** The whole plants and seeds of *Baccharoides anthelmintica* L. were collected and macroscopic and microscopic features were documented for identification. Physicochemical parameters such as moisture content, ash value, and extractive values were determined using standard protocols. Preliminary phytochemical screening identified bioactive compounds, while HPTLC analysis fingerprinted alkaloids and diterpenes. **Results:** All parameters examined in this study, revealed distinctive features aiding in identification, differentiating from potential adulterants, provided essential data for standardization and quality control. Preliminary phytochemical screening confirmed the presence of alkaloids and diterpenes, supporting its traditional uses. HPTLC fingerprinting validated the presence of these compounds, highlighting the plant's pharmacological potential. **Conclusion:** This comprehensive data enhances our understanding of *Baccharoides anthelmintica* L. through pharmacognostical, physicochemical, and phytochemical perspectives. It provides crucial data for authentication, quality assessment, and utilization in traditional medicine and pharmaceutical sectors, supporting sustainable healthcare practices.

Keywords: *Baccharoides anthelmintica* L., HPTLC, Pharmacognosy, Phytochemistry.

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INTRODUCTION

Herbal remedies offer a host of advantages in healthcare, characterized by their safety, affordability, and minimal side effects, making them an appealing choice for those seeking alternative and holistic healing. However, the field of herbal medicine faces a significant challenge related to adulteration, primarily due to issues of misidentification and the shared use of vernacular names for distinct plant species. To address these issues, it is crucial to use accurate identification methods, set clear physical criteria, and enhance our knowledge of the anatomical features, the primary phytochemical compounds targeted, and the physicochemical parameters of the medicinal plant in use.

One such plant of considerable importance is *Baccharoides anthelmintica* L., which is known by various names, including Wild cumin, Iron weed or Purple Flea-Bane. It belongs to the Asteraceae family and has been recognized under different botanical synonyms, such as *Vernonia anthelmintica* Willd., *Centratherum anthelminticum* Kuntze, *Ascaradia indica*, *Conyza ascaradia*, and *Serratula anthelmintica*.[1,2] The diversity in its nomenclature reflects the plant's significance in various cultural and traditional systems of medicine.

This versatile plant contains a wide array of preliminary phytochemical constituents. These include glycosides, carbohydrates, phenolic compounds, tannins, flavonoids, proteins, saponins, sterols, lipids, fats, sesquiterpenes, lactones, alkaloids, terpenoids, diterpenoids and steroids. These compounds offer a rich source of bioactive molecules with diverse pharmacological activities, making *B. anthelmintica* a potential goldmine for green medicine practices.[3]

The reported pharmacological activities associated with *B. anthelmintica* are extensive and encompass anti-inflammatory, anti-arthritic, anti-pyretic, anti-filarial, anti-cancer, anti-microbial, anti-malarial, anti-viral, anthelmintic, anti-diabetic, melanogenesis regulation, wound healing, anti-bacterial, anti-fungal, anti-hypoglycemic, diuretic,

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analgesic, larvicidal, cytotoxic, insecticidal and anti-implantation activities. Its versatile applications across various health concerns underscore its significant role in natural medicine and traditional healing practices.[3]

In Ayurveda, the seeds of this plant are considered hot, acrid, astringent, and anthelmintic. They are used to treat ulcers, balance *vata* and *kapha* doshas, and manage skin diseases, leukoderma, and fever.[4] In the Unani system, the seeds are valued for their anthelmintic and purgative properties and are used to address asthma, kidney troubles, hiccough, inflammatory swellings, liver-related issues, sores, and eye itching. The plant's multifaceted applications in diverse systems of traditional medicine highlight its broad spectrum of therapeutic potential.[1]

This study represents a pivotal effort to standardize the pharmacological, physicochemical, and phytochemical parameters of both the whole plant and its seeds. Alkaloids and diterpenoids are widely recognized for their ethnomedicinal properties among all secondary metabolites. Therefore, efforts are made to identify and understand the presence of these compounds for their potential enhanced medicinal applications with the help of HPTLC. By doing so, it provides a reliable and comprehensive tool for the precise identification and effective utilization of *B. anthelmintica* L. in the realm of green medicine. This research not only enhances our understanding of the plant but also contributes to its safe and effective application in healthcare practices, ultimately benefiting individuals seeking natural remedies and holistic healing options.

MATERIALS AND METHODS

Collection and authentication of plant material

Baccharoides anthelmintica L. whole plant and seed material used in this study were cultivated within the premises of Jai Hind College (Autonomous), Churchgate, Mumbai and authenticated by Blatter's Herbarium at St. Xavier's College, Mumbai, India. The material was marked as B.a. (WH) for *Baccharoides anthelmintica* whole plant and B.a.(Sd) for *Baccharoides anthelmintica* seed for further study.

Determination of pharmacognostical parameters of whole plant

Macroscopy

The morphological features and organoleptic characters of freshly collected whole plant material were evaluated, which were visible to our naked eyes or magnifying glass as per standard procedures.[5]

Microscopy

Cross-sections were manually cut from recently gathered material. After that, sections were stained with safranin, examined at 10X magnification using an Olympus compound microscope. Microphotographs were captured showing various anatomical characteristics using a digital camera.[6]

Leaf Constants

Applying the methods described by,[7] a thorough analysis of stomatal number and stomatal index was conducted. Stomatal types were identified in accordance with.[8] With a few minor adjustments, the leaf clearance method was carried out in order to analyse leaf architecture in accordance with.[9] Palisade ratio was calculated using[9,7] methodology. Trichome type and density were evaluated using a standard procedure proposed by.[9-11]

Determination of Physicochemical Constants of whole plant and seed

The Ash value, Extractive value, and Moisture content of both the entire plant and the seeds were determined separately. The assessment of Ash value involved the examination of Total cash value, Acid-insoluble ash value, and Water-soluble ash value, as specified in.[5] Additionally, the Sulphated ash value was determined following the method outlined by.[12] Alcohol extractive value, Water extractive value, and Moisture content were analysed in accordance with the guidelines provided by.[5]

Fluorescence analysis of whole plant and seed

The powdered drugs of both the whole plant and seeds underwent fluorescence analysis by exposing them to ultraviolet radiation. The fluorescence was then observed by following the procedure outlined in the works of[13] as well as.[14]

Powder study of whole plant and seed

Chloral hydrate was used to clear the powdered drug. Unstained powder was examined for calcium oxalate crystals, Phloroglucinol and hydrochloric acid - stained powder, helped in identification of lignified tissues using method.[15]

Determination of Preliminary Phytochemical Screening of whole plant and seed

Ethanolic extracts were used for Phytochemical analysis of lactone, lipids, coumarin, glycoside, tannin, terpenoid, alkaloids, and more following[16] standard protocol for testing.

HPTLC Fingerprinting for presence of Alkaloids and Diterpenes

The stationary phase in Thin Layer Chromatography (TLC) consisted of MERCK™ Fine particle size TLC Silica gel 60 F254 Aluminium sheets (20 cmX20 cm), heated at 100°C for 15-20 min to eliminate absorbed water. CAMAG® Automatic TLC Sampler loaded samples at varying concentrations. The CAMAG® TTC (Twin Trough Chamber) (20 cmX20 cm) was prepared by methanol rinsing, then saturated with the respective mobile phase on Whatman no. 1 filter paper for 20 min. After complete saturation, the mobile phase ran up to 115 mm.

