Evaluation Of Antibacterial And Antioxidant Properties Of Different Varieties Of Grape Seeds(Vitis Vinifera L.)

A.Kulastic Jassy, S.Dillwyn, M.M. Pragalyaashree, D. Tiroutchelvame

Abstract—Grape seeds are the by-products from fruit juice and wine industries and are generally disposed as waste. These seeds have abundance of phytochemicals and can be utilized as a potential raw material from which dietary supplements can be produced. The present study investigated the influence of solvent (ethanol and water at different concentration) on different varieties of grape seeds (Sauvignon blanc, Medika, Symphony, Shiraz) in extracting the antioxidants and comparing the antioxidant activities of the varieties. The grape seeds were made into powder form and the fatty material was extracted using petroleum ether at 80°C for 6 h in a soxhlet extractor. The defatted powder was extracted with solvent extraction method using water and ethanol in various concentrations (50%, 60% and 70%) at a temperature of 60°C on different varieties of grape seeds. Antibacterial activity was tested for these extracts by disc diffusion method against Escherichia coli and Staphylococcus aureus. Among the various varieties, ethanolic extract of Symphony showed better zone of inhibition in Escherichia coli whereas ethanolic extract of Shiraz and Sauvignon blanc showed better zone of inhibition in Staphylococcus aureus. Antioxidant activity was determined using DPPH assay and it was found that the results were highly dependent on the variety of grape seeds. All the extracts proved remarkable antioxidant activity ranging from 53% to 76.5%. It was concluded that the Shiraz variety extracted with ethanol (60% concentration) showed higher antioxidant value compared to the other varieties.

Index Terms—phytochemicals, soxhlet extraction, anti-bacterial activity, zone of inhibition, DPPH assay

1 INTRODUCTION

Grape (Vitis vinifera L.) fruit is the largest produced fruit crop in the world and is mostly used in food processing industries for the production of fruit juices and wine. It is reported that the skin, pulp and seeds of grapes contain several phenolic compounds [1] and possess antioxidant properties. These properties have a significant role in lowering the risk of heart diseases and cancer prevention by reducing the oxidation of low-density protein [2,3]. It was reported that the variety of grapevine, geographical and climatic factors, cultural practices and ripening stages have a greater influence on the amount of phenolic content of grapes [4,5]. The by-product produced after wine making process is mostly grape pomace and seeds. In that, grape pomace makes about 10 to 20% weight of the grapes [6]. Large amounts of grape seeds are obtained from the wastes of this industry and represent about 50% to 60% of the raw fruit. Grapes seed contains oil, which is rich in Vitamin E hence it is used for nutritional supplement preparation and pharmaceuticals purposes [7]. Grape seeds are rich sources of monomeric phenolic compounds such as, (-)-epicatechin, (+)-catechins, (-)gallocatechin, (+)-gallocatechins and their dimeric, trimeric, tetrmeric and oligomeric proanthocyanidin [8,9].

Solvent extraction method was used in order extract the antioxidants effectively. The factors that influence the extraction of phenolic compounds are the nature and type of solvent, solid-liquid ratio, temperature, extraction time, extraction method, particle size, flow rate, time and conditions of sample storage and the presence of interfering substances. Selection of solvent plays a major role in extraction process as it influence the solubility of compounds present [10]. According to literature, the most frequently used solvents with different efficiency in extraction of plant phenolic are ethanol, methanol, acetone, propanol, dimethylformamide, ethyl acetate, their mixture and their mixture with water[11]. The current study was carried out to investigate the efficiency of different concentration of solvent (different aqueous solutions of ethanol: 50, 60 and 70% with water) on the extraction of anti-oxidant compounds from different varieties of grape seeds and its action against pathogenic microorganism.

