**Original article:**

Comparative phyto toxicological and anti inflammatory effects of leaves extracts of *holoptelea integrifolia*

Yasin H¹, Khalid S², Abrar H³, Rizwani GH⁴, Perveen R⁵, Fatima K⁶

**Abstract**

**Objective:** Plants play valuable role in the new drug discovery and significantly used to treat different diseases. Nowadays scientists are investigating the therapeutically active phytochemical constituents that are safe and producing lesser side effect in comparative to other standard drugs.

**Methods:** The plant *Holoptelea integrifolia* is medicinally important and this study was carried out to evaluate the anti inflammatory activity of aqueous extract of the leaves of *Holoptelea integrifolia* in male albino rats wistar stain treated with acetic acid to induced paw edema.

**Results:** Result indicated the significant anti-inflammatory activity while compared with standard drug (diclofenac sodium). Brine shrimp bioassay (cytotoxicity), phytotoxicity, insecticidal and enzyme inhibition activity was performed in different extracts of the leaves of *H. integrifolia*. Results of brine shrimp bioassay indicating positive lethality at high dose in BuOH and H₂O only. While the results of phytotoxicity in all crude extracts displayed mild phytotoxicity(46.3 µg/ml) in high concentrations (1000 µg/ml) except H₂O extract showed no phytotoxicity. Result of insecticidal activity revealed that BuOH extract were found more effective against *Rhyzoperthadamini*ca, the EtOH extract expressed major while EtOAC extract showed mild activity against *Callosobruchusalanis*. Aqueous extract possessed no insecticidal activity.

**Conclusion:** Results of Urease inhibition activity suggested that EtOAC and BuOH extracts of this plant expressed no activity while EtOH and H₂O possessed mild inhibiting activity.

**Keywords:** *Holoptelea integrifolia*; phytotoxicity; insecticidal activity; enzyme inhibition activity; Anti-inflammatory activity; Brine Shrimp Bioassay

---

**Introduction**

*Holoptelea integrifolia* (Roxb) Planch. is one of medicinally important traditional plant belongs to family Ulmaceae, commonly known as Indian Elm, indigenous to tropical regions of Asia, also found in different areas of Pakistan especially in Karachi¹-⁴. All parts of this plant used to treat inflammation, bacterial infections, diarrhea, tumor, diabetes and wound, etc.⁵-¹⁰. Bark and leaves of *H. integrifolia* have been used as bitter, astringent, anthelmintic, acid, thermogenic, anti-inflammatory, digestive, carminative and laxative to treat various diseases. Seeds and stem bark applied against ringworms externally. Seeds have been used to cure ulcers and as body deodorizer⁴,¹¹-¹⁴. Leaves of *H. integrifolia* possess analgesic, anti inflammation and anti diabetes properties also used for various skin infections, piles and gastrointestinal diseases¹⁵,¹⁶. Alkaloids, tannins, cardiac glycosides, saponin glycosides, cyanogenetic glycoside and anthracene derivatives.

---

1. Hina Yasin, Baqai institute of Pharmaceutical Sciences, Baqai Medical University & Dow College of Pharmacy, Dow University of Health Sciences, Pakistan
2. Shaukat Khalid, Baqai institute of Pharmaceutical Sciences, Baqai Medical University, Pakistan.
3. Hina Abrar, Department of Pharmacology, Baqai institute of Pharmaceutical Sciences, Baqai Medical University
4. Ghazala H. Rizwani, Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Pakistan.
5. Rehana Perveen, Baqai institute of Pharmaceutical Sciences, Baqai Medical University
6. Kaneez Fatima, Baqai institute of Pharmaceutical Sciences, Baqai Medical University

**Correspondence to:** Hina Abrar, Department of Pharmacology, Dow College of Pharmacy, Dow University of Health Sciences, Suparco Road, Off Main University Road, Gulzar-e-Hijri, Scheme 33, Karachi, Pakistan.
Email: hina.abrar@duhs.edu.pk
Hexacosanol, octacosanol, β-sitosterol and β-amyrin have been obtained in alcoholic extract of leaves of *Holoptelea integrifolia*. This research has been conducted for the anti-inflammatory activity with aqueous extract of plant *H. integrifolia* in comparison to standard drug (Diclofenac sodium). While different extracts of *H. integrifolia* were selected to evaluate the cytotoxicity, phytotoxicity, insecticidal and enzyme inhibition activities of this medicinally valuable plant.

