Larvicidal and Antibacterial Activities of Different Solvent Extracts of Solanum nigrum LINN

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Abstract: Solanum nigrum is an important ingredient in traditional Indian medicines. In the present paper deals the level of larvicidal activity of ethanol, hexane and chloroform extracts of stem and leaf of Solanum nigrum at different concentrations viz., 500, 1000 and 1500 ppm has been investigated. The results obtained show that this plant material exhibited significant activity and could be considered as potent natural larvicidal agent. The antibacterial activities of ethanol, hexane and chloroform extracts of stem and leaf, Solanum nigrum were screened against various pathogenic bacteria by ‘agar well diffusion’ method. The plant extracts showed various levels of activity on different test organisms among that ethanol extract of Solanum nigrum stem was found to be the most potent extract.

Key words: Solanum nigrum • Larvicidal activity • Culex quinquefasciatus • Antibacterial activity

INTRODUCTION

Mosquitoes are well known group of insects, which transmit many dreadful diseases causing serious health problems to human beings. Controlling of these vectors is being achieved for a long time, by using synthetic chemicals. But the chemicals may cause pollution problem and help to develop resistance in mosquito species [1]. The microorganisms and insecticides have developed resistance against many antibiotics due to the indiscriminate use of antimicrobial drugs [2]. Antibiotics are sometimes associated with side effects [3] whereas there are some advantages of using antimicrobial compounds of medicinal plants, such as often fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature [4]. All these data high lights the need for new alternative drug regimens. Studies on the natural plant products for their efficacy as larvicides and antimicrobials during the last decade have indicated them to be possible alternatives to synthetic chemical insecticides and antibiotics [5-7]. Solanum nigrum L. (Black night shade) a member of the Solanaceae, has a wide range of medicinal values. The herb is antiseptic, antisynderic and antidiuretic used in the treatment of cardiac, skin disease, psoriasis, herpesvirus and inflammation of kidney. The root bark is laxative, useful in the treatment of ulcers on the neck, burning of throat, inflammation of liver and chronic fever. Berries are bitter and pungent useful in the heart disease, piles, dysentery [8].

MATERIALS AND METHODS

Preparation of Stock Solution of Plant Extract: The plant parts, stem and leaf of Solanum nigrum were collected from 3-5 months old mature plants growing in the garden of Ragavendra Medical Institute of Electropathy, Senathipalayam, Erode, Tamil Nadu, India and washed with sterile water and then chopped into small fragments.
The materials were then shade dried at ambient temperature (32°C) for 10 to 15 days and the drying operation was carried out under controlled conditions to avoid chemical changes. The dried samples were crushed into fine powder using an electronic blender. The fine powder of the stem and leaf was extracted at 47°C by using soxhlet apparatus using three different solvents like Ethanol, Hexane and Chloroform. After extraction, the solvents were removed by the extracts were dried at 50°C in hot air oven for 2 hours and they were stored properly for further studies.

Larvicidal Activity

Test Organism: The larvae used to test for the larvicidal activity were obtained from colonies of Culex quinquefasciatus mosquitoes cultured and maintained in the laboratory at a temperature of 28°C ± 2°C and 80 - 90% relative humidity. The larvae were fed with mice feed and yeast powder in the ratio of 3:1. They were transferred to another clean bowl for three days at 24hrs interval and the water was aerated with the aid of an air pump.

Bioassay: Larvicidal activity of the mosquito C. quinquefasciatus was assessed by following the standard WHO method [9]. The ethanol, hexane and chloroform extracts of stem and leaf of Solanum nigrum for assayed against larvicidal activity was carried out at different concentrations ranging from 500, 1000 and 1500 ppm in distilled water. Ten second, third and fourth instar larvae of C. quinquefasciatus were collected separately and transferred gently to the test medium and simultaneously a control was maintained with ethanol-fresh tap water mixture. The larval mortality in both treated and control were recorded after 24 h. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. The experiments were replicated three times and conducted under laboratory conditions at 25-30°C and 80-90% relative humidity.

