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Isolation and Characterization of Triterpenoids in Cuticular wax of leaves of *Helicanthus elasticus* Linn. (Loranthaceae) parasitic on *Memecylon umbellatum* Burm.f. (Melastomataceae)

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Abstract

The triterpenoid pattern of cuticular wax of leaves of *Helicanthus elasticus* Linn. (Loranthaceae) parasitic on *Memecylon umbellatum* Burm.f. (Melastomataceae) was studied and compare with standard triterpenoids such as lupeol, olenolic acid and betulin. From HPTLC analysis further study focus on isolation of some triterpenoids from cuticular wax of leaves of *Helicanthus elasticus* Linn. (Loranthaceae). The cuticular extract was subjected to column chromatography and isolated compound characterized by UV, IR, HPTLC and MS studies and compare with standards. From the results cuticular wax of *Helicanthus elasticus* Linn shows presence of lupeol. In future it may help in tracing some major triterpenoids because it having promising pharmacological activity.

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Key words:

Helicanthus elasticus, cuticular wax, column chromatography, lupeol.

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Introduction:

Chemotaxonomic tracing of genus *Loranthus,* endemic to Western Ghat, is expected to yield useful pharmacological activities. Even as some literature has accumulated on the medicinal properties of Loranthaceae plants [1, 2, 3, 4, 5, 6] as such very rare information available about *Helicanthus elasticus*. This hemiparasite is found on different host plants

such as *Mangifera indica, Citrus maxima, Syzium cumini, Memecylon umbellatum*. In indigenous system of medicine the leaves of plant is used to check abortion; also in vesicle calculi and kidney affections [7]. Nevertheless, plant has been investigated for its cytotoxic activity [8].

All primary aerial surfaces of plants are covered by a cuticle. One of the major functions of the cuticle is the formation of an efficient barrier against unregulated water loss [9]. Cuticle is a thin, hydrophobic, and flexible membrane (0.1–10 μm) that is composed of a polymer matrix (cutin) and associated solvent-soluble lipids (cuticular waxes). The cuticular waxes consist of species-, organ- and tissue-specific homologous series of very-long-chain aliphatics (e.g. fatty acids, alcohols, aldehydes, alkanes, and esters) with characteristic chain length distributions and varying proportions of cyclic compounds (e.g. pentacyclic triterpenoids or phenyl propanoids) [10].

Significance of cuticular wax

Cuticular waxes constitute the main barrier limiting the transport across the plant atmosphere interface. This property allows waxes to control cuticular transpiration [11], foliar uptake of xenobiotics [12] and resistance against fungi [13]. Permiability of cuticles differs greatly among plant species and both the chemistry and the structure of cuticular waxes are responsible for these differences [14].

Triterpenoids

Triterpenoids are abundant in nature, particularly in resins and may occur as either esters or glycosides [15]. Chemically triterpenoids are compounds with a carbon skeleton based on six isoprene units which are derived biosynthetically from the acyclic C_{30} hydrocarbon, squalene. They have relatively complex cyclic structures, most being either alcohols, aldehydes or carboxylic acids. Triterpenoids can be divided into at least four groups of compounds, true triterpenes, steroids, saponins and cardiac glycosides [16].

2. Materials and Methods

2.1 Collection of plant material

The mistletoe, *Helicanthus elasticus* Linn. Parasitic on *Memecylon umbellatum* Burm.f. (Melastomataceae) were collected from Western Ghats (Latitude 17° 55' 0N and Longitude 73° 40' 0E and at altitude 1352 m) in February 2006. From this infected and damaged leaves were discarded carefully and select those leaves with fully mature, completely dried with uniform size and shape for cuticular extraction. The specimens of host and parasite plants were authenticated from Botanical Survey of India (BSI), Pune (Maharashtra). The plant specimen (Voucher no. LOT-1) was authenticated by Dr. P.S. N. Rao (Botanical Survey of India, Pune).

2.2 Extraction Methodology of cuticular wax of leaves [17]

Approximately 10 g dried leaves were used for the cuticular wax study. Total cuticular waxes were obtained by dipping leaves/ scales in chloroform for 30 sec at room temperature and subsequently in hot chloroform for 120sec. The combined extract was filtered by Whatman filter paper. Finally the organic solvent was evaporated at room temperature and waxes were stored at room temperature until further study. The yield of waxy material was 2.4009 gm.

