GCMS analysis and antimicrobial action of latex of *Euphorbia caducifolia*

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**Abstract**

*Euphorbia caducifolia* is conspicuously and regularly represented in the flora of the Rajasthan state. Latex of *E. caducifolia* is used to cure skin infections, cutaneous eruption, leucoderma and applied to cuts and wounds for speedy healing. The GCMS analysis of fraction isolated from latex showed presence of methyl palmitate, 5,9-heptadecadienoate, methyl 11 octadecenoate, methyl octadecenoate and 3,7,11,15-tetramethyl-2-hexadecene-1-ol. Isolated fraction of *E. caducifolia* (IFEC) and latex of *E. caducifolia* (ECL) were tested against *S. aureus*, *M. luteus*, *B. subtilis*, *E. coli*, *S. typhi*, *A. niger* and *C. albicans*. IFEC was found to be more effective against fungal species, and MIC was found to be 150 µg/ml against *A. niger*.

**INTRODUCTION**

Ethnomedicinal plants are one of the most important sources of finding new therapeutic agents. Some of the most outstanding medicines have been developed from ethnomedicinal plants [1]. Rich flora of the Thar Desert has many unexplored and underutilized ethnomedicinal plants. One such plant *Euphorbia caducifolia* is conspicuously and regularly represented in the flora of the Rajasthan state. This plant is common throughout the state on rocky habitat [2]. *Euphorbia caducifolia* is considered poisonous and not used as food or fodder except for the juicy leaves, which can be consumed as a vegetable. Latex is present in great abundance in the entire plant. The latex content appears to be the highest compared to that of the entire desert flora in this area. The latex of *Euphorbia caducifolia* (ECL) is considered to have medicinal value and used by shepherds and local inhabitants for treating bleeding wounds caused by accidental injury. ECL is also used to cure the skin infections, leukoderma, earache and to expel guinea worms [3].

Latex of many euphorbia species such as *Euphorbia abyssinica gmel* and *Euphorbia antiquorum* have been reported to have antimicrobial property [4-5]. No report has been published regarding biological activity of latex of *Euphorbia caducifolia*. this prompted us to investigate the phytoconstituent present in latex by using GCMS and to perform antimicrobial screenings of latex as well as an isolated fraction.

**MATERIAL AND METHOD**

**Plant material**

The *Euphorbia caducifolia* was collected from Jodhpur, India and was identified by Taxonomist of Botanical survey of India, Jodhpur. The voucher specimen (MGEC) was deposited for future reference.
Latex was collected by making incisions on the stems of the plant.

Preparation of sample for GCMS

Latex (2kg) was obtained from Euphorbia caducifolia and kept at a low temperature. The latex was extracted with ether, chloroform and methanol by liquid-liquid extraction. Ether extract was further purified by column chromatography using acetonitrile and methanol (1:1); the terpenoid mixture so obtained (7.5%) was subjected to GCMS and antimicrobial activity along with fresh latex.

GCMS analysis

The GCMS analysis was carried out at Sophisticated Analytical Instrument Facility, Indian Institute of Technology, Madras using a JEOL GC MATE II Gas Chromatograph coupled to a mass detector. Electron impact mode was used with ionization voltage of 70eV.

Identification of Phytoconstituents

The identification and interpretation on mass-spectrum GC-MS was conducted using the database of National Institute Standard and Technology NIST Ver.2.0 MS, AOCS Lipid Library, Mass. library and research papers.

Test Microorganisms

Microbial strains obtained from Institute of Microbial Technology, Chandigarh, India were used. Microbial strains includes: Gram-positive bacteria- Staphylococcus aureus MTCC-96, Micrococcus luteus MTCC-106, Bacillus subtilis MTCC-441, Gram-negative bacteria- Escherichia coli MTCC-443, Salmonella typhi MTCC - 734 and fungi Aspergillus niger MTCC- 282 and Candida albicans MTCC-227. Bacterial cultures were prepared by transferring two to three colonies into a tube containing 20 ml of nutrient broth and grown overnight at 37°C. The turbidity of the culture was adjusted with sterile saline solution to match 0.5 Mc Farland standard 10⁸ colony forming units/ml (CFU/ml)

