IN VITRO ANTI-INFLAMMATORY ACTIVITY OF SOME COMMON FRUITS OF WEST COAST OF INDIA.

Srividya*¹ and Chandra M.²

¹Department of Biotechnology, Manipal Institute of Technology, Manipal, Karnataka, India.
²Department of Post graduate studies and Research in Biosciences, Mangalore University, Mangalagangothri, Karnataka, India.

ABSTRACT

The objective of the study is to evaluate Anti-inflammatory activity of different fruits viz Annona reticulata, Vitis vinifera, Punica granatum, Malus domestica, Citrus sinensis, Ananas comosus, Carica papaya, Manilkara zapota, Citrullus lanatus and Musa cavendishii. Anti-inflammatory activity was evaluated using Albumin denaturation assay and Proteinase inhibitory activity at different concentrations. Diclofenac sodium was used as a reference drugs for the study of Anti-inflammatory activity. Among ten common fruits, Vitis vinifera showed highest inhibition in both Albumin denaturation assay and Proteinase inhibitory activity with IC₅₀ values 114.03µg/ml and 120.69µg/ml respectively. Fruit extract of Musa cavendishii showed lowest inhibition with IC₅₀ values 360.88µg/ml and 367.37µg/ml for Albumin denaturation and Proteinase inhibitory activity respectively. In Albumin denaturation and Proteinase inhibition activity assay, Diclofenac sodium showed highest inhibition with IC₅₀ values 67.44µg/ml and 70.95µg/ml respectively.

KEYWORDS: Albumin denaturation, Anti-inflammatory activity, Diclofenac sodium, Proteinase inhibition.

INTRODUCTION

Inflammation is part of the body's immune response to harmful stimuli, infection or trauma.¹ Symptoms of inflammation include redness, swelling, pain, joint stiffness, loss of joint function as a result of infection, irritation or injury.² Various aspects of inflammatory responses like functions of neutrophils, the metabolic products of arachidonic acid and the

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¹Correspondence for Author
Srividya
Department of Biotechnology, Manipal Institute of Technology, Manipal, Karnataka, India.
role played by reactive oxygen species which have been utilized for screening of anti-inflammatory compounds.[3] Rheumatoid arthritis is a common chronic and systemic autoimmune disorder characterized by inflammation of the synovial joints and concomitant destruction of cartilage and bone.[4]

Drugs which are used presently for the management of pain and inflammatory conditions are either steroidal like corticosteroids (prednisolone) or non steroidal like ibuprofen. These drugs influences toxic and side effects like allergic reactions, hearing loss, renal failure or they may increase the risk of haemorrhage by affecting platelet function.[5] Now a day the usages of herbal remedies are increased when compared to allopathic drugs.[6] Some medicinal herbs were used as raw materials for the production either directly for crude drugs or as the bioactive components in the formulation of Pharmaceuticals and cosmetics.[7]

The parts of the plants like Leaf, stem and bark are given as natural medicine as a cure for various diseases.[8],[9],[10] Anti-inflammatory foods include most colorful fruits and vegetables, oily fish (omega -3 fatty acid), nuts, seeds, and certain spices, such as ginger, garlic and cayenne. World Health Organization motivates the developing countries for the use of safe and effective herbal medicines because of the great potential they hold in prevention of various diseases.[11] Hence, the present study was undertaken to evaluate in vitro anti-inflammatory activity of methanol extracts of some common fruits of west coast of India.

MATERIALS AND METHODS

Plant materials

Samples of fresh ripe fruits were purchased from the local market of west coast (Udupi district) of Karnataka state. The fruits comprised of Annona reticulata, Vitis vinifera, Punica granatum, Malus domestica, Citrus sinensis, Ananas comosus, Carica papaya, Manilkara zapota, Citrullus lanatus and Musa cavendishii. The fruit samples were authenticated by K. Gopalkrishna Bhat, a taxonomist.

Extraction procedure

Each Sample of fresh fruit was washed under running tap water followed by washing with distilled water to remove the surface debris. Edible portions of the fruit pulp (100g) were weighed and minced using a mixer grinder. Then it was extracted in methanol for 72 hours in dark at 37°C incubator shaker. After 72 hours, the fruit extracts were filtered and then centrifuged to obtain clear extract. The filtrate was concentrated using Rotary vacuum
evaporator and lyophilized to obtain dry powder.\cite{12} The yielded crude extracts were preserved in a deep freezer (-20° C) for further use.

