In vitro Evaluation of Anti Inflammatory activity of Methanolic and Ethanolic leaf extracts of five Indigenous Plants in South India

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Abstract: Methanol and ethanol leaf extracts of five indigenous plants of Piper betle L., Punica granatum L., Psidium guajava L., Gloriosa superb, L. and Mangifera indica, L. were investigated for their in vitro anti-inflammatory proprieties by Human Red Blood Cell membrane stabilization method. The prevention of hypotonocity induced HRBC membrane lysis was taken as a measure of anti inflammatory activity. The anti inflammatory activity of methanol and ethanol leaf extracts of Piper betle L., Punica granatum L., Psidium guajava L., Gloriosa superb, L. and Mangifera indica, L. were compared to that of standard drug Diclofenac. The % prevention of lysis by standard Diclofenac at 100µg/ml is 24.1% and at concentration 200µg/ml is 32.30%. The percentage of anti-inflammatory activity of methanol extracts differs from ethanol extracts. Among the five plants of methanol extracts, Mangifera indica, L., at100µg/ml is 15.2% and at a concentration 200µg/ml is 19.2% shows the highest anti inflammatory activity. Among the five plants of ethanol extracts, Gloriosa superb, L. at 100µg/ml is18.5% and at a concentration 200µg/ml is 19.8% shows the highest Anti-inflammatory activity. All the five plant species exhibits strong invitro anti inflammatory activity suggested to use in production of anti inflammatory drugs.

Key words: Anti-inflammatory activity, Human Red Blood Cell (HRBC) membrane stabilization, indigenous plants.

INTRODUCTION:

Inflammation is a dynamic and multifactorial process involving with many systems in the body. Rheumatic diseases are common inflammatory diseases in the world [1].There is a great disadvantage of present anti-inflammatory synthetic drugs like Steroidal and non steroidal drugs lied in their toxicity and reappearance of symptoms after discontinuation of treatment [2]. Plant based traditional medicine system play a vital role in
healthcare [3] and various plant extracts and their isolated compounds are excellent anti-inflammatory agents [4].

*Piper betel* L. leaves is widely used as a mouth freshener after meal. This plant is extensively grown in Bangladesh, India, Sri Lanka, Malaysia, Thailand, Taiwan and other Southeast Asian countries. Its common name is betel in English, paan in India and Bangladesh [5]. Indian system of medicine and health has adopted the use of betel leaves in various ways. In Indian folkloric medicine, betel leaf is popular as an antiseptic and is commonly applied on wounds and lesions for its healing effects. Essential oil extracted from betel leaf may be used as an industrial raw material for manufacturing medicines, perfumes, mouth fresheners, tonics, food additives etc [6].

*Punica granatum* L., commonly known as pomegranate, is a fruit-bearing deciduous shrub or small tree, native to Asia and belongs to the family Lythraceae [7]. The leaves are shiny and about 7.6 cm long [8]. Different parts of the plant such as bark, leaves, immature fruits and fruit rind have medicinal significance [9]. *Punica granatum* L. has been extensively used as a traditional medicine in many countries for the treatment of dysentery, diarrhea, helminthiasis, acidosis, hemorrhage and respiratory pathologies [10]. Additionally, this plant is reported to have excellent antibacterial, antifungal, antiprotozoal and antioxidant properties [11, 12, 13]. Numerous phytochemical constituents have been reported to be present in different parts of the pomegranate plant making it pharmacologically precious [14].

*Psidium guajava* Linn, belonging to the Family Myrtaceae, is originated in the tropical South America [15] and grows wild in Bangladesh, India, Thailand, Brazil, Florida, West Indies, California and also in several other countries [16]. *Guajava* leaf extract contains guajava polyphenol that has an anti-oxidation action [17, 18] and flower and leaf of the plant have been reported to have antibiotic activity [19]. The leaves contain various constituents such as fixed oil 6%, volatile oil 0.365% 3.15% resin, 8.5% tannin, fat, cellulose, chlorophyll and mineral salts and a number of other fixed substances [20, 21].

