Chapter-2

A. Bioassay guide fractionation of Chloroform extract of the rhizomes of *Hedychium spicatum*:

As discussed in the previous chapter, initially the shade dried rhizomes powder of *Hedychium spicatum* was extracted with petroleum ether in a soxhlet apparatus for 48 hours. After the petroleum ether extraction, the plant material was further extracted with chloroform for 48 hours. The chloroform extract was concentrated under vacuum to obtain a dark brown gummy residue. The chloroform extract was screened for cytotoxic activity. The extract showed significant cytotoxic activity on various cell lines like Colo-205 (colon cancer), A-431 (skin cancer), MCF-7 (breast cancer), A-549 (lung cancer) and CHO (Chinese hamster ovary cells) cell lines. But it has shown moderate antimicrobial activity. The dark brown gummy chloroform extract (8.5 g), was subjected to column chromatography using silica gel (60-120 mesh) to obtain 8 compounds, A, B, C, D, E and F with \( R_f \) values 0.95, 0.85, 0.80, 0.70, 0.55 and 0.50 (TLC solvent system; chloroform: methanol 80:20) respectively. Their separation and purification is described in the experimental section. The flow diagram depicting their isolation is shown below.
Structure of compound A:

Compound A was obtained as white needles, mp 124°C, showed the molecular formula of \( \text{C}_{20}\text{H}_{28}\text{O}_{2} \), \((M^+ + H, 301)\). The physical and spectroscopic data of the compound was found to be identical with the known compound, labda-8(17),11,13-trien-16-olide\(^1\), a labdane diterpene previously isolated from the rhizomes of \textit{Hedychium coronarium}\(^1\). The occurrence of labda-8(17),11,13-trien-16-olide is reported for the first time from this species.

\[
\text{Labda-8(17),11,13-trien-16-olide}
\]

Structure of compound B:

Compound B was obtained as white crystal, m.p.140-142°C and analyzed for \( \text{C}_{15}\text{H}_{28}\text{O}_{2} \) \((M^+ 230)\). The physical and spectroscopic data of the compound was found to be identical with the known compound, cryptomeridiol previously isolated from the rhizomes of \textit{H. spicatum}\(^2\).

\[
\text{Cryptomeridiol}
\]
Structure of compound C:

Compound C was isolated as a yellow semi solid with the positive optical rotation $[\alpha]_D^{25} +98.64$ (c1.64, CHCl$_3$). The HRESI-MS (Fig-1) of compound C revealed a molecular ion peak corresponding to ($M^+ + H$) at $m/z$ 331.142 indicating the molecular formula C$_{20}$H$_{26}$O$_4$. The $^1$H NMR spectrum (Table.1) of compound C showed all the features of labdane diterpene. The IR spectrum (Fig-2) displayed absorption bands at 3418 cm$^{-1}$ (OH), 1649 cm$^{-1}$ ($\alpha, \beta$-unsaturated C=O) and 1736 cm$^{-1}$ ($\alpha, \beta$-unsaturated $\gamma$-lactone). The $^1$H NMR spectrum (Fig-3) displayed four quaternary methyl signals each integrating for three protons as singlets at $\delta$ 0.97, $\delta$ 1.16, $\delta$ 1.19, and $\delta$ 1.81. It has displayed a singlet at $\delta$ 2.09 for one proton (H-5) indicating the presence of one methine adjacent to the carbonyl (C-6) carbon atom. A sharp singlet integrated for 1 H at $\delta$ 5.88 is due to methine proton (H-14) in the lactone ring. A characteristic doublet for one proton at $\delta$ 2.94 (d, $J$=7.0 Hz) indicating the presence of methine (H-9) group adjacent to olefinic double bond. The presence of one trans double bond at $\delta$ 6.73 (1H, dd, $J$=15.8Hz, 9.8Hz,) and $\delta$ 6.28 (1H, d, $J$=15.8 Hz) was suggested by the $^1$H NMR, and NOESY spectrum. And Comparison of NMR data with that of yunnacoronarin D$^3$ indicates the presence of trans double bond at C-11/C-12 position. Another sharp singlet integrated for two protons at $\delta$ 4.83 (2H, s) is assigned to CH$_2$ group in the lactone ring. Furthermore, the $^1$H NMR spectrum also revealing that the trans double bond (C-11/C-12) is conjugated with lactone ring. The $^{13}$C NMR spectrum (Fig-4) of compound C showed the presence of 20 C-atoms. The DEPT experiment (Fig-5) indicated the presence of four methyls, four CH$_2$, five CH groups, and seven quaternary Carbons. The $^{13}$C NMR spectrum of compound C also showed all the features of labdane diterpene.
Table 1: $^1$H NMR and $^{13}$C NMR data of Compound A and C in CDCl$_3$