2.3 Chemicals and reagents

oleanolic acid, betulin, lupeol (Regional Research Laboratory, Jammu; India), n-Hexane, Dichloroethane, Methanol, Tolune, Ethyl acetate, Benzene, Ethyl acetate(all reagents of analytical grade, E-Merck) and Aluminium precoated silica plates (E-Merck).

2.4 Apparatus:

For chromatographic profile use HPTLC (Camag, Switzerland) with Linomet V applicator and scanner III (WinCAT software).

2.5 The Triterpenoid pattern:

The triterpenoid pattern of cuticular waxes of *Helicanthus elasticus* Parasitic on *Memecylon umbellatum* was recorded using HPTLC (Camag, Switzerland) using pre-coated silica gel plates (Merck). The chromatogram was evaluated densitometrically using win CAT software and for comparison oleanolic acid, betulin and lupeol was used as a reference standard and triterpenoids were traced after treatment with Anisaldehyde-sulphuric acid reagent. Following specifications used for triterpenoid pattern.

2.5.1 Preparation of Sample Solution: 1mg of cuticular extract of *Helicanthus elasticus* dissolved in 1 ml of chloroform to give conc.1000 µg/ml, filter by using, Whatman filter paper.

2.5.2 Preparation of Reference standard solution:

Oleanolic acid: 1mg oleanolic acid (Regional Research Laboratory, Jammu; India) dissolve in 1ml chloroform to give 1000 µg/ml.

Betulin: 1mg betulin (Regional Research Laboratory, Jammu; India) dissolve in 1ml chloroform to give 1000 µg/ml

Lupeol: 1mg lupeol (Regional Research Laboratory, Jammu; India) dissolve in 1ml chloroform to give 1000 µg/ml.

2.5.3 Development of HPTLC Techniques:

Alluminium precoated plates of dimension (10×10 cm, E-Merck) with 0.2cm thickness were used for application of both sample and standard solution. The plates were developed in specific solvent system in Twin Trough Chamber. Before develop all plates twin trough chambers saturated with solvents for 30min. All plates were developed as per standard procedure up to 8cm. The TLC plates were air dried and triterpenoids were traced by treatment with Anisaldehyde-sulphuric acid reagent. The plate was kept in oven at 110° C for 5min and detection was carried out by scanning at 366nm.

2.5.4 Estimation of Oleanolic acid, Betulin and Lupeol in cuticular waxes of *Helicanthus elasticus*.

Stationary Phase: Alluminium precoated plates of dimension (10×10cm, E-Merck), Mobile Phase: n-Hexane: Dichloroethane: Methanol (32:32.1.6), Reference standard**:** Oleanolic acid 1mg/ml (1000 µg/ml).Sample Preparation (cuticular wax): 1mg/ml (1000 µg/ml).

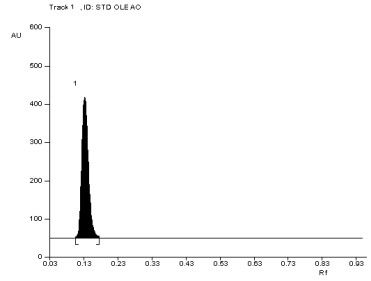
Stationary Phase: Alluminium precoated plates of dimension (10×10 cm, E-Merck), Mobile Phase: n Tolune: Ethyl acetate (90:10), Reference standard**:** Betulin 1mg/ml (1000 µg/ml).Sample Preparation (cuticular wax): 1mg/ml (1000 µg/ml).

Stationary Phase: Alluminium precoated plates of dimension (10×10 cm, E-Merck), Mobile Phase: Benzene: Ethyl acetate (9.7: 0.7), Reference standard**:** Lupeol 1mg/ml (1000 µg/ml).Sample Preparation (cuticular wax): 1mg/ml (1000 µg/ml).

Chromatography of cuticular waxes of *Helicanthus elasticus*

Fig 1: Peak and peak areas of HPTLC of std oleanolic acid and cuticular wax material of *Helicanthus elasticus* after derivatization.

