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Preliminary Phytochemical Screening of Methanolic Extracts of Green Grapes and Black Grapes (*Vitis vinifera* L.)

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Abstract: ***The dietary consumption of grapes and its products is associated with lower risks of cardiovascular diseases and certain types of cancers. In present research, we have focused on comparing bioactive components present in green and black grapes (Vitis vinifera). Extraction of grapes was done by maceration process with methanol. Phytochemical screening was performed to detect the presence of alkaloids, flavonoids, anthocyanin, anthraquinones, saponins, steroids & terpenoids, carbohydrates, glycosides, phenol compound, protein and amino acids respectively. Black grape was found to be phytochemically richer as compared to green grapes. Results reveal that green grape extract contained alkaloids, flavonoids, anthraquinones, saponins, carbohydrates and phenol compound. Steroids and glycosides were not found in preliminary (qualitative) analysis of black grapes.***

Keywords: Green and black grapes, *Vitis vinifera,* maceration, phythochemical screening, preminilary (qualitative) analysis

1. Introduction

Nature has been a source of medicinal agents for 1000's of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine. Thus, plants continue to plays a main role in traditional medicine system for health care. The abundance of plants on the earth's surfaces has led to the growing interest in correlating phytochemical constituents of a plant with its pharmacological activity[2]. The bioactive compounds present in plants like alkaloids, flavonoids, tannins and phenolic compounds are the reason of its medicinal value that produce a definite physiological action on the body[4]. Recently, natural phytochemicals have been used instead of synthetic drugs[12]. The plant-based drugs have been shown to have very few side effects, cheap and easy availability [1]. Plants are known as a large source of natural phytochemicals which are contained of biological activities [7, 5]. Grapes, one of the most popular fruits and the most widely cultivated throughout the world, contain large amounts of phytochemicals including anthocyanins and resveratrol, which offer health benefits[11]. Grapes are one of the major dietary sources of anthocyanins, which are responsible for the colouring of black, red and purple grapes; however, they are lacking in white grapes. The beneficial health-related effects of phenolics in grapes are of importance to consumers, breeders and the grape industry. The main objective of present study is to compare the phytochemical compounds present in green grapes and black grapes.

2. Materials and Methods

2.1 Plant Material

The berries of green and black grapes were collected from farms of Nasik and were brought to lab and then washed and were further processed for extraction.

2.2 Extraction

Extraction was done by using maceration process. Grape berries (500gm) were grounded and dipped in solvent (methanol) and the mixture was left for four days with occasional shaking or stirring. The extract was then taken out and allowed to dry in oven. This process was repeated with the left over grounded residue of berries until the solvent runs clear. Same process was done for making extracts of both green grapes and black grapes.

2.3 Phytochemical screening

Following tests were performed for preliminary phytochemical analysis of green grapes and black grapes respectively[6].

2.3.1 Test for alkaloids (Mayer's test)

A small amount of each extract was neutralized by adding 1 or 2 drops of dilute H_2SO_4. The resulting solution was treated with a very small amount of Mayer's reagent in a test tube. Appearance of dull, white precipitates confirms the presence of alkaloids.

2.3.2 Test for flavonoids (Shinoda test)

A few drops of concentrated HCl and 1-2 magnesium turnings were added to 1 ml of extracts. The presence of flavonoids was indicated by the development of pink or magenta red colour.

2.3.3 Test for anthocyanins (Sodium hydroxide test)

To 2ml of extract was taken, to it 1 ml of 2N NaOH was added and heated for 5 minutes at 100°C. Formation of bluish green colour indictes the presence of anthocyanin.

2.3.4 Test for anthraquinones (Borntranger's test)

To the extract 2-3 drops of dilute HCl were added. Then the mixture was boiled for 2 minutes (hydrolysis of glycosides). Then the mixture was filtered and cooled. The filtrate was

extracted with chloroform. The chloroform layer was separated and shaken vigorously with 10% ammonium hydroxide. Immediate appearance of rose pink or cherry red color in aqueous layer confirms the presence of anthraquinones.

