Original article

In vitro Study on Total Phenolics and Antioxidant Activities of the Fruits Crude Extracts of Annona squamosa Collected from Al Jabal Al Akhdar, Sultanate of Oman

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ABSTRACT
Abstract: The major sources of natural antioxidants are plants and plant products. Medicinal plants may have different physiological action on human health. Natural antioxidants are the reduce the risk of different illnesses such as cardiovascular diseases, intestinal cancer and infections symptoms. The aim of this work was to prepare different crude extracts from the fruits of Annona squamosa (AS) and evaluate their antioxidant activity and total phenolics content of the selected plant species collected from Jabal Al Akhdar, Sultanate of Oman. Soxhlet extraction method was used to prepare the crude extract from the fruits powder samples of the selected plant species with methanol. The prepared crude extract was completely defatted with water. Then it was extracted individually with different polarities of solvents with increasing polarity such as hexane, chloroform, ethyl acetate and butanol. The prepared different fruits crude extracts of AS were used for the determination of total phenolics content through Folin-Ciocalteu reagent method (FCR). Similarly, all fruits crude extracts from AS were used for the evaluation of antioxidant activity by using 2,2-Diphenyl-1-picyrylhydrazyl method (DPPH). The results of total phenolics content in the fruits crude extracts were in the range of 76.5 to 635.75 mg/100 gm of dry powder samples. The highest antioxidant activity of different polarities crude extracts isolated from the fruits of AS was in hexane crude extract and lowest was chloroform crude extract and the order was found hexane > methanol > butanol > water > ethyl acetate > chloroform crude extract. In conclusion, isolated all crude extracts from the fruits of AS showed significant amount of total phenolics and strong antioxidant activity. Therefore, it could be used as a medicine for the treatment of different diseases.

Introduction

Annona squamosa (AS) is a medicinal plant belonging to the family Annonaceae. The English name is Sugar Apple. In Oman, it is called Almofataf. This plant is native to America and the West Indies. This fruits of the plant is very tasty and high nutritive value. Now it is commercially cultivated all over the world such as Indonesia, Thailand, Taiwan and India [1-3]. Similarly, several varieties are available in Oman. Most of the varieties are found at Al Jabal Al Akhdar, Nizwa, Sultanate of Oman. It is a medium plant growing up to 3-7 meter in height. Its fruits are round to conical shape. The leaves occur singly and it the edges are without teeth. It is mainly grown in gardens for its fruits and ornamental value [4].

AS is consider a useful medicinal plant. Traditionally all parts of this plant are used for the treatment of different diseases [5]. It is used as food and as a flavouring agent (Anon, 1986). Mainly, it is used as medicine for the treatment of canceous tumours, insect bites, and other skin complaints [6]. In some countries they used it for the treatment of toothache [7]. The powdered seeds from the fruits are toxic so it is used to kill head-lice and fleas [8]. The tree is a good source of firewood [8].

Therapeutically, it is used for the treatment of wide variety of curable and incurable diseases. The leaves have been shown to have anti-diabetic properties [9]. It is also shown very high hepatoprotective activity [10]. The leaves and fruits are also used as anti-depressants, epilepsy and in spinal cord disorders [3-4]. The fruit of AS is very delicious, haematinic, cooling, act as sedative, stimulant as well as functioning as expectorant and tonic [11]. The seeds of AS are used as an abortificient and insecticidal [12-13]. The roots are powerful purgatives and are also used in dysentery [8]. The most important chemical compounds present in this plant are steroid, terpenoid, glycoside, alkaloid, flavonoids, saponin and phenolic compounds [3-10]. The literature search reveals that no scientific data is available on the Omani AS (Sugar Apple). Therefore, the aim of this work is to prepare different polarities crude extracts from the fruits of AS and evaluate their total phenolics content and antioxidant activity of the selected species collected from Al Jabal Al Akhdar, Sultanate of Oman.
2 Materials and methods

1.1 Materials

Hexane, chloroform, ethyl acetate and acetic anhydride were used in the present study obtained from Scharlau, European Union. Butanol, ammonium carbonate and DPPH were collected from Sigma-Aldrich, Germany. Methanol was obtained from Emsure, Germany. Heavy duty Blender machine (Japan, Super Deluxe, and India) was used for grinding of fruits samples of AS. Rotary evaporator (Yamato, Rotary Evaporator, and Model - RE 801) was used for the evaporation of different solvents. The absorbance was measured by UV-1800 Shimadzu spectrophotometer (Japan).

