HPTLC analysis and anti-inflammatory activity of *Plectranthus amboinicus* (Lour.) leaves

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**ABSTRACT**

*Plectranthus amboinicus* (Lour.) Spreng. are generally used in Chinese folk medicine for treating fever, cough, mumps, sore throats, and mosquito bite. The current study was therefore carried out to provide vital pharmacological details about the leaf. This study investigated the anti-inflammatory properties of ethanolic extract from *Plectranthus amboinicus*. The leaves of *Plectranthus amboinicus* were rinsed, air dried and then powdered using machine. The ethanol extract of the plant was used for phytochemical analysis to identify the constituents present in the plants. HPTLC analysis was carried out to investigate its secondary metabolite profile by using the solvent mixture. The anti-inflammatory effect was analysed by inhibition of protein denaturation assay. The hypotonicity induced haemolysis was observed from 100 g/ml to 500 g/ml of extract concentration. The results proved that the ethanolic leaf extract of *Ramboinicus* has antiinflammatory activity. The presence of flavonoid, quercetin in plant extract was confirmed by HPTLC analysis and these flavonoids may be responsible for the antiinflammatory activity. Further studies are needed to the development of potential drug that may be used for various pharmacological activities.

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**INTRODUCTION**

Inflammation is usual and mandatory protective biological response to the harmful stimuli like antigen-antibody reactions, infectious agents, ischemia, chemical, thermal and physical agents. It is triggered by various stimuli, comprising UV irradiation, physical damage, immune reactions and microbial infections. The key aspects of inflammation are warmness, swelling, redness and pain. Inflammation cascades can results in the progression of diseases including arthritis, psoriasis, chronic asthma, multiple sclerosis and inflammatory bowel disease. Most of these disorders are debilitating and are becoming progressively common in our ageing society. Degenerative and rheumatoid arthritis are the main inflammatory diseases afflicting people worldwide (Roh and Sohn, 2018). Even though many antiinflammatory drugs are available for therapy, the need for the new drug arises due to many adverse effects.

Medicinal plants form the basis of health care throughout the world since the earliest days of humanity. Plants are significant for pharmacological studies and for the drug development. Many of medicinal herbal drugs have been established depending on the ethnic information in human health care. Herbal formulations constitute vital
natural resource from which chemicals of probable interest for agriculture, medicine, industry and other areas can be detected and isolated (Saad et al., 2017).

Plectranthus amboinicus (Lour) Spreng, a succulent aromatic perennial herb belongs to Lamiaceae, family and is commonly called as country borage in English. It is a wide, shrubby below, tomentose or hispidly villous. It is observed all through India, Ceylon and Moluccas. Previously, it was claimed that the leaves were being conventionally used as a diuretic. Upon review of literature it was observed that the herb contains β-caryophyllene, butylanisole, quercetin, ursolic acids, α-pinene, β-pinene, thymol, triterpenic acids, eugenol, carvacrol, β-phellandrene, 1,8-cineole, p-cymene, crisilamaritin, salvigenin, and chrysoeriol. Various pharmacological actions have been reported comprising antitumor, antiepileptic, antimutagenic neuropharmacological antioxidant antimicrobial antibacterial and antifungal properties (Silva et al., 2017; Swamy et al., 2017). The design of the present work carried out to evaluate invitro anti-inflammatory activity and fingerprint profile of Plectranthus amboinicus using HPTLC analysis.

METHODOLOGY

Plant collection and identification
The plant samples of Plectranthus amboinicus leaves were obtained in and around Pattukkottai, Thanjavur District and authenticated by a Botanist. These plants were collected from their natural habitat and from identified herbalists.

Preparation of the extract
The leaves of plant species were cleaned using water until soil and other materials on them were removed. Thereafter, they were then shade dried for a week. The materials of plant were ground into fine powder and then wrapped in air-tight containers and placed in the laboratory at room temperature (25°C) prior to further analysis. The ethanolic extract of plant was made using Soxhlet extraction method. About 500g of plant material powder were uniformly packed into a thimble and extracted with 2L of ethanol. The extraction process persisted for about 8 hours. After that the extract were dried using rotary evaporator at 40-60°C. The dried extract was carefully removed and kept in refrigerator at 4°C awaiting phytochemical analysis.

High Performance Thin Layer Chromatography (HPTLC) analysis
Quercetin (10mg) was liqiudified in ethanol (10ml). From this 1ml was diluted to 10ml with ethanol. About 2μl to 6μl was dotted comprising concentration in the range of 200μg to 600μg/ml. For TLC analysis, the ethanolic extract (100mg) was liqiufied in 1ml of ethanol (Mobile phase: Toluene: Ethyl acetate: Formic acid (5:4:1); Stationary phase: Silica Gel 60 F 254). The standard solution (2μl to 6μl) and the extract (5μl to 10μl) were added on a precoated silica gel 60 F 254 HPTLC plate (E.Merck) of even thickness (0.2mm) using Linomat5 sample applicator. In the solvent system, the plate was developed to 8cm distance. The plate was scanned densitometrically using TLC Scanner at 254nm. The plate was detected at 254nm under UV light and observed at 366nm under CAMAG REPROSTAR3 (Amina et al., 2018).