Antimicrobial Activity

Microorganisms Tested: A total of eight bacterial cultures (Enterococcus faecalis, Escherchia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Shigella flexneri, Salmonella typhi, Staphylococcus aureus and Vibrio cholerae) were used in this study. The cultures were procured from Raja Muthiah Medical College, Annamalai Nagar, India. The bacterial strains were grown in nutrient broth at 37°C and they were stored on nutrient agar slants for future use.

Well-in Agar Method: Anti-bacterial activity of plant extracts was tested by a modified well-in agar method [10]. From the nutrient broth, the inoculum suspension was swabbed uniformly over the Muller Hilton Agar by using of sterile cotton swab. Subsequently, using a sterile borer, well of 0.5 cm diameter was made in the pathogen inoculated media. Different concentrations, i.e., 50 µl and 100 µl of each extract were 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 h at 37 °C. The results were recorded by measuring the diameter of inhibition zone at the end of 24-48 h.

RESULTS AND DISCUSSION

Larvicidal Activity: The larvicidal activity of ethanol, hexane and chloroform extracts of stem and leaf of Solanum nigrum against C.quinquefasciatus mosquito larvae were given in Tables 1. The larvicidal activity of

<p>| Table 1: Larvicidal activity of stem and leaf extracts of Solanum nigrum |
|-----------------|-----------------|-----------------|-----------------|
|                | Mortality%       |                  |                  |
|                | Stem extract     | Leaf extract     |                  |</p>
<table>
<thead>
<tr>
<th></th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>28.11±0.33</td>
<td>33.78±0.21</td>
<td>44.66±0.25</td>
<td>30.00±0.42</td>
<td>32.33±0.21</td>
<td>49.44±0.33</td>
</tr>
<tr>
<td>1000</td>
<td>46.66±0.42</td>
<td>55.22±0.42</td>
<td>76.55±0.47</td>
<td>50.55±0.21</td>
<td>64.11±0.30</td>
<td>85.66±0.30</td>
</tr>
<tr>
<td>1500</td>
<td>65.55±0.33</td>
<td>90.20±0.30</td>
<td>92.77±0.21</td>
<td>73.88±0.54</td>
<td>95.66±0.33</td>
<td>100.0±0.22</td>
</tr>
<tr>
<td>n-Hexane</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>20.33±0.34</td>
<td>27.00±0.40</td>
<td>32.53±0.21</td>
<td>26.66±0.22</td>
<td>31.00±0.22</td>
<td>41.10±0.30</td>
</tr>
<tr>
<td>1000</td>
<td>42.00±0.34</td>
<td>55.00±0.44</td>
<td>61.00±0.21</td>
<td>50.08±0.22</td>
<td>65.73±0.30</td>
<td>73.86±0.30</td>
</tr>
<tr>
<td>1500</td>
<td>60.66±0.68</td>
<td>70.66±0.50</td>
<td>73.86±0.40</td>
<td>71.10±0.30</td>
<td>75.40±0.42</td>
<td>82.16±0.21</td>
</tr>
<tr>
<td>Chloroform</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>500</td>
<td>30.55±0.33</td>
<td>60.55±0.16</td>
<td>64.55±0.33</td>
<td>33.33±0.42</td>
<td>69.2±0.40</td>
<td>72.66±0.22</td>
</tr>
<tr>
<td>1000</td>
<td>66.06±0.33</td>
<td>83.77±0.16</td>
<td>85.06±0.33</td>
<td>63.73±0.16</td>
<td>80.55±0.16</td>
<td>93.55±0.21</td>
</tr>
<tr>
<td>1500</td>
<td>83.77±0.16</td>
<td>96.00±0.0</td>
<td>98.77±0.16</td>
<td>80.53±0.47</td>
<td>94.88±0.16</td>
<td>100±0.0</td>
</tr>
</tbody>
</table>
ethanol extracts of stem and leaf of *Solanum nigrum* showed 92% and 100 of death with the use of 1500 ppm concentrations, respectively, after 3 days. The third day 1000 ppm concentration killed more than 90% of the larvae in both extracts. Hexane extracts of stem and leaf of *Solanum nigrum* causes 73 and 82% of death after 3 days with respect to 1500 ppm concentration of extract, respectively. The larval mortality was below 60% when 1000 and 500 ppm concentrations. Chloroform extract from stem and leaf of *Solanum nigrum* showed greater than 50% mortality when 500 and 1000 ppm concentrations were used. Only the highest concentration (1500 ppm) of stem and leaf extracts showed 98 and 100% mortality, respectively. Among the two extracts, the leaf extract of *Solanum nigrum* was found more lethal than stem extracts. This work demonstrates the potency of *Solanum nigrum* in the control of mosquito larvae. Leaf extract appeared as the most lethal as stem extracts tested. The high mortality recorded for leaf extract might be attributed to deficiency of dissolved oxygen in the water. Senthil Nathan *et al.*, [11] reported that the plant allelochemicals may be quite useful in increasing the efficacy of biological control agents because plants produce a large variety of compounds that increase their resistance to insect attack. Previous studies have shown that extracts of some plant parts do possess insecticidal effects. This result compared favourably with that from other species, for example, Elimam *et al.*, [12] reported that leaf extract of *Calotropis procera* has showed larvicidal activity against the mosquitoes *Anopheles arabiensis* and *C. quinquefasciatus*. Rajmohan and Ramaswamy, [13] evaluated the efficacy of *Ageratina adenophora* against *Culex quinquefasciatus*.

