INTRODUCTION

Ipomoea carnea subsp. fistulosa (Convolvulacea) is a plant native to South America and is also sparsely distributed in India and Bangladesh. Anticancer and anti HIV properties have been attributed to the extracts of the plant. A recent communication from this institute reported bioassay monitored isolation and characterization of the chief antifungal fraction of the plant as a mixture of (E) and (Z) isomers of octadecyl p-coumarate whose detailed structure elucidation, antifungal activity and isomerization have already been reported. The compounds were compared with alkyl coumarates and alkyl ferulates reported from other Convolvulaceae plants. It was found that octadecyl p-coumarate is the most frequently reported alkyl coumarate in the family. It was also observed that while the number carbon atoms in the alkyl groups of alkyl coumarates reported from Convolvulaceae is always even, in the case of alkyl ferulates, number of carbon atoms of the alkyl moiety can either be odd or even.

ABSTRACT: Three alkyl coumarates (hexadecyl coumarate, eicosyl coumarate and docosyl coumarate) and five alkyl ferulates (tetradecyl ferulate, pentadecyl ferulate, heptadecyl ferulate, octadecyl ferulate and nonadecyl ferulate) could be tentatively detected in the partially purified HPLC fraction of Ipomoea carnea subsp. fistulosa by high resolution electrospray ionization mass spectroscopy (HRESIMS). This is in addition to octadecyl p-coumarate whose detailed structure elucidation, antifungal activity and isomerization have already been reported. The compounds were compared with alkyl coumarates and alkyl ferulates reported from other Convolvulaceae plants. It was found that octadecyl p-coumarate is the most frequently reported alkyl coumarate in the family. It was also observed that while the number carbon atoms in the alkyl groups of alkyl coumarates reported from Convolvulaceae is always even, in the case of alkyl ferulates, number of carbon atoms of the alkyl moiety can either be odd or even.

KEYWORDS: Ipomoea carnea subsp. fistulosa, alkyl coumarates, alkyl ferulates, Convolvulaceae, HRESIMS

Co-occurrence of alkyl coumarates and alkyl ferulates in Convolvulaceae has been established.


Structure elucidation of naturally occurring compounds usually involves extensive chemical and spectral studies for which sufficient amounts of the compound have to be obtained in pure form. This is extremely difficult if the compounds are present in plants only in trace amounts. However, in high resolution electrospray ionization mass spectroscopy (HRESIMS), the molecular formula of a molecular ion can be determined directly by comparison of the ion masses at high resolution with possible composition using accurate masses of individual isotopes. Since the electrospray method is a soft ionization procedure, fragmentation of ions is less compared to electron impact method. Once the molecular formula is obtained,
it is possible to compare this with those of compounds already isolated from the plant or related species and arrive at logical inferences leading to the chemical nature of compounds. For this purpose, the mass accuracy of the compounds should be within reasonable limits. Mass accuracy ($M_a$) is given by the formula

$$M_a = \frac{(\text{Calculated mass} - \text{observed mass}) \times 10^6}{\text{Calculated mass}} \text{ (ppm)}$$

While mass accuracy is always preferred to below 5 ppm, values up to 58 ppm are often reported for natural compounds. Although such inferences can be considered as tentative only, they can be of importance for further research particularly from chemotaxonomic point of view.

From this standpoint, tentative detection of three alkyl coumarates and five alkyl ferulates in the partially purified HPLC fraction of *Ipomoea carnea* subsp. *fistulosa* by HRESIMS is being reported in this paper. This is in addition to octadecyl $p$-coumarates whose detailed structure elucidation by extensive spectral studies has already been reported. The compounds are being compared with alkyl coumarates and alkyl ferulates reported in different plants of Convolvulaceae family.

**MATERIALS AND METHODS**

Details of bioassay monitored isolation, HPLC purification, structure elucidation by spectral methods, isomerization and antifungal activity of octadecyl $p$-coumarates have been reported in the earlier paper. Column chromatography of the crude ethyl acetate extractive over silica gel with hexane-ethyl acetate mixture (7:3) gave a fraction which was subjected to HPLC. HPLC purification was performed using a Waters HPLC system (515 pump, 7725 Rheodyne injector, Waters 2487 Dual λ absorbance detector) with conditions as follows: Column Prep Nova Pak HR Silica 7.8 × 300 mm, flow rate 1 ml/min, UV detection at 254 nm, eluent CH$_2$Cl$_2$. The HPLC fraction corresponding to $t_R = 48$ minutes was collected. After the first stage of purification, it was further purified once more. This fraction after removal of solvent was subjected to TLC bioassay using *Cladosporium cucumerinum*. The fraction was also subjected to HRESIMS. The spectrum was obtained on Micromass Q-TOF apparatus.

**RESULTS AND DISCUSSION**

The fraction obtained after two stages of purification exhibited antifungal activity against the spore germination of *Cladosporium cucumerinum* by TLC bioassay at a dose of 0.3 mg. From the HRESIMS spectrum (Figure 1), it was clear that the fraction did not correspond to any single compound. Thus it was subjected to three more stages of HPLC purification (total five stages). Detailed study of this fraction using C, H analyses, HRESIMS, CIMS, IR, $^1$H NMR and $^{13}$C NMR revealed that it was octadecyl $p$-coumarate obtained in pure form. Once the structure of the major active principle was identified as octadecyl $p$-coumarates by extensive spectral studies, it was possible to arrive at logical inferences about other peaks in HRESIMS of the partially purified fraction after two stages in relation to published literature on alkyl coumarates and alkyl ferulates. It was found that the most of the peaks corresponded to compounds reported from other *Ipomoea* species or structurally related to octadecyl $p$-coumarates.

