# CHAPTER - I

## Isolation and characterization of bio-active molecules from medicinal plants

## 1. INTRODUCTION

Natural products are organic compounds that are formed by living systems. The elucidation of their structures and their chemistry, synthesis and biosynthesis are major areas of organic chemistry. These compounds may be divided in to three broad categories. Firstly, there are those compounds which occur in all cells and play a central role in the metabolism and reproduction of those cells. These compounds include the nucleic acids and the common amino acids and sugars. They are known as primary metabolites. Secondly, there are the high-molecular-weight polymeric materials such as cellulose, the proteins and the lignin's which form the cellular structures. Finally, there are those compounds that are characteristics of a limited range of species. These are secondary metabolites.

Secondary Metabolites are classified in to:

- **Polyketides and fatty acids**
- **Terpenoids and steroids**
- **Phenylpropanoids**
- **Alkaloids**
- **Specialized amino acids and peptides**
- **Specialized carbohydrates**

**Polyketides and fatty acids**: Polyketides are formed by the linear combination of acetate units derived from the "building block" acetyl co-enzyme A. Terpenoids and steroids are assembled in nature from isoprenoid $C_5$ units derived from isopentenyl (3-methylbut-3-en-l-yl) pyrophosphate. These $C_5$ units are linked together in a head-to-tail manner. They have a characteristic branched chain structure. A further group of natural products are those containing a phenylpropanoid ($C_6$-$C_3$) unit (i.e. Ph-$CH_2$-$CH_2$-$CH_3$).

The amino acids are the building blocks for peptides and proteins. Although the amino acids are normally considered as primary metabolites, there some unusual amino acids that are of restricted occurrence. Some antibiotics such as the penicillins are formed from small peptides. The alkaloids are a structurally diverse group of natural products containing nitrogen. The nitrogenous portions of the alkaloids are derived from amino acids such as ornithine, lysine, tyrosine or tryptophan.

Although sugars (carbohydrates) such as glucose are typical primary metabolites, there are other sugars that are of a much more limited occurrence. Some of these less common sugars are attached to natural products as part of a glycoside. The non-sugar portion is known as the aglycone, and maybe a terpenoid, alkaloid or polyketide.

**Isolation of Natural Products:**

Secondary metabolites, with some exceptions, occur in amounts that are less than 0.01% of the dry weight of the plant. Extraction of 1 kg of dry plant material is likely to yield less than 100 mg of a natural product. These compounds may be unstable and present as part of a complex mixture. The isolation, separation and purification of these natural products require considerable skill.

Natural products may be obtained from the crushed biological material by extraction with a solvent such as petroleum ether or hexane, chloroform, ethyl acetate or methanol. Several solvents of increasing polarity may be used. Thus lipid material (waxes, fatty acids, sterols, carotenoids and simple terpenoids) can be extracted with non-polar solvents, but more polar substances such as alkaloids and glycosides are extracted with methanol, aqueous methanol or even hot water. Many alkaloids are present as their salts with naturally occurring acids such as tartaric acid. Finally, the structure of the natural products can be established by using experimental data such as $^{1}$H-NMR, $^{13}$C-NMR Mass, IR and X- Ray Crystallography techniques followed by chemical methods as per the requirement.

**Phytochemical investigation and biological activity on evaluation of *Salvinia Natans***

Natural products isolated from higher plants arid microorganisms have been providing novel, clinically active drugs. The 20th century has witnessed much attention directed to synthetic compounds and the rise of combinatorial chemistry as an important part of the drug discovery process. Chemistry of natural products involves characterization of their composition and isolation of specific active component with different techniques, which lie in various fields of science specially chemistry.

The world health organization has estimated that more than 80% of the world's population in developing countries depends primarily on herbal medicine for basic healthcare needs.[1] *Salvinia natans*, which belongs to the family of *Salvinaceae*, is a free floating, rootless aquatic fern. Despite the large number of fern-like plants, aqueous ferns of the Salvinia family have only ten species. They all grow in freshwater aquifers of tropical and subtropical countries, mainly in Africa and South America.[2] Salvinia is a rare and disappearing plant that arrived during the preglacial period. The plant *Salvinia natans* (*Salvinaceae*) is small in size, floating aquatics with creeping stems, branched and bearing hairs but no true roots. Leaves in whorls of three, with two leaves green, sessile or short-petioled, flat, entire, and floating, one leaf finely dissected, petiolate, root like, and pendent. Submerged leaves bearing sori that are surrounded by basifixed membranous indusia (sporocarps); sporocarps of two types, bearing either mega sporangia that are few in number (ca. 10), each with single megaspore, or many micro sporangia, each with 64 microspores. The small, hair like growths, known as microgametical follicles, are not known to have any productive function, and are currently a biological mystery. Chemical constituents of this plant show anti-oxidant properties and anti-oxidant efficiency is proportional to the number of polyhydroxy benzoyl units.[3] Many anti-oxidant compounds possess anti-cancer or anti-cancerogenic properties.[4]

**Scientific Classificaiton**

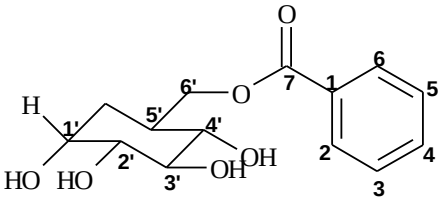
| | | |
|---|---|---|
| Kingdom | : | Plantae |
| Division | : | Pteridophyta |
| Class | : | Pteridopsida |
| Order | : | Salviniales |
| Height | | 1 cm – 3 cm |
| Width | | 5 cm - 10 cm |
| Temperature | | 12 - 30° C |
| $P^H$ | | 5.5 – 8 |

Usually, aquatic plants are considered as an effective strategy for decontaminating waste water. *Salvinia natans* was found to have a great potentiality for the removal of heavy metals such as cadmium (Cd), copper (Cu), chromium (Cr), nickel (Ni), lead (Pb), and mercury (Hg) from waste water. The understanding of chemistry of Salvinia plants can result in controlling their invasive growth and promote their utilization for useful purposes.[5] The phytochemical investigation on *Salvinia natans* showed that it consists 96% of amino compounds.[6] Though it is used in most of the systems of medicine including Homeopathy, no reports on medicinally important phytochemical compounds isolated from *Salvinia natans.* Several reports indicate that there is an inverse relationship between the incidence of human diseases and the dietary intake of antioxidant-rich foods.[7,8] Hence, search for new synthetic and natural antioxidants is essentially important. For the present study, the plants were collected from the Chidambaram area of TamilNadu, India.

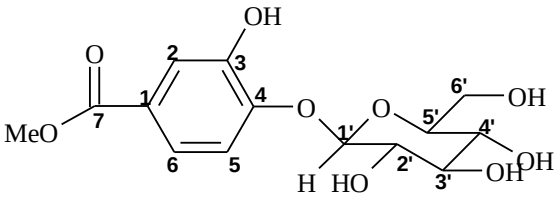
**Chemical Constituents of the family *Salvinaceae***

Methyl benzoate, hypogallic acid, caffeic acid, paeoniflorin and pikuroside and two glycosides are the major chemical constituents of *Salvinaceae* family of *Salvinia molesta*. Phospholipids, betaine lipid, Fatty acids are the major classes of compounds which have been isolated from the leaves of *Salvinia natans*. *O. A Rozentsvet et al.* isolated 1,2-diacylglyceryl-3-O-4′-(N,N,N trimethyl)homoserine (DGTS) from the aquatic plant *Salvinia natans* collected from South America.[2] *M. Iqbal Choudhary et al.,* isolated two glycosides,6′-O-(3,4-dihydroxybenzoyl)-β-D-glucopyranosylester **(1)** and 4-O-β-D-glucopyranoside-3-hydroxy methyl benzoate **(2)**, along with five known compounds from the fern *Salvinia molesta* collected from the *Haliji* Lake (*Sindh*, Pakistan).[5] Here we have

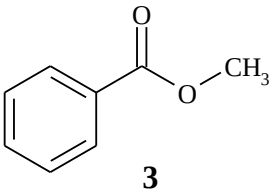
reviewed the literature of above mentioned compounds isolated from the family *Salvinaceae.*



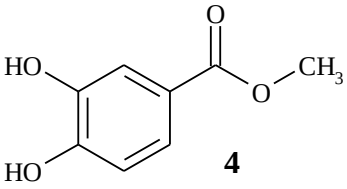
**1**



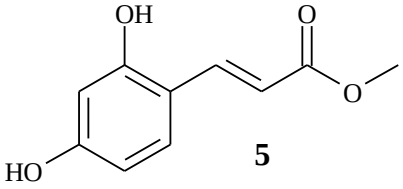
**2**



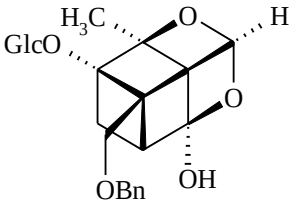
**3**



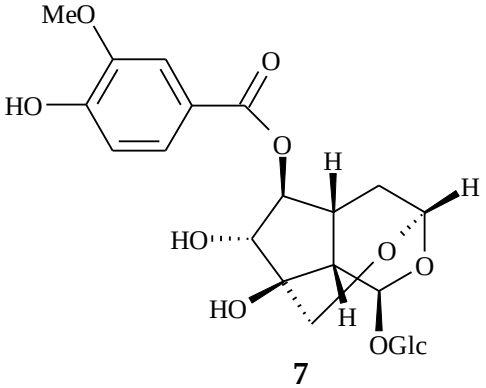
**4**



**5**



**6**



**7**

# PRESENT WORK

**Chemical examination of the aquatic fern *salvinia natans***

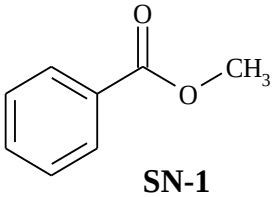
We have examined an aquatic plant, *Salvinia natans*, collected from the Chidambaram area of TamilNadu, India. The shade dried and finely powdered leaves of *S. natans* (2.5 kg) were defatted with hexane (3x3 L). The defatted leaves were further soaked in ethyl acetate (3x2 L) at room temperature for 24 h each time.

The details of the extraction and isolation of the compounds from the plant material has been described in the experimental section. Out of all the isolated compounds SN-3 **(8)** was found to be an unusual novel anti-oxidant dibenzoyl glycoside from *S. natans* and found to have anti-oxidative properties.[5] and its structure was established by means of spectroscopic analysis. The isolated compounds were listed in Table-1.

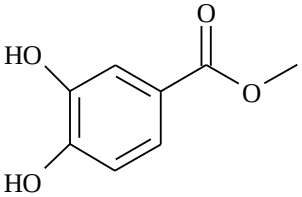
**Table-1: Isolated compounds from the plant *salvinia natans***

| Compound code | Compound Name | Nature | Mol. formula | Remark |
|---|---|---|---|---|
| SN-1 | Methylbenzoate | liquid | $C_8H_8O_2$ | Known compound |
| SN-2 | 3,4-dihydroxy methylbenzoate | liquid | $C_8H_8O_4$ | Known compound |
| SN-3 | Natansnin | colourless powder | $C_{20}H_{20}O_{13}$ | New Compound |

Structures of **SN-1** and **SN-2** will be shown as follows, and they were found to be known compounds after study of their experimental data.

