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Phytochemical and Pharmacognostic investigation on *Cardiospermum halicacabum* L. (Sapindaceae)

Bhagat AP and Bhuktar AS

Abstract
Cardiospermum halicacabum L. is a traditional medicinal plant. Commonly called as *Winter Cherry, Heartseed, Balloon vine, Kanphuti, Kapala-phodi, and Tejovati.* It is an annual or perennial; scrambling shrub or herbaceous climber by averages of tendrillar hooks. The drug also showed (transient) vasodepressant activity. Used in rheumatism, lumbago, skeletal fractures, nervous diseases, amenorrhea, hemorrhoids, erysipelas. The herb is used in hair oils for treating dandruff, alopecia and for darkening hair. The transverse section of stem showed aster shaped outline, slightly wavy, lenticels are present starch sheath, vascular cylinder is open, collateral, cambium bi-layered with inter-fascicular ring. Leaf lamina is dorsoventrally or partly centric, unicellular, trichome, long and pointed, uniserriate, Glandular trichome are bi- celled, short and bulbous, uniserriate. The stomata are anomocytic or rananculaceous, amphistomatic ca. average lower stomatal index 30.69+4.00, and ca. average upper stomatal index 28.70+7.07. Crud methanol extracts of whole plant were screened against the *Shigella dysenteriae* ATCC 9752 shows ca. average 8+0.50 mm zone of inhibition. Gas Chromatography fractions confirmed the presence of 15 major components that revealed the presence of 9-Octadecenoic acid-(Z)-,2-hydroxy-1-(hydroxymethyl) ethyl ester at 20.0 minutes retention time having C21H40O4 molecular formula and 356 molecular weight, Phthalic acid, isobutyl octadecyl ester at 12.3 minutes retention time having C30H50O4 molecular formula and 223 molecular weight, Stigmasterol at 23.2 minutes retention time having C29H48O molecular formula and 412 molecular weight.
These studies were undertaken to standardize the plants or plant parts on the basis of morphological, anatomical and phytochemical properties. The standardization of plant material will help in the correct identification of medicinal plant part and also will be helpful in finding out odd material or adulteration, when the herbal medicines are purchased or acquired from other commercial sources in crude form.

Keywords: *Cardiospermum halicacabum*, medicinal plants, phytochemistry and pharmacognocy

Introduction
Enumerations Vernacular Name: Mar: *Kanphuti, Kapala-phodi, Tejovati;* Eng: *Winter Cherry, Heartseed, Ballon vine, Blister Creeper, Heart pea*; Hin: *Kanphuti, Kapalphoti;* San: *Sakralata, Indravalli;*

Family: Sapindaceae *Cardiospermum halicacabum* L. Sp. Pl. 366. 1753; Hiern in Hook.f. Fl. Brit. India 1: 670.1875; Cooke, Fl. Pres. Bombay 1: 280. 1958 (Repr.); Naik, Fl. Marathwada 1:228.1998; Kulkarni in Singh *et al*. Fl. Maharashtra St. Dicot. 1: 571. 2000.
Annual or perennial; scrambling shrub or herbaceous climber by averages of tendrillar hooks; branches slender, striate, pubescent or glabrous. Leaves deltoid, biternate, petiole 2-3.8 cm. long; ultimate segments of the leaves lanceolate, glabrous or sparsely pubescent, inciso-serrate, very acute at the apex and narrowed at the base. Flowers zygomorphic; white, 3-4 mm long, in few-flowered umbellate cymes; peduncles slender, stiff, axillary, 3.8-10 cm. long, provided beneath the cyme with 2 opposite usually circinnate tendrils; pedicels very slender, 3-12 cm. long, Sepals 4, greenish, outer sepals rounded, obovate, usually with a few scattered hairs on the back just below the apical margin; inner sepals larger than the outer, rounded, membranous. Petals rounded at the apex. Style very short. Capsules shortly stalked, 2-3x2.0-2.4 cm, sub-globose or more commonly depressed-pyriform, trigonous, truncate at top, winged at the angles, bladdery, veined. Seeds globose, 4-6 mm. diam., smooth, black, with a small white heart-shaped aril.

Fls. and Frts: August-December.
Distribution: Growing as a weed in open sites, common in all districts.
Exsiccata: *Aurangabad District, APB* 133.
Parts Used: Whole plant.

Chemical Constituents: Whole plant contains alkaloids, *l*-tricontanol, and *n*-pentacosane, *n*-tricontane, *n*-tricontanol, *n*-non-acosane, pelargonidin-3-galactoside, glucocappasalin, *β*-sitosterol and phthalic acid (Prajapati *et al*. 2003) [12]. The leaves are contain flavones such as apigenin, acacetin, 7-O Me apigenin, 7, 4'-diOMe apigenin and 3',4,-diOMe luteolin, phenolic acids such as vanillic, syringic, melitotic, p-coumaric and ferulic acids, β-sitosterol, saponins and alkaloids. A cyanolipid is also reported from this plant. The seeds yield fatty oil (28%) unique in having 11-eicosenoic acid as the major fatty acid (42%) and oleic, linolenic, arachidic and linoleic acids as minor components (Daniel, 2006) [4]. The leaves contain beta-sitosterol and its D-glucoside, an alkaloid, oxalic acid and amino acids. The presence of a saponin and quebrachitol is reported in the plant (Khare, 2007) [2].

Uses: The roots are diuretic, diaphoretic, emetic, mucilaginous, laxative and emmenagogue and useful in strangury, fever, arthritis, amenorrhea, lumbago and neuropathy. The leaves are rubefacient and good for arthritis, otalgia and ophthalmodynia. The seeds are tonic and diaphoretic and used against the fever. The plant has sedative action on the central nervous system (Prajapati *et al*. 2003) [12]. Dried aerial parts are used in chronic bronchitis, phthisis, rheumatism, nervous diseases, piles and amenorrhea. Tincture of the plant is good in skin inflammation, rheumatism, arthritis, itching and psoriasis. Decoction of the unripe fruit juice is orally used as an emmenagogue (Acharya *et al*. 2008) [5]. The plant extract exhibited significant diuretic and anti-inflammatory effects as well as a sedative effect on the central nervous system. The roots and leaves are recommended for hair growth (Daniel, 2006) [4]. The plant extract showed significant analgesic and anti-inflammatory activity and sedative effect on CNS. The drug also showed (transient) vasodepressant activity. Used in rheumatism, lumbago, skeletal fractures, nervous diseases, amenorrhea, hemorrhoids, erysipelas. The herb is used in hair oils for treating dandruff, alopecia and for darkening hair. The alkaloid fraction from the seeds showed hypotensive activities and cardiac inhibition in anaesthetized dogs; blocked spasmogenic effects of acetylcholine, histamine and 5-HTon guinea pig ileum, biphasic effort on frog rectus abdominis muscle. The seeds also showed antibacterial activity (Khare, 2007) [2].