2 MATERIALS AND METHODS

2.1 Raw material and Sample preparation

Different varieties of grape seeds (Sauvignon blanc, Medika, Symphony, Shiraz) were collected from fruit shops in Coimbatore, Tamil Nadu. Laboratory grade petroleum ether (68%v/v), ethanol (99.9% v/v), nutrient agar, agar, sterile disk, 2,2-diphenyl-1-picrylhydrazyl were purchased from a scientific company (Coimbatore, Tamil Nadu). Different varieties of grape seeds were cleaned manually and washed with tap water to re drained. The seeds were then air dried in order to remove the moisture completely and to prevent spoilage. The dried seeds were then ground into fine powder in a mixer grinder for 5 minutes with a time interval of 30 s in order to avoid heating of sample [12]. The ground samples were then packed in air tight containers and stored at room temperature until further analysis.
2.2 Soxhlet extraction
About 10g of each type of grape seed powder were packed and defatted with 100ml of petroleum ether in a round bottom flask at 80°C for 5 hours. After the extraction process petroleum ether containing extracts were removed, the grape seeds were then dried to remove the residual petroleum ether. The seed powder was then packed in an air tight container until further process [13].

2.3 Solvent extraction
Grape seed powder (0.5g) of each variety was extracted with 20 ml of ethanol in a seal flask. The concentration of ethanol used was 50%, 60% and 70%, respectively. Each set of sample were mixed with various concentration of ethanol and kept in a water bath for 4 hrs at 70°C. After 4 hours, once the extraction process was done the extracts were centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected and then stored until further analysis[10].

2.4 Anti-bacterial activity
Anti-bacterial activity of solvent extracts was determined by disc diffusion method. Nutrient agar was used as growth medium for the bacterial strains like Staphylococcus aureus and Escherichia coli. The stock cultures of Staphylococcus aureus and Escherichia coli were grown in nutrient broth for 22hr at a temperature of 37°C. Corresponding concentration of ethanol was used as control. The prepared nutrient agar was poured into petri plates and allowed to solidify and then the cultures were inoculated through swab method followed by placing the sterile disc. Plates were incubated at room temperature for 24 hours. The zone of incubation if present is measured in mm [14,15].

2.5 Antioxidant activity using DPPH for free radical scavenging
The anti-oxidant activity of different varieties of grape seed was estimated using DPPH assay. About 0.1 mM solution of 1, 1- diphenyl-2-picrylhydrazyl (DPPH) were used for the free radical scavenging activity of different varieties of grape seeds and 39.4 mg of DPPH were dissolved in ethanol to produce 0.1mM. About 1ml of 0.1mM DPPH solution was added with 3ml of each type of sample. Control was made with corresponding concentration of ethanol. The mixture was then vortexed and then incubated at room temperature for 30 min in dark. Then the absorbance was taken at 517nm in a UV-VIS spectrophotometer. The percentage of DPPH scavenging effect was calculated by using the following equation.

\[
\text{Percentage cytotoxicity} = \frac{\text{absorbance of control} - \text{absorbance of test}}{\text{absorbance of control}} \times 100
\]

3 RESULTS AND DISCUSSION

3.1 Antibacterial activity of grape seed extract against E. coli
Anti-bacterial activity of different varieties of grape seeds (Standard, Medika, symphony, Shiraz and Sauvignon blanc) was determined by disc diffusion method. Zone of inhibition is used to determine the activity of extracts against gram negative E. coli and the results are tabulated in Table 1. It was observed from the results that almost all varieties showed considerable amount of anti-bacterial activity against E. coli as shown in Fig.1. The grape seeds extracted with 70 % ethanol showed greater anti-bacterial activity against all other concentrations (50 and 60%). Compared with four varieties, the variety Shiraz showed larger zone of inhibition (17mm). The other varieties recorded a zone of inhibition of about 14mm (Standard) and 15 mm (Medika, symphony and Sauvignon blanc). The results confirmed with the research of Shoko et al., (1999) [16] for the antimicrobial activity of methanol extract from grape seeds.