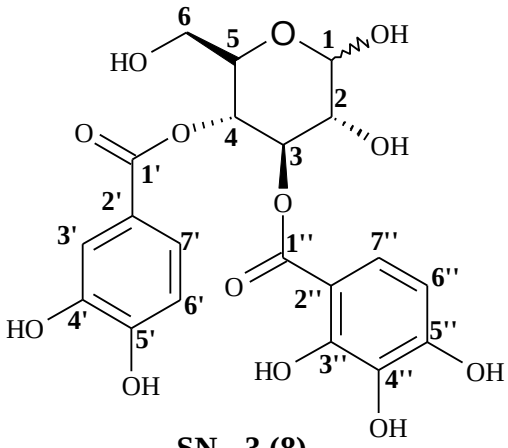



**SN-1**




**SN -2**

**Structural elucidation of SN-3 (8) :**




**SN - 3 (8)**

SN-3 **(8)** was obtained as colourless powder, m.p. $209^0$C, $[\alpha]_D^{25}$ +78.6 (c 0.011, MeOH) and analysed for $C_{20}H_{20}O_{13}$ by HREIMS m/z 468 (observed 468.1324; calculated 468.0898 for $C_{20}H_{20}O_{13}$). UV spectrum showing absorbance at $\lambda_{max}$ (log ε): 242 (0.4722), 351 (0.3227) and IR spectral data showing stretching frequencies at ν (KBr) ($cm^{-1}$): 3418, 2925, 177, 1617 indicates free hydroxyl functional group, phenolic group, aromatic double bond stretchings and ester functional group respectively. Further it's $^1$H (Fig.1.01, Table-2) and $^{13}$C-NMR spectra (Fig.1.02, Table-2) revealed two sets of signals attributable to the presence of α and β glycosides. SN-3 **(8)** is a homogenous mixture of α and β and glycosides which did not resolve on TLC and HPLC; however, their proton and carbon signals were resolved in the NMR time scale. Further, it was observed in $^1$H- and $^{13}$C-NMR spectra that the aromatic signals were not resolved for α and β- glycosides.

A close inspection of the $^1$H-NMR spectrum of SN-3 **(8)** revealed signals due to the presence of a 1,3,4-trisubstituted benzoyl group at δ 7.49 (1H, d, *J* = 2.2 Hz, H-3'), 7.43 (1H, dd, *J* = 8, 2.2 Hz, H-7') and 6.89 (1H, d, *J* = 8 Hz, H-6'), and a 1,2,3,4-tetra substituted benzoyl group at δ 7.72 (1H, d, *J* = 8 Hz, H-7'') and 6.85 (1H, d, *J* = 8 Hz, H-6''), respectively. Further, its $^1$H-NMR spectrum displayed several oxygenated signals (Table 2). Among them, two of the most conspicuous signals attributable to anomeric protons appeared at δ 5.20 (1H, d, *J* = 3.6 Hz, $H_\alpha$-1) ($\delta_c$ 93.93) and 4.63 (1H, d, *J* = 7.36 Hz, $H_\beta$-1) ($\delta_c$ 98.44), respectively, for α and β D-glucopyranose.[9]

Compound **(8)** afforded hexa-acetate as a single product upon acetylation with acetic anhydride in pyridine. Interestingly, the two anomeric protons shifted downfield by 1 ppm at δ 6.30 (1H, d, *J* = 3.6 Hz, H-1") and 5.78 (1H, d, *J* = 7.36 Hz, H-1"), respectively, in its hexa-acetate $^{1}$H-NMR spectrum.[10]

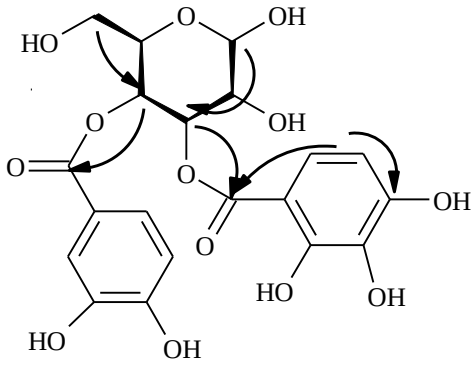



**Fig.-1**. Bold line represents $^{1}$H-$^{1}$H COSY, and arrows HMBC correlations.

At this stage, the structure of SN-3 **(8)** was established by the study of $^{1}$H–$^{1}$H COSY (Fig.1.04), HSQC (Fig.1.06) and HMBC (Fig.1.05, Table-2) correlations. In the $^{1}$H–$^{1}$H COSY spectrum, the signal at δ 5.2 showed linear correlation with a methine proton at δ 3.58 (1H, dd, *J* = 3.6, 9.5 Hz, H-2), which in turn showed correlation with δ 3.91 (1H, t, *J* = 9.5 Hz, H-3), having HMBC correlation with the carbonyl carbon at δ 167.65 (C-1"). Further, the signal at δ 3.91 showed linear correlation with a methine proton at δ 5.3 (1H, t, *J* = 9.5 Hz, H-4), which showed HMBC correlation with the carbonyl at δ 168.26 (C-1'). The signal at δ 5.3 showed linear correlation with the methine at δ 4.3 (1H, dt, *J* = 3.6, 10 Hz, H-5), which showed linear correlation with a methylene group 3.96 (1H, dd, *J* = 3.6, 10 Hz, Ha-6) and 5.01 (1H, dd, *J* = 10, 4.4 Hz, $H_b$-6). Similarly, the linear correlations for the β anomer were established by mapping from the anomeric proton at δ 4.63. Hence, the structure of the compound was established as SN-3 (**8**).

**Table -2. $^{1}H$, $^{13}C$-NMR and HMBC correlations of SN-3 (8)**

| Position | α -isomer | | ß-isomer | | HMBC |
|---|---|---|---|---|---|
| | $^{1}H$ NMR Chemical shift (δ,$D_4$-MeOH) Multiplicity (*J* in Hz) | $^{13}C$-NMR | $^{1}H$ NMR Chemical shift (δ, $D_4$-MeOH) Multiplicity (*J* in Hz) | $^{13}C$-NMR | |
| 1 | 5.2 (1H, d, *J* = 3.6 Hz) | 93.93 | 4.63 (1H, d, *J* = 7.3 Hz) | 98.44 | C-3 |
| 2 | 3.58 (1H, dd, *J* = 3.6, 9.5 Hz ) | 73.8 | 3.36 (1H, dd, *J* = 7.3, 9.5 Hz) | 76.36 | C-3 |
| 3 | 3.91 (1H, t, *J* = 9.5 Hz) | 71.15 | 3.66 (1H, t, *J* = 9.5 Hz) | 74.09 | C-1", C-2, C-4 |
| 4 | 5.3 (1H, t, *J* = 9.5 Hz) | 77.96 | 5.33 (1H, t, *J* = 9.5 Hz) | 77.38 | C-1', C-5, C-3 |
| 5 | 4.3 (1H, td, *J* = 3.6, 10.3 Hz) | 64.02 | 3.83 (1H, td, *J* = 4.4, 10.3 Hz) | 68.5 | |
| 6 | 3.96 (1H, dd, *J* = 3.6, 10.3 Hz) 5.01 (1H, dd, *J* = 10.3, 4.4 Hz) | 65.36 | 3.98 (1H, dd, *J* = 3.6, 10.3 Hz) 5.07 (1H, dd, *J* = 4.4, 10.3 Hz) | 65.95 | C-5, C-4 |
| 1' | - | 168.26 | - | 168.26 | |
| 2' | - | 148.19 | - | 148.19 | |
| 3' | 7.49 (1H, d, *J* = 2.2 Hz) | 114.5 | 7.49 (1H, d, *J* = 2.2 Hz) | 114.5 | C-2', C-4', C-5' |
| 4' | - | 146.21 | - | 146.21 | |
| 5' | - | 157.88 | - | 157.88 | |
| 6' | 6.89 (1H, d, *J* = 8.1 Hz) | 115.31 | 6.89 (1H, d, *J* = 8.1 Hz) | 115.31 | C-2', C-4' |
| 7' | 7.43 (1H, dd, *J* = 8.1, 2.2 Hz) | 121.06 | 7.43 (1H, dd, *J* = 8.1, 2.2 Hz) | 121.06 | C-5' |
| 1" | - | 167.65 | - | 167.65 | |
| 2" | - | 148.82 | - | 148.82 | |

| | | | | | |
|---|---|---|---|---|---|
| 3" | - | 129.19 | - | 129.19 | |
| 4" | - | 116.35 | - | 116.35 | |
| 5" | - | 143.16 | - | 143.16 | |
| 6" | 6.85 (1H, d, *J* = 8.1 Hz) | 111.65 | 6.85 (1H, d, *J* = 8.1 Hz) | 111.65 | C-2", C-4", C-5 |
| 7" | 7.72 (1H, d, *J* = 8.1 Hz) | 129.48 | 7.72 (1H, d, *J* = 8.1 Hz) | 129.48 | C-3", C-1" |

Further, the CD spectrum (Fig.1.07) showed first a negative Cotton effect ($\Delta_{\epsilon 240}$ = -19.25) followed by a positive Cotton effect ($\Delta_{\epsilon 209}$ = +12.48), which established that the two benzoates are present in vicinal positions and the sugar moiety is glucopyransose.[11] From the forgoing and spectraldata SN-3 **(8)** was established as Natansnin is a homogenous mixture of α and β-D-3,4-dibenzoyl glucopyranose which is separable in the NMR time scale.

## BIOLOGICAL ACTIVITIES

**Antioxidant activity of Natansnin**: Natansnin was tested *in-vitro* for its anti-oxidant activity using ascorbic acid as the standard.[12] The $IC_{50}$ value of natansnin is 25.28 µM, whereas for ascorbic acid, it is 25.89 µM. To the best of our knowledge very few free anomeric substituted sugars are available in nature, for example, 'coyolosa', a hypoglycaemic compound isolated from the methanol extract of the root of *Acrocomia Mexicana*.[13] Coyolosa is comprised of two hexopyranose units joined through an ether link at their 6-positions. To the best of our knowledge, this is the first report of the existence α and β-3, 4-dibenzoyl D-glucopyranose which is separable in the NMR time scale.

**Free radical scavenging activity on a DPPH radical of Natansnin (8):**

Assay for the scavenging of the stable free radical, DPPH, was done as reported earlier. In brief, in a 96-well micro plate, 25 µL of the test sample dissolved in DMSO (1 mg/mL) and 125 µl of 0.5mM DPPH dissolved in absolute ethyl alcohol were added. The reaction mixture was shaken well and incubated in the dark for 30 min. The absorbance was read at 517nm spectrophotometrically ($SPECTRA_{MAX}PLUS^{384}$, Molecular Devices Corporation, Sunnyvale, CA, USA).

The free radical scavenging potential was expressed as the percent change in color of the DPPH solution due to the test sample, and calculated as (1-B/A) is the multiple of 100, where ‘A’ represents absorbance of the DPPH solution without the test sample and B, the absorbance of the DPPH solution with the test sample. The $SC_{50}$ values (50% free radical scavenging activity) of the test sample were calculated by regression analysis. Ascorbic acid was taken as the reference standard as a free radical scavenger. Measurements were performed in triplicate.

## EXPERIMENTAL

### Collection and Identification

The plant material *Salvinia natans* (*Salvinaceae*) was collected from from the Chidambaram area of Tamil Nadu, India. Plants were collected in the month of November, 2005. Prof. R. Pannerselvam, Department of Botany, Annamalai University, Annamalai Nagar 608 002, TamilNadu, India, identified the plant.

### Extraction and fractionation procedure

The shade dried and powdered of root part of the plant (2.1 kg) *Salvinia natans* was extracted with n-hexane (3x5 L) followed by $CH_2Cl_2$: MeOH (3x5 L) at room temperature.

