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**Original Article**

# Preliminary Phytochemical Screening and Anti-bacterial Activity of Grape Seeds Methanolic Extract

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ABSTRACT

**Objective**: The aim of the study is to screen the phytochemicals present in the methanolic extract of grape seed and to determine its antibacterial activity against both gram-positive and gram-negative bacteria. **Methodology**: The present study involves extraction, preliminary photochemical analysis and antibacterial activity of grape seeds belonging to the family Vitaceae for its medicinal value. A qualitative photochemical analysis of methanol extract of grape seeds was performed to identify the presence of alkaloids, carbohydrates, cardiac glycosides, saponins, phenols, flavonoids, proteins, amino acids, tannins, anthraquinones and steroids. Disc diffusion method was conducted to determine the inhibitory effect of grape seed methanolic extract against four bacterial species such as gram positive, *Staphylococcus aureus; Streptococcus pyogenes* and gram negative, *Pseudomonas aeruginosa; Klebsiella pneumoniae*. **Results**: Preliminary phytochemical screening of methanolic extract of grape seed showed the presence of alkaloids, carbohydrate, tannins, flavonoids, proteins, terpenoids, anthraquinones and phenols. Whereas, cardiac glycoside, saponins, amino acids and steroids were absent. Maximum zone of inhibition was observed against *Streptococcus pyogene*(18mm), followed by *Klebsiella pneumoniae* (17mm) and *Pseudomonas aeruginosa* (16mm). It was also observed that grape seed methanolic extract showed only 14mm zone of inhibition against *Staphylococcus aureus*. Conclusion: As grape seeds found to have tannins, phenols and flavonoids, it can be a potent bioactive agent. The methanolic extract of grape seed was proven to be effective in inhibiting both Gram-positive and Gram-negative bacteria hence it was suggested that it is used in treating the infections as an antibiotics.

**Key words**: Grape seed, *Vitis vinifera,* methanolic extract, phytochemicals, antibacterial activity.

## 1. INTRODUCTION

Grape (*Vitis vinifera*), the member of Vitaceae family, is one of the widely cultivated and most important fruit crops in the world [1]. This plant is native to most of Europe and northern and central Asia, but has widely naturalized elsewhere in the world. Grape cultivation is believed to be originated in Armenia near the Caspian Sea in Russia, from where it spreads westward to Europe and eastward to Iran and Afghanistan. It was introduced in India in 1300 AD by Moghul invaders from Iran and Afghanistan[2]. Grapes can be

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categorized into grapes with edible seeds, seedless, wine grapes, table grapes, and raisin grapes. It is also one of the most commonly consumed fruits in the world both as fresh fruit and processed fruit. Grape therapy, also known as ampelo therapy is a form of naturopathic medicine or alternative medicine that involves heavy consumption of grapes, including seeds, and parts of the vine, including leave[4].About 80% of the grape production is used in juice-making, and also in this process seeds remained as by-product which was formulated for an animal feed. Grape seed is a well-known dietary supplement and contains vitamins, minerals, and polyphenols. They are also rich in sugars, flavonoids, anthocyanins and proanthocyanins, organic acids, tannin, mineral salts and vitamins [3]. In recent years, grape seed extract has become increasingly popular on the market as a nutritional supplement especially in the Australia, Korea, Japan and the United States because of their rich phenolic compounds and their beneficial effects on human health[5].The grape seed also shown to exhibit bioactivities such as antioxidant, anti-inflammatory, anti-bacterial, anti-cancer, antiviral, cardio protective, hepatoprotective, neuroprotective, antiaging and anti-diabetic. The oil extracted from grape seeds is also used in cosmetic, culinary, pharmaceutical and medical purposes [6,7].

Seeds, skin, and stem from grape exhibit different phenolic profiles. Seeds have the highest total phenolic content with flavanol as the most abundant compound[8]. As grape seeds are a natural source of flavanols, they can be added during wine fermentation to improve the antioxidant activity of wines. Catechin is usually the most important individual flavonol that present in both grape skin and seeds. The consumption of grape derived dietary flavonoids in the form of grape extracts and grape seeds powder has been shown to effectively suppress oxidative stress and prevent oxidative damage [9].

Rich phenolic compounds present in grape seeds have the ability to damage microbial cells by exerting an influence on the selective permeability of the plasma membrane, which results in the leakage of vital intracellular substances. Grape seed extract has exhibited as a promising source for the manufacture of new generation of antibacterial agents for dental use without exerting an influence on the biological equilibrium in the oral cavity [10].

The aim of this present study is to screen the phytochemicals present in the methanolic extract of grape seed and to determine its antibacterial activity against both gram-positive and gram-negative bacteria.

## 2. MATERIALS AND METHODS

**Collection of plant material:** The Grape (*Vitis vinifera*) was procured from local fruit market. Seeds were separated from grape fruits and dried in shadow. The dried seeds were ground to powder.

