Pharmacological evaluation of *Ziziphus nummularia* leaves for phytotoxic and molluscicidal bioassays

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Medicinal plants have curative properties due to the presence of various complex chemical substances of different composition which contain secondary metabolites. In the present study, the phytochemical screening of *Ziziphus nummularia* leaves were carried out to investigate their phytoconstituents like alkaloids, carbohydrates, protein, saponin, tannins, fixed oil, fats, volatile oil, glycosides, phenol and flavonoids. The phytotoxic and molluscicidal potential of *Z. nummularia* leaves were evaluated which exhibit that both ethanolic and n-hexane extracts were highly molluscicidal and good phytopromoters. The results indicate that *Z. nummularia* have growth promoting capability and can be used in fertilizer industries.

**Key words:** Phytochemical screening test, phytotoxic, molluscicidal, *Ziziphus nummularia*.

**INTRODUCTION**

Pharmacognosy is the study of chemical, physical and biochemical properties of natural drugs, their potential, drug substances, origin and the discovery of new drugs from natural sources (Tyler, 1999). The study of multicellular and unicellular organism including fungi, bacteria, aquatic organisms, aquatic plants, terrestrial plants and animals, all comes under pharmacognosy preview.

Pharmacognosy helps us to know the past history, collection, cultivation, drying, preservation, storage, commerce and uses of drugs obtained from biological source (Evans, 2002). Morph-histological study is the preliminary step towards identification and standardization of plants and plants derived drugs (Youenken, 1950).

*Ziziphus nummularia* (Family Rhamnaceae) commonly called Malla or Jher beri (Hindi) is used for its fruits. The fruit is cooling, astringent, appetizer, stomachic, cures mucous and increase biliousness effect (Oudhia, 2003). The bark has nematicidal, antihelminthic, antipyretic and anti-inflammatory properties but showed no spermatotoxicity (Bachayaa et al., 2009). The leaf paste is used in boils and skin diseases like scabies etc. (Singh et al., 2002). Herbicides and weedicides are used for the effective control of weeds in order to increase crop yield (Kim, 1994; Santos, 2009). But sometimes the misuse and overuse of these synthetic weedicides may create some problems like resistance against the pesticides, water, soil and air pollutions (Judith et al., 2001). Due to these harmful effects, it is needed to reduce the use of synthetic weedicides or herbicides instead of using natural herbicides to overcome these problems.

*Schistosomiasis* and *Fascioliasis* are two prevalent diseases that infect about 207 million people worldwide (King and Cha, 2008). The best method for the control of these diseases is the parasites life cycle breakage (Mello-Silva et al., 2006; Jigyasu and Sing, 2010). Snails are the intermediate host for the transmission of these diseases.

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By the control of snails it is possible to reduce and eliminate these diseases (Giovanelli et al., 2001; Mello-Silva et al., 2006). In the present study, the phytotoxic or weedcidal and molluscicidal potential of Z. nummularia was evaluated.

MATERIALS AND METHODS

Plant

Healthy and fresh leaves of Z. nummularia were collected from Peshawar University Campus, Pakistan. The collected leaves were washed with tap water to remove the dust particles. The leaves were shade dried for 10 to 15 days then powdered by electric grinder. The plant specimen with voucher number Bot. 20033 (PUP) was deposited in herbarium of Botany Department University of Peshawar, Pakistan.

Preparation of extracts

Two hundred grams of powder was soaked in absolute ethanol and n-hexane solvent for 72 h and then filtered through Whatman filter paper No. 1823. The process was repeated three times. The filtrate evaporated through rotary evaporator to get the extracts and preserved in refrigerator at 4°C for phytochemical screening analysis and pharmacological bioassays.

Phytochemical screening test

The ethanolic and n-hexane extracts were subjected to different qualitative chemical tests to find out the presence of different phytoconstituents that is, alkaloids, carbohydrate, phenolics and tannins, fats, fixed oils, proteins and amino acids, flavonoids, saponins by following detection methods.

Detection of carbohydrates, protein, alkaloids, phenol, tannins, saponins, fats and oil and flavonoids

Same volume of Fehling (copper sulphate in distilled water) and Fehling B (potassium tartarate and sodium hydroxide in distilled water) were added with few drops of sample solution of each extract and then boiled. The appearances of brick red precipitate of cuprous oxide confirm the presence of reducing sugar (Trease and Evans, 2002).