### Hexane extract

The combined hexane extract was filtered and concentrated under reduced pressure to obtained greenish gummy residue (30.2 g). The crude slurry was subjected to silica gel (100-200 mesh) column chromatography by gradient elution using with hexane through hexane-ethylacetate mixtures to ethylacetate. Fractions (250 ml) were collected and monitored on silicagel ($GF_{254}$) TLC. The visualization of spots on TLC was carried out either in UV light or by exposing TLC plates to iodine vapours or by spraying 10% sulfuric acid in methanol and heating at $110^0$C. Similar fractions were combined and the results borne out in the chromatography are recorded in the following Table-3.

**Table-3: Silica gel column chromatography of hexane extract of *Salvinia natans***

| Eluent (Hexane:EtOAc) | Fractions | Residue (g) | Remarks |
|---|---|---|---|
| 100:0 | 1-5 | 2.2 | Fatty oil |
| 90:10 | 6-15 | 4.6 | Fraction-I |
| 80:20 | 16-18 | 2.6 | Fatty solid |
| 70:30 | 19-23 | 5.2 | Fraction -II |
| 60:40 | 24-32 | 6.8 | Green slurry |
| 40:60 | 33-40 | 5.2 | Green pigment |
| 20:80 | 41-48 | 2.0 | Dark green matter |
| 0:100 | 49-52 | 1.6 | Dark green matter |

**Fraction-1**

It was obtained as a mixture of compounds as noticed from its silica gel TLC plates and hence it was re-chromatographed over silica gel (100-200mesh) column chromatography using hexane-ethylacetate mixtures as eluents. Fractions (20ml each) were collected and the results of the chromatography are recorded in the Table-4.

**Table-4: Silica gel column chromatography of Fraction-I**

| Eluent (Hexane:EtOAc) | Fractions | Yield (mg) | Remarks |
|---|---|---|---|
| 100:0 | 1-10 | 460 | Fatty oil |
| 95:5 | 11-14 | 80 | SN-1 |
| 80:20 | 14-20 | 250 | Fatty solid |
| 70:30 | 21-25 | 220 | Fattymaterial |
| 60:40 | 26-31 | 90 | Fatty material |
| 50:50 | 32-38 | 630 | Fatty material |

**Fraction-II**

It was obtained as a mixture of compounds as noticed from its silica gel TLC plates and hence it was re-chromatographed over silica gel (100-200 mesh) column chromatography using hexane-ethylacetate mixtures as eluents. Fractions (10ml each) were collected and the results of the chromatography are recorded in the Table-5.

**Table-5: Silica gel column chromatography of Fraction-II**

| Eluent (Hexane:EtOAc) | Fractions | Yield (mg) | Remarks |
|---|---|---|---|
| 100:0 | 1-10 | 260 | Fatty oil |
| 90:5 | 11-16 | 280 | Fatty solid |
| 90:10 | 17-25 | 450 | Fatty solid |
| 85:15 | 26-33 | 320 | Fatty solid |
| 80:20 | 34-36 | 40 | SN-2 |
| 70:30 | 37-42 | 570 | Fatty material |
| 60:40 | 42-48 | 1580 | Fatty material |
| 50:50 | 48-55 | 290 | Fatty material |

**Dichloromethane: Methanol extract**

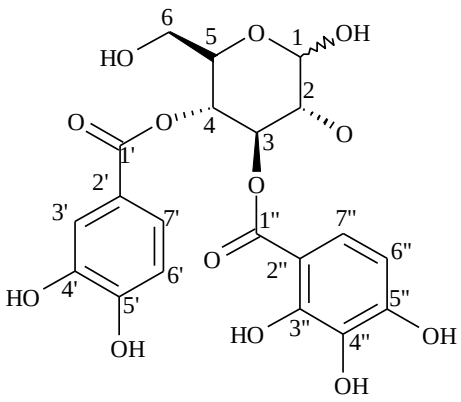
The combined $CH_2Cl_2$:MeOH extract was filtered and concentrated under reduced pressure to yield a dark greenish residue (80.4g). This crude solid was subjected to silicagel (100-200 mesh) column chromatography by gradient elution using with hexane through hexane-ethylacetate-methanol mixtures to methanol as eluents. Fractions (350 ml) were collected and monitored on silicagel ($GF_{254}$) TLC. The visualization of spots on TLC was carried out either in UV light or by exposing TLC plates to iodine vapours or by spraying 10% sulfuric acid in methanol and heating at $110^0$C. Similar fractions were combined and the results borne out in the chromatography are recorded in the following Table-6.

**Table-6: Silica gel column chromatography of hexane extract of *Salvinia natans***

| Eluent (Hexane:EtOAc:MeOH) | Fractions | Residue (g) | Remarks |
|---|---|---|---|
| 100:0:0 | 1-6 | 5.2 | Fatty oil |
| 90:10:0 | 7-13 | 4.6 | Fatty solid |
| 80:20:0 | 14-18 | 3.0 | Fatty solid |
| 70:30:0 | 19-25 | 10.1 | Fatty solid |
| 60:40:0 | 26-30 | 12.7 | Green slurry |
| 50:50:0 | 31-35 | 16.0 | Dark green matter |
| 40:60:0 | 36-41 | 8.2 | Dark green matter |
| 30:70:0 | 42-47 | 6.0 | Fraction-1 |
| 0:100:0 | 48-53 | 4.5 | Intractable gum |
| 0:80:20 | 54-59 | 3.5 | Intractable gum |
| 0:40:60 | 60-65 | 2.0 | Intractable gum |
| 0:20:80 | 66-70 | 2.4 | Intractable gum |
| 0:0:100 | 71-75 | 2.2 | Intractable gum |

**Fraction-1**

This fraction-1 was purified on HPLC using $C_{18}$ reversed phase column (24x2 cm, 10 mm), eluting with methanol and water (60: 40), to give compound SN-3 **(8)** (80mg).



**SN-3 (8)**

| | | |
|---|---|---|
| **Compound (SN-3)** | **:** | **Natansnin** |
| **Physical property** | **:** | colourless powder |
| **Amount isolated** | **:** | 80mg |
| **Melting point** | **:** | $209^0$C |
| **$[\alpha]_D^{25}$** | **:** | +78.6 (c 0.011, MeOH) |
| **IR (KBr) $\nu_{Max}$** | **:** | 3418, 2925, 177, 1617 |
| **UV $\lambda_{max}$ (log ε)** | **:** | 242 (0.4722), 351 (0.3227) |
| **Molecular formula** | **:** | $C_{20}H_{20}O_{13}$ |
| **HREIMS** | **:** | clacd. 468.0898 |
| **Obtd.** | **:** | 468.1324 |
| **$^1$H NMR of α-isomer** | **:** | ($CD_3OD$, 300Mz) : δ 5.20 (1H, d, *J* = 3.6 Hz, $H_\alpha$-1), 3.58 (1H,dd, *J* = 3.6, 9.5 Hz, H-2), 3.91 (1H, t, *J* = 9.5 Hz, H-3), 5.3 (1H, t, *J* = 9.5 Hz, H-4), 4.3 (1H, td, *J* = 3.6, 10.3 Hz, H-5), 3.96 (1H, dd, *J* = 3.6, 10.3 Hz, Ha-6), 5.01 (1H, dd, J = 10.3, 4.4 Hz, $H_b$-6), 7.49 (1H, d, *J* = 2.2 Hz, H-3'), 6.89 (1H, d, *J* = 8.1 Hz, H-6'), 7.43 (1H, dd, *J* = 8.1, 2.2 Hz, H-7'), 6.85 |

(1H, d, *J* = 8.1 Hz, H-6''), 7.72 (1H, d, *J* = 8.1 Hz, H-7'').

**$^{13}$C NMR of α-isomer** **:** ($CD_3OD$, 50 Mz) 93.93 (C-1), 73.8 (C-2), 71.15 (C-3), 77.96 (C-4), 64.02 (C-5), 65.36 (C-6), 168.26 (C-1'), 148.19 (C-2'), 114.5 (C-3'), 146.21 (C-4'), 157.88 (C-5'), 115.31 (C-6'), 121.06 (C-7'), 167.65 (C-1''), 148.82 (C-2''), 129.19 (C-3''), 116.35 (C-4''), 143.16 (C-5''), 111.65 (C-6''), 129.48 (C-7'').

**$^{1}$H NMR of β-isomer** **:** ($CD_3OD$, 50 Mz) δ 4.63 (1H, d, *J* = 7.3 Hz, $H_\beta$1), 3.36 (1H, dd, *J* = 7.3, 9.5 Hz, H-2), 3.66 (1H, t, *J* = 9.5 Hz, H-3), 5.33 (1H, t, *J* = 9.5 Hz, H-4), 3.83 (1H, td, *J* = 4.4, 10.3 Hz, H-5), 3.98 (1H, dd, *J* = 3.6, 10.3 Hz, Ha-6), 5.07 (1H, dd, J = 10.3, 4.4 Hz, $H_b$-6), 7.49 (1H, d, *J* = 2.2 Hz, H-3'), 6.89 (1H, d, *J* = 8.1 Hz, H-6'), 7.43 (1H, dd, *J* = 8.1, 2.2 Hz, H-7'), 6.85 (1H, d, *J* = 8.1 Hz, H-6''), 7.72 (1H, d, *J* = 8.1 Hz, H-7'').

**$^{13}$C NMR of β-isomer** **:** ($CD_3OD$, 50 Mz) 98.44 (C-1), 76.36 (C-2), 74.09 (C-3), 77.38 (C-4), 65.0 (C-5), 65.95 (C-6), 168.26 (C-1'), 148.19 (C-2'), 114.5 (C-3'), 146.21 (C-4'), 157.88 (C-5'), 115.31 (C-6'), 121.06 (C-7'), 167.65 (C-1''), 148.82 (C-2''), 129.19 (C-3''), 116.35 (C-4''), 143.16 (C-5''), 111.65 (C-6''), 129.48 (C-7'').

## SECTION–B: Phytochemical investigation of heartwood of *Decalepis hamiltonii*

The Indian subcontinent is rich in biodiversity with more than 2000 species of flowering plants. D*ecalepis hamiltonii* (*Asclepiadaceae*) known as swallow in biotechnology root is a monogeneric climbing shrub and a native of the forests of Deccan peninsula and Western Ghats of India. Its tubers are consumed as pickles and the juice for its alleged health promoting properties. It grows between the rocks and places where there is thick vegetation. The roots of *D. hamiltonii* are used as a flavoring principle, appetizer, and blood purifier and as a preservative. Similarly, the roots of this taxon are considered as "Sariva Bheda" in Ayurveda where these find use as an alternative to the roots of *Hemidesmus indicus* in the preparation of several herbal drugs like *Amrutamalaka taila* (hair tonic), *Drakshadi churna* (general vitalizer), *Shatavari rasayana* (adapatogenic) and *Yeshtimadhu taila* (mild analgesic, rheumatism).