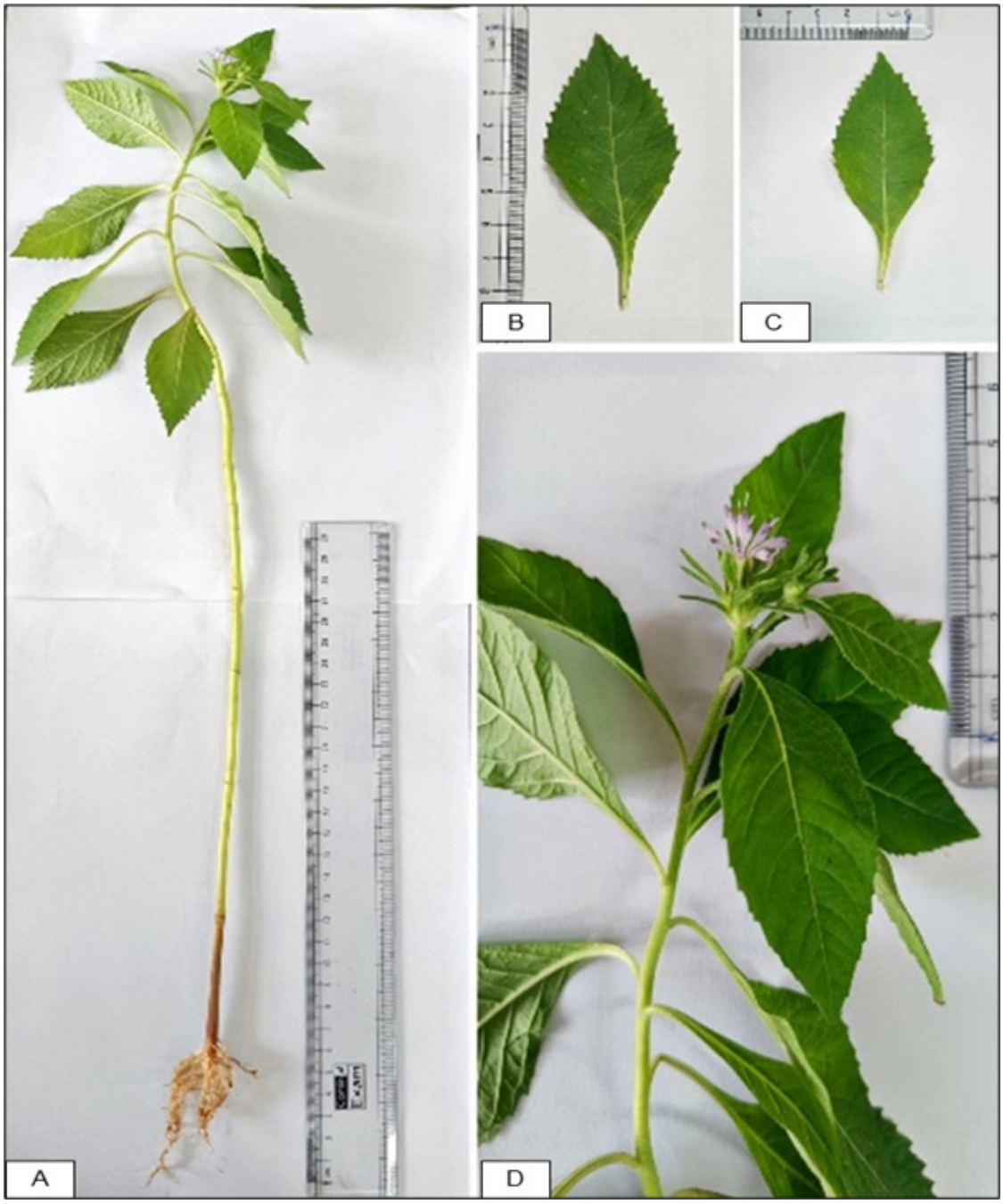

Figure 1: A: Macroscopy: entire plant, B: Macroscopy: leaf length, C: Macroscopy: leaf breadth, D: Macroscopy: inflorescence.

Chromatogram development was confirmed with a CAMAG® TLC Visualizer, followed by derivatization. The derivatized silica TLC plate was scanned at 254 nm, 366 nm, and visible light using the CAMAG® TLC Scanner, connected to data recording software, recording Rf values. The specific class of compounds were tested in ethanolic extracts from both whole plant material and seeds such as, Alkaloids were assessed using Toluene: Ethyl acetate: Diethylamine (70:20:10) as the mobile phase, employing Dragondroff reagent for derivatization.[17,18] Diterpenoids were separated using Chloroform: Toluene: Methanol (6:2.5:1.5) and derivatized with Anisaldehyde sulphuric acid.[19]

RESULTS

Determination of Pharmacognostical characters

Macroscopy

Systematic Description: (Figure 1)

It is an annual, robust, leafy and an erect plant. It has been widely distributed throughout India, Brazil, USA, Bangladesh and Nepal.[2] Stems are usually high, branched and pubescent with about 2-3 ft in height. Leaves are 2-3.5 in. long, 1-1.25 in. broad, lanceolate or elliptic-lanceolate, acute, coarsely serrate, pubescent on both surfaces, petiolate and tapering base.

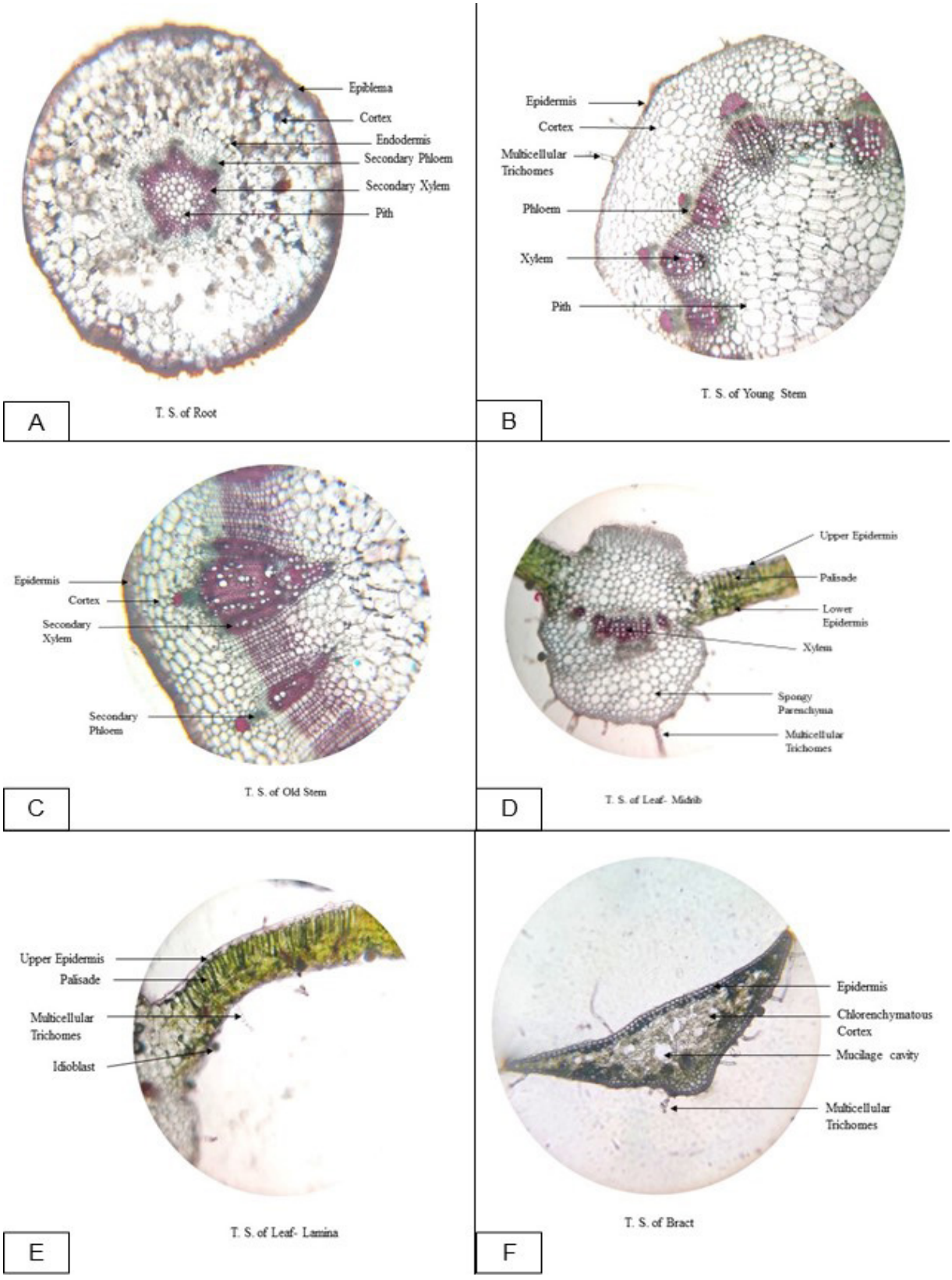

Figure 2: A: Transverse section of Root, B: Transverse section of young stem, C: Transverse section of old stem, D: Transverse section of Leaf Midrib, E: Transverse section of Leaf Lamina, F: Transverse section of Bracts.