3.2 Antibacterial activity of grape seed extract against Staphylococcus aureus
The results of anti-bacterial activity (zone of inhibition) of grape seed extracts at different concentration (50, 60 and 70%) against the gram positive Staphylococcus aureus is presented in the Table 2. It is clearly seen that the zone of inhibition for Staphylococcus aureus is low for the grape seeds extracted with 50% ethanol and 60% ethanol when compared with E. coli (Fig.2). The grape seed extracted with 70% ethanol showed larger zone inhibition except symphony. The variety Medika showed 22mm zone of inhibition, Shiraz and Sauvignon blanc exhibited 25mm of zone of inhibition [13]. The most effective concentration was 70% ethanol as reported by Gokturk-baydar (2003)[14]. Pure ethanol as a control exhibited comparatively low zone of inhibition on both bacterial cultures. The inhibitory effect of E. coli and Staphylococcus aureus by the different varieties of grape seed extracts was due to the presence of gallic acid, an active compound in grape seeds. The presence of three hydroxyl groups and the benzene rings substituent were found to be effective against the bacteria [17,18,19]. Similar results were reported by Shoko et al., (1999)[16] for the antimicrobial activity of methanol extract from grape seeds.

3.3 Antioxidant activity of the grape seed extracts
The antioxidant activity of grape seeds was determined using DPPH assay. Free radical scavenging activity of different varieties of grape seeds extracts (Standard, Medika, Symphony, Shiraz and Sauvignon blanc) were analysed using DPPH method and the results are given in Table 3. The scavenging activity of each variety at various concentrations was determined through observing the changes of absorbance caused due to reduction in DPPH [13,20,21].From the Fig.3, it is evident that the variety shiraz showed higher anti-oxidant activity among all the concentrations. Many studies on correlations between antioxidant activity and phenolic content have been reported. As a general rule the antioxidant capacity has been positively correlated with phenolic content [22,23,24,25]. The maximum scavenging activity of 76.57% was observed in shiraz (60%) and minimum scavenging activity of 53.69% was seen in Symphony (50%). The seed extracts attribute to the hydrogen donating ability and auto-oxidation of unsaturated lipids in food materials is caused by free radicals [26,27]. The antioxidants also interrupt the free radical chain of oxidation to provide hydrogen from the phenolic hydroxyl groups and thereby forming stable end product where further oxidation of lipid was not initiated. The data obtained from the research revealed that the extracts were free radical inhibitors and primary antioxidants that react with free radicals.

4 CONCLUSION
Different varieties of grape seeds were selected to determine
their antibacterial and antioxidant activity. The grape seeds were extracted with different concentration of ethanol (50%, 60%, and 70%) at 70°C was investigated. From the study on different varieties of grape seed extracts, it can be concluded that almost all type of seeds have considerable amount of antibacterial and anti-oxidant compound. Among the four varieties studied shiraz variety showed better antibacterial and antioxidant activity which might be due to the presence of more phenolic compounds in that variety compared to other varieties. Grape seeds which are considered as food industry waste can be converted into value added products like nutraceuticals, to exploit the major phenolic and antioxidant compounds.

### TABLE 1 ANTIBACTERIAL ACTIVITY OF GRAPE SEED EXTRACT AGAINST *E. COLI*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Concentration of extracts (%)</th>
<th>Zone of inhibition in (mm)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Standard</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>14</td>
</tr>
</tbody>
</table>

![Fig.1 Antibacterial activity of grape seed extracts against *E. coli*](image)

### TABLE 2 ANTIBACTERIAL ACTIVITY OF GRAPE SEED EXTRACT AGAINST *STAPHYLOCOCCUS AUREUS*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Concentration of extracts (%)</th>
<th>Zone of inhibition in (mm)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Standard</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>5</td>
</tr>
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<td>2</td>
<td>60</td>
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<td>3</td>
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Fig. 2 Antibacterial activity of grape seed extract against *Staphylococcus aureus*

**TABLE 3  ANTIOXIDANT ACTIVITY OF DIFFERENT VARIETIES OF GRAPE SEED BY DPPH ASSAY**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Grape varieties</th>
<th>Scavenging activity at various concentration of extracts</th>
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<tr>
<td></td>
<td></td>
<td>50%</td>
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<tr>
<td>1</td>
<td>Medika</td>
<td>71.60</td>
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<tr>
<td>2</td>
<td>Symphony</td>
<td>53.69</td>
</tr>
<tr>
<td>3</td>
<td>Shiraz</td>
<td>74.12</td>
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<tr>
<td>4</td>
<td>Sauvignon blanc</td>
<td>69.63</td>
</tr>
</tbody>
</table>

Fig. 3 Antioxidant activity of different varieties of grape seed
ACKNOWLEDGEMENT
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REFERENCES