**Scientific Classification**

| | |
|---|---|
| Kingdom: | Plantae |
| Order: | Gentianales |
| Family: | Apocynaceae |
| Genus: | ***Decalepis*** |
| Species: | ***D. hamiltonii*** |

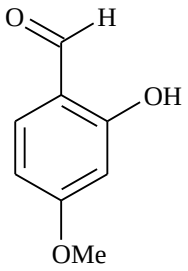
The roots contain 92% fleshy matter and 8% woody core. Of late, the highly aromatic roots have been subjected to over exploitation by destructive harvesting that has endangered the survival of this plant. A method for rooting of *D. hamiltonii* for field transfer is reported. In earlier reports, it was observed that the aromatic roots of *D. hamiltonii* possess bioinsecticide property on storage pests at lethal and sub-lethal levels. The extracts of these roots have also been shown to be potent antimicrobial agents as well. The antimicrobial properties of the roots of *D.hamiltonii* have been attributed to the presence of 2-hydroxy-4-

methoxy benzaldehyde and vanillin.[14] Earlier work has shown that the roots contain aldehyde, inositol, saponins, ketonic substances, sterols, amyrins and lupeols.[15-17] *Thangaduarai et al*.[18] and *Nagarajan et al*.[19] have reported several volatile flavor compounds including 4-methoxybenzaldehyde, vanillin, and salcilaldehyde in the essential oil extracts from the roots of *D. hamiltonii*. We have recently shown that the roots of *D. hamiltonii* possess antioxidant properties and hypothesized that antioxidants constituent present in the root extracts could contribute to the health-promoting potential.[20] We now report the isolation and characterization of antioxidant compounds from the methanolic extract of the roots of *D. hamiltonii.*

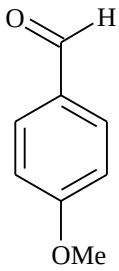
**Earlier chemical investigation of root of *Decalepis hamiltonii.***

The plant material was collected from the forest of Tirumala hills in Chitoor Dist, Andhra Pradesh, India in the month of July 2005 and identification was made by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati. Shade dried powdered heartwood (5 Kg) of *Decalepis hamiltonii* was extracted with hexane, chloroform and methanol at room temperature about 48 h.

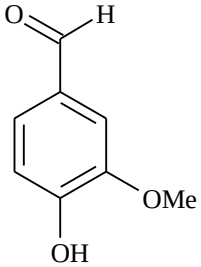
Previously reported that the methanolic extract of roots of *Decalepis hamiltonii* contained 2-hydroxy-4-methoxybenzaldehyd **9**, *p*-anisaldehyde **10**,vanillin **11**, borneol **12**, salicylaldehyde **13** and *bis*-2,3,4,6-galloyl α/β-D-glucopyranoside (Decalepin) **14** and also the aqueous extracts on the other hand were found to contain 4-hydroxyisophthalicacid **15**, 14-aminotetradecanoicacid **16**, 4-(1-hydroxy-1-methylethyl)-1-methyl-1,2-cyclohexanediol **17**, 2-hydroxymethyl-3-methaoxybenzaldehyde **18**, 2,4,8-trihydroxybicyclo[3.2.1] octane-3-one **19.** [21]



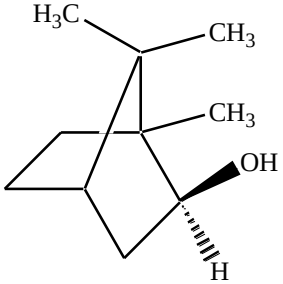

**9**



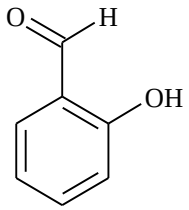

**10**



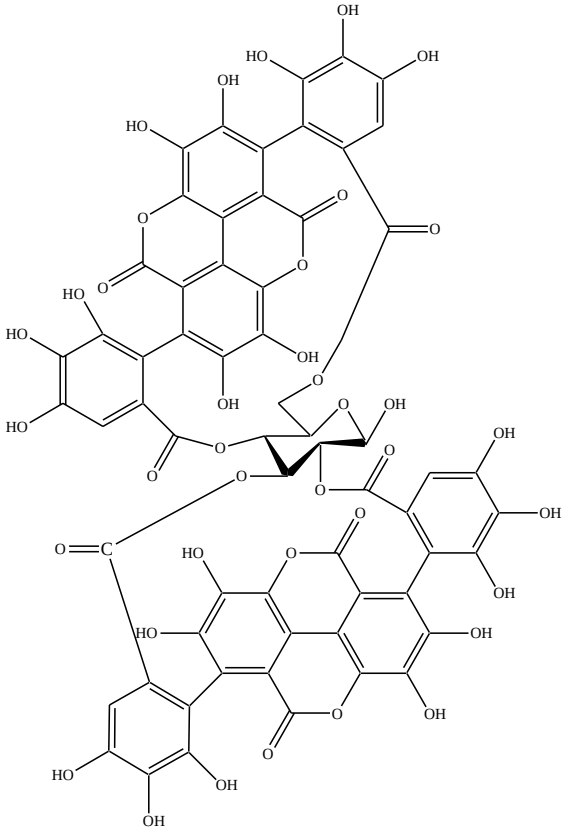

**11**



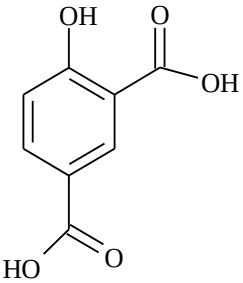

**12**



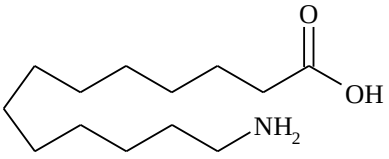
**13**



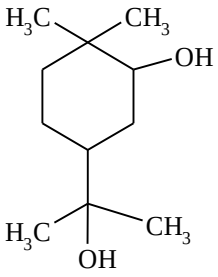
**14**



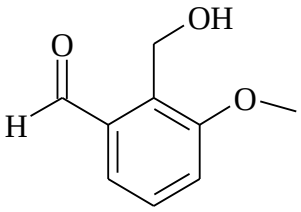
**15**



**16**



**17**



**18**

In the present study, the plant *Decalepis hamiltonii* yielded five new sources,out of which two new sources showing anti-inflammatory activities by down regulating TNF-α and IL-2 specific mRNA,besides up regulating the synthesis of mRNA of IL-10.[22]

## PRESENT WORK

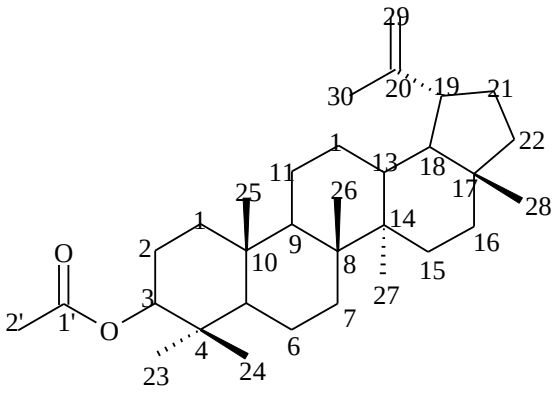
### Chemical investigation of *Decalepis hamiltonii*

The shade dried and powdered root of the plant *Decalepis hamiltonii* (*Asclepiadaceae*) extracted with hexane and in 1:1 DCM: MeOH at room temperature to afforded five new sources **DH-1** to **DH-5**. The details of the extraction and isolation of the compounds from the plant material has been described in the experimental section. Compounds **DH-1** to **DH-5** was found to be new sources. And their structures were established by means of spectroscopic analysis. The isolated compounds are listed in Table-7.

**Table-7: The isolated compounds from the plant *Decalepis hamiltonii***

| Compound code | Compound Name | Nature | Mol. formula | Remark |
|---|---|---|---|---|
| DH-1 | Lupeol acetate | white needles | $C_{32}H_{52}O_2$ | New source |
| DH-2 | Sesamin | White crystals | $C_{20}H_{18}O_6$ | New source |
| DH-3 | (*S*)- Naringenin | yellow needles | $C_{15}H_{12}O_5$ | New source |
| DH-4 | Milimorin | yellow crystals | $C_{16}H_{12}O_7$ | New source |
| DH-5 | (*S*)-Naringenin 4'-O--β - glucopyranoside | yellow needles | $C_{21}H_{22}O_{10}$ | New source |

**Structural elucidation of Compound DH-1 (19)**




**DH-1 (19)**

Compound DH-1 **(19)** was obtained as white needles, m.p. 127-129$^0$C, $[\alpha]^{20}$ +27.2 (c 4.8, $CHCl_3$). Its EI mass spectrum shows molecular ion peak $[M]^+$ at *m/z* 468 and analyzed for $C_{32}H_{52}O_2$, which required seven degrees of unsaturation. The $^1$H-NMR spectrum (Fig.1.08,Table-8 ) in $CDCl_3$ at δ 0.79 (3H,s), 0.83 (3H,s), 0.84 (3H,s), 0.85 (3H,s), 0.94 (3H,s), 1.03 (3H,s), 1.69 (3H,s) and 2.05(3H,s) indicated the presence of eight tertiary methyls and δ 4.57 and 4.69 (2H,s) representing an exocyclic double bond protons H-29a and H-29b, respectively. Its $^{13}$C NMR spectrum (Fig.1.09,Table-8) in $CDCl_3$ shows carbonyl group at δ 171.3 and the signal at δ 81.2 indicates C-3 carbon and the alkene carbons at δ 151.20 and 109.6. From the forgoing spectral data and the literature [23] survey revealed that the structure of compound DH-1 **(19)** was confirmed as lupeolacetate, this was first report of isolation from the plant *Decalepis hamiltonii.,* before it was found in *deertongue* leaf, [24]*Erythroxylum leal costae,*[25] stem-bark of *Artocarpus chaplasha* [26] and *Ficus hispida*.[27]

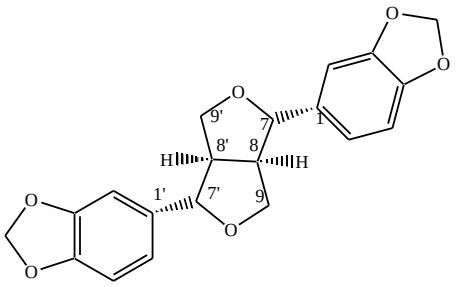
**Table-8. $^{1}H$ and $^{13}C$-NMR of DH-1 (19)**

| Position | Chemical shift values based on the reference [23 (b)] | | Chemical shift values of **DH-1 (19)** | |
|---|---|---|---|---|
| | $^{1}H$ NMR Chemical shift ($\delta$ in $CDCl_3$) Multiplicity ($J$ in Hz) | $^{13}C$-NMR | $^{1}H$ NMR Chemical shift ($\delta$ in $CDCl_3$) Multiplicity ($J$ in Hz) | $^{13}C$-NMR |
| 1 | - | 38.6 | - | 38.61 |
| 2 | - | 21.7 | - | 21.55 |
| 3 | 4.47 (1H, dd , $J$ = 4.4, 12.8 Hz) | 81.2 | 4.47 (1H, dd , $J$ = 4.4, 12.8 Hz) | 81.21 |
| 4 | - | 38.0 | - | 38.02 |
| 5 | - | 55.6 | - | 55.66 |
| 6 | - | 18.4 | - | 18.43 |
| 7 | - | 34.4 | - | 34.44 |
| 8 | - | 41.0 | - | 41.08 |
| 9 | - | 50.5 | - | 50.57 |
| 10 | - | | - | 37.31 |
| 11 | - | 21.1 | - | 21.17 |
| 12 | - | 24.0 | - | 23.94 |
| 13 | - | 36.2 | - | 36.80 |
| 14 | - | 43.0 | - | 43.05 |
| 15 | - | 25.3 | - | 25.33 |
| 16 | - | 35.8 | - | 35.80 |
| 17 | - | 43.2 | - | 43.22 |
| 18 | - | 48.5 | - | 48.52 |
| 19 | - | 48.2 | - | 48.23 |
| 20 | - | 151.2 | - | 151.19 |
| 21 | - | 30.0 | - | 30.06 |
| 22 | - | 40.2 | - | 40.22 |
| 23 | 0.85 (3H,s) | 27.6 | 0.80 (9H,s) | 27.66 |
| 24 | 0.84 (3H,s) | 16.7 | | |

| | | | | |
|---|---|---|---|---|
| 26 | 0.83 (3H,s) | 16.2 | | 16.72 |
| 25 | 1.03 (3H,s) | 16.4 | 1.03 (3H,s) | 16.40 |
| 27 | 0.79 (3H,s) | 14.7 | 0.79 (3H,s) | 14.70 |
| 28 | 0.94 (3H,s) | 18.2 | 0.94 (3H,s) | 18.23 |
| 29 | 4.57 (1H,s H-29a)<br>4.69 (1H,s,H-29b) | 109.6 | 4.57 (1H,s H-29a)<br>4.69 (1H,s,H-29b) | 109.58 |
| 30 | 1.69 (3H,s) | 19.5 | 1.69 (3H,s) | 19.51 |
| 1' | - | 171.3 | - | 171.26 |
| 2' | 2.05,(3H, s) | 28.2 | 2.05,(3H, s) | 28.17 |