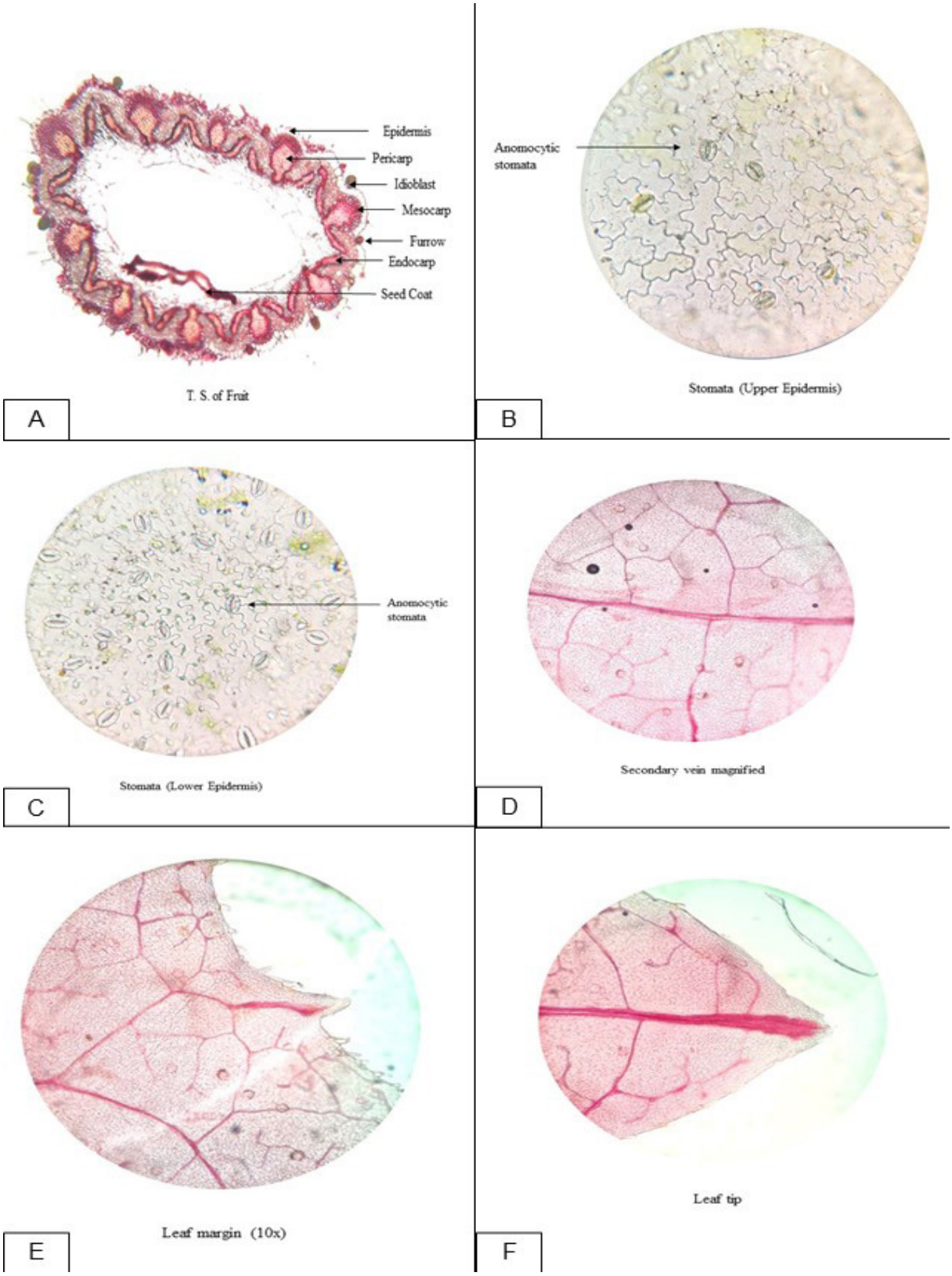

Figure 3: A: Transverse section of Fruit, B: Stomata on upper epidermis and its type, C: Stomata on lower epidermis and its type, D: Secondary veins magnified, E: Leaf Margin showing trichome, F: Leaf Tip.

Sub-corymbose inflorescence present with many or about 40 flowers present. Flowers usually consist of linear bracts near the top peduncle. Reddish pappus present, with exterior row very short, sub-paleaceous, persistent, with flattened inner hair and deciduous but much shorter than the glabrous corolla. Outer involucre bracts linear, hairy, herbaceous, shorter than those of the inner rows; intermediate bracts with herbaceous hairy tips, linear, acute or sub-obtuse, often constricted at the base of the herbaceous part, equalling or shorter (rarely longer) than the innermost; inner most bracts usually the longest, linear, subacute, scarious, often tipped with purple. Pappus reddish, the exterior row very short, sub-pale aceous, persistent, the inner hairs somewhat flattened, deciduous, much shorter than the glabrous corollas.[20] Achenes are long, oblong-cylindric, 10 ribbed, pubescent and abundant small silky hairs on outer surface. Externally fruits are brownish-black with extremely bitter taste and characteristic odour.[1] According to Ayurveda, seeds are hot, acrid, astringent, anthelmintic; cures ulcers, vata and kapha also to cure skin disease, leukoderma and fever.[4] According to Unani system, seeds are anthelmintic, purgative, used for asthma, kidney troubles, hiccough, inflammatory swellings, to remove blood from the liver, sores and itching of eyes.[1]

The macroscopic characters of root, stem, leaves, flower and fruit are noted down below in the Tables 1-5 respectively.

Microscopy

Transverse section of Root: (Figure 2(A))

Epiblema is single layered without root hair. Cortex is 5-6 layered, parenchymatous a few cells filled with brown matter and starch. Single layered endodermis strictly devoid of starch. Medullary region shows 5-6 radial vascular bundles with large pith in the centre. Secondary growth also appears normal with formation of secondary xylem on the inside and secondary phloem on the outside.

Transverse section of young Stem: (Figure 2(B))

Single layered epidermis with a few multicellular trichomes. Cortex consists of 9-10 layer of distinct parenchymatous endodermis. It is seen to be strictly devoid of starch. A ring small and large vascular bundle alternating with each other. Each vascular bundle is conjoint, collateral and open with hard bast. Xylem is endarch. Large parenchymatous pith occupies the centre.

Table 1: Macroscopical Characters of Root.

Sl. No.	Evaluating features	Characteristics of Stem
1.	Size	Length: 2.1 cm- 2.9 cm
		Breath: 0.65 cm -1.2 cm
2.	Type	Taproot
3.	Shape	Round

Transverse section of old Stem: (Figure 2(C))

It shows secondary growth with abnormalities. The inter-fascicular region shows presence of prosenchyma cells whereas the intra-fascicular region shows secondary xylem towards inside and secondary phloem towards outside.

Table 2: Macroscopical Characters of Stem.

Sl. No.	Evaluating features	Characteristics of Stem
1.	Size	Length: 20 cm-46 cm
2.		Breath: 0.95 cm-1.65 cm
3.	Shape	Circular
4.	Node	Many
5.	Internode	Many
6.	Odour	Leafy slightly bitter
7.	Taste	Bitter
8.	Colour	Green
9.	Texture	Smooth

Table 3: Macroscopical Characters of Leaf.

Sl. No.	Evaluating features	Characteristics of Leaf
1.	Size	Length: 5 cm-8.3 cm
2.		Breath: 1.85 cm-2.8 cm
3.	Shape	Ovate Lanceolate
4.	Apex	Acute
5.	Margin	Serrate
6.	Veins	Unicostate reticulate
7.	Veinlets	Quinternery
8.	Lamina	Both surfaces are slightly rough.
9.	Base	Acute
10.	Petiole	Short
11.	Odour	Characteristic, bitter
12.	Taste	Characteristic, bitter
13.	Colour	Green
14.	Texture	Slightly rough

Transverse section of Leaf: (Figure 2(D) and 2(E))

A typical dorsiventral leaf showing a single layered upper epidermis with a few uniseriate hair (trichomes) and well distributed stomata. The mesophyll is differentiated into one layered palisade cells and 3-4 layered spongy parenchyma. Lower epidermis is single layered small stalked glandular hair giving rise to pellucid dots. The leaves have a coating of varnish which is secreted by sub-epidermal idioblasts. The midrib shows a large parenchymatous region. The region also shows a few vascular bundles which are well developed. The region also shows mucilage ducts, uniseriate trichomes and glandular trichomes. Inulin crystals are present as per[21] it is also confirmed and16 observed in the midrib region.

