Evaluation of Anti-psoriatic Potential of the Fruit Rind of Punica granatum L.

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ABSTRACT
Background: Pomegranate (Punica granatum L.) is universally known for its therapeutic properties due to its potential bioactive compounds. However, there is no established scientific study on antipsoriatic activity of pomegranate fruit rind. The aim of the study is to evaluate the anti-psoriatic activity of the isolated compounds and the extract from the fruit rind of P. granatum. In our previous study, the isolated compounds were evaluated for antioxidant potential. In continuation to the previous investigation, the present study is taken up to evaluate the extract and compounds for in vitro anti-psoriatic activity. Methods: Chromatographic techniques were employed to isolate the compounds from the aqueous acetone extract and in vitro anti-psoriatic activity was determined by thymidine phosphorylase inhibition assay. Results: From previous phytochemical investigation, three compounds were identified as Punicalagin, 2,3(S)-hexahydroxydiphenoyl-D-glucose and Punicalin. In the present study, the extract and the compounds were evaluated for anti-psoriatic activity. The results reveal that the isolated three compounds showed inhibitory activity of 89% to 95% against thymidine phosphorylase. Aqueous acetone extract also exhibited 87% inhibition. Conclusion: Punica granatum is an ideal plant for further investigation to prove its anti-psoriatic activity. Key words: Anti-psoriatic activity, Punica granatum, Thymidine phosphorylase inhibition.

INTRODUCTION
Phytochemicals or bioactive constituents derived from the medicinal plants are commonly called as secondary metabolites and are considered as the natural source to protect various illness.1-4 In folk and traditional systems of medicines, Punica granatum (Lythraceae) has been used as a food supplement or medicine for its wide spread pharmacological activities due to the presence of its array of bioactive chemical constituents with different potency. The plant has been reported for antioxidant, antiangiogenic, antiproliferative, antibacterial and hepatoprotective activities. P. granatum reported to have polyphenols and tannins which are responsible for its potent antioxidant and anti-inflammatory activity.1-3 Flavonoids in plants are known for their antioxidant effects and possible use of natural antioxidant agent for control of psoriasis is of great interest since skin is a target of oxidative stress.6 Angiogenesis, the development of new blood vessels from a pre-existing vascular bed, is a vital component of the pathogenic mechanisms involved in psoriasis.7-8 Thymidine phosphorylase is an angiogenic enzyme that catalyzes the reversible phosphorylysis of thymidine into thymine and 2-deoxy-D-ribose 1-phosphate and then the 2-deoxy-D-ribose 1-phosphate undergo dephosphorylation to produce 2-deoxy-D-ribose which is important for the angiogenic activity of thymidine phosphorylase. Thymidine phosphorylase enzyme is reported to be present in high levels in the psoriatic lesion which is likely to induce psoriasis.9

Psoriasis is an autosomal, chronic inflammatory dermatitis affecting 2% of world population. It is present in all racial groups and has a strong genetic component. Plants play a significant role in the discovery of new agents for the treatment of psoriasis.10

The present study was aimed to evaluate the anti-psoriatic activity of aqueous acetone extract and compounds of the fruit rind of P. granatum.

MATERIALS AND METHODS
Instruments and chemicals
Shimadzu UV Spectrophotometer UV 1800. Standards (5-Nitouracil), chemicals and other reagents utilized were of AR grade purchased from Sigma (USA), Rankem (India) and Merck (Germany).

Plant material
Fresh fruits of Punica granatum were collected from Nilgiri District, Tamil Nadu, India and authenticated by the In-house Taxonomist, Natural Remedies Pvt Ltd., Bangalore. A voucher specimen PC/PN/TRM125 was preserved at the Agronomy Department of Natural Remedies Pvt. Ltd., Bangalore.