Assignments were made by comparison of earlier data[23 (b)]

**Structural elucidation of Compound DH-2** (**20**):




**DH-2 (20)**

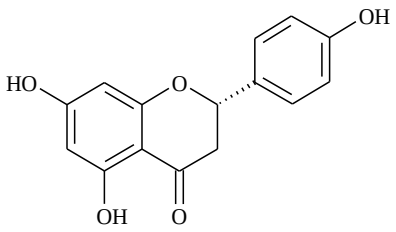
Compound DH-2 (**20**) was obtained as colorless prisms, m.p. 127-129$^0$C, $[\alpha]_D^{20}$ +64.5 (c 1.75, $CHCl_3$). Its EI mass spectrum showed molecular ion peak at *m/z* 354 $[M]^+$ and analyzed for $C_{20}H_{18}O_6$, which required twelve degrees of unsaturation. Its $^1$H-NMR spectrum (Fig.1.10,Table-9) indicated the presence of symmetric methylene dioxygroup which appeared as singlet at δ 5.92 (4H,s). Aromatic ring protons appears at δ 6.72 to δ 6.80 (6H, m) which shows that the presence of symmetrical aromatic rings within the structure. The $^{13}$C NMR spectrum (Fig.1.11, Table-9) of the compound **20** is shown in the following table. Based on the above data it is concluded as a lignan. Hence from the forgoing spectral data and literature survey reveled that compound **DH-2** is closely related to those of sesamin **(20)** which was earlier isolated from the dried peeled roots of *Glossostemon bruguierise.*[28] and this is first report of isolation from this plant *Decalepis hamiltonii*. Hence the structure of **DH-2** was established as sesamin **(20)**.

**Table-9. $^{1}$H and $^{13}$C-NMR of DH-2 (20)**

| Position | Chemical shift values based on the reference [28] | | Chemical shift values of **DH-2 (20)** | |
|---|---|---|---|---|
| | $^{1}$H NMR Chemical shift ($\delta$ in $CDCl_3$) Multiplicity (*J* in Hz) | $^{13}$C-NMR | $^{1}$H NMR Chemical shift ($\delta$ in $CDCl_3$+ $d_6$-DMSO) Multiplicity (*J* in Hz) | $^{13}$C-NMR |
| 1,1' | - | 135.1 | - | 134.48 |
| 2,2' | 6.76-6.84 (2H,m) | 119.4 | 6.72-6.80 (2H,m) | 118.42 |
| 3,3' | 6.76-6.84 (2H,m) | 108.2 | 6.72-6.80 (2H,m) | 107.30 |
| 4,4' | - | 147.1 | - | 146.26 |
| 5,5' | - | 148.0 | - | 147.19 |
| 6,6' | 6.76-6.84 (2H,m) | 106.5 | 6.72-6.80 (2H,m) | 105.74 |
| 7,7' | 4.71 (2H,m) | 85.8 | 4.60 (2H,m) | 84.80 |
| 8,8' | 3.04 (2H, m) | 54.4 | 2.90 (2H, m) | 53.64 |
| 9,9' | $H_a$-9 and $H_a$-9' 4.23 (2H, m) $H_b$-9 and $H_b$-9' 3.84 (2H, m) | 71.7 | $H_a$-9 $H_a$-9′ 4.15 (2H, m) $H_b$-9 and $H_b$-9' 3.75 (2H, m) | 70.74 |
| 10,10' | 5.92 (4H, s) | 101.1 | 5.92 (4H, s) | 95.43 |

Assignments were made by comparison of earlier data[28]

**Structural elucidation of Compound DH-3 (21):**




**DH-3 (21)**

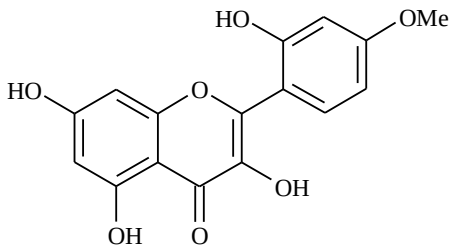
Compound DH-3 **(21)** was obtained as pale yellow needles, m.p. 245-247$^0$C $[\alpha]_D^{27}$ +15.8 (c 0.30, EtOH). Its ESI mass spectrum showed molecular ion peak $[M]^+$at m/z 272 (calcd. 272.26) and analyzed for $C_{15}H_{12}O_5$, which required eleven degrees of unsaturation. The IR spectrum of compound DH-3 **(21)** showed bands for hydroxyl and α,β-unsaturated carbonyl group at 3417,2912 and 1626 $cm^{-1}$. The UV absorption of compound DH-3 **(21)** was observed at $\lambda_{max}$ 226,292 nm and the $^1$H-NMR spectrum (Fig. 1.12,Table-10) of the compound revealed the presence of a methylene proton at δ 2.85 (1H, dd, *J* = 17.1, 12.8 Hz, H-3ax), 2.63 (1H, dd, *J* = 17.1, 2.2 Hz, H-3eq), and a methine proton at δ 5.42 (1H, *J*=12.08Hz, 2.2 Hz), further, in the downfield, an aromatic proton of ring 'B' at δ 6.20 (2H,d,*J* = 2.68Hz,H-6 and H-8) and the signals at δ 7.25 (2H, dd, *J*= 8.05Hz, *J* =1.34Hz,H-3'and H-5'), δ 6.82 (2H,dd,*J*= 8.05Hz, *J* = 1.34Hz,H-2' and H-6') due to aromatic protons of ring C and the presence of three hydroxyl groups appeared at δ 9.39 (2H,s, 7-OH,4'-OH) was assigned to phenolic hydroxyl groups and the other hydroxyl group appeared as a sharp singlet at δ 12.00 (1H,s,5-OH) indicative of a chelated hydroxyl proton. Its $^{13}$C-NMR spectrum (Fig. 1.13,Table-10) showed the presence of fifteen carbons consisting of seven quaternary carbons at δ 196.86, 167.11, 163.93, 163.39,158.17, 129.31 and 102.21 which were assigned to C-4, C-7, C-5, C-9,C-4',C-1' and C-10 respectively. The seven methane carbons of flavonoid ring carbons shows at δ 128.81, ,115.62, 96.25,95.43 and 78.88 was assigned to C-2' & C-6', C-3'& C-5',C-6, C-8 and C-2 respectively. The methylene carbon for C-3 appeared at δ 42.41. From the forgoing spectral data and a literature [29] survey compound DH-3 **(21)** was established as as (*S*)-naringenin, which was previously isolated from the plant *Streptomyces graminofaciens*, fruits of *Ficus benjamina* Linn. and the roots and rhizomes of *Asarum longerhizomatosum* and this is first report of isolation from the plant *Decalepis hamiltonii*.

**Table-10. $^{1}$H and $^{13}$C-NMR of DH-3 (21)**

| Position | Chemical shift values based on the reference [29] | | Chemical shift values of **DH-3 (21)** | |
|---|---|---|---|---|
| | $^{1}$H NMR Chemical shift (δ in $d_6$-DMSO) Multiplicity (*J* in Hz) | $^{13}$C-NMR | $^{1}$H NMR Chemical shift (δ in $D_6$-DMSO) Multiplicity (*J* in Hz) | $^{13}$C-NMR |
| 1 | - | - | - | - |
| 2 | 5.45 (1H, dd.*J* = 2.7,12.9 Hz) | 78.4 | 5.42 (1H, dd.*J* = 2.2,12.0 Hz) | 78.88 |
| 3 | 3.26 (1H, dd, *J* = 12.9,17.3, H-$3_{trans}$) 2.68 (1H, dd, *J* = 3.2 ,17.3, H-$3_{cis}$) | 41.9 | 2.85 (1H, dd, *J* = 12.8 , 17.1, H-$3_{trans}$) 2.63 (1H, dd, *J* = 2.2 , 17.1, H-$3_{cis}$) | 42.41 |
| 4 | - | 196.3 | - | 196.86 |
| 5-OH | 12.14 (1H,br,s) | 163.4 | 12.01 (1H,br,s) | 163.93 |
| 6 | 5.87 (2H,s, H-6,H-8) | 94.9 | 5.95 (1H,d, *J* = 2.6 Hz) | 96.25 |
| 7-OH | 9.59 (1H,br,s) | 166.6 | 9.62 (1H,br,s) | 167.11 |
| 8 | 5.87 (2H,s, H-6,H-8) | 95.8 | 5.95 (1H,d, *J* = 2.6 Hz) | 95.43 |
| 9 | - | 162.9 | - | 163.39 |
| 10 | - | 101.7 | - | 102.21 |
| 1' | - | 128.8 | - | 129.31 |
| 2',6' | 7.31 (1H,d, *J* = 8.6 Hz) | 128.3 | 6.82 (1H,d, *J* = 8.0,1.3 Hz) | 128.81 |
| 3',5' | 6.80 (1H,d, *J* = 8.6 Hz) | 115.1 | 7.40 (1H,d, *J* = 8.0,1.3 Hz) | 115.62 |
| 4'-OH | 9.59 (1H,br,s) | 157.7 | 9.62 (1H,br,s) | 158.17 |

Assignments were made by comparison of earlier data [29]

**Structural elucidation of Compound DH-4 (22)**




**DH - 4 (22)**

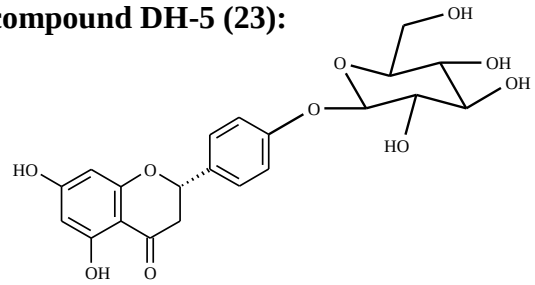
Compound DH-4 **(22)** was obtained as yellow needles, m.p. 235-237$^0$C. Its HRMS mass spectrum (Fig. 1.18) showed molecular ion $[M+1]^+$ peak at *m/z* 317 (calcd. 317.26) and analyzed for $C_{16}H_{12}O_7$, which required eleven degrees of unsaturation. Its $^1$H NMR spectrum (Fig. 1.14, Table-11) in acetone-$d_6$ of compound DH-4 **(22)** indicates the presence of four hydroxyl groups of which three appeared as a broad singlet at δ 9.45(1H, s, 2-OH) and 8.40 (2H, s, 7-OH, 2'-OH) was assigned to phenolic hydroxyl groups and the other hydroxyl group appeared as a shart singlet at δ 12.80 (1H, s, 5-OH) indicates the presence of a chelated hydroxyl group at C-5. Further, its $^1$H NMR spectrum displayed signals at δ 7.63 (1H, dd, *J* = 8.8 Hz, 1.6 Hz), δ 7.01 (1H, d, *J*= 8.8 Hz, 6.46 (1H, d, *J* = 1.6 Hz), δ 6.22 (2H, *J* = 1.60 Hz) and a singnal at δ 3.8 (3H, s). The signals at δ 7.63 and δ 7.01 were assigned to H-5' and H- 6' respectively. The signal at δ 6.46 was assigned to H-3'. Finally, the signal at δ 6.22 was assigned to H-6 and H-8 protons and signal at δ 3.8 was assigned to methaoxy ether. Its $^{13}$C-NMR spectrum (Fig.1.15, Table-11 ) in acetone-$d_6$ displayed sixteen carbons of assignments which were also made by DEPT (Fig.1.16) experiment The spectrum showed the presence of sixteen carbons consisting of ten quaternary carbons at δ 179.50, 149.08, 164.87, 157.81, 145.85, 163.1, 156.7, 139.26, 123.03 and 105.87 which were assigned to C-4, C-9, C-7, C-5, C-2,C-4',C-2',C-3,C-1' and C-10 respectively. Five methane carbons shows at δ 122.12, 99.37, 116.32, 94.48 and 94.42 was assigned to C-6', C-3'& C-5', C-6 and C-8 respectively, and a methoxy ether at δ 60.17. Further the position of methaoxy group of ring C was established by NOE spectrum (Fig.1.17). Foregoing and literature [30] survey revealed that compound DH-4 was found to be milimorin **(22)** which was previously isolated from the plant *Euphorbia milii*., and this is first report of isolation from the plant *Decalepis hamiltonii.* Hence the structure of DH-4 was established as milimorin **(22)**.