<table>
<thead>
<tr>
<th>Position</th>
<th><strong>Compound A</strong></th>
<th></th>
<th><strong>Compound C</strong></th>
<th></th>
</tr>
</thead>
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<tr>
<td></td>
<td>$\delta_c$</td>
<td>$\delta_H$ multiplicity</td>
<td>$\delta_c$</td>
<td>$\delta_H$ multiplicity</td>
</tr>
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<td>1.53 (2H, m)</td>
<td>18.08</td>
<td>1.48 (2H, m)</td>
</tr>
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<td>1.25 (2H, m)</td>
</tr>
<tr>
<td>4</td>
<td>33.84</td>
<td>---</td>
<td>32.50</td>
<td>---</td>
</tr>
<tr>
<td>5</td>
<td>54.93</td>
<td>2.08 (1H, t)</td>
<td>63.29</td>
<td>2.09 (1H, s)</td>
</tr>
<tr>
<td>6</td>
<td>23.65</td>
<td>---</td>
<td>199.65</td>
<td>---</td>
</tr>
<tr>
<td>7</td>
<td>37.04</td>
<td>2.44 (1H, ddd)</td>
<td>143.86</td>
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<tr>
<td>8</td>
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<td>---</td>
<td>156.10</td>
<td>---</td>
</tr>
<tr>
<td>9</td>
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<td>2.37 (1H, br d)</td>
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<td>2.94 (1H, d, $J$=7.0 Hz)</td>
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<tr>
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<td>39.53</td>
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<td>42.80</td>
<td>---</td>
</tr>
<tr>
<td>11</td>
<td>137.17</td>
<td>6.90 (1H, dd, $J$ = 15.8, 9.8 Hz)</td>
<td>134.62</td>
<td>6.73 (1H, dd, $J$ = 15.8, 9.8 Hz)</td>
</tr>
<tr>
<td>12</td>
<td>120.81</td>
<td>6.11 (1H, d, $J$ = 15.8 Hz)</td>
<td>123.24</td>
<td>6.28 (1H, d, $J$ = 15.8 Hz)</td>
</tr>
<tr>
<td>13</td>
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<td>---</td>
<td>128.83</td>
<td>---</td>
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<tr>
<td>14</td>
<td>142.71</td>
<td>7.15 (1H, t)</td>
<td>128.27</td>
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<tr>
<td>15</td>
<td>69.81</td>
<td>4.81 (2H, d)</td>
<td>172.11</td>
<td>---</td>
</tr>
<tr>
<td>16</td>
<td>172.62</td>
<td>4.81 (2H, s)</td>
<td>69.71</td>
<td>4.83 (2H, s)</td>
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<tr>
<td>17</td>
<td>108.62</td>
<td>4.76 (1H, s) &amp; 4.50 (1H, s)</td>
<td>21.65</td>
<td>1.81 (3H, s)</td>
</tr>
<tr>
<td>18</td>
<td>33.81</td>
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<td>0.87 (3H, s)</td>
<td>15.79</td>
<td>0.97 (3H, s)</td>
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</table>
The $^{13}$C NMR spectrum indicated the presence of α, β-unsaturated ketone (δ 199.65), trisubstituted olefin (δ 143.86 and 156.10) and four methyl signals (δ 22.90, 33.57, 15.79 and 21.65). Further, it also displayed signal at δ 172.11 is due to C=O of lactone ring, δ 128.83, 128.27 are corresponding to disubstituted olefin, and δ 69.71 is assignable to methylene carbon in the lactone ring.