*Gloriosa superba* Linn. is an important medicinal plant belonging to the family Liliaceae. Which is one of the endangered species among the medicinal plants [22, 23]. Being native form Indian especially Southern India it is known as glory lily and climbing lily-in English. In the world market glory lily considered as rich source of colchicines and gloriosine [24]. The flower has analgesic, anti-inflammatory potential, antimicrobial, larvicidal potential, antipoxviral potential, antitumor potential, enzyme inhibition potential, and also used in treatment of snake bite, Skin disease, respiratory disorders [25, 26, 27].

Leaves of *Mangifera indica*, L. contain alkaloids, anthracenosides, coumarins, flavonoides, sugars, tannins, steroids and saponins. These, together with chlorophyll, are responsible for the extract colours observed. The fruity or sweet smell of the extracts is due to the presence of esters and essential oils in the plant extracts [28, 29]. Some of these compounds have been reported to possess antimicrobial activity [30].

In this study, methanol and ethanol leaf extracts of above five plants, which had been described in herbal books and folklore medicine of India, were screened for their anti inflammatory activity by Human Red Blood Cell membranes stabilization method.


**MATERIALS AND METHODS**

The leaves of selected indigenous plants were collected from Prakasam district of Andhra Pradesh, South India. And they were identified and authenticated by k.Baburao, In charge scientist, Sai Lara Biotechnology, Hyderabad, Andhra Pradesh, India. The leaves of selected indigenous plants were separated and washed with sterile distilled water and dried using laminar air flow, ground in to fine powder using a blender and stored in air tight container till further analysis.

Extraction Procedure: Ten grams of each plant fine powder of indigenous plants weighed in to a 250 ml conical flask and 100 ml of methanol and ethanol was added separately for each plant powder then on a rotary shaker at 190 – 220 rpm for 24 hours [31]. This was filtered with whatman No1. Filter paper, the residue discarded, and the filter were evaporated to dryness in a water bath temperature at 80°C.

Preparation of stock solution for each extract of leaves selected indigenous plants powder: stock solution was prepared by weighing 10 mg of each dried solvent extract dissolved in 1 ml of dimethyl sulfoxide (DMSO) giving a final concentration of 10,000 µg/ml. The stock solution was kept in screw capped bottles for subsequent use Anti-Inflammatory Activity of Indigenous plant Extract by HRBC Method

In Vitro Anti-inflammatory Activity by Human Red Blood Cell membranes stabilization method [32]

Human red blood cell membrane method was used for the Invitro estimation of anti-inflammatory activity. Blood was collected from healthy volunteers and was mixed with equal volume of sterilized Alsevers solution (equal volume of (2% dextrose, 0.8% sodium citrate, 0.05% citric acid & 0.42% sodium chloride in water). This blood solution was centrifuged at 3000 rpm and the packed cells were separated. The packed cells were washed with isosaline solution and a 10% v/v suspension was made with isosaline. This HRBC suspension was used for the estimation of anti-inflammatory property. Different concentrations of extract, reference sample and control were separately mixed with 1ml of phosphate buffer, 2 ml of hyposaline and 0.5 ml of HRBC suspension. Instead of hyposaline 2 ml of distilled water used in the control. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged at 3000 rpm. The supernatant liquid was decanted and the hemoglobin content was estimated by spectrophotometer at 560 nm. The percentage of hemolysis was estimated by assuming the hemolysis produced in the control as 100%.

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\% \text{ Percentage Protection} = 100 - \frac{\text{Optical Density of sample}}{\text{Optical Density of Control}} \times 100
\]

Plant Materials:
1. *Piper betle* L. (Betel leaf) - leaf
2. *Punica granatum* L. (Pomegranate) – leaf,
3. *Psidium guajava* L. (Guava) - leaf,
4. *Gloriosa superb*, L. (glorylilly) – leaf and

RESULTS AND DISCUSSION:

Methanol and Ethanol leaf extract of *Piper betle* L., *Punica granatum* L., *Psidium guajava* L., *Gloriosa superb*, L. and *Mangifera indica*, L. exhibited membrane effect by inhibiting hypertonicity induced Lysis of erythrocyte membrane. The erythrocyte membrane is analogues to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membrane [33,34].Stabilization of lysosomal membrane is important in reducing the inflammatory response by preventing the release of lysosomal constituent of activated neutrophil such as bacterial enzymes and proteases which cause further tissue inflammation and damage.

