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Pharmacognosy and phytochemical evaluation of *Hygrophila auriculata* (Schumach.) Heine. root

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ABSTRACT

The genus *Hygrophila auriculata* (Schumach.) Heine belongs to family Acanthaceae found in India. Commonly known *Kokilaksa*, as in Sanskrit. Root and seeds used as tonic, for asthma and dysentery (Bhuktar, 2000). Decoction of the root is used as a diuretic in dropsy. The root is considered cooling, bitter, tonic and diuretic, and is used in rheumatism, urinary affections, and anasarca. The present study was carried out to investigate morphological, microscopical and phytochemical screening of root revealed that the presence of 2-furancarboxaldehyde,5-(hydroxymethyl), oleic acid, elaidic acid, isopropylester, 5-(hydroxymethyl)-2 (dimethoxymethyl) furan, methyl 2,6-difluorobenzoate. The result study was useful for drawing pharmacognostic parameters also detected phytoconstituent may proceed to find a novel drug for this species.

Keywords: *Hygrophila auriculata*, Pharmacognosy, Acanthaceae.

INTRODUCTION

Hygrophila auriculata (Schumach.) Heine belongs to family Acanthaceae found in India. It is distributed in tropical and subtropical region in India in literature. The plant is used in cancer and tubercular fistula (Yusuf *et al.* 2009)^[9]. Root and seeds used as tonic, for asthma and dysentery (Bhuktar, 2000). The leaf, root and seed of this plant are traditionally used for the treatment of inflammation, jaundice, hepatic obstruction, urinary infection, oedema, gout, diabetes, bacterial infection etc. (Chopra *et al.*, 1986; Nadkarni, 1978)^[2, 5].

Morphology of plants

Hygrophila auriculata (Schumach.) Heine Kew Bull. 16: 172. 1963 *Hygrophilaschulli* (Buch- Ham.) M. R. & S. M. Almeida in J. Bombay Nat. Hist. Soc. 83 (Suppl.): 221. 1986; Naik, Fl. Marathwada 674. 1998; Londhe in Singh *et al.*, Fl. Maharashtra St. Dicot. 2: 636. 2001. *Behelschulli* Buh.-Ham. in Trans. Linn. Soc. Lond. 14: 289. 1825. *Asteracantha longifolia* (L.) Nees in Wall. Pl. Asiat. Rar. 3: 90. 1832; Cooke, Fl. Pres. Bombay 2: 428. 1958 (Repr.). *Hygrophila spinosa* Anders. In Thw. Enum. Pl. Zeyl. 225. 1860; Cl. in Hook. f. Fl. Brit. India 4: 408. 1884.

Vernacular name

Marathi: *Kolshinda*, *Talimkhana*, Sanskrit: *Kokilaksa*, Bengali: *Kuliyakhara*, Gujrati: *Ekharo* Hindi: *Talmakhana* Kannada: *Kolavali*, Marathi: *Talikhana*, *Kalsunda* Tamil: *Golmidi*, Urdu: *Talmakhana*

Description

Herbs, 40-100 cm tall with unbranched, subquadrangular stems with numerous fasciculate, swollen node, hispid with long hairs. Leaves sub-sessile, lanceolate, 6-15×1.5-3 cm, acute, hairy, in whorls of 6 at each node, the two outer one much larger than the four inner ones. Thorns from the axils of leaves sharp, 2-3 cm long, yellowish-brown. Flowers in axillary clusters of eight at each node in 4 pairs. Bracts lanceolate, hairy and ciliate, like the leaves; bracteoles linear-lanceolate, 1.5-2 cm long, with hyaline margins in the lower part, hairy and ciliate with long white hairs. Calyx 4 partite; upper sepals broader unequal, longer than the other three, all linear lanceolate, 1.2-2 cm long, with hairy on the back and hyaline ciliate margin. Corolla purple-blue, 2-3 cm long, bilipped; tube 11-13 mm long, swollen at top; stamens didynamous 4; filaments glabrous. Ovary 2 celled with 4 ovules, capsules linear-oblong, 4 seeded 5-7 mm long, pointed. Seeds, ovate, compressed, hairy, hygroscopic, black.

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Soil type : Wet soil of Marshy places.
Locality : In all districts.
Flowers and fruits : June to February.
Exsiccata : Aurangabad. SDS 115.