A complete assignment of protons and carbons was assisted by HMBC, COSY and HSQC (Fig-6) experiments. The carbon skeleton suggested by several diagnostic correlations (H-5 /C-4, C-6, C-10; H-9 / C-8, C-10, C-11; H-11 / C-9, C-12; H-12 / C-11; H-14 / C-15 ; H-16 / C-13, C-15 ; H-17 / C-8 ; H-5, H-18, H-3, H-19 / C-4) and $^1$H-$^1$H COSY (H-11 / H-12; H-14 / H-15; H-1 / H-2; H-2 / H-3). The HMBC correlations (Fig-7) also suggested that α, β-unsaturated γ-lactone ring (δ 128.83, 128.27, 69.71 and 172.11) is attached to the decalone nucleus through the trans double bond (δ 134.62, 123.24). All the key HMBC correlations are depicted below.

![Key HMBC correlations of compound C.](image)

In addition, the relative configuration of compound C was proposed on biogenetic basis and by inspection of COSY (Fig-8) and NOESY spectrum (Fig-9), which showed the correlation between the following proton pairs (19-H$_3$ and 20-H$_3$; 5-H and 9-H, 18-H$_3$).
Based on these data, compound C was identified as 7-hydroxy, 6-oxo-7, 11, 13-labdatrien-16, 15-olide, a new labdane type diterpenoid named hedychialactone D.

\[\text{Hedychialactone D}\]

**Structure of compound D:**

Compound D was obtained as unstable colourless oil, and analysed for C\(_{20}\)H\(_{28}\)O\(_2\) (M\(^{+}\)+H 301). The physical and spectroscopic data of the compound was found to be identical with the known compound, yunnacoronarin A, a labdane diterpene previously isolated from the rhizomes of *Hedychium yunnanense*\(^4\). The occurrence of yunnacoronarin A is reported for the first time from this species.

\[\text{Yunnacoronarin A}\]
Structure of compound E:

Compound E was isolated as a pale yellow viscous liquid, with positive optical rotation $[\alpha]_D +96.9$ (c1.28, CHCl$_3$). The HRESI-MS (Fig-10) of compound E revealed a molecular ion peak corresponding to $(M^+ + H)$ at m/z 315.121 indicating the molecular formula C$_{20}$H$_{26}$O$_3$. The IR spectrum (Fig-11) showed absorption bands at 3425 cm$^{-1}$(OH), and 1663 cm$^{-1}$(C = O). The $^1$H NMR (Fig-12) and $^{13}$C NMR spectroscopic data of the compound E (Table-2) indicated labdane skeleton, which is similar to that of compound C; both have $\alpha$, $\beta$-unsaturated ketone (199.65 in compound C, 199.88 in compound E) and double bond ($\delta$ 134.62, 123.24 in C; $\delta$ 121.25, 128.39 in E) directly attached to the dacalin nucleus. The principal difference in 1D NMR spectra of compound E was that with the replacement of lactone ring with furan. In $^{13}$C NMR (Fig-13), DEPT (Fig-14) and HSQC experiments (Fig-15) $\delta$ 78.17 indicates hydroxy bearing carbon atom at C-9.

The structure was further confirmed by HMBC correlations (Fig-16) between H-5 / C-4, C-6, C-10; H-7 / C-6, C-8; H-11 / C-9, C-12; H-12 / C-11; H-14 / C-15; H-15 / C-14; H-16 / C-15, C-13; H-17 / C-8; H-5, H-18, H-3, H-19 / C-4) and $^1$H-$^1$H COSY (H-11 / H-12; H-1 / H-2; H-2 / H-3) respectively. Key HMBC correlations were depicted below.