Out of five plants tested for anti inflammatory activity all the five plant species showed anti-inflammatory activity by preventing lysis of RBC membrane. The results of the anti- inflammatory activity of plant extracts tested by Human Red Blood Cell membranes stabilization method are shown in Table -2and 3.Among the plants screened, the methanol extract of *Mangifera indica*, L., *Gloriosa superb*, L. show significant anti-inflammatory activity followed by *Mangifera indica>*gloriosa superb>*piper betle>*punica granatum>*psidium guajava..The ethanol extract of *Gloriosa superb*, L, *Punica granatum* L., show significant anti inflammatory activity followed by gloriosa superb>punica granatum>piper betle>psidium guajava>mangifera indica shown in figures.
From the above study it was concluded that the Methanol and ethanol leaf extract of *Piper betle* L., *Punica granatum* L., *Psidium guajava* L., *Gloriosa superb*, L. and *Mangifera indica*, L has significant membrane stabilization property.

**Table 1: Anti inflammatory activity of Standard Diclofenac at various concentrations**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Plant Extract and Standard Drug</th>
<th>Concentration µg/ml</th>
<th>% of Activity (Prevention of lysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Standard Diclofenac</td>
<td>100</td>
<td>24.18%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200</td>
<td>32.30%</td>
</tr>
</tbody>
</table>

**Table 2: Anti inflammatory activity of Methanol extracts of Five Indigenous plants at 100 and 200 µg/ml concentrations**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Plant Extract</th>
<th>% of Activity (Prevention of lysis) At 100 µg/ml Concentration</th>
<th>% of Activity (Prevention of lysis) At 200 µg/ml Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>P.B.</td>
<td>11.3%</td>
<td>17.1%</td>
</tr>
<tr>
<td>02</td>
<td>P.G.</td>
<td>13.8%</td>
<td>14.4%</td>
</tr>
<tr>
<td>03</td>
<td>Ps.G.</td>
<td>11.3%</td>
<td>12.6%</td>
</tr>
<tr>
<td>04</td>
<td>G.S.</td>
<td>14.8%</td>
<td>18.5%</td>
</tr>
<tr>
<td>05</td>
<td>M.I.</td>
<td>15.2%</td>
<td>19.2%</td>
</tr>
</tbody>
</table>

**Table 3: Anti inflammatory activity of Ethanol extracts of Five Indigenous plants at 100 and 200 µg/ml concentrations**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Plant Extract</th>
<th>% of Activity (Prevention of lysis) At 100 µg/ml Concentration</th>
<th>% of Activity (Prevention of lysis) At 200 µg/ml Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>P.B.</td>
<td>13.5%</td>
<td>16.2%</td>
</tr>
<tr>
<td>02</td>
<td>P.G.</td>
<td>15.1%</td>
<td>18.8%</td>
</tr>
<tr>
<td>03</td>
<td>Ps.G.</td>
<td>9.9%</td>
<td>10.2%</td>
</tr>
<tr>
<td>04</td>
<td>G.S.</td>
<td>18.5%</td>
<td>19.8%</td>
</tr>
<tr>
<td>05</td>
<td>M.I.</td>
<td>8.7%</td>
<td>11.3%</td>
</tr>
</tbody>
</table>

CONCLUSION

Methanol leaf extract of *mangifera indica*, *gloriosa superb* exhibited highest anti-inflammatory activity and ethanol leaf extract of *gloriosa superb*, *punicagranatum* exhibited highest anti-inflammatory activity, ethanol extract of *mangifera indica* exhibited lowest anti-inflammatory activity among five plants. The results give a justification for the use of these plants in anti-inflammatory medicine.

REFERENCES


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