PHARMACOGNOSTIC STUDIES ON LEAVES OF *GYMNANTHEMUM AMYGDALINUM* WITH SPECIAL REFERENCE TO A NEW ADDITION TO THE FLORA OF SOUTH INDIA

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ABSTRACT

There has been a tremendous increase in the demand for plant-based drugs over the last several years. Due to this, there is a need for standardization of the plant raw materials. Although modern techniques are being widely used for the standardization process, the pharmacognostic approach is still reliable for identifying the raw material. The current study deals with a detailed investigation of macro-and micromorphological characters, powder characteristics, organoleptic characters, HPTLC fingerprinting and preliminary phytochemical analysis on the leaves of *Gymnanthemum amygdalinum*. It belongs to the family Compositae. Commonly known as a bitter leaf, in Telugu "sugarchettu" it is found throughout Bihar, Madhya Pradesh, Odisha, West Bengal and has been recently reported in Sriramagiri Village, Mahabubabad District, Telangana, South India. The leaves are used ethnomedicinally and in folk-lore medicine, as antidiabetic and in treatment of gastritis and hepatitis. The present work can serve as a useful tool in identifying, authentication and standardization of the plant material and quality control in the pharmaceutical industry.

Keywords: *Gymnanthemum amygdalinum*, compositae, pharmacognosy, uniseriate filiform peltate hair

INTRODUCTION

Pharmacognosy is the study of crude drugs, obtained from plants, animals and mineral kingdom and their constituents; history, distribution, cultivation, collection, identification, selection, evaluation, preservation, use of drugs, production and commerce[1]. The present study reports a detailed account of the pharmacognostic investigation carried out on *G. amygdalinum* (Delile) Sch. Bip. ex Walp. This would be of immense value and diagnostic importance in the botanical identification and standardization of the drug in its crude form and may help us in distinguishing it from substitutes and preventing its adulteration.

G. amygdalinum, commonly known as bitter leaf, bitter-tea vernonia, in Telugu "sugarchettu", belongs to the family Compositae. Large shrubs or small trees to 3-5 m high with branches terete, densely glandular-pubescent are its features. In folk medicine, the aerial organs of *G. amygdalinum* are mainly used as antipyretic, laxative, anti-malarial, and anthelmintic[4-5,6-7], antioxidant[8-9], antimicrobial[10-12], antidiabetic[13-14], in gastritis, hepatitis, stomachache as diarrhea tea (decoction), tea (infusion)[15] and in gastrointestinal disorders[16].

Folk-lore uses in the present investigation (Diabetes): Fresh leaves are taken, then ground pasted to make small tablets of groundnut size and taken orally twice daily for 45 days and two spoons of leaf powder in one glass of milk are orally taken for the treatment of diabetes.

MATERIALS AND METHODS

Plant collection and preparation

G. amygdalinum was collected in the flowering and fruiting stage from Sriramagiri forest, Nellikudur Mandal, Mahabubabad district, Telangana, India. The collected material was poisoned and mounted on herbarium sheets, taxonomically identified by the Botanical Survey of India (BSI), Deccan Regional Center Hyderabad and deposited in Herbarium, Botany Department, Osmania University, Hyderabad Telangana (HY). (Voucher specimen No: OUID-002971).

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The leaves were boiled, fixed in F.A.A. (formaldehyde -acetic acid -alcohol), dehydrated through xylene -alcohol series embedded in paraffin wax. The sections were cut at 10-12 µm on Optica 1090A rotary microtome, stained with crystal violet and basic fuchsin combination, and mounted in canada balsam[17]. Epidermal peels were obtained by gentle scraping and peeling by razor blade, were stained with saffranine, and mounted in glycerine. The powder microscopy characters were studied by boiling the drug in distilled water, stained in saffranine, and mounted with glycerine. The photomicrography was done on Olympus BX-53 trinocular microscope attached to a digital Sony camera.

Stomatal index

The stomatal index was calculated by standard procedure[18-19].

Hot continuous successive extraction

Hot continuous successive extraction was carried out using the Soxhlet apparatus for the plant samples. An earlier study on phytochemical extractions suggested that the Soxhlet extraction process provided standard results. 20 g fine leaf powder in 200 mL of solvent was taken. Extraction was carried by increasing the polarity of the solvents, i.e., from petroleum ether to methanol. The leaf powder was extracted with petroleum ether, chloroform, ethyl acetate, acetone and methanol. Extraction temperatures were adjusted to boiling points of solvent to allow a faster rate of recycling of fresh solvent. Eight hours were allocated to each solvent for hot continuous and successive extraction or until the solvent in the extractor's siphon tube became colorless[20-21].

Preliminary phytochemical screening

Phytochemical tests were carried out adopting standard procedures[22-26]. Tests were carried out for alkaloids, carbohydrates, proteins, amino acids, flavonoids, saponins, steroids and terpenoids, phenols, tannins, glycosides, coumarins, anthraquinones, quinones, resins, gums and leuco-anthocyanins.