MATERIALS AND METHODS

The root of *Hygrophila auriculata* were collected from Aurangabad Maharashtra state the plant was authenticated and voucher specimen were deposited at Vivekanand College Sardar Dalipsingh Commerce and Science College Aurangabad Maharashtra state

Maceration

Root were studied by maceration techniques. The root pieces were boiled in Jeffery fluid (chromic acid 10% and nitric acid 10% in (1:1 proportion) (Khandelwal 2005)^[4]. The dimensions of the cells were measured with the help of microscope and by micrometry

Microscopy

Qualitative microscopic evaluation was carried out by taking free hand transverse section of fresh root. Section were dehydrated with different alcohol grade and stained with safranin and light green these permanent preparation were observed in microscope (Khandelwal 2005)^[4] of *Hygrophila auriculata*.

Plant sample extraction

25 gram of powder drug was extracted with methanol solvent using soxhlet extractor for 18 hours at 65 °C. The extracts were filtered through a Whatman filter paper no. 42 (125 mm) and concentrated at 40°C by using an evaporator and stored in amber color bottle at 4 °C. These extracts were send to *Sophisticated Analytical Instrumentation Facility, Indian Institute of Technology Bombay, Powai Mumbai, India*. For GC-MS (Gas chromatography mass spectroscopy)

GC-MS analysis

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. Inter face temperature is 250, Ion source is at 200, Analysis was done by using E+ ionization with 70ev, The MS is AccuTOF GCV, Column through the sample passes is HP-5. The MS detection was completed in 36 minutes. The detection employed the NIST Ver. 2.0-year 2005 library.

RESULT AND DISCUSSIONS

Transverse section of root shows circular in outline. Epidermis 2 - 3 layered composed of cubical to squarish thin walled cells ca 20 - 50 × 30 - 70 µm with unicellular hairs. Cortex composed of rounded thin walled cells ca 30 - 90 × 50 -100 µm forming large intercellular spaces most of these cells separated tangentially forming air chambers. Endodermis single layered composed of thin walled oval shaped cells ca 18 -20 × 20 - 50 µm. Pericycle single layered composed of circular to oval shape cells ca 18 - 20 × 20 - 28 µm. Vascular bundle tetra archorpena archxylem composed of vessels arranged in radial rows ca 30 - 70 × 30 - 80 µm with xylem fibres.

Phloem composed of phloem parenchyma small cells ca 10-12 × 10-13 µm. Medullary rays multiseriate runs up to the secondary cortex ca 20 - 40 × 20 -50 µm. Pith small composed of thin walled irregular parenchymatous cells ca 20- 900 × 20 - 120 µm. (Photo plate -12)

Maceration

Parenchyma cells

Cells are thick walled, squarish, rhomboidal, rectangular, pits many, circular, oval, distributed all over the cell, cell wall continuous ranges 40 - 90 x 30-70 µm and average 67 x 45 µm. Second type cells are thin walled, squarish, rectangular, pits few, circular to oval, distributed all over the cell, cell wall continuous, ranges 60 -180x 28-30 µm and average 116 x 30 µm (fig.5.a,b)

Fibres

Simple long, slender, tapering and sharply pointed at both ends, outline entire ranges 350 - 970 x 20-30 µm and average 642 x 26 µm (fig.5.c)

Tracheids

Tracheids long slender ends blunt or pointed or forked at one end, pits few many, elongated in one-many rows, alternate, outline entire ranges 180 - 500 x 20 - 32 µm 343 x 27 µm average (fig.5.d,e)