**Table-11. $^{1}H$ and $^{13}C$-NMR of Milimorin DH-4 (22)**

| Position | Chemical shift values based on the reference [30] | | Chemical shift values of **DH-4 (22)** | |
|---|---|---|---|---|
| | $^{1}H$ NMR Chemical shift ($\delta$ in $CDCl_3$) Multiplicity (*J* in Hz) | $^{13}C$-NMR | $^{1}H$ NMR Chemical shift ($\delta$ in Acetone-$d_6$) Multiplicity (*J* in Hz) | $^{13}C$-NMR |
| 1 | - | - | - | - |
| 2 | - | 153.3 | | 145.85 |
| 3 | OH | 142.1 | 9.62 (1H,br,s, OH-3) | 139.26 |
| 4 | - | 173.9 | | 179.50 |
| 5-OH | 12.82 (1H,br,s) | 161.0 | 12.80 (1H,br,s) | 157.81 |
| 6 | 6.25 (1H,d,*J* = 1.5 Hz) | 95.6 | 6.22 (1H,d,*J* = 1.6 Hz) | 94.42 |
| 7-OH | OH | 163.7 | 9.62 (1H,br,s, OH-7) | 164.87 |
| 8 | 6.25 (1H,d,*J* = 1.5 Hz) | 92.6 | 6.22 (1H,d,*J* = 1.6 Hz) | 94.48 |
| 9 | - | 158.8 | - | 149.08 |
| 10 | - | 109.8 | - | 105.87 |
| 1' | - | 112.8 | - | 123.03 |
| 2' | OH | 159.4 | 8.42 (1H,br,s, OH-2') | 156.75 |
| 3' | 6.39 (1H,d,*J* = 1.5 Hz) | 98.8 | 6.46 (1H,d,*J* = 1.6 Hz) | 99.37 |
| 4' | - | 162.6 | - | 163.17 |
| 5' | 6.54 (1H, dd, *J* = 8.5,1.5 Hz) | 104.7 | 7.63 (1H, dd, *J* = 8.8,1.6 Hz) | 116.32 |
| 6' | 7.36 (1H,d, *J* = 8.5 Hz) | 131.7 | 7.01 (1H,d, *J* = 8.8 Hz) | 122.12 |
| $OCH_3$ | 3.79 (3H, s) | 60.3 | 3.80 (3H, s) | 60.17 |

Assignments were made by comparison of earlier data [30]

**Structure elucidation of compound DH-5 (23):**




**DH -5 (23)**

Compound DH-5 **(23)** was obtained as yellow needels, $[\alpha]_D^{27}$ -22.5$^o$ (MeOH). Its ESI mass spectrum (Fig. 1.22) showed molecular ion peak at *m/z* 435.40 $[M+1]^+$ and analyzed for $C_{21}H_{22}O_{10}$, which required eleven degrees of unsaturation. The IR spectrum (Fig.1.23) showed bands for two kinds of hydroxyl groups and carbonyl group at 3417, 2912 and 1626 $cm^{-1}$ respectively. The UV absorption of compound DH-5 **(23)** was observed at $\lambda_{max}$ 332 and 286 and the $^1$H-NMR spectrum (Fig. 1.19,Table-12) of the compound revealed the presence of a methylene proton at δ 3.06 (1H, dd, *J* = 17.1, 12.8 Hz, H-$3_{ax}$), 2.63 (1H, dd, *J* = 17.1, 2.2 Hz, H-$3_{eq}$), and a methine proton at δ 5.42 (1H,d,*J* = 12.0 Hz, 2.2 Hz,H-2). The anomeric proton of glucose moiety shows at δ 4.72 (1H, d, *J* = 7.2 Hz, H-1'') finally, the remaining protons of glucose moiety shows at δ 3.21-3.50 (4H, m). Further, in the downfield, an aromatic proton of ring A at δ 6.20 (2H,d, *J* = 2.6 Hz,H-6 and H-8) and the signals at δ 7.25 (2H,d, *J*= 8.0 Hz , H-3' and H-5'), δ 6.82 (2H,d, *J*= 8.0 Hz, H-2' and H-6') due to aromatic ring protons of B and a chelated hydroxyl group at δ 12.00 (1H,br,s, 5-OH) were observed. The $^{13}$C-NMR spectrum (Fig-1.20, Table-12.) in $d_6$-DMSO and also the assignments were made by DEPT (Fig-1.21) experiment. Its $^{13}$C-NMR spectrum showed the presence of twenty-one carbons consisting of seven quaternary carbons at δ 196.18, 165.00, 162.90, 162.31, 157.52,130.00 and 103.02 which were assigned to C-4, C-7, C-5, C-9,C-4',C-1' and C-10 respectively. The seven methane carbons of flavonoid ring carbons shows at δ 128.09, ,114.98, 95.24,95.40 and 78.40 was assigned to C-2' & C-6', C-3' & C-5',C-6, C-8 and C-2 respectively. In addition to these signals, its $^{13}$C-NMR spectrum show signals due to δ 96.37, 72.58, 76.67, 69.16, 76.00 and 60.42 which were assigned to C-1'', C-2'',C-3'',C-4'',C-5'',and C-6'' respectively. The methylene carbon for C-3 appeared at 42.15.Based on the spectral data obtained and compared with published data [29] compound DH-5 **(23)** concluded to be (*S)*-Naringenin 4'-O--β –glucopyranoside (choerospondin), which was previously isolated from the plant

*Asarum longerhizomatosum* and this is first report of isolation from the plant *Decalepis hamiltonii*.

**Table-12. $^{1}$H and $^{13}$C-NMR of DH-5 (23)**

| Position | Chemical shift values based on the reference [29] | | Chemical shift values of **DH-5 (23)** | |
|---|---|---|---|---|
| | $^{1}$H NMR Chemical shift ($\delta$ in $d_6$-DMSO) Multiplicity ($J$ in Hz) | $^{13}$C-NMR | $^{1}$H NMR Chemical shift ($\delta$ in $d_6$-DMSO ) Multiplicity ($J$ in Hz) | $^{13}$C-NMR |
| 1 | - | - | - | - |
| 2 | 5.64 (1H, $J$ = 12.9,2.7 Hz) | 78.1 | 5.42 (1H, $J$ = 12.0,2.2 Hz) | 78.43 |
| 3 | 3.01 (1H, dd, $J$ = 17.3, 12.9 Hz , H-$3_{ax}$) 2.64 (1H, dd, J = 17.3, 2.7 Hz,H-$3_{eq}$) | 42.1 | 3.06 (1H, dd, $J$ = 17.1, 12.8 Hz , H-$3_{ax}$) 2.63 (1H, dd, J = 17.1, 2.2 Hz,H-$3_{eq}$) | 42.15 |
| 4 | - | 196.0 | - | 196.18 |
| 5 | 12.00 (1H,br,s,OH-5) | 163.0 | 12.00 (1H,br,s,OH-5) | 162.90 |
| 6 | 6.10 (1H,d, $J$ = 2.6 Hz) | 95.7 | 6.20 (1H,d, $J$ = 2.6Hz) | 95.40 |
| 7 | 9.03 (1H,br,s) | 166.3 | 9.39 (1H,br,s,OH-7) | 165.06 |
| 8 | 6.10 (1H,d, $J$ = 2.68 Hz) | 95.0 | 6.20 (1H,d, $J$ = 2.6 Hz) | 95.24 |
| 9 | - | 162.7 | - | 162.31 |
| 10 | - | 101.7 | - | 103.02 |
| 1’ | - | 131.8 | - | 128.09 |
| 2',6' | 7.30 (1H,d, $J$ = 9.2 Hz) | 128.0 | 7.25 (1H,d, $J$ = 8.0 Hz) | 127.66 |
| 3',5' | 6.82 (2H,d, $J$ = 9.2 Hz) | 116.1 | 6.82 (2H,d, $J$ = 8.0 Hz) | 114.98 |
| 4' | - | 157.4 | - | 157.52 |
| 1" | 4.80 (1H, d, $J$ = 7.5 Hz) | 100.2 | 4.72 (1H, d, $J$ = 7.2 Hz) | 99.48 |
| 2" | 3.20-3.50 (4H,m) | 73.1 | 3.21-3.50 (4H,m) | 72.58 |
| 3" | | 77.0 | | 76.67 |
| 4" | | 69.5 | | 69.16 |
| 5" | | 76.5 | | 76.00 |
| 6" | 3.68 (2H,d, $J$ =11.6 Hz) | 60.6 | 3.70 (2H,d, $J$ =11.6 Hz) | 60.42 |

Assignments were made by comparison of earlier data [29]

## EXPERIMENTAL

### Collection and Identification

The plant material *Decalepis hamiltonii* (*Asclepiadaceae*) was collected from Bhadrachalam forest, Andhra Pradesh, India in January 2007, and identified by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, and Tirupathi, India.

### Extraction and Isolation procedure

The shade dried and powdered of root part of the plant (3.1 kg) *Decalepis hamiltonii* was extracted with n-hexane (3x5 L) followed by $CH_2Cl_2$: MeOH (3x5 L) at room temperature.

### Hexane extract

The combined hexane extract was filtered and concentrated under reduced pressure to obtained greenish gummy residue (40.4g). The crude slurry was subjected to silicagel (100-200 mesh) column chromatography by gradient elution using with hexane through hexane-ethylacetate mixtures to ethylacetate. Fractions (250 ml) were collected and monitored on silicagel ($GF_{254}$) TLC. The visualization of spots on TLC was carried out either in UV light or by exposing TLC plates to iodine vapours or by spraying 10% sulfuric acid in methanol and heating at $110^0$C. Similar fractions were combined and the results borne out in the chromatography are recorded in the following Table-13.