HPTLC chromatograms of standard oleanolic acid after treatment with Anisaldehyde-H_2SO_4



HPTLC chromatograms of cuticular extract after treatment with Anisaldehyde-H_2SO_4

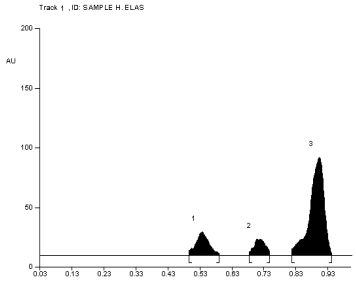


Table 1: Peak and peak areas of HPTLC of std olinolic acid and cuticular wax material of *Helicanthus* elasticus after derivatization

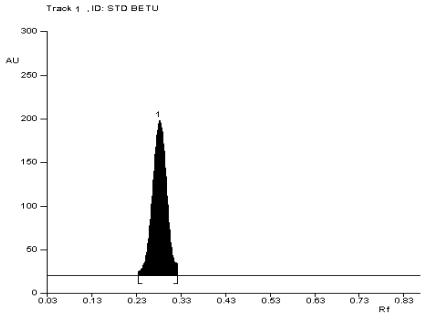
Name	Track no.	Max Rf of Peak	Area
Oleanolic acid	1	0.13	6028.3
Cuticular extract	1	0.53	668.4
	2	0.71	416.6
	3	0.90	3031.3

Table 2: Peak and peak areas of HPTLC of std betulin and cuticular wax material of *Helicanthus* elasticus after derivatization.

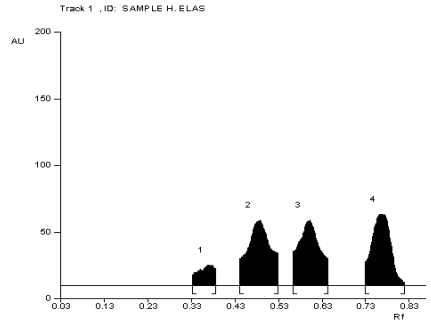
Name	Track no.	Max Rf of Peak	Area
Betulin	1	0.28	4704.8
Cuticular extract	1	0.38	482.7
	2	0.48	2169.9
	3	0.60	1984.5
	4	0.77	2003.9

Fig 2: Peak and peak areas of HPTLC of std betulin and cuticular wax material of *Helicanthus elasticus* after derivatization.

HPTLC chromatograms of standard betulin after treatment with Anisaldehyde-H_2SO_4



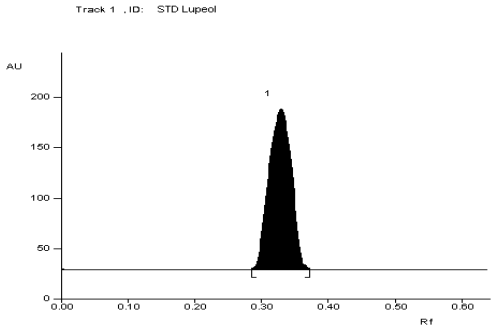
HPTLC chromatograms of cuticular extract after treatment with Anisaldehyde-H_2SO_4.



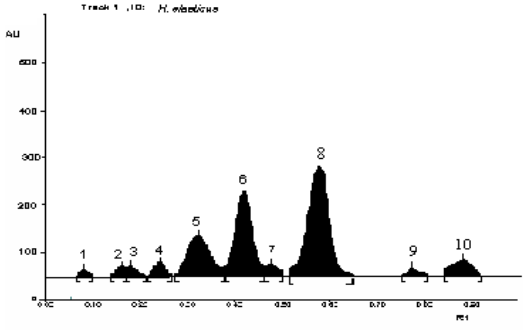
Name	Track no.	Max Rf of Peak	Area
Lupeol	1	0.33	4704.8
Cuticular extract	1	0.08	110.3
	2	0.16	165.3
	3	0.18	273.8
	4	0.23	561.4
	5	0.33	3928.7
	6	0.42	5257.6
	7	0.48	517.6
	8	0.58	8392.7
	9	0.77	306.5
	10	0.88	1005.9

Fig 3: Peak and peak areas of HPTLC of std Lupeol acid and cuticular wax material of *Helicanthus elasticus* after derivatization.

HPTLC chromatograms of standard Lupeol acid after treatment with Anisaldehyde-H_2SO_4



HPTLC chromatograms of cuticular extract after treatment with Anisaldehyde-H_2SO_4.