Girish *et al.* (2008) [8], tested *in vitro* evaluation of the efficacy of leaf and its callus extracts of *Cardiospermum halicacabum L.* on important human pathogenic bacteria. Ara *et al.* (2009) [1], tested hepatoprotective activity of *C. helicacabum* L. stem extracts against carbontetrachloride-induced hepatotoxicity in wistar rats. Rao *et al.* (2006) [13], reported anti-diarrheal activity of the extracts of *C. halicacabum* L. due to the presence of phytochemical constituents in it.

Material and Method

Cardiospermum halicacabum L. were collected from various localities of Marathwada region. This was identified using pertinent literature (Cooke 1958; Hooker 1874; Naik 1998; Singh *et al.* 2000, Almeida 1996-2008; and Bentham) [11] and by consulting BAMU Herbarium. The voucher specimens were deposited in Herbarium, Department of Botany, Vivekanand Arts, Sardar Dalipsingh Commerce and Science College, Aurangabad. Medicinally used parts of plant specimen (crud drug) as like root or tuber, bark, stem, leaves, flowers and fruits were taken for further investigation.

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, petiole, root k were taken with the help of razor-blades. These sections were dehydrated with alcohol and stained with safranine and light green (Khandelwal, 2005) [10]. The slides were observed under trinacular microscope (Cutler, 1978) [3]. Measurements were recorded by using ocular micrometer.

Epidermal Studies was carried out with trichrome were scraped from leaf surface with the help of razor, stained with safranin and mount in glycerin on a slide. The slide was observed under microscope and dimensions were measured with the help of ocular micrometer (Roy, 2006). Upper and lower epidermis were peeled separately and mounted in glycerin water on a slide. The stomata were observed under compound microscope and measurements taken. Stomatal index was calculated as I = [(S) / (E + S)] x 100, where, I = Stomatal index, S = No. of stomata per unit area, E = No. of epidermal cells in the same unit area.

Maceration: Maceration involves separation of tissue cells by disintegration of middle lamella (Esau 2006) [6] into vascular elements such as vessels, tracheid, xylem fibers, xylem parenchyma and etc. For this purpose fresh, preserved or dried stems, roots, leaves or bark were macerated in Jeffrey's maceration fluid (Khandelwal, 2005) [10] the types of vascular elements were observed under microscope, and measurements were taken.

Phytochemistry

Sample Preparations: The plant materials were collected at different times of the day, in the morning or afternoon. The plant material was dried in shade followed by oven (95 + 5° C) the dried plant material was ground to a fine powder. From 10-20 gm powdered sample was extracted with Methanol, Ethanol, Hexane, Benzene, Toluene and Petroleum ether using Soxhlet extraction method, as described by Harborne, (1973) [9] and Evans, (1996) [7]. Fractionation was done by Solvent extraction or Liquid - liquid extraction. The solvent evaporated and condensed extract was stored at -4^{0}C. The samples of the extracts were analyzed for volatile secondary metabolites by GC-MS at Indian Institute of Technology (IIT), Mumbai.

Analytical Method: For each sample the analytical method is same while the oven temperature is variable, Injection port temperature is 250, Carrier gas is Helium 1ml /sec, Interface temperature is 250, Ion source is at 200, Analysis was done by using E+ IONISATION WITH 70ev, The MS is AccuTOF GCV, Column through the sample passes is HP-5,

Antimicrobial Activity

Material: Antimicrobial activity was mainly focused on to investigate the antibacterial and antifungal potentials of various extracts from different plants / plant parts / plant organs. In order to evaluation of the prepared solvent extract of above mentioned plant species were screened against the following organisms and compared with their + ve and –ve controls.

In order to examine antimicrobial properties of crud extracts the selected human pathogens such as *Salmonella typhi* ATCC 10749, *Shigella dysenteriae* ATCC 9752, *Pseudomonas aeruginosa* ATCC9027 *Candida albicans* ATCC10231, *Cryptococcus neoformans* (Hospital Strain)

against the 50 µl concentration of plant extract. Then they are compared with the +ve control Amoxicillin and Amphotericine B while –ve control is methanol and ethanol. Obtained results are mentioned in the table no.

Result and Discussion

T.S. of Stem

The transverse section of stem showed aster shaped outline, slightly wavy, lenticels present, cuticle thick. Epidermis single layered, in primary stems and forms periderm in older stems, Cork cambium present, cells cutinized, tanniniferous, thin walled, parenchymatous, oval or rectangular, tangentially elongated, measured dimensions are 25x17.5-12.5x10µm. Stem consist of 3-4 layered outer cortex, cells tanniniferous, thick walled, inner wall circular, pentagonal to hexagonal, horizontal, 22.5x17.5-12.5x10µm. Inner cortex is 3-4 layered, cells parenchymatous, thin walled, isodimetric, rectangular to hexagonal, measured dimensions are 10x7.5-12.5x15µm. Starch sheath is single layered, cells parenchymatous, thin walled, oval, 50x17.5-37.5x12.5µm. Perivascular fibers 4-14 layered, cells sclerenchymatous, thick walled, compactly arranged, walls angular, straight, 32.5x25-12.5x10µm. Vascular bundle open, collateral, cambium bi-layered. Primary phloem with partitions, secondary phloem containing sieves tubes, companion cells, fibers and stone cells, parenchymatous, rectangular, irregular, 12.5x10-10x7.5µm. Xylem forms the continuous cylinder, xylem tissues arranged in 2-4 celled grouped, sometimes isolated, 112.5x17.5µm, with axial parenchyma, tracheids smaller. Pith large, parenchymatous, thin walled, sometimes including stone cells, crystals are solitary as well as clustered.

