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IN VITRO ANTIOXIDANT ACTIVITY ON ETHANOLIC EXTRACT OF BLACK GRAPES (*VITIS VINIFERA*) AND PRELIMINARY PHYTOCHEMICAL SCREENING

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Abstract:

The aim of study is to assess the preliminary phytochemical screening and in vitro antioxidant activity of ethanolic extract obtained from fruits of black grapes of Vitis vinifera. The assessment of antioxidant activity performed by in vitro method using DPPH (2,2'-diphenyl-1-picrylhydrazyl), reducing power and hydrogen peroxide. Black grapes have good source of reducing sugars, Flavanoids, Saponins and Monosaccharides. The ethanolic extract of black grapes has been reported to show high scavenging activity against the DPPH free radical generating system. The antiradical activity of test compound and ascorbic acid against DPPH ic_{50} values were found to be as 17.3±0.71, to 57.7±0.5 increased with respectively concentrations with that of reference standard, ascorbic acid (47.6±0.48 to 89.1±0.51). Antioxidant activity of black grapes was found to be good and about near to the standards.

Keywords Vitis Vinifera, Antioxidant activity

INTRODUCTION[1-7]

Grape is the single most abundant fruit harvested in the world from which a natural color is commercially obtained. Grapes are highly pigmented with anthocyanins.1**-2** The samples of black grape fruits procured from local fruit market Kothapet, Hyderabad and sent for authentification to Depatment of Pharmacognosy, Sree dattha Institute of Pharmacy, Sheriguda, Hyderabad for identification of species.

The Botanical classification of Black grape fruits is as

Classification

Kingdom : plantae

Sub kingdom : tracheobionta

Superdivison : spermatophyte

Division : magnoliophyta

Class : magnoliopsida

Subclass : rosidae

Order : rhamnales

Family: : *Vitaceae*

Genus : *Vitiss*

Species: : *vinifera*

EXPERIMENTAL

Chemicals

The chemicals used for study are Ethanol AR grade, Sulphuric acd LR, Fehling reagent A and B, Ninhydrin solution, Deionised water, Conc. nitric acid and Barfoed's reagent.

Extraction

The maceration method is used to prepare the extract from whole fruits of black grapes. Friuts were washed properly with water and chopped in small pieces. These pieces soaked in ethanol then left for overnight or longer. Filter of and save the liquid extract and throw away the solid waste. Dilute the extract by about 50% deionised water. Store the extract solution in an appropriate sized plastic bottle. Label and date the bottle store

the solution in the refrigerator.Grape juice does not need the extraction, but add ethyl alcohol or ethanol (about 25%ethyl alchol and 75% juice) to prevent microbial growth.

Preliminary Phytochemcal screening[8]

The following test were performed for phytochemical study

1. Test for Alkaloids

a. Mayer' s test To a few ml of plant sample extract, two drops of Mayer‟s reagent are added along the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids.

b. Wagner's test: A few drops of Wagner‟s reagent are added to few ml of plant extract along the sides of test tube. A reddish- Brown precipitate confirms the test as positive.

2. Test for Amino acids The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatmann No. 1 filter paper and the filtrate is subjected to test for Amino acids.

a. Ninhydrin test Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) are added to 2 ml of aqueous filtrate. Appearance of purple colour indicates the presence of amino acids.

3. Test for Carbohydrates

a. Molish' s test To 2 ml of plant sample extract, two drops of alcoholic solution of α-naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

b. Benedict' s test To 0.5 ml of filtrate, 0.5 ml of Benedict‟s reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Table 1: Results of preliminary phytochemical test

TEST	RESULTS
Amino Acids	-ve
Alkaloids	-ve
Reducing Sugars	+ve
Flavanoids	+ve
Proteins	-ve
Saponins	+ve
Monosaccharides	+ve

In Vitro Antioxidant Activity[9-11]

Assay of Reducing Power

In test tubes, different concentrations of plant extract solution (100 - 500 µg/ml) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%, w/v), then mixture was incubated at 50º C for 20 minutes. After incubation, 2.5 ml of trichloroacetic acid (100g/l) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml FeCl3 (1g/l) and absorbance measured at 700nm in UV Visible spectrophotometer. Ascorbic acid was used as standard and phosphate buffer used as blank solution. The absorbance of the final reaction mixture of three experiments was expressed as mean ± standard deviation. Increased absorbance of the reaction mixture indicates stronger reducing power.

