Antibacterial effects of *Emblica officinalis* and *Phyllanthus niruri* crude extracts against bacterial pathogens

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Abstract

The present study was undertaken to determine the antibacterial activity of aqueous crude extracts of *Emblica officinalis* and *Phyllanthus niruri* was screened by using agar well diffusion method, against five human bacterial pathogens such as *Bacillus* sp., *Lactobacillus* sp., *Pseudomonas* sp., *Proteus* sp., and *Streptococcus* sp. The different concentrations of plants extracts were used for this tests were 30µl, 60µl and 90µl. The crude extract of *Emblica officinalis* was effective against all the test bacterial strains while *Phyllanthus niruri* was found to be effective against *Lactobacillus* strain only. The data clearly shows that the aqueous crude extract of *Emblica officinalis* possess strong inhibitory action against all the test bacterial pathogens.

Key words: *Emblica officinalis*, *Phyllanthus niruri*, Antimicrobial activity, Agar well diffusion.

1. Introduction

In India, many plants are mostly used as traditional medicines for the treatment of various infective diseases. In small villages and rural areas, people use those medicinal plants for the treatment of some common infections [1]. The active ingredients present in those plants are highly used for curing the diseases. Hence, it is necessary to identify the potential use of those medicinal plants for the treatment of infectious diseases. Among the common medicinal plant *Emblica officinalis* and *Phyllanthus niruri* plays a crucial role in curing wide range of diseases and they were used in unani, siddha and in ayurveda treatments [2].

*Emblica officinalis* is a deciduous tree, commonly known as Indian gooseberry or amla and ‘Nelli’ in tamil. It belongs to the family Phyllanthaceae. It is widely grown in all over India. Their fruits are edible and are pale yellowish and fleshy in nature. It is the highest source of natural vitamin C [3]. The fruits are highly used in unani medicine to treat diseases like diarrhoea, dysentery, diabetes, asthma, bronchitis, cardiac disorder and haemorrhages [4, 5]. It is used for anti inflammatory [6] and anti pyretic treatments and also to treat disorders like Scurvy, Cancer and Heart diseases [7].

*Phyllanthus niruri* is an herbaceous plant with an average height of 50 cm. Its fruits are so tiny, found below the branches and it is structurally similar to the fruits of *E. officinalis* hence it is called ‘keela nelli’ in tamil. It also belongs to the same Phyllanthaceae family. It is found all over the tropical regions. It is widely used for the treatment of jaundice, syphilis, against constipation, gonorrhoea and kidney disorders [8, 9].

The wide use of *Emblica officinalis* and *Phyllanthus niruri* for various purposes prompted us to select for screening of antibacterial activity. The present study aimed at identification of antibacterial ability of these two plants. In vitro studies on these plants strongly help in the identification of the plant constituents with antimicrobial activity capable of exerting protective effects against human bacterial pathogens. This study involves the in vitro antimicrobial activity of these plant extracts against some gram positive and gram negative pathogenic micro organisms and to ascertain the chemical constituents that may be present in those plant extracts.

2. Materials and methods

2.1 Selection of plants

In the present work, *Emblica officinalis* (Fig 1 a) and *Phyllanthus niruri* (Fig 1 b) plants were screened for its
potent antibacterial activity.

2.1.1 Parts used:

Figure 1: Plants (a) Phyllanthus emblica - fruits (b) Phyllanthus niruri - leaves

2.2 Plant materials collection

Fresh and healthy Emblica officinalis fruits and leaves of Phyllanthus niruri were collected in the campus of Sri Paramakalyani Centre for Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi, India. The fruits and leaves were ensured that they were healthy and uninfected and they were thoroughly washed in tap water and rinsed with sterile distilled water and air dried in room temperature for few hours.

2.3 Preparation of aqueous extract

The two separate plant extracts were prepared. 50g of the samples were taken and homogenized well with the help of mortar and pestle and extracted with 100ml of sterile distilled water. Thereafter, it was filtered with the help of Whatman No. 1 filter paper. The filtrate was collected and stored at 4°C for further use. The final concentration of the extracts were 500mg/ml.

2.4 Antibacterial activity

2.4.1 Preparation of culture inoculums for test

The test bacterial strains such as Bacillus sp., Lactobacillus sp., Pseudomonas sp., Proteus sp., and Streptococcus sp. were obtained from stock culture in the Department of Microbiology, Sri Paramakalyani College, Alwarkurichi, Tirunelveli, India. The test bacterial strains were prepared on Nutrient agar medium and incubated for 24 hrs at 37°C. Then the cultures were stored at 4°C till test as stock cultures. Active cultures for experiments were prepared by transferring a loopfull of cells from the stock cultures to test tubes of Nutrient broth that were incubated in shaker at 37°C for 24 hrs that were used as the inoculums.

2.4.2 Agar well diffusion assay

The effects of these two plant extracts on the five bacterial strains were assayed by agar well diffusion method [10]. Mueller Hinton agar medium was poured into the petriplates aseptically and that were allowed to solidify. The lawns of the test bacterial strains were prepared by inoculation of 24 hrs culture of bacterial strains by spread plate method. Petriplates were allowed to remain for few minutes and the excess of inoculum were removed aseptically using micropipettes. Wells were made with the help of sterile cork borer (6mm) and the cuts agar discs were removed aseptically with sterile forceps, then the aqueous plant extracts were added in 30 µl, 60 µl and 90 µl concentrations. The test plates were incubated aerobically at 37°C depending on the incubation time required for a visible growth. Control wells containing sterile distilled water (negative control) were also incubated. After incubation, the results were recorded, as the presence or absence of inhibition zone. The inhibitory zone around the well indicates the absence of bacterial growth that shows positive result and the absence of zone indicates the negative result. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well [11]. The diameters of the zones of inhibition (ZOI) were measured in millimeters.