HPTLC analysis

The concentrated methanolic extract was spotted in the form of bands of length 5 mm with a 25 µL syringe on pre-coated silica gel aluminum plate 60 F254 (5 x 10 cm with 0.25 mm thickness; Merck, Darmstadt, Germany) and the plates were washed with methanol before use. The TLC development chamber was saturated with mobile phase using filter paper. The sample and standard solutions were applied as bands of 6 mm wide and 10 mm apart using an Automatic TLC Sampler 4 applicator (CAMAG, Muttenz, Switzerland, supplied by Anchrom Technologists, Mumbai) fitted with a 25 µL Hamilton syringe supplied with nitrogen flow. A constant application rate of 15 µL sec^{-1} was employed. The space between the bands was fixed as 20 mm sec^{-1}. The slit dimension was kept at 4 × 0.20 mm, and a scanning speed was 20 mm sec^{-1} was employed. The mobile phase consisting of a saturated mixture of chloroform: methanol: butanol (7: 2:1 V/V/V) was found to be suitable for separation of the phytoconstituents in the species studied, and chromatography was performed using 10 mL of mobile phase in a 10 × 10 cm twin-trough glass chamber (CAMAG, Muttenz, Switzerland) with linear ascending development[27]. The optimized chamber saturation time for the mobile phase was 20 min at room temperature with a chromatographic run the length of 8.5 cm. After development, the TLC plates were dried in a current of air with the help of a hot air dryer in a wooden chamber with adequate ventilation. Densitometric scanning was performed with a CAMAG TLC Scanner III in the absorbance–reflectance mode at 254 nm and 366 nm with a slit dimension of 4 × 0.20 mm and a scanning speed 20 mm/sec was employed. All the instruments were operated by winCATS software (v. 1.4.3 CAMAG) resident in the system. The source of radiation utilized was a deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm, and the concentrations of the compounds chromatographed were determined from the intensity of diffusely reflected light. Further, for digital documentation, the DigiStore 2 documentation system (CAMAG) consisting of the illuminator, Reprostar 3 and digital camera power shot G2 (Canon, Tokyo, Japan) were used.

OBSERVATION AND RESULTS

Macroscopic characters

Leaves of *G. amygdalinum* are alternate, elliptic, elliptic-lanceolate, ovate, obovate, highly variable, cuneately attenuate at base, acuminate, mucronulate, serrate, adaxial surface dark green when dry, sparsely puberulent; abaxial surface pale green, glandular, densely puberulent, and hairy along veins; lateral veins 5-15 pairs, slightly curved; petioles up to 4.5 cm long, densely puberulent (Figs. 1A-1F, 2A, 3).

Microscopic characters

Microscopical evaluation is indispensable in the initial identification of herbs, in identifying small fragments of crude or powdered herbs, and in the detection of adulterants (e.g., insects, animal feces, mold, fungi, etc.)

Fig. 1A-F : *G. amygdalinum* A- Habit, B- Branch, C-D- Flowering twigs, E- F- Inflorescence

as well as identifying the plant by characteristic tissue features.

Leaf: In surface view, epidermal cells are 5-6 sided, polygonal isodiametric to diametric, sides thin, mostly straight to curved,rarely curved to wavy, surface smooth, contents slightly dense on adaxial side. The abaxial surface studies are similar to the adaxial surface studies except in epidermal cells sides curved to wavy and wavy to sinuate, sinuses deep "U" shaped. Costal cells are 6-8 sided, polygonal anisodiametric to linear, sides thin, straight to curved, surface smooth, contents dense, oriented in parallel and present on primary, secondary veins. Stomata occur on either side, anomocytic, subsidiaries 4-5, monocytic, indistinct, mostly of f-type; guard cells reniform to linear, surface striated. Trichomes are of two types, namely i) Uniseriate filiform peltate hair, ii) Uniseriate macro form cylindrical hair is present on both surfaces. Epidermal cell frequency 1046 per sq.mm., stomatal frequency 146 per. sq. mm and stomatal index 12.2 (adaxial surface) and E.C.F 1170 per sq.mm., S.F. 466 per sq.mm. S.I. 22.2., (abaxial surface) (Figs. 4A, 4B, 4C, 4D, 4E, 4F & Figs. 5A, 5B, 5C, 5D, 5E).

In the transverse section, the leaf is ribbed on either side but prominently ribbed on the abaxial at midvein 621-915 (735) µm and lamina wings are flat, 85-200 (140) µm in thickness. The epidermis is single-layered, and composed mostly of barrel-shaped, few oval-shaped

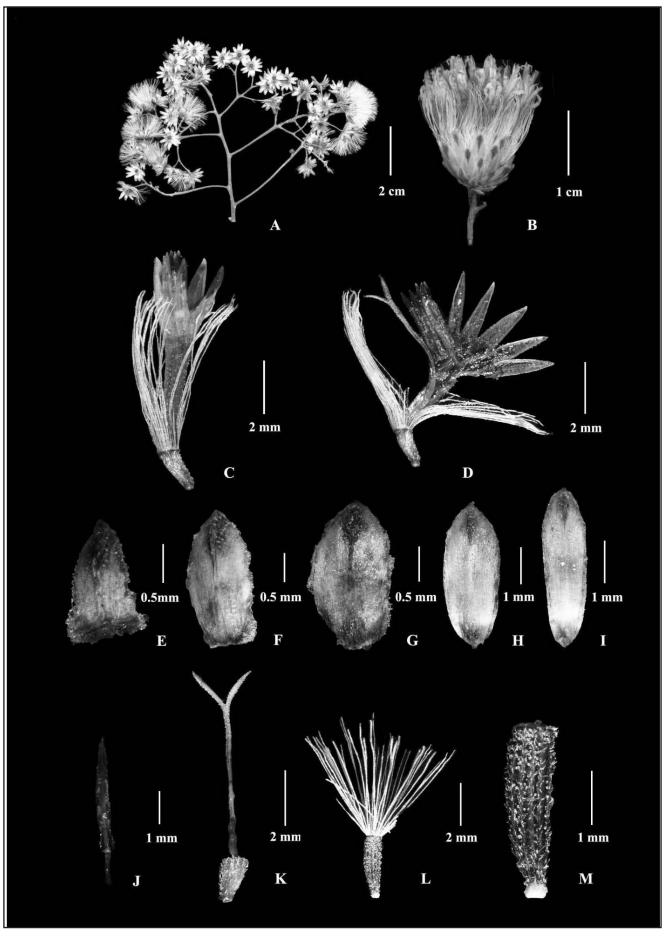


Fig. 2A-M: *G. amygdalinum*, A- Inflorescence, B- Head, C- D- Floret, E-I- Phyllaries, J- Stamen, K- Gynoecium, L- Cypsela, M- Achene