Transverse section of Bracts: (Figure 2(F))

The bracts are covered by glandular and non-glandular, multicellular uniseriate trichomes. Epidermis is uniseriate made up of parenchyma cells. Absence of cuticle is very evident. Just beneath the epidermis 1-2 layered chlorenchyma cells make up the hypodermis cortex which is loosely arranged parenchyma cells showing chloroplast. Many large mucilage cavities are also seen in cortex.

Transverse section of Fruit: (Figure 3(A))

Cypsela is circular in shape with prominent thick ridges and furrows. The ridges are wide and semicircular, alternating with furrows. The Pericarp consists of regions such as epidermis, mesocarp and endocarp. The epidermis is thin walled. Inner to the epidermis is a wide zone of parenchymatous mesocarp. The cells of the mesocarp are fairly thick walled, angular, compact and wide. The inner layer of pericarp extends all around the fruit; along the ridges the endocarp extends in the form of a semicircular body with narrow stalk. The cells of the endocarp are sclerenchymatous with thick walls and reduced lumen. The epidermal cells of the pericarp produce dilated, spherical bulbous bodies and regular intervals.[22]

Leaf Constants

Stomatal Number

The stomatal number was calculated from three different fields on upper and lower epidermis separately. The results were recorded in Table 6 and types of stomata were identified and shown in Figure 3(B) and 3(C).

Stomatal Index

The ratio of stomata and total epidermal cells was calculated as I=S/E+S, and the observations are given in Table 7.

Table 4: Macroscopical Characters of Flower.

Sl. No.	Evaluating features	Characteristics of Flower
1.	Size of Bracts	Length: 0.75 cm
		Breath: 0.1 cm
3.	Size of petals	Length: 0.6 cm
4.	Inflorescence	Capitulum with only disc florets which are bisexual.
5.	Pedicel	Sessile
6.	Taste	Bitter
7.	Colour	Bracts green, petals lilac.
8.	Odour	None

Table 5: Macroscopical Characters of Fruit.

Sl.No.	Evaluating features	Characteristics of Fruit
1.	Size	Length: 5.0 mm
		Breath: 1.2 mm
2.	Shape	Small, Truncate, Oblong and Cylindrical.
3.	Colour	Dark brown
4.	Odour	Characteristic
5.	Taste	Bitter
6.	Type	Cypsela (Achene)
7.	Nature	Pubescent
8.	Pappus	Copious and reddish; Inner is long and outer is short.
9.	Ridges/Furrows	Ribbed alternating

Table 6: Stomatal Number.

Field	Number of Stomata per sq. mm	
	Upper Surface	Lower surface
1.	8	25
2.	7	23
3.	7	19
Average	7.33	22.33

Table 7: Stomatal Index.

Field	Upper Epidermis		Lower Epidermis	
	Stomatal Number	Epidermal cell Number	Stomatal Number	Epidermal cell Number
1	8	47	25	10
2	7	54	23	13
3	7	52	19	18
Stomatal Index	12.57		62.03	

Table 8: Palisade Ratio.

Field	Number of Palisade cells beneath on epidermal cell per sq. mm		
	Number Epidermal cells	Number of Palisade cells	Palisade Ratio
1	6	21	3.5
2	6	18	3.0
3	6	23	3.83
Palisade Ratio (Average)	3.0 - 3.83		

Table 9: Trichome Density.

Field	Number of Trichomes beneath epidermal cell per sq. mm	
	Number Epidermal cells	Number of Trichome
1	71	1
2	75	2
3	73	1
Trichome Density (Average)	1.79	

Leaf Architecture: (Figure 3(D), 3(E), and 3(F))

Leaf organization is simple. With respect to leaf shape and size, the length of the whole leaf is 54 mm and the width is 18 mm. The lamina is symmetrical; base is symmetrical; form is ovate lanceolate; apex is acute and base is acute normal. The margin is serrate. The leaf texture is coriaceous. There are no glands and the petiole is normal. The type of venation is pinnate craspedodromous. Primary vein (1^0) is massive; its course is straight and unbranched. Secondary veins (2^0) are present; angle of divergence is acute wide. The variation in the angle of divergence is nearly uniform. The relative thickness of secondary veins is moderate; its course is curved and unbranched. Intersecondary veins are composite. Intramarginal vein is absent. Tertiary veins (30) are present; angle of origin exmedial to admedial side is AR/AO/AA. The pattern is orthogonal reticulate and the course is simple and forked. The higher order venation forming a reticulum in which vein orders are distinct. Quaternary veins (4^0) are thin; its course is reticulate. Quintenary veins (5^0) are thin; its course is reticulate. The highest vein order of leaf is 5^0. The marginal ultimate venation is looped. Areoles are well developed; arrangement is random, and shapes are triangular, quadrangular, pentagonal. Veinlets are simple, linear or once branched.

Palisade Ratio: The palisade ratio was calculated and the results were recorded in Table 8.

Table 10: Physicochemical Parameter Values for Whole and Seed.

Evaluative parameter	Whole Plant (% w/w)	Seed
Total Ash Value	17.73±0.625	6.408±0.251
Acid Insoluble Ash	1.233 ±0.251	0.416±0.028
Water Soluble Ash	9.33 ±0.305	1.183±0.230
Sulphated Ash	29.06±0.351	9.2±0.1
Alcohol-Soluble Extractive	12.08±0.4	33.033±0.602
Water-Soluble Extractive	22.±0.4	18.666 %±0.61
Moisture Content	11.4%±0.754	4.36%±0.378

Table 11: Fluorescence Test for Whole Plant and Seed.

Test	Whole Plant		Seed	
	Short U. V. Light (254 nm)	Long U. V. Light (365 nm)	Short U. V. Light (254 nm)	Long U. V. Light (365 nm)
I.	yG27	yO45	-	yG26
II.	G48	yF47	-	-
III.	G27	oF47	-	-
IV	g27	g27	-	-
V	yG17	gO36	-	yG25
VI	G26	y36	-	-
VII	G26	oF36	-	gY16
VIII	-	-	-	-
IX	yG36	-	-	-

Trichome density: The trichome density was calculated and the results were recorded in Table 9.

Determination of Physicochemical Character for whole plant and seed: The detection of Ash value, Extractive value and Moisture content was carried out and recorded in the Table 10 for whole plant and seed separately.

Fluorescence test for whole plant and seed: The analysis was carried and results were recorded in Table 11 and the key is provided for the same.

Powder Study of Whole Plant and Seed (Figures 4 and 5).

The powder shown in the Figure 4 shows presence of unicellular and multicellular trichomes, crystal fibres, fibres showing presence of fixed oils. Along with pollen grains, starch grains, oil globules, mesophyll cells, stomata, spiral pitting and simple fibres. The powder study of the seeds Shown in Figure 5 exhibits presence of cells from endosperm and embryo, fibres and xylem vessel showing spiral pitting.

Determination of Preliminary Phytochemical Screening of Whole Plant and Seed: The preliminary phytochemical screening was carried out using standard protocols and the results were noted down in Tables 12 and 13 for secondary metabolites and primary metabolites respectively.

Determination of HPTLC Fingerprinting (Figures 6, 7 and 8): For Alkaloids, whole plant and seed extract shows 61 and 29 different spectral peaks in 254 nm respectively and 62 and 23 different spectral peaks in 366 nm respectively. Spectral profiling is listed in Table 14. Diterpenoids show 49 and 22 different spectral peaks in 254 nm and 52 and 22 different spectral peaks in 366 nm were found for whole plant and seed extract respectively and are noted in Table 15.

DISCUSSION

Baccaroides anthelmintica L. was earlier also known as *Centratherum anthelminticum* Kuntze, *Vernonia anthelmintica* Wild; this too could lead to wrong identification and create confusion.

Many researchers have carried out pharmacognostical investigations restricted to the seed which is commercially important. Transverse section of seed has been described by

Table 12: Preliminary Phytochemical Screening for Secondary Metabolites of Whole Plant and Seed.