T.S. of Leaf lamina

Cuticle thin, continuously covers the epidermal layer and interrupted by stomata. Outline of the leaf is winged, thin and flat, the lower surface is papillose, leaf lamina dorsiventral or partly centric. upper epidermis single layered, interrupted by glandular and non- glandular trichome and stomata, cells parenchymatous, thin walled, tangentially rectangular to oval, 50x27.5-25x5µm. Mesophyll usually dorsiventral or partly centric and consists of palisade parenchyma, columnar in shape, chlorophyllous, elongated, isobilateral, thin walled, rectangular, measured dimensions are 32.5x5-25x5µm. Vascular bundles are open, collateral, spongy mesophyll cells surrounds the vascular bundles, cells small, thin, chlorophyllous, circular to oval, with air spaces, measured dia. 10-12.5µm. Lower epidermis single layered, cells as above, measured dimensions are 25x12.5-17.5x12.5µm.

T.S. of Midrib

Transverse section of midrib showing the presence of thick cuticle. Midrib vertically transcurrent, surrounded by a ring of sclerenchyma, smaller veins embedded in mesophyll. Upper epidermis single layered, interrupted by trichome, cells parenchymatous, thin walled, circular to oval, isobilateral, measured dimensions are 22.5x12.5-17.5x12.5µm. Below that 3-4 layered collenchymas girders are present, cells thick walled, circular, filled with crystals, measured range15x10-10x7.5µm. Cortex 6-10 layered cells parenchymatous, thin walled, circular to oval, measured dimensions are 30x22.5-10x7.5µm, filled with clustered crystals. Vascular bundles are open, collateral, composed of xylem vessels, cells thick walled, circular to polygonal, dia. 32.5-12.5µmm, surrounded by 6-7 layers of phloem, consists of companion cells and sieve tubes, cells indistinct, abaxial epidermis same as above.

T.S. of Petiole

Outline aster shaped due to ribs or slightly wavy, cuticle thin and continuously covers the epidermis and interrupted by trichome and stomata. Epidermis is single layered, cells parenchymatous, thin walled, measured dimensions are 30x7.5-12.5x10 µm. Outer cortex is 4-6 layered made up of collenchymas, cells thick walled, compactly arranged, pentagonal or hexagonal, measured dimensions are 37.5x15-15x12.5µm. Inner cortex is 2-3 layered parenchymatous, cells thin walled, irregularly rectangular to squarest, measured dimensions are 57.5x30-32.5x27.5µm. Below that single layered starch sheath surrounds internal core of vascular bundles, cells thin walled, parenchymatous, horizontally oval, measured dimensions are 62.5x42.5-42.5x30µm. Perivascular fibers are 2-4 layered, cells sclerenchymatous, pentagonal to hexagonal, very thick walled, measured dimensions are 25x22.5-10x7.5µm. Vascular bundles are placed at 4 corners and they are open, collateral, composed of outer phloem consists of companion cells, sieves tubes, surrounding the xylem. Xylem consists of vessels, 47.5x32.5-30x17.5µm. Pith central, large, parenchymatous, filled with prismatic crystals measured dia. 12.5-10µmm, clustered crystals measured dia. 25-17.5µm.

Trichome: Leaf shows unicellular, trichome, long and pointed, uniseriate, pointed at terminal end, basal cell rhomboid and bulbous or swollen, showing lumen from base to the apex, observed range 80x10-340x50µm. Glandular trichome are bi- celled, short and bulbous, uniseriate, stalk or basal cell oblong, observed range 30x15-37.5x15µm.

Stomata: The stomata are anomocytic or ranunculaceous, amphistomatic, each stomata surrounded by 3-5 epidermal cells, anticlinal wall thin, irregular or undulate, cells measured length range 17.5-56.25µm. Upper stomatal no. is 31-47 and ca. average stomatal index 30.69+4.00, lower stomatal no. is 12-19 and ca. average stomatal index 28.70+7.07.

Table 1: Types Upper S.D. SI Lower

	Types	SI (Mean) Upper	S.D.	SI (Mean) Lower	S.D.
Trichome	Unicellular Uniseriate	238.5	178.83	25	11.40
Stomata	Anomocytic or Ranunculaceous	30.69	4.00	28.70	7.07
Epidermal Cells	Irregular Shape	Undulate Anticlinal Wall	17.5-56.25	42.37	9.44

Stem maceration

Stem maceration shows thick walled with pits, opposite or alternate, measured dimensions are 50 x 15 - 287.5 x 25 µm. Thick walled parenchyma are showing pits large, opposite or alternate, measured dimensions are 50 x 15 - 287.5 x 25 µm. Simple vessels are showing pits small and simple, perforations plates simple, measured dimensions are240 x 20 – 700 x 60µm. Spiral vessels are long, perforations simple, circular or oblique, beaks very short or absent, measured dimensions are 430 x 15 – 750 x 30µm. Reticulated vessels are oblong, conical, cup shaped and drum shaped, perforation simple, measured dimensions are 160 x 20 – 330 x 250µm. Trachieds are slender or tapering, pits minute, ends blunt or sharply pointed, slightly forked, measured dimensions are 210 x 10 – 420 x 40 µm. Fiber trachieds forked at one end, pitted, measured dimensions are 520 x 20 – 910 x 20µm. Fibers are moderately thickened, elongated and sharply pointed at both ends, lumen present, longer than fiber trachieds, measured dimensions are 520 x 20 – 1600 x 20µm.

Table 2: Maceration

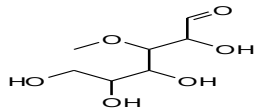
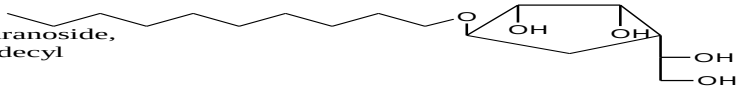
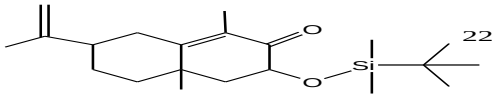
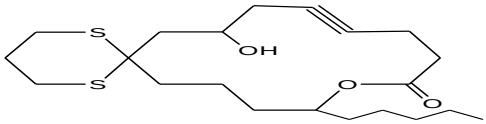
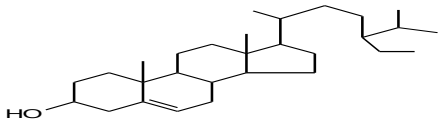
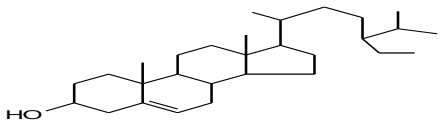
Sr. No.	Cell Type	Range(µ)	Mean(µ)	S.D.
1)	Thin wall Parenchyma Length	180-287.5	232	35.36
2)	Thin wall Parenchyma Breadth	25-27.5	25.75	1.14
3)	Thick wall parenchyma Length	40-200	108.25	41.93
4)	Thick wall parenchyma Breadth	25-37.5	29.25	4.47
5)	Simple Vessles Length	240-700	430.5	153.30
6)	Simple Vessles Breadth	20-60	35.5	13.68
7)	Spiral Vessles Length	430-750	578	115.56
8)	Spiral Vessles Breadth	15-30	20	5.47
9)	Reticulated Vessels Length	160-330	233.25	57.11
10)	Reticulated Vessels Breadth	20-250	110.25	167.60
11)	Pitted Trachied Length	210-420	32.25	71.22
12)	Pitted Trachied Breadth	10-40	18.25	9.87
13)	Fiber Trachied Length	520-910	707.75	105.90
14)	Fiber Trachied Breadth	20-20	20	0.00
15)	Gelatinous Fiber Length	520-1600	1054	345.75
16)	Gelatinous Fiber Breadth	20-20	20	0.00