elongated, lamina adaxially larger, elongated cells 15-28 (22) µm long, 12-17 (15) µm in width, isodiametric cells 10-18 (14) µm in diameter on lamina adaxial. Abaxial epidermal cells are smaller 15-25 (20) µm long and 10-15 (12) µm wide and 7-15(11), cells over midvein adaxial mostly barrel-shaped 10-18 (15) µm long, 7-14 (10) µm in width, isodiametric cells 8-17 (11), abaxially barrel-shaped, few oval shaped 12-22 (16) µm long, 7-15(12) µm in width, isodiametric cells 10-18 (13), achlorophyllous, contents scanty. Cuticle slightly thick over the surface.

Stomata: Amphistomatic, flushed with epidermal cells. The epidermis is interrupted by trichome bases at some places (Fig. 6A, 6B). The mesophyll is heterogenous, differentiated into palisade and spongy tissues.

Palisade: one-layered, throughout, extending into midvein cells columnar, perpendicular to epidermis 35-63 (45) µm in long, 5-12 (9) wide, cells loosely arranged with intercellular spaces with chloroplasts; interspersed

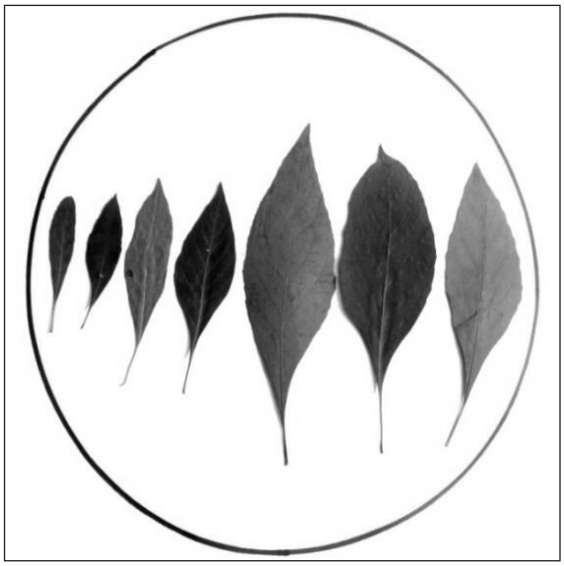


Fig. 3: Leaf macroscopy

with sphaero crystalliferous idioblasts, 18-68 (32) µm in diameter.

Spongy tissue: 4-5 layered, cells circular, loosely arranged with large intercellular spaces, contents dense with chloroplasts 7-25 (16) µm in diameter; often interspersed with sphaero crystalliferous idioblasts, about 18-68 (32) µm in diameter (Fig. 7A, 7B).

Ground tissue is differentiated into collenchyma, palisade and parenchyma tissues.

Collenchyma: As a group of cells in the adaxial ridge and 1-2 layered abaxially, cells are polygonal, oval to circular, lamellar, about 5-20 (13) µm in diameter on adaxial and 8-24 (18) µm in diameter on abaxial side, contents scanty. **Palisade**: one-layered beneath the adaxial collenchyma. **Parenchyma:** is beneath the palisade 5-6 celled on adaxial, 4-5 layered on abaxial, cells mostly polygonal oval to circular, about 15-50 (36) µm in diameter on adaxial and 12-45 (34) µm in diameter on abaxial, walls thin with small intercellular spaces, contents scanty, interspersed with sphaero crystalliferous idioblasts, 10-28 (18) µm in diameter (Figs. 6A, 6B).

Vascular tissue of midvein consists of two large and one small arc-shaped vascular bundles arranged at the center. Large vascular bundle about 198-320 (242) µm in diameter and smaller 115-210 (168) µm in diameter. Vascular bundles collateral, conjoint, endarch, xylem consists of tracheary elements 50-60 in number, arranged in radial rows, cells polygonal, oval to circular 10-37 (22) µm in diameter. Secondary wall thickenings of tracheary elements in L.S. mostly helical and few annular, xylem parenchyma in between tracheary elements. Phloem consists of phloem parenchyma, companion cells, sieve tubes/cells and fibers.

In the transverse section, the petiole is broadly ovate, 1144-2158 (1782) µm in diameter. The epidermis is one layered cell oval to barrel-shaped, 12-27 (20) µm tangentially long and radially 7-15 (11) µm in wide. Isodiametric cells 7-20 (12) µm in diameter, walls thin, few cells with dense dark contents, cuticle slightly thick over the surface (Fig. 6C).

