Research Article

SYNERGISTIC ANTIBACTERIAL EFFECT OF METHANOLIC EXTRACT OF CERTAIN INDIAN MEDICINAL PLANTS

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Abstract

Aerial parts of Eclipta alba (L.), Centella asiatica (L.) and Phyllanthus amarus (L.) are used in treating various ailments skin, antinociceptive, stomach pain, anti-inflammatory, antiviral, rejuvenator, antimitotoxic, antihyperglycemic, antioxidant and cancer. In this study, three plants are used individually to check their antimicrobial activity against Salmonella Typhi, Escherichia coli and Bacillus subtilis. P. amarus has exhibited maximum inhibition activity against E.coli (30±0.5 mm) followed by B. subtilis (28± 0.32 mm) and S. Typhi (24±0.16 mm). For the first time, we report the synergistic potential of these plants in the ratio of (1:1:1) against the test pathogens, which showed excellent antibacterial activity when compared with individual plant extract.

Keywords: Antibacterial activity, Eclipta alba, Centella asiatica, Phyllanthus amarus

INTRODUCTION

Indian medicinal plants represent a rich source of antibacterial agents (Mahesh and Satish, 2008). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine (Mann et al., 2008). Plant products used in traditional medicine still remain the principal source of pharmaceutical agents (Ibrahim, 1997; Ogundipe et al., 1998). In common, with most traditional phytotherapeutic agents C. asiatica is claimed to possess a wide range of pharmacological effects, being used for human wound healing, mental disorders, atherosclerosis, fungicidal, antibacterial, antioxidant and anticancer purposes. C. asiatica has also been reported to be useful in the treatment of inflammations, diarrhea, asthma, tuberculosis and various skin lesions and ailments like leprosy, lupus, psoriasis and keloid. In addition, numerous clinical reports verify the ulcer-preventive and antidepressive sedative effects of C. asiatica preparations, as well as their ability to improve venous insufficiency and microangiopathy (Zheng and Qin, 2007).

Various biological activities are possessed by E. alba, such as memory disorder treatment, general tonic, edema, fever, rheumatic joint pain treatment, digestion, hepatitis, enlarged spleen, antioxidant activity and skin disorders (Chopra et al., 1956; Karnick and Kulkarni, 1990; Karthikumar et al., 2007).

P. amarus leaves and whole plants are usually used for the treatment of gonorrhea, jaundice, rickets and asthma (Schlage et al., 1992) which is mostly used by traditional healers (Leaman et al., 1995). The Phyllanthus genus is a source of active chemicals. Extracts of Phyllanthus have secondary metabolites like alkaloid, flavonoid, lignin, phenol, tannin and terpene. Many of the active constituents like lignin, glycosides, flavonoids, alkaloids, ellagilannins, phenyl propanoids, sterols and flavonols are found in the leaf, stem and roots of the plant. Infectious diseases account for high proportion of health problems in the developing...
countries (Sashi et al., 2003). Plants are an important source of potentially useful structures for the development of new chemo therapeutic agents (Gomathi et al., 2011). Phytochemicals such as tannins and phenol from P. amarus have been reported to associate with antimicrobial activity (Cordell, 1995). From this study, an ingredient for the control of the test pathogenic microbes can be obtained for the benefit of mankind.

MATERIALS AND METHODS

Collection of plants

E. alba, C. asiatica and P. amarus (Figure 1) plants were collected from agricultural lands, Vaniyambadi, Vellore district. Fresh plant materials were first washed under running tap water and surface sterilized by 0.01% of HgCl$_2$ for 2 minutes subsequently soaked with double distilled water. After that the leaves were air dried under shade at room temperature for one week. The dried leaves are later ground with an electric grinder into fine powder which was stored in an air tight container at room temperature.

Extraction

The 100g powder of individual plants and three plants powder in equal ratio was simultaneously extracted with methanol by keeping in a shaker (200 rpm) for 24 hrs at room temperature in separate flasks. The extracts were filtered using Whatman No. 1 filter paper and then concentrated in the vacuum rotary evaporator at 40°C. The concentrated crude extract (blackish-green) was stored at 4ºC for further use.

Drug preparation

The solvent free methanolic extract of E. alba, C. asiatica and P. amarus was used for further investigation. All the plant extracts were prepared at the concentration of 50 mg in 1 ml of 10% DMSO in a sterile vial.