DPPH scavenging activity procedure:[12-15] - DPPH radical scavenging activity was measured using the method of Cotelle et al., with some modifications. 3 ml of reaction mixture containing 0.2 ml of DPPH (100 µm in methanol) 2.8 ml of test solution, at various concentrations (5, 10, 20, 40, 80, 160 320 µg/ml) of the synthetic compound was incubated at 37°C for 30 min absorbance of the resulting solution was measured at 517 nm using Beckman model DU-40 spectrophotometer. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control using the following equation:

% scavenging activity=absorbance of blank–absorbance of test / Absorbance of blank x 100+ IC_{50} will obtain from a plot between concentration of test compounds and % scavenging. Ascorbic acid is used as standard for comparison.

IN VITRO METHOD:

Experimental animals: 20 adult male albino rats weighing 140-160g were used for the study were procured. They were housed in polypropylene cages and were maintained at room temperature of 23°c ± 2°c and relative humidity 50%. They were maintained in 12h: 12hr light: dark cycle throughout the period of acclimatization and experimental study. Animals were provided with standard rodent pellet diet. Food and water was allowed *ad libitum.*

Table 2:DPPH radical by various concentrations of test compound and ascorbic acid

Concentrations of test compound and ascorbic acid (µg/ml)	**Percentage inhibition of dpph radical (ic_{50})**	
	***Test compound* (EETC)**	**Ascorbic acid**
5	17.3±0.71	47.6±0.48
10	25.2±0.31	56.15±0.65
20	31.3±1.0	65.6±0.48
40	38.4±0.7	70±1.33
80	40.7±0.35	77.8±0.82
160	47.6±0.7	84.9±1.1
320	57.7±0.5	89.1±0.51

The test compounds have been reported to show high scavenging activity against the DPPH free radical generating system. The antiradical activity of test compound and ascorbic acid against DPPH was shown in table and the ic_{50} values were found to be as 17.3±0.71, to57.7±0.5 increased with respectively concentrations with that of reference standard, ascorbic acid (47.6±0.48 to 89.1±0.51).

The results clearly indicate the free radical scavenging activity of test compound in vitro and this activity comparable with that of standard drug ascorbic acid.