Ground tissue is heterogenous, consisting of collenchyma and parenchyma tissues. **Collenchyma**: Hypodermal 2-4 layered, throughout, cells polygonal, oval to circular, lamellar 12-58 (25) µm in diameter, intercellular spaces absent. **Parenchyma**: The rest of the ground tissue is parenchymatous, between collenchyma and the vascular bundles; cells polygonal, oval to circular, 18-125 (68) µm in diameter, walls thin, intercellular spaces narrow, contents dense with chloroplasts.Vascular bundles 5, arranged in an arc, oval to spherical largest vascular bundle 180-345 (205) µm in diameter and smallest 60-140 (96) µm in diameter, endarch, conjoint, collateral. Xylem consists of tracheary elements arranged in radial rows, polygonal, oval to circular, 10-37 (22) µm in diameter, walls thick, secondary wall thickenings of tracheary elements in L.S. mostly helical and few annular, helices both double and single; xylem parenchyma in between tracheary elements, contents slightly dense. Phloem consists of phloem parenchyma, companion cells, sieve tubes/cells, and fibers. Phloem parenchyma cells are compactly arranged without intercellular spaces, thinwalls, contents slightly dense (Fig. 6C).

ORGANOLEPTIC CHARACTERS

Colour- Green, Odour-Pungent, Taste- Very bitter, Touch-Smooth.

POWDER MICROSCOPY

The powdered crude drug analysis was aimed to study and also to assess the quality of herbal drugs for therapeutic value which are generally studied by classical pharmacognostical studies. The authenticity of the herbal drug was confirmed by a comparison of its powder characteristics. Fragments of the epidermis are with straight to curved sides; epidermis showing anomocytic stomata; uniseriate macro form cylindrical hair; costal cells with polygonal to linear cells in parallel rows;sphaero-crystaliferous idioblasts; uniseriate filiform peltate hairs;

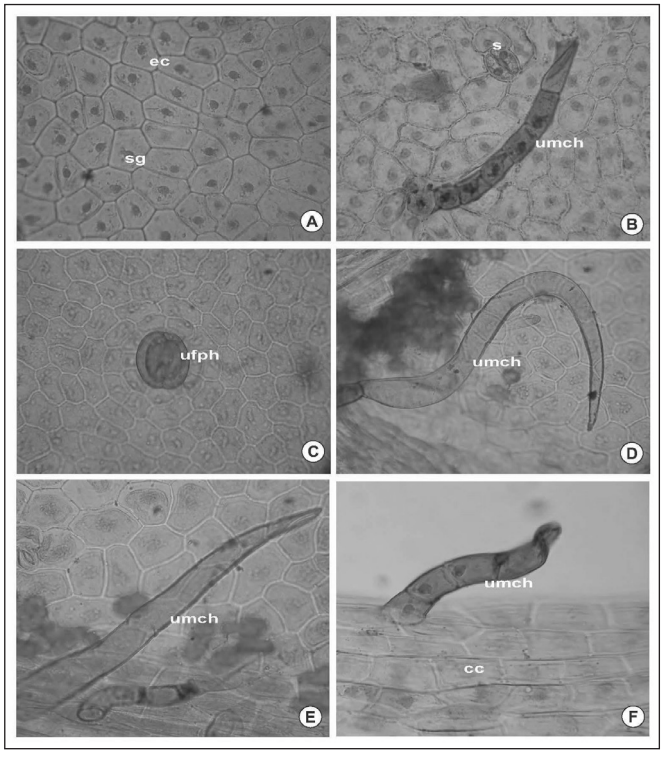


Fig. 4A-F: Leaf adaxial surface epidermis: 4A- with starch grains X 180; 4B- with stomata X 195; 4C- with ufph X 206; 4D- with umch X 312; 4E- with umch X 377; 4F- costal cell with umch X 288

Abbrevations: ec-epidermal cells; s-stomata; cc- costal cells; umch-Uniseriate macroform conical hair; ufph-Uniseriate filiform peltate hair

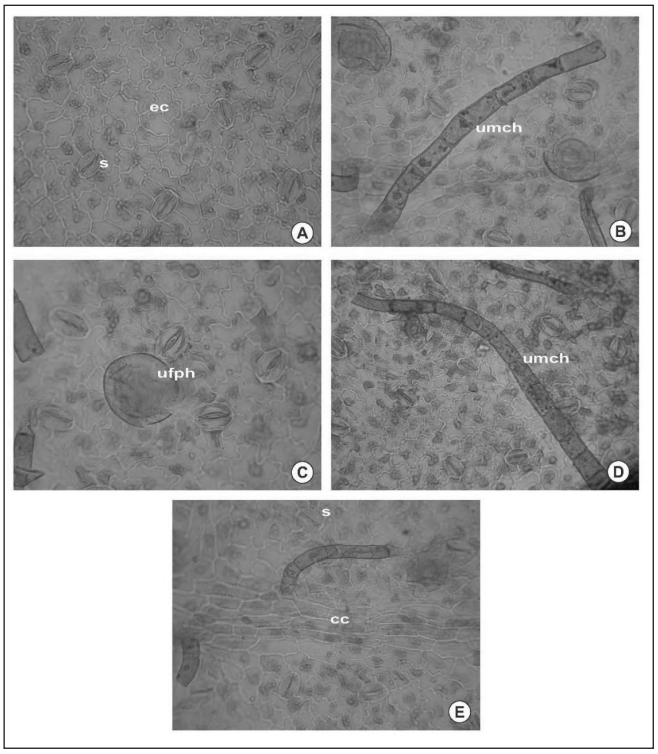


Fig. 5A-E: Leaf abaxial surface epidermis: 5A- with stomata X 84; 5B- with umch X 198; 5C- with ufph X 233; 5D- with umch X 255, 5E- with costal cells X 164

Abbrevations: ec-epidermal cells; s-stomata; cc- costal cells; umch-Uniseriate macroform conical hair; ufph-Uniseriate filiform peltate hair

tracheary elements showing helical thickenings; tracheary elements with annular thickenings (Fig.7A, 7B, 7C, 7D, 7E, 7F, 7G, 7H).