Invitro Anti-inflammatory Activity

Effect on protein denaturation
Various concentrations of ethanol leaves extracts (100-500μg/ml) were added on to egg albumin solution (1ml) and kept for 15 minutes at 27°C. The solution mixture was incubated in a water bath at 70°C for 10 minutes to trigger the denaturation process. After that the mixture was cooled in room temperature and the turbidity was measured using a spectrophotometer at 660 nm (Kumar and Kumar, 2020).

Percentage anti-denaturation effect was calculated from control without the extract.

\[
\% \text{Anti-denaturation activity} = \frac{Abs \text{sample} - Abs \text{control}}{Abs \text{control}} \times 100
\]

Effect on Heat induced haemolysis
The reaction mixture (2ml) have test sample (1ml) of various doses and RBCs suspension (1ml of 10%). The saline was taken in the control test tube instead of test sample. The standard drug used was Diclofenac sodium. The reaction mixtures inside the centrifuge were incubated for 30min at 56°C in water bath. After that, the tubes were placed under running tap water. Centrifugation of the test mixture was done for 5 min at 2500 rpm and the absorbance was measured at 560 nm. The experiment was performed in triplicates for each and every sample.

The haemolysis inhibition percentage was measured as follows:

\[
\text{Percentage inhibition} = \frac{(Abs \text{ control} - Abs \text{ sample}) \times 100}{Abs \text{ control}}
\]

Effect on Hypotonicity-induced haemolysis
Various doses of extract, control and reference standard were separately assorted with hyposaline (2ml), phosphate buffer (1ml), and HRBC suspension (0.5ml). The standard drug used was
Diclofenac sodium. The test mixtures were kept for 30 minutes at 37°C and centrifugation was carried out (3000 rpm). The liquid supernatant was transferred and the haemoglobin content was taken using a spectrophotometer (560 nm). The hemolysis percentage was assessed by taking the haemolysis in the control as 100% (Prabakaran et al., 2020).

\[
\text{Percentage protection} = 100 - \left( \frac{OD \text{ sample}}{OD \text{ control}} \right) \times 100
\]

**Statistical Analysis**

Results were measured as Mean ± Standard Deviation. The statistical studies were done with the help of SPSS statistical package (version 22.0).

**RESULTS AND DISCUSSION**

Natural products belonging to plant origin are used by various races of people all through the world. A study has assessed that about more than 500,000 of plants exist on earth in which only 10% are used for food products and medicinal uses for humans. Seeing the therapeutic use and functions of herbal plants help in disclosing the therapeutic effects and would be supportive in treating various diseases. Plants make wide array of bioactive molecules belonging to several molecular families and makes them a rich source of herbal medicines (Parvez, 2018). Thus natural products having biological and pharmacological properties play an important role in modern medicines which are important in treating life-threatening conditions. In the present study the HPTLC analysis was carried out for the ethanolic extract of *Plectranthus amboinicus* to investigate its secondary metabolite profile. The total number of bands developed (Figure 1) was found and quercetin bands in the ethanol extract was evaluated by comparing the UV-Vis absorption spectra with those of standards (Table 1). The use of standard ensures the concentration and ratio of the test compound in the leaves extract (Figures 2 and 3). The standard quercetin showed the Rf value of 0.52. The quantity of Quercetin in ethanolic extract was found to be 0.3376% w/w.

Similar to the observations, the HPTLC profile for ethanol extract, and it was shown that secondary metabolites are made by a large variety of plants, of which 10-20% are flavonoids and are belongs to natural products (EL-Hefny et al., 2020). Flavonoids are reported to have various useful functions, such as enzyme inhibition, anti-inflammatory, anticancer, antimicrobial, antioxidant, and antiallergy effects (Maleki et al., 2019).

The therapeutic use of herbal medicine increased from past decades due to the side effects of syn-
thetic drugs. Herbal drugs as a vital remedy in the traditional system of medicine are used in medical practice from ancient timings (Yatoo et al., 2018). Ayurveda is the holistic alternative science from India. Herbs are staging a comeback over the globe. The product made from herbs are safe when compared to synthetic drugs that are regarded as unsafe to the human body and environment (Schink et al., 2018). Inflammation is the protective mechanism of the body that helps to protect itself from injury, allergens, toxins, irritants and chemicals. Now a day whole world moves towards herbal medicines with minimum side effects and cost effective for the treatment of such ailments.