Well-in agar method

Anti-bacterial activity of latex (ECL) and isolated fraction (IFEC) was tested by a modified well-in agar method [6]. The inoculum suspension was spread uniformly over the agar plates using a sterile glass rod spreader to get uniform distribution of bacteria. Subsequently, using a sterile borer, a well of 0.7 cm diameter was made in the inoculated media. Addition of 0.2 ml of each extract was aseptically filled into the well. Later, the plates were placed at room temperature for an hour to allow diffusion of extract into the agar. Then the plates were incubated for 24 hours at 37°C. The results were recorded by measuring the diameter of the inhibition zone at the end of 24 hours.

Determination of MIC by serial dilution technique

Anti-bacterial activity of ECL and IFEC was carried out by broth micro-dilution method. Serial dilutions of the test fractions, isolated compounds and reference drugs were prepared in DMSO to attain a final concentration of 1 mg/ml. Further progressive dilutions with Mueller-Hinton agar were performed to obtain the required concentrations of 1, 2, 4, 16, 31.25, 62.5, 125, 250 and 500 µg/ml. The tubes were inoculated with 10⁸ cfu/ml (colony forming unit/ml) of each microorganism and incubated at 37°C for 18 hours. To ensure whether solvent had any effect on the bacterial growth, a respective parallel control was performed. Minimum inhibitory concentration (MIC) of the fractions was determined. Ciprofloxacin and fluconazole were used as standards to compare the antibacterial activity of the fractions of the plant.

RESULTS

GCMS analysis

Gas chromatogram of the isolated fraction is presented as Figure-1. The mass spectra of all major peaks shown in gas chromatogram were analyzed and seven compounds were identified.

The identified compound are presented in Table 1 according to their retention time, compounds identified include 3-Oxo-25, 26, 27-trisnor (5a, 13a,14b,17a) lanost-8-en-24-ol, methyl palmitate, 5,9-heptadecadienoate, methyl 11 octadecenoate, methyl octadecenoate, 3,7,11,15-tetramethyl- 2-hexadecene-1-ol (Phytol) and lanost-8-en-24-al. 

Antimicrobial study

Table 2 shows the antimicrobial effect of the isolated fraction of Euphorbia caducifolia (IFEC) and latex of Euphorbia caducifolia (ECL), there were significant differences in their activities depending on the microorganism tested. Diameters of inhibition zones ranged between 8.1 and 20.5 mm. C. albicans and A. niger were the most susceptible microbes by IFEC showed with inhibition zones of 18.3 and 20.5 mm, respectively.

Minimum inhibitory concentration (MIC) of IFEC and ECL against all tested microorganisms is shown in Table 3. The MIC of ECL against S. aureus, M. luteus, B. subtilis, E. coli and S. typhi were found to be 458, 450, 475, 525 and 500 µg/ml respectively. MIC against A. niger and C. albicans were found to be 237 and 225 µg/ml. IFEC was found to be more effective against fungal species, MIC was found to be 150 µg/ml against A. niger and maximum MIC 275 µg/ml against S. typhi.
Figure 1. GCMS chromatogram of isolated fraction of latex of Euphorbia caducifolia

Table 1. Results of analysis of mass spectra obtained by GCMS analysis of isolated fraction of latex of Euphorbia caducifolia

<table>
<thead>
<tr>
<th>S. No.</th>
<th>RT</th>
<th>M+</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>10.86</td>
<td>398</td>
<td>3-Oxo-25,26,27-trisnor(5a,13a,14b,17a)lanost-8-en-24-ol</td>
</tr>
<tr>
<td>2.</td>
<td>15.69</td>
<td>270</td>
<td>Methyl Palmitate</td>
</tr>
<tr>
<td>3.</td>
<td>17.13</td>
<td>280</td>
<td>5,9-heptadecadienoate</td>
</tr>
<tr>
<td>4.</td>
<td>17.42</td>
<td>296</td>
<td>Methyl 11 octadecenoate</td>
</tr>
<tr>
<td>5.</td>
<td>17.65</td>
<td>298</td>
<td>Methyl octadecenoate</td>
</tr>
<tr>
<td>7.</td>
<td>20.29</td>
<td>442</td>
<td>Lanost-8-en-24-ol</td>
</tr>
</tbody>
</table>

RT (Retention Time) M+ (Molecular ion peak)

Table 2. Preliminary antimicrobial activity of latex of Euphorbia caducifolia and isolates fraction against bacterial and fungal species.