PRELIMINARY PHYTOCHEMICAL STUDIES

The phytochemical screening revealed the presence of alkaloids in chloroform and ethyl acetate extracts, whereas carbohydrates and tannins were present in ethyl acetate and methanol extracts. Proteins, phenols, amino acids, anthraquinones, quinones, and leuco-anthocyanins were absent in all extracts, flavonoids were identified in ethyl acetate, acetone and methanol extracts, while saponins, coumarins and glycosides were found in acetone and methanol extracts. Steroids and terpenoids were obtained in all extracts, except petroleum ether extracts,resins were found in all extracts but except in chloroform extract and gums in chloroform extract only (Table I). The percentage (%) yield of petroleum ether, chloroform, ethyl acetate, acetone and methanol crude extracts were 10.5 %, 12.8 %, 31.5 % 29.4 % and 52.6, % respectively (Fig. 8).

Profile of HPTLC fingerprinting of *G. amygdalinum*

The profile of chromatographic separation of leaf methanol extract scanned at 254 nm reveals twelve spots (Fig. 9), out of which spot 12 possesses maximum composition with R_f at 0.90. The densitogram scanned at 366 nm revealed nine spots R_f 0.91 with spot 9 showing maximum composition (Fig. 10). It is evident from the data that these are characteristics of the studied drug, which will help in the identification and authentication of the drug. These can be considered as valuable standards in the pharmacopeia. At 254 nm, twelve spots appear at R_f 0.18, 0.21, 0.37, 0.39, 0.48, 0.52, 0.53, 0.56, 0.68, 0.69,0.81 and 0.90 (all brown) (Fig. 9) with various concentrations while at 366 nm, nine spots appear at R_f 0.15 (yellow), 0.36 (yellow), 0.38 (blue), 0.40 (blue), 0.49 (blue), 0.53 (blue), 0.57 (blue), 0.68 (blue), 0.81 (yellow) and 0.91 (red) (Fig. 10). This is a vital fingerprint parameter to ensure the reliability and reproducibility of the drug.

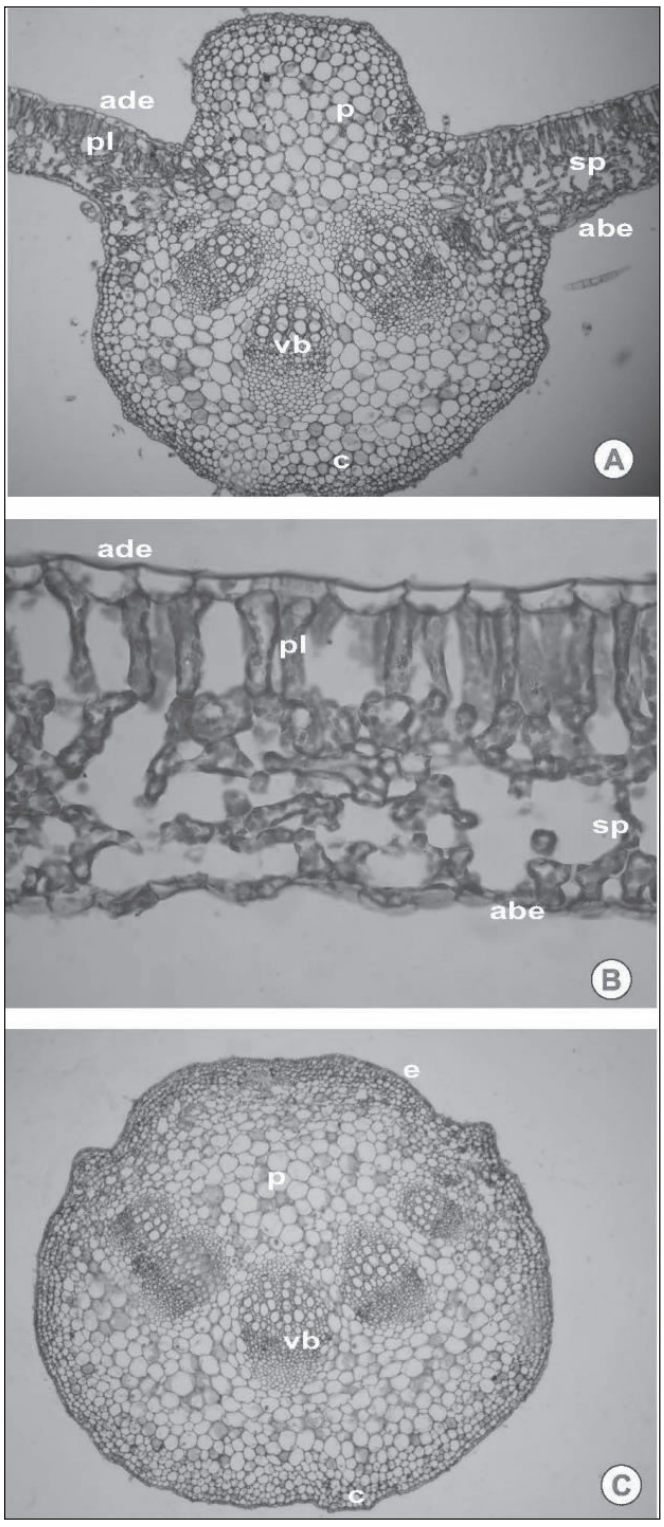


Fig. 6A-C: T.S. of 6A - leaf midvein X 238; 6B - leaf lamina X 240; 6C - petiole X 201

