



PHARMACOGNOSY AND PHYTOCHEMICAL INVESTIGATION OF *CASSINE ALBENS* (RETZ.) KOSTERM.

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Abstract

During present investigation attempts were made to collect *Cassine Albens* (Retz.) Kosterm. plant material from Marathwada region of Maharashtra state. There anatomical features were studied by taking sections and observing them under compound microscope. Besides this, dermatological studies were undertaken to study the nature of epidermis, trichomes and stomata. The plant parts were macerated for the observation of dead cells. In addition, phytochemical analysis was undertaken by following standard methods. In order to investigate their medicinal properties.

The standardization of plant material will help in the correct identification of medicinal plant part, and will also be helpful in finding out odd material or adulteration, when the herbal medicines are purchased or acquired from other commercial sources, in crude form. Small glabrous trees, 3-6 m tall. Leaves elliptic, oblong-ovate, acute or rounded at base. Flowers greenish, calyx very deeply divided; lobes 4-5, unequal, orbicular, with membranous margins. Petals 4-5, greenish-white; Drupes obovate to ovoid, 8-12 mm, apiculate, stalked, apiculate. Transverse section of stem showing the presence of 4-7 layered cork. Cells thick walled, tubular, parenchymatous, cuticularized, dark-brown, squarest to rectangular, flattened, tanniniferous, or with phenolic contents. Large sclerieds, oval, whitish, sclerenchymatous cells and with thick walled rectangular bast fibers, horizontal, flattened, elongated.

Keywords: *Cassine Albens*, Anatomy, Epidermal Studies, Maceration Phytochemistry

Introduction:

During present investigation attempts were made to collect plant material from Marathwada region of Maharashtra state. The bark and the leaves has 8-15% tannin respectively. Astringent, anti-inflammatory, emetic. Powdered leaves have a sternutatory action and are used as snuff to relieve headache and for fumigation to cure the patient's suffering from hysteria. There anatomical features were studied by taking sections and observing them under compound microscope. Besides this, epidermal studies were undertaken to study the nature of epidermis, trichomes and stomata. The plant parts were macerated for the observation of dead cells. In addition, phytochemical analysis was undertaken by following standard methods. In order to investigate their medicinal properties.

The standardization of plant material will help in the correct identification of medicinal plant part, and will also be helpful in finding out odd material or adulteration. These studies were undertaken to standardize the plants or plant parts on the basis of morphological, anatomical and phytochemical properties. Medicinally used parts of plant specimen (crud drug) as like bark, stem and leaves were taken for further investigation.

Material and Methods:

Plants are collected in crude form. Those were identified using pertinent literature (Naik 1998; Singh *et al*; 2000,) and by consulting BAMU Herbarium. The voucher specimens were deposited in Herbarium, Department of Botany, Vivekanand Arts, Sardar Dalipsingh Commerce and Science College, Aurangabad. The plant materials were separately collected in sterile polyethylene bags and immediately brought to laboratory. For anatomical studies, free hand transverse sections of leaf, stem and petiole were taken with the help of razor-blades. These sections were dehydrated with alcohol and stained with safranin and light green (Khandelwal, 2005). The slides were observed under trinocular microscope (Cutler, 1978). Measurements were recorded by using ocular micrometer. The study of gross internal structure of plant organs by the technique of section cutting is called plant anatomy. Various plant organs viz, root, stem, leaves, flower, seed show their typical anatomical structure in sections.

In present work anatomy of drug plants is carried out. Anatomical studies included observations of transverse sections of crude drug at various regions to understand the nature of different cell arrangements in the plant. The crude drug is perhaps anatomically the most varied organ of angiosperms and its anatomical variation often concur closely with generic and specific occasionally familial-line (Carlquist, 1961). It is therefore assumed that crude drug anatomical features can be a good source for standardizing a crude drug. For describing plant anatomy, a hand section of crude drug was taken and observed. Systematically valuable histological features and references dealing with study of specific plant anatomy

Enumeration: *Cassine albens* (Retz.) Kosterm.

Synonyms: *Cassine dichotoma* (Roxb.) M.R.Almeida; *Cassine roxburghii* (Wight & Arn.) Ramamoorthy;

Family: Celastraceae

***Cassine albens* (Retz.) Kosterm.** O Ktze. Rev. Ge. Pl. 1:114. 1891; Ding Hou in Steenia, Fl. Males.1,6:286. 1962; Londhe in Singh et al. Fl. Maharashtra St. Dicot. 1: 520. 2000. *Mangifera glauca* Rottb. Nye. Samml. Kongel. Norske Vid. Selsk. Skr. 2: 534, t. 4.f.1. 1783. *Elaeodendron glaucum* (Rottb.) Pers. Syn. 1: 241. 1805; Law. in Hook.f. Fl. Brit. India 1: 623. 1875, p.p.; Cooke, Fl. Pers. Bombay 1: 248. 1958 (Repr.). *Cassine albens* (Retz.) Kosterm. In Garf. Bull. Singapore 39(2): 178, 1. 1986; Naik, Fl. Marathwada 1: 211. 1998.