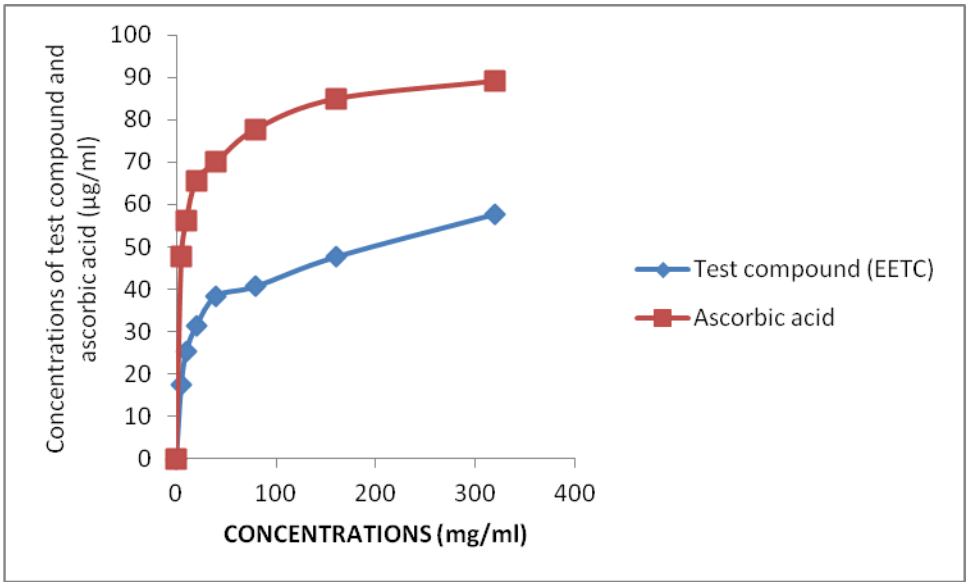


Figure 1: In vitro concentration dependent percentage inhibition of DPPH radical by EETC and ascorbic acid

Estimation of Superoxide Dismutase (SOD)

Superoxide dismutases are the enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. As such, they are an important antioxidant defense in nearly all cells exposed to oxygen. The enzyme superoxide dismutase (SOD) was determined in erythrocytes using photo oxidation method (Misra and Fridowich, 1977; Arutla *et al.,* 1998), which is briefly described below.

Principle: In this assay free radicals are generated by photo-oxidation of o-dianisidine sensitized by riboflavin. The photo oxidation of O-dianisidine involves a complex series of free radical chain reactions involving the superoxide anion ($O_2^{\bullet -}$) as the propagating series A general free radical scavenging compound has a inhibitory effect on this reaction leading to a decrease in the oxidized dianisidine measurable by UV/visible spectrophotometer. In contrast, any compound which specifically scavenges $O_2^{\bullet -}$ will remove the $O_2^{\bullet -}$ from step 3 and 4 in Figure 5.1 thus increasing the amount of oxidized dianisidine and hence will have an augmentary effect in this reaction. This assay can thus be used to determine whether a compound is a general, free radical or a scavenger specific for the super oxide anion. A substance with no free radical scavenging activity.

Photo-oxidation of *o*-dianisidine

1. $Rb + h\nu \longrightarrow Rb^{\bullet}$
2. $Rb^{\bullet} + DH_2 \longrightarrow rbh^{\bullet} + DH^{\bullet}$
3. $Rbh^{\bullet} + O_2 \longrightarrow Rb + O_2^{\bullet -} + H^+$
4. $DH^{\bullet} + O_2^{\bullet -} + H^+ \longrightarrow DH_2 + O_2$
5. $DH^{\bullet} + DH^{\bullet} \longrightarrow D + DH_2$
6. $O_2^{\bullet -} + O_2^{\bullet -} \xrightarrow{SOD} H_2O_2$

Rb = Riboflavin

Hν = energy of photon light

$Rb^{\bullet}$ = exited riboflavin

DH_2 = *o*-dianisidine

$O_2^{\bullet -}$ = superoxide anion

D = product formed by photo oxidation measured at 460nm

Reagents preparation:

For SOD estimation, 0.01M phosphate buffer (ph 7.5) was prepared.

Potassium phosphate buffer preparation:

Weight 1.741gms of K_2HPO_4, and dissolved in 1000ml distilled water, and 680.45mg of KH_2PO_4, and dissolved in 50ml individually, then the ph of K_2HPO_4 was observed. To K_2HPO_4, add aliquots of KH_2PO_4 until 7.5 ph attained which was measured with ph meter.

Preparation of riboflavin solution:

Riboflavin (5mg) was weighed, and dissolved in 1lit of potassium phosphate buffer, to attain concentration of $1.3*10^{-5}$ M.

Preparation of o-dianisidine solution:

O-dianisidine solution was prepared by weighing of 122mg and dissolved in 50ml of ethanol.

Extraction procedure: 3ml of packed blood cells were lysed by the addition of equal volume of cold deionized water. Hemoglobin was then precipitated by the addition of chloroform: ethanol (1.5:1). This was diluted with 500µl of water and centrifuged for 15 minutes at 3000 rpm. The supernatant containing SOD was taken for the measurement of its activity.