1.2 Plant Samples

The ripe fruits samples of AS were collected from Al Jabal Al Akhdar, Nizwa, Sultanate of Oman. The harvesting date and time were on December, 2013 and at 4 pm. The collected ripe fruits samples of AS was packed in polyethylene bags and transported to the house for cleaning. Then the whole ripe fruits samples were kept at room temperature for processing. The morphological identification was done by Prof. Zahirul Islam, Department of Botany, University of Dhaka, Dhaka, Bangladesh and the voucher number (239) was deposited at the herbarium of the Institute of Biological Sciences, Faculty of Science, University of Dhaka, Dhaka, Bangladesh.

1.3 Preparation of samples

At first the ripe fruits samples were washed with clean water. The water was dried at room temperature and cut into small pieces by knife. The whole small pieces of fruits of AS were put on newspaper and dried at room temperature for two week. The dried samples were transported to the laboratory for grinding. The dry fruits samples were grinding by a heavy duty blender machine. The obtained powder samples were used for the extraction by using Soxhlet extractor.

1.4 Extraction procedure from fruits samples

Dry powder fruits samples (201.26 gm) were extracted with methanol solvent (500 ml, 48 h) by using Soxhlet extractor. After extraction from the fruits samples, the solvent part was collected from the round bottom flask and evaporated at low temperature and pressure using rotary evaporator to give crude methanol extract (22.19 gm). The methanol crude extract (8.36 g) was dissolved in water and extracted with different polarities the crude extracts of AS was analyzed by the Folin-Ciocalteu method [14].

1.6.1 Total Phenolic Acid Analysis

The determination of total phenolic content of different polarities the crude extracts of AS was analyzed by the Folin-Ciocalteu method [14].

1.6.2 Preparation of Standards

Gallic acid standard was used to calculate the total phenolic content, which was expressed as gallic acid equivalent (GAE). Gallic acid standard curve was constructed through the known concentrations of gallic acid. Gallic acid stock solution was prepared by dissolving 25 mg of gallic acid in 100 ml of methanol. The standard solutions were prepared in five different concentrations, which were 12.5, 25, 50, 100 and 200 µg/ml. For the preparation of gallic acid standard curve, each concentration (1 ml) was pipetted into a separate test tube. Then 10% Folin-Ciocalteu reagent (1.5 ml) was added and mixed well by hand for about 1 min. The mixture was kept at room temperature for 3-8 minutes and 6% anhydrous Na2CO3 solution was added to it. Again the mixture was mixed thoroughly for another 1 min and incubated in water bath at 45°C for 15 min. The absorbance of the gallic acid standard solution was measured by UV-visible spectrophotometer at fixed wavelength 760 nm.

1.6.3 Total phenolics content

All six crude extracts from the fruits of AS were used for the determination of total phenolics content through Folin-Ciocalteu reagent with external calibration with gallic acid. Each crude extract was taken in a separate test tube and added 1.5 ml of Folin-Ciocalteu reagent. The whole contents mixed thoroughly by hand. Then the test tube kept in dark place for 3-8 min. After that, 1.5 ml of 6% sodium carbonate was added to it. The mixture was incubated in water bath at 45°C for 15 min. The absorbance was measured at 760 nm using UV-visible spectrophotometer. The concentration of the total phenolics was determined as mg of gallic acid equivalent by using an equation obtained from gallic acid calibration curve. The estimation of phenolics compounds in the fractions was carried out in triplicate and the results were averaged.

3 Results

The fruits powder samples of AS were used for the extraction with methanol through Soxhlet method. Methanol crude extract was dissolved in water and extracted with different polarities of solvents like hexane, chloroform, ethyl acetate, butanol and methanol to give hexane (1.56 gm), chloroform (1.79 gm), ethyl acetate (1.98 gm), butanol (0.93 gm) crude extracts. The
remaining water part was evaporated by rotary evaporator to give the water extract (2.45 gm). All the six crude extracts were used for the determination of total phenolics and antioxidant activity by using FCR and DPPH methods.

The antioxidant activity of different crude extract such as hexane, ethyl acetate, chloroform, butanol and methanol obtained from the fruit of AS was determined through DPPH method [15]. The results of antioxidant activity of different polarities crude extract from fruits of AS are presented in Table 1 and Figure 1. All the crude extracts showed different percentage of inhibition. The highest antioxidant activity was found in hexan crude extract.

