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Phytochemical Composition and Antioxidant Activity of Fresh and Dried Grape (*Vitis vinifera)* Fruit Proportions

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Abstract:- Phytochemicals and antioxidants are naturally occurring compounds found in plants, especially fruits that have anti-inflammatory properties and protect against a variety of diseases. Foods derived from plants play an important role in avoiding oxidative stress-related diseases like cancer, cardiovascular disease, and diabetes. Antioxidants are substances that can avoid or delay cell damage caused by free radicals, which are unstable molecules generated by the body in response to environmental and other stresses. Antioxidants are believed to be abundant in many plant-based foods. Grape (Vitis vinifera) fruits are rich in phytochemicals and antioxidants, and are one of the world's most commonly eaten fruits. Grape skin and seed extracts have potent free radical scavenging and inhibit lipid oxidation in a number of food and cell models in vitro. Hence the objectives of this present study was to identify and quantify the phytochemicals and to identify the antioxidant activity of the fresh and cabinet dried grape fruit proportions such as skin, pulp and seeds. Preliminary qualitative phytochemical analysis was carried out by the standard methodology to identify the secondary metabolites like alkaloids, flavonoids, quinons, phlobatannin, phenol, saponin, tannin, terpenoids and steroids. Quantitative phytochemical test such as Alkaloids, Flavonoids and Total Phenol were also done. Tests were done in triplicates and the results were interpreted in tables. It can be concluded that the fresh grape seed had the better profile of phytochemical and antioxidant activity.

Keywords:- *Phytochemicals, Defense mechanism, Free Radical Scavenging, and Antioxidants.*

I. INTRODUCTION

Natural remedies and plant extracts have been used for over a millennium. Despite the substantial advancement of synthetic pharmaceuticals, interest in herbal medicine based on various plant extracts has skyrocketed in recent decades. The resistance of some microbes to existing antimicrobial agents, as well as some particular unwanted effects, was among the reasons for these evidence (Leal *et al.*, 2020). Plants are also used for food processing, pharmaceutical, and medicinal purposes, in addition to being an essential source of nutrition. Orthodox medicinal herbs' phytochemicals have long been recognised for their therapeutic value in the treatment of a wide range of serious health problems, including cancer. Plant-derived bioactive molecules are now being used to develop new treatments. (Ahmad *et al.*, 2017). Fruits, seeds, leaves, roots, and tubers are all examples of plant foods. In prospective cohort studies or randomised controlled trials, certain fruits and vegetables have been tested separately. Phytochemical content of fruits and vegetables, such as polyphenols, phytoestrogens, and antioxidants, is usually of concern.(Slavin and Lloyd, 2012).Grapes, one of the most common fruits and the most widely grown around the world, contain a lot of phytochemicals including tannins, anthocyanins, flavonols, flavan-3-ols, epicatechin, epigallocatechin, catechin, gallocatechins, and epicatechin gallate, as well as proanthocyanidins (typically hexamers) or procyanidins, all of which have been documented for their bioactive compounds (Pezzuto, 2008). Grape seeds and skins contain functional components that can be consumed by humans. Since phenols are primarily derived from the skins and seeds of red grapes (Suresh *et al.*, 2014). As a result, rather than replacing conventional drugs with more effective natural compounds, there is an increasing appetite for natural compounds that can be used in the manufacture of new medicines or food products. Grape fruit proportions contain polyphenolic material and other potent phytochemicals, promoting long-term growth with benefits for both the climate and corporate profits. Hence, the objective of this study was to screen the phytochemicals, quantify the phytochemicals and to evaluate the free radical scavenging activity in fresh and dried proportions of fruits.

II. MATERIALS AND METHODS

Selection and Collection of Fruits

The grape fruits were collected from various organic cultivators in the Dindigul Districts and were fresh and well ripened. The Bangalore blue variety was chosen, and the grapes have large, oval-shaped grains with slightly raised longitudinal ribs. The skin is dark violet in colour, medium dense, and delicate. The flesh has a mild muscat taste and is crunchy and palatable (with good ripening). The clusters range in size from medium to massive.