2.3.5 Test for saponins
To 5ml of the extracts, 5ml of distilled water was added and shaken for the formation of froth which confirms the presence of saponins.

2.3.6 Test for steroids and terpenoids
A small portion of extract was dissolved in 1 ml of chloroform and filtered. To the filtrate on ice, 1 ml of acetic acid was added and then a few drops of concentrated H_2SO_4 were run down the side of the test tube. The appearance of blue, bluish-green or a rapid change from pink to blue colours indicates the presence of steroids, the appearance of pink or pinkish-brown ring/color indicates the presence of terpenoids and a combination of pink and these colours indicates the presence of both steroids and terpenoids.

2.3.7 Test for carbohydrates (Fehling's test)
To the extracts, equal quantities of Fehling's solution A and B were added and on heating, formation of a brick red precipitate indicates the presence of carbohydrates.

2.3.8 Test for glycosides (Borntranger's test)
To the extract 2-3 drops of concentrated HCl were added. Then the mixture was boiled for 2 minutes (hydrolysis of glycosides). Then the mixture was filtered and cooled. The filtrate was extracted with chloroform. The chloroform layer was separated and shaken vigorously with 10% ammonium hydroxide. Appearance of pink color in aqueous layer confirms the presence of glycosides.

2.3.9 Test for phenol compound (Lead acetate test)
The extract was dissolved in 5ml of distilled water. To this, 3ml of 10% lead acetate was added. Appearance of bulky white precipitate indicated the presence of phenol compound.

2.3.10 Test for protein and amino acids (Biuret test)
The extract (100mg) was dissolved in 10ml of distilled water and filtered though whatmann no.1 filter paper and further an aliquot of filtrate was treated with one drop of 2% $CuSO_4$ solution. To this, 1 ml of ethanol (95%) was added, followed by excess of sodium hydroxide pellets. Pink color in the ethanolic layer indicates the presence of proteins and amino acids.

3. Result

	Green grape methanolic extract	Black grape methanolic extract
Alkaloids	+	+
Flavonoids	+	+
Anthocyanins	-	+
Anthroquinones	+	+
Saponins	+	+
Steriods	-	-
Terpenoids	-	+
Carbohydrates	+	+
Glycosides	-	-
Phenol compound	+	+
Protein and amino acid	-	+

(+) indicates presence and (–) indicates absence

Above table shows the results of phytochemical analysis of both green and black grape methanolic extracts.

4. Discussion

Many kinds of fruits, vegetables, spices and medicinal plants have been reported to be good sources of phytochemicals. The phytochemical analysis conducted on methanolic extract of green grape revealed the presence of alkaloid, flavonoid, anthraquinones, saponins, carbohydrates and phenol compounds whereas phytochemical analysis of methanolic black grape extract revealed the presence of alkanoids, flavonoids, anthocyanins, anthraquinones, saponins, terpenoids, carbohydrates, phenol compound and protein and amino acid. These phytochemicals have been found to play protective roles against chronic degenerative diseases[13]. The phytochemicals including polyphenols, flavonoids and vitamins were found to be more important for study and interested as they are effective on human health[9]. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and acts as natural anticancer agents[10]. Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane bound enzymes such as the ATPase and phospholipase A2[8]. Flavonoids serve as health promoting compound and results in anion radicals[3]. Phenols have been found to be useful in the preparation of some antimicrobial compounds such as dettol and cresol. Grape plant is used routinely among many tribes in Africa for the treatment of various diseases.

5. Conclusion

From the above results it can be concluded that black grape are rich source of phytochemicals. The enormous amount of phytochemicals present in both grapes depicts that in future grapes can be used as an effective drug against various chronic diseases like cancer, diabetes. The data found in this work might be useful for further study of the green and black grape extracts on various applications such as health supplement and pharmaceutical benefits. These drugs will be economic and poor men friendly.

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