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Determination of Antioxidant and Genoprotective Potential of Grape Seed Extract.

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ABSTRACT

Background: Awareness of antioxidantpotential of natural fruit, vegetables, medicinal plants etc. is rapidly developing world wide. Free oxidative radicals produced in our body by the process of natural metabolism cause the damage of healthy cells in our body. Antioxidants scavenge the free oxidative radicals and protect our healthy cells. Present study is an attempt to evaluate the antioxidant potential of Grape seed by estimating the total phenolic contents.Gallic acid, the specific bioactive compound present in grape seed, identified by HPLC technique, showed to have a genoprotective potential for prevention of oxidative DNA damage.

Materials & methods: Fresh grape seeds in form of powder were used for methanol, ethanol and acetone solvent extraction. The specific bioactive compound present in grape seed extract (Gallic acid) was analytically separated and identified by HPLC technique. Antioxidant potential of Grape seed was checked on Yeast Model by Viable Cell Count method, which showed that grape seed extract prevents oxidative cell damage. Total Phenolic compounds present in grape seed was determined by Folin-Ciocalteu assay. The potential of scavenging oxidative radicals was proved by DPPH assay. FRAP assay determined the potential of grape seed extract to reduce ferric compounds to ferrous, which is considered to be an important property of being an anti-oxidant. Protection of oxidative DNA damage by grape seed extract was also shown by Agarose Gel Electrophoresis technique using plasmid DNA.

Results: The data obtained through various chemical and biological methods indicated that grape seed has high potential of scavenging oxidative radicals which can cause oxidative DNA damage and the above extract can also prevent oxidative cell death by reducing the effects of UV radiation evidenced by using yeast model.

Conclusion: Now-a-days air pollution, chemicals, food additives, industrial pollution, fertilizers, pesticides etc., increase the chances of diseases related to DNA and cell damages which can cause cancer and neurodegenerative complications. Hence searching for natural substances which can prevent and reduce the damage of healthy cells in human body is a vital requirement. Grape seed with high antioxidant value, genoprotective potential and low cost therefore can make it a highly demandable supplement.

Keywords: Grape seed, Gallic acid, Antioxidant, Oxidative stress, DNA damage.

Introduction

Scientific evidence has suggested that under oxidative stress conditions, oxygen radicals such as superoxide anions (O2-), hydroxyl radical (OH) and peroxyl radicals (H2O2) are produced in biological system. These reactive oxygen species can damage DNA which causes mutation and chromosomal damage (Halliwell and Gutteridge, 1999)[15]. Moreover, the production of excessive free radicals

stimulates the oxidative damage and such situation contribute to more than one hundred disorder in humans including atherosclerosis, coronary heart disease, neurodegenerative disorder, cancer and they playing major role in the aging process (Pong,2003)[16]. Therefore, antioxidants are vital substances which possess the ability to protect the body from damage caused by free radicals induce oxidative stress (Ozsoy et al, 2008)[17]. Toxicities of the synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylatedhyroxytoluene (BHT), increased attention towards natural antioxidants (Naimiki, 1990)[18]. There is growing interest in the therapeutic potential of medicinal plants as antioxidants properties in reducing such free radical induced damages rather than looking for synthetic ones (McClements and Decker, 2000)[19].The search for plant-derived antioxidants has been received much attention and effort in order to identify the compounds that has high capacity in scavenging free radicals related to various diseases (Silva et al., 2007)[20].Generally, antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide of lipid hydroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Epidemiologists have observed that a diet rich in polyphenolic compounds may result in a positive health effect attributed to its antioxidants properties (Frankel et al., 1996; Hertog et al., 1993)[21]. OPCs in grape seed are powerful antioxidants shown to be far more potent than the common antioxidants vitamins C, E and beta-carotene. In fact, their usefulness in fighting free radicals and helping people lead a longer, healthier life is becoming well established, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols (Sies, Helmut,1997)[1].

Material & methods:

Production of Grape Seed Powder and Solvent Extraction

Fresh red seeded grapes (Peru product) obtained from market of Pune city, Maharashtra, India were washed with water thoroughly then dried. Seeds were grinded finely with coffee grinder machine and kept at -18 °C for methanolic, acetonic and ethanolic solvent extraction. 10g powder of grape seed was added in each of the three flasks and mixed with 20ml of 70% methanol, acetone and ethanol separately and kept on shaker for two hours, then filtered with Whatman filter paper no.1 and centrifuged at 10000 rpm at 4 'C. The same procedure was done on residues of filtration as second extraction step. All the supernatant was collected in dark color glass bottles. The extracts were concentrated by using Rotary Evaporator apparatus at 30 rpm and 50 °c. All the extracts were stored at 4 °C.