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Processing of Fruits

Grapes have a short shelf life and are only available for a limited time during the summer. It was made more usable during the year and more flexible for use in various product processing techniques. The tray dryer system is used to dry grains, fruits, and vegetables in a cabinet dryer. The nutrient content after drying with a cabinet dryer is of high quality. (Satwase *et al.*, 2013). Grapes were washed and held at room temperature for 8 hours to extract moisture from their surface and skin. Grape pulp and seeds were extracted and put in separate trays in a cabinet drier, where the temperature was kept at 60^0 for 7 hours. It was cooled, ground into a coarse powder, and placed in a tightly sealed container away from environmental climatic change after the heat treatment.

Qualitative Phytochemical analysis

The presence of various phytochemicals in fresh and dried grape fruit was analyzed. Phytochemical such as alkaloids, saponin, tannin, terpenoids, quinons, flavonoids, phenol, phlobatannins and steroids were analysed by standard procedures with methanol extracts. Qualitative Phytochemical screening procedure is given below:

Alkaloids (Mayer's Test): A drop of Mayer's reagent was applied along the sides of the test tube with 2 ml of the collected sample, and the formation of white precipitate indicated that the test was positive.

Flavonoids (Alkaline reagent test): Flavonoids was determined by treating 2 ml of extract with 1 ml of 10% ammonium hydroxide solution. Yellow fluorescence suggested the presence of flavonoids.

Phenols (Ferric chloride test): A dark green colour shows the presence of phenolic compounds when 2 ml of the extract was dissolved in 2 ml of distilled water and a few drops of neutral 5 percent ferric chloride solution were added.

Saponins (Frothing test): After 15 minutes of shaking, 2 ml of the extract was diluted with 3 ml of distilled water and then diluted again with 5 ml of distilled water. Two layers of foam showed the presence of saponins.

Tannins: 2 mL extract, 1 mL water, and 1-2 drops ferric chloride solution were applied to the mixture. Gallic tannins have a blue colour, while catecholic tannins have a green black colour.

Terpenoids: 0.5 ml acetic anhydride and 0.5 ml chloroform were added to 2 ml of extract. Slowly, concentrated sulphuric acid was applied to the test tube's surfaces. Terpenoids were found to have a red violet colour.

Phlobatanin: Phlobatanin was determined by treating 2ml of extract with 1ml of 1 percent HCL and observing the formation of red precipitate.

Steroids (Libermann-Burchard reaction): 0.5 ml acetic anhydride and 0.5 ml chloroform were added to 2 ml of the extract. Then, gradually adding concentrated sulphuric acid, a green bluish colour for steroids was found.

Quantitative Phytochemical Analysis

Quantitative phytochemical analysis of alkaloids, flavanoids and total phenol which possess anticancer activity in fresh and dried fruit samples was done with methanol extract since the qualitative analysis have shown the appearance of tested Phytochemicals in methanol extract of fresh fruit proportions when compared to the other extracts. The procedure followed to quantify the alkaloids, flavanoids and total phenol is given below:

Alkaloids determination by using Harborne (1973) method

5g of the sample was weighed into a 250 ml beaker, and 200 ml of 10% acetic acid in ethanol was applied, covered, and set aside for 4 hours. This was diluted, and the extract was condensed to one-fourth of its original volume in a water bath. Drops of concentrated ammonium hydroxide were applied to the extract one at a time until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, was dried and weighed.

Flavanoid determination by the method of Bohm and Kocipai- Abyazan (1994)

A volume of 0.25 ml of the sample was diluted to 1.25 ml with distilled water. 75 µl of 5% sodium nitrite was added and after six minutes 5 ml of 0.1 % aluminium chloride solution was added. 0.5 ml of 0.1M NaOH was added after 5 minutes and made up to 2.5 ml with distilled water. The solution was mixed well and the absorbance was read in ultraviolet spectroscopy at 510 nm along with standard quercetin at 5 - 25 µg concentration.

Total phenol determination by the method of Kumaran (2006)

Determination of total phenolic content Folin–Ciocalteu procedure given by Yu *et al..*, (2002) was used to estimate the total phenolic contents in the methanol extract of the plants. Following this method, 0.1 ml of fractions was diluted to 1 ml with distilled water. To this solution 0.5 ml of Folin–Ciocalteu reagent (2N, 1:1) and 1.5 ml of 20% sodium carbonate solution was added. The mixture was incubated for 2 hours at room temperature. The volume of the mixture was raised to 10 ml with distilled water and the absorbance of blue colored mixture was measured at 765 nm in ultraviolet spectroscopy. All determinations were carried out in triplicates.

Estimation Antioxidant Activity

Antioxidant activity is characterised as the prevention of oxidation of proteins, lipids, DNA, or other molecules by blocking the propagation stage of the oxidative chain. Primary antioxidants directly scavenge free radicals, while secondary antioxidants indirectly prevent free radical formation (Pour *et al.*, 2012). Antioxidants generally scavenge the radicals thus it is important to measure the free radical scavenging activity by using different methods. Hence antioxidant activity of the fresh and dried grape fruit proportions was measured by DPPH radical scavenging assay (DPPH), Hydroxyl radical scavenging assay (OH^-), Superoxide radical scavenging assay (O_2^-) and Hydrogen peroxide scavenging assay (H_2O_2).

DPPH radical scavenging activity was measured according to the method of Mensor *et al.*, (2001). Hydroxyl

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radical scavenging assay (OH^-) was determined by following the method of Kunchandy and Rao, (1990).Superoxide radical scavenging activity was determined by the method of McCord and Fridovich, (1969). Hydrogen peroxide scavenging activity was determined according to the method described by Ruch *et al*., (1989).

III. RESULTS AND DISCUSSION

Table- 1
Qualitative Phytochemical Analysis of Fresh Grape Fruit Proportions with Various Solvent Extract

Fruits	Fresh fruit Proportions	Alkaloids	Flavonoids	Phenol	Saponin	Tannin	Terpenoids	Quinons	Phlobatannin	Steroids
Methanol	Skin	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	- - -	+ + +	+ + +
	Pulp	+ + +	+ + +	+ + +	+ + +	+ + +	- - -	+ + +	+ + +	+ + +
	Seed	+ + +	+ + +	+ + +	- - -	+ + +	+ + +	- - -	+ + +	+ + +
Ethanol	Skin	+ + +	+ + +	- - -	+ + +	+ + +	- - -	+ + +	+ + +	+ + +
	Pulp	+ + +	+ + +	+ + +	- - -	+ + +	- - -	- - -	+ + +	+ + +
	Seed	+ + +	+ + +	+ + +	- - -	- - -	- - -	- - -	+ + +	+ + +
Chloroform	Skin	+ + +	+ + +	- - -	+ + +	- - -	- - -	+ + +	- - -	+ + +
	Pulp	+ + +	- - -	+ + +	+ + +	- + +	+ + +	- - -	- - -	- - -
	Seed	+ + +	- - -	- - -	+ + +	- - -	- - -	- - -	- - -	- - -
Acetone	Skin	+ + +	- - -	- - -	- - -	+ + +	+ + +	- - -	- - -	+ + +
	Pulp	- - -	- - -	- - -	- - -	+ + +	+ + +	+ + +	- - -	- - -
	Seed	+ + +	- - -	- - -	- - -	+ + +	+ + +	- - -	+ + +	- - -
Petroleum ether	Skin	+ + +	- - -	- - -	- - -	- - -	+ + +	+ + +	+ + +	+ + +
	Pulp	+ + +	+ + +	- - -	- - -	+ + +	- - -	- - -	+ + +	+ + +
	Seed	+ + +	+ + +	- - -	- - -	+ + +	- - -	- - -	+ + +	- - -
Aqueous	Skin	+ + +	+ + +	- - -	+ + +	+ + +	+ + +	- - -	+ + +	+ + +
	Pulp	- - -	- - -	- - -	- - -	+ + +	+ + +	+ + +	- - -	- - -
	Seed	+ + +	- - -	- - -	- - -	+ + +	+ + +	- - -	+ + +	- - -

Table – 1 depicts the qualitative phytochemical analysis of fresh fruit proportions of grape with various solvent extract. Methanol extract of grape skin reveals that it had all the tested phytochemicals except quinons. In grape pulp except terpenoids other tested phytochemicals are present. Same results were observed from Mathew *et al*., (2012) the presence of flavonoids, alkaloids, steroids, terpenoids, saponins, cardiac glycosides, and reducing sugars. Hanna *et al*, (2015) results found that the phytochemical constituents of grape seed extract revealed the presence of steroids, terpenoids, anthocyanins, emodins, glycosides, flavonoids and phenols in acetone, methanolic, ethanolic and water extracts but steroid and terpenoids were absent in water whereas saponins were absent in methanol and water extracts.

Ethanol extract of fresh grape fruit proportions shows that grape skin had alkaloids, flavonoids, saponin, tannin, quinons, phlobtannin and steroid. Pulp contains alkaloids, flavonoids, phenol, tannin, phlobtannin and steroid. Seed comprise of alkaloids, phenol and steroid. Chloroform extract of fresh grape skin shows the presence of alkaloids, flavanoids, saponin, quinons, and steroid. Whereas it's Pulp contains alkaloids, phenol, saponin, tannin and terpenoids. In seed alkaloids and saponin are present.

Acetone extract found that the alkaloids**,** tannin, terpenoids and steroids were present in grape skin extract. Tannin, terpenoids and quinons are present in grape pulp. Grape seed was found with phlobtannin, tannin and terpenoids.

Petroleum Ether extractof fresh grape fruit proportions such as its skin, pulp and seed shows that the alkaloids, terpenoids, quinons, phlobtannin and steroid present in skin; alkaloids; flavonoids, tannin, phlobtannin and steroid are present in pulp; alkaloids, flavonoids, tannin, and phlobtannin are present in its seed.

Aqueous extract Grapes skin had alkaloids, flavonoids, saponin, tannin, terpenoids, phlobtannin and steroid. Tannin, terpenoids and quinons were present in pulp. Seed contains alkaloids, tannin, terpenoids and phlobtannin.

Majority of the tested phytochemical were present in the methanol extract of fresh grape fruit proportions.

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Table -2
Qualitative Phytochemical Analysis of Dried Grape Fruit Proportions with Various Solvent Extract

Fruits	Dried fruit Proportions	Alkaloids	Flavonoids	Phenol	Saponin	Tannin	Terpenoids	Quinons	Phlobatannin	Steroids
Methanol	Skin	+ + +	+ + +	- - -	- - -	- - -	+ + +	- - -	- - -	- - -
	Pulp	+ + +	+ + +	+ + +	- - -	- - -	- - -	+ + +	+ + +	+ + +
	Seed	+ + +	+ + +	+ + +	- - -	+ + +	+ + +	- - -	- - -	- - -
Ethanol	Skin	+ + +	+ + +	- - -	+ + +	+ + +	+ + +	- - -	+ + +	+ + +
	Pulp	+ + +	+ + +	+ + +	- - -	+ + +	+ + +	+ + +	- - -	- - -
	Seed	+ + +	- - -	- - -	- - -	- - -	- - -	- - -	+ + +	- - -
Chloroform	Skin	+ + +	+ + +	- - -	+ + +	+ + +	+ + +	- - -	+ + +	+ + +
	Pulp	+ + +	+ + +	+ + +	- - -	+ + +	+ + +	+ + +	+ + +	+ + +
	Seed	+ + +	+ + +	- - -	- - -	+ + +	+ + +	+ + +	+ + +	+ + +
Acetone	Skin	+ + +	- - -	- - -	- - -	+ + +	- - -	- - -	- - -	+ + +
	Pulp	- - -	- - -	- - -	- - -	+ + +	+ + +	+ + +	- - -	- - -
	Seed	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	- - -	- - -	- - -
Petroleum ether	Skin	+ + +	- - -	- - -	- - -	- - -	+ + +	+ + +	+ + +	+ + +
	Pulp	- - -	+ + +	- - -	- - -	+ + +	- - -	- - -	+ + +	+ + +
	Seed	+ + +	+ + +	- - -	- - -	+ + +	- - -	- - -	- - -	- - -
Aqueous	Skin	+ + +	+ + +	- - -	- - -	+ + +	+ + +	- - -	+ + +	- - -
	Pulp	- - -	- - -	- - -	- - -	+ + +	+ + +	- - -	- - -	- - -
	Seed	+ + +	- - -	- - -	- - -	+ + +	+ + +	- - -	+ + +	- - -

Table -2 shows the qualitative phytochemical analysis of dried grape fruit proportions with various solvent extract. Methanol extract of dried grape skin powder contains the phytochemicals like alkaloids, flavonoids and terpenoids; Pulp had alkaloids, flavonoids, phenol quinons, phlobtannin and steroid; Seed shows the presence of alkaloids, flavonoids, phenol, tannin and terpenoids.

Ethanol extract dried grapes skin powder shows the presence of alkaloids, flavanoids, tannin, terpenoids and phlobtannin. Alkaloids, flavanoids and phenol were present in grapes pulp. Seed has containing alkaloids and phlobtannin.

Chloroform extract of dried grape proportions such as skin, pulp and seed was reported with the presence of alkaloids, flavonoids, saponin, tannin, terpenoids, phlobtannin, and steroid in skin; Alkaloids, flavonoids, phenol, tannin, terpenoids, quinons, phlobtannin, and steroid in pulp; Whereas, alkaloids, flavonoids, tannin, terpenoids, quinons, phlobtannin and steroid present in seed.

Acetone extract of dried grape skin contains alkaloids, tannin and steroid. Tannin, terpenoids and quinons were present in pulp. Alkaloids, flavonoids, phenol, saponin, tannin and terpenoids were present in seeds. Rekha and Bhadkar (2013) also revealed the presence of phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids. Watermelon skin had flavonoids, terpenoids and steroids. Alkaloids, flavonoids and steroid present in its pulp. Seed sows that the presence of flavonoids, phenol, saponin and tannin.

Petroleum Ether extracts of dried grape proportions reveals the presence of alkaloids, terpenoids, quinons, phlobtannin and steroid in skin powder. Flavonoids, tannin, phlobtannin and steroid were present in its pulp. Alkaloids, flavonoids and tannin were present in seed.

Alkaloids, flavonoids, saponin, tannin and phlobtannin were found in aqueous extract of dried grapes skin powder. Tannin and terpenoids were present in its pulp. Alkaloids, tannin, terpenoids and phlobtannin were present in seed.

Majority of the tested phytochemical were present in the chloroform extract of dried grape fruit proportions.

Table – 3
Quantitative Phytochemical Analysis of Fresh and Dried Grape Fruit Proportions of Methanol Extract

Fruits	Proportions	Alkaloids	Total Phenol	Flavanoids
Fresh Grapes	Skin	58.79 ± 0.58	43.02 ± 0.38	66.10 ± 0.37
	Pulp	87.99 ± 0.63	74.17 ± 0.63	72.41 ± 0.07
	Seed	88.05 ± 0.17	87.29 ± 0.28	96.31 ± 0.58
Dried Grapes	Skin	72.59±0.18	66.37±4.11	83.11±0.72
	Pulp	44.70±3.11	67.47± 0.18	52.38±0.27
	Seed	70.66±3.35	85.15±0.15	82.26±5.39