Assay procedure: 0.88ml of riboflavin solution (1.3×10^{-5} M in 0.01M potassium phosphate buffer, ph 7.5) was added to 60µl of O-dianisidine solution (10^{-2} M in ethanol) and to this 100µl of clear separated SOD was added and optical density was measured at 460nm. Then the cuvette containing reaction mixture was transferred to the illuminating box, illuminated for 4min., and optical density was remeasured against blank containing ethanol in place of enzyme. The change in the optical density was determined. The SOD content was determined from the standard graph prepared using pure bovine SOD.

Evaluation of antioxidant activity using azathioprine induced oxidative stress in rats

Superoxide dismutase:

Superoxide dismutase is class of enzyme that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. It is an important antioxidant defence in nearly all cells exposed to oxygen. Superoxide dismutase activity was estimated in tissue homogenate with help of pure bovine superoxide dismutase standard. The values were shown in below table, and figure.

Table 3: standard graph values of superoxide dismutase

Sod(µu)	**Absorbance**
1000	0.015
3000	0.017
10000	0.039
30000	0.062
100000	0.16

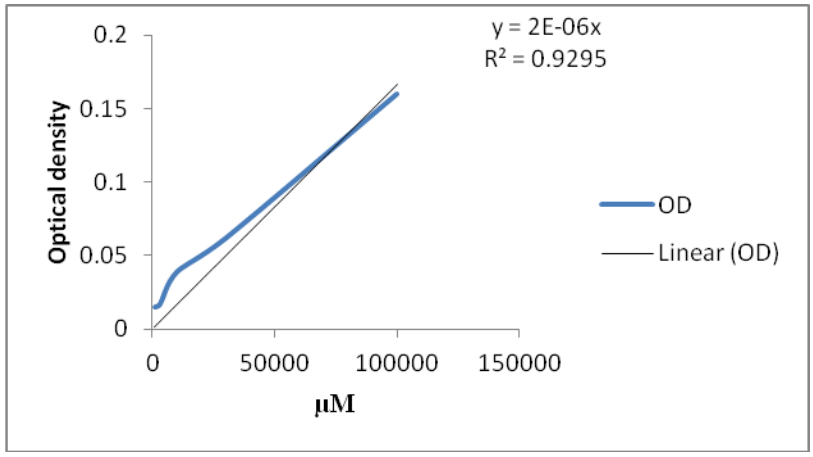


Figure 2: standard graph of superoxide dismutase

Table 4: superoxide dismutase levels in kidney tissue homogenate

Group	Sod(u/mg) in kidney
Normal group	98.6±0.95
Toxic control (20mg/kg)	13.2±0.22
EETC low dose(100mg/kg)	39.7±0.6**
EETC high dose (30mg/kg)	57.6±1.1***
Standard ascorbic acid(10mg/kg)	80.2±0.84***

All the values are expressed as mean ±sd (n=6); ** indicates p<0.001, *** indicates p<0.0001 vs toxic control.