Abbrevations: e-epidermis; ade- adaxial epidermis; abe- abaxial epidermis; c- collenchyma; p-parenchyma; vb-vascular bundle; pl-palisade; sp-spongy tissue

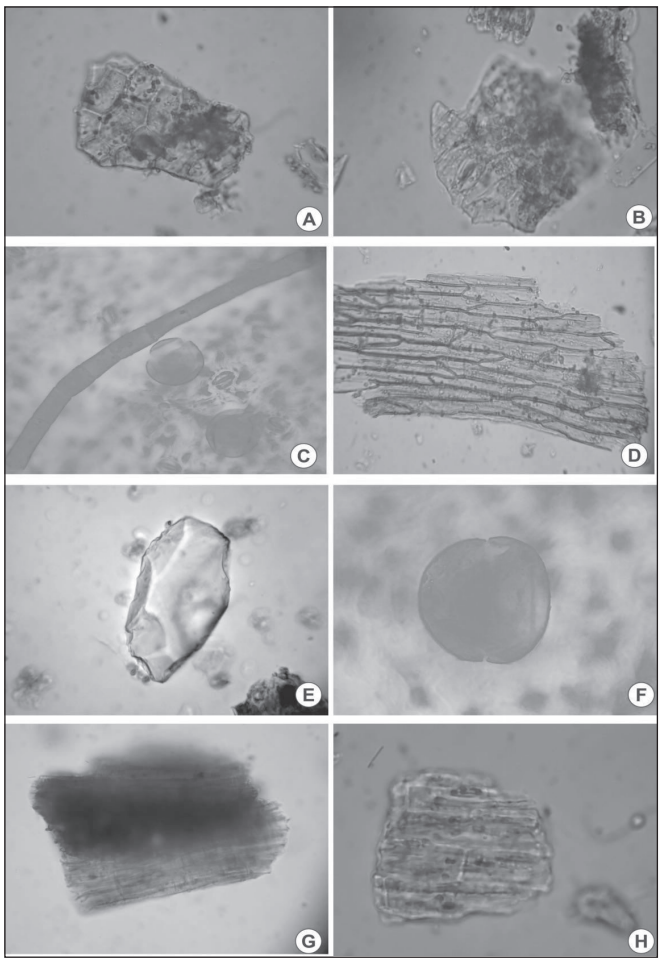


Fig. 7A-H: 7A-Fragments of epidermis with straight to curved sides x 40; 7B-Epidermis showing anomocytic stomata x 73, 7C-Uniseriate macroform cylindrical hair x 118; 7D-Costal cells with polygonal to linear cells in parallel rows x 84; 7E- Sphaero-crystaliferousi dioblasts x 75; 7F- Uniseriate filiform peltate hairs x 90; 7G-Tracheary elements showing helical thickenings x 68; 7H-Tracheary elements with annular thickenings x 54

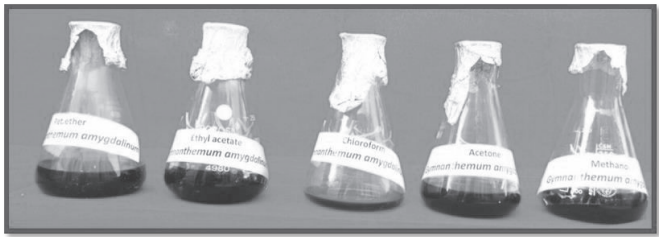


Fig. 8: *G. amygdalinum* phyto-chemical crude extracts

DISCUSSION

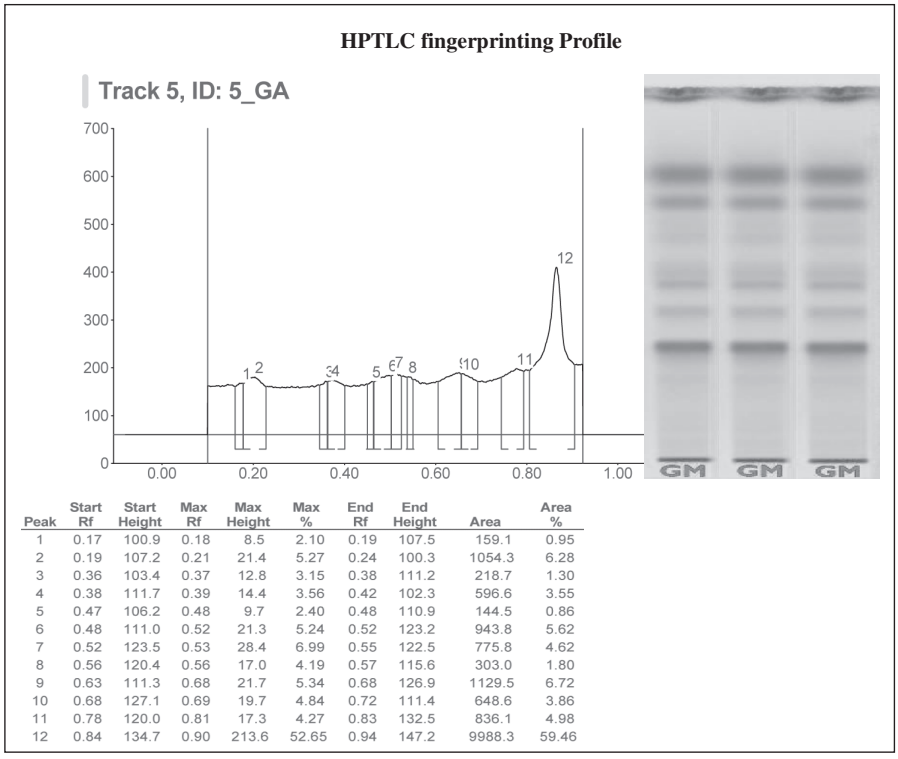
G. amygdalinum (Asteraceae), locally known as "sugarchettu" leaves were collected from Sriramgiri Village, Nellikudur Mandal, Mahabubabad District, Telangana. Locally the leaves are popularly used in the