Antimicrobial Activity

Similarly the 50 µl crud methanol extracts of whole plant were screened against the three bacterial human pathogens and two fungal human pathogens and from that *Shigella dysenteriae* ATCC 9752 shows average 8+0.50 mm zone of inhibition. Simultaneously the results obtained show that the crude methanol extract of whole plant of *Cardiospermum halicacabum* L. does not shown any zone of inhibition against the *Salmonella typhi* ATCC 10749, *Pseudomonas aeruginosa* ATCC9027, *Candida albicans* ATCC10231, and *Cryptococcus neoformans*. Thus they are resistant against the plant extract. Crud methanol extracts of whole plant were screened against the *Shigella dysenteriae* ATCC 9752 shows ca. average 8+0.50 mm zone of inhibition. Simultaneously the obtained results does not shown any zone of inhibition.

Phytochemistry

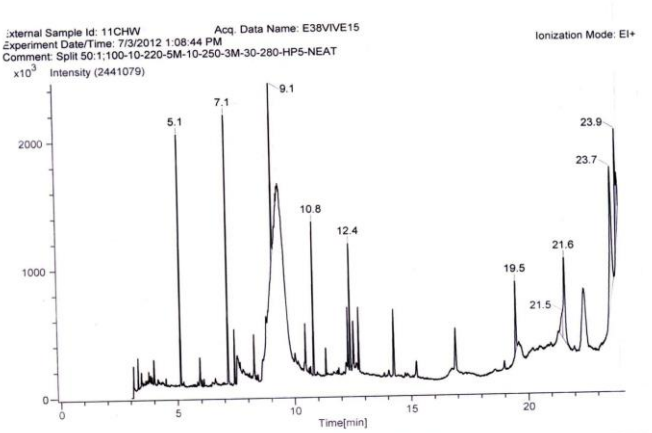
The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractions of the ethanolic extract. These compounds were identified through mass spectrometry attached with GC. Phytocomponents identified in *C. halicacabum* L.

Name of compound	Structure of compound	Retention time	Molecular formula	Molecular weight
3-O-Methyl-d-glucose		9.3	$C_7H_{14}O_6$	194.18
Mannofuranoside, 1-O-decyl		9.3	$C_{16}H_{32}O_6$	318.24
2(3H)-Naphthalenona, 3[{(1,1-dimethylethyl)dimethylsilyl]4,4a,5,6,7,8-hexahydro-1,4a-dimethyl-7		22.4	$C_{21}H_{36}O_2Si$	348.25
18-hydroxy-10-pentyl-11-oxa-1,5-ditha-spirononadec-one		22.4	$C_{21}H_{34}O_3S_2$	398.19
β-Sitosterol		23.9	$C_{29}H_{50}O$	414.39
r-Sitosterol		23.9	$C_{29}H_{50}O$	414.39

The compound prediction is based on Dr. Duke's Phytochemical and Ethnobotanical Databases. The results revealed that the presence of 3-O-Methyl-d-glucose, Mannofuranoside, 1-O-decyl, 2(3H)-Naphthalenona, 3[{(1,1-dimethylethyl)dimethylsilyl]4,4a,5,6,7,8-hexahydro-1,4a-dimethyl-7, 18-hydroxy-10-pentyl-11-oxa-1,5-ditha-spirononadec-one, ß-Sitosterol, ɤ-Sitosterol. The spectrum profile of GC-MS confirmed the presence of nine major components with the retention time 5.1, 7.1, 9.3, 10.8, 12.4, 19.5, 21.6, 22.4, 23.9 respectively. The individual fragmentation patterns of the components were illustrated. The mass spectrum of the compound with retention time 9.3 (Hit 1) -O-Methyl-d-glucose gave 8 major peaks (m/z) at 57, 73, 87, 103, 145, 163, 177, 194. The mass spectrum of the compound with retention time 9.3 (Hit 2) Mannofuranoside, 1-O-decyl gave 11 major peaks (m/z) at 57, 73, 87, 101, 116, 127, 141, 163, 213, 242, 259. The mass spectrum of the compound with retention time 22.4 (Hit 1) 2(3H)-Naphthalenona, 3[{(1,1-dimethylethyl)dimethylsilyl]4,4a,5,6,7,8-hexahydro-1,4a-dimethyl-7 gave 12 major peaks (m/z) at 55, 75, 91, 105, 143, 207, 222, 249, 276, 291, 317, 333. The mass spectrum of the compound with retention time 22.4 (Hit 2) 18-hydroxy-10-pentyl-11-oxa-1,5-ditha-spirononadec-one gave 16 major peaks (m/z) at 55, 67, 81, 107, 145, 167, 183, 207, 228, 245, 291, 305, 323, 341, 380, 398. The mass spectrum of the compound with retention time 23.9 (Hit 1) □-Sitosterol gave 18 major peaks (m/z) at 55, 69, 81, 105, 119, 145, 159, 173, 199, 213, 231, 255, 273, 303, 329, 354, 396, 414. The mass spectrum of the compound with retention time 23.9 (Hit 2) □-Sitosterol gave 18 major peaks (m/z) at 55, 69, 81, 95, 119, 145, 161, 173, 199, 213, 231, 255, 273, 303, 329, 354, 396, 414.

In the present study we characterized the chemical profile of *C. halicacabum* L. 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. halicacabum* L. 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. halicacabum* L. for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.



