In vitro and In vivo Anti-inflammatory Activity of the Extracts of Whole Plant Argyreia imbricata (Roth) Sant. & Patel

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ABSTRACT

Plants of the genus Argyreia have ethnomedicinal importance, and several pharmacological activities are also reported. In this study, the anti-inflammatory activity of different extracts of Argyreia imbricata was evaluated by in vitro and in vivo methods. In both evaluations, standard, Diclofenac sodium was used for comparative evaluation. In this study, extraction of powdered whole plant material was done with different solvents viz., petroleum ether, chloroform, ethyl acetate and methanol by soxhletation. In vitro anti-inflammatory activity of all the prepared extracts was evaluated by stabilization of human red blood cell (HRBC) membrane in different temperature and tonicity conditions. Among the six different concentrations of four tested extracts, the ethyl acetate and methanol extracts (1000 g/ml) showed significant activity in the in vitro evaluation. They were selected for the in vivo evaluation on the paw oedema induced by carrageenan on Wistar albino rats. Two doses, 200mg.kg⁻¹ and 400mg.kg⁻¹ of the test extracts were subjected to evaluation. Both the tested extracts showed the activity, particularly, the methanol extract in the dose of 400mg.kg⁻¹ showed significant activity. Results of this study strongly supported the anti-inflammatory activity of the tested extracts. Further, studies on toxicity, identification, isolation of the active constituents may give useful results.
this genus is commonly found in south India at an altitude up to 300m mean sea level which has not been previously explored scientifically to our knowledge was selected for our research. In our previous effort, a preliminary phytochemical screening and the spectral characterization of active constituents present in the different dried extracts of whole plant material of *Argyreia imbricata* was done successfully (Sebastian et al., 2019). As a part of an investigation of different pharmacological activities, previously, the anti diabetic potential of the extracts was evaluated in vitro and in vivo. Now, it was focused on the evaluation of anti-inflammatory activity by in vitro and in vivo methods.

**MATERIALS AND METHODS**

**Plant materials — Collection and extraction**

The whole plant of *Argyreia imbricata* was collected from Mekkarai, the village located close to the hillocks of the Western Ghats in the Tirunelveli District of Tamil Nadu, India. After proper identification and authentication, the collected material was dried, powdered and extracted by soxhlet apparatus assembly by using the solvents of increasing polarity viz., petroleum ether, chloroform, ethyl acetate, and methanol (Sebastian et al., 2019). The dried extracts obtained were used for the experiments.

**Anti-inflammatory activity — In vitro**

It was done by stabilization of human red blood cell (HRBC) membrane with different approaches such as heat-induced and hypo-tonicity induced membrane-lysis. All the prepared extracts were subjected to in vitro assays in triplicate, and the results were expressed as mean ± standard deviation.

**HRBC membrane stabilization method**

The study was designed about the procedure of (Seema et al., 2011; Anosike et al., 2012; Chowdhury et al., 2014; Patel and Desai, 2016). Whole human blood collected freshly from the healthy volunteers was mixed with an equal volume of sterile Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid, 0.42% sodium chloride, and 100ml distilled water) and centrifuged at 3000rpm for 10min., and the packed cells obtained were washed and reconstituted with sterile isosaline (0.85% sodium chloride in water, sterilized by autoclaving) as 10%v/v suspension. HRBC suspension (1ml) and each test extracts (1ml) in different concentration (100, 200, 400, 600, 800, 1000μg/ml) was taken in individual tubes. Normal control tube contains HRBC suspension and Alsever solution only. Standard control tube contains diclofenac sodium 200μg/ml instead of test extracts. All the tubes were subjected to incubation (37°C for 30min) and then centrifugation. The supernatant was collected, and its haemoglobin content was estimated spectrophotometrically (560nm), and the percentage haemolysis and protection were calculated by

\[
\text{Percentage of haemolysis} = \frac{\text{O.D of test}}{\text{O.D of control}} \times 100
\]

\[
\text{Percentage of protection} = 100 - \frac{\text{O.D of test}}{\text{O.D of control}} \times 100
\]

**Heat-induced haemolysis**

Test extracts were dissolved in isotonic phosphate buffer solution. The reaction mixture contains test extracts (5ml) in different concentration (100, 200, 400, 600, 800, 1000μg/ml) and 10%v/v HRBC suspension (0.1ml). Normal control tube contains saline, and the standard control tube contains diclofenac sodium 200μg/ml instead of test extracts. A batch of prepared tubes was kept at 54°C for 20min, in a regulated water bath. Another batch tube was kept at −10°C for 20min in a freezer. Then, all were centrifuged at 3000rpm for 3min, the supernatant was collected, and the haemoglobin content was estimated spectrophotometrically (540nm), and from this, the percentage inhibition of haemolysis by the tests was calculated by

\[
\text{Percentage of haemolysis} = 1 - \frac{\text{O.D.1}}{\text{O.D.2}} \times 100
\]

