IN VITRO ANTIBACTERIAL EFFICACY OF A SESQUITERPENE LACTONE, PARTHENIN FROM PARTHENIUM HYSTEROPHORUS L (COMPOSITAE) AGAINST ENTERIC BACTERIAL PATHOGENS

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ABSTRACT
A sesquiterpene lactone, Parthenin was isolated from Parthenium hysterophorus L (Compositae). The chemical structure was established based on the data obtained from nuclear magnetic resonance (1H NMR and 13C NMR) and Mass spectroscopic analysis. Invitro antibacterial activity of parthenin against enteric bacterial pathogens was established. Out of the tested enteric bacterial pathogens, S. typhi, E. coli and P. aeruginosa was more sensitive to the compound (MIC = 50 μg/ml). Parthenin is moderately active against S. typhimurium, K. pneumoniae, S. epidermidis and S. aureus with a minimum inhibitory concentration of 100 μg/ml.

Key words: Antibacterial; Diarrhoea; Enteric bacteria; Parthenin;

INTRODUCTION
Parthenium hysterophorus Linn grows abundantly as a weed in India and was collected from Machilipatnam region, Andhar Pradesh, India and identified. Phytochemical investigations reported the isolation of parthenin as the major constituent of the plant along with some related pseudoguaianolides1-5. Parthenin is known to possess significant allelopathic and cytotoxic properties6. C. Ramesh et al.,7 studied the Antibacterial activity of parthenin and its analogues. We targeted the isolated sesquiterpene lactone, parthenin, towards diarrhoeal pathogens. Infectious diarrhoeal diseases are responsible for considerable morbidity and mortality, especially in developing countries8. According to World Health Organization (WHO) bulletin, diarrhoeal infections are major public health problems in developing countries and contribute to the death of millions of children annually9. Among the bacterial enteric-pathogens, Vibrio cholerae, Salmonella spp. and Shigella spp. are of special concern because of the severity of the illness they cause and their association with various outbreaks. The problem of antimicrobial resistance in bacterial pathogens causing diarrhoeal diseases continues to be alarming10-12. Even though a number of reports were reported on various biological activities of parthenin, the antibacterial activity of the compound against enteric bacterial pathogens was not yet established. In this regard, in order to discover alternative natural products against infective agents, the present study is focused on the in vitro antienterobacterial activity of parthenin isolated from an orbonox weed of India, P. hysterophorus.
MATERIALS AND METHODS

Extraction and Isolation

Air dried plant material of *P. hysterophorus* was powdered and macerated with petroleum ether for 72 h at room temperature. After evaporation of solvent to dryness, residue obtained was 9 g. The crude extract (8.5 g) was then fractionated over an open 100-200 mesh silica gel column using EtOAc/petroleum ether as eluents with increasing polarities. Six fractions were collected and the major fraction was subjected to spectroscopy (1H NMR, 13C NMR and Mass) and the chemical structure (Figure 1) of the major compound was determined by comparing spectroscopic data with that of the literature.

Spectroscopy

1H NMR (300 MHz) and 13C NMR (150 MHz) spectra were measured with Bruker UXNMR/XWIN-NMR (300 MHz) instrument. Chemical shifts (δ) are reported in ppm downfield from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI positive ion trap detector.

Microorganisms

The test bacterial cultures were procured from microbial type culture collection (MTCC), IMTECH, Chandigarh, India. The bacterial cultures were maintained on Mueller-Hinton Agar and stored in a refrigerator at 4°C.

In vitro Antibacterial activity

Agar cup bioassay was employed for testing preliminary antibacterial activity of the compound following the standard procedure. The Minimum Inhibitory Concentrations were determined using broth dilution method according to the protocols of National Committee for Clinical Laboratory Standards (NCCLS) against various enteric bacterial pathogens.

RESULTS AND DISCUSSION

NMR spectral data of Parthenin

1H NMR (300 MHz, CDCl3): δ 7.46 (1H, d, J = 6.4 Hz), 6.25 (1H, d, J = 2.1 Hz), 6.14 (1H, d, J = 6.4 Hz), 5.54 (1H, d, J = 2.1 Hz), 4.76 (1H, d, J = 9.4 Hz), 3.45 (1H, m), 2.33-2.13 (2H, m), 2.11-2.08 (1H, m), 1.83-1.65 (2H, m), 1.23 (3H, s), 1.13 (3H, d, J = 7.2 Hz)

13C NMR (150 MHz, CDCl3): δ 208.7, 172.4, 162.8, 139.2, 130.1, 120.8, 85.1, 78.2, 60.1, 44.9, 39.6, 30.8, 27.6, 19.6, 16.2; MS (ESI): m/z 263 [M+H]+.

Agar cup bioassay was employed for testing preliminary antibacterial activity of the compounds and tabulated (Table 1). Parthenin (100 µg/ml) was highly active against *S. typhi*, *E. coli* and *P. aeruginosa* with zone of inhibition diameter (IZD) of 20mm. At a concentration of 100 µg/ml of parthenin, moderate antibacterial active was observed against the bacteria, *S. typhimurium*, *K. pneumoniae*, *S. epidermidis*, *S. aureus*, *V. cholerae* and *S. flexneri* with IZD value in between 16 - 18mm. *E. aerogenes* and *P. vulgaris* were least sensitive to parthinin with a zone of inhibition diameter of 12 mm at the concentration of 100 µg/ml.

From the results of Minimum inhibitory concentration can be interpreted that the parthenin was active against all the tested bacterial strains with degree of variation. Parthenin was highly active against *S. typhi*, *E. coli* and *P. aeruginosa* with MIC value of 50µg/ml. Against *S. typhimurium*, *K. pneumoniae*, *S. epidermidis*, *S. aureus*, *V. cholerae* and *S. flexneri* the MIC value is of 100 µg/ml. *E. aerogenes* and *P. vulgaris* were least sensitive to parthenin with an MIC value of >200 µg/ml. From the biological activity it can be noticed that parthenin is significantly active against the bacterial strains, *S. typhi*, *E. coli* and *P. aeruginosa*. Biological activities including Antidiarrheal activity of many sesquiterpene lactones was clearly stated by Anna K. Picman and Eloy Rodriguez et al. In silico analysis of the parthenin biological activity, synthesis of its analogues and identifying mode of action of the compound against the enteric bacterial pathogens is further warranted to get best results in the area of drug discovery from phytochemicals.

ACKNOWLEDGEMENTS

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Table 1: Antibacterial activity of parthenin against enteric bacterial pathogens

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Zone of inhibition</th>
<th>Minimum Inhibitory Concentration (MIC) μg/ml</th>
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<tbody>
<tr>
<td></td>
<td>50 μg/ml</td>
<td>100 μg/ml</td>
</tr>
<tr>
<td><em>Salmonella typhi</em> (MTCC 733)</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em> (MTCC 98)</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em> (MTCC 111)</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> (MTCC 1109)</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td><em>Escherichia coli</em> (MTCC 739)</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em> (MTCC 744)</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> (MTCC 424)</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em> (MTCC 435)</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (MTCC 96)</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em> (MTCC 3905)</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td><em>Shigella flexneri</em> (MTCC 1457)</td>
<td>12</td>
<td>16</td>
</tr>
</tbody>
</table>

Zone of Inhibition diameters are in mm; DMSO – no activity; MIC - Minimum Inhibitory Concentration.

![Chemical structure of Parthenin](image)

Fig. 1: Chemical structure of Parthenin

REFERENCES


8. Thapar N and Sanderson IR. Diarrhoea in children: an interface between developing and developed