Table 1. Result of antioxidant activity of different fruits crude extracts of AS

<table>
<thead>
<tr>
<th>Concentration of crude extract (μg/ml)</th>
<th>Water</th>
<th>Methanol</th>
<th>Hexane</th>
<th>Ethyl acetate</th>
<th>Chloroform</th>
<th>Butanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>78.35±0.24</td>
<td>84.73±0.12</td>
<td>84.41±0.23</td>
<td>86.50±0.30</td>
<td>82.14±0.09</td>
<td>82.08±0.09</td>
</tr>
<tr>
<td>25</td>
<td>90.66±0.12</td>
<td>94.00±0.12</td>
<td>94.95±0.39</td>
<td>88.27±0.65</td>
<td>89.16±0.21</td>
<td>91.68±0.14</td>
</tr>
<tr>
<td>50</td>
<td>94.46±0.11</td>
<td>94.41±0.29</td>
<td>95.43±0.12</td>
<td>89.09±0.14</td>
<td>91.68±0.36</td>
<td>93.38±0.20</td>
</tr>
<tr>
<td>100</td>
<td>94.47±0.14</td>
<td>94.41±0.52</td>
<td>95.56±0.32</td>
<td>89.91±0.23</td>
<td>91.34±0.13</td>
<td>94.41±0.32</td>
</tr>
<tr>
<td>200</td>
<td>94.61±0.20</td>
<td>95.09±0.19</td>
<td>95.43±0.22</td>
<td>91.27±0.34</td>
<td>92.16±0.55</td>
<td>95.02±0.76</td>
</tr>
</tbody>
</table>

The antioxidant activity in different polarities crude extracts such as hexane, chloroform, ethyl acetate, water, butanol and methanol crude extracts from the fruits samples of AS at different concentrations was determined through DPPH using UV-visible spectrophotometer method [15]. The experimental results showed that the absorbance was increasing with increasing concentration of different polarities fruits crude extracts. The significant percentage of inhibition was observed all the crude extracts (Table 1 and Figure 1). The highest antioxidant activity was found in hexane crude extract among the six different polarities fruits crude extracts of AS. However, the lowest antioxidant activity was found in chloroform crude extract and order of antioxidant activity was found hexane>methanol>butanol>water>ethyl acetate>chloroform crude extract (Table 1 and Figure 1). The literature search reveals that there is no work has been done on the Omani AS species. Therefore, we are unable to compare our findings. But, several authors have been conducted on the mentioned study of other species all over the world and their findings are almost similar to our trend [3-7,8-12].

The total phenolics content of all crude extracts from the fruits of AS was determined through FCR method [14]. The results of total phenolics content in our present study in different polarities crude extracts are present in Table 2 and Figure 2. All the crude extracts from the fruits of AS contains significant amount of total phenolic compounds. The maximum amount of total phenolics was found in ethyl acetate crude extracts and the minimum was in water crude extract (Table 2 and Figure 2).

Table 2. Results of total phenolics contents from the fruits crude extracts of AS

<table>
<thead>
<tr>
<th>Different polarities crude extracts</th>
<th>Total Phenolics content (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>76.50±0.74</td>
</tr>
<tr>
<td>Chloroform</td>
<td>95.56±0.12</td>
</tr>
<tr>
<td>Hexane</td>
<td>159.50±0.33</td>
</tr>
<tr>
<td>Butanol</td>
<td>256.00±0.99</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>655.75±0.23</td>
</tr>
<tr>
<td>Methanol</td>
<td>1095±0.19</td>
</tr>
</tbody>
</table>

Figure 2. Results of total phenolics content from the fruits crude extracts of AS

W=water extract; C=Chloroform; H=Hexane; B=Butanol; E=Ethyl acetate; M=Methanol extract

4. Discussion

Since the ancient time, plants are considered as good sources for the discovery new drugs such as pharmaceutical ingredients and medicines [16]. AS is an indigenous plant which was chosen for this study belongs to the family Annonaceae. Various information on this plant facilitates the study due to its medicinal value [2-11]. Firstly, the crude extract was prepared from the fruits powder samples with methanol. Then the obtained methanol crude extract was dissolved in water and mixed well by hand until dissolved. The water dissolved part was used for fractionation with different polarities solvents such as hexane, ethyl acetate, chloroform and butanol to give hexane, chloroform, ethyl acetate and butanol crude extracts. The water was evaporated from the remaining water part to give the water crude extract. The highest yield of extraction from the fruits of AS was in hexane and lowest was in ethyl acetate.

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Conclusion

All the crude extracts from the fruits showed very high content of total phenolics and antioxidant activity. Therefore, according to our experimental findings all the crude extracts could be used as medicine for the treatment of different diseases. Further study will be needed for the isolation and characterization of bioactive compounds from fruits crude extracts of AS.

Acknowledgement

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Reference


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