O.D.1—Optical density of tests cooled; O.D.2—Optical density of tests heated; O.D.3—Optical density of normal control heated

**Hypotonicity induced haemolysis**

Test extracts were dissolved in hypotonic solution (0.2% NaCl) and isotonic solution (0.9% NaCl) separately. Both hypotonic and isotonic reaction mixture contains 5ml of test extracts (5ml) in different concentration (100, 200, 400, 600, 800, 1000μg/ml) and 10%v/v HRBC suspension (0.1ml). Normal control tube contains distilled water, and the standard control tube contains diclofenac sodium 200μg/ml instead of test extracts. The mixtures were kept in incubation (37°C; 1hr) and then, centrifuged (3000rpm; 3min), the supernatant was collected, and its haemoglobin content was estimated spectrophotometrically (540nm), and the percentage inhibition of haemolysis was calculated by

\[
\text{Percentage of haemolysis} = 1 - \frac{\text{O.D.1}}{\text{O.D.2}} \times 100
\]

O.D.1—Optical density of tests in isotonic solution; O.D.2—Optical density of tests in hypotonic solution;
All animal experiments were carried out as per the guidelines of a committee for control and supervision on experiments on animals (OECD 423). The selected animals were housed in suitable temperature (25±1°C), humidity (55±3%) and 12h light/dark cycle and fed with commercial diet and water ad libitum. Experimental animals were grouped into 7 with six animals of each. Group I is normal control administered with saline (5 ml.kg⁻¹). Group II is inflammatory control treated with carrageenan. Group III is standard control treated with Diclofenac sodium inj. (10 mg.kg⁻¹). Group IV and V are treatment control treated with ethyl acetate extract 200mg.kg⁻¹ and 400 mg.kg⁻¹ respectively. Group VI and VII are treatment control treated with methanol extract 200 mg.kg⁻¹ and 400 mg.kg⁻¹ respectively. 30min before the administration of Diclofenac sodium/test, extracts 1%w/v inj. Carrageenan in saline (0.1ml) was given to the subplantar region in the right hind paw of the animals of the entire group except for normal control. The standard and the test extracts were administered

Table 1: Effect of test extracts on the cell membrane protection.

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>Pet. Ether</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>02.15±0.28</td>
<td>04.69±0.53</td>
<td>07.40±0.70</td>
<td>06.13±1.26</td>
</tr>
<tr>
<td>200</td>
<td>05.92±0.50</td>
<td>08.63±0.10</td>
<td>11.05±0.40</td>
<td>18.15±0.70</td>
</tr>
<tr>
<td>400</td>
<td>09.60±0.23</td>
<td>12.45±0.36</td>
<td>29.84±0.78</td>
<td>27.26±1.25</td>
</tr>
<tr>
<td>600</td>
<td>24.54±0.45</td>
<td>30.56±1.31</td>
<td>47.50±1.61</td>
<td>39.28±0.51</td>
</tr>
<tr>
<td>800</td>
<td>37.61±0.62</td>
<td>43.88±1.20</td>
<td>60.94±0.86</td>
<td>58.37±0.88</td>
</tr>
<tr>
<td>1000</td>
<td>46.21±0.72</td>
<td>51.03±1.75</td>
<td>71.33±0.60</td>
<td>63.16±0.79</td>
</tr>
<tr>
<td>200 (Diclofenac)</td>
<td></td>
<td></td>
<td></td>
<td>68.56±1.47</td>
</tr>
</tbody>
</table>

Table 2: Effect of test extracts on the protection of cell membrane in temperature variation.

<table>
<thead>
<tr>
<th>Con.(μg/ml)</th>
<th>Heat</th>
<th>% inhibition of haemolysis</th>
<th>Cold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control Extracts</td>
<td>26.62±0.64</td>
<td>28.18±0.61</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>14.17±1.47</td>
<td>15.23±1.47</td>
<td>24.35±1.47</td>
</tr>
<tr>
<td>200</td>
<td>19.52±1.47</td>
<td>23.20±1.47</td>
<td>26.18±1.47</td>
</tr>
<tr>
<td>400</td>
<td>26.70±1.47</td>
<td>34.50±1.47</td>
<td>33.46±1.47</td>
</tr>
<tr>
<td>600</td>
<td>31.83±1.47</td>
<td>43.23±1.47</td>
<td>37.60±1.47</td>
</tr>
<tr>
<td>800</td>
<td>38.79±1.47</td>
<td>49.52±1.47</td>
<td>45.30±1.47</td>
</tr>
<tr>
<td>1000</td>
<td>44.63±1.47</td>
<td>53.40±1.47</td>
<td>54.10±1.47</td>
</tr>
<tr>
<td>200 (Std.)</td>
<td>67.83±1.26</td>
<td>66.70±0.87</td>
<td></td>
</tr>
</tbody>
</table>

