ABSTRACT
The present study was performed with the objective of dose determination and evaluation of Anti-inflammatory and Analgesic activity of Ethanolic extracts of Inularacemosa root and Albizia amara. The dose determination studies were carried out according to OECD guidelines and the safe dose determined was 2000 mg/kg. Hence, 200 mg/kg was given for the models for anti-inflammatory (carrageenan induced paw edema) and analgesic (hot plate) activity. The percentage reduction in paw volume observed against Carrageenan induced paw oedema for Inula was found to be 34% whereas in Albizia it was 15%. In hot plate method, the percentage inhibition was 42% and 61.91% respectively. The data were found statistically significant by using one way ANOVA (P< 0.05). Thus, both the Extracts were able to show anti-inflammatory & analgesic activity as compared with standard drug Aspirin 100 mg/kg. But the effect of Albiziaamara extract was more statistically significant compared to extracts of Inularacemosa.

Keywords: anti-inflammatory, analgesic, Inularacemosa root, Albiziaamara.

INTRODUCTION
Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants, and the treatment that reduces this inflammation is called as anti-inflammatory. Currently there are huge variety of anti-inflammatory drugs are available in the market. These anti-inflammatory drugs are associated with some or the other side effects. Therefore, the development of potent anti-inflammatory drugs with fewer side effects is necessary. Alternatively, we can use the herbal plant extracts for the treatment of inflammation, because these herbal plants shows the better activity and lesser side effect. Inularacemosa (Pushkamoola) root is available as cylindrical, straight; surface rough due to longitudinal striations and cracks, scars of lateral rootlets and rhytidoma present, externally brownish-grey and internally yellowish-brown. The roots are used for the various treatments like angina pectoris, ischemic heart disease, decreases cholesterol, anti bacterial, Asthma, bronchial disease, dyspnea. Albiziaamara grows throughout southern India and in some parts of Madhya Pradesh. This plant is a small to moderate-sized, much-branched deciduous tree with smooth, dark green, scaly bark. It resembles the acacias but lacks thorns. Leaf and flowers of this plant shows anti-inflammatory activity usually used for boils and ulcers. Leaves are used for erysipelas. Seeds showastringent, anti diarrhoeal and antibacterial effect whereas the extract showed DNA binding activity, antibacterial, and they inhibit platelet aggregation and human lymphocyte transformation. They also show anti-inflammatory and cytotoxic activity. The oil from the seeds is said to cure leprosy and leucoderma. No scientific report is available to date to validate these folkloric uses, so, we now report the anti-inflammatory and analgesic activities of Ethanolic extract of Inularacemosa and Albiziaamara.

MATERIALS AND METHODS
Plant material
Roots of Inula were purchased from local market in Aurangabad and Authenticated at the Agharkar Research centre, Pune. A voucher specimen of the root of Inularacemosa was deposited at the Agharkar Research centre, Pune. Plant of Albiziaamara were collected from local market of Shirpur and Authenticated at Jai Hind Education Trust’s, ZulalBhilajiraoPatil College Dhule, Extraction of both the material was done at School of Pharmacy and Technology Management, NMiMs, Shirpur, Dhule.

Drugs and chemicals -
Aspirin was obtained as a gift sample from the Cadila pharmaceuticals Ltd., Gujarat. Carrageenan and Carboxy methyl cellulose was purchased from SD-Fine chem, Mumbai and CDH Lab, Mumbai respectively. Ethanol was used as solvent for extraction, and was obtained from Rankem, Mumbai.

Processing of the plant material and extraction -
The roots & the plant were separately dried in shade and were grounded to get a coarse powder and subjected to Soxhlet extraction using ethanol. Dried and powdered

*Author for Correspondence
Email: parikhrima1@gmail.com
cages with not more than six animals per cage and (110–180 g). The animals were obtained from the Animal Inularacemosa & Albiziaamara. The qualitative chemical tests of Ethanolic extracts of Preliminary phytochemical investigation Committee (IAEC). The experiments were performed as Institutional Animal Ethical and protocols used in this study were approved by the

Animals were placed in a Soxhlet apparatus, submerged with 95% ethanol and kept overnight at 50°C for 20 h. The percolate was collected and filtered. Ethanol was distilled off using Rota-evaporator under reduced pressure at 50°C. The final drying was done initially in vacuum desiccators. The yield of different extracts was calculated. The powder extracts obtained were then subjected to phytochemical analysis to detect the chemical constituents present in each extracts.

**Animals**

Studies were carried out using female Wistar albino rats (110–180 g). The animals were obtained from the Animal house of SPTM, grouped and housed in polyethylene cages with not more than six animals per cage and maintained under standard conditions with 12 hr natural light and dark cycle. They were fed with standard pellet diet and water ad libitum. All the experimental process and protocols used in this study were approved by the Institutional Animal Ethical Committee (IAEC). The experiments were performed as per the norms of CPCSEA.

