ANTIMICROBIAL ACTIVITY OF GRAPE SEED EXTRACT

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ABSTRACT
Grape pomace (skin and seed) is produced during winemaking and is considered to be a waste product of wine industries. Grape seed is known to exhibit various bioactivities. In the present study, we determined antimicrobial activity of grape seed extract (GSE). Agar well diffusion assay was conducted to determine inhibitory effect of GSE against Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae isolated from urinary tract infection. S. aureus and E. coli was inhibited to high and least extent respectively by GSE. Poisoned food technique was performed to screen antifungal effect of GSE against Colletotrichum capsici. GSE was effective against mycelia growth of C. capsici (>65% inhibition). GSE can be used in the treatment of urinary tract infections and in the management of anthracnose of chilli.

Key words: Grape seed extract, Agar well diffusion, Urinary tract infection, Poisoned food technique, Colletotrichum capsici.

INTRODUCTION
Grape (Vitis vinifera L.) is one among the important and leading fruit crops grown worldwide. About 80% of the harvest is used in industries for wine making. The solid grape residues (pomace) namely skin and seeds are produced in large quantities by the winemaking industries. These are typically waste byproducts of wine industries. Grape seeds are around 15% of the solid waste produced in wine industries. Grape seed is a well-known dietary supplement and contains vitamins, minerals, and polyphenols. The abundant phenolic compounds from grape seed are catechins, epicatechin, procyanidin, and some dimmers and trimers. The polyphenols of grape seeds have been recognized for their beneficial role in...
human health. The grape seed is shown to exhibit bioactivities such as antioxidant, anti-inflammatory, anti-bacterial, anti-cancer, antiviral, cardioprotective, hepatoprotective, neuroprotective, antiaging and anti-diabetic. The oil extracted from grape seeds is used in cosmetic, culinary, pharmaceutical and medical purposes[1-6]. In the present study, we determined antimicrobial activity of grape seed extract (GSE) against urinary tract bacteria and *Colletotrichum capsici*.

**MATERIALS AND METHODS**

**Preparation of GSE**

Fully ripe grapes were purchased from a local shop in market of Shivamogga city. The grapes were crushed and seeds were separated. The seeds were washed well using clean water and dried in oven at 60°C. The seeds were powdered in a blender. 25g of grape seed powder was added to a conical flask containing 100ml of methanol and stirred well. The flask was left aside for 48 hours and occasionally stirred. The content of flask was filtered through Whatman No. 1 and evaporated to dryness in oven a 50°C[7].

**Antibacterial activity of GSE**

Agar well diffusion assay was performed to investigate antibacterial efficacy of GSE against three bacteria *viz.*., *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* from urinary tract infections. 24 hours old Nutrient broth (HiMedia, Mumbai) cultures of test bacteria were swabbed uniformly on sterile Nutrient agar (HiMedia, Mumbai) plates. Using sterile cork borer, wells of 8mm diameter were punched in the inoculated plates. 100µl of GSE (20mg/ml of 25% Dimethyl sulfoxide [DMSO; HiMedia, Mumbai]), reference antibiotic (Chloramphenicol, 1mg/ml of sterile distilled water) and DMSO (25%, in sterile water) were added to labeled wells and the plates were incubated for 24 hours at 37°C. The zones of inhibition around the wells were measured using a ruler[7].

**Antifungal activity of GSE**

In order to determine antifungal effect of GSE against *C. capsici*, we employed poisoned food technique with minor modifications[8]. The spore suspension of test fungus was inoculated at the centre of control plates (without extract) and poisoned Potato dextrose agar (HiMedia, Mumbai) plates (1mg extract/ml of medium) by point inoculation method using sterile inoculation needle. The plates were incubated for 5 days. The diameter of fungal colonies on control and poisoned plates was measured on 5th day. Antifungal activity of GSE was mentioned as
% Inhibition of mycelial growth = (C - T / C) x100, where C is the diameter of colonies on control plates and T is the diameter of colonies on poisoned plates.