Con. – Concentration; Std. – Standard (Diclofenac); 1 – Petroleum ether; 2 – Chloroform; 3 – Ethyl acetate; 4 – Methanol

O.D.3– Optical density of normal control in a hypotonic solution

**Anti-inflammatory activity– In vivo**

Based on the results of in vitro anti-inflammatory evaluation, the extracts selected were subjected to in vivo evaluation in paw oedema of the experimental animals induced by carrageenan. The evaluation was designed about the procedure of (Mohan et al., 2013; Arul and Smith, 2017; Gupta and Gupta, 2017). Healthy young adult male Albino Wistar rats (about 180–200g) used in the experiments were obtained from the Central animal house of Srinivas College of Pharmacy, Mangalore, Karnataka, India. The institutional animal ethics committee approved the study (SCP/IAEC/F150/P148/2018). All animal experiments were carried out as per the guidelines of a committee for control and super

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Percentage inhibition of inflammation was calculated by metrically and continued at the interval of one hour started to measure from zero hours by plethysmometrically except for normal and inflammatory conditions to the animals of the entire group by intraperitoneal injection except for normal and inflammatory control. The paw volume of the injected animal was started to measure from zero hours by plethysmometrically and continued at the interval of one hour for five hours. The percentage inhibition of inflammation was calculated by

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

Where, Vc–mean an increase in paw volume in a control group of rats; Vt–mean an increase in paw volume in rats treated with test extracts. The results were expressed in mean ± SEM (Standard Error Mean) 6 experimental animals in each group. ANOVA and Dunnett’s test assessed statistical significance. P-values < 0.05 were considered significant.

RESULTS AND DISCUSSION

Results of HRBC membrane stabilization assay are presented in Table 1. In this study, all the four tested extracts showed a concentration based activity; notably, the ethyl acetate extract (1000 μg/ml) showed significant activity (71.33 ± 0.60) comparing with other tested extracts and standard drug. Of course, the methanol extract also showed a significant concentration-dependent cell protection activity. Still, it was observed that its highest concentration (1000 μg/ml) showed a less percentage (63.16 ± 0.79) comparing with the ethyl acetate extract in

<table>
<thead>
<tr>
<th>Group</th>
<th>0h</th>
<th>1h</th>
<th>2h</th>
<th>3h</th>
<th>4h</th>
<th>5h</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.37±0.05</td>
<td>0.48±0.14</td>
<td>0.56±0.23</td>
<td>0.53±0.02</td>
<td>0.50±0.03</td>
<td>0.45±0.06</td>
</tr>
<tr>
<td>II</td>
<td>0.47±0.13</td>
<td>0.52±0.07</td>
<td>0.65±0.04</td>
<td>0.69±0.11</td>
<td>0.67±0.07</td>
<td>0.65±0.05</td>
</tr>
<tr>
<td>III</td>
<td>0.45±0.05</td>
<td>0.51±0.02</td>
<td>0.46±0.02</td>
<td>0.44±0.04</td>
<td>0.40±0.08</td>
<td>0.37±0.09</td>
</tr>
<tr>
<td>IV</td>
<td>0.43±0.04</td>
<td>0.50±0.02</td>
<td>0.47±0.03**</td>
<td>0.45±0.02**</td>
<td>0.43±0.05**</td>
<td>0.40±0.03**</td>
</tr>
<tr>
<td>V</td>
<td>0.44±0.03</td>
<td>0.48±0.06</td>
<td>0.45±0.07***</td>
<td>0.42±0.05***</td>
<td>0.40±0.04***</td>
<td>0.40±0.02***</td>
</tr>
<tr>
<td>VI</td>
<td>0.45±0.06</td>
<td>0.48±0.04</td>
<td>0.46±0.05***</td>
<td>0.44±0.05***</td>
<td>0.43±0.03***</td>
<td>0.41±0.02***</td>
</tr>
<tr>
<td>VII</td>
<td>0.43±0.09</td>
<td>0.46±0.11</td>
<td>0.43±0.12***</td>
<td>0.42±0.18***</td>
<td>0.39±0.15***</td>
<td>0.38±0.21***</td>
</tr>
</tbody>
</table>

