Antioxidant and Antimicrobial Activity of Grape Seed Extract

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ABSTRACT
Recent studies have shown that procyanidins in grape seeds possess anti-inflammatory, anti-arthritic, anti-allergic and anticancer activities. It is also reported that it prevents heart disease and skin aging Besides inhibiting carrageen in dextran-induced hindpaw edema which stabilizes the capillary wall and improves visual performance in humans. The concentration of phenolic compounds in grapes depends on the variety of grapevine and is influenced by viticultural and environmental factors. The synthesis of flavonoid and non-flavonoid plant polyphenols such as stilbenes is increased in plant tissues following wounding or infection by pathogenic organisms. The present study is aimed at extracting red grapes seed with methanol and perform the Phytochemical tests using qualitative analysis. Furthermore Total Phenolic content by Quantitative analysis, Column Chromatography by GC-MS to confirm the secondary metabolites for seed, Antioxidant activity by DPPH, Hydrogen Peroxide and Total antioxidant capacity and Antibacterial activity for grape seed extract is also assessed.

Keywords: Grape seed, Procyanidins, Phytochemical Analysis, Antioxidant and Antimicrobial Property

ARTICLE INFO

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1. Introduction
Red grape is a fruiting berry of the deciduous woody vines of the botanical genus Vitis (Figure 1). Grapes can be eaten raw or they can be used for making wine, jam, juice, jelly, grape seed extract, raisins, vinegar, and grape seed...
oil (Patrice et. al., 2006). A study conducted to investigate the antibacterial activity of Vitis vinifera seed extracts against Gram positive bacteria Staphylococcus aureus and Bacillus subtilis and Gram negative bacteria such as Pseudomonas aeruginosa and Escherichia coli revealed the assay against the bacterial strains (E.Q. Xia et. al., 2010). Polyphenols can also reduce damage to DNA and production of free radicals in the body (BUB et. al., 2003). Many of the flavonoids found in grape juice, such as catechin, epicatechin, quercetin, and anthocyanins are known to have antioxidant, anti-inflammatory, and platelet inhibitory effects, as well as for being able to reduce LDL oxidation and oxidative damage to DNA, both in vitro and in animal studies (Frankel et. al., 1998 and Singletory et. al., 2003).

![Figure 1: Vitis vinifera (Red grapes)](image1)

The antimicrobial properties of plant extracts have shown promise for development of new drugs (Hakkinen, S, 2000). A study was conducted to measure the antibacterial activity of grape (Vitis vinifera) seed extract against Streptococcus mutans and Aggregatibacter actinomycetemcomitans (Mahkameh Mirkarimi, et. al., 2012). Vitis vinifera is used in prescriptions for cough, respiratory tract catarrh, subacute cases of enlarged liver and spleen, as well as in alcohol-based tonics (Wang, L, et. al., 2014). Using the sap of grapevines, European folk healers sought to cure skin and eye diseases (Monagas M et. al., 2003). Other historical uses include the leaves being used to stop bleeding, pain and inflammation of hemorrhoids (R. Carpenter, M. N, et. al., 2007). Unripe grapes are used for treating sore throats, and raisins given as treatments for consumption (tuberculosis), constipation and thirst. Ripe grapes are used for the treatment of cancer, cholera, smallpox, nausea, skin and eye infections as well as kidney and liver diseases (Shi J, Yu J, et. al., 2003).

2. Materials and Methods
   Preparation of Extract
   The Seeds were collected and dried in shade for over two weeks. The dried seeds were then ground into powder. 30grams of the dried seed powder was weighed and immersed in 300 ml of the solvents – methanol for 48hrs. After 48 hours, the extract was filtered and the filtrates were used for further phytochemical analysis.

   Phytochemical Test
   Preparation of Reagent
   1. 20% Ethyl Alcohol -20ml of Ethyl alcohol in 80ml of distilled water.

   Figure 2: Grape Seed Filtrate

   Figure 3: Mass Spectrometry analysis.

   Antioxidant Activity of the Extracts
   Test for Carbohydrates, Proteins, Alkaloids, Flavonoids, Terpenoids, Saponins and Renin were conducted using standard protocols.

   Antimicrobial Activity of the Extracts
   The seeds were collected and dried. The dried seeds were ground into powder and dissolved in methanol solvent and incubated for 48 hours and the extract were filtered and the filtrates were used for further phytochemical analysis (Figure 2).

   Phytochemical test
   Test for carbohydrates - reduction of fehling’s solution
   Brick red precipitate indicates the presence of carbohydrates (Figure 3)
Test for Proteins-Ninhydrin Test
Appearance of violet colour indicates the presence of proteins (Figure 4)

Test for Glycosides - Keller –Killani Test
A reddish brown colour is formed at the junction of two layers and the upper layer turns bluish green indicating the presence of glycosides (Figure 5).