Extract solution of each sample was taken in test tube and 0.2% of Ninhydrin solution added in it and then boiled. The appearances of violet colour indicate the presence of protein otherwise not (Kumar and Kiladi, 2009).

For the detection of alkaloids, few drops of Hager reagent were added in sample of each extract. The formation of yellow precipitate confirms the presence of alkaloids (Khandelwal, 2004).

2 ml of ferric chloride solution was taken in a test tube to which 2 ml of extract solution was added. The bluish green colour of the solution indicates the presence of phenol otherwise none (Dahiru et al., 2006).

To an extract solution, sodium hydroxide was added. Tannins were detected by the appearance of yellow to red precipitate (Kokate, 1994).

Phytotoxic activity

Phytotoxic activity of the extracts were carried out against Lemma minor following the standard procedure of Atta-ur-Rehman et al. (2001).

Materials used were Lemma minor fronds, extracts flask 250 ml, distilled water, micropipettes (10 to 100 µl, 100 to 1000 µl), filter paper, glass vials, laminar flow hood, brush, oven etc.

The medium was prepared in distilled water and autoclaved at 121°C for about 20 min, and by adding KOH pellets the pH was adjusted to 5.4 to 5.5. Stock solutions were prepared by dissolving 10 mg extracts in 40 ml ethanol and n-hexane. Then three different concentrations that is, 10, 100 and 1000 µl were prepared from stock solutions by taking 5, 50 and 500 µl from stock solutions, respectively. The solvents were allowed to evaporate. In each Petri dish, 20 ml of medium were separately added and 10 plants of L. minor each with 2 or 3 fronds were added in each Petri dish. Methanol and pararue were used as positive and negative controls, respectively. For seven days the Petri dishes were placed in growth chamber at 28°C. On day seven, the numbers of fronds in each Petri dish were counted. Percent growth promotion in both extracts was calculated by using the following formula:

\[
\% \text{Regulation} = 100 - \frac{\text{Number of fronds in test sample}}{\text{Number of fronds in positive control}} \times 100
\]

Molluscicidal activity

The molluscicidal activity of ethanol and n-hexane leaves extracts of Z. nummularia was carried out by following the procedure of Atta Ur Rahman et al. (2001).

The materials used were V.Lymnea acuminata, extracts, distilled water, micropipettes (10, 100 and 1000 µl), Petri dishes, pinching needle, beakers (500 ml).

Aquatic snails (L. acuminata) were collected from different fresh water bodies of Peshawar, Pakistan. The snails were kept in de-chlorinated tap water aquaria with 25±2°C. The same size snails were chosen for the activity. 10, 100 and 1000 ppm concentrations of crude extracts of leaves were prepared. Each concentration was replicated three times of both extract. snails were placed in each concentration and covered the flask with perforated aluminum foil to allow air circulation in flask. After 24 h all the snails were brought out from the test solution. The mortality of the snails were evaluated by punching needle and counted as dead when they did not show any movement or action. The control series contained only distilled water. With reference to positive control, the percent mortality of both extracts were calculated by the following formula:

\[
\text{Percentage mortality} = 100 - \frac{\text{No of snails alive in test}}{\text{No of snails alive in control}} \times 100
\]
Table 1. Phytochemical study of *Ziziphus nummularia*.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Test name</th>
<th>Ethanol extract</th>
<th>n-Hexan extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>Fehling’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Protein</td>
<td>Ninhydrine</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Hagers test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenol</td>
<td>Ferric chloride test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Alkali test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Alkali test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>Frothing test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Killaer killini</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fixed oil and fats</td>
<td>Spot test</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Volatile oil</td>
<td>Spot test</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = present and - = absent.

Table 2. *Lemna* Phytotoxicity of *Ziziphus nummularia* leaves extracts.

<table>
<thead>
<tr>
<th>Extracts used</th>
<th>Concentration (μg/ml)</th>
<th>No. of fronds</th>
<th>% growth promotion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial reading</td>
<td>After 7 days</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>1000</td>
<td>30</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>30</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>0 (-ve control)</td>
<td>30</td>
<td>32</td>
</tr>
<tr>
<td>n-hexane</td>
<td>1000</td>
<td>30</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>30</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>30</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>0 (-ve control)</td>
<td>30</td>
<td>33</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Phytochemical screening test

In the present study, the ethanol and n-hexane extracts of *Z. nummularia* leaves were screened for phytochemical analysis. Both the extracts showed the same result for carbohydrates, protein, alkaloids, phenol, flavonoids, tannins, saponins and glycosides, while fixed oil, fats and volatile oil were present in n-hexane and absent in ethanol extract. The results are presented in Table 1.