In this way, hexadecyl coumarate, tetradecyl ferulate, pentadecyl ferulate, heptadecyl coumarate, octadecyl ferulate, nonadecyl ferulate and docosyl coumarate could be tentatively detected. Details are given in Table 1. Since this paper deals with only a tentative detection of alkyl coumarates and alkyl ferulates of the plant, mass accuracy values up to 45 ppm were considered valid. Because these compounds are structurally related to octadecyl $p$-coumarates, they may be exhibiting isomerization as reported in the case of octadecyl $p$-coumarates. However, structures of only ($E$) isomers are given in Table 1. Further, position of the hydroxyl group, methoxyl group and straight chain nature of the alkyl groups are presumed based on the structures of majority of the compounds isolated from Convolvulaceae.

The results are being compared with alkyl $p$-coumarates and alkyl ferulates reported from other plants of Convolvulaceae family in Table 2. It may be noted that octadecyl $p$-coumarate is the most widely reported alkyl $p$-coumarate. It is also noteworthy that while number carbon atoms in the alkyl groups of alkyl coumarates reported from Convolvulaceae...
Table 1: Alkyl coumarates and alkyl ferulates tentatively detected in *Ipomoea carnea* subsp. *fistulosa*

<table>
<thead>
<tr>
<th>No.</th>
<th>Obs. Mass -Na</th>
<th>Mol. formula</th>
<th>Calc. mass</th>
<th>Mass accuracy</th>
<th>Compound</th>
<th>R¹</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>388.3153</td>
<td>C₂₅H₄₀O₃</td>
<td>388.2978</td>
<td>45</td>
<td>Hexadecyl Coumarate</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>390.2816</td>
<td>C₂₄H₃₈O₄</td>
<td>390.2770</td>
<td>12</td>
<td>Tetradecyl Ferulate</td>
<td>OCH₃</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>404.3055</td>
<td>C₂₅H₄₀O₄</td>
<td>404.2927</td>
<td>32</td>
<td>Pentadecyl Ferulate</td>
<td>OCH₃</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>416.3341</td>
<td>C₂₇H₄₄O₃</td>
<td>416.3291</td>
<td>12</td>
<td>Octadecyl Coumarate*</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>432.3102</td>
<td>C₂₇H₄₄O₄</td>
<td>432.3240</td>
<td>32</td>
<td>Heptadecyl Ferulate</td>
<td>OCH₃</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>444.3572</td>
<td>C₂₉H₄₈O₃</td>
<td>444.3604</td>
<td>7</td>
<td>Eicosyl Coumarate</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>446.3300</td>
<td>C₂₉H₄₈O₄</td>
<td>446.3396</td>
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<td>Octadecyl Ferulate</td>
<td>OCH₃</td>
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</tr>
<tr>
<td>8</td>
<td>460.3373</td>
<td>C₂₉H₄₈O₄</td>
<td>460.3553</td>
<td>39</td>
<td>Nonadecyl Ferulate</td>
<td>OCH₃</td>
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</tr>
<tr>
<td>9</td>
<td>472.3769</td>
<td>C₃₁H₅₂O₃</td>
<td>472.3917</td>
<td>31</td>
<td>Docosyl Coumarate</td>
<td>H</td>
<td></td>
</tr>
</tbody>
</table>

Mₐ = Mass accuracy  *Confirmed compound

Table 2: Alkyl coumarates and alkyl ferulates reported from Convulvulaceae

<table>
<thead>
<tr>
<th>No.</th>
<th>Plant</th>
<th>Coumarates/Ferulates</th>
<th>Alkyl groups</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Ipomoea carnea</em> subsp. <em>fistulosa</em></td>
<td>Coumarates</td>
<td>Methyl, Octyl and dodecyl. Hexadecyl, octadecyl, eicosanyl and docosyl</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ferulates</td>
<td>Tetradecyl, pentadecyl, heptadecyl, Octadecyl and nonadecyl</td>
<td>2 and the present study</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Present study</td>
</tr>
<tr>
<td>2</td>
<td><em>Ipomoea batatas</em></td>
<td>Coumarates</td>
<td>Hexadecyl, octadecyl and eicosyl</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ferulates</td>
<td>Hexadecyl, heptadecyl and octadecyl</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td><em>Ipomoea digitata</em></td>
<td>Coumarates</td>
<td>Octadecyl</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td><em>Argyreia populifolia</em></td>
<td>Coumarates</td>
<td>Octadecyl</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td><em>Cascutta chinensis</em></td>
<td>Coumarates</td>
<td>Octadecyl</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td><em>Merremia tuberosa</em></td>
<td>Coumarates</td>
<td>Hexadecyl and octadecyl</td>
<td>1</td>
</tr>
</tbody>
</table>

is always even, in the case of alkyl ferulates, number of carbon atoms of the alkyl moiety can either be odd or even. The report on the presence of alkyl coumarates and alkyl ferulates in several Convulvulaceae plants may be of interest from the chemotaxonomic point of view. The wide range of biological activities attributed to alkyl coumarates and alkyl ferulates will be of interest from the pharmacological use of Convulvulaceae plants also.
REFERENCES