Phytochemical Analysis and in vitro Anti-Inflammatory and Anti-Bacterial Activities Curcuma Pseudomontana J.Graham

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Abstract

Aim: The present study is aimed to evaluate the Phytochemical analysis and In-vitro Anti-inflammatory and Anti-bacterial activity of Curcuma pseudomontana J.Graham. Materials and methods: In the present investigation, the rhizomes of curcuma pseudomontana powder was extracted by successive soxhlation extraction method with ethyl acetate and methanol. The ethyl acetate extracts responded positively to all the tests for carbohydrates and also to the tests for flavonoids and glycosides and methanolic extracts produced positive test for the presence of carbohydrates, proteins, steroids, flavonoid glycosides, tannins and phenol compounds. The extracts were used for testing the in-vitro anti-inflammatory activity by using albumin denaturation assay, proteinase inhibitory activity at a concentration of 20, 40, 60, 80 and 100mg/ml and Anti-bacterial activity against two gram positive microorganisms (Bacillus subtilis and Staphylococcus aureus) and two gram negative microorganisms (Salmonella typhi and Escherichia coli) at concentrations 100 mg/ml and 200 mg/ml by adopting cup plate method.

Results: The extracts exhibited significant in-vitro anti-inflammatory effect and inhibited the growth of both Gram positive and Gram-negative microorganisms at 100 mg/ml and 200 mg/ml concentrations. Conclusion: The findings of this study showed that the effectiveness of methanol extract shows more Anti-inflammatory and Anti-bacterial activity compared to ethyl acetate extract. Because of methanol extract contains more bioactive compounds comparatively then ethyl acetate extract and bioactive components justifying its traditional use.

Keywords
Curcuma pseudomontana, Anti-inflammatory activity, Anti-bacterial activity, Phytochemical analysis.

INTRODUCTION
Curcuma pseudomontana J. Graham is an extremely rare Zingiberaceae species found so far only in the Naikongchhari forest area of Bandarban district in the southeastern hilly area of Bangladesh1. C. pseudomontana is endemic to the Western and Eastern Ghats, of peninsular India, the species found in Karnataka, Maharashtra and Andhra Pradesh in English it is known as hill turmeric2. Dried rhizomes of C. pseudomontana J. Grahm., used in skin diseases and impurities of blood3. Rhizomes boiled in oil and used as an application to sprain and useful on snake bite4. Rhizome powder are useful in leucoderma, scabies, smallpox, and intestinal worms as well as
juice strong remedy against rheumatism and in combination of ginger used for smooth delivery in North East India\^5. Boiled tubers along with a pinch of salt in oral administration increase the secretion of milk among new mothers and lactating woman in Andhra Pradesh\^6, The Bagata and Valmiki tribes of Munchingiputtu Mandal, Visakhapatnam district, Andhra Pradesh use C. _pseudomontana_ rhizome in the treatment of jaundice and diabetes 7. The rhizome are used for skin problems and coughs by the tribals of Achampet Forest Division in Nallamalais, Telengana, India\^8. The Kattunaikan tribe of Malappuram district in Kerala, India, uses the rhizomes for cardiac disorders\^9. The rhizomes are used for muscle pain, leprosy and debility by tribal communities residing in Gundlabrahmeswaram Wildlife Sanctuary (Eastern Ghats), Andhra Pradesh, India\^10.

**RESULTS**

**In- vitro Anti-inflammatory**

**Inhibition of albumin denaturation**

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen’s egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of the extract was added to reach final concentrations (20, 40, 60, 80, and 100 mg/ml). Similar volume of double distilled water served as control. Then the mixtures were incubated at 37±2ºC in an incubator for 15 minutes and then heated at 70ºC for 5 minutes. After cooling down, their absorbance was measured at 660 nm using vehicle as blank. The Diclofenac sodium at the final concentration of (20, 40, 60, 80, and 100 mg/ml) was used as reference drug and treated similarly for determination of absorbance\^21.

**Anti-proteinase action**

The reaction mixture (2 ml) include 0.06 mg trypsin, 1 ml 20Mm Tri HCL buffer (pH 7.4) and 1 ml test sample of different concentrations (20-100 µl/ml). The mixture was incubated at 37ºC for 5 minutes and then 1 ml of 0.8% casein was added. The mixture was incubated for an additional 20 minutes. 2ml of 70% perchloric acid was added to arrest the reaction. The cloudy suspension was centrifuged, and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory was calculated\^22.