Small glabrous trees, 3-6 m tall. Leaves elliptic, oblong-ovate, 6-15 x 2.5-6 cm., acute or rounded at base, crenate-serrate or sub-entire, acute or acuminate, twisted at apex; petioles 1-2 cm long. Inflorescence in divaricates, axillaries or extra axillaries, paniculate, dichotomously branched cymes; pedicels slender; glabrous, bracts minute, ovate, acute. Flowers greenish, calyx very deeply divided; lobes 4-5, unequal, orbicular, with membranous margins. Petals 4-5, greenish-white, obtuse, distant, oblong 4-5 mm long, obtuse; Stamens much shorter than the petals, 4-5, anthers roundish. Stigma 2-5 lobed, disk thick and fleshy, 4-5 angled or lobed. Drupes obovate to obovoid, 8-12 mm, apiculate, stalked, apiculate. Stone 1-3 locular, 3-6 or 2 seeded.

Fls. and Frts: February-June.

Distrib: On hill-tops of forest area of Aurangabad, Nanded.

Exsiccata: Gautala Forest, Aurangabad. APB 110

Vernacular Name:

Eng.: Ceylon Tea; **Ayur:** Krishnamokshaka;

Mar: Bhutkeshi; **Sidd/Tam:** Selluppaimaram;

Folk: Kaalaa-mokhaa, Ratangaruur, Jamrasi(gum);

Parts Used: Leaves, Bark

Chemical Constituents: The bark and the leaves 8-15% tannin respectively.

Uses: Astringent, anti-inflammatory, emetic. Powdered leaves have a sternutatory action and are used as snuff to relieve headache and for fumigation to cure the patients suffering from hysteria (in folk medicine it is believed that the smoke wards off ghosts) (Khare, 2007). 50g of fresh leaves taken in 200 ml of water with 1 spoonful of turmeric powder, boiled to prepare decoction. 5-10 ml of decoction is taken orally twice a day to get relief from cough with phlegm, for about a fortnight. (Kumar et al; 2008)

Anatomy:**T.S. of Stem:**

Transverse section of stem showing the presence of 4-7 layered cork. Cells thick walled, tubular, parenchymatous, cuticularized, dark-brown, squarish to rectangular, flattened, tanniniferous, or with phenolic contents, measured dimensions are $30 \times 22.5 - 17.5 \times 12.5 \mu\text{m}$, cork cambium 2-layered, indistinct, cells tangentially flattened. Outer cortex 4-layered, cells thick walled, parenchymatous, squarish to rectangular, tanniniferous, or with phenolic contents, compactly arranged, measured dimensions are $32.5 \times 25 - 17.5 \times 12.5 \mu\text{m}$. Inner cortex 4-5 layered, cells thick walled, parenchymatous, circular to oval, $75 \times 17.5 - 17.5 \times 15 \mu\text{m}$, tanniniferous, intercellular spaces present, Perivascular fibers discontinuous 1-2 layered, moderately thick, cells sclerenchymatous, pentagonal, vertically elongated, dia. $3.75 - 2.5 \mu\text{m}$, measured dimensions are $25 \times 12.5 - 17.5 \times 7.5 \mu\text{m}$. Large sclerieds, oval, whitish, sclerenchymatous cells, measured dimensions are $50 \times 30 - 37.5 \times 25 \mu\text{m}$, and with thick walled rectangular bast fibers, horizontal, flattened, elongated.

T.S. of Leaf:

Transverse section of leaf lamina showed thickened cuticle, continuously covers the epidermal layer and interrupted by stomata. Leaf lamina dorsiventral, epidermis is followed by hypodermis. cells parenchymatous, squarish to rectangular, cuticularized, measured dimensions are $50 \times 30 - 25 \times 20 \mu\text{m}$, Hypoderm is present only at upper side, with several crystal or idioblasts of calcium oxalate. Palisade parenchyma 2-layered, cells small, tanniniferous, thin walled, brownish, compactly arranged, squarish to rectangular, or irregular, measured dimensions are $27.5 \times 10 - 20 \times 10 \mu\text{m}$, below that 4-5 layered, parenchyma, cells thin walled, horizontally flattened, rectangular, filled with phenolics substances yellowish to brownish in color, measured dimensions are $37.5 \times 15 - 25 \times 10 \mu\text{m}$, spongy parenchyma 3-4 layered, cells small, tanniniferous, thin walled, brownish, compactly arranged, squarish to rectangular, irregular, $22.5 \times 7.5 - 20 \times 7.5 \mu\text{m}$, lower epidermis single layered, cuticle thick, cells squarish to rectangular, interrupted by stomata, measured dimensions are $25 \times 20 - 20 \times 7.5 \mu\text{m}$, storing druses of calcium oxalate dia. $12.5 \mu\text{m}$.

