Antibacterial and Antioxidant activity of Benincasa hispida using Hydrogen peroxide scavenging model

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ABSTRACT
The seeds of Benincasa hispida commonly known as ash gourd belonging to Cucurbitaceae family is employed as a main ingredient in kusmanda lehyam in Ayurvedic system of medicine. The seeds of Benincasa hispida mashed with milk or the various preparations from the pulp of fruit in the form of sweetmeats, like Kusmanda paka and petha are commonly used as a general tonic, aphrodisiac, rejuvenative and also a brain tonic. The plant of Benincasa hispida was collected from (Central Council for Research in Ayurveda & Siddha, Dept. of ayush, Ministry of Health & F.W, Govt. of India New Delhi) Govt. Central Pharmacy Annex, Ashoka Pillar, Jayanagar Bangalore. The extracts were subjected to Antioxidant activity by In hydrogen peroxide-scavenging model, model acetone extract showed scavenging (80.1%) of hydrogen peroxide and chloroform extract showed ( 79%) in comparison with ascorbic acid ( 94.5% ). Antioxidant activity was carried out for extract (acetone, chloroform, and aqueous extracts) with Hydrogen peroxide-scavenging model. Acetonic extract showed more scavenging (80.1%) of Benincasa hispida and least scavenging was done by aqueous extract of (43%). After comparison with the standard drug (Ascorbic acid) showed (94.5%). From this it was concluded that acetone extract of Benincasa hispida showed high scavenging activity. Conclusively, the result revealed that Benincasa hispida seeds has antimicrobial activity & antioxidant activity which may be due to the presence of alkaloids, phenolic compounds, flavonoids constituents present in the sample.

1. Introduction

Benincasa hispida (Thunb.) of cucurbitaceae family commonly known as kushmanda, winter melon, wax gourd is used in Ayurvedic system of medicine. It is cultivated throughout the plains of India & on the hills up to 1200 meter altitude as a vegetable. White gourd, the best vegetable fruit, & the medicine for Pitta personality has remarkable therapeutic value in Pitta ailments, epilepsy, bleeding & insanity [1]. The continuous formation of free radicals in humans’ body can be controlled naturally by different beneficial compounds known as antioxidants[2]. Oxidative stress can be caused in result of free radicals formation [3]. Aging and different chronic diseases including diabetes, cancer and cardiovascular diseases could be caused by oxidative stress[4]. Free radicals are stabilized or deactivated by antioxidants before they attack cells. Antioxidants are important factor to maintain optimal cellular and human body health. Plants are reported to contain flavonoids, triterpenes, vitamin-c which are responsible for the antioxidant activity. Highly reactive free radicals and oxygen species those are present in human body that causes degenerative processes by oxidising nucleic acid in the cells. In addition, involvement of oxygen derive free radicals such as superoxide anions, hydrogen peroxide and hydroxyl radicals are well established in the injury of gastric mucosa and in the other models of gastric mucosal damage induced by nonsteroidal anti-inflammatory drugs and H.pylori ethanol and feeding restriction stress[5]. On the other hand, the expectorant effect of Benincasa hispida seeds extract due to facility of mucus secretion which prevents gastric ulcer was pointed out by Kim and Shin and Grover et al., [6-7]. In addition, Benincasa hispida seeds extract also could enhance immunoreactions result in histamine secretion inhibition [8-9].

2. Materials & methods

2.1 Plant authentication


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2.2 Extraction

The extraction was done by successive solvent extraction method using the various solvents like Acetone, chloroform, water. The seeds of *Benincasa hispida* were dried under shade & than powdered with a mechanical grinder. The powder was passed through sieve no. 40 & store in an airtight container for further use. The dried powdered seed of *Benincasa hispida* was defatted with petroleum ether (60-80°C) in a soxhlet apparatus. The defatted powder material these obtained was further extract with chloroform, acetone, water. The solvent were removed by distillation under reduced pressure & the resulting semisolid mass was flask evaporated[10].

2.3 Antimicrobial Activity

**Preliminary screening for Antibacterial activity**

<table>
<thead>
<tr>
<th>Preparation of Assay Media</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef extract</td>
<td>4.0</td>
</tr>
<tr>
<td>Peptone</td>
<td>5.0</td>
</tr>
<tr>
<td>Agar</td>
<td>20.0</td>
</tr>
<tr>
<td>Distilled water</td>
<td>q. s. 1000 ml</td>
</tr>
</tbody>
</table>

The above mentioned quantities of different ingredients were accurately weighed and dissolved in appropriate amount of distilled water. The prepared media was sterilized by autoclaving at 121°C for 15 minutes.

**Procedure**

The petridishes were thoroughly washed and sterilized in hot air oven at 160°C for one hr. Inoculum was added to 30 ml of sterile nutrient agar medium and was poured into sterile petridishes for solidifying. Bores were made on the medium using sterile borer. 0.1 ml of test solution was added to the respective bores, 0.1ml of the Ampicillin at a concentration of 100 μg/ml 0.1 ml was taken as standard reference. A control having only DMSO in the cup was maintained in each plate.

The petridishes were kept in the refrigerator at 40°C for 45 minutes for diffusion to take place. After diffusion, the petridishes were incubated at 37°C for 24 hr and zones of inhibition were observed and measured using a scale.