**Antibacterial Activity:** The result of the anti-bacterial activity tests of *Solanum nigrum* stem and leaf extracts are presented in Fig 1. Totally eight bacterial strains (two Gram positive and six Gram negative bacteria) were used in this investigation. The ethanol extracts of stem and leaf of *Solanum nigrum* showed better growth inhibition against all tested pathogens excluding *S. aureus* and *P. aeruginosa*. The ethanolic extracts exhibited least activity against the strains employed. In chloroform extract of stem and leaf, the maximum zone of inhibition i.e., 6.23mm and 5.50 mm was registered against *Vibrio cholerae*. The antibacterial activity of hexane extract of stem and leaf exhibited least antibacterial activity against *S. flexneri*, *S. aureus*, *K. pneumoniae* and *P. aeruginosa*. Among these two extracts, the stem extracts showed the maximum antibacterial activity against *E.coli* (5.36 mm), followed by *P. mirabilis* (3.46 mm) than leaf extract. Generally two concentrations of chloroform extract of stem and leaf showed activity against the strains employed. In chloroform extract of stem and leaf, the maximum zone of inhibition was observed for the species *V. cholerae* (6.43 and 5.56mm, respectively), *P. aeruginosa* (4.16 and 3.95 mm, respectively) and *E. faecalis* (4.10 and 2.70 mm, respectively) at a concentration of 100 µl. It was observed that *Solanum nigrum* has a low antimicrobial activity against
K. pneumoniae (0.40±0.11 mm) and S. typhi. There are several reports stating that other Solanum species extracts exhibit anti-bacterial activity. Solanum torvum showed activity against Pseudomonas aeruginosa and Staphylococcus aureus [14], while Solanum nigrum was active against Salmonella typhi [15]. Solanum trilobatum was able to reduce bacterial load in aquaculture system [16] and Solanum incanum could inhibit the growth of Staphylococcus aureus [17]. Solanum trilobatum showed activity against Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi and E.coli [18]. Similarly Suffredini et al., [19] reported the aqueous extract of Physalis minima was active against Staphylococcus aureus and Enterococcus faecalis.

REFERENCES