In protein denaturation process the proteins miss their secondary and tertiary structure by applying external stimuli or compound, a concentrated inorganic salt, like strong base or acid, heat or an organic. Protein denaturation results in the losing of their biological function which is due to result of inflammation. As part of the findings on the anti-inflammation mechanism, ability of plant extract to hinder denaturation of protein was studied. It was effective in hindering heat induced denaturation of albumin Percentage inhibition of denaturation of protein was exhibited depending on the

Table 1: Rf values of standard and ethanolic extract of *P. amboinicus*

<table>
<thead>
<tr>
<th>Peak</th>
<th>Start Height</th>
<th>Start Height</th>
<th>Max Rf</th>
<th>Max Height</th>
<th>End Height</th>
<th>Area</th>
<th>Assigned Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std</td>
<td>0.49</td>
<td>1.0</td>
<td>0.53</td>
<td>68.6</td>
<td>0.2</td>
<td>1145.6</td>
<td>Quercetin</td>
</tr>
<tr>
<td>Extract</td>
<td>0.49</td>
<td>0.2</td>
<td>0.52</td>
<td>24.6</td>
<td>0.6</td>
<td>279.8</td>
<td>Quercetin</td>
</tr>
</tbody>
</table>

Table 2: Effect of ethanol extract of *P. amboinicus* leaves on heat induced protein denaturation

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration (μg/ml)</th>
<th>% inhibition of protein denaturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>100</td>
<td>18.15±0.5</td>
</tr>
<tr>
<td>2.</td>
<td>200</td>
<td>22.05±0.28</td>
</tr>
<tr>
<td>3.</td>
<td>300</td>
<td>24.2±0.23</td>
</tr>
<tr>
<td>4.</td>
<td>400</td>
<td>32.75±0.4</td>
</tr>
<tr>
<td>5.</td>
<td>500</td>
<td>38.2±0.2</td>
</tr>
<tr>
<td>6.</td>
<td>Standard</td>
<td>76.4±0.14</td>
</tr>
</tbody>
</table>

Table 3: Effect of ethanol extract of *Plectranthus amboinicus* leaves on heat-induced hemolysis

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration (μg/ml)</th>
<th>% inhibition of Hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>100</td>
<td>19.9±0.6</td>
</tr>
<tr>
<td>2.</td>
<td>200</td>
<td>22.5±0.7</td>
</tr>
<tr>
<td>3.</td>
<td>300</td>
<td>25.45±0.1</td>
</tr>
<tr>
<td>4.</td>
<td>400</td>
<td>30.75±1.2</td>
</tr>
<tr>
<td>5.</td>
<td>500</td>
<td>34.2±0.5</td>
</tr>
</tbody>
</table>

Table 4: Effect of ethanol extract of *Plectranthus amboinicus* leaves on Hypotonicity induced hemolysis

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration (μg/ml)</th>
<th>% inhibition of haemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>100</td>
<td>17.3±0.7</td>
</tr>
<tr>
<td>2.</td>
<td>200</td>
<td>21.35±0.6</td>
</tr>
<tr>
<td>3.</td>
<td>300</td>
<td>24.4±0.2</td>
</tr>
<tr>
<td>4.</td>
<td>400</td>
<td>27.5±0.3</td>
</tr>
<tr>
<td>5.</td>
<td>500</td>
<td>31.9±0.4</td>
</tr>
</tbody>
</table>
concentration. The percentage inhibition of protein denaturation was 38.2% for 500µg/ml of the extract. The antiinflammatory drug, Diclofenac sodium, displayed the maximum inhibition 76.4% at of 100 µg/ml concentration compared with control (Table 2).

**Heat and Hypotonicity induced hemolysis**

The secretion of lysosomal constituents like proteases and bactericidal enzymes by activated neutrophils during inflammatory response, triggers several reactions, which, in turn, induce biomolecule damage, protein denaturation and membrane lipid peroxidation. Thus, lysosomal membrane stabilization is crucial in limiting the inflammatory reaction (Yesmin et al., 2020). On the other hand, the release of proteases during inflammatory response denatures the proteins, resulting in the production of auto-antigens and lead to the development of auto-immune disorders (Bamdad et al., 2017).

The ethanol extract of *P. amboinicus* was also active in reducing the hypertonicity and heat induced hemolysis in concentration dependent manner. The results showed that maximum inhibition was with ethanol extract of at 500µg/ml while minimum in 100µg/ml). The extracts were effectively hindering the heat-triggered hemolysis involving stabilization of RBC membrane (Tables 3 and 4). The IC50 values for heat induced and hypotonicity induced hemolysis is 0.622±0.02 and 0.692±0.53µg/ml.

A similar study by Prasanna et al. (2019) also showed effective hindrance of heat-induced hemolysis by extract of *Wedelia trilobata*. The maximum inhibitions were 78.11% from leaf extract followed by the stem (74.17%) and flower (58.74%).

**CONCLUSION**

From the results it can be said that the ethanolic leaf extract of *Pamboinicus* has antiinflammatory activity. HPTLC analysis established the presence of flavonoid quercetin in plant extract and these flavonoids may be responsible for the antiinflammatory activity. Further studies are needed to the development of potential drug that may be used for various pharmacological activities.

**Conflict of Interest**

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

**Funding Support**

The authors declare that they have no funding support for this study.

**REFERENCES**