Peak Numb	Time [min]	Type	Peak Width [min]	Area [Intens. * se	Height	Description	Start Point Time[min]	Start Point Height	End Point Time[min]	End Point Height
1	5.1	BB	0.0221	2800928.46	1964500.28		5.1	87009	5.2	93241
2	7.1	BB	0.0240	3219561.12	2100801.02		7.1	99199	7.2	101741
3	9.1	BB	0.0245	2190278.74	1381389.24		9.0	909295	9.1	1149889
4	10.8	BB	0.0249	1910818.54	1204367.24		10.8	150742	10.9	153078
5	12.4	BB	0.0268	1697008.42	992549.20		12.3	190421	12.4	185475
6	19.5	BB	0.0404	1447492.30	551380.33		19.4	233266	19.5	352250
7	21.5	BV	0.1007	1047436.59	207270.88		21.4	452944	21.5	406474
8	21.6	VB	0.0664	3334206.70	658821.13		21.5	406474	21.8	329025
9	23.7	BB	0.0858	6367551.67	1005459.02		23.5	510084	23.8	881160
10	23.9	BV	0.0540	2500615.46	921612.25		23.8	947679	23.9	1212107
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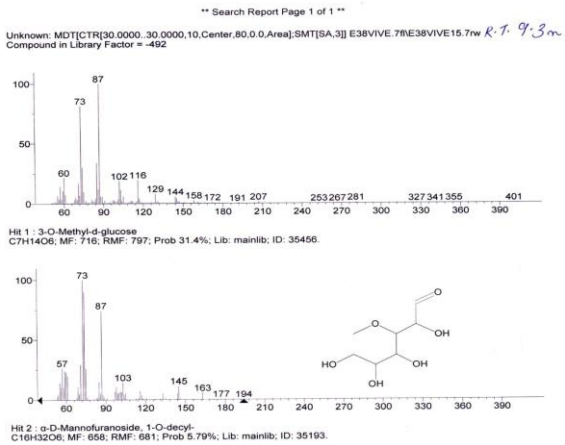
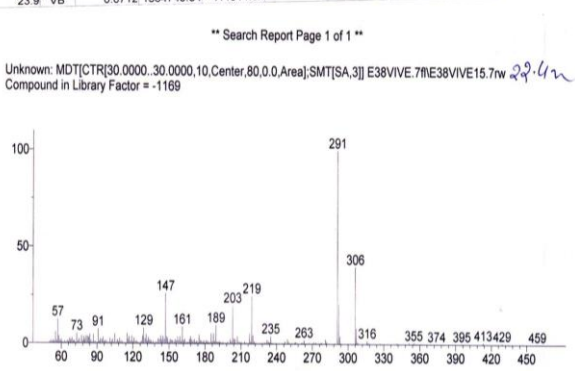
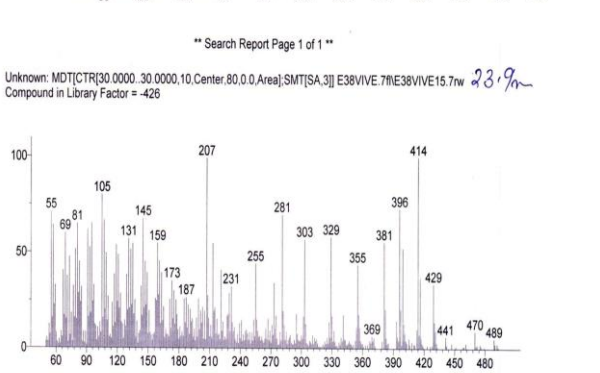
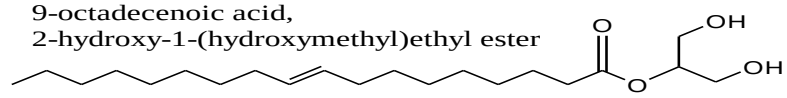
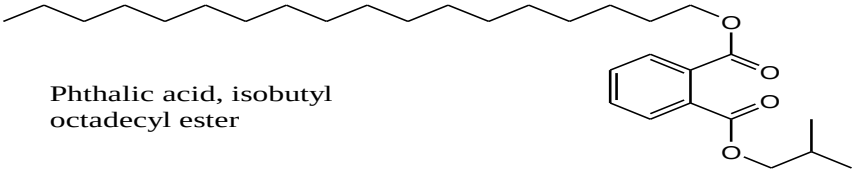
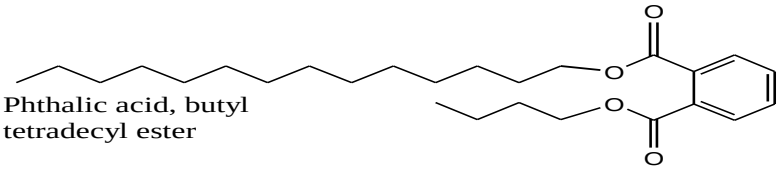
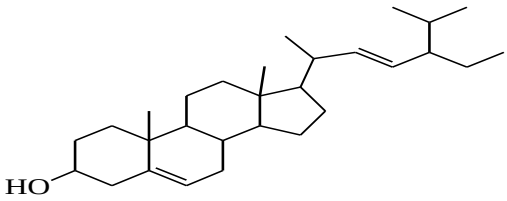
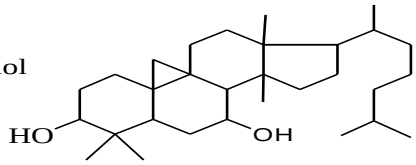






Cardiospermum halicacabum L. Toulene Fraction:
The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractions of the toulene extract of C. *halicacabum* L. These compounds were identified through mass spectrometry attached with GC. The results of the present study were tabulated in Table.
Table Phytocomponents identified in *C. halicacabum* L.