RESULTS

Antibacterial activity of GSE

The result of antibacterial efficacy of GSE is shown in Table 1. GSE was found to inhibit all test bacteria but to a varied extent. Among bacteria, marked inhibition was observed in case of S. aureus whereas E. coli was inhibited to least extent. Inhibitory activity of reference antibiotic was higher than that of GSE. DMSO was not effective in inhibiting test bacteria.

Table 1: Inhibitory activity of GSE against test bacteria

<table>
<thead>
<tr>
<th>Test bacteria</th>
<th>Zone of inhibition in cm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GSE</td>
</tr>
<tr>
<td>E. coli</td>
<td>1.1±0.0</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>S. aureus</td>
<td>1.8±0.0</td>
</tr>
</tbody>
</table>

Antifungal activity of GSE

The GSE was found to inhibit mycelial growth of C. capsici and the result is shown in Table 2. The growth of C. capsici was drastically reduced on plates poisoned with GSE (with an inhibition of 68%) when compared to control plates.

Table 2: Inhibitory activity of GSE against C. capsici

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Colony diameter (cm)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.2±0.1</td>
<td>-</td>
</tr>
<tr>
<td>GSE</td>
<td>1.0±0.0</td>
<td>68.75</td>
</tr>
</tbody>
</table>

DISCUSSION

Urinary tract infections (UTIs) are one among the most common infections in community and hospital settings affecting individuals of all age groups. Several bacteria are known to cause UTIs among which E. coli is involved in most cases. The infections may involve single species or sometimes it may be polymicrobial in nature. UTIs are treated using antibiotics. However, most strains have already developed resistance against commonly used antibiotics. Hence, research on alternative disease treatment strategies is of much interest. It has been already shown that plants exhibit marked inhibitory efficacy against uropathogenic bacteria[9-16]. In the present study, we screened the efficacy of GSE against three bacterial isolates recovered from urinary tract infections. The GSE was more effective against S. aureus followed by K. pneumoniae and E. coli i.e., susceptibility to GSE was high in case of Gram positive bacterium when compared to Gram negative bacteria. Similar result was
observed in the study of Jayaprakasha et al.[17] where solvent extracts of grape seed powder inhibited Gram positive bacteria at lower concentration while Gram negative bacteria were inhibited at high concentration. In another study, Corrales et al.[18] showed the efficacy of GSE against food borne pathogens. It was observed that Gram positive pathogens were inhibited by GSE while Gram negative pathogens remained unaffected.

The term chilli refers to ripe fruit of the genus *Capsicum* (Solanaceae). Chilli is extensively grown for consumption, nutritional and economy purposes. Anthracnose is an important disease of chilli (*Capsicum annuum* L.). The disease results in drastic loss in yield and deterioration of fruit quality. The typical symptoms of anthracnose include sunken necrotic tissues with concentric rings of acervuli on chilli fruit. The disease is caused by various species of genus *Colletotrichum* among which *C. capsici* is an important pathogen. The management of disease is mainly based on the use of synthetic fungicides. However, the pathogen is reported to exhibit resistance against synthetic fungicides. Hence, search for cheaper, ecofriendly alternative strategies for disease control is of interest. Plants are one of the best alternatives for management of fungal diseases[8,19-21]. In the present study, we evaluated antifungal efficacy of GSE against *C. capsici* isolated from anthracnose of chilli. GSE was found to exhibit marked inhibition of mycelial growth of fungus. An inhibition of >65% of fungus by extract was observed in this study. In an earlier study, Mendoza et al.[22] showed inhibitory effect of anthocyanin rich extracts from grape pomace against phytopathogenic fungus *Botrytis cinerea*.

**CONCLUSION**

Grape pomaces are the major byproduct, often considered as waste, generated during winemaking process. The utilization of grape seeds is advantageous in terms of waste reduction and production of added value products. In the present study, the GSE was found to exhibit marked antibacterial and antifungal activity. The GSE can be used to treat urinary tract infections and to control anthracnose disease in chilli.

**ACKNOWLEDGEMENTS**

Authors are thankful to Head, Department of Microbiology, Principal, S.R.N.M.N College of Applied Sciences, Shivamogga and N.E.S, Shivamogga for providing facilities to conduct work.
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