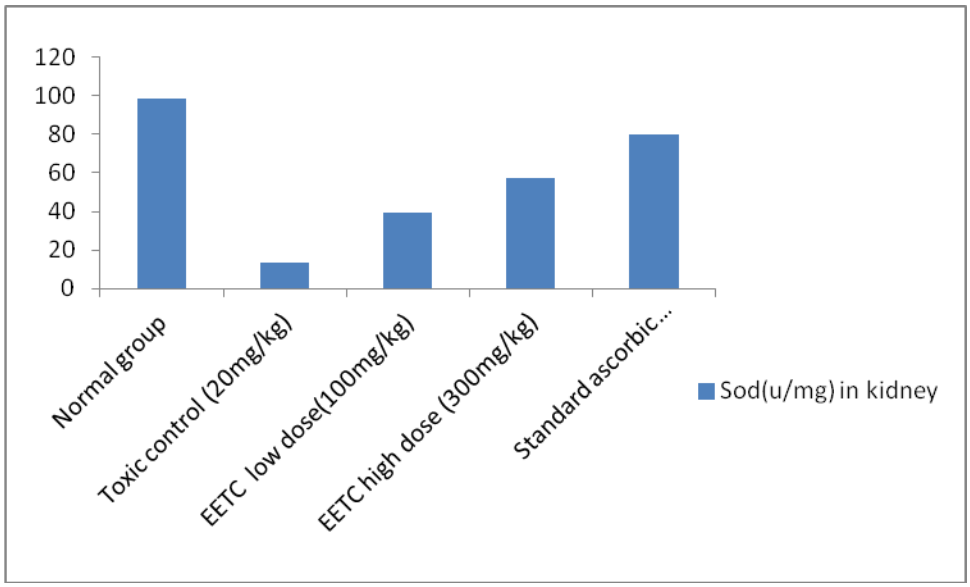


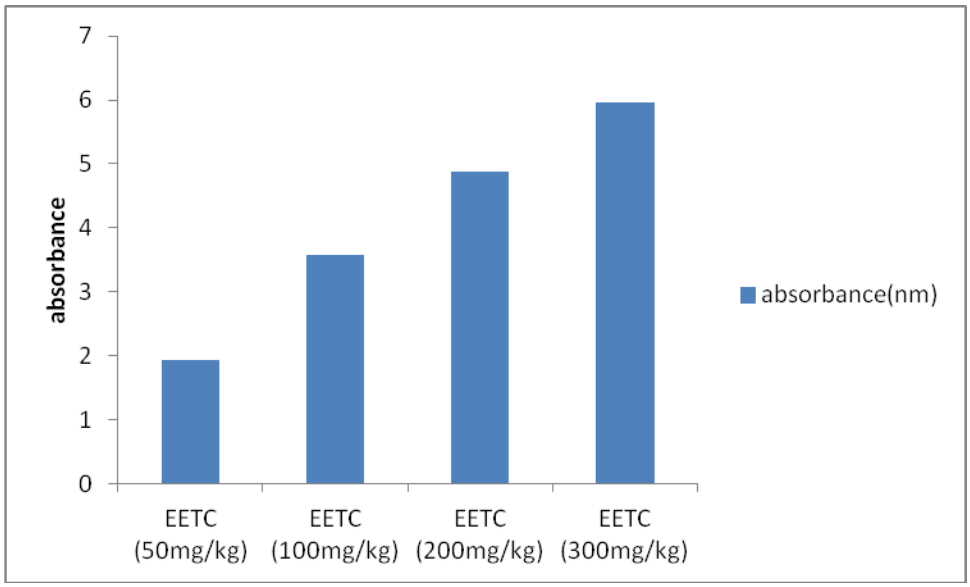
Figure 3:Effect of EETC on superoxide dismutase levels in kidney tissue homogenate in rats treated with azp.

In this study, we found that 20mg/kg dose of azathioprine causes significant ($p<0.001$) decrease in superoxide dismutase levels. This reduction indicates that oxidative stress and toxicity is produced with azathioprine. Post treatment with test compound at the dose of 100mg/kg and 300mg/kg after a 20mg/kg dose of azathioprine administration, shown a significant ($p<0.001$, $p<0.0001$) dose dependent increase in levels compared to toxic control group.

REDUCING POWER

Table 5: Reducing power of different concentrations of test compound

Group	Absorbance(nm)
EETC (0.50mg/kg)	1.925
EETC (0.100mg/kg)	3.568
EETC (0.200mg/kg)	4.869
EETC (0.300mg/kg)	5.965



RESULTS AND DISCUSSIONS

The present study for in vitro antioxidant activity was carried out on *Vitis vinifera* (Muscat variety) fruit extracts. These revealed the presence of phytochemicals with biological activity that can be of valuable medicinal value for phytochemicals such

as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids. The phytochemical screening of grape showed the presence of alkaloids, flavonoids, carbohydrates, saponins, tannins, triterpenoids, catechol, steroids, phlobatannins and acidic compounds. Glycosides, phytosterol were absent in both grape fruit extracts (Table 1). In in vitro antioxidant study black grapes fruits shown good activity with compared to standard.