Phytochemical analysis

Table I: Phytochemical analysis of *G. amygdalinum*

S. No.	Pyto. Name	Pet. ether	Chloroform	Ethyl acetate	Acetone	Methanol
1	Alkaloids	-	++	+	-	-
2	Carbohydrates	-	-	++	-	+++
3	Proteins	-	-	-	-	-
4	Amino acids	-	-	-	-	-
5	Flavonoids	-	-	++	+	+
6	Saponins	-	-	-	++	+++
7	Steroids & Terpenoids	-	++	++	+	+
8	Phenols	-	-	+	-	-
9	Tannins	-	-	++	-	++
10	Glycosides	-	-	-	+	++
11	Coumarins	-	-	-	++	++
12	Anthraquinones	-	-	-	-	-
13	Quinones	-	-	-	-	-
14	Resins	+	-	+	+	+
15	Gums	-	+	-	-	-
16	Leuco-anthocyanins	-	-	-	-	-

"+" = present; "-"= absent

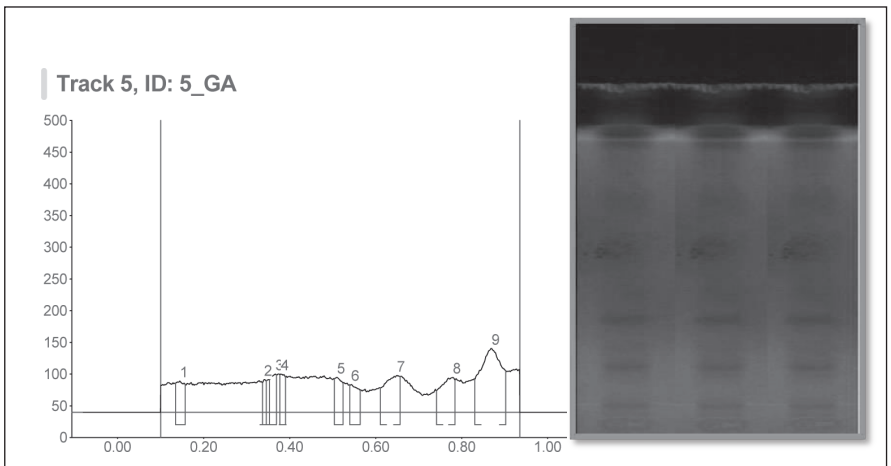


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.17	100.9	0.18	8.5	2.10	0.19	107.5	159.1	0.95
2	0.19	107.2	0.21	21.4	5.27	0.24	100.3	1054.3	6.28
3	0.36	103.4	0.37	12.8	3.15	0.38	111.2	218.7	1.30
4	0.38	111.7	0.39	14.4	3.56	0.42	102.3	596.6	3.55
5	0.47	106.2	0.48	9.7	2.40	0.48	110.9	144.5	0.86
6	0.48	111.0	0.52	21.3	5.24	0.52	123.2	943.8	5.62
7	0.52	123.5	0.53	28.4	6.99	0.55	122.5	775.8	4.62
8	0.56	120.4	0.56	17.0	4.19	0.57	115.6	303.0	1.80
9	0.63	111.3	0.68	21.7	5.34	0.68	126.9	1129.5	6.72
10	0.68	127.1	0.69	19.7	4.84	0.72	111.4	648.6	3.86
11	0.78	120.0	0.81	17.3	4.27	0.83	132.5	836.1	4.98
12	0.84	134.7	0.90	213.6	52.65	0.94	147.2	9988.3	59.46

Fig. 9: HPTLC densitogram of methanolic extract of *G. amygdalinum* scanned at 254 nm by using chloroform: ethanol: butanol (7: 2: 1 V/V/V)

treatment of diabetes. During the course of the literature survey, it was observed that the plant species is not reported in peninsular India. *G. amygdalinum* is reported here as a new addition to the flora of Peninsular India. The present work is its first pharmacognostical report from India. The pharmacognostical studies of this plant were reported by others groups[28-29]. The anatomical characteristics of the leaf were reported[28] and the report is lacking in micro measurements, leaf surface studies and phytochemical examinations. However, the plant was collected by the authors from the spontaneous flora in Palotina, state of Paraná, Brazil.