T.S. of Midrib:

Transverse section of midrib showing the presence of thick cuticle continuously covers the epidermal layer. Epidermis bi-layered, cells thick walled, parenchymatous, squarish to rectangular, horizontally elongated, measured dimensions are $45 \times 20 - 25 \times 10 \mu\text{m}$, Collenchymas girders are 2-3 layered, cells thick walled, hexagonal, cuticularized, with tannins filled with brownish and yellowish to golden colored phenolic substances, $35 \times 20 - 25 \times 17.5 \mu\text{m}$, Vascular bundles 2, bicollateral, arc-shaped, central one is larger and smaller one at left side, composed of outer phloem consists of sieve tubes and companion cells, squarish to rectangular, measured dimensions are $17.5 \times 10 - 10 \times 7.5 \mu\text{m}$, surrounding by xylem, cells thick walled, consists of fiber, trachieds and vessels. Vessels are hexagonal, $30 \times 20 - 17.5 \times 12.5 \mu\text{m}$, metaxylem at periphery and protoxylem towards the center, and smaller veins are vertically transcurrent. Medulary rays uniserrate, vascular cambium present above and below the xylem patch, internal phloem present as above. Ground tissue parenchymatous, 7-10 layered, cells thin walled, circular to hexagonal, irregular, filled with crystals, some cells are tanniniferous, and some are filled with brownish, yellowish to golden color phenolic substances, measured dimensions are $57.5 \times 50 - 30 \times 17.5 \mu\text{m}$. Lower epidermis single layered, cells thick walled, cuticularized, squarish to rectangular, horizontally elongated, measured dimensions are $30 \times 25 - 25 \times 17.5 \mu\text{m}$.

T.S. of Petiole:

Transverse section of petiole showing the presence of thin grooved cuticle continuously covers the epidermal layer. Outline hemispherical. Epidermis is single layered, cells parenchymatous, thick walled, squarish, measured dimensions are $30 \times 22.5 - 25 \times 17.5 \mu\text{m}$. Ground tissue 9-11 layered, cells parenchymatous, thin walled, squarish, pentagonal to hexagonal, tanniniferous, intercellular spaces storing druses of calcium oxalate, measured dimensions are $80 \times 62.5 - 35 \times 32.5 \mu\text{m}$. Endodermis is indistinct or crushed, cells parenchymatous, thin walled, measured dimensions are $67.5 \times 12.5 - 20 \times 10 \mu\text{m}$. Perivascular fibers arranged in 1-2 layered, cells thick walled breadth $5 - 2.5 \mu\text{m}$, rectangular, square, or pentagonal, sclerenchymatous, discontinuous ring, measured dimensions are $42.5 \times 27.5 - 25 \times 12.5 \mu\text{m}$. Vascular cylinder is curved, single, present at central portion, open, bi-collateral. It is composed of phloem with 7-10 layered and it consists of companion cells, sieve tubes, tanniniferous, measured dimensions are $22.5 \times 17.5 - 17.5 \times 5 \mu\text{m}$. Vascular cambium 1-2 layered, cells tangentially rectangular, parenchymatous, surrounding xylem. Xylem consist of 4-6 vessels in each radial row, they are uni-serrate to bi-serrate, thick walled,

measured dimensions are $47.5 \times 22.5 - 12.5 \times 10 \mu\text{m}$, alternate to medullary rays. Medullary rays 1-2 cell wide, cells parenchymatous, thin walled, filled with clustered crystals and idioblasts, tanniniferous cells and secretory canals with yellow contents situated in vascular bundles, internal phloem present.

Stomata:

The stomata are anomocytic or ranunculaceous, hypostomatic, each stomata surrounded by 3-5 epidermal cells, isodiametric, anticlinal wall thick, straight or angled, measured length range $27.5-45\mu\text{m}$, ca. average length $29.3 \pm 8.78\mu\text{m}$. Guard cell bean shaped, measured length range $22.5-30\mu\text{m}$, ca. average length $26.25 \pm 2.56\mu\text{m}$. Stomatal pore oval, measured length range $7.5-12.5\mu\text{m}$, ca. average length $10.37 \pm 3.5\mu\text{m}$. Lower stomatal no. is 22-34 and ca. average stomatal index 29.61 ± 4.13 .

