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Screening and Evaluation of medicinal properties of Grape fruit varieties in Theni District

G.Renuga[1] and M. Hemapriya[2]

[1] *Principal & Research Co-ordinator, Dept of Biochemistry,*
[2] *Research scholar Dept of Biochemistry, Sri Adi Chunchanagiri women's College, Cumbum, Theni (Dt), Affiliated to Mother Teresa Women's University, Kodaikanal, Tamil Nadu, India.*

Abstract--*Theni districts could be considered one of the highest grapes producing area also export best quality of Paneer dhrakshai Indian table grapes in Tamil Nadu. The research work focused to identify the nutritive compounds in the grapes (Vitis vinifera) species which are a wonderful fruit having pulp content, vibrant color and exert an immense health benefits. Grape fruits were found to be rich source of Anthocyanin content and vitamin C also macromolecules contents such as carbohydrate contents of 29mg, Protein 18.7mg and fat content 3.7mg /100 g of f. wt. Micronutrients were estimated by AAS which showed that calcium content was 3.1mg/g.f.wt, iron content 2.7 mg/g.f.wt, potassium 2.6 mg/g.f.wt found to be more than other minerals in fresh extracts of grape fruits. The comparative results of revealed considerable amount of phytoconstituents in grape fruits in which anthocyanins alone has been taken to evaluate therapeutic effects on breast cancer cell MCF-7 cell viability test was carried out to determine cell proliferation. The results revealed presence of anthocyanins in high quantity in the process of purification through various biochemical methods that could ultimately be used for preventing cancerous cell proliferation which reduces the development of cancer diseases. On comparison Grapes (Vitis vinifera) may serve as functional food with vital pharmaceutical and biological value.*

Keywords: *Anthocyanin, Grape fruits, phytochemicals, Cancer treatment, Minerals.*

I.INTRODUCTION

The grape has been well recognized worldwide for over 2,000 years as one among the edible sweet fruits and recognized for its wide spectrum of biological properties. The grape belongs to the berry family as it is found attached to the stem. Many berries make up a cluster or bunch of grapes. The fruit of the grape is one of the most palatably edible foods, having many established nutritional and medicinal properties for consumers. The essential parts of the berry include skin, pulp, and seeds also the composition of the grape in terms of nutrients are reported in many varieties grown at Pune, Bangalore etc. Oxidative stress is a hallmark of various health problems. The grape is one of the most valued conventional fruits, worldwide. Although most of the parts of the grapevine are useful, primarily, the grape is considered as a source of unique natural products not only for the development of valuable medicines against a number of diseases, but also for manufacturing various industrial products. Fruits and vegetable processing in India generates substantial quantities of waste. It had been previously reported that these wastes and by-products of fruits are an abundant source of antioxidant polyphenol [1].

Moreover, skin and seeds of grape are good sources of phytochemicals like gallic acid, catechin, and epicatechin, which are appropriate raw substrates for the production of anti-oxidative dietary supplements. On the basis of these observations the consumption of fruit of the grape and its constituents may be part of therapeutic regimens to suppress oxidative stress-related threats. Another important group of components present in grapes is the anthocyanins, which belong to the flavonoid family. Resveratrol (3,5,40 -trans-trihydroxystilbene) is a natural phytoalexin abundantly found in grapes and red wine, which has potent antioxidant property. Extensive epidemiological evidence suggesting that dietary intake reduces cardiovascular mortality due to the presence of variety of polyphenol compounds, including flavonoids, phenolic acids, and resveratrol in Grapes [2] Kris-Etherton]

Polyphenol bioactivity is further concluded by variable bioavailability due in part to differences in food matrix and human gut absorption (Erdman].In addition to developing better dietary recommendations, emerging studies showing cardiovascular benefits of polyphenol rich foods have also prompted interest by the pharmaceutical industry. It is possible that purified formulations of specific grape constituents, e.g. resveratrol, might be efficacious as drugs for cardiovascular disease treatment or prevention [3].