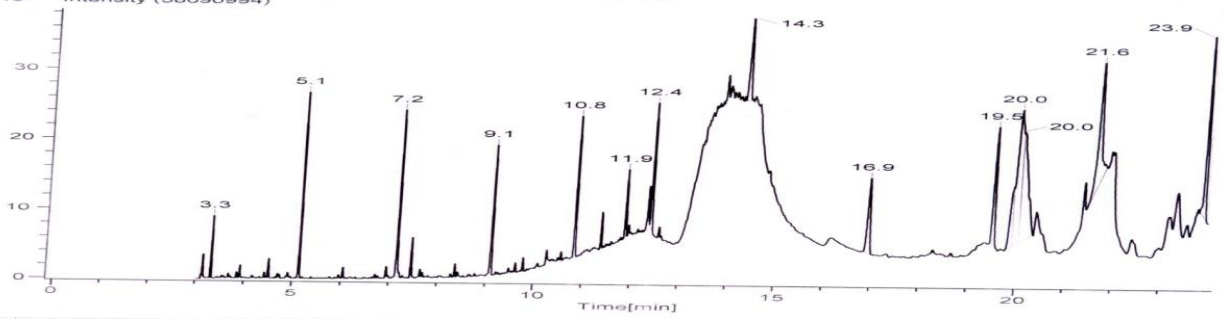
Name of compound	Structure of compound	Retention time	Molecular formula	Molecular weight
9-octadecenoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester		20.0	$C_{21}H_{40}O_4$	356.11
Phthalic acid, isobutyl octadecyl ester		12.3	$C_{30}H_{50}O_4$	474.37
Phthalic acid, butyl tetradecyl ester		12.3	$C_{26}H_{42}O_4$	418.33
Stigmasterol		23.2	$C_{29}H_{48}O$	412.56
9,19-Cyclolanostane-3,7-diol		23.2	$C_{30}H_{52}O_2$	444.37

The compound prediction is based on Dr. Duke's Phytochemical and Ethnobotanical Databases. The results revealed that the presence of 9-Octadecenoic acid-2-hydroxyester, Phthalic acid, isobutyl octadecyl ester, Phthalic acid, butyl tetradecyl ester, Stigmasterol, 9,19-Cyclolanostane-3,7diol. The spectrum profile of GC-MS confirmed the presence of six major components with the retention time 12.4, 14.3, 19.5, 20.0, 21.6, 23.9 respectively. The individual fragmentation patterns of the components were illustrated. The mass spectrum of the compound with retention time 20.0 (Hit 1) 9-Octadecenoic acid-2-hydroxyester gave 8 major peaks (m/z) at 55, 69, 81, 98, 109, 137, 264, 356. The mass spectrum of the compound with retention time 12.3 (Hit 1) Phthalic acid, isobutyl octadecyl ester gave 6 major peaks (m/z) at 57, 149, 167, 205, 223, 419. The mass spectrum of the compound with retention time 12.3 (Hit 2) Phthalic acid, butyl tetradecyl ester gave 6 major peaks (m/z) at 57, 149, 167, 205, 223, 363. The mass spectrum of the compound with retention time 23.2 (Hit 1) Stigmasterol gave 10 major peaks (m/z) at 55, 69, 83, 95, 133, 159, 255, 271, 394, 412. The mass spectrum of the compound with retention time 23.2 (Hit 2) 9,19-Cyclolanostane-3,7diol gave 15 major peaks (m/z) at 57, 69, 81, 87, 95, 109, 121, 133, 149, 201, 261, 313, 393, 411, 426, 444.

In the present study we characterized the chemical profile of *C. halicacabum* L. 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. halicacabum* L. 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. halicacabum* L. for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

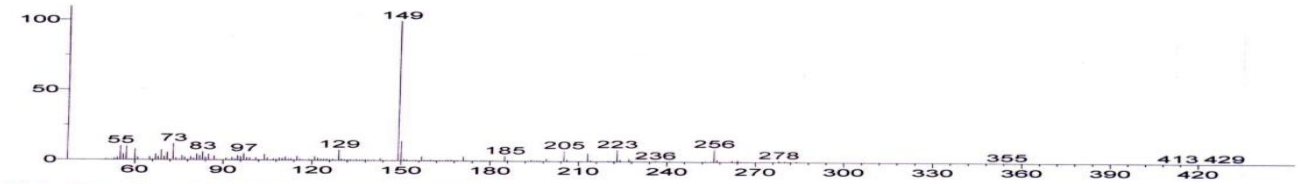
External Sample Id: 24CT Acq. Data Name: E38VIVE25A
Experiment Date/Time: 7/5/2012 10:53:30 AM
Comment: Split 50:1;100-10-220-5M-10-250-3M-30-280-HP5-Toluene
Ionization Mode: EI+



Peak Numb	Time [min]	Type	Peak Width([min]	Area [Intens. * se	Height	Description	Start Point Time[min]	Start Point Height	End Point Time[min]	End Point Height
1	3.3	BB	0.0187	10498401.4	8916790.88		3.3	159976	3.4	118344
2	5.1	BB	0.0241	40203908.2	26656346.1		5.1	245173	5.2	203747
3	7.2	BB	0.0253	40010124.9	23909791.6		7.1	486199	7.2	629427
4	9.1	BB	0.0264	31513781.3	18593020.9		9.0	971045	9.1	944054
5	10.8	BB	0.0270	34802152.6	20223977.0		10.8	3492456	10.9	3702218
6	11.9	BB	0.0255	16108395.6	9694610.75		11.8	6191916	11.9	6738068
7	12.4	BB	0.0285	32003258.5	18020437.2		12.4	8440796	12.4	7002680
8	14.3	BB	0.0463	36148249.3	11797745.5		14.2	26307549	14.4	26271802
9	16.9	BB	0.0628	42014476.6	10653301.5		16.8	4967986	17.0	4542457
10	19.5	BB	0.0458	49002860.1	17010817.3		19.4	6251431	19.6	5256843
11	19.9	BV	0.0691	36330584.9	8627325.75		19.8	5362277	19.9	5872965
12	20.0	VV	0.1131	121771998	19231631.3		19.9	5872965	20.0	6383493
13	20.0	VB	0.1442	136308155	15757253.9		20.0	6383493	20.3	7481201
14	21.6	BB	0.0660	93579828.4	17820237.8		21.4	11615407	21.8	17261121
15	23.9	BB	0.0397	67683323.7	25985107.6		23.8	11159520	24.0	9494607

** Search Report Page 1 of 1 **

Unknown: MDT[CTR[30.0000..30.0000,10,Center,80,0.0,Area];SMT[SA,3]] E38VIVE.7fl\E38VIVE25A.7rw 19.3
Compound in Library Factor = -1032