<table>
<thead>
<tr>
<th>Micro-organisms</th>
<th>ECL</th>
<th>IFEC 500 µg/ml</th>
<th>Ciprofloxacin, 60 µg/ml</th>
<th>Flucanazole 100 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>9.3 ± 0.21</td>
<td>16.2 ± 0.22</td>
<td>23.1 ± 0.58</td>
<td>-</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>9.2 ± 0.31</td>
<td>14.4 ± 0.33</td>
<td>21.8 ± 0.64</td>
<td>-</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>8.1 ± 0.25</td>
<td>14.5 ± 0.26</td>
<td>24.4 ± 0.42</td>
<td>-</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>9.4 ± 0.24</td>
<td>15.1 ± 0.41</td>
<td>21.6 ± 0.34</td>
<td>-</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>8.2 ± 0.33</td>
<td>16.4 ± 0.51</td>
<td>26.4 ± 0.21</td>
<td>-</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>18.7 ± 0.52</td>
<td>20.5 ± 0.23</td>
<td>-</td>
<td>22.5 ± 0.26</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>15.6 ± 0.41</td>
<td>18.3 ± 0.32</td>
<td>-</td>
<td>18.4 ± 0.35</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM of six replicates and inhibition zone including the diameter of the bore (7 mm).
Table 3. Minimum inhibitory concentration of latex of *Euphorbia caducifolia* and isolates fraction against bacterial and fungal species.

<table>
<thead>
<tr>
<th>Micro-organisms</th>
<th>ECL (micrograms)</th>
<th>IFEC (micrograms)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>458</td>
<td>262</td>
</tr>
<tr>
<td><em>Micrococcus luteus</em></td>
<td>450</td>
<td>212</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>475</td>
<td>187</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>525</td>
<td>225</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>500</td>
<td>275</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>237</td>
<td>150</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>225</td>
<td>175</td>
</tr>
</tbody>
</table>

MIC (Minimum inhibitory concentration)

**DISCUSSION**

Euphorbia species have been reported for antibacterial and anti-fungal activities. Crude extracts of *Euphorbia hirta* were found to be effective against gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Salmonella typhi* and *Proteus mirabilis* [7]. *Euphorbia thymifolia* is active against *Bacillus Subtilis*, *Staphylococcus Aureus* and *Escherichia coli* [8] and *Euphorbia segetalis* also possess antiviral and antimicrobial properties [9]. In the present study, latex of *Euphorbia caducifolia* showed antibacterial and anti-fungal activities against the tested bacteria and fungal strains.

The diverse composition of the latex, which includes toxic compounds as well as other interesting and potentially bioactive molecules such as diterpenes and triterpenes could be responsible for antibacterial and anti-fungal activity [10-11].

IFEC found to have a lower MIC value than ECL, and is indicative of activity of terpenes. Furthermore, a number of studies have been reported on the antibacterial and anti-fungal activity of terpenes of natural origin [12]. The possible mechanism of antimicrobial activity of terpenes is by reducing the synthesis of ergosterol, a specific fungal cell membrane component [13]. The inhibition of synthesis ergosterol causes defective cell wall formation and leakage of cellular contents. Terpenes increase the permeability of bacterial and mammalian cell by inserting themselves into the lipid layer of the cell membrane and thus influencing the selective permeability of the cell to foreign substances [14].

**CONCLUSIONS**

It could be concluded that the results observed are in line with the therapeutic use of the plant in traditional medicine. Latex and compounds isolated from latex of *Euphorbia caducifolia* have potential antimicrobial effects. The study of ECL and separated compound for wound healing potential is underway.

**ACKNOWLEDGMENT**

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**REFERENCES**


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