3. Isolation and characterization of compound (CW1) from cuticular wax of *Helicanthus elasticus*

The cuticular wax (10gm) obtained from leaf of *Helicanthus elasticus* obtained from above procedure was subjected to column chromatography (200g silica gel, #60-120) packed in hexane. Elution was started with hexane and polarity was gradually increased with chloroform. The collected fractions (50ml each) were monitored by TLC using n-Hexane:Chloroform (3:7) solvent system and visualization was done by Anisaldehyde-H2SO4. From all, fractions numbers (22-47) were combined and processes for preparative chromatography. For preparative chromatography the glass plates were coated with the silica gel G. The plates were activated at 110°c. The sample was applied in the form of bands and eluted in the TLC chamber. Fractions were concentrated and single bands were applied on plate. After development of plate; developed band was scraped correspondence to the particular R_f value. After separation of single desirable compound from the silica it is dried and crystallized using Chloroform.

The pure compound note as cw1 (55mg) obtained from preparative chromatography was subjected to UV, IR and MS studies. The UV absorption spectrum of the compound was recorded in range of 200-400 nm on Shimadzu UV-2450 spectrophotometer. The IR spectrum was recorded on FT-IR 8400S by press pellet technique with AR grade KBr. The R_f and retention time data of both, isolated compound and standard reference lupeol were recorded by using HPTLC. The R_f of compound and was recorded on HPTLC (Camag, Switzerland) with Linomet V applicator and scanner III (WinCAT software) using solvent system of benzene: ethyl acetate (9.7:0.7). The MS spectra were obtained using, turbo spray ionization source (ESI), at electron energy of 70V, at source temperature 190°C.

FTIR spectrum: The IR spectrum of the compound was recorded on FT-IR 8400S by press pellet technique with AR grade KBr. IR (KBr), ν_{max} 3326.98, 2937.38, 2856.38, 1637.45, 1465.8, 1450.37, 1379.01, 1305.72 cm^{-1}. An IR spectrum was matched with the standard lupeol IR spectra. (Fig. 8)

4.4 Mass analysis: The Turbo spray MS spectra were obtained using, turbo spray ionization source (ESI), at electron energy of 70V, at source temperature 190°C. The ion energy at 1.7V and at pressures < 1.7e − 4 Torr and 1.1e − 5 Torr penning was used for MS analysis. The LC-MS shows its protonated molecular ion at m/z 426 (M+ H^+).

Table 4: Interpretation of Mass spectra of compound CW1

Sr. No	m/e	Fragments
1	107	C_8H_{11}+
2	121	C_9H_{13}+
3	135	$C_{10}H_{15}$+
4	161	$C_{12}H_{17}$+
5	175	$C_{13}H_{19}$+
6	207	$C_{14}H_{23}O$+
7	271	$C_{19}H_{27}O$+
8	315	$C_{22}H_{35}O$+
9	339	$C_{24}H_{35}O$+
10	358	$C_{25}H_{42}O$+
11	383	$C_{27}H_{43}O$+
12	426	$C_{29}H_{50}O$+

Fig. 4 HPTLC chromatogram std, lupeol

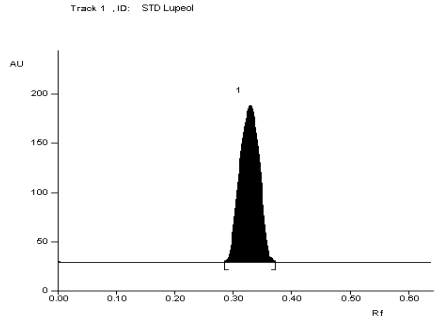


Fig. 5 HPTLC chromatograms of cuticular extract after treatment with Anisaldehyde-H_2SO_4.

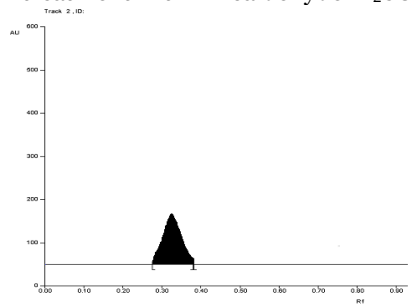


Fig.6 UV spectra of reference standard, lupeol

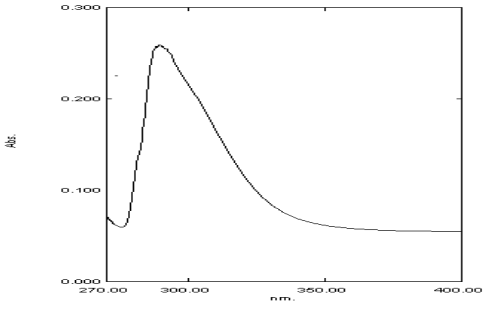


Fig. 7 Overlay spectra of isolated and reference standard, lupeol

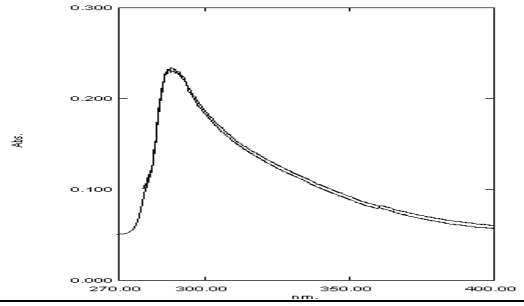


Fig.8 Overlay IR of reference standard lupeol and isolated compound from cuticular wax of *Helicanthus elasticus*

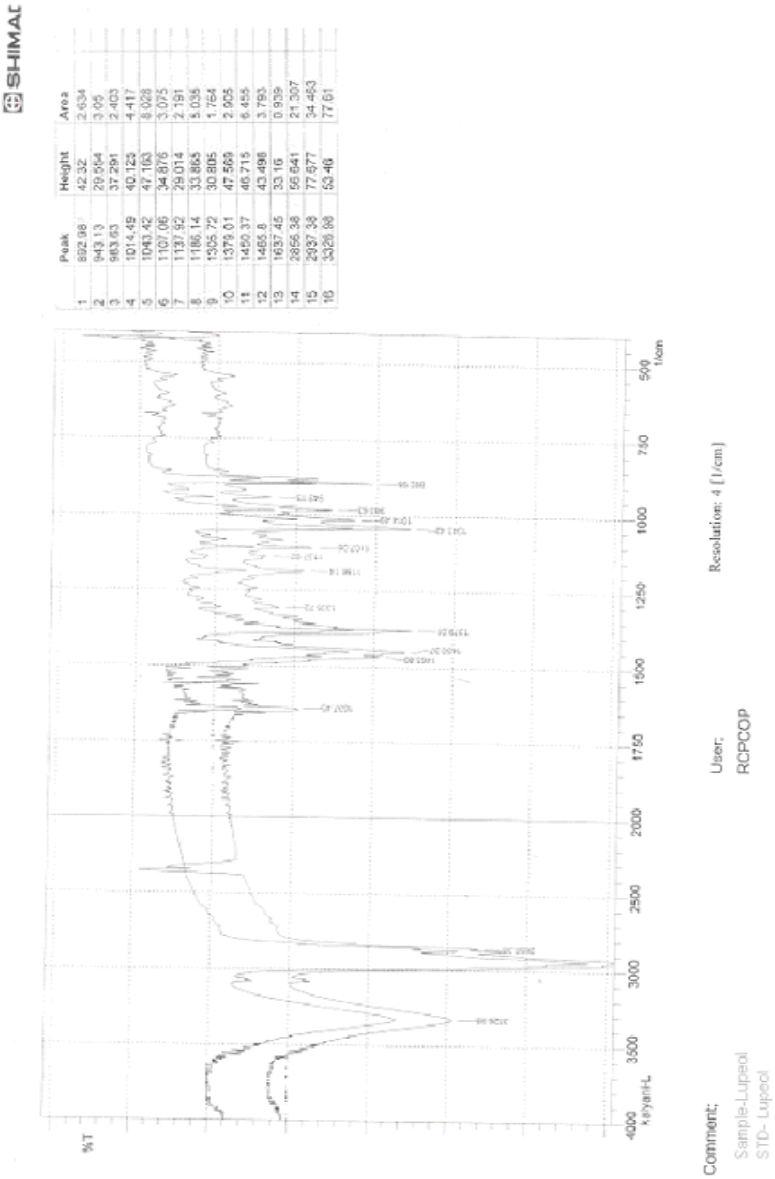
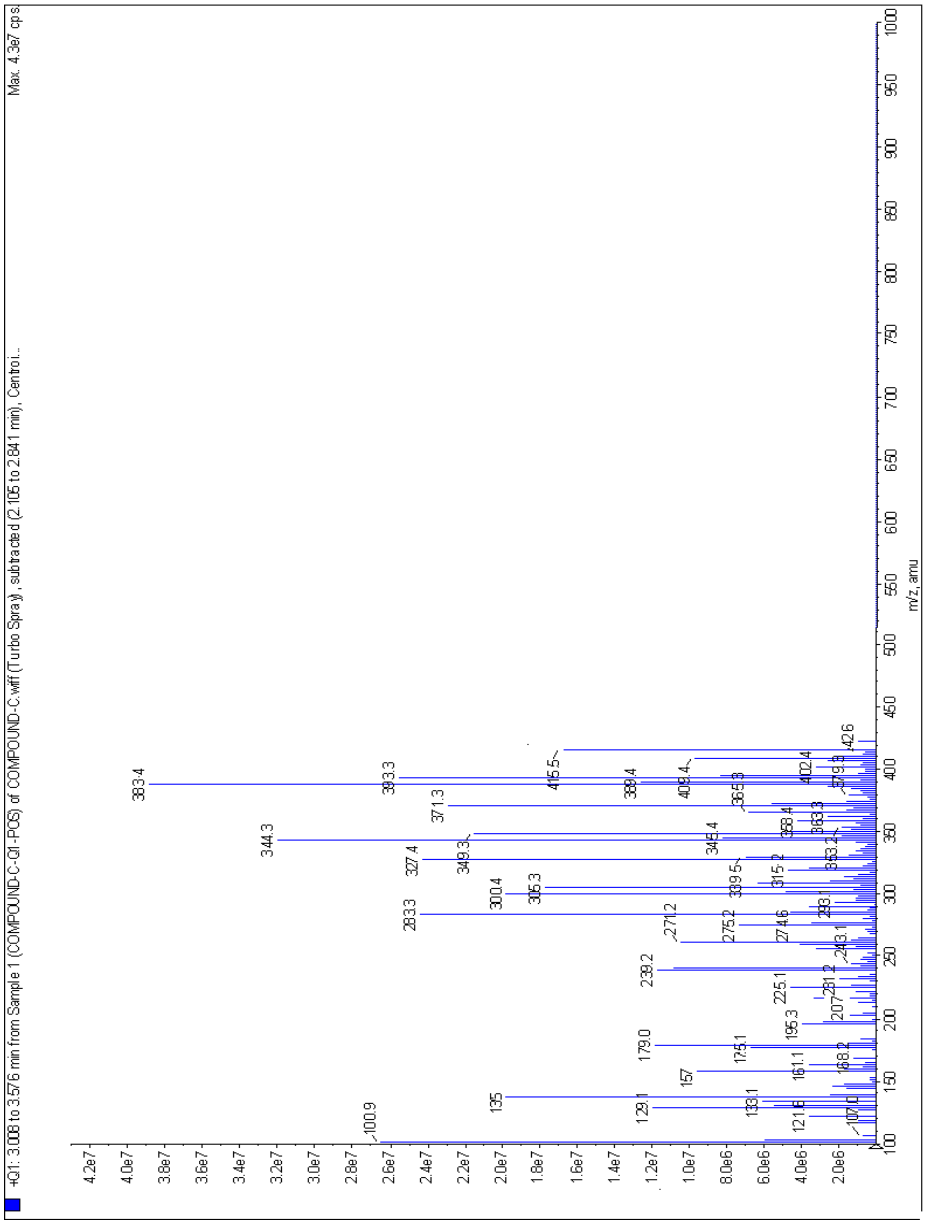