Test for Tannins: A dark blue or green black colour appears which indicates the presence of tannins (Figure 6)

Test for Alkaloids: A yellow precipitate or yellow solution indicates the presence of alkaloids (Figure 7).

Test for Flavonoids: Appearance of white or yellow precipitate indicates the presence of flavonoids (Figure 8).

Test for Terpenoids
A reddish brown colouration formed in the interface shows positive results for the presence of Terpenoids (Figure 9).

Test for Saponins: Formation of stable foam indicates the presence of saponins (Figure 10).
Antioxidant activity of the extracts
DPPH free radical scavenging activity
The percentage inhibition of the DPPH radical by the samples was calculated according to the formula
\[
IP = \frac{[(AC(0) - AA(t))/AC(0))] \times 100
\]
Where, AC(0) is the absorbance of the control at t = 0 min; and AA(t) is the absorbance of the antioxidants at t = 16 min. (Table 2)

Hydrogen Peroxide Scavenging Activity
The percentage of H₂O₂ scavenging by the extract and standard compounds was calculated as follows:
\[
\% \text{ of inhibition} = \frac{(A \text{ of control} - A \text{ of test})}{A \text{ of control}} \times 100
\]
Where A of control is the absorbance of the control reaction and A of test is the absorbance of the sample extracts (Table 3)

Total Antioxidant Capacity
The absorbance of the aqueous solution of each was measured at 695 nm against a blank. Ascorbic acid was used as the standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid (Table 4).

Antimicrobial Activity of Grape Skin and Seed Extracts
Petriplates containing Muller Hinton Medium were seeded with 24 hours culture of bacterial cultures. Wells were made in each of these plates using sterile cork borer. Crude extracts were added into the wells and allowed to diffuse. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed with Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, by measuring the diameter of the inhibition zone formed around the well (Figures 12 – 15 & Table 5).

Column Chromatography
Samples were purified for GC-MS Analysis in order to determine compounds present in the purified sample (Figure 16).

Gas chromatography-MASS Spectroscopy
GC-MS identified the secondary metabolites present in grape seed (Table 6 & Figure 17). The peak area percentage and peak area coverage of the Grape Seed (GSD) is given below.
Table 1: Phytochemical Tests

<table>
<thead>
<tr>
<th>Phytochemical tests</th>
<th>Grape seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>-</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Resins</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 2: Antioxidant Assay by DPPH

<table>
<thead>
<tr>
<th>Name of the Sample</th>
<th>Control OD</th>
<th>Sample OD</th>
<th>Antioxidant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grape Seed</td>
<td>0.612</td>
<td>0.283</td>
<td>53.70</td>
</tr>
</tbody>
</table>

Table 3: Hydrogen Peroxide Antioxidant Activity

<table>
<thead>
<tr>
<th>Name of the sample</th>
<th>Control OD</th>
<th>Sample OD</th>
<th>Antioxidant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grape Seed</td>
<td>0.86</td>
<td>0.56</td>
<td>34.8</td>
</tr>
</tbody>
</table>

Table 4: Total Antioxidant Capacity

<table>
<thead>
<tr>
<th></th>
<th>Grape seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control OD</td>
<td>0.54</td>
</tr>
<tr>
<td>Sample OD</td>
<td>0.53</td>
</tr>
<tr>
<td>Antioxidant (%)</td>
<td>1.85</td>
</tr>
</tbody>
</table>

Table 5: Zone of Inhibition

<table>
<thead>
<tr>
<th>S. aureus (mm)</th>
<th>E. coli (mm)</th>
<th>B. subtilis (mm)</th>
<th>P. aeruginosa (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grape Seed</td>
<td>2mm</td>
<td>2mm</td>
<td>10mm</td>
</tr>
</tbody>
</table>

Table 6: GCMS for Grape Seed

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>RT (Min.)</th>
<th>Compound Name</th>
<th>Peak Area</th>
<th>Peak Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.17</td>
<td>16-Hexadecanoyl hydrazide</td>
<td>24649440</td>
<td>41.51</td>
</tr>
<tr>
<td>2</td>
<td>7.22</td>
<td>d-Glucitol, 2,5-anhydro-1-O-octyl-</td>
<td>3976000</td>
<td>6.70</td>
</tr>
<tr>
<td>3</td>
<td>8.33</td>
<td>Palmitic anhydride</td>
<td>24546096</td>
<td>41.34</td>
</tr>
<tr>
<td>4</td>
<td>8.82</td>
<td>Cholestan-3-ol,2-methylene-,[3a,5a]-</td>
<td>6210816</td>
<td>10.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>59382352</td>
<td>100.00</td>
</tr>
</tbody>
</table>

5. References