Hit 1 : Phthalic acid, isobutyl octadecyl ester
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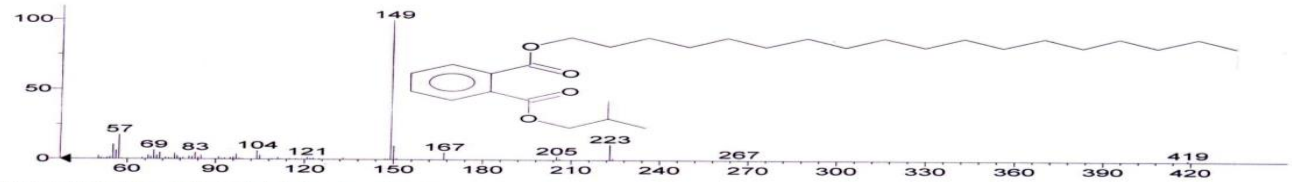

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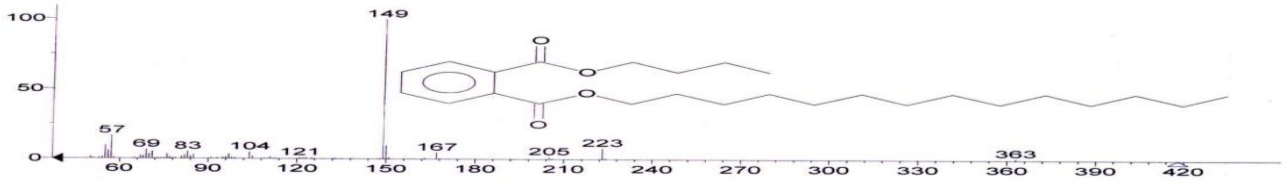

** Search Report Page 1 of 1 **

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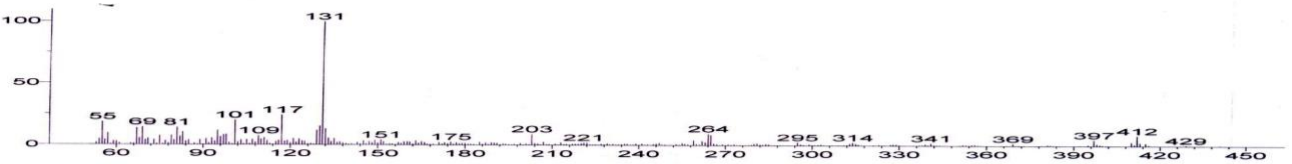

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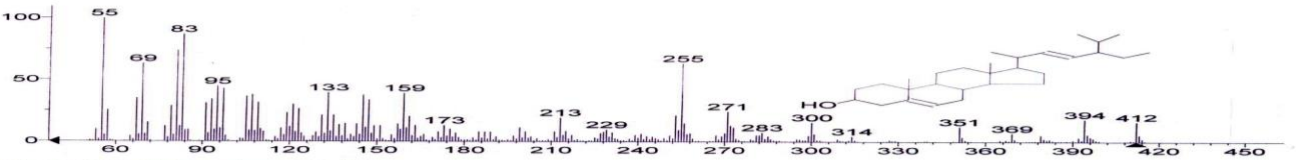

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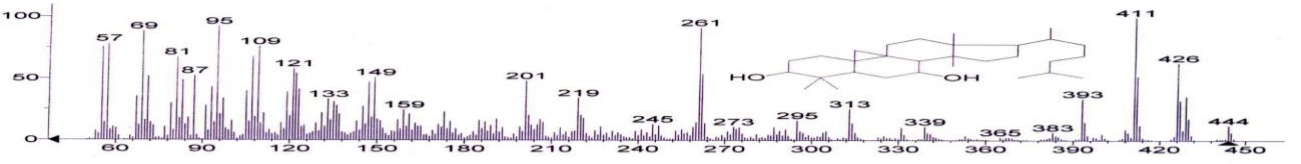
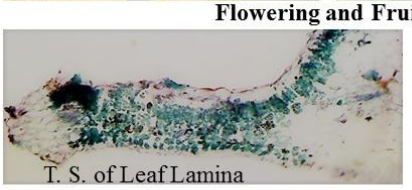
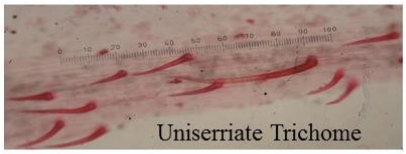
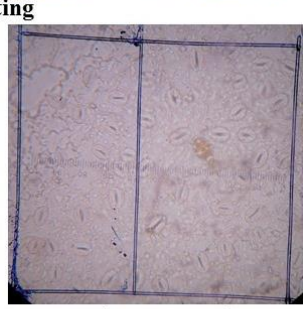




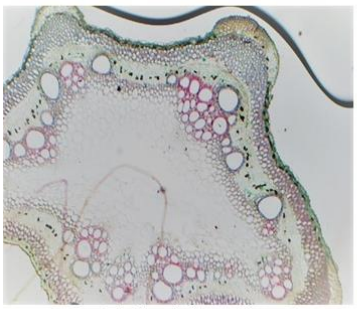
Flowering and Fruiting



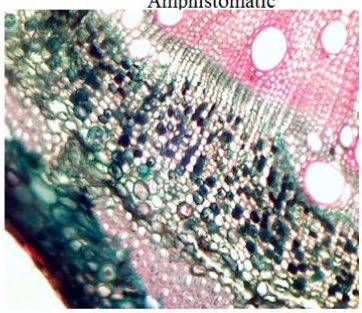




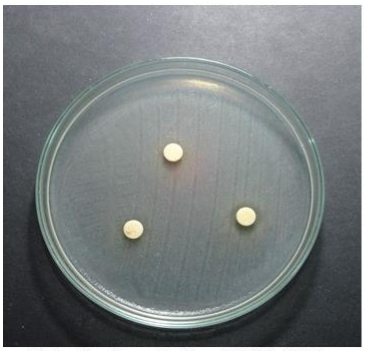
Anomocytic Or Rananculaceous, Amphistomatic