Limited research has been performed to identify the benefits of grape intake for type 2 diabetics. Because both the glycemic index (GI) and glycemic load (GL) GL fall within the low range, this fruit is appropriate for inclusion in a diet targeting low glycemic foods, such as a diet for a diabetic individual. Grapes contain an abundance of phytochemicals with antioxidant and anti -inflammatory activities.

A small number of studies have been conducted that suggest grapes or constituents of grapes may have some protective effects against the metabolic disturbances observed in type 2 diabetics [4]. Cultivation of fruit crops plays a vital role in the agricultural development of a nation. Fruits are the chief source of vitamins and minerals, without which human body cannot maintain proper health and resistance to disease. Currently a large part of the adult population worldwide is suffering from diabetes, and prevalence of the disease is increasing day by day. Until now no therapy has been available to treat diabetes, and various therapeutic strategies have also been applied to suppress its increasing prevalence. Even then, grapes are cultivated to be the one of the major fruit crop in Tamil Nadu due to its demand by consumer as a table fruit.

Phytochemicals found in grapes or grape plant-derived products have shown inhibitory effects in the chemically induced diabetic models. Rats fed red wine polyphenols had reduced hypertension, cardiac hypertrophy, and production of reactive oxygen species in cardiac tissue. In both of these studies, the authors used pharmacological doses of the red wine polyphenols. Grapes are rich in resveratrol, one of the world's best known anti-oxidants that can combat cancers of colon, prostrate, Alzheimer's and various viral and fungal infections.

In the present study, grape varieties grown at Cumbum, Theni Dt, Tamil Nadu were evaluated for physico-chemical characteristics and medicinal properties which will provide valuable information on the potential use of grape products may provide health benefits to cancer disease.

II.MATERIALS AND METHODS

2.1. Experimental Samples

A total of four varieties of grape fruits were collected in and around cumbum belonging to the district Theni of Tamil Nadu state. The grape fruit species were identified and authenticated by Tamil Nadu Agricultural University Research Station, Anaimalayanpatty, Theni District, Tamil Nadu, India. Fruits were collected and washed under running tap water to eliminate all the unwanted foreign particles.
Then weighed 100g of samples used for extracted as follows: 100g of sample (Pulp) was homogenized with electric blender and filtered using whatman no.1 filter paper and the recovered extracts were centrifuged at 5000 rpm for 10 minutes and the supernatant was discarded and the residues were preserved in refrigerator condition till further uses.

2.2. Analysis of macro & micronutrients potentials

Fresh grapes juice samples were subjected for phytochemical screening according to the standard methods as described by Trease and Evans [5**]** and Harper [6**].** Vitamin C was analysed by dichlorophenol indophenols method by chinoy and singh [7**]** and polyphenol was analysed by spectrophotometric method [8**]** and quantitative analysis of anthocyanin was performed according to the modified procedure **[9]**.Macromolecules such as carbohydrate was analysed by Anthrone method **[10]**, protein was measured by lowrey's method **[11**[1] and fat content was estimated by Soxhlet method [12]. The micro nutrients such as iron, phosphorus, calcium, magnesium, sulphate, sodium, potassium, copper, zinc, nitrogen and manganese were analyzed by Atomic Absorption Spectrophotometer [13].

2.3. Extraction of Anthocyanin

The extraction of anthocyanins was performed according to Liang *et al*. with some modification [14] 20 mL methanol with 5% (v/v) formic acid was added into 100 mL Erlenmeyer flasks that contained 1 g of grape skin powder. Anthocyanins were extracted at 30 °C for 30 min in a dark environment; this was repeated five times to collect the extract solution. The extraction was concentrated under vacuum at 30 °C using a rotary evaporator until dryness. The dry extraction was resolved in 5 mL solvent of 2% formic acid in distilled water. About 1 mL of extracted solution was strained through a 0.45 μm millipore filter for further experimental use or analysis.