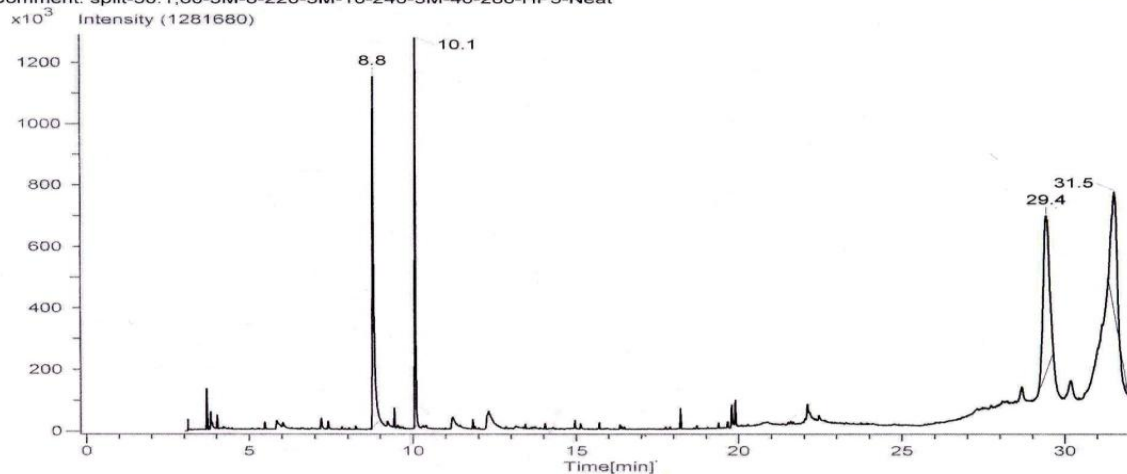
Vessels

Pitted – Vessel element short to long, end wall horizontal or oblique with simple perforation pits alternate, circular to oval, beaked at one or both ends or absent ranges 230 - 530 x 40-70 µm and average 371 x 46 µm (fig.5 f,g,h)

GC-MS analysis

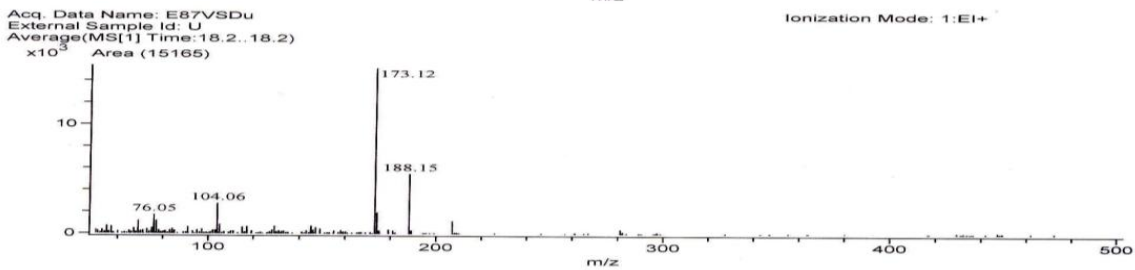
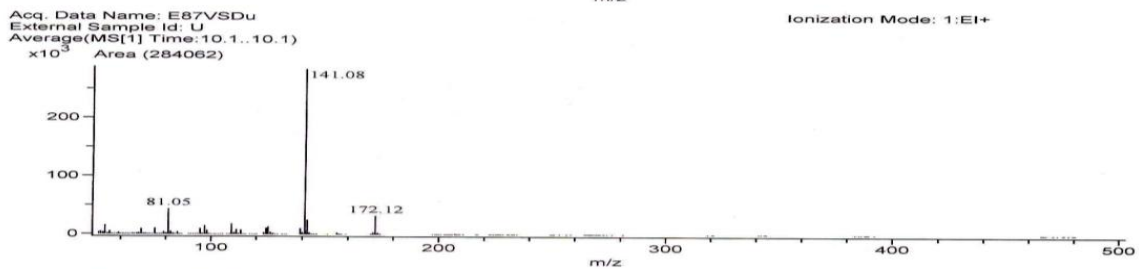
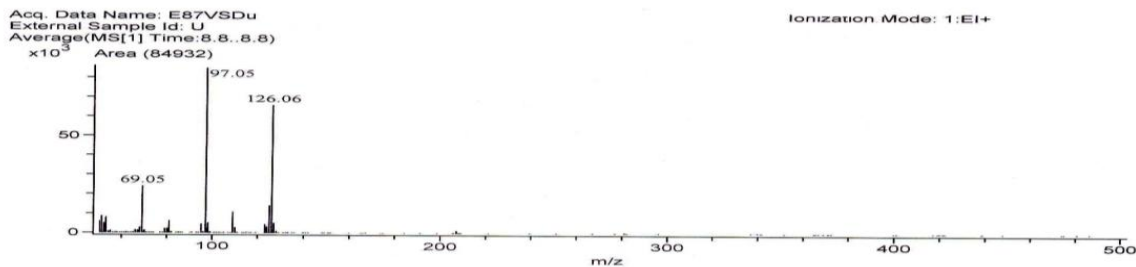
The results revealed that the presence of d- The compound prediction is based on Dr. Duke's Phytochemical and Ethnobotanical Databases. The results revealed that the presence of 2-furancarboxaldehyde, 5-(hydroxymethyl), oleic acid, elaidic acid, isopropylester, 5-(hydroxymethyl)-2 (dimethoxymethyl) furan, methyl 2,6-difluorobenzoate. The spectrum profile of GC-MS confirmed the presence of four major components with the retention time 8.8, 10.1, 29.4, 31.5 respectively (Figure 16A). The individual fragmentation patterns of the components were illustrated in Figure 16 B-F. The mass spectrum of the compound with retention time 8.8 (Hit 1) gave 6 major peaks (m/z) at 53, 69, 81, 97, 109, 126 (Figure 16B). The mass spectrum of the compound with retention time 10.1 (Hit 1) gave 9 major peaks (m/z) at 53, 69, 75, 81, 95, 109, 124, 141 (Figure 16C). The mass spectrum of the compound with retention time 10.1 (Hit 2) gave 14 major peaks (m/z) at 50.63, 68, 74, 81, 87, 93, 101, 113, 127, 141, 153, 172 (Figure 16D). The mass spectrum of the compound with retention time 22.1 (Hit 1) gave 11 major peaks (m/z) at 55, 69, 83, 97, 111, 125, 151, 180, 222, 264, 282 (Figure 16E). The mass spectrum of the compound with retention time 22.1 (Hit 1) gave 16 major peaks (m/z) at 55, 69, 83, 97, 111, 125, 139, 165, 193, 222, 245, 264, 282, 324 (Figure.16.F).