Fig.9 Mass spectra of isolated compound CW1 from cuticular wax of leaves of *Helicanthus elasticus*



Result and Discussion:

In present study the triterpenoid pattern of cuticular wax was studied. The common triterpenoids such as olenolic acid, betulin and lupeol. From this olenolic acid and betulin were not detected. Further cuticular wax subjected to isolation and isolated unknown compound (cw1) was characterized by using UV, IR, HPTLC and Mass spectroscopy and compare with standard reference compound. The compound isolated was white crystalline powder having melting point 212^{O}C, UV λmax (log ε) 288.40 (0.201), IR (KBr), ν_{max} 3326.98, 2937.38, 2856.38, 1637.45, 1465.8, 1450.37, 1379.01, 1305.72 cm^{-1}. An IR spectrum was matched with the standard lupeol IR spectra. (Fig.8). The LC-MS shows its protonated molecular ion at m/z 426 (M+ H^{+}) corresponds to molecular formula C_{30} $H_{50}O$.

From observations Lupeol was found to be major triterpenoid of cuticular wax of leaves of

Helicanthus elasticus. This is first report stating the presence of lupeol in cuticular wax of leaves of *Helicanthus elasticus*. It is in agreement with previous report on lupeol in members of Loranthaceae. Thus these results can be used for identification of plant material. It was also evident from HPTLC study that plant contain higher amount of triterpenoids, which correlate it, at least in part, for diverse pharmacological activities.

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