**Material and methods**

**Plant collection:** The fresh leaves of *H. integrifolia* were collected from the Faculty of Pharmacy, University of Karachi, Pakistan and the plant material was dried (500 g), chopped and macerates with methanol (MeOH) for 15 days after identification. Solvent was evaporated at reduced pressure and controlled temperature to obtained MeOH extract. This extract was subjected for step by step extraction with *n*-hexane, ethanol (EtOH), ethyl acetate (EtOAC), butanal (BuOH) and H₂O then concentrated in rotary evaporator to obtained different fractions. Brine shrimp bioassay, phytotoxicity, insecticidal activity and enzyme inhibition activity (indophenol method) were performed. For the determination of insecticide activity and toxicity different methods are being used on various surfaces (Insecticide impregnated dust on grain, direct spray on grain, impregnated filter paper test). Anti-inflammatory activity has performed with aqueous leave extract *H. integrifolia*. % Inhibition of paw volume = (Control mean – treated mean) / Control mean×100. Ethical approval was taken prior the study.

**Results and discussion**

**Anti-inflammatory activity:** Aqueous extract of *H. integrifolia* has significant anti-inflammatory activity. Active constituents of plants play a major role for the discovery of new pharmaceutical products for the treatment of various ailments due to its efficacy and safety. Inflammatory diseases are one of the most common causes of different health disorders. Anti-inflammatory studies have been conducted using aqueous extract of the leaves of *H. integrifolia* and outcomes were analyzed in comparison with standard anti-inflammatory drug diclofenac sodium. Paw edema was calculated at different time interval using plethysmometer (Table 2). New bould method was used to evaluate percentage inhibition of paw edema (Table 3). The statistically significant results has been produced in group that is treated with aqueous extract of the leaves of *H. integrifolia* after 3 hours in comparison to group that treated with diclofenac sodium that shown % of inhibition after 4 hours. The significant (*P*< 0.05) anti-inflammatory activity of aqueous extract of the leaves of *H. integrifolia* markedly reduced the paw edema in comparison to standard drug i.e. Diclofenac sodium against acetic acid induced paw edema in albino rats at a dose of 250mg/kg /body weight and outcomes have been mentioned in Fig. 1 and 2 (Table 1).

**Table 1: Anti-inflammatory Activity of the Aqueous Extract of *H. integrifolia***

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean increase in Paw volume with SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hr</td>
</tr>
<tr>
<td>(NC)</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>2.90±0.19</td>
</tr>
<tr>
<td>(AA)</td>
<td>2.79±0.19</td>
</tr>
<tr>
<td>(DS)</td>
<td>2.52±0.12</td>
</tr>
<tr>
<td>(AEH)</td>
<td>2.38±0.04</td>
</tr>
</tbody>
</table>

(Where: NC= Normal control; AA= Acetic acid (positive control); DS= Diclofenac sodium, AEH= Aqueous extract of *Holoptelea integrifolia* (Roxb) Planch.; * = significant value *P* values < 0.05 as compared with acetic acid (positive control))

The data were subjected to statistical analysis using one-way analysis of variance (ANOVA). *P* values < 0.05 were considered significant.

**Percentage Inhibition of Paw edema (on 5th day):**

<table>
<thead>
<tr>
<th>Table 2: Percentage Inhibition of Paw Edema.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage inhibition (hr)</td>
</tr>
</tbody>
</table>
Toxicity Studies

Brine Shrimps Bioassay (LD₅₀): Beside the therapeutic activity of plants and their constituents, some of these are detrimental effects to humans and other living organisms. Shrimp larvae (Artemiasolina) are susceptible to various harmful constituents and are used to evaluate the toxicity. Different extracts (EtOH, EtOAC, BuOH and H₂O) of the plant H.integrifolia were subjected for the determination of LD₅₀ activity using brine-shrimps (Artemiasolani). Brine shrimp bioassay was conducted in triplicate in three different concentrations i.e. 10, 100, 1000 µg/ml of EtOH, EtOAC, BuOH and H₂O extracts of H.integrifolia and Etoposide was selected as control (Table 3,4,5 and 6). Results revealed positive lethality at high dose with LD₅₀ 629.5723 and 100,000 µg/ml in BuOH and H₂O extract respectively and compared with the standard drug Etoposide at high concentration dose³(Finney, 1971). While EtOH and EtOAC extracts were not showed such toxicity against brine shrimps although at high concentration.