![Key HMBC correlations of compound E](image-url)
Table 2: $^1$H NMR and $^{13}$C NMR data of Compound D and E in CDCl$_3$
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<th>Position</th>
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<td>(\delta_H) multiplicity</td>
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<td>42.79</td>
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<td>4</td>
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<td>32.36</td>
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</tr>
<tr>
<td>5</td>
<td>63.29</td>
<td>2.09 (1H, s)</td>
<td>55.67</td>
<td>2.78 (1H, s)</td>
</tr>
<tr>
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<td>---</td>
<td>199.88</td>
<td>---</td>
</tr>
<tr>
<td>7</td>
<td>143.86</td>
<td>---</td>
<td>128.89</td>
<td>5.82 (1H, s)</td>
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<td>8</td>
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<td>154.78</td>
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</tr>
<tr>
<td>9</td>
<td>61.73</td>
<td>2.94 (1H, d, (J = 7.0) Hz)</td>
<td>78.17</td>
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<td>10</td>
<td>42.80</td>
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<td>45.93</td>
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</tr>
<tr>
<td>11</td>
<td>134.62</td>
<td>6.73 (1H, dd, (J = 15.8), 9.8 Hz)</td>
<td>121.25</td>
<td>6.71 (1H, d, (J = 15.8), 9.8 Hz)</td>
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<tr>
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<td>128.39</td>
<td>6.02 (1H, d, (J = 15.8) Hz)</td>
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<tr>
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<td>128.44</td>
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<td>5.88 (1H, s)</td>
<td>143.72</td>
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<td>140.71</td>
<td>7.48 (1H, s)</td>
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<tr>
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<td>4.83 (2H, s)</td>
<td>107.37</td>
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<td>1.16 (3H, s)</td>
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<td>1.19 (3H, s)</td>
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<td>22.91</td>
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<td>15.79</td>
<td>0.97 (3H, s)</td>
<td>19.47</td>
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</table>
Similarly, the doublet attributed to H-12 showed $J = 15.8$ Hz, which is an indicative of *trans* configuration at C-11/C-12. Further the C-11/C-12 trans double bond was confirmed by COSY (Fig-17) and NOESY (Fig-18) spectrum. Thus, compound E was identified as 9-hydroxy-15,16-epoxy-7,11,13(16)14-labdatetraen-6-one, another new labdane type diterpenoid named 9-hydroxy hedychenone.

Structure of 9-hydroxy hedychenone

**Structure of compound F:**

Compound F was isolated as a colorless oil and analysed for $C_{20}H_{30}O_{3}$ ($M^+ 314$). The physical and spectroscopic data of the compound was found to be identical with the known compound, hedychilactone A, a labdane diterpene previously isolated from the rhizomes of *Hedychium coronarium*\(^5\). The occurrence of hedychilactone A is reported for the first time from this species.

![Hedychilactone A](image-url)
B. Biological significance of the constituents from the Chloroform extract of rhizomes of *Hedychium spicatum*:

Cytotoxic activity:

All the isolates obtained in this investigation of *Hedychium spicatum* were tested for their in vitro cytotoxicity against Colo-205 (colon cancer), A-431 (skin cancer), MCF-7 (breast cancer), A-549 (lung cancer) and CHO (Chinese hamster ovary cells) cell lines. The cytotoxicity data and IC\(_{50}\) values in μg/mL are shown in table-3. According to results obtained, IC\(_{50}\) values ranged between 7.69 and 49.29 μg/mL for labdane diterpenes, and between 20.36 and 54.21 μg/mL for isoflavonoids. The small structural differences of labdane diterpenes influenced the cytotoxic activity. As evident from cytotoxic activity results (table 3) compound C exhibited significant activity on colo-205, A-431 and MCF-7 and CHO cell lines and moderate activity on A-549 cell lines. Compound E also shown moderate activity colo-205, A-431 and significant activity on CHO cell lines. It is interesting to note that compound 4 and 5 are inactive against A-549 cell lines.

On comparing the activity of compound C with remaining isolates, it can be concluded that the presence of an α, β-unsaturated γ-lactone ring and α, β-unsaturated ketone (C-7/C-8, C-6) enhances the resultant cytotoxicity for the tested cell lines. Even with α, β-unsaturated ketone (C-7/C-8, C-6) cytotoxicity is slightly decreased due to lack of α, β-unsaturated γ-lactone in compound E. Compound A also exhibited good activity, due to presence of an α, β-unsaturated γ-lactone ring. It is interesting to note that all the isolates are significantly active on CHO cell lines. Due to lack of α, β-unsaturated γ-lactone and α, β-unsaturated ketone cytotoxicity is reduced in compounds D and F. Compound B also shown considerable activity on tested cell lines. Hence, the compound C was found to possess significant cytotoxic activity among all the isolates. In summary, *Hedychium*
spicatum, of the Zingiberaceae family has been used as a traditional medicine for the treatment of cancer. The compounds responsible for this activity have yet to be determined. The cytotoxic experiments were repeated in triplicate, and the IC\textsubscript{50} values were expressed as mean±standard deviation.