**Preparation of methanolic extract:** 5g of grape seed powder was dissolved in 100ml of methanol and kept in orbital shaker overnight at 120rpm. Then the mixture was filtered using whatmann No.1 filter paper and the filtrate was evaporated at room temperature to get dried powder. The yield of dried extract was calculated using the formula,

Yield (%) = (W1 X 100)/W2

Where, W1 was the weight of extract after evaporation and W2 was the dry weight of the sample.

**Preliminary phytochemical Screening:**

Phytochemical examinations were carried out for the methanolic extract as per the standard methods[11,12,13].

**Detection of Carbohydrates:**

**Fehling's Test:**

1ml of crude extract was hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

**Benedict's test:**

1ml of crude extract was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

**Detection of Proteins (Xanthoprotein Test):**

1ml of crude extract was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

**Detection of Amino acids (Ninhydrin Test):**

1ml of crude extract when boiled with 0.2% solution of Ninhydrin, would result in the formation of purple colour suggesting the presence of free amino acids.

**Detection of Alkaloids (Wagner's Test):**

1ml of crude extract was dissolved in dilute HCl. and filtered. Filtrates were treated with Wagner's reagent (iodine in potassium iodide). Formation of brownish/ reddish precipitate indicates the presence of alkaloids.

**Detection of Cardiac glycosides (Keller-Killani test):**

1ml of crude extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring on the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

**Detection of Saponins (Froth Test):**

1ml of crude extract was diluted with distilled water to 2ml and this was shaken vigorously for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

**Detection of Flavonoids (Lead Acetate test):**

1ml of crude extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

**Test for Tannins (Ferric chloride test):**

1ml of crude extract when mixed with ferric chloride solution would give blackish red colour which indicates the presence of tannins.

**Test for Terpenoids (Salkowshi test):**

1ml of crude extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 minutes. Development of a greyish colour indicates the presence of terpenoids.

**Detection of steroids (Liebermann Burchard test)**

1ml of crude extract was treated with few drops of acetic anhydride, boiled and cooled. To this conc. $H_2SO_4$ was added from the sides of the test tube. A brown ring at the junction of two layers and the upper layer turns green colour showed the presence of steroids.

**Detection of Anthraquinones (Ammonium hydroxide test):**

One drop of concentrated ammonium hydroxide was added to 1ml of crude extract. After two minutes, formation of red colour indicated the presence of anthraquinone

**Detection of phenols:**

1ml of crude extract was dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution was added. A dark green colour indicated the presence of phenolic compounds.

**Anti-bacterial activity**

The agar disc diffusion method was employed for the determination of antibacterial activities of the methanolic extract of grape seed powder [14]. The test organisms, *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*was spread on Mueller Hinton Agar plates. Filter paper discs (6 mm in diameter) were impregnated with 20 µl of 5mg/ml methanolic extract and placed on the inoculated plates. The plates were incubated at 37°C for 24h. The diameter of the inhibition zones were measured in millimeters. Streptomycin antibiotic discs were used as positive control and were evaluated for their antibacterial activities and their results were compared with grape seeds methanolic extract. Methanol was served as negative control.

## 3. RESULTS AND DISCUSSION

**Preparation of crude extract:**

The yield of grape seed methanolic extract was calculated and found to be **19.3%.**

The influence of different plant residues on extraction yield were evaluated and observed that the type of residue was more influential than the solvent system on extraction yield[15].The yield of ethanolic extract of lyophilized grape seed powder showed only 7.92%[16].

**Phytochemical screening:**

Preliminary phytochemical screening of methanolic extract of grape seed showed the presence of alkaloids, carbohydrate, tannins, flavonoids, proteins, terpenoids, anthraquinones and phenols.Whereas, cardiac glycoside, saponins, amino acids and steroids were absent (Table-1).

**Table 1: Phytochemical screening of grape seed methanolic extract**

| Phytochemical tests | Grape seed (Methanol extract) |
|---|---|
| Alkaloids | + |
| Carbohydrate | + |
| Cardiac glycoside | - |
| Tannins | + |
| Flavonoids | + |
| Proteins | + |
| Amino acids | - |
| Terpenoids | + |
| Saponins | - |
| Steroids | - |
| Anthraquinones | + |
| Phenols | + |

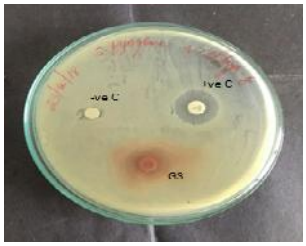
The ethanolic grape seed extract showed the presence of alkaloids, flavonoids, glycosides, polyphenol and flavonoids, tannins, and sterols [17]. The phytochemicals such as alkaloids, flavonoids, saponins and tannins are implicated as important bioactive agents and might be involved in the therapeutic use of grapes. Flavonoids are known to be synthesized by plants in response to microbial attack. Hence, it should not be surprising that they have been found to be effective antimicrobial substances against a wide array of microorganisms, when tested *in-vitro*[18]. The biological activities of the grape seed such as hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial, anticholinergic activities etc., are mainly due to the significant contribution of these secondary metabolites[19]. Tannins are also reported to have various physiological effects like anti-irritant, anti-secretolytic, antiphlogistic, antimicrobial and anti-parasitic effects. Phytotherapeutically, tannin containing plants are used to treat nonspecific diarrhoea, inflammations of mouth and throat and slightly injured skins[20]. Plant phenols are vital compounds used in eliminating the causes and effects of skin aging, skin diseases, and skin damage, including wounds and burns [21]. Phenols and flavonoids becomes very important plant constitutes because of their antimicrobial activity [22]. As grape seeds found to have tannins, phenols and flavonoids, it can be a potent bioactive agent.