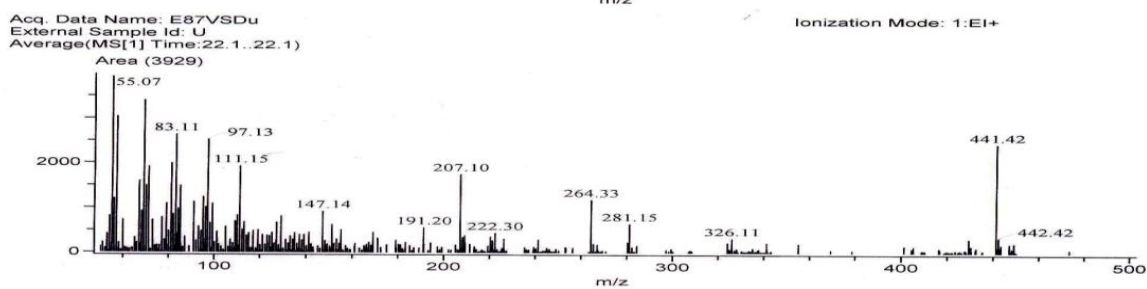
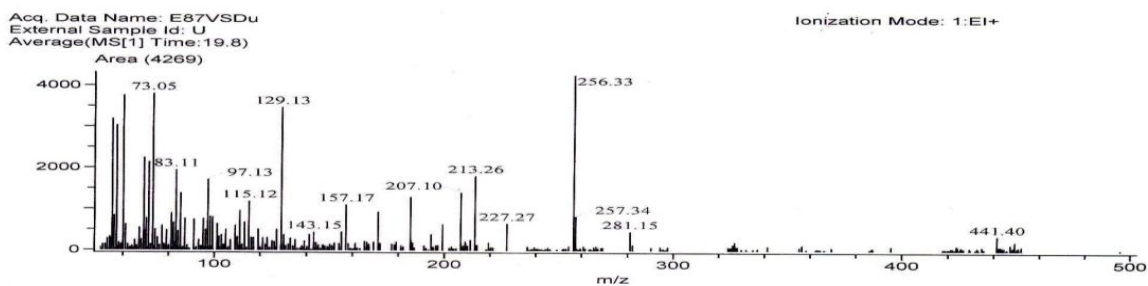
External Sample Id: U Acq. Data Name: E87VSDu
 Experiment Date/Time: 4/2/2012 11:48:26 AM Ionization Mode: EI+
 Comment: split-50:1,80-3M-8-220-5M-10-240-3M-40-280-HP5-Neat