**Analysis of in-vitro anti-inflammatory activity**

**Inhibition of albumin denaturation**

Inhibition of albumin denaturation was determined by the method Mizushima et al.\cite{13} with slight modifications. 2ml of the reaction mixture consisting of aqueous solution of bovine serum albumin and different concentrations of the fruit extract. 1N HCl was used to change the pH(6.3) of the reaction mixture. The reaction mixture was incubated at 37°C for 20 min and then heated at 57°C for 30 min. After cooling the samples, 1ml of Phosphate buffer saline (pH 6.3) was added to the sample tubes. The optical density of the reaction mixture was measured spectrophotometrically at 660 nm against blank. The test was performed in triplicate. Inhibition of protein denaturation was calculated using following formula:

\[
\text{Inhibition (\%)} = \left( \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100
\]

**Proteinase inhibitory activity**

Proteinase inhibitory activity was determined by the method Oyedepo et al.\cite{14} with slight modifications. Different concentrations of the fruit extracts were taken in a series of test tubes, added 80µg trypsin and 20 mM Tris HCl buffer (pH 7.4). The reaction mixture was incubated at 37°C for 5 min and added 0.5 ml of 1% casein. The mixture was incubated further 15 minutes. Added 1 ml of 70% perchloric acid to stop the reaction. The reaction mixture was centrifuged and the optical density of the supernatant was read at 210 nm against blank. The test was performed in triplicate. The proteinase inhibitory activity was calculated using following formula:

\[
\text{Inhibition (\%)} = \left( \frac{\text{Optical density of control} - \text{Optical density of sample}}{\text{Optical density of control}} \right) \times 100
\]

**Statistical analysis**

The results are expressed as the mean±SD for three replicates. IC50 values are calculated using linear regression analysis.
RESULTS AND DISCUSSION

Inhibition of albumin denaturation

All fruit extracts exhibited albumin denaturation with percentage inhibition values between 62% to 23% at concentration of 200µg/ml. Inhibition of albumin denaturation at 200µg/ml was found to be highest in Vitis vinifera followed by, *Annona reticulata, Punica granatum, Malus domestica, Citrus sinensis, Ananas comosus, Manilkara zapota, Carica papaya, Citrullus lanatus and Musa cavendishii* and the respective values were 62.43±1.66%, 50.83±1.66%, 48.44±1.94%, 46.78±3.04%, 45.67±1.68%, 44.75±1.10%, 43.65±1.65%, 42.73±0.85%, 42.54±3.31% and 23.76±1.65%. Percentage inhibition value for the standard diclofenac sodium at 200µg/ml was found to be 92.64±2.30%. The values are displayed in the graph (Fig.1).

In Inhibition of albumin denaturation, the IC$_{50}$ value for common fruit extracts were found to be highest in Vitis vinifera followed by, *Annona reticulata, Punica granatum, Malus domestica, Citrus sinensis, Ananas comosus, Manilkara zapota, Carica papaya, Citrullus lanatus and Musa cavendishii* and values were 114.03µg/ml, 162.44µg/ml, 171.08µg/ml, 183.09µg/ml, 192.19µg/ml, 206.38µg/ml, 219.37µg/ml, 235.05µg/ml, 240.46µg/ml and 360.88µg/ml respectively. IC$_{50}$ value for the standard diclofenac sodium was found to be 67.44µg/ml. Bovine serum albumin denaturation is a well documented cause of inflammation. [2]

![Fig. 1. Inhibition of Albumin denaturation of common fruit extracts.](image_url)
Proteinase inhibitory activity

In Proteinase inhibitory activity percentage inhibition value ranges between 59% to 20% at concentration of 200µg/ml. The Proteinase inhibitory activity at 200µg/ml was found to be highest in Vitis vinifera followed by, Annona reticulata, Punica granatum, Malus domestica, Citrus sinensis, Ananas comosus, Manilkara zapota, Carica papaya, Citrullus lanatus and Musa cavendishii and the values were 59.55±1.59%, 44.62±1.83%, 42.54±1.31%, 40.8±1.83%, 40.1±1.05%, 38.54±1.04%, 37.50±1.57%, 37.33±2.86%, 36.46±1.56% and 20.66±1.83% respectively. The values are displayed graphically (Fig.2). Proteinase inhibitory value for the standard diclofenac sodium was found to be 90.08±2.56%.