**Table-13: Silica gel column chromatography of hexane extract of *Decalepis hamiltonii***

| Eluent (Hexane:EtOAc) | Fractions | Residue (g) | Remarks |
|---|---|---|---|
| 100:0 | 1-5 | 2.1 | Fatty oil |
| 90:10 | 6-15 | 4.6 | Fatty solid |
| 80:20 | 16-18 | 3.1 | Fraction-I |
| 70:30 | 19-23 | 5.2 | Fraction –II |
| 60:40 | 24-32 | 6.8 | Green slurry |
| 40:60 | 33-40 | 5.2 | Green pigment |

| | | | |
|---|---|---|---|
| 20:80 | 41-48 | 6.4 | Dark green matter |
| 0:100 | 49-52 | 7.0 | Dark green matter |

**Fraction-1**

It was obtained as a mixture of compounds as noticed from its silica gel TLC plates and hence it was re-chromatographed over silica gel (100-200 mesh) column chromatography using hexane-ethylacetate mixtures as eluents. Fractions (20ml each) were collected and the results of the chromatography are recorded in the Table-14.

**Table-14: Silica gel column chromatography of Fraction-I**

| Eluent (Hexane:EtOAc) | Fractions | Yield (mg) | Remarks |
|---|---|---|---|
| 100:0 | 1-10 | 160 | DH-1 |
| 95:5 | 11-14 | 140 | DH-2 |
| 90:10 | 14-20 | 400 | Fatty solid |
| 80:20 | 21-25 | 820 | Fatty solid |
| 70:30 | 26-31 | 340 | Fatty material |
| 60:40 | 32-38 | 1160 | Fatty material |
| 50:50 | 39-42 | 80 | Fatty material |

**Fraction-II**

It was obtained as a mixture of compounds as noticed from its silica gel TLC plates and hence it was re-chromatographed over silica gel (100-200 mesh) column chromatography using hexane-ethylacetate mixtures as eluents. Fractions (10ml each) were collected and the results of the chromatography are recorded in the Table-15.

**Table-15: Silica gel column chromatography of Fraction-II**

| Eluent (Hexane:EtOAc) | Fractions | Yield (mg) | Remarks |
|---|---|---|---|
| 100:0 | 1-10 | 560 | Fatty oil |
| 90:5 | 11-16 | 480 | Fatty solid |
| 90:10 | 17-25 | 650 | Fatty solid |
| 80:20 | 26-33 | 140 | DH-3 |
| 85:25 | 34-36 | 520 | Fatty material |
| 70:30 | 37-42 | 870 | Fatty material |

| | | | |
|---|---|---|---|
| 60:40 | 42-48 | 1490 | Fatty material |
| 50:50 | 48-55 | 490 | Fatty material |

**Dichloromethane: Methanol extract**

The combined $CH_2Cl_2$: MeOH extract was filtered and concentrated under reduced pressure to yield a dark greenish residue (80.5g). This crude solid was subjected to silicagel (100-200 mesh) column chromatography by gradient elution using with hexane through hexane-ethylacetate-methanol mixtures to methanol as eluents. Fractions (350 ml) were collected and monitored on silicagel ($GF_{254}$) TLC. The visualization of spots on TLC was carried out either in UV light or by exposing TLC plates to iodine vapours or by spraying 10% sulfuric acid in methanol and heating at $110^0$C. Similar fractions were combined and the results borne out in the chromatography are recorded in the following Table-16.

**Table-16: Silica gel column chromatography of hexane extract of *Salvinia natans***

| Eluent (Hexane:EtOAc:MeOH) | Fractions | Residue (g) | Remarks |
|---|---|---|---|
| 100:0:0 | 1-6 | 5.2 | Fatty oil |
| 90:10:0 | 7-13 | 4.6 | Fatty solid |
| 80:20:0 | 14-18 | 10.1 | Fatty solid |
| 70:30:0 | 19-25 | 3.0 | Fraction -I |
| 60:40:0 | 26-30 | 12.7 | Green slurry |
| 50:50:0 | 31-35 | 16.0 | Dark green matter |
| 40:60:0 | 36-41 | 8.2 | Green pigment |
| 20:80:0 | 42-47 | 6.0 | Green pigment |
| 0:100:0 | 48-53 | 4.5 | Dark green matter |
| 0:80:20 | 54-59 | 3.5 | Fraction-II |
| 0:40:60 | 60-65 | 2.0 | Intractable gum |

| | | | |
|---|---|---|---|
| 0:20:80 | 66-70 | 2.4 | Intractable gum |
| 0:0:100 | 71-75 | 2.3 | Intractable gum |

**Fraction-1**

It was obtained as a mixture of compounds as noticed from its silica gel TLC plates and hence it was re-chromatographed over silica gel (100-200 mesh) column chromatography using hexane-ethylacetate mixtures as eluents. Fractions (5 ml each) were collected and the results of the chromatography are recorded in the Table-17.

**Table-17: Silica gel column chromatography of Fraction-I**

| Eluent (Hexane:EtOAc) | Fractions | Yield (mg) | Remarks |
|---|---|---|---|
| 100:0 | 1-10 | 660 | Fatty oil |
| 95:5 | 11-14 | 280 | Fatty solid |
| 80:20 | 14-20 | 800 | Fatty solid |
| 85:25 | 21-25 | 80 | DH-4 |
| 70:30 | 26-31 | 290 | Fatty material |
| 60:40 | 32-38 | 730 | Fatty material |
| 50:50 | 39-42 | 160 | Fatty material |

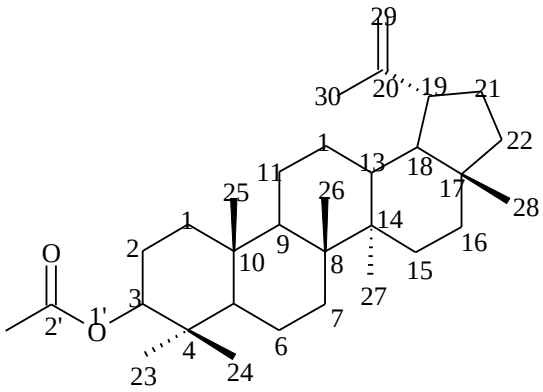
**Fraction-1I**

It was obtained as a mixture of compounds as noticed from its silica gel TLC plates and hence it was re-chromatographed over silica gel (100-200 mesh) column chromatography using hexane-ethylacetate mixtures as eluents. Fractions (5 ml each) were collected and the results of the chromatography are recorded in the Table-18.

**Table-18: Silica gel column chromatography of Fraction-II**

| Eluent (Hexane:EtOAc) | Fractions | Yield (mg) | Remarks |
|---|---|---|---|
| 100:0 | 1-10 | 920 | Fatty oil |
| 95:5 | 11-14 | 40 | Fatty solid |
| 80:20 | 14-20 | 500 | Fatty solid |
| 85:25 | 21-25 | 440 | Fatty material |
| 70:30 | 26-31 | 1320 | Fatty material |

| | | | |
|---|---|---|---|
| 60:40 | 32-38 | 180 | DH-5 |

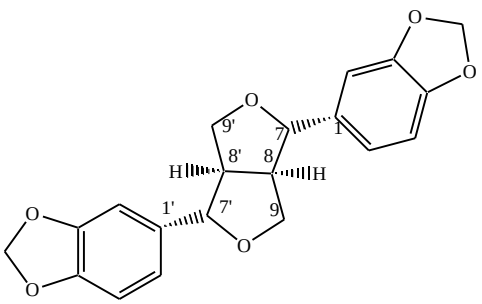



| | | | |
|---|---|---|---|
| 50:50 | 39-42 | 100 | Fatty material |

**DH-1 (19)**

| | | |
|---|---|---|
| **Compound DH-1** | **:** | **Lupeol acetate** |
| **Physical property** | **:** | white needles |
| **Amount isolated** | **:** | 0.18 g |
| **Melting point** | **:** | 127-129$^0$C |
| **$[\alpha]_D$ $^{25}$** | **:** | +27.2 (c 4.8, $CHCl_3$) |
| **Molecular formula** | **:** | $C_{32}H_{52}O_2$ |
| **EIMS** | **:** | 468 |
| **$^1$H NMR** | **:** | ($CDCl_3$,200 MHz) δ 4.69 (1H, s, H-29b), 4.57 (1H, s, H-29a), 4.47 (1H, dd, *J* = 4.4, 12.8 Hz, H-3), 2.05 (3H, s, H-2'), 1.69 (3H, s, H-30), 1.03 (3H, s, H-25), 0.90 (3H, s, H-28), 0.80 (9H, s, H-23,H-24,H-26), 0.79 (3H, s, H-27). |
| **$^{13}$C NMR** | **:** | ($CDCl_3$, 75 Mz) δ 171.26 (C-1'), 151.19 (C 20), 109.58 (C-29), 81.21 (C-3), 55.66 (C-5), |

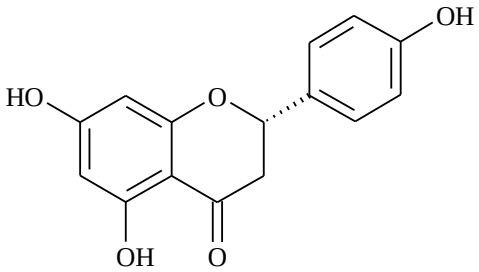
50.57 (C-9), 48.52 (C-18), 48.23 (C-19), 43.22 (C-17), 43.05 (C-14), 41.08 (C-8), 40.22 (C-22), 38.61 (C-1), 38.02 (C-4), 37.31 (C-10), 36.80 (C-13), 35.80 (C-16), 34.44 (C-7), 30.06 (C-21), 28.17 (C-2'), 27.66 (C-23), 25.33 (C-15), 23.94 (C-12), 21.55 (C-2), 21.17 (C-11), 19.51 (C-30), 18.43 (C-6), 18.23 (C-28), 16.72 (C-24, C-26), 16.40 (C-25), 14.70 (C-27).




**DH-2 (20)**

| | | |
|---|---|---|
| **Compound DH-2** | **:** | **Sesamin** |
| **Physical property** | **:** | colorless prisms |
| **Amount isolated** | **:** | 80mg |
| **Melting point** | **:** | 127-129$^0$C |
| **$[\alpha]_D^{25}$** | **:** | +64.5 (c 1.75, $CHCl_3$) |
| **IR (KBr) $\nu_{max}$** | **:** | 1033 $cm^{-1}$ |
| **Molecular formula** | **:** | $C_{20}H_{18}O_6$ |
| **EI-MS** | **:** | m/z 354 $[M]^+$ |
| **$^1$H NMR** | **:** | ($CDCl_3$+DMSO-$d_6$, 200 MHz): δ 6.72-6.80 ( 2H, m, H-1, H-1'), 6.72-6.80 ( 2H, m, H-3, H-3'), 6.72-6.80 ( 2H, m, H-6, H-6'), 5.92 (4H, s, H-10, H-10'), 4.60 (2H, m, H-7, H-7'), 4.15 (2H, m, $H_a$-9 $H_a$-9'), 3.75 (2H, m, $H_b$-9 and $H_b$-9'), 2.90 (2H, m, H-8, H-8') |

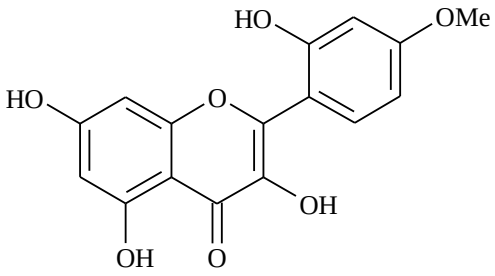
| | | |
|---|---|---|
| **$^{13}$C NMR** | **:** | ($CDCl_3$+ DMSO-d6, 75 MHz): δ 53.64 (C-8 & C-8′ ), 70.74 ( C-9 & C-9′), 84.80 ( C-7 & C-7′), 95.43 [(-O-$CH_2$-O-)$_2$], 105.74 ( C-6 & C-6′), 107.30 ( C-3 & C-3′), 118.42 ( C-2 & C-2′), 134.48 (C-1 & C-1′), 146.26 (C-4 & C-4′) and 147.19 (C-5 & C-5′) |