Peak Num	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	8.8	BB	0.0416	3822746.04	1141286.25		8.7	5700	9.0	37300
2	10.1	BB	0.0290	2457463.84	1267046.94		10.0	7917	10.1	23361
3	29.4	BB	0.2153	6834380.97	505733.67		29.1	112581	29.6	255954
4	31.5	BV	0.2241	4094124.32	396065.17		31.4	493914	31.9	161101
5	31.9	VB	-0.0426	-74832.54	-24946.95		31.9	161101	32.0	99473

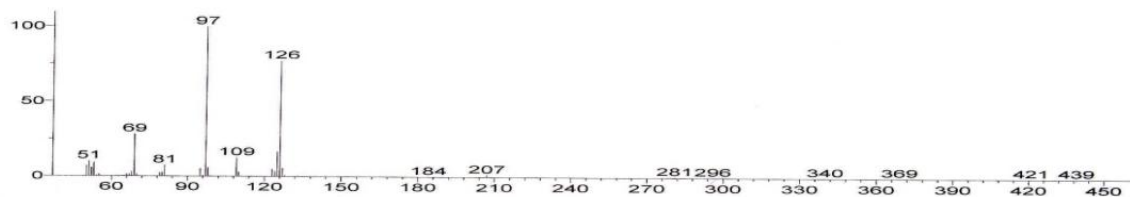
Fig 16 A



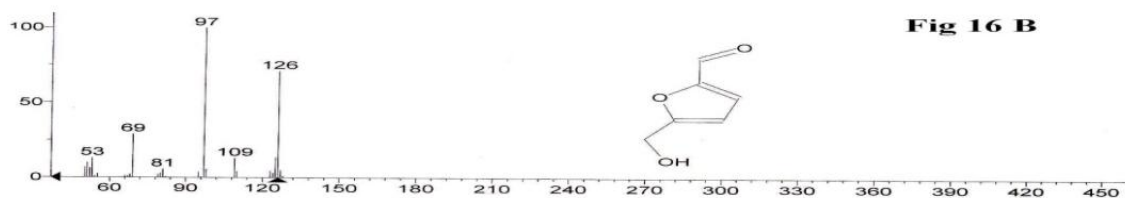


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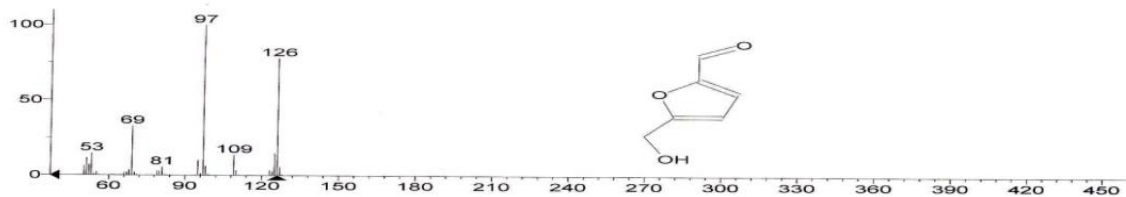
Unknown: MDT[CTR[30.0000..30.0000,10,Center,80,0,0,Area];SMT[SA,3]] E87VSD.7f\E87VSDu.7rw 8.8 min
 Compound in Library Factor = 671



Hit 1 : 2-Furancarboxaldehyde, 5-(hydroxymethyl)-
 C6H6O3; MF: 946; RMF: 963; Prob 97.4%; CAS: 67-47-0; Lib: replib; ID: 12795.

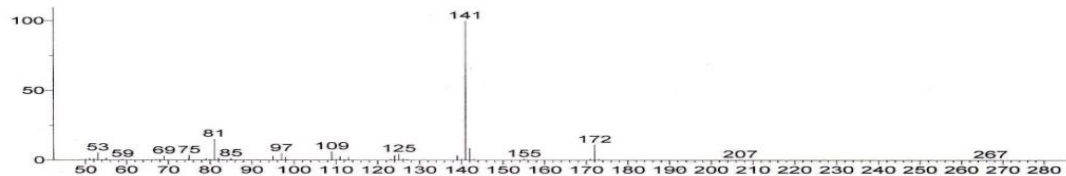


Hit 2 : 2-Furancarboxaldehyde, 5-(hydroxymethyl)-
 C6H6O3; MF: 911; RMF: 922; Prob 97.4%; CAS: 67-47-0; Lib: mainlib; ID: 60271.