2.4. Measure of Viability

This procedure can be performed along with the cell counting procedure but cell density may require adjustment in order to obtain approximately 10^6 cells per milliliter. Mix 1 drop of trypan blue with one drop of the cell suspension and allow 1 - 2 minutes for absorption. Prepare haemocytometer and load chambers as described in "Cell Quantitation". Count both the total number of cells and the number of stained (dark) cells. Calculation: percent viability= (Total cell counted – Stained cells) X 100 / Total cells counted [15].

III.RESULTS AND DISCUSSION

Grapes are a non-climacteric fruit that grows on the perennial and deciduous woody climbing vine. Tamil Nadu is third largest producer of grape in India after the states of Maharashtra followed by Karnataka and Tamil Nadu. Cumbum valley is known as Grapes city of South India. Grapes are a non-climacteric fruit that grows on the perennial and deciduous woody climbing vine. The commercial grape cultivation in India is about 60 years old and mainly confined to

cultivation of *Vitis vinifera* cultivars in the tropical and sub-tropical areas comprising of Tamil Nadu. A unique type of staggered pruning in the **"Paneer dhrakshai"** [other name: Muscat Hamburg]is practiced in the Cumbum valley of Theni district which together with the congenial climatic conditions prevailing in this tract favors harvesting of five crops in two years. Grape is harvested almost all the year round. If not all the varieties, one or more varieties are always available at any given time of the year.

3.1.Description of samples: V1- Paneer dhrakshai [*Vitis vinifera*]; V2 -Panner Hybrid [*Vitis Rotundifolia*]; V3- Green seedless [Sonaka]; V4 -black seedless [*Vitis labrusa*].Table 1 revealed comparison of fruits physical parameters such as moisture content, colour, texture and pulb which could suggested **Paneer dhrakshai** as suitable table variety .

Table 1: Analysis of Grape fruits Quality based on Physical Parameter

Varieties	**Parameters**			
	Juice content	Colour	Texture	Pulb Content
Vitis Vinifera	Very High	Brownish	Soft	High & soild
Vitis Rotundifolia	High	Black	Moderate soft	High & semisolid
Thompson Seedless	Less	Green	Rough	Medium & semisolid
Vitis Labrusca	Very less	Pale Black	Rough	Low & solid

It is a small round berry grows in clusters on a perennial and deciduous woody vines. The fruit features a semi-translucent flesh encased by smooth skin. Some varieties contain edible seeds, while others are seedless. Presence of poly-phenolic pigments in grape decides the colour as red or purple which is due to anthocyanins while white colour is due to catechin. Fortunately, these antioxidants are densely concentrated in the skin and seeds.

Table: 2 Analysis of phytochemicals in selected Grapes varieties

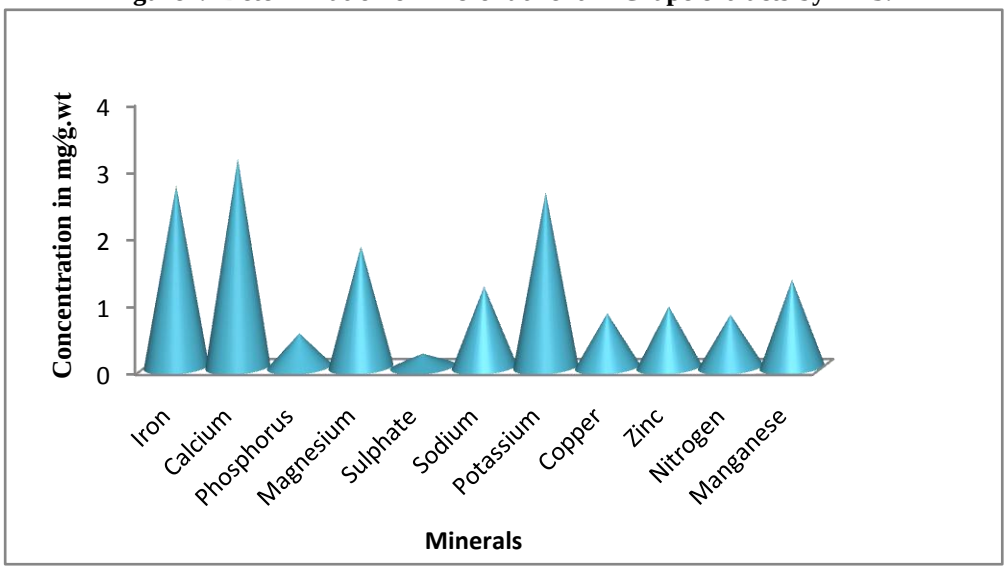
S.No	**Phytochemical Parameters**	**Grapes Varieties**			
		V1	V2	V3	V4
1	Alkaloids	-	-	-	-
2	Flavonoids	+	+	+	+
3	Glycosides	+	+	+	+
4	Saponins	-	-	-	-
5	Phenols	+	+	+	+
6	Tannins	-	-	-	+
7	Steroids	+	+	-	-
8	Amino acids	+	+	+	+
9	Anthocyanin	+	+	+	+
10	Carbohydrates	+	+	+	+
11	Protein	+	+	+	+
12	Lipids/Fats	-	-	-	-
13	Vitamin C	+	+	+	+

Legends: + Positive symbol indicates the presence of the compound; - Negative symbol indicates the absence of the compound.

Table 2 showed the phytochemical characterization of Grape fruits extract which revealed the presences and absences of various secondary metabolites synthesis in fruits development stages. Results were explained phytochemical substances found in selected experimental samples of grape fruit varieties, almost all samples revealed the presence of compound similarly on comparison of four different varieties. Fresh fruit extracts has screened to identify compound of therapeutic potential source for the drug against human illness.

Microelements present in Grapes extracts analyzed by Atomic Absorbance Spectrophotometer (**Figure 1**) revealed presences of essential minerals were found high content particularly Fe, Ca, Mg, K, and trace elements also showed its presence which are considered rich source of nutrients has medicinal properties. Phyto-pharmaceuticals should be validated from cultivation in the farm to manufacturing in the industry for quality, safety, efficacy, and regulatory requirements. Grapes are loaded with potassium, which helps lower blood pressure by balancing out the negative effects of salt. A low sodium diet is beneficial for people struggling with problem of high blood pressure.

Figure1: Determination of Micronutrient in Grape extracts by AAS.

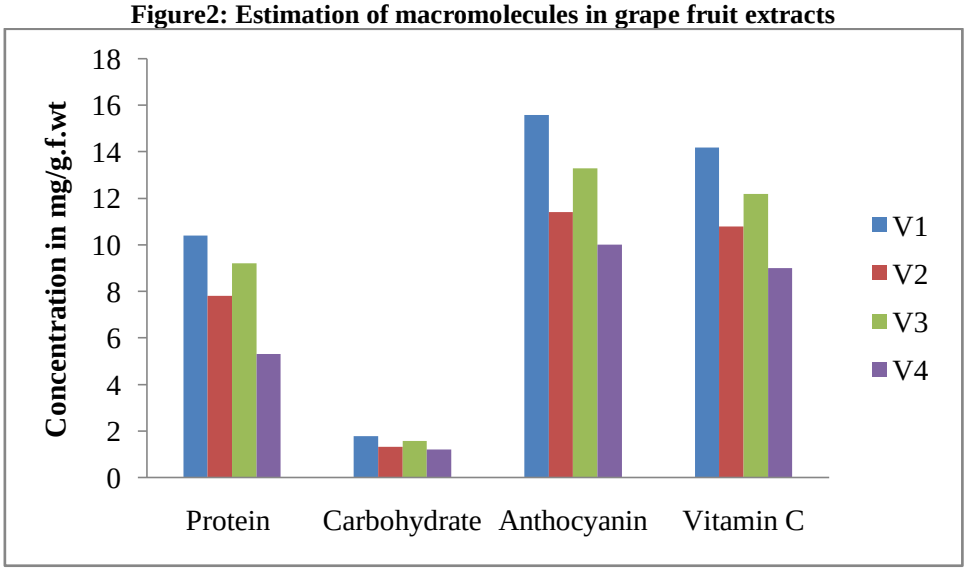


Legends: Each cone indicated different amount of minerals. Each value was represents with five average experimental results of estimation by AAS.

Grapes result in lower levels of inflammatory proteins and higher amounts of protective proteins in the retinas (it is the part of the eye that contains the cells that respond to light, known as photoreceptors). Vitamin C in Grapes: 100 grams of grapes have14.2 mg of vitamin C in paneer grapes belongs to origin of cultivation in and around cumbum, Theni Dt. which is essential for strengthening immunity and promoting skin health. Phosphorous, which accounts to 5percent of Phosphorous works with vitamin D to support bone strength.

Grapes might have preventive properties when it comes to diabetes that found greater consumption of whole fruits, particularly grapes, blueberries and apples was significantly associated with a lower risk of type 2diabetes. Proteins are too large to pass freely across the membranes separating the compartments, but since proteins attract water, they act to maintain proper fluid balance. Proteins in the body are constantly broken down and re-synthesized. Our bodies reuse most of the released amino acids, but a small portion is lost and must be replaced in the diet. Consumption of paneer grapes as table fruit was associated with effective prevention of diabetes. Carbohydrates and lipids are intermediate in complexity between nucleic acids and proteins. Carbohydrates hold a special place in human nutrition providing the largest single source of energy in the diet and satisfying instinctual desire for sweetness. High carbohydrate content is a major source of readily available energy. Proteins are fundamental elements for metabolism of enzymes, hormones and many other molecules essential for life. Vitamin C found in higher level played an essential role for improved the metabolism of carbohydrates, protein, and other minerals thereby produced ATP, NADH also other activated molecules in cells which can adapt to continue function during disease condition or even eliminate particular reaction pathway, energy derived from macromolecules are served as fuel for cell to carry out reactions in various metabolic pathways thereby used as the raw materials for biosynthesis.

Figure2: Estimation of macromolecules in grape fruit extracts



Legends: Macromolecules contents were expressed as mg/g.f.wt of sample. Each value was represents with five average experimental results of grape extracts. V1, V2, V3 &V4 were given in description of samples:

The Samples were subjected to partial purification then these results were taking into account that the anthocyanin enriched fraction was concentrated that contributed the action of antioxidant capacity showed considerable contents of vitamin and anthocyanin are major contributors. Anthocyanins are widely known as nutraceticals and are a group of soluble vacuolar pigments which are polyphenols explore the significance of anthocyanins as chemopreventive agents and the promising possibilities for development as potential anticancer drugs. Anthocyanins offer protection against certain chronic illnesses including hyperglycemia [16] and inhibit the growth of tumor cells in humans [17].

Purities of both purified and unpurified anthocyanin extracts were determined by spectroscopic scanning. The spectral character of the unpurified sample demonstrates the highest peak at 520 nm (peak 2) which is confirmed as the anthocyanin absorption peak, and a lower peak at 320 nm (peak 1) which is recognized as the hydroxycinnamate absorption peak [HPLC data not given]. The quality and nutritional value of fruits and their products is commonly associated with the color that is derived from anthocyanins. Anthocyanins possess medication and anticarcinogenic activity and are ameliorative in CVD and neurodegenerative disorders. They are also useful in weight management and polygenic disorders [18]. Anticancer activity of this compound is mainly due to induction of apoptosis via several pathways, as well as alteration of gene expressions, all leading to a decrease in tumor initiation, promotion, and progression. Hence, anthocyanin possesses good potential to be used as an adjunctive or alternative therapy for cancer and inflammatory diseases.

To evaluate the possible cytotoxic effects of anthocyanin fractions in MCF-7 cells, an MTT assay was performed (3-4,5 dimethylthiazol -2-yl)-2,5 diphenyl tetrazolium bromide). Cells were incubated with different ranges of concentration of extract and the purified anthocyanin fraction for 24 hrs. After treatment cell viability measured and observed it was not significantly affected at concentrations up to 200µg⁄ml, but revealed cytotoxicity at higher concentrations.

Figure3a: Effects of Adriamycin on BCF-7 Cell viability

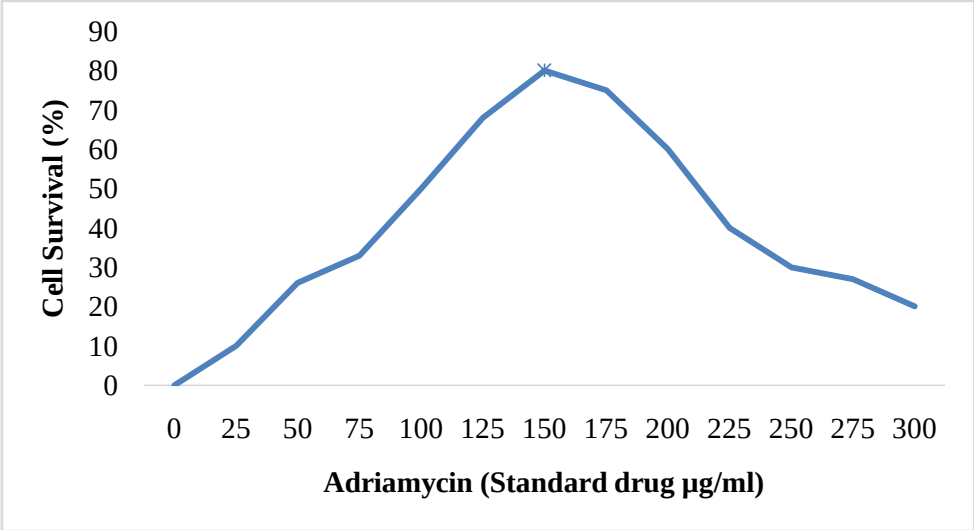
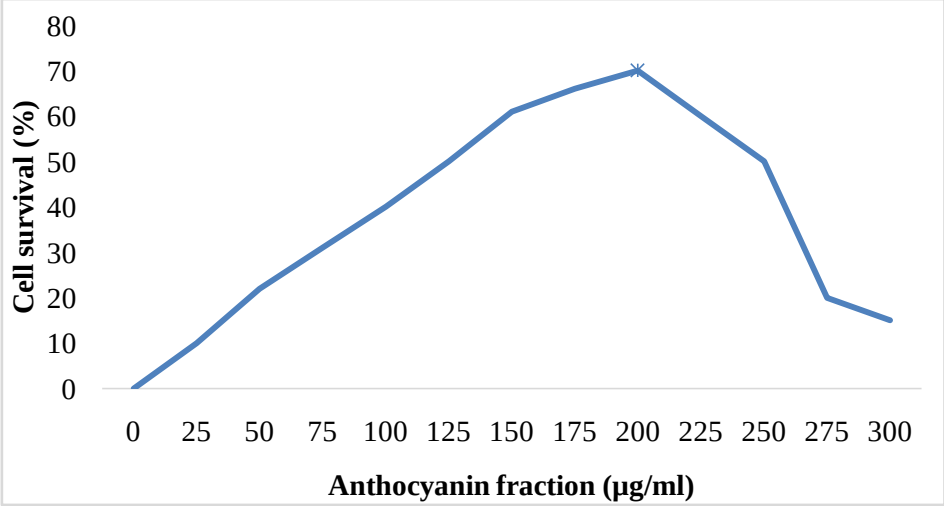


Figure3b. Effects of Anthocyanin on BCF-7 Cell viability



Legend: Viability of MCF-7 Cells after treatment with Adriamycin and Anthocyanin were given in Fig 3a & 3b. Standard drug and Anthocyanin enriched fraction of grape extracts has used for treatment with various concentration

indicated maximum of cell growth reduction at 200µg/ml for 24 hrs. Values are expressed as the mean ± SD of fine replicates (n=5). The Asterisk indicates the concentration from which significant differences ($p<0.05$) were observed when compared to the control.

Results given in figure 3 revealed pharmaceutical efficiency as inhibit tumor cell growth by cell arrest and lead to cell apoptosis with IC50 upto 200 µg/ml.The isolated anthocyanin fraction expressed to arrest cell proliferation ultimately confirm its effect to suppress cancer cell development which might help to exerts its anticancer activity of anthocyanin at 200µg/ml by decrease in expression of TNF receptor.Adriamycin used as a positive control composed as 150µg/ml was used as a known cytotoxic agent that gives 80%. Lethality under the same conditions.Standard drugs were used against the experimental test samples to compare the activity of the natural extracts expressed 70%. Fruits having high antioxidant activity in vitro may exhibit many functional properties like antimicrobial, antidiabetic, anti-inflammatory, cardio protective properties. It is important to understand how grape coloration is affected by genetic and environmental factors, as this knowledge may contribute to more stable production of high-quality grapes. The color of berry skins is determined mainly by the quantity and composition of anthocyanins. The inhibitory effects of red wine polyphenolics on human breast cancer cells have been demonstrated to be due to inhibition of cell proliferation by flavonoids, which in turn could be related to the inhibition of calcium=calmodulin-associated phosphodiesterase activity, indicating that flavonoids interfere with the function of the second messenger calcium [19]. Thus, certain grape wine ingredients that have anticancer properties may be helpful for developing functional neutraceuticals with anticancer properties.

IV.CONCLUSION

Grape (*Vitis sp.*) belonging to Family Vitaceae is a commercially important fruit crop of India. This crop occupies fifth position amongst fruit crops in India with a production of 1.21 million tonnes (around 2% of world's production of 57.40 million tonnes) from an area of 0.05 million ha. Tamil Nadu is third largest producer of grape in India after the states of Maharashtra followed by Karnataka and Tamil Nadu. Cumbum valley is known as Grapes city of South India. Grape is known as the Queen of the fruits as it is treasure of the health benefitting nutrients as poly phenolic antioxidants, vitamins and minerals. Many cultivars of grapes are grown for different purposes either eating as a table fruit, fresh or dried as Raisin or in wine production.

The grapes as **Paneer Dhrakshai** (Vitis vinifera), (Vitis labrusca and Vitis rotundifolia) and their hybrids are grown for different purposes. Although the differences in anthocyanin and flavonol content between the varieties analyzed were significant, the levels of these compounds are such that extracting polyphenols from grape skin might be economically viable. Grape skin could then be used (i) as a natural alternative to the synthetic antioxidants used in the food industry to prolong the shelf life of food or (ii) as a good antioxidant source in dietary supplements. The most important variety as Paneer Dhrakshai and Thompson Seedless cover almost eighty five percent of the area in India. Fruits are usually used as edible food for their nutritional substances such as minerals, vitamins, amino acids, proteins, sugars, polyphenols, and anthocyanins. Breast cancer cell MCF-7 was used to study the efficiency of anthocyanin isolated from grape fruit extract which showed significant inhibition on cancer cell MCF-7 in vitro condition.

The Anthocyanin fraction has been shown to exhibit inhibition of cell proliferation well known for its anticancer properties.Grapes are good source of phyto-chemicals which reduces the risk of chronic diseases, such as certain types of cancer diseases. Anthocyanin was subjected for purification through classical biochemical process and the eluted fraction was estimated for anthocyanin and evaluated obtaining anthocyanin with purity 86-90% when the traditional sephatex-25 method of solid phase extraction was employed. In future, studies will be focused on increasing productivity as well as the tonnages of export of best quality grapes, so that it can play an important role in feeding the world with Indian table grapes according to consumer's demand worldwide.

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