**Table 3:** Cytotoxicity effects of CHCl\textsubscript{3} extract and constituents from *H. spicatum*.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cell lines (IC\textsubscript{50} µg/ml)</th>
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<th></th>
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<tr>
<td></td>
<td></td>
<td>Colo-205</td>
<td>A-431</td>
<td>MCF-7</td>
<td>A-549</td>
</tr>
<tr>
<td>CHCl\textsubscript{3} extract</td>
<td>43.14±1.12</td>
<td>37.45±0.9</td>
<td>58.08±0.15</td>
<td>63.21±1.19</td>
<td>39.52±0.06</td>
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<tr>
<td>A (Labda-8(17),11,13-trien-16-olide)</td>
<td>33.12±1.11</td>
<td>22.45±0.80</td>
<td>42.08±0.05</td>
<td>35.21±1.09</td>
<td>22.96±0.04</td>
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<tr>
<td>B (cryptomeridiol)</td>
<td>49.56±0.02</td>
<td>68.51±0.04</td>
<td>77.29±0.80</td>
<td>65.26±0.83</td>
<td>51.36±0.08</td>
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<tr>
<td>C (hedychialactoneD)</td>
<td>12.03±0.03</td>
<td>16.01±0.05</td>
<td>21.05±0.04</td>
<td>37.98±0.91</td>
<td>7.69±1.12</td>
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<tr>
<td>D (yunnacoronarin A)</td>
<td>27.14±0.03</td>
<td>29.02±0.05</td>
<td>28.05±0.08</td>
<td>46.56±1.01</td>
<td>17.89±1.18</td>
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<td>E (9-hydroxy hedychenone)</td>
<td>24.02±0.01</td>
<td>26.09±0.09</td>
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<td>F (hedychilactone A)</td>
<td>29.81±0.06</td>
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<td>33.49±0.02</td>
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<td>21.34±1.11</td>
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</table>

NA = Not active, each value represents the mean± standard deviation
EXPERIMENTAL

A. Chemical examination of rhizomes of *Hedychium spicatum*:

Extraction

The rhizomes of *H. spicatum* were collected from Itanagar forests in the month of October and were identified by Department of Taxonomy, Indian Medicines Pharmaceutical Corporation Ltd. (IMPCL), Almora (district), Uttaranchal. A voucher specimen of the plant was deposited in the Herbarium of Taxonomy Department with an accession No. 143. The shade dried rhizomes (1 kg) were powdered and extracted successively with petroleum ether for 30 hrs, followed by chloroform in a Soxhlet apparatus for 30 hrs. The chloroform extract was concentrated under vacuum to obtain a dark brown residue (8.5 g). It has shown thirteen prominent spots on TLC (solvent system: Chloroform : methanol 8 : 2) with $R_f$ values of 0.95, 0.90, 0.85, 0.80, 0.78, 0.74, 0.70, 0.65, 0.60, 0.55, 0.50, 0.45 and 0.40 corresponding to compounds A, B, C, D, E, F, G, H and I.

Chromatographic separation of the chloroform extract

The dark brown gummy residue (8.5 g) was dissolved in chloroform (50 ml) and silica gel (30 g) was added. The chloroform was removed under vacuum and the powder obtained was transferred to a silica gel column set in chloroform (60-120 mesh, 500 g). The column was eluted with successively with chloroform, chloroform : methanol 95: 5, chloroform : methanol 90 : 10, chloroform : methanol 85 : 15 and chloroform : methanol 80 : 20. Fractions of 50 ml were collected and concentrated. Monitoring by TLC the fractions were grouped as shown in Table 4.
Table 4

<table>
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<tr>
<th>Eluant</th>
<th>Fraction No.</th>
<th>Group No.</th>
<th>Compound</th>
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<td>1-9</td>
<td>I</td>
<td>-</td>
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<tr>
<td>Chloroform: Methanol 95 : 5</td>
<td>10-20</td>
<td>II</td>
<td>A and B</td>
</tr>
<tr>
<td>Chloroform: Methanol 90 : 10</td>
<td>21-30</td>
<td>III</td>
<td>C and D</td>
</tr>
<tr>
<td>Chloroform: Methanol 85 : 15</td>
<td>31-50</td>
<td>IV</td>
<td>E and F</td>
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<tr>
<td>Chloroform: Methanol 80 : 20</td>
<td>51-60</td>
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<td>-</td>
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</table>

**Group I:**

The residue obtained from these fractions did not show any prominent spots and suggested waxy nature. It was not examined further.

**Group II:**

The brown coloured fractions under group II were combined and the solvent was removed under vacuum to yield a dark brown residue (2.0 g). The residue on TLC showed two major different spots (chloroform : methanol 85 : 15) at R_f values 0.95 and 0.85.