Information on leaf epidermis on the surface is scanty, with epidermal cells in the surface reported as the adaxial side and sinuous on the abaxial surface[28]. We report that, the epidermal cells are 5-6 sided,



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.14	45.9	0.15	8.1	3.96	0.16	44.1	194.0	2.56
2	0.35	47.0	0.36	15.4	7.55	0.36	49.8	192.5	2.54
3	0.37	51.8	0.38	24.8	12.17	0.39	59.5	562.4	7.43
4	0.39	59.3	0.40	25.3	12.42	0.41	56.0	502.2	6.64
5	0.52	53.3	0.53	23.3	11.43	0.55	46.4	641.4	8.48
6	0.56	43.7	0.57	12.7	6.24	0.59	35.1	342.8	4.53
7	0.64	38.7	0.68	29.7	14.58	0.68	56.3	1530.8	20.23
8	0.77	34.5	0.81	18.0	8.82	0.82	51.0	807.3	10.67
9	0.87	52.3	0.91	46.4	22.81	0.94	65.8	2792.4	36.91

Fig. 10: HPTLC densitogram of methanolic extract of *G. amygdalinum* scanned at 366 nm by using chloroform: methanol: butanol (7: 2: 1 V/V/V)

polygonal isodiametric to diametric, sides thin, mostly straight to curved, rarely curved to wavy on either side, except sides curved to wavy and wavy to sinuate, sinuses deep "U" shaped on the abaxial side only. The epidermal cell frequency is higher on leaf abaxial at 1170 per sq.mm., while lower towards adaxial at 1046 per sq.mm. The leaves are reported amphistomatic with anomocytic[28-30], which is confirmed in the present studies. Besides subsidiaries are 4-5, monocyclic, indistinct, mostly of f-type guard cells reniform to linear, surface striated on either surface except mostly of a-type, few f-type on the abaxial side. In frequency, as mentioned earlier, the stomata are larger in number 466 per sq.mm., on abaxial while less on adaxial 146 per sq.mm—the stomatal index is 22.2 on abaxial and 12.2 on adaxial. The non-glandular trichomes have been study reported earlier[28-30]. In the present study, two types of trichomes are confirmed on both surfaces. This is new information, namely i) uniseriate filiform peltate hair ii) uniseriate macro form cylindrical hair. The trichomes distribution is common, all over, confined more to primary, secondary, and tertiary veins.

In the transverse section of the leaf, a review of the literature reveals no earlier information on the leaf anatomy. In T.S., the leaf was found to be ribbed on either side but prominently ribbed on the abaxial at midvein 621-915 µm thick while lamina wings were flat, 85-200 µm in thickness. The epidermis is single-layered, with cells mostly barrel-shaped, oval-shaped elongated being larger on adaxial and smaller on abaxial and covered by a slightly thick cuticle over the surface. The stomata are amphistomatic and flushed with epidermal cells. The epidermis is interrupted by trichome bases in some places. The mesophyllis found as heterogeneous, and differentiated into palisade and spongy tissues. Palisade is one layered, throughout, extending into midvein, cells columnar, loosely arranged with intercellular spaces with sphaerocrystalliferous idioblasts. The spongy tissue is in the form of stacks of 4-5 layered, cells circular, loosely arranged with large intercellular spaces, sphaerocrystalliferous idioblasts.The ground tissue at midveinin the studied species is differentiated into collenchyma, palisade, and parenchyma tissues. Collenchyma is a group of cells in the adaxial ridge and 1-2 layered abaxially, palisade one layered beneath the adaxial collenchyma. The ground parenchyma is beneath the palisade 5-6 celled on adaxial, 4-5 layered on abaxial, cells mostly polygonal oval to circular, walls thin with small intercellular spaces with sphaero crystalliferous idioblasts. The vascular tissue mid vein consists of two large and one small arc-shaped vascular bundle arranged at the center. Large vascular bundle is about 198-320 µm in diameter and the smaller bundle 115-210 µm in diameter. The xylem consisted of 50-60 tracheary elements in number, arranged in radial rows.

In T.S., the petiole is broadly ovate about 1144-2158 µm in diameter. The epidermis is layered, ground tissue is heterogeneous, consisting of collenchyma and parenchyma tissues. The hypodermal collenchyma is 2-4 layered. The rest of the ground tissue is parenchymatous, between collenchyma and the vascular bundles. The vascular tissue consists of 5 vascular bundles arranged in an arc, oval to spherical, largest vascular bundle about salient 180-345 µm in diameter and the smallest 60-140 µm in diameter. Overall, the features of the leaf and petiole presented are diagnostic for the plant.

The phytochemical screening revealed the presence of alkaloids, carbohydrates, tannins, saponins, coumarins, glycosides, flavonoids, steroids and terpenoids, resins, and gums in various extracts namely petroleum ether, chloroform, ethyl acetate, acetone and methanol. Proteins, phenols, amino acids, anthraquinones, quinones, and leuco-anthocyanins were absent in all extracts. The highest percentage (%) of crude yield is 52.6 % in methanol and the lowest 10.5 %, extract in the petroleum ether.

The HPTLC profile of methanolic extract of the leaf shows the presence of 12 prominent spots scanned at 254 nm and nine spots at 366 nm. At 254 nm, spot with R_f 0.90 is characteristic and maximum composition with a percentage of the area of 59.46, while at 366 nm, R_f 0.91 is characteristic and possesses maximum concentration with a percentage area of 36.91. These parameters may be helpful to identify the biomarker and may be considered as fingerprint parameters.

CONCLUSION

Pharmacognostical analysis provides valuable information about the morphology, microscopical leaf epidermis, leaf and petiole anatomy, powder analysis preliminary phytochemical analysis and HPTLC fingerprinting aspects of crude drugs, as well as scientific information about the purity and quality of crude drugs, which may aid in the prevention of adulteration.

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