**In vitro Anti-bacterial activity**

**Test organisms**

Two strains of gram-negative bacteria _Escherichia coli_, _Klebsiella pneumoniae_, and two strains of gram-positive bacteria _Bacillus subtilis_ and _Staphylococcus aureus_ were used in our experiment to evaluate the Anti-bacterial activity.

**Disc diffusion Method**

Disc diffusion method for antimicrobial susceptibility testing was carried out to assess the presence of antibacterial activities of the ethyl acetate and methanol extract\^9,10.

**RESULTS**

**In- vitro Anti-inflammatory activity effect of Curcuma pseudomontana on heat induced protein denaturation**

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Diclofenac sodium (standard)</th>
<th>Ethyl acetate extract of <em>Curcuma pseudomontana</em></th>
<th>Methanol extract of <em>Curcuma pseudomontana</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>58.24±3.12</td>
<td>33.14±3.16</td>
<td>35.48±5.18</td>
</tr>
<tr>
<td>40</td>
<td>65.32±1.24</td>
<td>35.18±2.35</td>
<td>38.74±3.24</td>
</tr>
<tr>
<td>60</td>
<td>72.40±2.24</td>
<td>48.90±2.04</td>
<td>59.20±2.42</td>
</tr>
</tbody>
</table>
Each value represents the mean ± SD. N=3, Experimental group were compared with control **p <0.01 considered extremely significant; *p < 0.05, non-significant.

In-vitro anti-inflammatory activity Curcuma psedomontana on heat induced protein denaturation

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Diclofenac sodium (standard)</th>
<th>Ethyl acetate extract of Curcuma psedomontana</th>
<th>Methanol extract of Curcuma psedomontana</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>60.25±3.64</td>
<td>34.44±2.52</td>
<td>35.98±.84</td>
</tr>
<tr>
<td>40</td>
<td>70.02±4.62</td>
<td>22.4±1.48</td>
<td>26.5±2.88</td>
</tr>
<tr>
<td>60</td>
<td>71.49±2.54</td>
<td>31.6±3.62</td>
<td>33.0±2.84</td>
</tr>
<tr>
<td>80</td>
<td>76.35±4.12</td>
<td>42.2±2.84</td>
<td>48.1±2.60</td>
</tr>
<tr>
<td>100</td>
<td>84.10±2.20</td>
<td>54.6±1.84</td>
<td>58.8±2.64</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD. N=3, Experimental group were compared with control **p <0.01 considered extremely significant; *p < 0.05, non-significant.

STATISTICAL ANALYSIS
Results are expressed as Mean ± SD. The difference between experimental groups was compared by One Way Analysis of Variance (ANOVA) followed by Dunnet Multiple comparison test (control Vs test).

In-vitro anti-bacterial activity of Curcuma psedomontana

<table>
<thead>
<tr>
<th>Name of organism</th>
<th>Zone of inhibition (mm)</th>
<th>Streptomycin</th>
<th>Ethyl acetate extract of Curcuma psedomontana</th>
<th>Methanol extract of Curcuma psedomontana</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100µg/ml</td>
<td>200µg/ml</td>
<td>100mg/ml</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
<td>10.8±0.22</td>
<td>12.2±0.27</td>
<td>6.6±0.24</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td></td>
<td>9.5±0.4</td>
<td>11.2±0.12</td>
<td>6.1±0.24</td>
</tr>
<tr>
<td>B. subtilis</td>
<td></td>
<td>10.4±0.32</td>
<td>12.6±0.12</td>
<td>5.6±0.12</td>
</tr>
<tr>
<td>S. aureus</td>
<td></td>
<td>10.6±0.74</td>
<td>11.8±0.44</td>
<td>5.5±0.22</td>
</tr>
</tbody>
</table>

ZI were expressed as mean± standard deviation of three replicates.
Low activity (1-6 mm), moderate activity (7-10mm), high activity (11-15 mm).
Represents mean ± S.D. mm; p < 0.05.

CONCLUSION
The above results of preliminary phytochemical analysis and anti-inflammatory and anti-bacterial activity of Curcuma psedomontana rhizomes extracts confirmed as a useful Anti-inflammatory and Antimicrobial agent. The present study provides evidence that Curcuma psedomontana rhizomes extracts contains medicinally important bioactive compounds like carbohydrates, proteins, alkaloids, glycosides, flavonoids and phenol compounds and this justifies the use of plant species as traditional medicine for treatment of inflammation and bacterial infections.

REFERENCES