	Phytochemical Screening	Interpretation of *B. anthelminticum*					
No.	Phytochemical Test	Whole Plant			Seed		
		Hexane	Ethanol	Ethyl acetate	Hexane	Ethanol	Ethyl acetate
1	Lactones						
a.	Legal's Test	-	-	-	-	+	-
b.	Baljet Test	-	-	-	-	+	-
3	Coumarins						
a.	Fluroscence Test	+	+	+	-	+	+
4	Tannins						
a.	Lead Acetate	-	-	-	-	+	+
b.	Ferric Chloride	-	-	-	-	+	-
c.	Potassium Dichromate	+	+	+	-	+	-
5	Glucosides						
a.	Picric acid Test	-	-	-	-	-	-
6	Flavonoids						
a.	$FeCl_3$ Test	+	+	+	-	+	-
b.	Shinoda Test	-	-	-	-	+	-
7	Triterpenoid						
a.	Salkowski's Test	+	+	+	+	+	-
b.	Lieberman Buchard's Test	-	-	-	-	+	+
c.	Tschugajen's Test	-	-	-	-	+	-
8	Terpenoids						
a.	Thionyl Chloride Test	-	-	-	-	-	-
9	Steroids						
a.	Salkowski's Test	+	+	+	+	+	-
b.	Lieberman Buchard's Test	-	-	-	-	+	+
10	Diterpenes						
a.	Copper Acetate Test	+	+	+	-	+	+
11	Glycosides						
a.	NaOH Test	-	-	-	-	-	-
b.	Keller Kiliani's Test	-	-	-	-	-	-
12	Quinones						
a.	Conc. H_2SO_4 Test	+	+	+	+	+	-
13	Alkaloids						
a.	Dragondroff's test	+	+	+	-	-	-
b.	Wagner's Test	+	+	+	-	+	-
c.	Mayer's Test	-	-	-	-	+	-
14	Phenols						
a.	$FeCl_3$ Test	-	+	-	-	+	-
15	Saponins						
a.	Foam Test	+	+	-	-	-	-
16	Anthraquinones						
a.	Borntrager's Test	-	-	-	-	-	-

Table 13: Preliminary Phytochemical Screening for Primary Metabolites of Whole Plant and Seed.

	Phytochemical Screening	Interpretation of *B. anthelminticum*					
No.	Phytochemical Test	Whole Plant			Seed		
		Hexane	Ethanol	Ethyl acetate	Hexane	Ethanol	Ethyl acetae
1	Lipids						
a.	Soap Test	-	-	-	-	-	-
2	Reducing sugar						
3	Carbohydrate						
a.	Mollisch's Test	+	+	-	-	+	+
4	PROTIEN						
a.	Biuret Test	-	-	-	-	-	-
b.	Millon's Test	-	-	-	-	+	-
c.	Sulphosalicylic acid Test	-	+	-	-	+	-
d.	Ninhydrin Test	-	-	-	-	-	-

Table 14: R_f Values of Alkaloids in the Extract.

Sl. No.	R_f Max- B.a (WH)		R_f Max- B.a (Sd)		Sr. No.	R_f Max- B.a (WH)	
	254 nm	366 nm	254 nm	366 nm		254 nm	366 nm
1	0.01	0.02	0.02	0.04	32	0.52	0.51
2	0.03	0.03	0.04	0.07	33	0.54	0.52
3	0.06	0.05	0.07	0.11	34	0.56	0.54
4	0.08	0.06	0.11	0.13	35	0.57	0.56
5	0.09	0.08	0.13	0.16	36	0.59	0.57
6	0.11	0.09	0.17	0.17	37	0.6	0.59
7	0.12	0.11	0.22	0.21	38	0.62	0.6
8	0.14	0.12	0.25	0.25	39	0.63	0.62
9	0.16	0.14	0.29	0.29	40	0.65	0.63
10	0.17	0.16	0.3	0.31	41	0.66	0.65
11	0.19	0.17	0.35	0.35	42	0.68	0.66
12	0.22	0.2	0.37	0.39	43	0.69	0.68
13	0.23	0.22	0.39	0.43	44	0.71	0.69
14	0.25	0.23	0.42	0.5	45	0.72	0.71
15	0.26	0.25	0.5	0.66	46	0.74	0.72
16	0.28	0.26	0.55	0.71	47	0.76	0.74
17	0.29	0.28	0.59	0.74	48	0.77	0.76
18	0.31	0.29	0.67	0.78	49	0.79	0.77
19	0.32	0.31	0.71	0.8	50	0.8	0.79
20	0.34	0.32	0.74	0.82	51	0.82	0.8
21	0.36	0.34	0.78	0.87	52	0.83	0.82
22	0.37	0.36	0.8	0.88	53	0.85	0.83

Sl. No.	R_f Max- B.a (WH)		R_f Max- B.a (Sd)		Sr. No.	R_f Max- B.a (WH)	
	254 nm	366 nm	254 nm	366 nm		254 nm	366 nm
23	0.39	0.37	0.82	0.9	54	0.86	0.85
24	0.4	0.39	0.84	-	55	0.88	0.86
25	0.42	0.4	0.87	-	56	0.89	0.88
26	0.43	0.42	0.88	-	57	0.91	0.89
27	0.45	0.43	0.91	-	58	0.92	0.91
28	0.46	0.45	0.94	-	59	0.94	0.93
29	0.48	0.46	0.96	-	60	0.96	0.94
30	0.49	0.48	-	-	61	0.97	0.96
31	0.51	0.49	-	-	62		0.97

Table 15: R_f Values of Diterpenoids in the Extract.

Sl. No.	R_f Max- B.a (WH)		R_f Max- B.a (Sd)		Sr. No.	R_f Max- B.a (WH)		R_f Max- B.a (Sd)	
	254 nm	366 nm	254 nm	366 nm		254 nm	366 nm	254 nm	366 nm
1	0.03	0.03	0.02	0.04	27	0.63	0.59	-	-
2	0.13	0.06	0.04	0.1	28	0.65	0.6	-	-
3	0.17	0.1	0.1	0.23	29	0.66	0.62	-	-
4	0.28	0.16	0.23	0.25	30	0.68	0.63	-	-
5	0.29	0.26	0.25	0.39	31	0.69	0.65	-	-
6	0.31	0.28	0.39	0.43	32	0.71	0.66	-	-
7	0.32	0.29	0.42	0.44	33	0.72	0.68	-	-
8	0.34	0.31	0.44	0.5	34	0.74	0.69	-	-
9	0.36	0.32	0.5	0.55	35	0.76	0.71	-	-
10	0.37	0.34	0.55	0.64	36	0.77	0.72	-	-
11	0.39	0.36	0.59	0.67	37	0.79	0.74	-	-
12	0.4	0.37	0.67	0.71	38	0.8	0.76	-	-
13	0.42	0.39	0.71	0.74	39	0.82	0.77	-	-
14	0.43	0.4	0.74	0.78	40	0.83	0.79	-	-
15	0.45	0.42	0.8	0.8	41	0.85	0.8	-	-
16	0.46	0.43	0.83	0.82	42	0.86	0.82	-	-
17	0.48	0.45	0.84	0.85	43	0.88	0.83	-	-
18	0.49	0.46	0.87	0.87	44	0.89	0.85	-	-
19	0.51	0.48	0.88	0.88	45	0.91	0.86	-	-
20	0.52	0.49	0.91	0.9	46	0.93	0.88	-	-
21	0.54	0.51	0.94	0.94	47	0.94	0.9	-	-
22	0.56	0.52	0.96	0.96	48	0.96	0.91	-	-
23	0.57	0.54	-	-	49	0.97	0.93	-	-
24	0.59	0.54	-	-	50	-	0.94	-	-
25	0.6	0.56	-	-	51	-	0.96	-	-
26	0.62	0.57	-	-	52	-	0.97	-	-