In Proteinase inhibitory activity, the IC\textsubscript{50} value for common fruit extracts were found to be highest in Vitis vinifera followed by Annona reticulata, Punica granatum, Malus domestica, Citrus sinensis, Ananas comosus, Manilkara zapota, Carica papaya, Citrullus lanatus and Musa cavendishii and values were 120.69µg/ml, 187.03µg/ml, 192.73µg/ml, 204.47µg/ml, 212.86µg/ml, 222.47µg/ml, 231.91µg/ml, 243.18µg/ml, 247.94µg/ml and 367.37µg/ml respectively. IC\textsubscript{50} value for the standard diclofenac sodium was 70.95µg/ml. The IC\textsubscript{50} values for common fruits were displayed in (Table.1). Plasma protein-derived mediators play important role in the activation, vasodilation of white blood cells and attempts at healing during inflammation by connective tissue replacement of damaged tissue with angiogenesis and fibrosis.

![Graph showing Proteinase inhibitory activity of common fruit extracts.](image-url)
Table 1: IC50 Values Of Common Fruit Extracts

<table>
<thead>
<tr>
<th>Fruit extracts Denaturation</th>
<th>Albumin (IC50 µg/ml)</th>
<th>Proteinase Inhibition (IC50 µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. vinifera</em></td>
<td>114.03</td>
<td>120.69</td>
</tr>
<tr>
<td><em>A. reticulata</em></td>
<td>162.44</td>
<td>187.03</td>
</tr>
<tr>
<td><em>P. granatum</em></td>
<td>171.08</td>
<td>192.73</td>
</tr>
<tr>
<td><em>M. domestica</em></td>
<td>183.09</td>
<td>204.47</td>
</tr>
<tr>
<td><em>C. sinensis</em></td>
<td>192.19</td>
<td>212.86</td>
</tr>
<tr>
<td><em>A. comosus</em></td>
<td>206.38</td>
<td>222.47</td>
</tr>
<tr>
<td><em>M. zapota</em></td>
<td>219.37</td>
<td>231.91</td>
</tr>
<tr>
<td><em>C. papaya</em></td>
<td>235.05</td>
<td>243.18</td>
</tr>
<tr>
<td><em>C. lanatus</em></td>
<td>240.46</td>
<td>247.94</td>
</tr>
<tr>
<td><em>M. cavendishii</em></td>
<td>360.88</td>
<td>367.37</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>67.44</td>
<td>70.95</td>
</tr>
</tbody>
</table>

Linear regression analysis was used to calculate IC50 value.

The previous phytochemical studies proved that the methanolic extract of above fruits contain carbohydrates, proteins, glycosides, steroids, terpenoids, polyphenols, flavonoids and tannins.[12] Previous studies have also reported that some of the bioactive compounds of the fruits like flavonoids, terpenoids, tannins and related polyphenols contribute significantly to the antioxidant activity.[12][15] Biochemical investigations have also shown that flavonoids can inhibit both cyclooxygenase and lipoxygenase pathways of arachidonic metabolism depending upon their chemical structures.[16],[17] Terpenoids may affect different mechanism relevant to inflammations arising in response to varied etiological factors.[18]

**CONCLUSIONS**

In Inhibition of albumin denaturation activity, the IC50 values were found to be highest in *Vitis vinifera* followed by, *Annona reticulata*, *Punica granatum*, *Malus domestica*, *Citrus sinensis*, *Ananas comosus*, *Manilkara zapota*, *Carica papaya*, *Citrullus lanatus* and *Musa cavendishii*. The same trend was seen in proteinase inhibitory activity of ten fruit extracts. The results obtained in the present investigation indicate that all the fruits were a potential source of Anti-inflammatory agents. The bioactive compounds present in these fruits may function as blockers, suppressors, or modulators of the inflammatory response. Since the ancient times, plants have played a significant role in human health care and disease management. Traditional plants exerts great role in discovery of new drugs. Majority of human population worldwide is getting affected by inflammation related disorders. In the present study, the presence bioactive compounds such as flavonoids, terpenoids, tannins and polyphenols might be helpful in the treatment of inflammation related disorders. Hence the
A detailed study is required for the isolation of single compound and development of suitable formulation which would be beneficial against inflammatory disorders.

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REFERENCES