CONCLUSION

Phytochemical screening suggests that black grapes have good source of reducing Sugars, Flavanoids, Monosaccharides and Saponins. The outcome of study suggest that black grapes have natural source of antioxidants which give economical benefits to poor people for antioxidants. Grapes are easily available in market.

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REFERENCES

1. G. Mazza and E. Miniati, Anthocyanins in Fruits, Vegetables and Grains, Boca Raton., FL: CRC Press, 1993, 1-6.
2. F.J. Francis, Food colorants: anthocyanins, Crit. Rev. Food. Sci. Nutr. 1989, 28, 273-314.
3. Weil, J.A. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem*.2003, 84, 551-562.
4. Arts, I.C.; Hollman, P.C. Polyphenols and disease risk in epidemiologic studies. *Am*. *J*. *Clin*. *Nutr*.2005, 81, 317S– 325S.
5. Arvanitoyannis, I.S., D. Ladas and A. Mavromatis: Potential Uses and Applications of Treated Wine Waste: A Review. *Int. J. Food Sci. Tech*. 2006, 41, 475-487.
6. Bagchi, D.; Bagchi, M.; Stohs, S.J.; Das, D.K.; Ray, S.D.; Kuszynski, C.A.; Joshi, S.S.; Pruess, H.G. Free radicals and grape seed proanthocyanidin extract: Importance in human health and disease prevention. *Toxicology*. 2000, 148, 187– 197.

7. Bajaj, S., A. Urooj and P. Prabhasankar: Effect of Incorporation of Mint on Texture, Colour and Sensory Parameters of Biscuits. *Int. J. Food Prop*. 2006, 9, 691-700.
8. Kokate C. K.,Practical Pharmacognosy, Vallabh Prakahan, Fourth Edtion 1994, chapter no. 6, page no. 108-111.
9. Bakkalbasi, E., O.Yemi, D. Aslanova and N. Art: Major Flavan-3-ol Composition and Antioxidant Activity of Seeds from Different Grape Cultivars Grown in Turkey. *Eur. Food Res. Technol.* 2005, 221, 792-797.
10. Bravo, L. Polyphenols: Chemistry, Dietary Sources, Metabolism and Nutritional Significance. *Nutr. Rev.* 1998, 56, 317-333.
11. Cano, A. and M.B. Arnao: Hydrophilic and Lipophilic Antioxidant Activity in Different Leaves of Three Lettuce Varieties. *Int. J. Food Prop*. 2005, 8, 521-528.
12. Dröge, W. Free radicals in the physiological control of cell function. *Physiol*. *Rev*. 2002, 82, 47–95.
13. Halliwell, B. Free radicals, antioxidants, and human disease: Curiosity, cause, or consequence? *Lancet* 1994, 344, 721–724.
14. L.S. Einbond, K.A. Reynertson, X.-D. Luo, M.J. Basile, E.J. Kennelly Anthocyanin antioxidants from edible fruits. *Food Chemistry*, 2004, 84, 23–28.
15. Moure, A., J.M. Cruz, D. Franco, J.M. Domínguez, J. Sineiro, H. Domínguez, M.J. Núñez and J.C. Parajo. Natural Antioxidants from Residual Sources. *Food Chem*, 2001, 72,145-171.
16. Pokorny, J., N.Yanishlieva, and M.Gordon. Introduction of Antioxidants in Food. *CRC Press and Woodhead Publishing Limited, Cambridge, England,* 2001, 1-3.

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