**DH-3 (21)**

| | | |
|---|---|---|
| **Compound DH-3** | **:** | **Naringenin** |
| **Physical property** | **:** | pale yellow needles |
| **Amount isolated** | **:** | 0.14g |
| **Melting point** | **:** | 245-247$^0$C |
| **$[\alpha]_D^{25}$** | **:** | +15.8 (c 0.30, EtOH) |
| **Molecular formula** | **:** | $C_{15}H_{12}O_5$ |
| **$^1$H NMR** | **:** | (DMSO-$d_6$, 300 MHz): δ 12.01 (1H, br, s, 5-OH), 9.62 (2H,br,s,7-OH, 4'-OH), 7.40 (1H,d, *J* = 8.05, 1.34 Hz), 6.82 (1H,d, *J* = 8.05, 1.34 Hz), 5.95 (2H,d, J = 2.68 Hz, H-6 & H-8), 5.42 (1H, dd., *J* = 2.2, 12.08 Hz ), 2.85 (1H, dd, J = 12.8 , 17.1, H-3$_{trans}$), 2.63 (1H, dd, J = 2.2 , 17.1, H-3$_{cis}$), |
| **$^{13}$C NMR** | **:** | (DMSO-$d_6$, 75MHz): δ 196.86 (C-4), 167.11(C-7), 163.93 (C-5), 163.39 (C-9), 158.17 (C-4′), 129.31 ( C-1′), 128.81(C-2′ & |

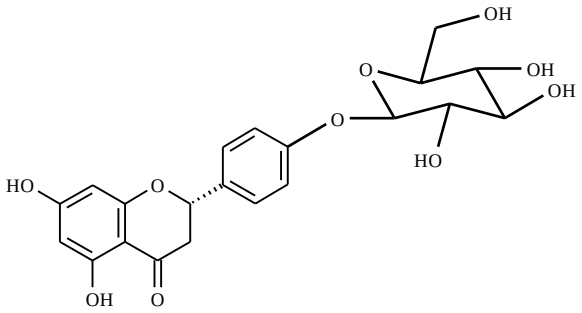
C-6′),115.62 (C-3' & C-5′), 102.21( C-10), 96.25 ( C-6 ), 78.80 (C-2), 42.41(C-3 )




**DH-4 (22)**

| | | |
|---|---|---|
| **Compound DH-4** | **:** | **Milimorin** |
| **Physical property** | **:** | yellow needles |
| **Amount isolated** | **:** | 0.14 g |
| **Melting point** | **:** | 235-237$^0$C |
| **UV $\lambda_{max}$** | **:** | 260,304,346 cm$^{-1}$ |
| **Molecular formula** | **:** | $C_{16}H_{12}O_7$ |
| **EI-MS** | **:** | m/z 317 $[M]^+$ |
| **$^1$H NMR** | **:** | (Acetone-$d_6$, 300 MHz): δ 12.80 (1H, br, s,OH ), 9.62 ( 1H,br,s,3- OH), 8.42 (1H,br,s, 2'- OH), 7.63 (1H, dd, *J* = 8.8,1.6 Hz), 7.01 (1H,d, *J* = 8.8 Hz), 6.46 (1H,d, *J* = 1.6 Hz), 6.22 (2H,d, *J* = 1.6 Hz, H-6 & H-8 ), 3.80 (3H, s) |

| | | |
|---|---|---|
| **$^{13}$C NMR** | **:** | (Acetone-$d_6$, 75MHz): δ 179.50 (C-4), 164.87 (C-7), 157.81 (C-5), 149.08 (C-9), 163.17 (C-4′ ), 123.03 (C-1′), 156.75 (C-2'), 122.12 (C-6′), 99.37 (C-3'), 116.32 (C-5′ ), 105.87 (C-10), 94.42 (C-6), 145.85 (C-2 ), 139.26 (C-3 ), 60.17 ($OCH_3$) |




**DH-5 (23)**

| | | |
|---|---|---|
| **Compound DH-5** | **:** | **Choerospondin** |
| **Physical property** | **:** | pale yellow needles |
| **Amount isolated** | **:** | 0.16 g |
| **$[\alpha]_D^{25}$** | **:** | -22.5$^o$ (MeOH). |
| **Molecular formula** | **:** | $C_{21}H_{22}O_{10}$ |
| **$^1$H NMR** | **:** | (DMSO-$d_6$, 200 MHz): δ 12.00 (1H, br, s, 5 – OH), 9.39 (1H,br,s,7-OH), 6.20 (1H,d, *J* = 2.6Hz, H-6,H- 8), 5.42 (1H, *J* = 12.0,2.2Hz, H-2), 3.06 (1H, dd, *J* = 17.1, 12.8 Hz , H-$3_{ax}$),2.63 (1H, dd, *J* = 17.1, 2.2 Hz,H-$3_{eq}$), 7.25 (1H,d, *J* = 8.0 Hz, H-2' and H-6'), 6.82 (2H,d, *J* = 8.0 Hz, H-3' and H-5'), 4.72 (1H, d, |

| | | |
|---|---|---|
| | | *J* = 7.2 Hz, H-1"), 3.21-3.50 (4H,m, H-2" - 5"), 3.70 (2H,d, *J* =11.6 Hz, H-6") |
| **$^{13}$C NMR** | **:** | (DMSO-$d_6$, 75 MHz): δ 196.0 ( C-4 ), 165.06 (C-7), 162.90 (C-5), 162.31 (C-9), 157.52 (C-4′), 128.09 (C-1′), 127.66 (C- 2′ & 6′), 114.98 (C- 3'& 5′ ), 103.02 (C-10), 99.48 (C-1"), 95.40 (C-6), 95.24 (C-8), 78.43 (C-2), 76.67 ( C-3" ), 76.00 (C-5" ), 72.58 ( C-2"), 69.16 ( C-4"), 60.42 (C-6" ), 42.15 (C-3). |

**REFERENCES:**


1. Vines G: Herbal harvests with a future: towards sustainable sources for medicinal plants, Plantlife International. **2004** [http://www.Plantlife.Org.uk].
2. Rozentsvet, O.A., Rezanka, T., Bosenko, E.S., Uzhametskaya, E.A., Dembitskii, .M., *Chem. Nat. Compd.*, **2005**, *41*, 487–490.
3. Narasimhulu M.; Ashalatha K.; Sri Laxmi P.; Sarma A. V. S.; Rama Rao B.; Kavi Kishor P.B.; David Krupadanam G.L.; Zehra Ali A.; Asok Tiwari K.; Panneer Selvam A.; Venkateswarlu Y. *Nat. Prod. Res.*, **2010**, 24, 1390–1394.
4. (a) Dragsted, L.O., Strube, M., & Larsen, J.C. *Pharmacology and Toxicology*, **1993**, 72,116–135. (b) Johnson, I.T., Williamson, G., & Musk, S.R. *Nutrition Reviews*, **1994**, 7, 175–204. **(c)** Owen, R.W., Giacosa, A., Hull, W.E., Haubner, R., Spiegelhalder, B., & Bartsch, H. *European Journal of Cancer*, **2000**, 36, 1235–1247.
5. Iqbal Choudhary M, Naheed Nadra, Abbaskhan Ahmed, Musharraf Ghulam Syed, Atta-ur-Rahman Siddiqui Hina; *Phytochemistry* **2008**, *69*,1018-1023.
6. Lahdesmaki P; *Physiol Plantarum* **1968**,*21*,1097-1103.
7. Sies H. *Eur j Biochem*, **1993**, *215*, 213-219.
8. Halliwell B. *Advances in pharmacology*, **1997**, *38*, 3-7.
9. Ravikanth, V.; Reddy, V.L.N.; Reddy, A.V.; Ravinder, K.; Rao, T.P.; Siva Ram, T., et al. *Chemical and Pharmaceutical Bulletin*, **2003**, *51*, 431–434.
10. (a) Debruyn, A.; Antieunis, M. *Acta Ciencia Indica*, **1974**, *1*, 83–88
    (b) Vignon, M.R., & Vottero, J.A. *Tetrahedron Letters*, **1976**, *17*, 2445–2448.
11. Harada, N.; & Nakanishi, K. *Accounts of Chemical Research*, **1972**, *5*, 257–263.
12. Rao, R.J.; Tiwari, A.K.; Kumar, U.S.; Reddy, S.V.; Ali, A.Z. & Rao, J.M. *Bioorganic and Medicinal Chemistry Letters*, **2003**, *13*, 2777–2780.
13. Perez, G.S.; Perez, G.R.M.; Perez, G.C.; Zavala, S.M.A.; & Vargas, S.R. *Pharmaceutica Acta Helvetiae*, **1997**, *72*, 143–146.
14. Phadke, N. Y.; Gholap, K.; Ramakrishnan; Subbulakshmi,G. *J. Food Sci. Technol*. **1994**, *31*, 472-475.
15. Murti, P. B. R.; Seshadri, T. R. *Proc. Indian Acad. Sci.* **1941a**, *A13*, 221-232.
16. Murti, P. B. R.; Seshadri, T. R. *Proc. Indian Acad. Sci*. **1941b**, *A13*, 399-403.

17. Murti, P. B. R.; Seshadri, T. R. *Proc. Indian Acad. Sci*. **1941c**, *A14*, 93-99.
18. Thangadurai, D.; Anitha, S.; Pullaiah, T.; Reddy, P. N.; Ramachandraiah, O.*J. Agric. Food Chem*. **2002**, *50*, 3147-3149.
19. Nagarajan, S.; Jagan Mohan Rao, L.; Gurudutt, K. N. *Flavour Fragrance J*., **2001**, *16*, 27-29.
20. Shereen; Srivastava, A.; Shivanandappa, T. 71st Annual Meeting of the Society of Biological Chemists, Hyderabad, India, **2001**; Abstract P2B28, p 176.
21. R. Harish; S. Divakar; Anup Srivastava and T. Shivanandappa, *J. Agric. Food Chem.* **2005,** *53*, 7709-7714.
22. Ashalatha K.; Venkateswarlu.Y; Moushumi Priya A., Lalitha P.; Krishnaveni, M.; S.Jayachandran, *Journal of Ethnopharmacology*, **2010**, *130*, 1,167-170
23. (a) Pakrashi, S.C.; Bhattacharyya, J.; Mookerjee, S.; Samatan, T.B. & Vorbrüggen, H. *Phytochemistry*, **1968**, *7*, 461–466. (b) A.K. Jamal*, W.A. Yaacob and Laily B. Din; *Journal of Physical Science*, **2008**,*19*, 45–50.
24. Appleton, R.A. & Enzell, C.R. *Phytochemistry*, **1971**,*10*, 447–449.
25. Chavez, J.P.; Dos Santos, I.D.; Cruz, F.G. & David, J.M., *Phytochemistry*, **1996**, *41*, 941– 943.
26. Mahato, S.B.; Banerjee, S.K. & Chakravarti, R.N. *Phytochemistry*, **1971**,*10*, 1351–1354.
27. Wang, S. & Coviello, D.A. *Tetrahedron*, **1975**, *31*,929–932,
28. Meselhy R. Meselhy et al., *Molecules*, **2003**, *8*, 614-621
29. Shu-xiang zhanga; Tadato tanib; Seiichi yamajib; Chao-mei maa; Min-chuan wanga; Shao-qing caia and Yu-ying zhaoa, *Journal of Asian Natural Products Research*, **2003**,*5*, 25–30
30. Khosa RL; Bhatia N; Sahai M. Milimorin., *Chemistry and Industry*, **1984**, *24*, 881.