Table 1: Thymidine phosphorylase inhibition activity of isolated compounds and aqueous acetone extract of Punica granatum fruit rind

<table>
<thead>
<tr>
<th>Sample name</th>
<th>10 µg/ml</th>
<th>30 µg/ml</th>
<th>100 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Punicalin</td>
<td>79.30± 0.65</td>
<td>90.68± 0.63</td>
<td>94.61± 0.39</td>
</tr>
<tr>
<td>2,3(S)-HHDPP-D-glucose</td>
<td>33.33± 0.71</td>
<td>74.21± 0.34</td>
<td>89.01± 0.35</td>
</tr>
<tr>
<td>Punicalin</td>
<td>76.99± 0.35</td>
<td>89.27± 0.22</td>
<td>94.61± 0.51</td>
</tr>
<tr>
<td>Aqueous acetone extract</td>
<td>35.00± 0.12</td>
<td>76.15± 0.64</td>
<td>87.23± 0.54</td>
</tr>
</tbody>
</table>

* The values are represented as mean ± SD (n=3)

**Extraction and isolation**

One kg of fruit rind of *P. granatum* was air-dried and coarsely powdered, then extracted with 75% acetone/water at 60°C for 1 hour for three times (3L) by reflux method. The extracts were filtered and concentrated at 60°C under vacuum. The aqueous acetone extract (350 g) was chromatographed over Diaion HP-20 resin with decreasing polarity using acetone and water. The enriched fractions were repeatedly subjected on Sephadex LH-20 with water/ acetone mixtures. The repeated purification afforded Punicalagin, 2,3(S)-hexahydroxydiphenoyl-D-glucose and Punicalin which was identified by spectral data. Detailed isolation process and characterization of the isolated compounds were described in our previous study.16

**Anti-psoriatic activity**

**Thymidine phosphorylase inhibition assay**

The aqueous acetone extract and the isolated compounds were evaluated for their in vitro thymidine phosphorylase inhibition. This assay was carried out as per Krenitsky et al.24 with slight modification. Total reaction mixture comprising Potassium phosphate buffer (200Mm, pH 7.4), different concentration of test solutions (in buffer), thymidine phosphorylase enzyme (5U/ml) and thymidine (3mM) were mixed and incubated at 25°C for 10 min and the absorbance was read at 290 nm in UV-spectrophotometer. 5-Nitouracil was used as a standard inhibitor.

**Statistical analysis**

The data were analyzed by GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA). Each experiment was performed in triplicates. The values are expressed as mean ± standard deviation (SD).

**RESULTS AND DISCUSSION**

The fruit rind of *P. granatum* was extracted with 75% acetone/water which was subsequently purified over Sephadex LH-20, yielded Punicalagin, 2,3(S)-hexahydroxydiphenoyl-D-glucose and Punicalin. The extract and the isolated compounds were screened against thymidine phosphorylase. The investigation was carried out with different concentrations (10, 30 and 100 µg/ml) of the test samples and the results demonstrated a dose dependent activity with increase in the concentration [Table 1]. The inhibition is expressed in percentage (%). The compounds and extract showed a good activity against thymidine phosphorylase [Figure 1]. The plant has been reported to contain phenolic constituents which may be responsible for the anti-psoriatic activity. There is no detailed established scientific study reported earlier on antipsoriatic activity of pomegranate fruit rind.

**CONCLUSION**

Pomegranate is used by the traditional healers for the ethnomedical preparations for various ailments including Psoriasis. The present study reveals that the fruit rind of *P. granatum* has anti-psoriatic potential which has been used in the traditional system of medicine or ethnomedicine by traditional healers or tribes. It is a promising anti-psoriatic source that warrants detailed investigation for treating or controlling the psoriasis disease.

**ACKNOWLEDGEMENT**

The authors thank M/s. Natural Remedies Pvt. Ltd., Bangalore, India, for providing all necessary facilities to carry out the research work.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**ABBREVIATIONS**

HHDPP: Hexahydroxydiphenoyl; UV: Ultra violet.

**REFERENCES**