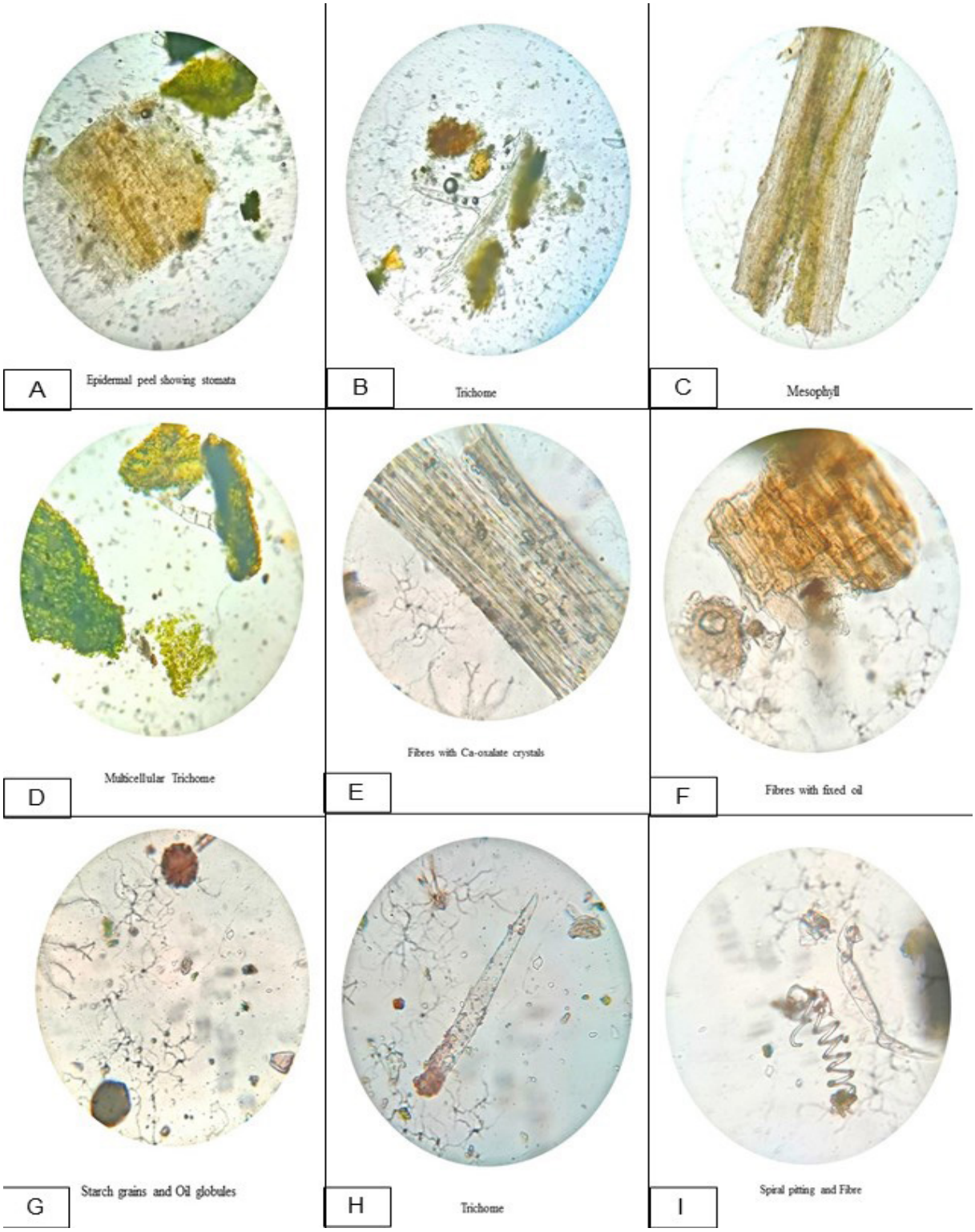

Figure 4: Powder Study Images of Whole Plant; A: Epidermal peel showing stomata, B: Trichome, C: Mesophyll, D: Multicellular trichome, E: Fibres with Ca Oxalate crystals, F Fibers with fixed oils, G: Starch grain and oil globules, H: Single unicellular trichome, I: Spiral pitting and fibre.

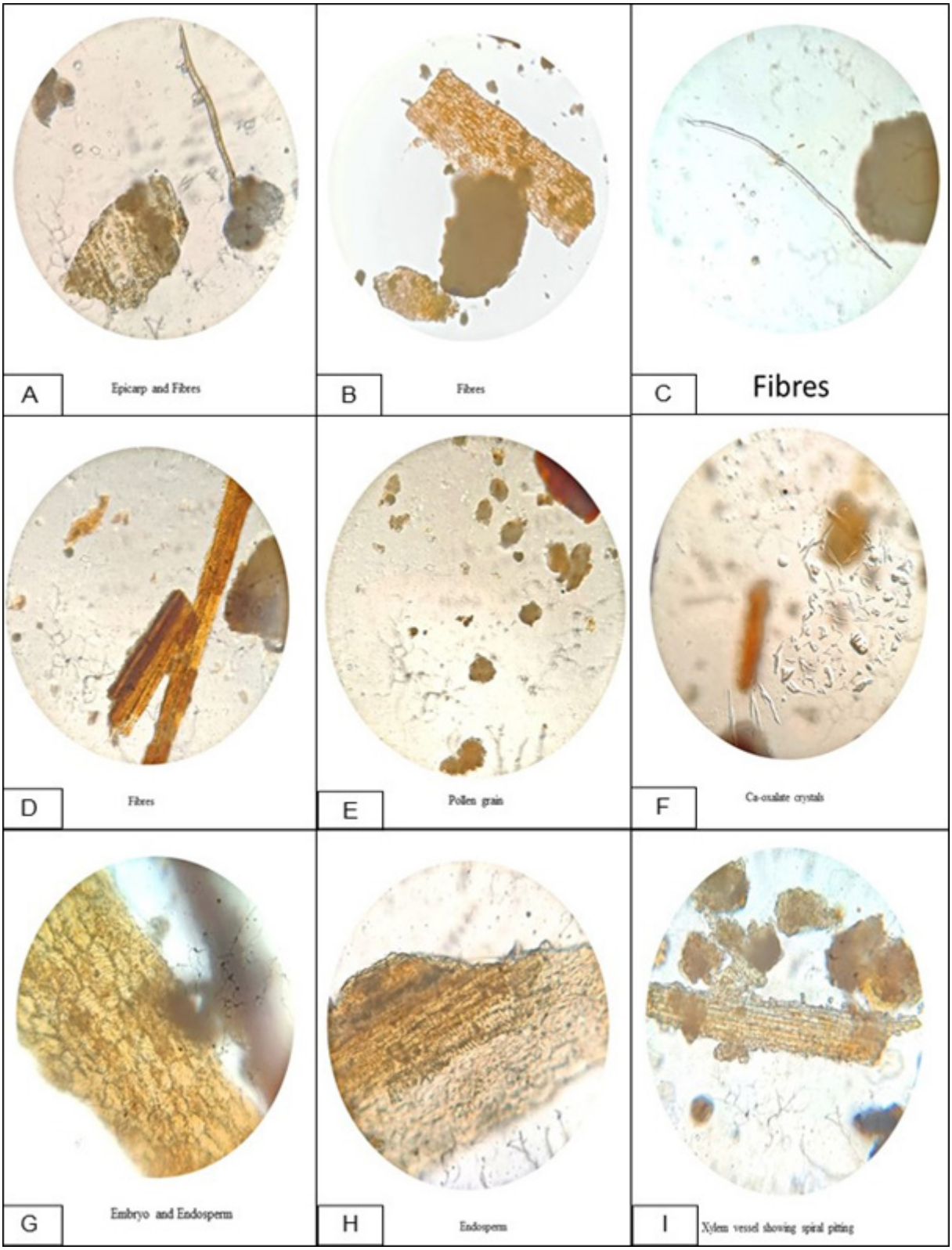

Figure 5: Powder Study Images of Seed; A: Epicarp and fibres, B: Fibres with oil deposition, C: Single fibre, D: Fibres, E: Pollen grain, F: Ca Oxalate crystals, G: Embryo and Endosperm, H: Endosperm, I: Xylem vessel showing spiral pitting.