Phytotoxicity bioassay

Herbicides and weedicides are used for the effective control of weeds in order to increase crop yield (Kim, 1994; Santos, 2009). Due to their harmful effects, it is needed to reduce the use of synthetic weedicides or herbicides instead of using natural herbicides to overcome these problems. In the present study, the ethanolic and n-hexane extracts of *Z. nummularia* leaves were tested for their phytotoxicity against *L. minor*. The results indicated that both the extracts posses growth promotion potential. At 1000 μg/ml both ethanol and n-hexane extracts exhibited high growth promotion 20 and 30%, respectively, while at 100 μg/ml, both the extracts showed 13 and 23% growth regulation. Both the ethanol and n-hexane at concentration 10 μg/ml revealed 10 and 13% growth regulation, respectively. Ali et al. (2009) *Euphorbia wallichii*; Khan et al. (2011) *Euphorbia prostrate* and Bashir et al. (2011) studied ethyl acetate, methanolic extracts and various other fractions of *Ziziphus jujuba* against *L. minor* and reported that *Z. Jujuba* has growth regulating potential. So our results are strongly supported by their research findings. Our results as shown in Table 2 indicate that as compared to control, the ethanolic and n-hexane extracts of *Z. nummularia* leaves are good growth promoter and can be used as a fertilizer for growth regulation.
Table 3. Molluscidal activity of Ziziphus nummularia leaf.

<table>
<thead>
<tr>
<th>Part</th>
<th>Extract conc. (mg/ml)</th>
<th>Total No. of individuals</th>
<th>No. of dead snails</th>
<th>% mortality</th>
<th>LD₅₀ (95% CI)</th>
<th>Least square line</th>
<th>χ² (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>5</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>3.7674</td>
<td>Y = 3.19 +1.12X</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>13</td>
<td>52</td>
<td>6.136</td>
<td>13.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>15</td>
<td>60</td>
<td>2.613</td>
<td>13.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>25</td>
<td>19</td>
<td>76</td>
<td>6.550</td>
<td>15.71</td>
<td></td>
<td>0.037</td>
</tr>
<tr>
<td>Leaf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>n-Hexane</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>6.550</td>
<td>Y = 3.91 +0.94X</td>
<td>0.037</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>16</td>
<td>64</td>
<td>15.71</td>
<td>2.613</td>
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<tr>
<td>10</td>
<td>25</td>
<td>19</td>
<td>76</td>
<td>13.52</td>
<td>6.136</td>
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<td></td>
</tr>
<tr>
<td>15</td>
<td>25</td>
<td>22</td>
<td>88</td>
<td>6.550</td>
<td>15.71</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Molluscidal bioassay

Snails are the intermediate host for the transmission of Fascioliasis disease. Control of snails will lead to the reduction and elimination of this disease (Giovanelli et al., 2001). The use of plant derived compounds is safe, inexpensive and easily available for mollusk control (Singh and Singh, 2010).

In the present study, the ethanolic and n-hexane extracts of Z. nummularia leaves were evaluated for their molluscicidal potential. The ethanolic extract produced 52% mortality at a concentration of 5 mg/ml and 60% at 10 mg/ml while significant lethality of 88% at a concentration of 15 mg/ml with LD₅₀ value of 3.7674 mg/ml. As compared to ethanolic extract, the n-hexane extracts also exhibited high lethality of 88% at 15 mg/ml and moderate lethality of 76% at 10 mg/ml while poor lethality of 64% at 5 mg/ml with LD₅₀ value of 6.550 mg/ml. Many researchers studied different plants for molluscicidal potential like Sharma et al. (2009) who have studied the effect of ethanolic extracts of Ricinus communis and Acalypha indica against L. acuminate snails. Hassan et al. (2011) Enterolobium contortisiliquum and Jaiswal et al. (2009) studied the effect of nutmeg and mace of Myristica fragrance against vector snail L. acuminate. The results displayed in Table 3 indicate that both the extracts are highly molluscicidal but n-hexane extract of Z. nummularia showed good results for the control of snails and further can be used for the breaking of Fascioliasis disease cycle.

REFERENCES