The brown residue was dissolved in chloroform (20 ml) and a small amount of silica gel was added (5 g). The powder obtained after removal of solvent under vacuum was transferred to a column of silica gel (60 g) set up with chloroform. The column was eluted with successively with chloroform, chloroform : methanol 98 : 2, chloroform : methanol 96 : 4 and chloroform : methanol 95 : 5. Fractions of 25 ml were collected, monitored by TLC and concentrated separately.
TLC examination of chloroform showed no prominent spots and hence was discarded.

Chloroform: methanol 95 : 5 eluate:

TLC examination of the fractions 11-17 chloroform : methanol 95 : 5 eluate have shown bright spot in UV light and pink spot when sprayed with 3% sulfuric acid in methanol at R_f value 0.55 (TLC solvent system: chloroform : methanol 85 : 15). The fractions on concentration yielded white solid, which upon crystallization from petroleum ether gave white needles (0.040 g) m.p. 124^0C. It was designated as compound A.

TLC examination of the fractions 18-20 of chloroform : methanol 95 : 5 have shown bright spot in UV light and pink spot when sprayed with 3% sulfuric acid in methanol at R_f value 0.50 (TLC solvent system: chloroform : methanol 85 : 15). The fractions upon crystallization from petroleum ether yielded white crystal (0.025 g) m. p. 140-142^0C. It was designated as compound B.

Group III:

Fractions obtained (21-26) under chloroform : methanol 90 : 10 eluate were combined and concentrated to yield light brown residue (1.5 g). The residue on TLC examination showed a pink in UV light and a dark brown spot when sprayed with 3% sulfuric acid in methanol at R_f 0.6 and 0.45 (TLC solvent system: chloroform : methanol 85 : 15).

The residue was dissolved in chloroform (10 ml) and a small amount of silica gel (0.200 g) was added. The powder obtained after the removal of solvent under vacuum was transferred to a column of silica gel (60-120 mesh, 20 g) set up chloroform. The
column was successively eluted with chloroform : methanol 95 : 5, chloroform : methanol 93 : 7 and chloroform : methanol 90 : 10.

As the fractions of chloroform : methanol 95 : 5 did not show any prominent spot they were discarded.

TLC examination of the fractions of chloroform : methanol 93 : 7 have shown a bright spot in UV light at R_f 0.45 (TLC solvent system: chloroform : methanol 85 : 15) which are concentrated to yield 0.025 g of yellow semi solid. It was designated as compound C.

The fractions 27-30 obtained under chloroform : methanol 90 : 10 eluate were combined and concentrated under vacuum to yield green residue (0.065 g). The TLC examination of the residue has revealed a bright spot on TLC in UV light at R_f 0.50.

The residue was dissolved in chloroform (10 ml), a small amount of silica gel (0.130 g) was added and the solvent was removed under vacuum to yield a powder. The powder was transferred to a column of silica gel (60-120 mesh, 15 g) set in petroleum ether. The column was successively eluted with chloroform : methanol 95 : 5 and chloroform : methanol 85 : 15.

As the fractions eluted with chloroform : methanol 95 : 5 did not show any prominent spot on TLC they were discarded.

TLC examination of the fractions eluted with chloroform : methanol 85 : 15 have shown bright spot in UV light at R_f 0.50 (TLC solvent system: chloroform : methanol 80 : 20) and were combined and concentrated to yield colourless oil (0.0020 g). It was designated as compound D.

**Group IV:**
The fractions obtained under chloroform : methanol 85 : 15 eluate were concentrated to obtain brown residue (2.0 g). The residue was dissolved chloroform (30 ml), 5 g of silica gel was added and the solvent was removed under vacuum. The powder then obtained was transferred to a column of silica gel (60-120 mesh, 100 g) set in chloroform. The column was successively eluted with chloroform : methanol 90 : 10, chloroform : methanol 88 : 12, chloroform : methanol 86 : 14 and chloroform : methanol 85:15.

The fractions eluted with chloroform : methanol 90 : 10 did not show any prominent spot and hence were discarded.

The fractions 31-35 eluted with chloroform : methanol 88 : 12 have shown a spot in UV light and a dark spot when sprayed with 3% sulfuric acid in methanol on TLC at \( R_f \) 0.60 (TLC solvent system chloroform : methanol 80 : 20). The combined fractions were concentrated to obtain pale pale yellow viscous liquid (0.015 g). It was designated as compound E.