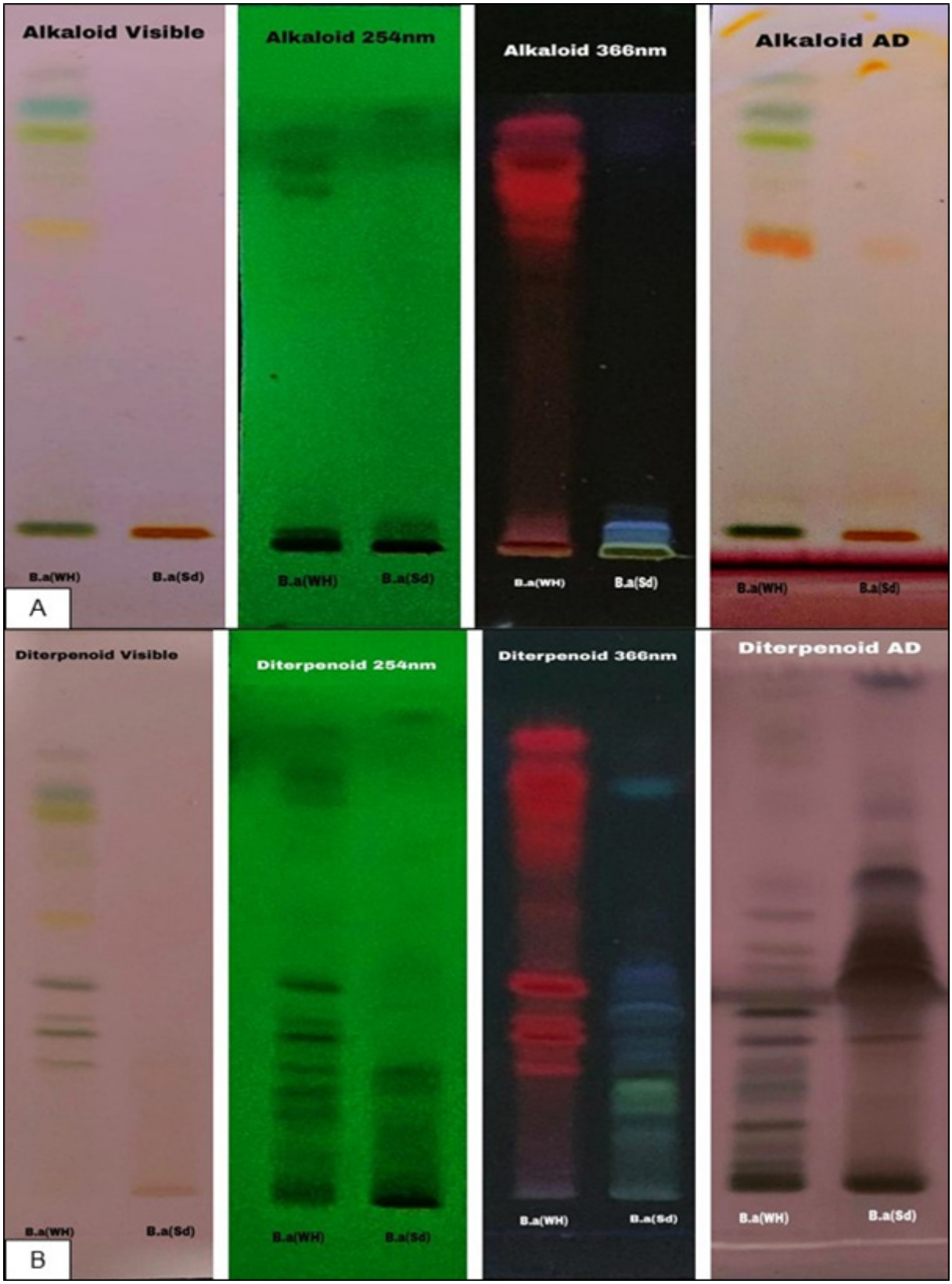

Figure 6: A: HPTLC Plate for detection of Alkaloids at Visible light, 254 nm, 366 nm, After Derivatization, B: HPTLC Plate for detection of Diterpenoids at Visible light, 254 nm, 366 nm, After Derivatization.

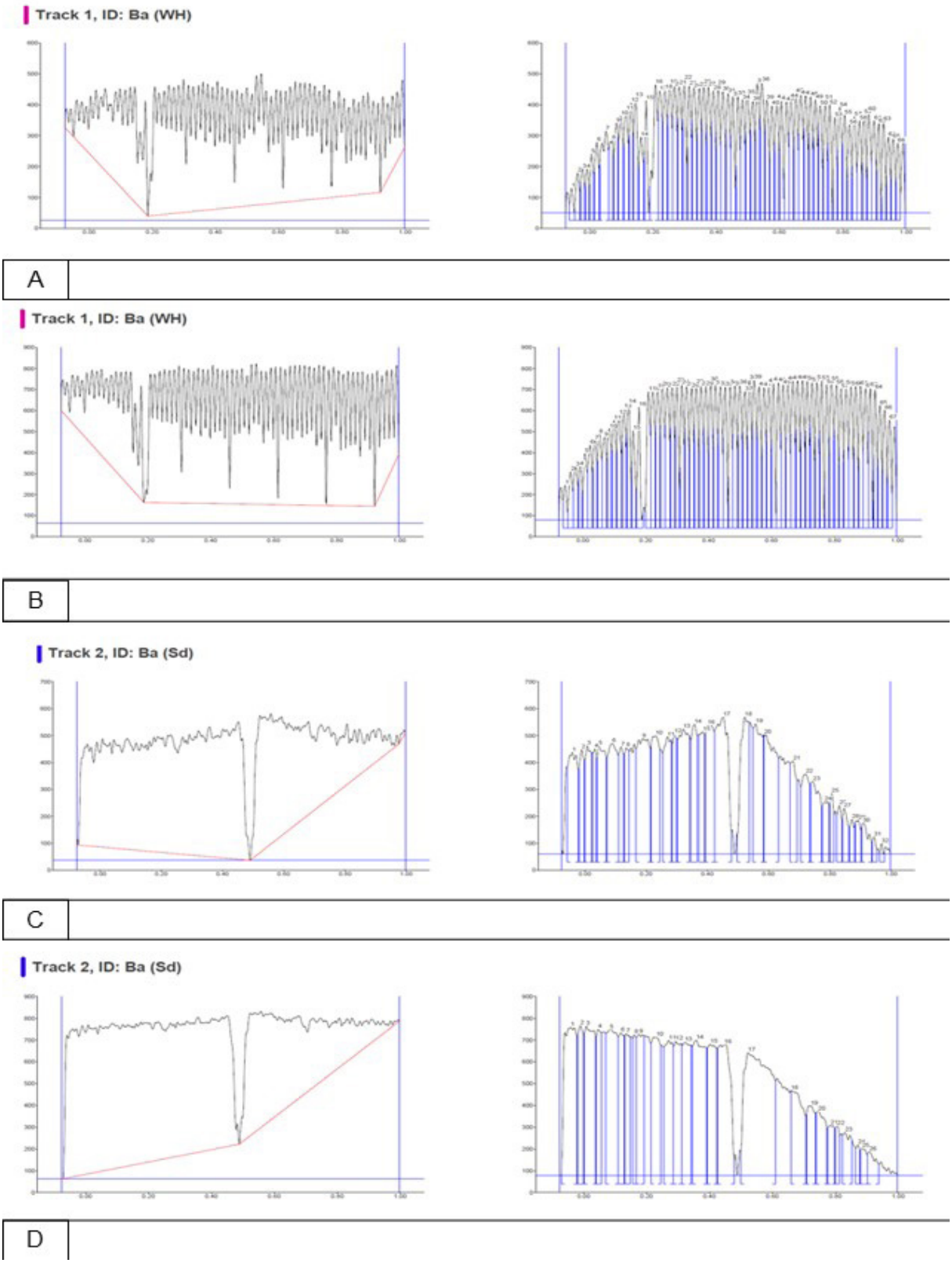

Figure 7: A: HPTLC Profiling of Alkaloids of Whole plant at 254 nm, B: HPTLC Profiling of Alkaloids of Whole plant at 366 nm, C: HPTLC Profiling of Alkaloids of Seed at 254 nm, D: HPTLC Profiling of Alkaloids of Seed at 366 nm.