Table No.1 Stomatal Index of Different Plant Species:

Type of Stomata	SI(Mean)Lower	S.D.
Anomocytic or Ranunculaceous	29.61	4.13

Table No. 2 Epidermal Cell Types and Dimensions

Shape	Anticlinal Wall Type	Range Length μm	Mean	S.D.
Squared to Pentagonal, Isodiametric	Straight	27.5-45	29.3	8.78

Table no. 3 Stomatal Cell Types and Dimensions

Lower Stomata Length Range μm	Mean \pm S.D	Lower Guard cell Length Range μm	Mean \pm S.D
7.5-15	10.375 ± 3.5	22.5-30	26.25 ± 2.56

Maceration:

Maceration is an artificial separation of cells of a tissue by causing a disintegration of the middle lamella (Esau's, 2006) such as vessels, trachieds, xylem fibers, xylem parenchyma etc. This type of cells are identified by the authentic literature and books (Cutler, 1978, Metcalfe and Chalk 1957, Schweingruber *et al*; 2011) That various type of cells were observed under microscope with their dimensions and obtained results were enlisted to find out the variation at cellular level as well as at species level. Stem maceration shows thin and thick walled parenchyma and sclerieds, simple, and spiral vessels, trachieds and fibers.

Parenchyma:

a) Thin Parenchyma: These types of parenchyma cells are showing thin walls, irregularly squired to rectangular in shape, dis-continuous due to the presence of pits on walls, pits large, many, oval, and oblique, alternate or paired, measured dimensions are $35 \times 15-140 \times 22.5\mu\text{m}$, ca. average length is $83.15\mu\text{m}$ and ca. average breadth is $20\mu\text{m}$.

b) Thick Parenchyma: These types of parenchyma cells are showing thickened walls, dis-continuous, squired to rectangular, circular to oval or irregular, walls pitted, pits bordered, few in no., measured dimensions are $62.5 \times 50-100 \times 75\mu\text{m}$, ca. average length is $83.6 \pm 11.37\mu\text{m}$ and ca. average breadth is $62.75 \pm 9.25\mu\text{m}$, wall thickness measured range $10-15\mu\text{m}$, ca. average $20.90 \pm 2.00\mu\text{m}$.

d) Simple vessels: These types of vessels are oblong and narrow at end, beaks small and pointed, intre-vascular pitting alternate, pits small and broader, sometimes present on beaks, perforations are vertical to oblique and sometimes scalariform and horizontal, perforation plates 1-4 on each beak, measured dimensions are $240 \times 20-580 \times 50\mu\text{m}$, ca. average length is $406 \pm 97.38\mu\text{m}$ and ca. average breadth is $34 \pm 11.13\mu\text{m}$.

e) Spiral Vessels: These types of vessels are oblong and longer, primary walls thin and cylindrical, narrow at end, secondary wall thickened annular to spiral, perforations simple, circular or oblique, beaks very short, measured dimensions are $125 \times 10-520 \times 20\mu\text{m}$, ca. average length is $333.5 \pm 122.67 \mu\text{m}$ and ca. average breadth is $14.25 \pm 5.28\mu\text{m}$.

f) Trachieds: These types of trachieds are longer and narrowing, walls thick and continuous, slender and narrow at end, ends blunt or pointed and sometimes forked, pits many, paired and opposite, arranged in

vertical rows, present throughout the wall, small to broader, measured dimensions are 220x10-570x30µm, ca. average length is 430.5±98.79 µm and ca. average breadth is 19.5±4.71µm.

i) Fiber: These types of fibers are longer, walls thickened, slender, irregular, ends tapering and sharply pointed or forked, pits many and minute, opposite and paired, simple and oblique, internal lumen short and narrowing, measured dimensions are 770x10-2050x20µm, ca. average length is 1252.5±455.95 µm and ca. average breadth is 17.5±4.03µm.

Maceration Table No.4

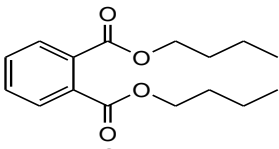
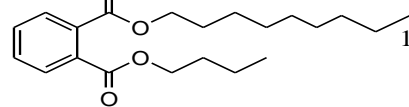
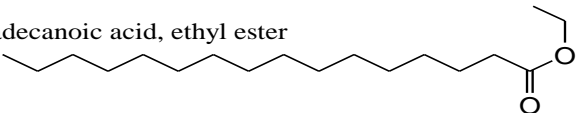
No	Cell Type	Range(µ)	Mean(µ)	S.D.
1)	Thin wall Parenchyma Length	35-140	83.15	31.14
2)	Thin wall Parenchyma Breadth	15-22.5	20	2.5
3)	Thick wall parenchyma Length	62.5-100	83.6	11.37
4)	Thick wall parenchyma Breadth	50-75	62.75	9.25
5)	Simple Vessles Length	240-580	406	97.38
6)	simple Vessles Breadth	20-50	34	11.13
7)	Spiral Vessles Length	125-520	333.5	122.67
8)	Spiral Vessles Breadth	10-20	14.25	5.28
9)	Trachieds Length	220-570	430.5	98.79
10)	Tracheid Breadth	10-30	19.5	4.71
11)	Fiber Length	770-2050	1252.5	455.95
12)	Fiber Breadth	10-20	17.5	4.03

Phytochemistry:

Plant-based natural constituents can be derived from any part of plant like bark, leaves and etc. There is growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity. Screening active compounds from plants has lead to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases. A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. A majority of the rich diversity of Indian medicinal plants is yet to be scientifically evaluated for such properties. With this background, the present study was aimed to identify the phyto-constituents like saponin, tannins, anthraquinone, alkaloids, oils, flavonoids, glycosides and terpenoids present in selected plants using GC-MS analysis.