Antibacterial activity of all the Extracts was carried out against all two microorganisms. The same media was used both for subculturing and for estimating antibacterial activity. All the reading was taken in triplicate and is reported in Standard Error Mean (± SEM). [11].

2.4 Antioxidant Activity

**Scavenging of Hydrogen Peroxide**

The antioxidant activity of the different extracts of *Benincasa hispida* was assessed on the basis of their hydrogen peroxide scavenging ability. The standard ascorbic acid and the extracts were prepared in phosphate buffer, pH 7.4. Sample and standard (0.5) were taken in the different test tubes and to each test tube; 0.6 ml hydrogen peroxide solution (2mM hydrogen peroxide in phosphate buffer, pH 7.4) was added. A control was prepared by replacing the sample/standard with phosphate buffer. These solution were kept at room temperature for 10 min. The absorbance was measured at 230nm against the blank solution containing phosphate buffer without hydrogen peroxide. All the samples were prepared and assayed in triplicate and averaged. The percentage inhibition was calculated using the below formula

\[
\text{Percentage inhibition} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}} \times 100}{\text{Absorbance}_{\text{control}}}
\]

Where:

Control – Hydrogen peroxide in phosphate buffer
Sample – Standard or extract solution in methanol

The antioxidant activity of the plant extracts was expressed as IC50. The IC50 value is defined as the concentration (in pg/ml) of extracts that inhibits the formation of hydrogen peroxide radicals by 50 %. All the tests were performed in triplicate and the graph was plotted with average of three observations [12].
3. Result

*Antibacterial Activity*

Table 3.17 Evaluation of antibacterial activity

<table>
<thead>
<tr>
<th>Plant</th>
<th>Treatment</th>
<th>Dose (mg/ml)</th>
<th>Zone of inhibition (mm) (Mean±SEM)</th>
<th>Gram negative bacteria</th>
<th>Gram positive bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benincasa hispida</td>
<td>Acetone extract</td>
<td>300</td>
<td>5.33±0.76**</td>
<td>5.33±0.90**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>400</td>
<td>6.66±0.76**</td>
<td>6.43±0.90**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>8.66±0.76**</td>
<td>8.76±0.90**</td>
<td></td>
</tr>
<tr>
<td>Benincasa hispida</td>
<td>chloroform extract</td>
<td>300</td>
<td>3.66±0.76**</td>
<td>3.66±0.83**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>400</td>
<td>4.33±0.76**</td>
<td>4.0±0.83**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>5.66±0.76**</td>
<td>6.33±0.83**</td>
<td></td>
</tr>
<tr>
<td>Benincasa hispida</td>
<td>Aqueous Extract</td>
<td>300</td>
<td>4.66±0.76**</td>
<td>4.33±0.73**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>400</td>
<td>5.0±0.76**</td>
<td>6.0±0.73**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>6.66±0.76**</td>
<td>7.5±0.73**</td>
<td></td>
</tr>
<tr>
<td>Benincasa hispida</td>
<td>Standard (cefotasime sodium)</td>
<td>25 µg/ml</td>
<td>24.6±0.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2ml</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Benincasa hispida</td>
<td>Blank</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The value represents mean ±S.E.M, One-way ANOVA followed by Dunnet test through Instat software, compare all vs. standard applied.
Graph 1 – Graphical representation of Antibacterial activity against *E. Coli*

Zone of inhibition of *Benincasa hispida* extracts against *E. coli*

Acetone extract

Chloroform extract

Aqueous extract
Zone of inhibition *S. aureus*

Graph 2– Graphical representation of Antibacterial activity against *S. Aureus*

Zone of inhibition of *Benincasa hispida* extracts against *S. Aureus*

Acetonic extract
Table 3.18 Antioxidant activity of *Benincasa hispida* using Hydrogen peroxide scavenging model

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>% Inhibition of Antioxidant activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ascorbic acid</td>
</tr>
<tr>
<td></td>
<td>Acetone extract</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>20</td>
<td>20.5</td>
</tr>
<tr>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>60</td>
<td>54.6</td>
</tr>
<tr>
<td>80</td>
<td>68.5</td>
</tr>
<tr>
<td>100</td>
<td>94.5</td>
</tr>
</tbody>
</table>

% Antioxidant activity using Hydrogen peroxide-scavenging model

Graph 3 - Graphical representation of Hydrogen peroxide-scavenging model

Conclusion

Antioxidant activity was carried out for extracts (acetone, chloroform, and aqueous extracts) with Hydrogen peroxide-scavenging model. Acetone extract showed more scavenging (80.1%) of *Benincasa hispida* and least scavenging was done by aqueous extract of (43%). After comparison with the standard drug (Ascorbic acid) showed (94.5%). From this it was concluded that acetone extract of *Benincasa hispida* showed high scavenging activity. Conclusively, the result revealed that *Benincasa hispida* seeds has antimicrobial activity & antioxidant activity which may be due to the presence of alkaloids, phenolic compounds, flavonoids constituents present in the sample.

Conflict of interest statement

We declare that we have no conflict of interest.

Reference:

11. Lin Jiang B.S., Fujian Agricultural and Forestry University, China, 2009 August 2011.