Test organisms

Three human pathogenic bacterial strains like Salmonella Typhi (MTCC 733), Escherchia coli (MTCC 443) and Bacillus subtilis (MTCC 441), were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India and were maintained on nutrient agar slants at refrigerated condition was used in the present study.

![Figure 1: Morphology of collected traditional plants](image)

A. Phyllanthus amarus  
B. Centella asiatica  
C. Eclipta alba

Culture media and inoculum preparation

Muller Hinton Agar (Himedia) is used to check the antibacterial activity and Nutrient broth (NB) is used to prepare inoculums. A loopful of gram positive and gram negative bacterial strains such as Salmonella Typhi, Escherchia coli and Bacillus subtilis were inoculated in 5 ml of nutrient broth in a test tube and incubated for 18 hrs to activate the strain.

Agar well diffusion method

The extracts obtained from three plants were studied for their antimicrobial activity. In agar well diffusion method, the media and the test bacterial cultures were inoculated into Petri dishes. The test strain 0.1 ml was inoculated into the media. Adequate care was taken to ensure proper homogenization. The experiment was performed under strict aseptic conditions. After the medium solidified, a well was made in the plates with sterile borer (6mm). The methanolic extract at the concentration of (1, 1.5, 2, 2.5 and 3mg) was introduced into the well, the 10% DMSO served as control and the plates were incubated at 37 °C for 24 hrs. The antibacterial activity was determined by measuring the diameter of the zone of inhibition.

RESULTS

Three methanolic leaves extracts of E. alba, C. asiatica and P. amarus were examined for their
Table 1: Antibacterial activity of methanolic extract of *E. alba, C. asiatica* and *P. amarus*.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Test organisms</th>
<th>Zone of inhibition (mm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.25 mg</td>
</tr>
<tr>
<td>1</td>
<td><em>E. coli</em></td>
<td>18±0.54</td>
</tr>
<tr>
<td>2</td>
<td><em>S. Typhi</em></td>
<td>08±0.45</td>
</tr>
<tr>
<td>3</td>
<td><em>B. subtilis</em></td>
<td>08±0.51</td>
</tr>
</tbody>
</table>

*Values are mean of three replicates.

Figure 2: Antibacterial activity of methanolic extract of *E. alba, C. asiatica* and *P. amarus*.

Table 2: Synergistic antibacterial activity of methanolic extract of *E. alba, C. asiatica* and *P. amarus*.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Test organisms</th>
<th>Zone of inhibition (mm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.100 mg</td>
</tr>
<tr>
<td>1</td>
<td><em>E. coli</em></td>
<td>10±0.55</td>
</tr>
<tr>
<td>2</td>
<td><em>S. Typhi</em></td>
<td>10±0.26</td>
</tr>
<tr>
<td>3</td>
<td><em>B. subtilis</em></td>
<td>9±0.33</td>
</tr>
</tbody>
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*Values are mean of three replicates.

DISCUSSION

There is a need to discover new drugs to treat pathogenic infections because of the resistant potential of the pathogens to the existing drugs. Plant plays a pivotal role in treating all kinds of disease. The plants used in this study are based on the prior information in treating diseases. Prabu et al., 2011 reported that *E. alba* showed significant antibacterial activity in methanolic extract. This report supported our claim. Jagtap et al. (2009) reported that, the aqueous extract of *C. asiatica* did not show any antibacterial effects at lower concentrations and petroleum ether shows moderately activity. However, our methanolic extract was effective at the concentration below 1 mg/mL against *E. coli* followed by *S. Typhi* and *B. subtilis*. Flora Oluwafemi and Folasade Debiri, 2008 has reported *P. amarus* showed good inhibitory activity against *S. Typhi* for ethanol and hot water extract, but the methanolic extract showed effective activity against *E.coli* and *S. Typhi*, similar results was reported by Komuraiah et al., 2009. With reference to these reports, the plants *E. alba, C. asiatica* and *P. amarus* were chosen in the present investigation. This attempt has showed good antibacterial activity in single and synergistic effect. The synergistic effect exhibited pronounced antibacterial activity.

REFERENCE


Calixto JB, Santos ARS, Cechinel Filho V and Yunes RA. 1998. A review of the plants of the genus Phyllanthus: their chemistry, pharmacology and