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractions of the methanolic extract of *C. albens* (Retz.) Kosterm. These compounds were identified through mass spectrometry attached with GC. The results of the present study were tabulated in Table.

Table 35: Phytocomponents identified in *C. albens* (Retz.) Kosterm.

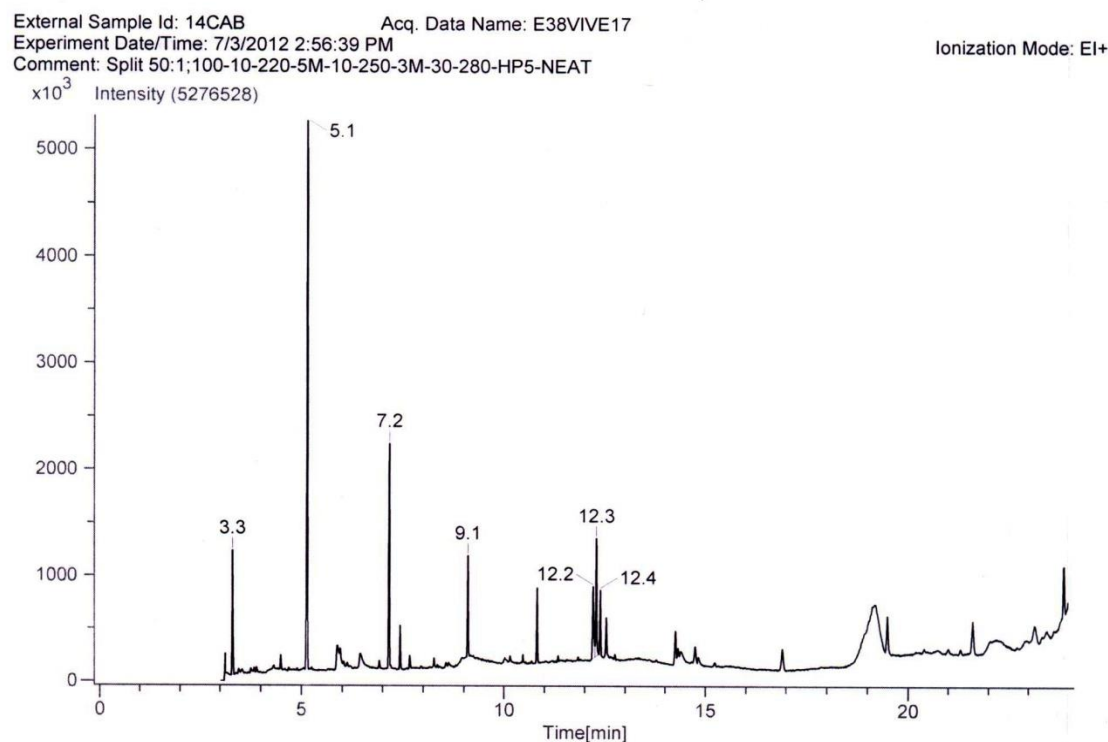
Name of compound	Structure of compound	Retention time	Molecular formula	Molecular weight
Dibutyl phthalate		12.3	C ₁₆ H ₂₂ O ₄	278.15
Phthalic acid, butyl nonyl ester		12.3	C ₂₁ H ₃₂ O ₄	348.24
hexadecanoic acid, ethyl ester		12.5	C ₁₈ H ₃₆ O ₂	284.12

The compound prediction is based on Dr. Duke's Phytochemical and Ethno-botanical Databases. The results revealed that the presence of Dibutyl phthalate, Phthalic acid, butyl nonyl ester, hexadecanoic acid, ethyl ester. The spectrum profile of GC-MS confirmed the presence of eight major components with the retention time 3.3, 5.1, 7.2, 9.1, 10.8, 12.2, 12.3, 12.4 respectively. The individual fragmentation patterns of

the components were illustrated. The mass spectrum of the compound with retention time 12.3 (Hit 1) Dibutyl phthalate gave 10 major peaks (m/z) at 56, 76, 93, 104, 121, 149, 160, 205, 223, 278. The mass spectrum of the compound with retention time 12.3 (Hit 2) Phthalic acid, butyl nonyl ester gave 10 major peaks (m/z) at 55, 76, 104, 121, 149, 167, 205, 223, 275, 293. The mass spectrum of the compound with retention time 12.5 (Hit 1) gave 9 major peaks (m/z) at 55, 69, 88, 101, 115, 157, 199, 239, 284.