Table 3: Brine shrimp toxicity bioassay of EtOH extract of H. integrifolia

<table>
<thead>
<tr>
<th>Dose µg/ml</th>
<th>No. of Shrimps</th>
<th>No. of Survivors</th>
<th>% of Survivors</th>
<th>LD₅₀ µg/ml</th>
<th>Std. Drug</th>
<th>LD₀ µg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>30</td>
<td>1 2 3 Av.</td>
<td>70%</td>
<td>-</td>
<td>Etoposide</td>
<td>7.4625</td>
</tr>
<tr>
<td>100</td>
<td>30</td>
<td>26</td>
<td>84%</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>26</td>
<td>90%</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Brine shrimp toxicity bioassay of EtOAC extract of H. integrifolia

<table>
<thead>
<tr>
<th>Dose µg/ml</th>
<th>No. of Shrimps</th>
<th>No. of Survivors</th>
<th>% of Survivors</th>
<th>LD₅₀ µg/ml</th>
<th>Std. Drug</th>
<th>LD₀ µg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>30</td>
<td>26</td>
<td>90%</td>
<td>-</td>
<td>Etoposide</td>
<td>7.4625</td>
</tr>
<tr>
<td>100</td>
<td>30</td>
<td>26</td>
<td>84%</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>26</td>
<td>90%</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Brine shrimp toxicity bioassay of n-BuOH extract of H. integrifolia (Roxb) Planch.

<table>
<thead>
<tr>
<th>Dose µg/ml</th>
<th>No. of Shrimps</th>
<th>No. of Survivors</th>
<th>% of Survivors</th>
<th>LD₅₀ µg/ml</th>
<th>Std. Drug</th>
<th>LD₀ µg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>30</td>
<td>13</td>
<td>47%</td>
<td>629.5726</td>
<td>Etoposide</td>
<td>7.4625</td>
</tr>
<tr>
<td>100</td>
<td>30</td>
<td>19</td>
<td>50%</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>19</td>
<td>63%</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Comparative phyto toxicological and anti inflammatory effects of leaves extracts of *holoptelea integrifolia*

Table 6: Brine shrimp toxicity bioassay of H$_2$O extract of *H. integrifolia* (Roxb) Planch.

<table>
<thead>
<tr>
<th>Dose µg/ml</th>
<th>No. of Shrimps</th>
<th>No. of Survivors</th>
<th>% of Survivors</th>
<th>LD$_{50}$ µg/ml</th>
<th>Std. Drug µg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>30</td>
<td>11</td>
<td>10 12 11</td>
<td>37%</td>
<td>100.00</td>
</tr>
<tr>
<td>100</td>
<td>30</td>
<td>15</td>
<td>14 16 15</td>
<td>50%</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>19</td>
<td>18 20 19</td>
<td>63%</td>
<td>-</td>
</tr>
</tbody>
</table>

**Phytotoxic activity or Lemna bioassay:** Growth inhibition and promotion of plants has been screened by Lemna bioassay and results for phytotoxicity of the crude extracts i.e. EtOH, EtOAC and BuOH has been expressed (Table 7,8, 9 and 10). In this study, level of toxicity (0.015 µg/m) was determined against *Lemna minor* using Paraquat as standard drug. All of these crude extracts exhibited limited phytotoxicity i.e. 46.3 µg/ml in high concentrations (1000 µg/ml) while H$_2$O extract was displayed no phytotoxicity at any concentration.

New herbicidal agents are now demanding to discover due to increasing in herbicide resistant weeds and alteration of synthetic herbicides are limitedly acceptable and effective against the resistant weed biotypes with reference to environmental and health related issues. Plants play a significant role to discover new herbicides which might be more convenient, safe, effective and biodegradable, so have fewer threat to the environment and used as substituent to the presently used synthetic agrochemicals. Different plants material, their extracts and / or their isolated active compounds may use as allelochemicals to other plants and be utilized for cultivation. The results obtained from this study showed that *H. integrifolia* leaves extracts of EtOH, EtOAC and BuOH could be useful as natural herbicides in comparison with other related herbal species at high concentration and could be deliberated as a source of bioactive agrochemical.

Table 7: Phytotoxic activity of EtOH extract of *H. integrifolia*

<table>
<thead>
<tr>
<th>Name of Plant</th>
<th>Conc. of Sample µg/ml</th>
<th>No. of Fronds Survived</th>
<th>% of Growth Regulation</th>
<th>Conc. of St. Drug µg/ml Paraquat</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lemna minor</em></td>
<td>1000</td>
<td>10</td>
<td>46.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>12</td>
<td>35.5</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>17</td>
<td>8.7</td>
<td></td>
</tr>
</tbody>
</table>

Table 8. Phytotoxic activity of EtOAC extract of *H. integrifolia*.

<table>
<thead>
<tr>
<th>Name of Plant</th>
<th>Conc. of Sample µg/ml</th>
<th>No. of Fronds Survived</th>
<th>% of Growth Regulation</th>
<th>Conc. of Standard Drug µg/ml Paraquat</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lemna minor</em></td>
<td>1000</td>
<td>10</td>
<td>46.3</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>15</td>
<td>19.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>18</td>
<td>3.3</td>
<td></td>
</tr>
</tbody>
</table>

Number of Fronds in control = 40.