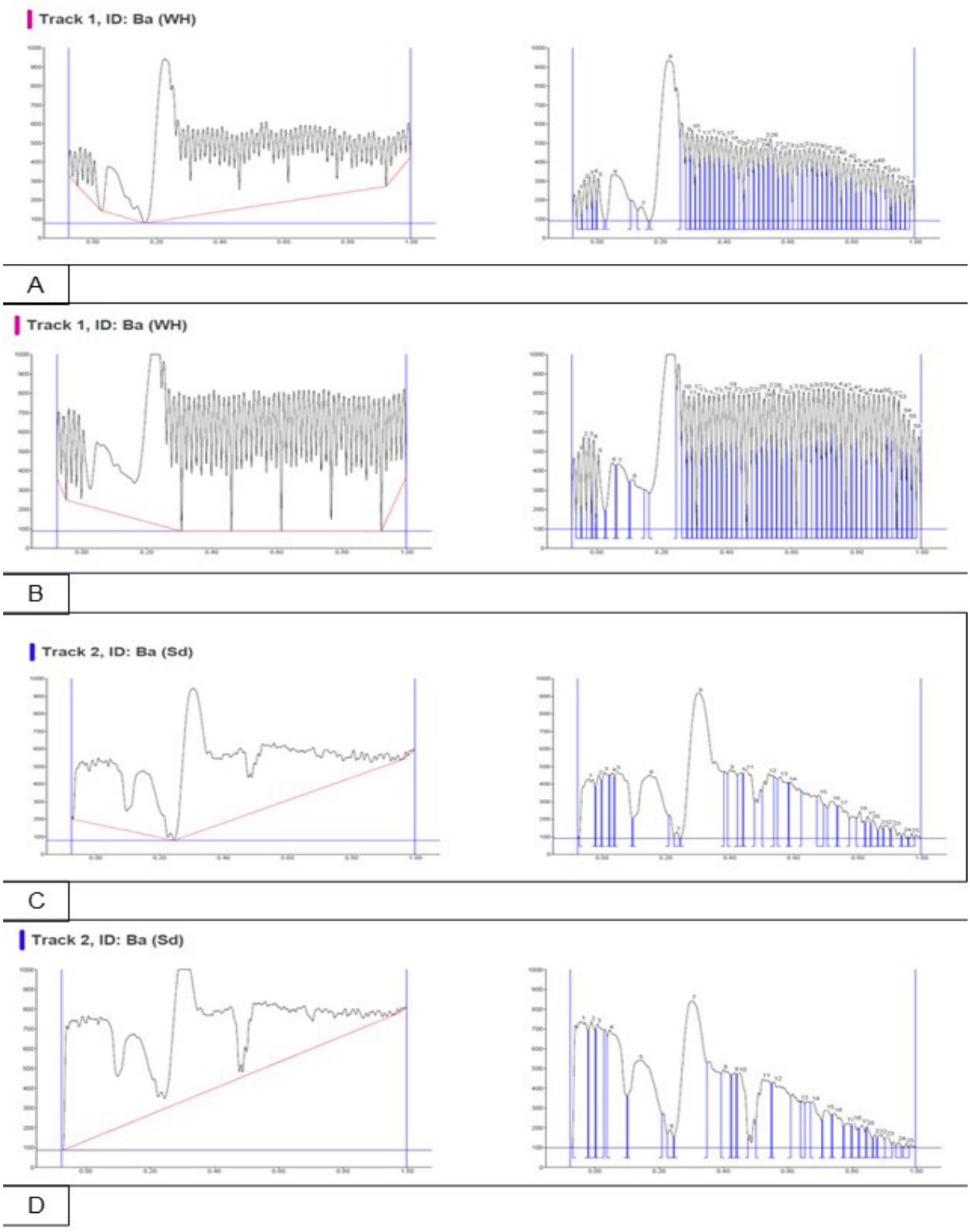

Figure 8: A: HPTLC Profiling of Diterpenoids of Whole plant at 254 nm, B: HPTLC Profiling of Diterpenoids of Whole plant at 366 nm, C: HPTLC Profiling of Diterpenoids of Seed at 254 nm, D: HPTLC Profiling of Diterpenoids of Seed at 366 nm.

Senniappan P., *et al*., 2016 and Bhatia D. *et al*., 2008. Current studies show the presence of idioblasts reported in this study. Microscopic studies on other plant parts have been carried out for the first time. Proximate analysis in the form of ash values, extractive value, moisture content, etc. was also carried out but the current study shows that the extractive value for water and ethanolic extracts is almost double than the previous studies. Sulphated ash value was performed for the first time on this material and very little difference was observed in the results of ash value while comparing with other papers. Fluorescence analysis was also carried out for the first time.

Preliminary phytochemical screening showed the presence of saponins, phenols, alkaloids, quinones, diterpenes, triterpenes, steroids, flavonoids, tannins and coumarins in the present study. Whereas the previous studies did not detect the presence of alkaloids, tannins, coumarins and quinones. HPTLC Fingerprinting was also carried out by Bhatia D. *et al*., 2008; but not specific phytochemical was detected. In the present study Alkaloids and Diterpenes were detected using HPTLC technique.[23]

CONCLUSION

The exploration of *B. anthelmintica* L. has brought forth an abundance of phytopharmacological insights. In the context of medicinal herbs, this research emerges as a pivotal milestone, contributing essential information for the accurate identification, standardization, and procurement of this botanical resource. It addresses a pressing issue within the field, namely the pervasive problems of adulteration and misuse that have been aggravated by the lack of comprehensive data on this plant. The fruit of the plant has been extensively studied and recognized for its therapeutic properties. However, there has been a notable absence of comprehensive research on the entire plant, despite its known medicinal value. This study aimed to bridge this gap by providing detailed data on the whole plant and conducting a comparative analysis between the fruit and whole plant.

Pharmacopoeial standards were established for the entire plant for the first time, facilitating a comparative assessment with the fruit. The comparison aimed to determine if the whole plant could be as effective as its fruit, thereby enhancing the utilization of the entire plant for medicinal purposes. This approach aligns with sustainable practices by reducing waste and expanding the sources of important phytochemicals. The findings of this study contribute significantly to our understanding of the plant's therapeutic potential and highlight the importance of considering the whole plant in medicinal applications. By demonstrating the efficacy of the whole plant, this research opens up new avenues for sustainable utilization and drug development, ultimately benefiting both the pharmaceutical industry and herbal medicine practices. This in turn serves as a foundational pillar upon which the future of medicinal herb utilization can be built. With a firm understanding of *Baccharoides anthelmintica* L., researchers can now make more informed decisions about its collection, processing, and application in traditional and modern medicine. This newfound knowledge offers the potential to reduce the risks associated with adulterated or misused herbal products and enhance the overall safety and efficacy of medicinal treatments.

In essence, this research empowers the field of herbal medicine with a more robust and scientific basis. Ultimately benefiting the health and well-being of individuals who rely on herbal remedies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This study marks the first comprehensive analysis of *Baccharoides anthelmintica* L. using the whole plant material, while also providing new insights into its seeds. Known as Iron weed or Kali Jeeri, this traditional medicinal plant is used to treat ailments such as asthma, sores, inflammation and may more. Despite its extensive therapeutic applications, detailed anatomical and chemical analysis has been limited until now. This research addresses this gap by examining macroscopic, microscopic, physicochemical, and HPTLC fingerprinting aspects to authenticate and prevent the adulteration of *B. anthelmintica* L. in both whole plant and seed forms. The study aims to thoroughly analyse *Baccharoides anthelmintica* L., focusing on pharmacognostical, physicochemical, and phytochemical parameters, and employing HPTLC to identify key alkaloids and diterpenes contributing to its medicinal properties.

Whole plants and seeds material were collected for macroscopic and microscopic documentation, physicochemical parameter determination, preliminary phytochemical screening, and HPTLC analysis. Results revealed distinctive macroscopic and microscopic features of the root, stem, leaves, flowers, and fruits, aiding in accurate identification and differentiation from adulterants. Physicochemical analyses provided specific moisture content, ash, and extractive values. Preliminary phytochemical screening confirmed the presence of alkaloids and diterpenes, supporting the plant's traditional uses. HPTLC fingerprinting validated the presence of these compounds, with the whole plant

extract showing 61 and 62 spectral peaks for alkaloids at 254 nm and 366 nm, and 49 and 52 peaks for diterpenes. The seed extract showed 29 and 23 spectral peaks for alkaloids and 22 peaks for diterpenes at both wavelengths.

This study delivers essential data for the authentication, quality assessment, and utilization of *B. anthelmintica* L. in traditional medicine and the pharmaceutical sector. It underscores the medicinal potential of the whole plant, supporting sustainable healthcare practices by reducing waste and maximizing phytochemical sources. The comprehensive data enhances the understanding of *B. anthelmintica* L., contributing to its safe and effective application in healthcare. This research lays a foundation for future exploration and standardization of this valuable medicinal plant, benefiting both the pharmaceutical industry and herbal medicine practices.

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