In the present study we characterized the chemical profile of *C. albens* (Retz.) Kosterm. using GC-MS. The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. This report is the first of its kind to analyze the chemical constituents of *C. albens* (Retz.) Kosterm. using GC-MS. In addition to this, the results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant.

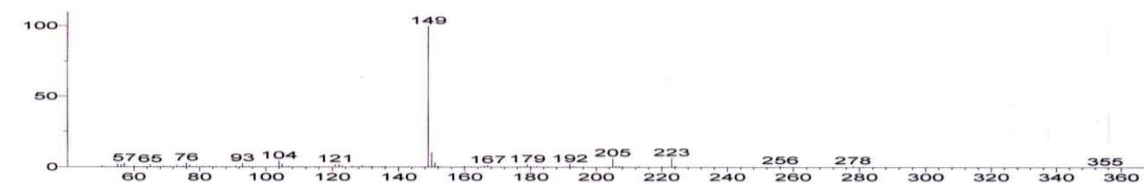
GC-MS analysis showed the existence of various compounds with different chemical structures. The presence of various bioactive compounds confirms the application of *C. albens* (Retz.) Kosterm. for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.



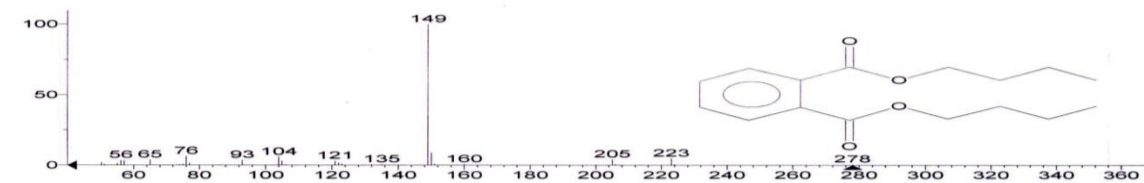
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2	5.1	BB	0.0260	8475132.70	5165744.50		5.1	110193	5.2	111374
3	7.2	BB	0.0246	3368355.05	2126543.29		7.1	118791	7.2	125267
4	9.1	BB	0.0236	1447636.33	956976.49		9.0	222958	9.1	243462
5	10.8	BB	0.0239	1099368.89	713607.10		10.8	174088	10.9	180004
6	12.2	BV	0.0299	1466725.49	686123.42		12.1	199626	12.2	234887
7	12.3	VB	0.0270	2020212.91	1114764.57		12.2	234887	12.3	264278
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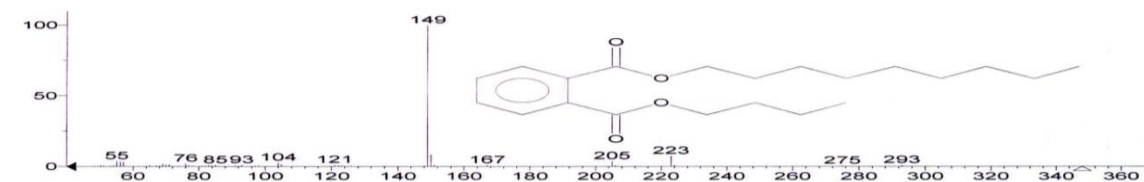
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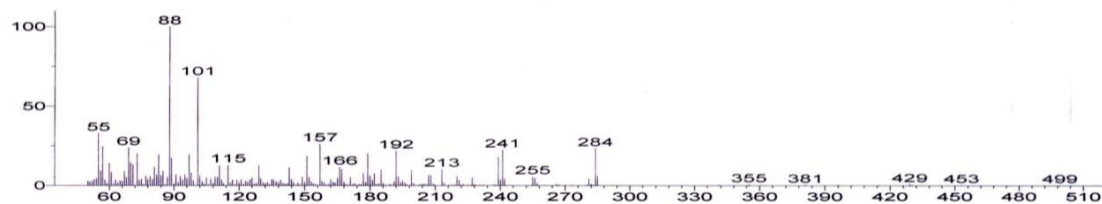


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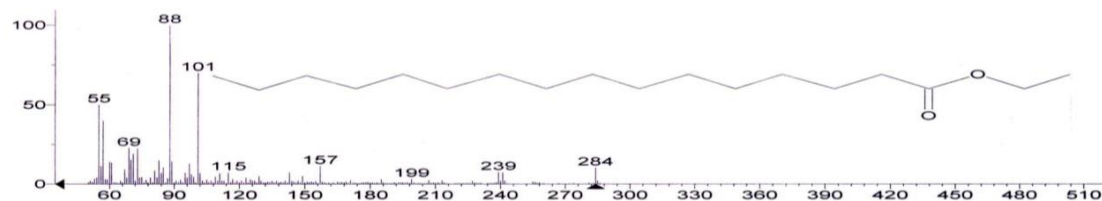