Group I–Normal control; Group II–Inflammatory control; Group III–Standard control; Group IV–Ethyl acetate extract 200mg.kg⁻¹; Group V–Ethyl acetate extract 400mg.kg⁻¹; Group VI–Methanol extract 200mg.kg⁻¹; Group VII–Methanol extract 400mg.kg⁻¹. All values are expressed as mean ± SEM for 6 animals in each group; *P<0.05, **P<0.01 compared.
showed significant activity (70.10\% inhibition) in the heated condition, but, the same concentration of the ethyl acetate extract showed very significant inhibition (71.27\%) in a heated condition, but, the same concentration of the methanol extract showed very significant inhibition (77.29\%) in cold condition. Diclofenac sodium (200 \(\mu\)g/ml) exhibited 67.83\% inhibition and 66.70\% inhibition in heated and cold condition, respectively. The results indicated that the ethyl acetate and methanol extracts showed a significant activity comparing with other extracts in the different temperature conditions of the experiment. Stabilization of HRBC under different tonicity was presented as the percentage inhibition of haemolysis in Table 2. In both the conditions, the extracts showed a concentration depended on the rise of activity. The results showed that the ethyl acetate extract (1000 \(\mu\)g/ml) showed a very significant inhibition (71.27\% inhibition) in a heated condition, but, the same concentration of methanol extract showed very significant inhibition (77.29\% inhibition) in cold condition. Diclofenac sodium (200 \(\mu\)g/ml) exhibited 67.83\% inhibition and 66.70\% inhibition in heated and cold condition, respectively. The results indicated that the ethyl acetate and methanol extracts showed a significant activity comparing with other extracts in the different temperature conditions of the experiment.

Stabilization of HRBC under different tonicity was presented as the percentage inhibition of haemolysis in Table 2. In both the conditions, the extracts showed a concentration depended on the rise of activity. The results showed that the ethyl acetate extract (1000 \(\mu\)g/ml) showed a very significant inhibition (71.27\% inhibition) in a heated condition, but, the same concentration of methanol extract showed very significant inhibition (77.29\% inhibition) in cold condition. Diclofenac sodium (200 \(\mu\)g/ml) exhibited 67.83\% inhibition and 66.70\% inhibition in heated and cold condition, respectively. The results indicated that the ethyl acetate and methanol extracts showed a significant activity comparing with other extracts in the different temperature conditions of the experiment. Stabilization of HRBC under different tonicity was presented as the percentage inhibition of haemolysis in Table 2. In both the conditions, the extracts showed a concentration depended on the rise of activity. The results showed that the ethyl acetate extract (1000 \(\mu\)g/ml) showed a very significant inhibition (71.27\% inhibition) in a heated condition, but, the same concentration of methanol extract showed very significant inhibition (77.29\% inhibition) in cold condition. Diclofenac sodium (200 \(\mu\)g/ml) exhibited 67.83\% inhibition and 66.70\% inhibition in heated and cold condition, respectively. The results indicated that the ethyl acetate and methanol extracts showed a significant activity comparing with other extracts in the different temperature conditions of the experiment. Stabilization of HRBC under different tonicity was presented as the percentage inhibition of haemolysis in Table 2. In both the conditions, the extracts showed a concentration depended on the rise of activity. The results showed that the ethyl acetate extract (1000 \(\mu\)g/ml) showed a very significant inhibition (71.27\% inhibition) in a heated condition, but, the same concentration of methanol extract showed very significant inhibition (77.29\% inhibition) in cold condition. Diclofenac sodium (200 \(\mu\)g/ml) exhibited 67.83\% inhibition and 66.70\% inhibition in heated and cold condition, respectively. The results indicated that the ethyl acetate and methanol extracts showed a significant activity comparing with other extracts in the different temperature conditions of the experiment. Stabilization of HRBC under different tonicity was presented as the percentage inhibition of haemolysis in Table 2. In both the conditions, the extracts showed a concentration depended on the rise of activity. The results showed that the ethyl acetate extract (1000 \(\mu\)g/ml) showed a very significant inhibition (71.27\% inhibition) in a heated condition, but, the same concentration of methanol extract showed very significant inhibition (77.29\% inhibition) in cold condition. Diclofenac sodium (200 \(\mu\)g/ml) exhibited 67.83\% inhibition and 66.70\% inhibition in heated and cold condition, respectively. The results indicated that the ethyl acetate and methanol extracts showed a significant activity comparing with other extracts in the different temperature conditions of the experiment.
cated, dried, powdered and extracted by soxhela-
tion with solvents of ascending order of polarity viz., petroleum ether, chloroform, ethyl acetate, and methanol. Dried extracts obtained were subjected to anti-inflammatory evaluation by in vitro and in vivo methods. Among the four tested, two, the ethyl acetate and methanol extracts showed significant activity in the in vitro evaluation. Based on this result, these two extracts were selected for the in vivo evaluation. In this evaluation also, the selected methanol and ethyl acetate extracts exhibited a significant activity, particularly, the methanol extract which suggested that some compounds from these extracts appeared to be promising for the treatment of inflammation. Our future studies in the direction of toxicity evaluation, identification and isolation of the active constituents from these extracts may give significant results valuable for further researches.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

REFERENCES