**Antibacterial activity:**

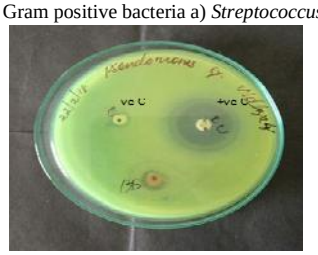
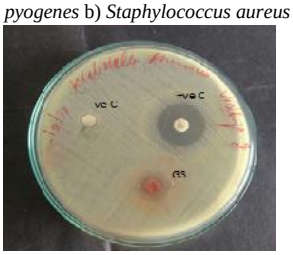
In the present investigation, the antibacterial activity of methanolic extracts of grape seed was performed against four bacterial species, *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Klebsiella pneumoniae.* Maximum zone of inhibition was observed against *Streptococcus pyogene*(18mm), followed by *Klebsiella pneumoniae* (17mm) and *Pseudomonas aeruginosa* (16mm). It was also observed that grape seed methanolic extract showed only 14mm zone of inhibition against *Staphylococcus aureus*(Table-2)(Fig-1)

**Table 2: Antibacterial activity of methanolic extract of grape seed**

| Bacterial species | Zone of inhibition | |
|---|---|---|
| | Streptomycin | Grape seed extract |
| *Staphylococcus aureus* | 23mm | 14mm |
| *Streptococcus pyogenes* | 16mm | 18mm |
| *Pseudomonas aeruginosa* | 21mm | 16mm |
| *Klebsiella pneumoniae* | 20mm | 17mm |




Gram positive bacteria a) *Streptococcus pyogenes* b) *Staphylococcus aureus*

Gram negative bacteria c)*Pseudomonas aeruginosa* d) *Klebsiella pneumonia*

**Fig 1: Antibacterial activity of methanolic extract of grape seeds**

GS - Methanolic extract of grape seed
+ve C - Streptomycin (standard)
-ve C - Methanol (control)

Plant polyphenols have been demonstrated as a potent antibacterial agent. The effect of grape seed extract has been evaluated against two most important bacterial strains in dental pathologies. *A. action mycetemcomitans* was first identified as a possible periodontal pathogen in 1975 in Localized Aggressive Periodontitis (LAP) and it's highly association with periodontal disease in adolescents has been established [23]. Various bacterial species exhibit different sensitivities towards phenolic compounds. The antibacterial activity of grape seed extract against *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans*, the two most important bacterial strain in pathologies. MIC and MBC for *Aggregatibacter actinomycetemcomitans* was 3.84 mg/mL and 7.68 mg/mL respectively [24].When testing the different efficacy of grape seeds, gram positive bacteria were more susceptible to grape seed extract than gram negative bacteria [25]. The inhibition of bacterial growth by grape seed extracts could be due to the presence of some active compounds which may act alone or in combination to inhibit bacterial growth [26]. Grape seed extract was effective in inhibiting *H. pyloriin vitro*, with highest efficacy and it was believed that asynergistic mode of action is more likely responsible for the extract's antimicrobial activity[27]. A study on antibacterial effect of grape seed extracts in common clinical and drug resistant isolates produced moderate zone of inhibition ranging between 11-15 mm among the 35 test common clinical isolates namely *Staphylococcus aureus, Escherichia coli, Klebsiella sp* and *Pseudomonas aeruginosa. E.coli* showed the highest susceptibility with zone ranging from 12-14 mm [28]. The antibacterial activity of grape seed extract against different *Campylobacter* strains were also observed[29] and it showed that 3 mg/ml of proanthocyanidin grape seed extract can be used for the inhibition of methicilin-resistant *Staphylococcus aureus*. This proves the high utilisation of grape seed extract in medicine [30].

## 4. CONCLUSION

This study showed that the methanolic extract of grapeseed possess various phytochemicals such as alkaloids, carbohydrate, tannins, flavonoids, proteins, terpenoids, anthraquinones and phenols. Grape seed extract was said to be natural antimicrobial compound. According to the results obtained from this study, it was suggested that grape seed must be used in treating the infections as an antibiotic for both gram positive and gram negative bacteria. Further research is needed to characterize isolated components and search for bioactive constituents with antimicrobial, antioxidant and other health- promoting activities.

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