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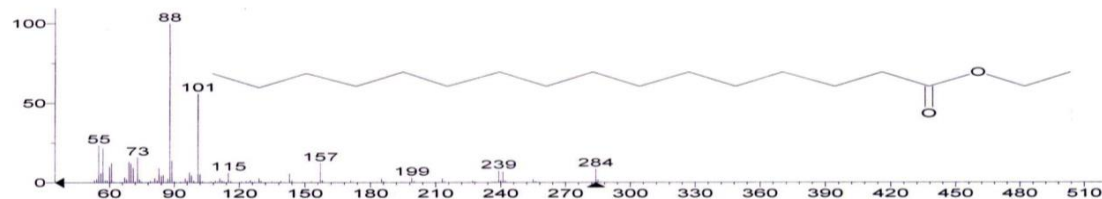
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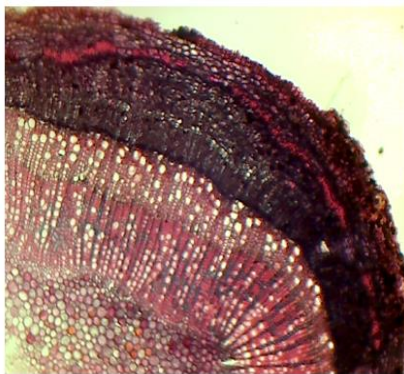
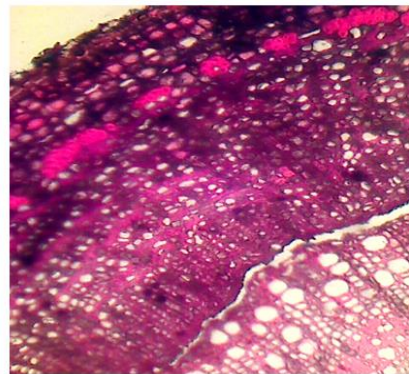
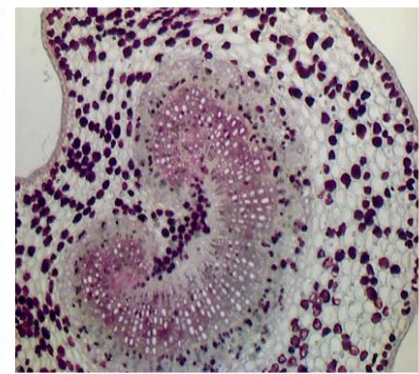
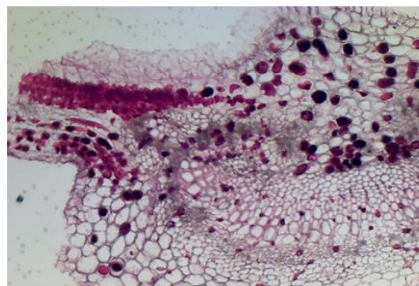
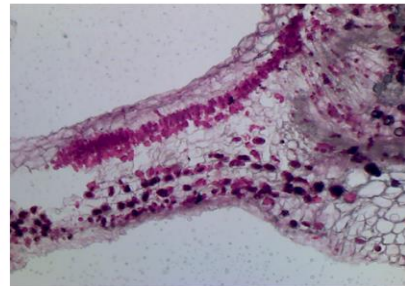
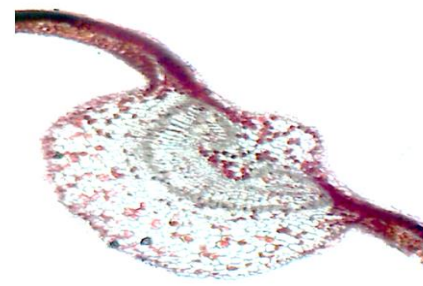
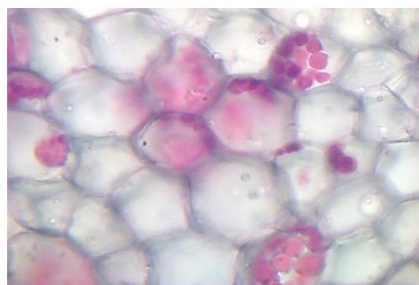
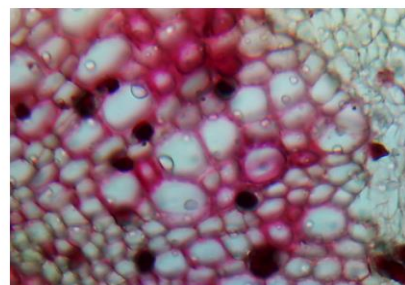
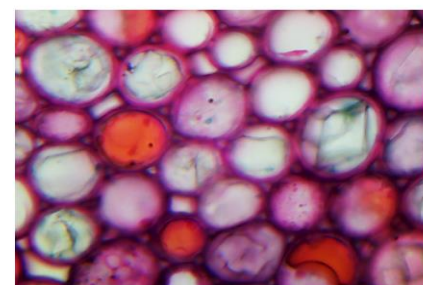