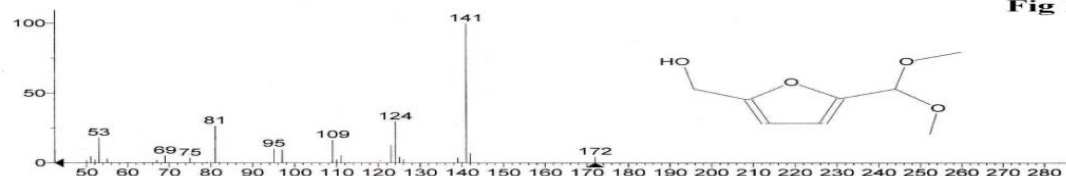
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Compound in Library Factor = -207



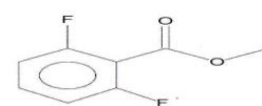
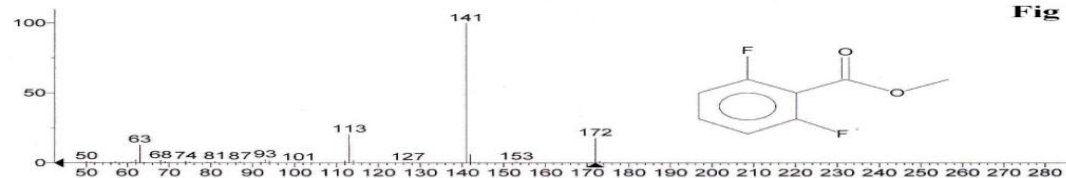
Hit 1 : 5-(Hydroxymethyl)-2-(dimethoxymethyl)furan
C8H12O4; MF: 764; RMF: 793; Prob 51.8%; CAS: 90200-14-9; Lib: mainlib; ID: 103719.

Fig 16 C



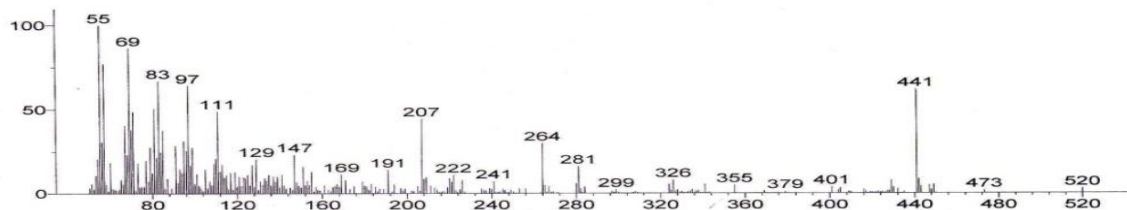
Hit 2 : Methyl 2,6-difluorobenzoate
C8H6F2O2; MF: 668; RMF: 772; Prob 4.12%; CAS: 13671-00-6; Lib: replib; ID: 19171.

Fig 16 D



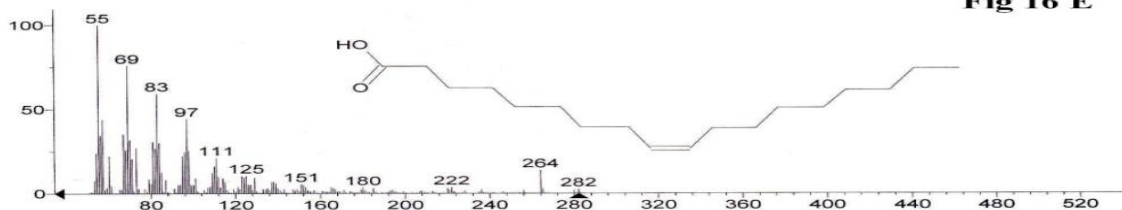
** Search Report Page 1 of 1 **

Unknown: MDT[CTR[30.0000..30.0000,10,Center,80,0,0,Area];SMT[SA,3]] E87VSD.7f1E87VSDu.7rw 22.1m
Compound in Library Factor = -1679



Hit 1 : Oleic Acid
C18H34O2; MF: 644; RMF: 757; Prob 4.58%; CAS: 112-80-1; Lib: replib; ID: 4483.

Fig 16 E



Hit 2 : Elaidic acid, isopropyl ester
C21H40O2; MF: 642; RMF: 766; Prob 4.23%; CAS: 22147-34-8; Lib: mainlib; ID: 17860.