**Preliminary phytochemical investigation**

The qualitative chemical tests of Ethanolic extracts of Inularacemosa & Albiziaamara was carried out using standard procedure.29

**Acute Oral Toxicity**

Acute oral toxicity studies were performed for Ethanolic extracts of Inularacemosa & Albiziaamara according to the toxic class method 423 as per OECD guidelines. Firstly the dose of 2000mg/kg dose was given and studies were carried out. Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. Additional observations will be necessary if the animals continue to display signs of toxicity10.

**Pharmacological Screening**

After the successful determination of the dose, the animals were subjected for Anti-inflammatory and analgesic activity. For the same, animals were divided into 5 groups containing 6 animals each. The groups are as:

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>No. of animals</th>
<th>Mortality Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 2000mg/kg.</td>
<td>03</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>2. 2000mg/kg A.</td>
<td>03</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Group</th>
<th>No. of animals</th>
<th>No. of animals</th>
<th>Mortality Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>03</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>2.</td>
<td>Test – I</td>
<td>03</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 min</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.94 ± 3.28</td>
<td>6.03 ± 1.89</td>
<td>6.14 ± 1.7</td>
<td>7.00 ± 3.42</td>
<td>6.0 ± 2.21</td>
</tr>
<tr>
<td>Standard</td>
<td>6.01 ± 1.74</td>
<td>34.16 ± 3.14</td>
<td>55.13 ± 1.95</td>
<td>62.12 ± 1.81</td>
<td>65.47 ± 1.92</td>
</tr>
<tr>
<td>Test-I</td>
<td>6.76 ± 2.32</td>
<td>17.03 ± 3.60</td>
<td>21.51 ± 3.66</td>
<td>33.08 ± 2.26</td>
<td>42.99 ± 1.95</td>
</tr>
<tr>
<td>Test-II</td>
<td>3.76 ± 1.34</td>
<td>28.83 ± 5.04</td>
<td>50.58 ± 2.68</td>
<td>56.7 ± 1.97</td>
<td>61.91 ± 1.9</td>
</tr>
<tr>
<td>Test-III</td>
<td>8.99 ± 2.35</td>
<td>20.07 ± 2.50</td>
<td>37.52 ± 3.13</td>
<td>38.77 ± 6.99</td>
<td>47.35 ± 9.42</td>
</tr>
</tbody>
</table>

Results were determined as the percentage inhibition by comparing with the control. Percentage inhibition of

<p>| Table 1: Phytochemical estimations on Ethanolic extract of Lraceroma &amp; A.amara |
|----------------|-------------------------------|</p>
<table>
<thead>
<tr>
<th>Chemical constituent</th>
<th>Inularacemosa</th>
<th>Albiziaamara</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>Glycoside</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>Essential Oil</td>
<td>+ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>Steroids</td>
<td>+ ve</td>
<td>- ve</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Amino Acids</td>
<td>+ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

+ve sign indicates Presence and –ve sign indicate Absence of compound.

**Table 2: Mortality Data of acute oral toxicity studies on Ethanolic extracts of Lraceroma and A. amara**
latency time between treated and control group was calculated as follows:

\[
\text{Percentage inhibition} = \left(\frac{V_c - V_t}{V_c}\right) \times 100
\]

Where, \(V_c\) and \(V_t\) represent mean increase in latency time in control and treated groups respectively.

**Statistical analysis**

The data are reported as mean ± standard error of the mean (SEM) and were compared using one way analysis of variance (ANOVA), followed by the Dunnett’s multiple comparison test using Graph Pad PRISM software, and \(p\)-values < 0.05 was considered significant.

**Pharmacological Screening**

**Analgesic Activity (Hot Plate Method)** - Analgesic studies were determined by using hot plate method. The analgesic studies of both the ethanolic extract and the combination of both the extract has been carried out at dose level of 200 mg/kg body wt. The dose of both extracts has shown significant analgesic activity (\(P<0.05\)) when compared with standard drug aspirin. Among the 3 test groups, the Ethanolic extract of Albizia amara has significantly higher activity compared to other groups. The percentage inhibition of I. racmosa and A. amara are 42.99 and 61.91 respectively. Table 3 shows the details of the analgesic activity as shown by the extracts.

**Anti-inflammatory Activity** - Anti-inflammatory activity was carried out by Carrageenan induced rat paw oedema method. The anti-inflammatory studies of both the ethanolic extracts individually and the combination of both the extracts has been carried out by Carrageenan induced rat paw oedema method at dose level of 200 mg/kg body wt. The dose of both extracts has shown significant analgesic activity \((P<0.05)\) when compared with standard drug aspirin. Among the 3 test groups, the Ethanolic extract of Albizia amara has significantly higher activity compared to other groups. The percentage reduction in

**Table 4:** Effect of Ethanolic extracts on Carrageenan Induce Paw Oedema (values are expressed in volume and they are shown in Mean ± SEM, \(n=6\))