Table 9: Phytotoxic activity of n-BuOH extract of *H. integrifolia*

<table>
<thead>
<tr>
<th>Name of Plant</th>
<th>Conc. of Sample µg/ml</th>
<th>No. of Fronds Survived</th>
<th>% of Growth Regulation</th>
<th>Conc. of Standard Drug µg/ml Paraquat</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lemna minor</em></td>
<td>1000</td>
<td>10</td>
<td>46.3</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>14</td>
<td>24.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>18</td>
<td>3.3</td>
<td></td>
</tr>
</tbody>
</table>

215
Table 10: Phytotoxic activity of H$_2$O extract of H. integrifolia

<table>
<thead>
<tr>
<th>Name of Plant</th>
<th>Conc. of Sample µg/ml</th>
<th>No. of Fronds Survived</th>
<th>% of Growth Regulation</th>
<th>Conc. of St. Drug µg/ml Paraquat</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lemna minor</em></td>
<td>1000</td>
<td>15</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>18</td>
<td>3.3</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>19</td>
<td>-2</td>
<td></td>
</tr>
</tbody>
</table>

Insecticide Activity: The extracts of *H. integrifolia* (EtOH, EtOAC, BuOH and H$_2$O) are used to conduct insecticidal activity against *Tribolium castaneum*, *Sitophilus oryzae*, *Rhyzopertha dominica*, *Callosbruchus analis*, and *Trogoderma granarium*. All test were carried out in control condition compared with standard drug i.e., Permethrin (Table 11). All four extracts were selected for insecticidal activity by contact method on three stored grain pests i.e. *Tribolium castaneum*, *Rhyzopertha dominica*, and *Callosobruchus analis*. Sample concentration was taken 1019.10 µg/cm$^2$. Permethrin was employed as standard sample with 235.9 µg/cm$^2$ concentration and experiment were performed with sample in 1019.10 µg/cm$^2$ concentration. Some activity against *Rhyzoperthadominica* was observed in EtOH extract, while major activity was shown against *Callosobruchus analis*. EtOAC extract was found to be only moderately activated against C. analis and significant activity was examined in BuOH extract against *Rhyzoperthadominica*; while in similar concentration of both extracts other pests were found ineffective. Aqueous extract not displayed effect against all pests or no insecticidal activity was observed (Table 11).

Table 11: Insecticidal activity of different extracts *H. integrifolia*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of insects</th>
<th>% Mortality</th>
<th>Sample</th>
<th>Sample</th>
<th>Sample</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>+ve control</td>
<td>-ve control</td>
<td>EtOH</td>
<td>EtOAC</td>
</tr>
<tr>
<td>1</td>
<td><em>Tribolium castaneum</em></td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td><em>Sitophilus oryzae</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td><em>Rhyzopertha dominica</em></td>
<td>100</td>
<td>40</td>
<td>0</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td><em>Callosobruchus analis</em></td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Concentration of test sample = 1572.7 µg/cm$^2$, Concentration of standard drug = 235.9 µg/cm$^2$, +ve control = Permethrin (Copex) standard drug, -ve control = Solvent.

Enzyme inhibition activity: The results showed that crude extracts of *H. integrifolia* (EtOH and H$_2$O) possessed very weak urease inhibiting activities while EtOAC and BuOH extracts of plant were found ineffective (Table 12).

Table 12. In-vitro urease inhibition activity of different crude extracts of *H. integrifolia*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Sample Name</th>
<th>% Inhibition</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Crude extract (EtOH)</td>
<td>10%</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl acetate (EtOAC)</td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>n-Butanolic fraction (BuOH)</td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Aqueous fraction (H$_2$O)</td>
<td>11%</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Standard drug Thio urea</td>
<td>94%</td>
<td>+++</td>
</tr>
</tbody>
</table>

(-No activity, + Low activity, ++ Moderate, +++ High)

This urea was used as control to perform the enzyme inhibition activity with the leaves extracts of *H. integrifolia* against Jack bean. For the discovery of new antiulcer drugs urease inhibitors have shown effectiveness nowadays 37. Activity of urease has employed majorly as virulence determinant in the pathogenesis of various diseases in human being and animal health and for agriculture 38,39. So far, designed based on urease inhibition is studied as tool first time, for evaluation of infections originated by using urease producing bacteria. This result indicated that EtOAC and BuOH extracts of this plant showed no activity while EtOH and H$_2$O exhibited mild inhibiting activity.
**Conclusion**

This aqueous extract of *Holoptelea integrifolia* has shown potent anti-inflammatory activity without any liver and kidney damage. This study showed that *H. integrifolia* leaves extracts of EtOH, EtOAC and BuOH could be useful as natural herbicides in comparison with other related herbal species at high concentration and could be deliberated as a source of bioactive agrochemical.

**References**