Fig 16 F

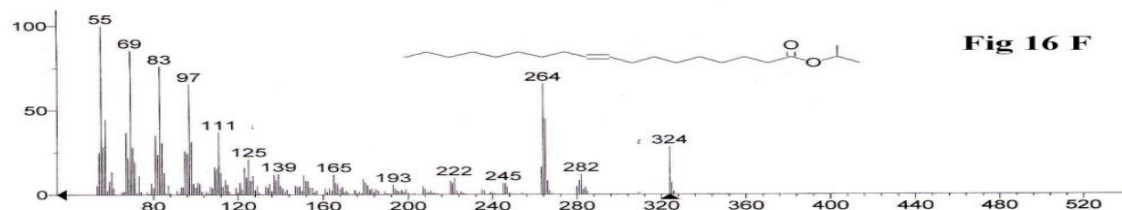


Figure 1: GC-MS Chromatogram of *Hygrophilauriculata* (Schumach.) Heine

Name of compound	Structure of compound	Retention time	Molecular formula	Molecular weight
2-furancarboxaldehyde, 5(hydroxymethyl)		8.8	C ₆ H ₆ O ₃	126.03
5-(Hydroxymethyl)-2-(dimethoxymethyl)furan		10.1	C ₈ H ₁₂ O ₄	172.18
Methyl, 2,6-difluorobenzene		10.1	C ₈ H ₆ O ₂ F ₂	172.14
elaidic acid, isopropyl ester		22.1	C ₂₁ H ₄₀ O ₂	324.54
oleic acid		22.1	C ₁₈ H ₃₄ O ₂	282.44

Figure 2: Components identified in roots of *Hygrophila auriculata* (Schumach.) Heine



Figure 3: photograph of *Hygrophila auriculata* (Schumach.) Heine.

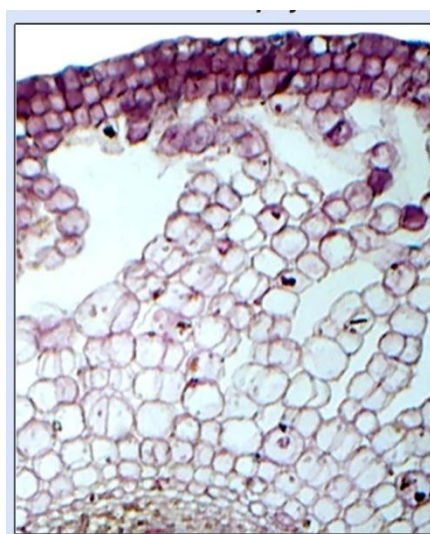
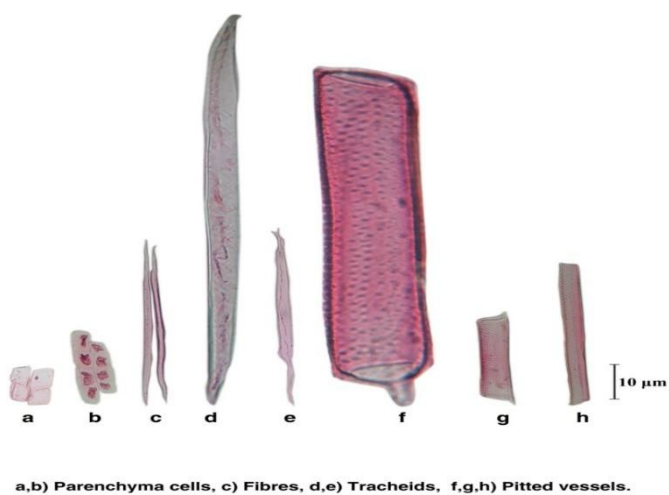


Figure 4: T. S of root of *Hygrophila auriculata* (Schumach) Heine



a,b) Parenchyma cells, c) Fibres, d,e) Tracheids, f,g,h) Pitted vessels.

Figure 5: Maceration of root of *Hygrophila auriculata* (Schumach) Heine.

Discussion

In the present investigation various standardization parameters such as morphology, anatomy, maceration, phytochemical study could be help in authentication of root drug of *Hygrophila auriculata* the result of present study will also serve as reference material in preparation of monograph. However isolation of detected phytoconstituent may proceed to find a novel drug.

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