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EVALUATION OF PHYTOCHEMICALS SCREENING AND ANTIOXIDANT ACTIVITY OF *VITIS VINIFERA* (GRAPES) FRUIT EXTRACT USING FENTON REACTION

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ABSTRACT
Grapes (*Vitis vinifera* L.) are a major fruit crop in the world. Grapes seem to confer health benefits due to their antioxidant activity. In traditional medicine system it is used especially for anticancer, blood purification, and as an antispasmodic. This plant was also useful in the health benefits associated with natural compounds and have been demonstrated with the emphasis on antioxidants. We have evaluated the phytochemical analysis and antioxidant potential of selected grape variety from India. The phenolic compound in fruits, vegetables, herbs and spices possess potent antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic activities. The present study focused on the phytochemical screening and antioxidant activity of *Vitis vinifera* fruit Extract in vitro conditions. The berries of *Vitis vinifera* was extracted with methanol using a Soxhlet extractor. Phytochemical screening of the methanol extract of *Vitis vinifera* showed the presence of glycosides, phytosterol, saponins, tannins, flavonoids and terpenoids. The total phenolics content of leaf as determined by Fenton reaction and was found to be good antioxidant activity as different dose concentrations. The antioxidant activity of extract was carried out with ascorbic acid as a standard reducing agent. All the analysis was made with the use of UV-Visible Spectrophotometer. In this plant *Vitis vinifera* Extract there was a remarkable concentration dependent free radical scavenging and reducing power was exhibited. These findings demonstrated that *Vitis vinifera* Extract possess free radical and hydroxyl radical scavenging activity as well as antioxidant activity in vitro. In conclusion the present Study indicates that *Vitis vinifera* Extract may be a potential source of natural antioxidant. The results suggested that *Vitis vinifera* Extract could serve as a potential source of natural phytochemicals and antioxidant and can be used in any preparations for combating free radical mediated damage to the body.

KEYWORDS: Antioxidant activity, Phytochemicals, Fenton Reaction, Hydroxyl radical, ascorbic acid, Vitis vinifera, TBARS.

1. INTRODUCTION
Grape is one of the world's largest fruit crops widely cultivated because of its economic importance in making wine, juice, jam, and raisins. The origin of various grape cultivars like Catawba, Concord, Niagara, Ontario, Delaware, and Thomson Green Seedless has been from the early 20th century (Read and Gu, 2003; Chiou, *et. al.*, 2007)). According to Macheix *et al.*, grapes are among the fruits containing the highest content of phenolic substances (Macheix, 1990). The grape phenolic compounds are mainly found in skins, pulps, and seeds that are partially extracted during winemaking (Revilla, *et. al.*, 2000). The phenolic compounds in fresh grapes and commercial grape juices may also be beneficial in the prevention of coronary heart disease as they have strong antioxidant activities toward human LDL oxidation in vitro (Meyer, *et. al.*, 1998). The quality of grapes juice depends on the type of the cultivar, the climate, and site factors like harvesting and post harvesting periods (Gu, *et. al.*, 2002). Phenolics are one of the most diverse groups of phytochemicals that are universally distributed in fruits, vegetables, and herbs. These compounds may be classified into phenolic acids, flavonoids, proanthocyanidins, stilbenes, and lignans (Luthria, *et. al.*, 2006; Keli, et. al., 1996). Flavonols are the most ubiquitous flavonoids in foods, and the main representatives are quercetin and kaempferol (Mira, *et al.*, 2002). Over the past years, researchers and food manufacturers had become increasingly interested in polyphenols from grapes because of their antioxidant properties and great abundance in diet (Kähkönen, *et. al.*, 1999; Filipe, *et. al.*, 2001). They play a probable role in the prevention of various diseases associated with oxidative stress, such as cancer, cardiovascular, type-2 diabetes mellitus, and neurodegenerative diseases. They are also important for various activities like antiplatelet,

anti-inflammatory, antiallergic, antiulcer, and antimutagenesis activities (Bomser, *et. al*., 2000; Hollman, *et. al*., 2001; Montonen, *et. al*., 2004). Flavonoids have also generated interest because of their broad pharmacological effects such as vasoprotective, antiviral, and antifungal actions (Carlo, *et. al*., 1999). The high phenolic content of the red wine is due to the incorporation of the grape skins into the fermenting grape juice during production. Kanner et al. showed that the black seedless grapes and red wines contain high concentrations of phenolics (Kanner, 1994). Furthermore, Day et al. showed that the commercial grape juice is effective in inhibiting the oxidation of LDL, isolated from human subjects (Day, *et. al*., 1997). Based on the above facts the objectives of our work was to quantify phenolics and flavonoids from fractions of twenty different grape cultivars that are available in Korea and to determine the antioxidant activities of the phenolic fractions of different grape cultivars. Such studies have great importance because the polyphenols have been shown to differ considerably in their bioavailabilities and to exert different biological activities *in vitro* and *in vivo*. Thus, this data may contribute to the selection of grape variety as a suitable plant material for the extraction of phytochemicals as ingredients of functional foods, and it is an important fruit as it is directly consumed by human and because of the potential use of the grape varieties in wine production (specially the grape varieties from *Vitis vinifera* L. species).

2. MATERIALS AND METHODS

2.1 Plant material – *Vitis vinifera* (Grapes) fruits was collected from local Market, Raipur (Chhattisgarh), India.

2.2 Chemicals and Reagent samples – The reagents used were of highest purity (>99.95%) and were purchased from Sigma Chemical Co. (Germany) and other. Sample absorbances were read using a Lambda 532 nm, UV Spectrometer made by Varian.

2.3 Preparation of extract - Dried powdered of *Vitis vinifera* (10 g) were extracted by continuous mixing in 100 ml 50% methanol, 24 h at room temperature. After the filtration process, methanol was evaporated until only water remained through evaporation on water bath at 60-70^0 C temperature. The final extract was kept in air tight box.

2.4 Phytochemical screening of the extract

The portion of the dry extract was subjected to the Phytochemical screening using the method adopted by Trease, Evans and Harbourne. Phytochemical screening was performed to test for alkaloids, saponins, glycosid, proteins, phytosterols, flavonoids, triterpenoids, tannins fixed oil and Fats.

2.4.1 Test for Alkaloids: A small portion of the extract was stirred separately with 1 ml of dilute Hydrochloric acid and filtered. The filtrate was treated with Dragandroff's reagent. Appearance of organic precipitate shows the presence of alkaloids.

2.4.2 Test for Saponin: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

2.4.3 Test for Glycosides: Small quantity of the extract o was hydrolyzed with 5ml Hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to Fehling's test. To 2ml of Fehling's solution (1ml of Fehling's A and 1 ml of Fehling's B solution), 2ml of extract was added, mixed well and boiled. Appearance of yellow or red color precipitate indicates the presence of reducing sugars.

2.4.4 Test for Proteins: Small quantity of the extract was dissolved in 5 ml of water and subjected to Xantho protein test. To 3 ml of the extract, 1ml of concentrate Nitric acid was added. A white precipitate was obtained. The solution was heated for 1minute and cooled under tap water. It was made alkaline by excess of 40% NaOH. Appearance of orange precipitate indicates the presence of protein.

2.4.5 Test for Phytosterol: Salkowski test was done for the detection of phytosterols. In this test, 1 ml of concentrated Sulphuric acid was added to the 1g plant extract and allowed to stand for 5 minutes. After shaking, formation of golden yellow color in the lower layer indicates the presence of phytosterols.

2.4.6 Test for Flavonoids: The extract was treated with concentrated Sulphuric acid. Appearance of yellowish orange show the presence of anthocyanins, yellow to orange color show the presence of flavones, and orange to crimson show the presence of flavonones.

2.4.7 Test for terpenoids (Salkowski test): 5 g of each extract was mixed in 2 ml of chloroform, and concentrated H_2S0_4 (3ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

2.4.8 Test for tannins: About 0.5 g of the dried powdered sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for browrish green or a blue-black colouration.

2.5 Deoxyribose assay to assess OH -radical scavenging activity

The OH- radical scavenging activity of *Vitis vinifera* extract (10–100 ug/ml) was determined according to the

deoxyribose method reported of Halliwell, *et al*., (1987). In the protocol the presence of 100 lM EDTA. $FeCl_3$, H_2O and ascorbic acid were prepared in degassed H_2O prior to use. The reaction tube contained (final concentrations) 3.6 mM deoxyribose, 100 lM EDTA, 1 mM H2O2, 100 lM L- ascorbic acid, 100 lM $FeCl_3$, H_2O in 25 mM phosphate buffer, pH 7.4 in 1.0 ml total volume. Samples was kept in incubation at 38º C, 1 hrs, 1.0 ml 1.0% TBA in 0.05 M NaOH and 1.0 ml 10% TCA were added to the reaction mixture after that samples was heated in a boiling water bath for 15 min. After the samples were cooled, the absorbance's were read at 532 nm. The IC50 value of the plant extract was compared with that of ascorbic acid, which was used as the standard. The lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percentage of inhibition of hydroxyl radical was calculated as follows:

$$\%inhibition = \left(\frac{Control\ Abs\ at\ 532\ nm - Sample\ Abs\ at\ 532\ nm}{Control\ Abs\ at\ 532\ nm}\right) \times 100$$

Antioxidant capacity of test compounds was expressed as IC_{50}, the concentration necessary for 50% inhibition concentration of TBARS.

2.6 Statistical Analysis

To evaluate associations between variables, the data were analyzed statistically using mean ±standard deviation and standard error.

3. RESULT

3.1 Phytochemicals Screening

The plant extract were screened for the presence of major secondary metabolite classes such as Alkaloides, Flavonoides, Saponin, Terpenoide, Tannin, Glycosides, Phytosterol, and Proteins, according to common phytochemical methods. The tests were based on visual observation of the change in color or formation of precipitate after the addition of specific reagent. The results of phytochemical tests carried out for *Vitis vinifera* with methanolic solvents are presented in Table 1. The present Study exhibited the presence and absence of phytochemical compounds in the extract. It was found that Methanol extract of the V. viifera was showed maximum number of phytochemicals.

Table 1: Phytochemicals screening of *vitis vinifera* extract.

Sl	Test	Result
01	Alkaloides	+
02	Flavonoides	+
03	Saponin	+
04	Terpenoide	+
05	Tannin	+
06	Glycosides	+
07	Phytosterol	-
08	Proteins	+

Where: + Present, - Absent

3.2 Antioxidant Activity

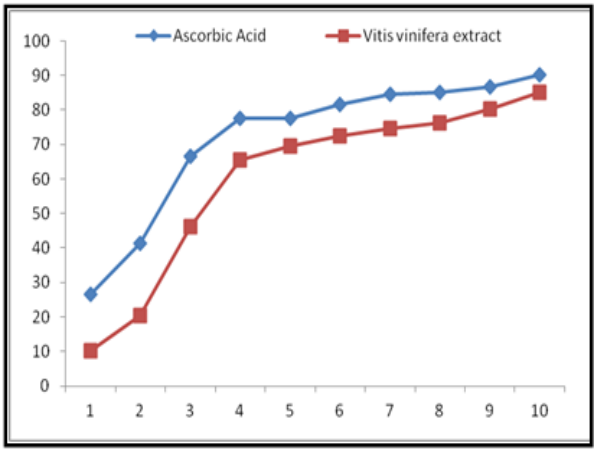
The result of the effect of the examined *Vitis vinifera* extract as well as control solution on OH- radical production. They show that all extract of *Vitis vinifera* extract and control solution as an Ascorbic acid inhibited the production of OH- radicals. The % of free radicals scavenging activity of hydro-methanol extract of *Vitis vinifera* presented reducing power, the free radical OH-scavenging activity of the extract increase with increasing the concentration. Extent of hydroxyl radical scavenged was determined the increasing in intensity of light pink coloured, which was determined at 532 nm. The oxidant activity was compared with Ascorbic acid as a positive control.

Table 2: Antioxidant activity of *Vitis vinifera* extract and ascorbic acid.

Concentration (in µl)	Ascorbic acid (Mean ± SE)	*Vitis vinifera* extract (Mean ± SE)
10	26.56±0.72	10.20±1.12
20	41.40±0.11	20.33±0.95
30	66.46± 0.14	46.22±0.24
40	77.58±0.19	65.52±0.58
50	77.52±0.27	69.39±0.58
60	81.63±0.38	72.57±0.56
70	84.46±0.29	74.59±0.13
80	85.17±0.14	76.10±0.55
90	86.58±0.14	80.21±0.33
100	90.11±0.10	84.97±0.86
Blank: 0.283		

IC_{50} Values

Sl	Group	IC_{50} Value
1.	**Ascorbic acid**	24.00 µg/ml
2.	***Vitis vinifera***	33.00 µg/ml



Graph 1: Shows the antioxidant activity of ascorbic acid and *vitis vinifera* extract.

Vitis vinifera fruits.

Drying process of *Vitis vinifera.*

Final powder of *vitis vinifera.*

4. DISCUSSION

The traditional medicaments have, therefore, been derived from rich traditions of ancient civilizations and scientific heritage. This is a term of recent origin and comprises nutritionally or medicinally enhanced foods with health benefits. These include engineered grain, cereals supplemented with vitamins or minerals or genetically manipulated soyabean and canola oil with fatty acids, etc. Many pharma and biotech companies have moved into this area since it does not involve regulatory clearances and offers large markets. These companies have extended the term nutraceutical to include pure compounds of natural origin like levitating, docosahexaenoic acid, sterols, curcumin, etc. Plants are able to produce a large number of diverse bioactive compounds. High concentrations of phytochemicals, which may protect against free radical damage, accumulate in fruits and vegetables (Carocho, *et. al*., 2013). Plants metabolites have biological properties such as antioxidant activities, antimicrobial effects, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human diseases (Beatty, *et. al*., 2000).

Grapes are the source of large number of nutraceuticals including resveratrol and have been suggested to have cardiovascular benefits, cancer chemo preventive activity, skin cancer prevention and protective action against other less prevalent but devastating illnesses such as Alzheimer's disease and urinary bladder dysfunction (Choi, *et. al*., 2012).

The plant is also beneficial in treating cardiovascular disease and in preventing liver toxicity, thus improving functions of heart and liver. In the our experiment the plant extract were screened for the presence of major secondary metabolite classes such as Alkaloides, Flavonoides, Saponin, Terpenoide, Tannin, Glycosides, Phytosterol, and Proteins, according to common phytochemical methods. The tests were based on visual observation of the change in color or formation of precipitate after the addition of specific reagent. The present Study exhibited the presence and absence of phytochemical compounds in methanol extract. Another set of experiment the effects of *Vitis vinifera* extract was evaluated on OH-radical production which was generated using Fenton reaction. They show that all extract concentration of *Vitis vinifera* and control solutions as an Ascorbic acid inhibited the production of OH-radicals. The free radicals scavenging activity of hydro-methanolic extract of *Vitis vinifera* presented reducing power, the free radical OH-scavenging activity of the extract increase with increasing the concentration. Extent of hydroxyl radical scavenged was determined the increasing in intensity of light pink coloured, which was determined at 532 nm. The oxidant activity was compared with Ascorbic acid as a positive control.

Vitis vinifera extract have a rich amount of phytochemicals and act as free radical hunter and avoid free radical mediated oxidation of biological molecules. Fruit extract showed strong antioxidant capacity in vitro and the extract can be considered as a good source of natural phytochemicals and antioxidant. Thus, *Vitis*

vinifera berries extract b could also become a potential source of phenolics for pharmaceuticals or functional foods.

5. SUMMARY

Grape (*Vitis vinifera L.*) is one of the world's largest fruit crop with apple, watermelon and banana. In 2014, the worldwide production was approximately 75 million tons, of which 41% was produced in Europe, 29% in Asia and 21% in the Americas. In addition, grapes contain a wide number of health promoting compounds, like polyphenols: flavonoids, and flavanols, flavonols and anthocyanins are the most representative in red varieties. In more detail, grape skins and leaves contain anthocyanins and flavonols, while pulp and seeds contain mainly proanthocyanidins and non-flavonoid compounds. Grape seed is available as a dietary supplement in capsules, tablets and liquid extracts. Among other beneficial effects, the active compounds in grape are believed to have different types of pharmacological activities. Black grape peel possesses a substantial amount of polyphenolic antimicrobial compounds that can be used for controlling the growth of pathogenic microorganisms. The purpose of this Study was to assess antibacterial and antifungal activity of black grape peel extracts against antibiotic-resistant pathogenic bacteria and toxin producing moulds, respectively. In the our experiment the plant extract were screened for the presence of major secondary metabolite classes such as Alkaloides, Flavonoides, Saponin, Terpenoide, Tannin, Glycosides, Phytosterol, and Proteins, according to common phytochemical methods. The antioxidant activities of *Vitis vinifera* extract scavenge OH' radical was assessed using the fenton reaction assay. Extent of hydroxyl radical scavenged was determined by the decrease in intensity of pink colored. The *Vitis vinifera* extract was found good antioxidant activity as compared with positive control as Ascorbic acid. This work provides as insight to understanding some prelim antioxidant properties of *Vitis vinifera* in traditional medicine. Further more, detailed studies on the isolation and characterization of the plant extract as well as *in vivo* assays will be necessary in discovering new biological antioxidants.

The grape skins and pulps have higher total phenolic contents and antioxidant capacities. Tremendous progress has been obtained for the extraction, analysis, and biological activities of polyphenols in grape. The bioactive compounds were usually extracted from grape using the liquid-liquid extraction, and high-performance liquid chromatography with UV or MS detection could be applied to the analysis of active components in grape. The grape and its main components like phenolics and flavonoids have a variety of bioactivities, such as antioxidant, cardioprotective, anticancer, anti-inflammation, antiaging, and antimicrobial activities. Thus, the presence of phytochemicals and other bioactive compounds present in grape skins and pulps may serve as a new potential source nutraceuticals and functional foods.

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