Table –3 depicts the quantitative phytochemical analysis of fresh and dried grape fruit proportions of methanol extract. Fresh grape proportions reveals that the phytochemical such as alkaloids, total phenol and flavanoid shows that the gape seed contains the high amount of alkaloids (88.05%), total phenol (87.29%) and flavanoid (96.31%) followed by grape pulp alkaloids (87.29%), total phenol (74.17%) and flavanoid (72.41%) and skin had alkaloids 58.79%, total phenol 43.02% and flavanoid was 66.10% and was moreover similar to the grape seed. Canals *et al.,* (2008) also reported the same results as in the present study that is grape seeds were richer in phenols than skins or pulp.

Grapes seed was noticed with high amount of alkaloids (70.66%), total phenol (85.15 %) and flavanoids (82.26%) followed by its skin alkaloids (72.59%), total phenol (66.37%)flavanoids (83.11%) and pulp alkaloids (44.70%), total phenol (67.47%) and Flavanoid (52.38%) respectively.

Table –4
Antioxidant Activity of Fresh and Dried Grape Fruit Proportions

Assay	Proportions	Antioxidant activity	
		Fresh	Dried
DPPH Radical Scavenging Assay	Skin	74.00±2.30	68.18±0.25
	Pulp	55.08±0.51	55.74±1.17
	Seed	84.39±3.15	72.75±3.18
Hydroxyl Radical Scavenging Assay	Skin	36.93±0.39	32.25±0.03
	Pulp	45.34±0.05	41.03±0.50
	Seed	57.04±1.48	54.25±0.32
Superoxide Radical Scavenging Assay	Skin	23.50±0.26	30.25±0.20
	Pulp	47.02±0.25	34.10±0.41
	Seed	53.10±0.54	50.49±0.50
Hydrogen Peroxide Scavenging Assay	Skin	29.25±0.62	28.10±0.05
	Pulp	33.08±0.40	34.25±1.43
	Seed	65.10±1.33	48.53±0.20

Table – 4 antioxidant activity of fresh and dried grape fruit proportions. DPPH radical scavenging assay in fresh grape skin was (74.00%), pulp (55.08%) and seed (84.39%).A study by Alrasheid *et al.,* (2019) reveals in fresh grape peel extract showed DPPH scavenging activity at (55%).Dried grapes skin powder had 68.18 %, pulp contains 55.74 % and the seed shows 72.75 % of antioxidant activity.

Hydroxyl radical scavenging assay in fresh grapes antioxidant activity was 36.93 % in skin, 45.34 % in pulp and 57.04 % in seed. Dried grapes skin powder contains 32.25 %, pulp 41.03 % and seed 54.25 % of antioxidant activity.

Superoxide radical scavenging assay (O2 −) of Fresh fruits grapes skin had 23.50%, pulp contains 47.02% and the seed shows 53.10% of antioxidant activity. When compared with grape pulp (34.10%) and skin (30.25%) its seed was reported high (50.49%) antioxidant activity.

Hydrogen peroxide scavenging assay (H_2O_2) of fresh grape skin had 29.25%, pulp contains 33.08% and the seed shows 65.10% of antioxidant activity. When compared with grape pulp (34.25) and skin (28.10) its seed was reported high (48.53) antioxidant activity.

IV. CONCLUSION

Grapes (*Vitis vinifera L.)* contain exclusive therapeutics, owing to their antioxidant and phytochemicals perspective. The present study revealed that the fresh grapefruit proportions have major phytochemical constituent and antioxidant activity when compared to dried grape fruit proportions; fresh grape fruit proportions had the high phytochemicals especially flavanoids followed by alkaloids and total phenol. The fresh grape seed shows high rate of antioxidant activity in DPPH radical scavenging assay, Hydroxyl radical scavenging assay, Superoxide radical scavenging assay and Hydrogen peroxide scavenging assay.

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