**Group V:**

The fractions obtained under chloroform: methanol 80: 20 eluates were concentrated to obtain brown residue (2.0 g). The residue was dissolved chloroform (30 ml), 5 g of silica gel was added and the solvent was removed under vacuum. The powder then obtained was transferred to a column of silica gel (60-120 mesh, 100 g) set in chloroform. The column was successively eluted with chloroform : methanol 85:15, chloroform : methanol 83:16, chloroform : methanol 82 : 18 and chloroform : methanol 80 : 20.
The fractions eluted with chloroform : methanol 85:15, chloroform : methanol 83:16, chloroform : methanol 82 : 18 and chloroform : methanol 80 : 20 did not show any prominent spot and hence were discarded.

**Spectral data :**

**Compound A: Labda-8( 17),11,13-trien- 16)-olide**

**MP**

124°C

**IR (KBr) \( \nu_{\text{max}} \)**

1664, 1639, 1502, 1381, 1372, 1225, 1164 and 785 cm\(^{-1}\).

**\(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \)**

7.15 (1H, t, \( J = 2.1, 0.6 \) Hz, H-14), 6.90 (1H, dd, \( J = 15.8, 10.0, 0.6 \) Hz, H-11), 6.11 (1H, d, \( J = 15.8 \) Hz, H-12), 4.81 (2H, d, \( J = 2.0, 0.7 \) Hz, H-15), 4.76 (1H, s, H-17), 4.50 (1H, s, H-17), 2.44 (1H, ddd, \( J = 13.6, 4.4, 2.3 \) Hz, H-7), 2.37 (1H, br d, \( J = 10.0 \) Hz, H-9), 2.08 (1H, t, \( J = 13.4, 5.3, 1.0 \) Hz, H-7), 1.70 (2H, ddt, \( J = 12.9, 5.2, 2.5 \) Hz, H-6), 1.53 (2H, m, H-2), 1.45 (2H, m, H-1), 1.39 (2H, m, H-6), 1.18 (1H, m, H-3), 1.09 (1H, dd, \( J = 12.6, 2.8 \) Hz, H-5), 0.89 (3H, s, H-18), 0.87 (3H, s, H-20), 0.84 (3H, s, H-19).

**\(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \)**

172.62 (C-16), 149.63 (C-8), 142.71 (C-14), 137.17 (C-11), 129.73 (C-13), 120.81 (C-12), 108.62 (C-17), 69.81 (C-15), 54.93 (C-5), 62.42 (C-9), 42.51 (C-3), 41.08 (C-1), 33.84 (C-4), 39.53 (C-10), 37.04 (C-7), 23.65 (C-6), 33.81 (C-18), 19.30 (C-2), 22.26 (C-19),15.34 (C-20).

**ESI-MS**

301 (M\(^+\)+H).
Compound B: Cryptomeridiol

MP
140-142°C.

IR (KBr) $\nu_{\text{max}}$
3385, 3300, 2980, 2950, 2915, 1480, 1385, 1370, 1230, 1160, 1070, 1025, 915 cm$^{-1}$.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$
1.20 (6H, s, (CH$_3$)$_2$-11), 1.12 (3H, s, H$_3$-4), 0.86 (3H, s, H$_3$-4).

$^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$
73.27 (C-4), 72.92 (C-11), 54.83 (C-5), 49.93 (C-7), 44.62 (C-9), 43.58 (C-3), 41.19 (C-1), 34.53 (C-10), 27.32 (C-13), 27.14 (C-12), 22.61 (C-14), 22.53 (C-6), 21.54 (C-8), 20.12 (C-2), 18.63 (C-15).

ESI-MS
231 (M$^+$+H).

Compound C: hedychialactone D

MP
108-109°C.

IR (KBr) $\nu_{\text{max}}$
3450, 1665, 1639, 1513, 1380, 1372, 1225, 1165 and 788 cm$^{-1}$.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$
Table.1

$^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$
Table.1
ESI-MS
315 (M⁺+H).

**Compound D: yunnacoronarin A**

**MP**

Colourless oil.

**IR (KBr) v max**

3450, 2925, 1654, 1621, 1016 cm⁻¹.