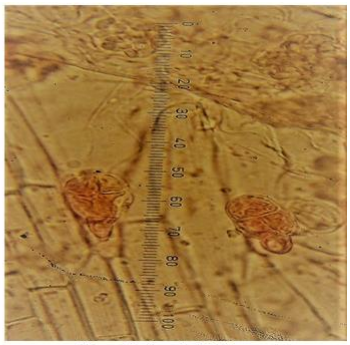
T. S. of Stem



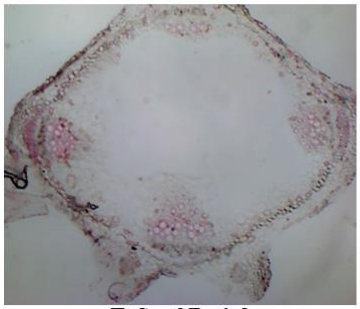
T. S. of Root



Shigella dysenteriae ATCC 9752

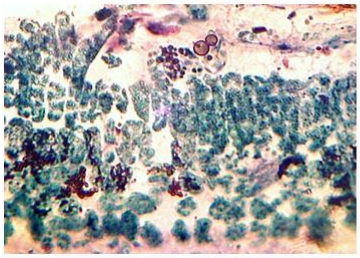


Glandular Trichome

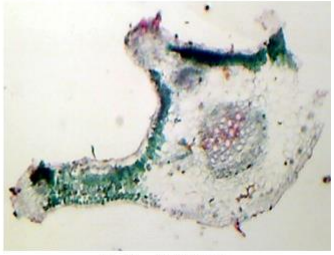


T. S. of Petiole

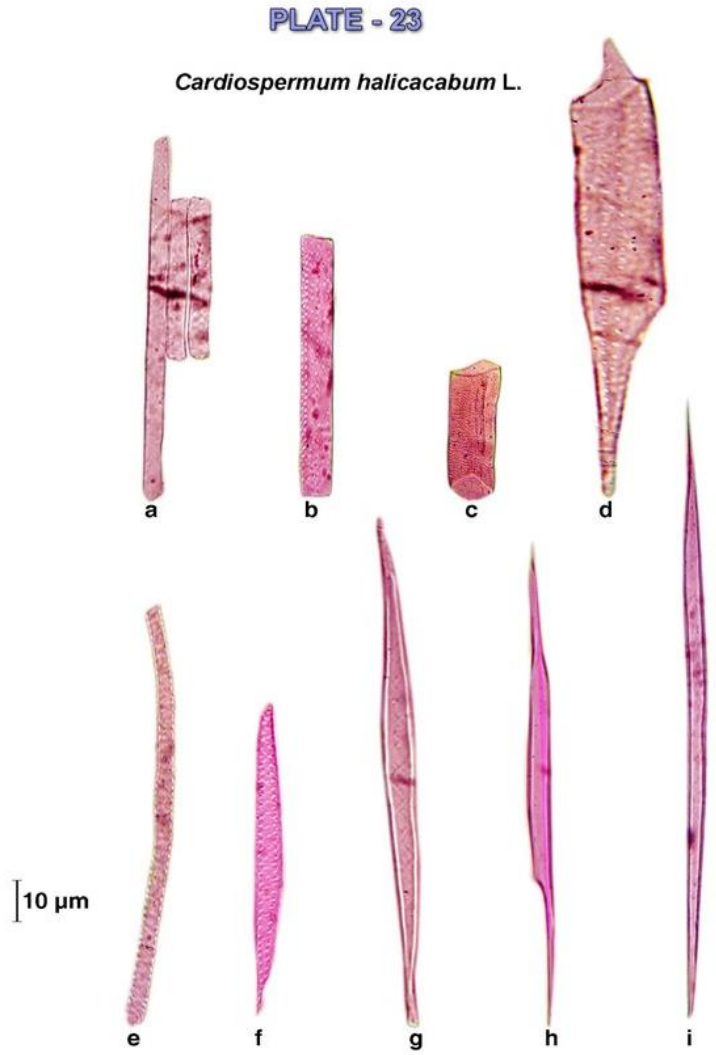
T. S. of stem on 40X



T. S. of leaf Lamina Mesophyll 40X



T. S. of Midrib



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

Conclusion

C. halicacabum is polymorphic taxa and it get confused with 3- different species of it. Adulteration is the major problem which can be get overcome by this type of dimension and measurements that could not get mixed with substitution also. Anatomical arrangements are specific and stomatal index with there types are also distinguish them from other species as anomocytic or ranunculaceous, amphistomatic, upper stomatal index 30.69+4.00, lower stomatal stomatal index 28.70+7.07. Likewise maceration gives definite dimension of vascular elements so the both qualitative and quantitative data is useful in finding out adulteration in herbal products. While phytochemical data such as identification of natural bio-compounds ß-Sitosterol, ɤ-Sitosterol, Stigmasterol with peak area, retention time, mass spectrum and structural elucidation is valuable information to make it possible herbal medicine as compaired with HRD-MS library data. Antimicrobial activity is final goal to check it out whether it is giving any resistance over tested pathogen such as *Shigella dysenteriae* ATCC 9752 shows ca. average 8+0.50 mm zone of inhibition.

Acknowledgment

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References

1. Ara *et al.* Ara Arjumand, Srinivas Reddy. K, C.S. Reddy, International Journal of Pharmaceutical Sciences and Nanotechnology. 2009; 2(1):487-492.
2. Khare CP. Indian Medicinal Plants An Illustrated Dictionary, Springer, 2007.
3. Cutler DF. Applied Plant Anatomy, Longman Inc., New York, 1978.
4. Daniel M Daniel. Medicinal Plants Chemistry and Properties, Oxford and IBH Publication, 2006.
5. Deepak Acharya, Anshu Shrivastava. Indigenous Herbal Medicines: Tribal Formulation and Traditional Herbal Practices, Aavishkar Pub. Distributers Jaypur, India, 2008.
6. Esau K. *Plant Anatomy II-edi.* John wiley New York, 1965.
7. Evans WC. Trease and Evans Pharmacognocy, 14th edn. W. B. Saunders, London, 1996.
8. Girish *et al.* Girish H.V., M.S. Sudarshana and E. Rati Rao, Advances in Biological Research. 2008; 2(1-2):34-38.

9. Harborne JB. Phytochemical *Methods*, Champman and Hall, New York, USA, 1984, 33-119, 182-195.
10. Khandelwal KR. *Practical Pharmacognocy* Techniques and Experiments, Nirali Prakashan, 14th Edn, 2005, 30-51.
11. Naik VN Naik. Flora of Marathwada. Amrut Prakashan, Aurangabad (M.S.) India. Fl. Marathwada. 1998; 1(2):228.
12. Prajapati Narayan Das SS Purohit, Arun K Sharma, Tarun Kumar. A Handbook of Medicinal Plants A Complete Source Book, Agrobios India, 2003.
13. Rao *et al*. Roy Piyush, Plant anatomy, New Central Book Agency, Pvt. Ltd. Kolkata, India, 2006.
14. Roy Piyush. Plant anatomy, New Central Book Agency, Pvt. Ltd. Kolkata, India, 2006.