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 min</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
<th>300 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.5 ± 1.70</td>
<td>7.5 ± 1.70</td>
<td>14.17 ± 2.02</td>
<td>19.17 ± 2.0</td>
<td>40.83 ± 10.83</td>
<td>47.5 ± 7.04</td>
<td>45.83 ± 7.12</td>
<td>40.83 ± 3.27</td>
</tr>
<tr>
<td>Standard</td>
<td>24.14 ± 2.38</td>
<td>32.5 ± 1.11</td>
<td>25.83 ± 1.53</td>
<td>25.67 ± 0.95</td>
<td>17.5 ± 1.70</td>
<td>12.5 ± 1.70</td>
<td>8.33 ± 1.05</td>
<td>4.16 ± 0.83</td>
</tr>
<tr>
<td>Test-I</td>
<td>10 ± 1.82</td>
<td>19.17 ± 2.713</td>
<td>23.33 ± 3.33</td>
<td>26.67 ± 1.66</td>
<td>34.17 ± 6.76</td>
<td>28.38 ± 3.65</td>
<td>21.67 ± 3.5</td>
<td>17.5 ± 2.5</td>
</tr>
<tr>
<td>Test-II</td>
<td>18.33 ± 2.47</td>
<td>31.16 ± 3.08</td>
<td>37.5 ± 5.73</td>
<td>33.17 ± 1.04</td>
<td>15 ± 4.47</td>
<td>12.5 ± 3.81</td>
<td>6.66 ± 3.03</td>
<td>5 ± 2.23</td>
</tr>
<tr>
<td>Test-III</td>
<td>8.33 ± 1.66</td>
<td>27.5 ± 2.14</td>
<td>33.33 ± 1.66</td>
<td>36.64 ± 2.108</td>
<td>21.67 ± 1.2</td>
<td>27.5 ± 4.03</td>
<td>19.17 ± 3.0</td>
<td>13.33 ± 4.04</td>
</tr>
</tbody>
</table>
paw volume observed against Carrageenan induced paw oedema for Inula was found to be 34% whereas in Albizia it was 15%. Table 4 depicts the results of Anti-inflammatory studies as studied by Carragenan induced paw oedema in rats.

RESULTS AND DISCUSSION

Preliminary phytochemical investigation - The preliminary phytochemical investigation of Ethanolic extracts of Inularacemosa and Albiziaamara are as shown in Table 1.

Acute Oral toxicity

Both the Ethanolic extracts were found to be safe at MTD >2000mg/kg as observed according to the OECD 423 guidelines. The data of the test is as shown in Table 2.

MTD>2000mg/kg, drug was found to be safe and nontoxic, no mortality occurs. The extract was found to be safe and nontoxic, no behavioral changes were observed. Hence the drug can be considered as safe at 2000mg/kg.

Thus, the final dose selected was 200mg/kg. (ED₅₀)

DISCUSSIONS

After successful Ethanolic extraction, the Percentage yield found for Inularacemosaa and Albiziaamara are 11.5% w/w and 6.72% respectively. The phytochemical studies performed in the present study confirmed that the extract of Inularacemosa and Albiziaamara possess Saponins, Steroids, flavonoids, alkaloids and glycosides.

After completion of Oral acute toxicity as per OECD guideline 423, it was found that MTD found is more than 2000mg/kg and drug was found to be safe. Thus, final dose selected was 200mg/kg.

For the pharmacological screening, Anti-inflammatory and Analgesic models were studied. From Table 3, it was seen that pretreatment by Albiziaamara plant increased the response latency in the hot plate test, which was significant. On the other hand pretreatment by Inularacemosaroot extract slightly increase the response latency in the hot plate but less in comparison to the response that was produced by the Albiziaamara.

And the combination of both the extract produces the intermediate response. From Table 4, it was seen that pretreatment by Albiziaamara plant produce more reduction in paw volume, in Carrageenan induce model right from 120 min which was significant. On the other hand pretreatment by Inularacemosaroot extract produce slight reduction in paw volume but less in comparison to the response that was produced by the Albiziaamara. And the combination of both the extract produces the intermediate response.

Statistically significant anti-inflammatory & analgesic activity was shown by the Ethanolic extract of Albiziaamara in comparison to the activity shown by Inularacemosaa and combination.

Thus, further work needs to be carried for the exact mechanism responsible in Ethanolic extracts of A. amara for the anti-inflammatory & analgesic activity.

ACKNOWLEDGEMENTS

Authors are thankful to Cadila Pharmaceuticals Ltd., Ahmedabad for providing gift samples of Aspirin and SPTM, NMiMS, Shirpur for providing the necessary facilities to conduct the experiments.

REFERENCES

15. Lignans and sesquiterpene lactones from Artemisia Sieversiana and inularacemosa, to check the anti fungal activity, TANG, SHUAI, Elsevier science Ltd, Phytochemistry\ Vol[ 38\ No[ 0\ pp[ 046050]:088.
28. Harish Chandra Pal a, Irum Sehar a, Shashi Bhushan a, Bishan Gupta b, Activation...