Table 1: Antimicrobial effects of crude extract of Phyllanthus emblica Measured by zone of inhibition

<table>
<thead>
<tr>
<th>Volume of aqueous extracts used</th>
<th>Bacillus sp.</th>
<th>Streptococcus sp.</th>
<th>Lactobacillus sp.</th>
<th>Pseudomonas sp.</th>
<th>Proteus sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 µl</td>
<td>13</td>
<td>16</td>
<td>16</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>60 µl</td>
<td>15</td>
<td>17</td>
<td>16.5</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>90 µl</td>
<td>16</td>
<td>19</td>
<td>17</td>
<td>13</td>
<td>14.5</td>
</tr>
</tbody>
</table>

– Sign indicates the absence of zones.

Table 2: Antimicrobial effects of crude extract of Phyllanthus niruri measured by zone of inhibition

<table>
<thead>
<tr>
<th>Volume of aqueous extracts used</th>
<th>Bacillus sp.</th>
<th>Streptococcus sp.</th>
<th>Lactobacillus sp.</th>
<th>Pseudomonas sp.</th>
<th>Proteus sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 µl</td>
<td>-</td>
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<td>12</td>
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<tr>
<td>60 µl</td>
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<td>-</td>
</tr>
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<td>90 µl</td>
<td>-</td>
<td>-</td>
<td>16</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

– Sign indicates the absence of zones.

3. Result

The *in vitro* antibacterial study of the two aqueous extracts of fruits of *E. officinalis* and leaves of *P. niruri* was done by simple agar diffusion method against the test bacterial cultures and their activity potentials were qualitatively evaluated by the presence or absence of inhibition zones and zone diameters. The results of antibacterial assay of the two plant extracts are presented in the Table 1 and 2. The results specified in the Table 1 predicted that the *E. officinalis* had greater potential as an antibacterial agent against the tested bacterial cultures than did *P. niruri* extract. It was found a regular increase in the zone of inhibition size with the increase in the concentration of extracts in all bacterial strains. Zones of inhibition of *E. officinalis* against the test bacteria *Bacillus* sp., *Lactobacillus* sp., *Pseudomonas* sp., *Proteus* sp., and
Streptococcus sp. were 16, 17, 13, 14.5 and 19 mm respectively at 90µl volume with 500mg/ml concentration of the aqueous extract (Table 1). Zone of inhibition of P. niruri against the test bacteria Lactobacillus sp., was 16mm at 90µl volume with 500mg/ml concentration of the aqueous extract (Table 2). And there was no inhibition zone formation against the other test bacteria such as Bacillus sp., Pseudomonas sp., Proteus sp., and Streptococcus sp. And the zones of inhibition found in different organisms were shown in Figure 2a and 2b. The aqueous extract of E. officinalis is found to be effective against all the test bacterial cultures (Figure 2a) but aqueous extract of P. niruri shows ZOI only in the Lactobacillus sp., and there is absence of inhibition zone in the other test bacterial cultures (Figure 2b). This may be due to the presence of certain Tanin, Alkaloids and Phenolic compounds present in the fruit extract of E. officinalis [12].

Fig 2a: Preliminary screening for antibacterial activity of aqueous fruit extract of P. emblica.
a - Bacillus sp. b - Streptococcus sp. c - Lactobacillus sp. d - Pseudomonas sp. e - Proteus sp. f – Control

Fig 2b: Preliminary screening for antibacterial activity of aqueous fruit extract of P. niruri.
a - Bacillus sp. b - Streptococcus sp. c - Lactobacillus sp. d - Pseudomonas sp. e - Proteus sp. f – Control

4. Discussion
The plant based traditional medicines were proven highly effective for their utilization as a source of antimicrobial compounds [12]. The plants are potentially useful for the development of chemotherapeutics. Many reports are available on the anti bacterial properties of E. officinalis and P. niruri [13, 14]. The aqueous fruit extracts of E. officinalis showed the antibacterial activity against all the five test bacterial strains which supports the potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. It was clear from the present results that the aqueous fruit extracts of E. officinalis exhibited pronounced activity against the five tested bacteria namely Bacillus sp., Lactobacillus sp., Pseudomonas sp., Proteus sp. and Streptococcus sp. Previous study conducted by Hossein et al., suggested that the ethanol and acetone extracts of E. officinalis posses antibacterial activity [15]. Saheb et al., assessed the antibacterial activity in tannins isolated from the leaves and fruits of E. officinalis [16]. The aqueous leaf extracts of P. niruri shows inhibitory action towards Lactobacillus sp. only and it does not show any inhibition on other test bacteria cultures. A more recent work conducted by Manas et al., [17] revealed that the methanolic extracts of various parts of Phyllanthus niruri have antibacterial activity against five bacterial strains - E. cloacae, S. aureus, P. aeruginosa, E. coli and S. viridans and two fungal strains - A. niger and T. viridae. It was found a regular increase in diameter of zones of inhibitions with the advancement of concentrations in all sensitive bacterial strains [18]. This tends to show that the active ingredients present in E. officinalis and P. niruri can be extracted and it was confirmed that the fruit extract and leaf extract of E. officinalis and P. niruri respectively, possess potential antimicrobial property which can be used as an active principle for any antibiotic preparations.

5. Conclusion
In this study, both the aqueous extracts were found to posses high antibacterial activity against Lactobacillus sp., however the E. officinalis posses antibacterial activity against all the test bacterial culture. The present study provides data for supporting the use of E. officinalis and P. niruri as natural broad spectrum antimicrobial agents against a wide range of microbes. And also the present study disclosed the importance of natural medicinal plant extracts to control pathogenic bacteria which pose threat to human health and can act as safe and effective medicines.

6. References
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