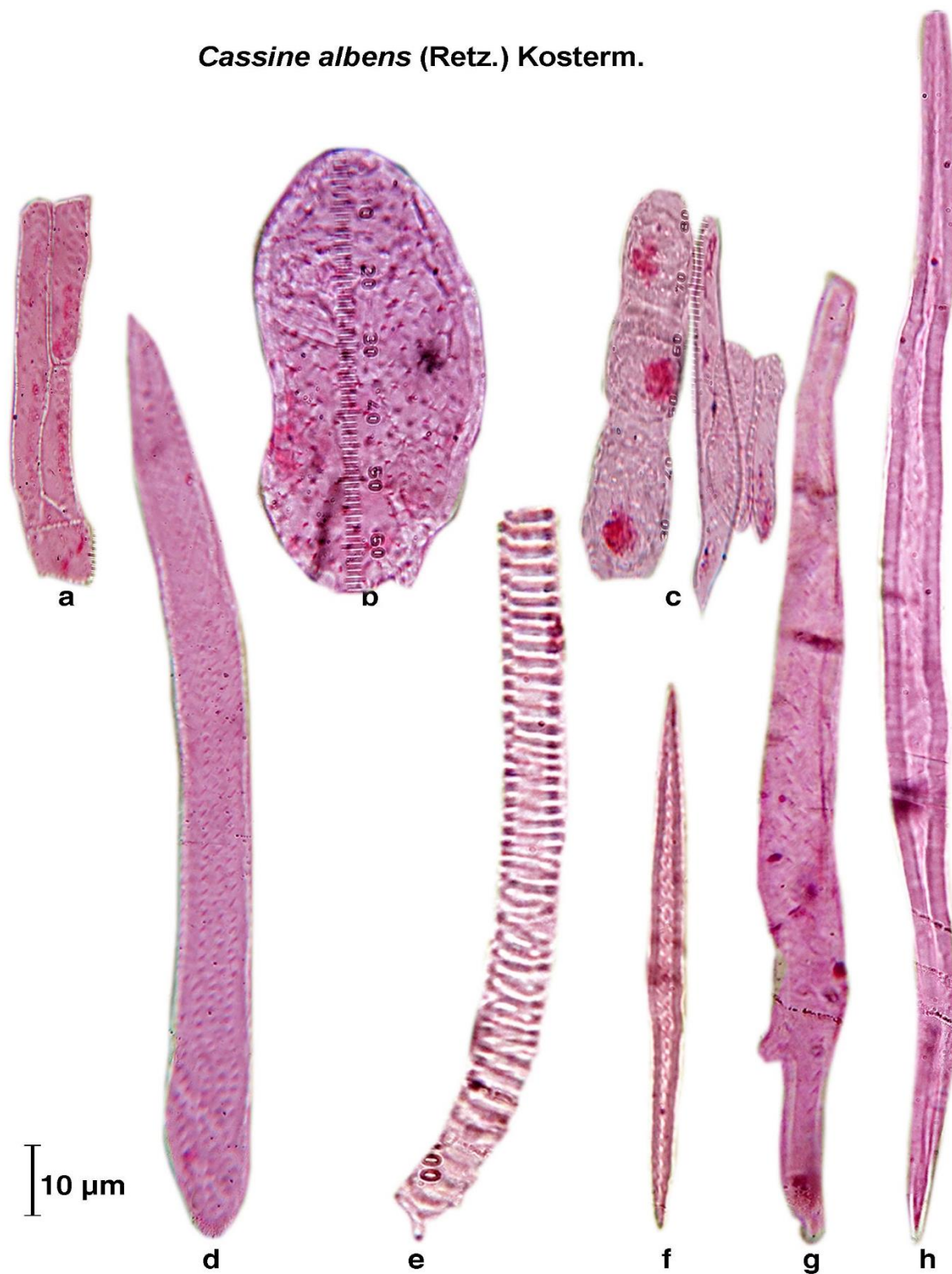
Hit 1 : Hexadecanoic acid, ethyl ester
C18H36O2; MF: 767; RMF: 779; Prob 81.6%; CAS: 628-97-7; Lib: replib; ID: 11116.



Hit 2 : Hexadecanoic acid, ethyl ester
C18H36O2; MF: 690; RMF: 769; Prob 81.6%; CAS: 628-97-7; Lib: mainlib; ID: 49485.



***Cassine albens* (Retz.) Kosterm.****Habit****Fruits****Inflorescence****T.S. of Stem****T.S. of Stem****T.S. of Petiole****T.S. of Leaf****T.S. of Leaf Lamina****T.S. of Midrib****Phenols****Phenols****Crystals**

***Cassine albens* (Retz.) Kosterm.**

a) Thin Parenchyma, b-c) Thick Parenchyma, d) Simple Vessels, e) Spiral Vessels, f-g) Pitted Trichied, i) Pitted Fiber.

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