**¹H NMR (300 MHz, CDCl₃) δ**

7.35 (1H, s, H-16), 7.34 (1H, s, H-15), 6.54 (1H, s, H-14), 6.19 (1H, d, J = 9.8 Hz, H-12), 5.76 (1H, dd, J = 9.8, 15.6 Hz, H-11), 4.86 (1H, m, H-6), 4.76 (1H, dd, J = 1.8, 3.7 Hz, H-17), 4.53 (1H, dd, J = 1.8, 3.7 Hz, H-17), 2.40 (1H, d, J = 9.8 Hz, H-9), 1.75-1.06 (m, CH₂’s), 0.90, 0.85 and 0.84 (3H each, all s, 20, 19, 18-H₃).

**¹³C NMR (75 MHz, CDCl₃) δ**

146.04 (C-8), 143.24 (C-16), 139.92 (C-15), 127.39 (C-12), 124.33 (C-13), 122.28 (C-12), 111.53 (C-17), 107.68 (C-17), 68.92 (C-6), 61.93 (C-9), 56.98 (C-5), 46.63 (C-7), 44.13 (C-3), 43.72 (C-1), 40.23 (C-10), 34.42 (C-4), 33.63 (C-18), 23.80 (C-19), 19.32 (C-2), 17.94 (C-20).

**ESI-MS**

269 (M⁺+H).
Compound E: 9-hydroxy hedychenone

**MP**

Viscous liquid.

**IR (KBr) \( \nu_{\text{max}} \)**

3425, 1691 cm\(^{-1} \).

**\(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \)**

Table.2

**\(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \)**

Table.2

**ESI-MS**

337 (M\(^+\)+Na).

**Compound F: hedychilactone A**

**MP**

Colorless oil.

**IR (KBr) \( \nu_{\text{max}} \)**

3393, 1752, 1676 cm\(^{-1} \).

**\(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \)**

6.68 (1H, m, H-12), 5.20, 4.58 (1H each, both br s, H\(_2\)-17), 4.39 (2H, t-like, H\(_2\)-15), 4.01 (1H, dd-like, H-7), 2.88 (2H, m, H\(_2\)-14), 2.40, 2.29 (1H each, both m, H\(_2\)-11), 2.11 (1H, ddd, \( J = 2.4, 5.5, 12.8 \) Hz, H-6\( \beta \)), 1.81 (1H, br d, \( J = 11 \) Hz, H-9), 1.69 (1H, br d, \( J = 13.2 \) Hz, H-1\( \beta \)), 1.58, 1.54 (1H each, both m, H-2\( \beta \)), 1.45 (1H, br d, \( J = 13.0 \) Hz, H-3\( \beta \)), 1.29 (1H, ddd, \( J = 11.4, 11.9, 12.8 \) Hz, H-6\( \alpha \)), 1.21 (1H, m, H-3\( \alpha \)), 1.18 (1H, dd, \( J = 5.5, 11.9 \) Hz, H-4\( \alpha \)).
Hz, H-7), 1.06 (1H, ddd, J = 4.0, 12.5, 12.8 Hz, H-1α), 0.92, 0.83, 0.72 (3H each, both s, 20, 19 and H₃-18).

1³C NMR (75 MHz, CDCl₃) δ

171.23 (C-16), 150.12 (C-8), 141.54 (C-12), 124.92 (C-13), 104.38 (C-17), 73.69 (C-7), 65.32 (C-15), 54.41 (C-9), 53.02 (C-5), 41.87 (C-3), 39.26 (C-10), 39.11 (C-1), 33.56 (C-4), 33.55 (C-18), 33.51 (C-6), 25.74 (C-11), 25.35 (C-14), 21.61 (C-19), 19.35 (C-2), 14.42 (C-20).

ESI-MS

319 (M⁺+H).

**Determination of cytotoxic activity**

The cell lines used in this study were Colo-205 (colon cancer), A-431 (skin cancer), MCF-7 (breast cancer), A-549 (lung cancer) and CHO (Chinese hamster ovary cells) cancerous cell lines. All the cells were obtained from National Center for cellular Sciences (NCCS)-Pune, India. Cells were maintained in DMEM supplemented with 10% fetal bovine serum, 2mM glutamine, 100U/mL penicillin, 100μg/mL streptomycin, at 37°C with 5% CO₂. The cells were seeded at 1x10⁴ cells/well. After 48 h, cells were treated with the test compound and IC₅₀ values were calculated in μg/mL.
References:


