ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 8, Issue 5, 2015

ISSN - 0974-2441

Research Article

QUANTIFICATION OF ANTIOXIDANT - PHYTOCHEMICAL STUDIES IN *VITIS VINIFERA*.L. VARIETIES

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Received: 23 June 2015, Revised and Accepted: 03 August 2015

ABSTRACT

Objective: To quantify the antioxidant - Phytochemicals in *Vitis vinifera* varieties.

Methods: In this study, different antioxidant phytochemicals in *V. vinifera* varieties was determined in Thompson seedless, flame seedless, Kismis chorni, Pusa navrang, and Rizamat by spectrophotometric method.

Results: Antioxidant is a compound that can delay or prevent the oxidation of lipids or other molecules by inhibiting the initiation or propagation of an oxidizing chain reaction .The Quantification levels of various antioxidants in selected grape varieties which possess predominant quantities of antioxidants are phenols, flavonols, flavonoids, ascorbic acid, anthocyanin, catalase, peroxidase, polyphenol oxidase, radical scavenging assay (FRAP, ABTS, DPPH) respectively, phenols was recorded more in Rizamat (25.7±0.52 mg/g dry wt), followed by FRAP radical scavenging activity was maximum in Kismiss chorni variety (34±0.167µM/g dry wt) among all the varieties Kismis chorni , Pusa navrang were showing maximum antioxidant activity. Fruits have an important function in maintaining the physiological balance, optimal levels of antioxidants such as vitamin C and phenol compounds ,Anthocyanin, , and phenol acids, being the most important for the human body .Vitamin C present in grapes, fights against reactive oxygen species and polyphenols are associated with the prevention of diseases caused by oxidative stress. Phenolic content of these fruits is significantly correlated with antioxidant capacity. These findings revealed that phytochemicals in the selected grapes have potent antioxidant activities.

Conclusions: This study shows that maximum antioxidant activity levels were in Kismis chorni followe0d by other varieties. The present experiments on *Vitis* varieties were designed to find out the important antioxidants and nutrient potential Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. From above study it is concluded that, *Vitis vinifera* promising antioxidant and free radical scavenging activity. Thus can function as powerful free radical scavengers.

Keywords: *Vitis vinifera,* Antioxidant - Phytochemical studies, Antioxidant scavenging activity (ferric reducing antioxidant property, 2,2'-azinobis3-ethylbenzthiazoline-sulphonic acid, 2,2-Diphenyl -2-picryl hydrazyl), Spectrophotometric method.

INTRODUCTION

Vitis vinifera (Common grape vine) commonly known as in (English - Grapes, Hindi - Angur, Telugu - Draksa) present study, five varieties have been used for the antioxidant studies (Thompson seedless, flame seedless, Kimis chorni, Pusa navrang, Rizamat).

Grapes are commonly available in different varieties and studies based on nutritional point of view much work has been done, but my aim and principle is to quantify the antioxidants and phytochemicals present in the varieties and to analyze the levels of chemicals present in the varieties and how much nutritional content can a human get from consuming the fruits in day to day life.

Grapes contain large amounts of phytochemicals including phenols, flavonoids, flavonols, proanthocyanin, ascorbic acid, anthocyanins, and with good health benefits. Antioxidants are the most important chemical substances and exhibit a substantial amount of antioxidant activity [1-6]. Fruits have an important function in maintaining the physiological balance, optimal levels of antioxidants such as vitamin C and phenol compounds, anthocyanin, and phenol acids, being the most important for the human body [7]. Vitamin C present in grapes, fights against reactive oxygen species (ROS) [8] and polyphenols are associated with the prevention of diseases caused by oxidative stress [9,10]. Oxidation is a basic part of the aerobic life and metabolism. The potential reactive derivatives of oxygen species, attributed as ROS, are continuously generated inside the human body as a result of an excess of oxidative stress [11]. ROS donate a collection of oxygen radicals and some derivatives of oxygen like singlet oxygen [12]. Free radicals and other ROS such as superoxide radical anion, hydroxyl radical, and hydrogen peroxide are constantly generated through many biological processes and may be considered as a measure of biological inefficiency [13]. ROS-mediated oxidative damage to macromolecules namely lipids, proteins, and DNA have been implicated in the pathogenicity of major diseases such as cancer, rheumatoid arthritis, post-ischemic reperfusion injury, degeneration process of aging, myocardial infarction, and cardiovascular diseases [14,15]. An antioxidant is a compound that can delay or prevent the oxidation of lipids or other molecules by inhibiting the initiation or propagation of an oxidizing chain reaction. Although physical exercise is known to have many beneficial effects, there is also much evidence that free radical production increases during exercise and that oxidative damage occurs in the muscles, liver, blood and other tissues [16]. The human body uses an antioxidant defense system to neutralize the excessive levels of ROS. This system consists of enzymatic and non-enzymatic antioxidants. Some of the enzymes that are found to provide protection against ROS are superoxide dismutases, catalases, and glutathione peroxidases [17] in addition to numerous non-enzymatic small molecules distributed widely in the biological system and capable of scavenging free radicals [18]. The antioxidants may be of either the natural ones or the synthetic ones. Polyhydroxy flavones, flavanones, flavanols, isoflavones, chalcones, and many members are of these groups of natural substances proved to have a high degree of antioxidant activity.

METHODS

Grapes

Grapes were grown in red soil, and the ripened fruits were procured during February from Grape Research Station, Rajendernagar, and Hyderabad.

Chemicals and reagents required

Ferric reducing antioxidant property (FRAP), 2,2-Diphenyl -2-picryl hydrazyl (DPPH), Folin–Ciocalteau, 2,4,6-Tri (2-pyridyl)-*s*-triazine(TPTZ), nicotinamide adenine dinucleotide phosphate, 2,2′-azinobis3-ethylbenzthiazoline-sulphonic acid (ABTS), aluminum chloride, was obtained from Sigma-Aldrich Co., St. Louis, USA. 2, 6-dichlorophenolindophenol, methanol Folin–Ciocalteau, phenol, sodium carbonate, ethlenediaminetetraacetic acid (EDTA) disodium salt EDTA. Ascorbic acid, pyrogallol, metaphosphoric acid (MPA), acetic acid, potassium persulfate, acetone, sodium hydroxide, all chemicals used were of G R grade only.

Extraction of phenols, flavonols, flavonoids

10 g of sample (fruit) was thoroughly crushed and homogenized in mortar pestle with 10 ml of 80% acetone and the extract was centrifuged at 10,000 rpm (rotation parts per million) for 15 minutes at 40°C and the supernatant was combined with initial extract, and this was used for the estimation of phenols [19].

Determination of phenols

Total phenol contents in the extracts were determined by the modified Folin–Ciocalteau method [20]. An aliquot of the extract was mixed with 5 ml Folin–Ciocalteau reagent (diluted with water 1:10 v/v) and 4 ml of sodium carbonate. The tubes were vortexed for 15 seconds and allowed to stand for 30 minutes at 40°C for color development. Absorbance was then measured at 765 nm using Shimadzu 160A ultraviolet-visible (UV-VIS) double beam spectrophotometer.

Determination of flavonoids

The aluminum chloride colorimetric method was used for the determination of flavonoids [21]. Each plant extracts (0.5 ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. It remained at room temperature for 30 minutes the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu 160A UV-VIS double beam spectrophotometer.

Determination of flavonols

A volume of 2.0 ml of the sample, 2.0 ml of 2% aluminum chloride, ethanol, and 3.0 ml sodium acetate solutions were added [22]. The absorption was read at 440 nm by Shimadzu 160A UV-VIS double beam spectrophotometer was read after 2.5 hrs at 20°C.

Determination of proanthocyanidins

0.5 ml extract solution was mixed with 3 ml of 4% vanillin-methanol solution and 1.5 ml of HCl [23]. The absorbance was measured by Shimadzu 160A UV-VIS double beam spectrophotometer at 500 nm.

Determination of total anthocyanins content

The total anthocyanins content of each sample was dissolved in potassium chloride-hydrochloride acid buffer solution pH 1.0 and sodium acetate trihydrate ($CH_3COONa.3H_2O$) buffer solution pH 4.5 [24]. The absorbance was determined at 510 nm in a Shimadzu 160A UV-VIS double beam spectrophotometer.

Determination of ascorbic acid

10 g of the fruit sample were sliced, frozen into liquid nitrogen and stored at −80°C until the analyses were carried out. Frozen pulverized samples were weighed and mixed with 2.5 ml of the extract solution (3% MPA and 8% acetic acid for MPA - acetic acid extraction and 0.1% oxalic acid for oxalic acid extraction). The mixture was homogenized in a high-speed blender at 18,000 g (in ice and darkness) for 1 minute and then centrifuged at 9000 rpm (refrigerated at 4°C) for 20 minutes. This procedure was repeated twice, and the two resulting supernatants were mixed together [25].

Extraction of antioxidant enzymes (catalase, peroxidase, polyphenol oxidase and glutathione reductase)

Catalase activity was estimated as per the method of Barber [26]. The reaction mixture consists of 1 ml of the enzyme, 2 ml of hydrogen peroxide and 3 ml of 0.05 M Tris-Hcl buffer (pH 7.0). The reaction was stopped by 1 ml of 2.5 N H_2SO_4. After 5 minutes of incubation at 20°C, the residual H_2O_2 was titrated with 0.01 $KMNO_4$. A blank was prepared by adding 1 ml of 2.5 NH_2SO_4 initially to the reaction mixture at zero time.

Determination of peroxidase activity

Peroxidase activity was estimated as per the method of Kar [27]. The reaction mixture consisted of 2 ml of Tris-HCl buffer 0.1 M (pH 7.0), 1 ml of pyrogallol (0.01 M), 1 ml of H_2O_2 (0.05 M) and 1 ml enzyme, 1 ml of 0.05 M H_2O_2 and 1 ml of enzyme extract. The reaction mixture was incubated at 25°C for 5 minutes. The reaction was stopped by adding 1 ml of 2.5 NH_2SO_4. The amount of purpurogallin formed was estimated by measuring the absorbance at 425 nm in Shimadzu 160A UV-VIS double beam spectrophotometer.

Determination of polyphenol oxidase activity

Polyphenol oxidase was estimated as per the method of Kar [27]. The reaction mixture consists of, 2 ml of Tris-HCl buffer 0.1 M (pH 7.0), 1 ml of pyrogallol (0.01 M) and 1 ml of enzyme extract. The assay mixture was incubated for 5 minutes at 25°C. The reaction was stopped by adding 1 ml of 2.5 N H_2SO_4. The absorbance at 425 nm was recorded using Shimadzu 160 A UV-VIS double beam spectrophotometer.

Determination of glutathione reductase activity

Glutathione reductase activity was determined according to the method of Beutler [28]. 0.2 ml of sample, 1.5 ml of 0.3 M phosphate buffer, pH 6.8. 0.5 ml of 25 mM EDTA, 0.2 ml of 12.5 mM oxidized glutathione, and 0.1 ml of 3 mM nicotinamide adenine dinucleotide phosphate was added a decrease in absorbance was measured against that of blank at 340 nm.

Extraction for the FRAP, DPPH and ABTS assay

Soxhlet extraction method was employed for the preparation of 50% alcoholic extracts of the fruit or leaf powered sample was extracted for 6 hrs. The collected solvent extract was evaporated, dried and stored at 4°C [29].

Determination of FRAP assay

The FRAP reagent was prepared from sodium acetate buffer (300 mM, and pH 3.6), 10 mM TPTZ solution (40 mM HCl as a solvent) and 20 mM iron (Fe^{+3}) [30]. Chloride solution in a volume ratio of 10:1:1, respectively. The FRAP reagent was prepared freshly and warmed to 37°C in a water bath before use, 100 µl of the diluted sample was added to 3 ml of the FRAP reagent, the absorbance of the reaction mixture was then detected at 593 nm by using Shimadzu 160 A UV-VIS double beam spectrophotometer.

Determination of ABTS radical scavenging activity ABTS

The ABTS• cation radical scavenging activity of the extracts was determined according to the modified method of Re *et al*. [31]. A stock solution of ABTS was produced by mixing 7 mM aqueous solution of ABTS• with potassium persulfate (2.45 mM) in the dark at ambient temperature for 12-16 hrs before use. The radical cation solution was further diluted until the initial absorbance value of 0.7±0.005 at 734 nm was reached. For assaying test samples, 0.98 ml of ABTS solution was mixed with 0.02 ml of the plant extracts. The decrease in absorbance was recorded at 0 minute and after 6 minutes scavenging ability relative to the reaction control (without plant extract as 100%) was calculated by using the formula:

ABTS• radical scavenging activity (%) = ([Initial reading-final reading]/ Initial reading) × 100,

Where, initial reading is absorbance at 0 minute and final reading is absorbance at 6 minutes.

Determination of DPPH radical scavenging activity (DPPH)

The DPPH• radical scavenging activity was estimated by measuring the decrease in the absorbance of the methanolic solution of DPPH• [32]. In brief, to 5 ml DPPH• solution (3.3 mg of DPPH in 100 ml methanol), 1 ml of each plant extracts were added, incubated for 30 minutes in the dark and the absorbance (A1) was read at 517 nm. The absorbance (A0) of a reaction control (methanol instead of plant extract) was also recorded at the same wavelength. Ascorbic acid (5-50 µg/ml) was used as a standard. Scavenging ability (%) was calculated by using the formula:

DPPH• radical scavenging activity (%) = ([A0−A1]/A0) × 100,

Where, A0 was the absorbance of reaction controls and A1 was the absorbance of extracts or standards.

Statistical analysis

All results are expressed as mean±standard error mean. All results are meant of three replicates. The data were correlated using Pearson correlation coefficient at $p<0.05$ correlations among data obtained were calculated using Pearson's correlation coefficient (r) and $^{**}p<0.05$ was considered significantly different; SPSS 15 version was used for the statistical analysis.

RESULTS AND DISCUSSION

Phenol

Total phenol content of five varieties of *V. vinifera* species extracts were determined and are presented in Fig. 1. Rizamat represented the highest phenolic content (25.7±0.52 mg/g dry wt.) followed by Kismis chorni, flame seedless, Pusa navrang, and least in Thompson seedless. The total phenolic content of Kismis chorni, flame seedless, Pusa navrang, Thompson seedless grape was significant from each other. The results indicate that Rizamat contain high concentrations of phenol, and the composition of phenolic content in grapes vary with variety, species, season, environmental and management factors such as soil conditions and climate. Phenols have the antioxidant ability of fruits by phenolic compounds, and their antioxidant nature is important in disease prevention in plants and animals [33]. Phenols scavenge ROS by donating an electron, antioxidant effectivity depends on the stability in different systems, as well as number and location of hydroxyl groups. *In vitro* studies, of phenolic compounds had higher antioxidant activity than antioxidant vitamins and carotenoids [34]. These antioxidants can act against various degenerative diseases such as Alziemers, Parkinsons, and cancer. Phenolic compounds and their antioxidant nature are important in disease prevention in plants and animals. Antioxidant activity is dependent on the structure of the free radical scavenging compounds, the substituent present on the rings of the flavonoids and the degree of polymerization. Similar work was done in grape wine varieties were the antioxidant activity was high (93-146 mg/L GAE) [35].

Flavonol content of five varieties of *V. vinifera* species extract are presented in Fig. 1. Among all the grape varieties analyzed Pusa navrang (13.8±0.05 mg/g dry wt.) and had the highest flavonol content followed by flame seedless, Kismis chorni, Rizamat, Thompson seedless (8.4±0.05 mg/g dry wt.) A significant difference in total flavonoids content was observed between highest and lowest flavonol content in grape varieties.

Flavonoid content of five varieties of *V. vinifera* species extract is presented in Fig. 1. Among all the grape varieties analyzed Pusa navrang (60.7±0.2 mg/g dry wt.) had the highest flavonoid content followed by Kismis chorni, Thompson seedless, flame seedless, and least content in Rizamat (22.2±0.52 mg/g dry wt.). There was a significant difference in total flavonoids content between Pusa navrang, Kismis chorni, and Rizamat. Significant differences in total flavonoids content was not observed between Thompson seedless and flame seedless. In this study, there was a twofold difference in total flavonoid content between the highest and lowest ranked varieties, Pusa navrang and Rizamat. Flavonoid content was reported similar in Cabernet Gernischt (60±24.3 mg/100 g) [36]. Especially, flavonoids have been reported to be anticancer, anti-inflammatory, antifungal, antimicrobial, antibacterial, and antiviral. Flavonoids commonly constitute 85% of the phenolic content. Their dietary coronary heart disease mortality [37,38]. The most common flavonoids in white and red wines are flavonols, catechins (flavan-3-ols), and anthocyanidins, the latter being found only in red wine. Small amounts of free leucoanthocyanins also occur. Flavonoids exist free or bound to other flavonoids, sugars, non-flavonoids, or combinations of these compounds. Proanthocyanin content of five varieties of *Vitis vinefera* species grape extract are presented in Fig. 1. Among all the grape varieties analyzed Kismis chorni, (2.1±0.23 mg/g dry wt.) had the highest proanthocyanin content followed by Flame seedless, Rizamat, Pusa navrang, and least content in Thompson seedless (0.89±0.005 mg/g dry wt.). However, significant differences in total flavonoids content were observed between highest and lowest ranked flavonol content in grape varieties.

Anthocyanin content of five varieties of *V. vinifera* species grape extract are presented in Fig. 2. Among all the grape varieties analyzed Kismis chorni, (16.4±0.1%) had the highest anthocyanin content followed by flame seedless, Pusa navrang, Thompson seedless and

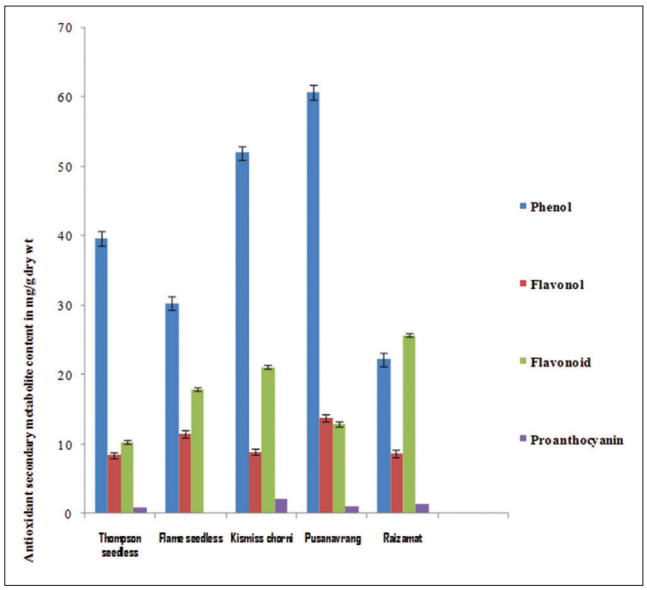

Fig. 1: Antioxidant secondary metabolite content in *Vitis vinifera* varieties

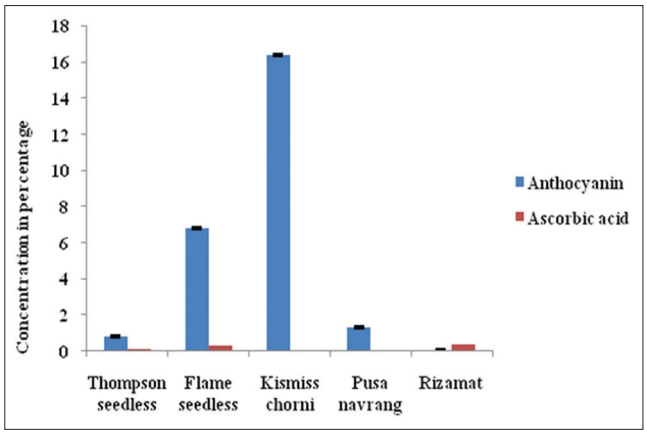

Fig. 2: The ascorbic acid and anthocyanin content in *Vitis vinifera* varieties

Rizamat (0.05±0.10%). However, significant differences in total flavonoids content were observed between highest and lowest ranked flavonol content in grape varieties. Similar studies on different grape varieties was done in milk grape variety and was showing (0.3±0.008 mg/100 g) [39].

Ascorbic acid content in five varieties of *V. vinifera* species grape extract is high in Rizamat (0.3±0.46%) followed by flame seedless, Thompson seedless, pusa nava rang, and least in kismiss chorni (0.01±0.05%) and statistically significant [40] the ascorbic acid content was high in wine varieties. Vitamin C content was very low in grapes (1.7 mg/g tissue), whereas in gooseberry and orange its concentration ranges from 40 to 57 mg/g vitamin C has received growing attention it is known to function as chemoprotective agents against oxidative stress mediated diseases. The importance of vitamin C is an antioxidant is indespensible in a biological system. This vitamin is reputed for scavenging the harmful; free radicals produced in the body and also enhance the antioxidant defense mechanism in the body. It has been reported that a dose of 1 g of vitamin C per day is sufficient to counteract the ill effects of low-density lipoprotein. Recent studies have shown that intake of sufficient amount of vitamin C is highly protective to prevent stroke and heart attack. Consuming diet rich in vitamin C fruits provides protection against cancer (Fig. 2).

Catalase enzyme activity of five varieties of *V. vinifera* species grape extract was maximum in Thompson seedless (3.7±0.005 U/g Fr wt.) followed by Pusa navrang, Rizamat, flame seedless and Kismis chorni (0.07±0.005 U/g Fr wt.) (Fig. 3). Enzymatic activity on studies were not done much work catalase is antioxidant enzyme which plays an important role in the body defense mechanism against the harmful effects of the ROS and free radicals in biological systems There was a significant catalase activity compared with highest and lowest enzyme activity. Catalase activity of increased with increased in the time interval and maximum catalase activity.

The peroxidase enzyme activity in five varieties of *V. vinifera*, the grapes were showing less peroxidase activity, similar studies were done in oranges were the activity was more in oranges than grapes (13.24 units/mg protein) in grapes (2.62 units/mg protein) [41].

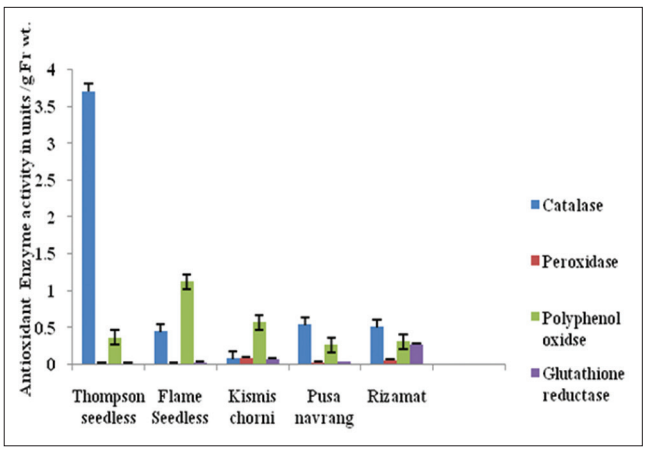

Fig. 3: The antioxidant enzyme activity in *Vitis vinifera* varieties

Species extracts of antioxidant enzymes namely catalase and peroxidase have been shown to increase when subjected to stress condition peroxidase reduces H_2O_2 to water while oxidizing a variety of substrates. Thus, peroxidases are oxidoreductases which use H_2O_2 as an electron acceptor for catalyzing different oxidative reactions. Showed a significant peroxidase activity and the maximum peroxidase activity of five varieties of grape extract was found to be peroxidase enzyme activity is high in Kismis chorni and (Fig. 3).

Polyphenol oxidase in five varieties of *V. vinifera* species grape extract is more in Flame seedless (1.12±0.01 U/g Fr wt.) and followed by Kismis chorni, Pusa navrang, Thompson seedless, Rizamat, less in Pusa navrang (Fig. 3). (0.26±0.001 U/g Fr wt.). Antioxidant enzyme which plays an important role in the body defense mechanism against the harmful effects of the ROS and free radicals in biological systems. Polyphenol oxidase has showed a significant enzyme activity and not much has been taken on enzyme activity.

The glutathione reductase activity in five varieties of *V. vinifera* species grape extract highest enzyme activity was in Rizamat (0.27±0.36 mg/g Fr wt.). Followed by Kismis chorni, Thompson seedless, Pusa navrang and least recorded in Flame seedless (0.005±0.005) (Fig. 3) much work on glutathione activity in grapes has been not done.

The five varieties of *V. vinifera* species grapefruit extracts exhibited higher antioxidant capacities and the values ranged from maximum to minimum and the maximum FRAP scavenging assay was in Flame seedless (34±0.167 µM/g dry wt.) and least in Thompson seedless(9.1±0.11 µM/g dry wt.). The antioxidant capacity of extracts on the FRAP assay decreased in the order of flame seedless > Pusa navrang > Rizamat > Kismis chorni > and Thompson seedless. Reported on the antioxidant properties of Norton (*Vitis aestivalis*) and Cabernet Franc (*V. vinifera*) are the wine grape varieties [42] studied on the antioxidant activity and phenol content of 16 raisin grape (*V. vinifera* L.) cultivars. Analysis of total phenols, total flavonoids, total anthocyanin and resveratrol in wine grapes and table grapes, was studied [43]. Quantitative analysis of wine varieties and (Cabernet Sauvignon, Chardonnay, Pinot Noir, Zinfandel, and Carignane), table grapes Emperor, Tokay, Perlette and Ribiero was studied by Wada *et al*. [44]. Ferric reducing antioxidant power is an antioxidant capacity assays. The FRAP assay is often used to measure the antioxidant capacity of foods, beverages and nutritional supplements containing polyphenols. The FRAP assay may offer putative index of antioxidant activity and measure the ability of antioxidants to reduce the ferric 2, 4, 6-tripyridyl-*S*-triazine complex [Fe (III)-(TPTZ) 2]$^{2+}$ to intensely blue colored ferrous complex [Fe (II)-(TPTZ)2]2+ in acidic medium. The antioxidant capacities of extracts from different samples using the FRAP assay are shown in. Further, FRAP exhibited a significant correlations with ABTS radical scavenging activities. Recently [45] a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species. It has been reported that the FRAP of Kei apple fruit juice correlated well with the polyphenol concentrations and the authors suggested that higher antioxidant activity of fruit is due to the presence of phenols.

The antioxidant capacities of five varieties of *V. vinifera* species grape extract using the ABTS assay are shown in Table 4, in decrease order Kismis chorni (11±0.5%) had highest scavenging activity followed by

Table 1: Antioxidant secondary metabolite content in *V. vinifera* varieties

S. No.	*V. vinifera* L. varieties	Phenols (mg/g dry wt.)	Flavonols (mg/g dry wt.)	Flavonoids (mg/g dry wt.)	Proanthocyanin (mg/g dry wt.)
1	Thompson seedlless	10.3±0.06	8.4±0.05	39.6±0.52	0.89±0.005
2	Flame seedless	17.9±0.05	11.5±0.06	30.3±0.28	1.9±0.003
3	Kismis chorni	21.1±0.289	8.9±0.05	51.9±0.58	2.1±0.23
4	Pusa navrang	12.9±0.058	13.8±0.05	60.7±0.2	1.1±0.04
5	Rizamat	25.7±0.52	8.6±0.05	22.2±0.52	1.4±0.17

V. vinifera: Vitis vinifera

> Thompson seedless > Pusa navrang > Rizamat > and least in Flame seedless (1.1±0.002%). Similar studies on antioxidant activities ABTS is frequently used to measure the antioxidant capacities of foods. ABTS method determines the antioxidant activity of hydrogen donating antioxidants and of chain breaking antioxidants. The ABTS assay is applicable to both lipophilic and hydrophilic compounds. The antioxidant capacities five varieties of grape extract using the ABTS assay are shown in decrease order Kismis chorni > Thompson seedless > Pusa navrang > Rizamat > Flame seedless (Fig. 4). In present study, the extract of all the samples showed notable ABTS·+ cation radical scavenging activity. Most fruits tested with high antioxidant capacity in the DPPH model, also showed a high antioxidant capacity in ABTS model. A significant correlation also exists between the total antioxidant capacity and total phenolics and between total antioxidant capacity and total flavonoids. According to Bao *et al*. [46] the highest ABTS scavenging activity of bayberry was attributed to the presence of higher levels anthocyanins, flavonoids, and total phenolic compounds.

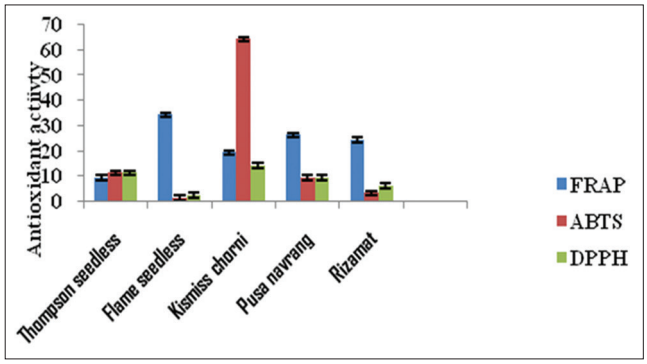

Fig. 4: Antioxidant radical scavenging activity in *Vitis vinifera* varieties

Table 2: Ascorbic acid and anthocyanin content in *V. vinifera* varieties

S. No.	*V. vinifera* L. varieties	Ascorbic acid (%)	Anthocyanin (%)
1	Thompson seedlless	0.1±0.01	0.8±0.05
2	Flame seedless	0.29±0.3	6.8±0.03
3	Kismis chorni	0.01±0.05	16.4±0.173
4	Pusa navrang	0.03±0.07	1.3±0.004
5	Rizamat	0.3±0.46	0.05±0.10

V. vinifera: Vitis vinifera

The scavenging effect of five varieties of *V. vinifera* species grape extracts the DPPH radical scavenging percentage was maximum in Kismis chorni (14.2±0.4%) followed by > Thompson Seedless > Pusa navrang > Rizamat > and least scavenging percentage of activity in Flame seedless (2.3±0.23%) (Fig. 4). Antioxidant effectives is measured by monitoring the inhibition of oxidation of a suitable substrate. In biological systems, it is classified into two group's evaluation of lipid peroxidation. Various methods are available for determine free radical scavenging activity DPPH method is recommended as easy and accurate with regard to measuring the antioxidant activity of fruits and vegetables. In this assay, the radical compound is stable and does not have to be generated as in other radical scavenging assays [47]. The assay is based on the measurement of the scavenging ability of antioxidants toward the stable radical-DPPH. The free radical DPPH is reduced to the corresponding hydrazine when it reacts with hydrogen donors.

The DPPH radical was widely used as the model system to investigate the scavenging activities of several natural compounds such as phenolic and anthocyanins or a crude mixture such as the ethanol extract of plants. DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The electrons become paired off and solution losses colors stochiometrically depending on the number of electrons taken up. The maximum percent of DPPH radical scavenging activity was found to be 95.46% in DPPH assay is an excellent tool for monitoring of chemical reactions involving radicals. DPPH is a stable free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers [23] DPPH is also a well-known free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Similar studies were studied on ABTS and DDPH activity as a rapid and simple measure of antioxidant activity; the DPPH radical scavenging capacity has been widely used. The DPPH assay was based on the reduction of the stable radical DPPH to yellow colored diphenyl picrylhydrazine in the presence of a hydrogen donor [48,49]. Kismis chorni was reported to have sustainable hydrogen donating and radical scavenging ability. It has been reported that the antioxidant activity of many compounds of botanical origin is proportional to their phenolic contents, suggesting a causative relationship between total phenolic content and antioxidant activity. Interestingly, which exhibited the highest total flavonoid and total anthocyanin, registered the highest DPPH radical scavenging potential activity [50]. Respectively the higher reactivity of ABTS reagent with phenolic compounds is the most important factor dietary intake of grape antioxidants helps to inhibit the production of ROS.

CONCLUSIONS

The present experiments quantification of the antioxidant - phytochemicals in *V. vinifera* varieties were designed to find out the

Table 3: Antioxidant enzyme activity in *V. vinifera* varieties

S. No.	*V. vinifera* varieties	Catalase (U/g Fr wt.)	Peroxidase (U/g Fr wt.)	Polyphenol oxidase (U/g Fr wt.)	Glutathione reductase (U/g Fr wt.)
1	Thompson seedless	3.7±0.005	0.006±0.001	0.36±0.058	0.005±0.005
2	Flame seedless	0.44±0.46	0.07±0.05	1.12±0.01	0.02±0.004
3	Kismis chorni	0.07±0.005	0.09±0.02	0.56±0.08	0.07±0.005
4	Pusanavrang	0.53±0.03	0.02±0.01	0.26±0.001	0.03±0.01
5	Rizamat	0.5±0.006	0.05±0.01	0.3±0.005	0.27±0.36

V. vinifera: Vitis vinifera

Table 4: Antioxidant radical scavenging activity in *V. vinifera* varieties

S. No.	*V. vinifera* varieties	FRAP (µM/g dry wt.)	ABTS inhibition (%)	DPPH inhibition (%)
1	Thompson seedless	9.1±0.11	11±0.5	11.5±0.05
2	Flame seedless	34±0.167	1.1±0.002	2.3±0.23
3	Kismis chorni	19.2±0.058	64±1.42	14.2±0.4
4	Pusanavrang	26.6±0.173	9.1±0.11	9.0±0.11
5	Rizamat	24.9±0.52	3.4±0.57	6.3±0.03

V. vinifera: Vitis vinifera

important antioxidants and nutrient potential, which are useful as a strategy of preventing and protecting the human populations from various stress, nutrient and age related diseases and disorders. Nature has gifted us with rich varieties and diversities of fruits which have to be carefully and judiciously used in a proper way for the benefit of human which are easily available and affordable and easy for the benefit of human welfare by implementing for the better health.

ACKNOWLEDGMENTS

I sincerely thank the Department of Botany, University College of Science, Osmania University, Hyderabad, Telangana state, India, for sponsoring the RFSMS (UGC-Delhi) fellowship.

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