Analytical separation and identification of Gallic acid from grape seed extract by HPLC

A Chemito LC 6600 HPLC apparatus type with Isocratic liner program was used. Methanolic extract sample and 20% methanolic solution of Gallic acid as standard was analyzed by C18 column with temperature of 25 °C, injection volume 10 µl, pressure of 15 psi, flow rate 1 ml/min, UV detector at 280 nm and methanol**:** acetic acid mobile phase with ratio of 90**:**10.The column was run for 20 minutes in case of sample and 10 minutes in case of standard.

Viable cell count assay using yeast model

By streaking technique pure yeast cells without any microbial contamination was obtained and preserved on prepared slants then kept at 4 °C for future use. To study the effect of free radicals, oxidative stress was given to yeast cells by adding hydrogen peroxide to the cells and exposure to UV light for three different time periods.30% stock hydrogen peroxide was diluted in 3 different ways to produce following doses:-Low dose: [1ml of H2O2 in 9ml of D/W (10%)] Medium dose: [2ml of H2O2 in 8ml of D/W (20 %)] and High dose: [3ml of H2O2 in 7ml of D/W (30 %)].0.25% methylene blue stain was used to observe the cells under the microscope.

1 ml of confluent yeast growth was added to 9 ml of D/W to reduce the number of cell for counting them easily (1/10 dilution). 250 µl Hydrogen peroxide was added to 500 µl yeast cells in the above doses and the cells were incubated for 15 minutes at room temperature, then were stained with 0.25% methylene blue and observed under microscope(at 40X power) to check its viability (25 µl + 50 µl). The viable cells were counted using a haemocytometer in less than 4 minutes because methylene blue is toxic to the cells and the number of dead cells will increase sharply. As showing in the picture (Fig.1) live cells are transparent or partially stained but dead cells are fully stained. Homogenized distribution of cells while counting is important and it is done by mixing

well the sample before observation under microscope. For all three doses, cells were counted three times and average was taken.

For UV stress test, UV lamp of laminar air flow was used as light source. 15 ml of 1/10 diluted yeast broth in a petriplate was placed with 15cm distance under the UV lamp for three doses: Low exposure dose: 15 minutes, Medium exposure dose, 30 minutes and High exposure dose: 60 minutes.For each exposure, the cells were counted three times and averages were taken. In this test also mixing before sampling and counting under microscope is important. The same volumes were used for staining the sample.

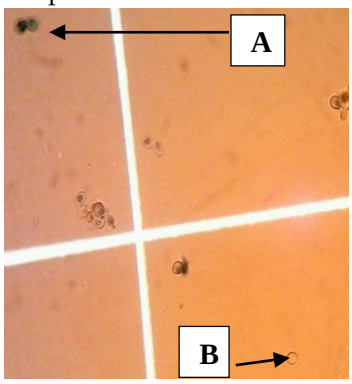


Fig.1 Cell counting with methylene blue stain under Haemocytometer (40X) A: Dead cell, B:Transparent (Live) cell.

DPPH assay

The DPPH (2, 2'-diphenyl-1-picrylhydrazyl radical) was used to check the scavenging activity of the samples. 6 mg of DPPH was dissolved in 100ml methanol; the tube was covered with aluminum foil to protect the reagent from light. 2ml of DPPH solution was added to 1 ml methanol and absorbance was taken immediately at 517nm for control. 1ml of extract was added into the test tube containing 2ml of DPPH solution and the same procedure was done for ascorbic acid as standard. The mixture was shaken and left in dark. Absorbance was taken after 30 minutes at 517nm using methanol as blank on UV-visible spectrophotometer and scavenging activity percentage was found by the formula:

$$\% \text{ scavenging} = \frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

FRAP assay

FRAP (Ferric Reducing Antioxidant Power) is used to check the antioxidant potential of a particular sample. 300mM Acetate buffer was prepared of pH 3.6, along with 40mM diluted HCl and 10mM TPTZ(2,4,6-tri[2-pyridyl]-s-triazine) and mixed in the following volumes to prepare FRAP reagent. 200ml acetate buffer, 20 ml TPTZ solution, 20ml FeCl3 solution and 24ml distilled water were kept at 37 °C in water bath. For blank 30µl distilled water was added to 1ml of FRAP reagent. 30µl of the sample and 1ml of the FRAP reagent were mixed into the cuvette and the absorbance at 593nm was recorded immediately. The cuvette was placed in water bath 37°C for 4 minutes and absorbance was recorded for the second time. The samples are then placed in water bath at 37°C for 4 minutes and again absorption was measured at 593nm. 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mM ascorbic acid were used to make the standard curve. The formula (FRAP value of standard is 2) used to calculate the ferric reducing power of the sample is:

$$\frac{\text{change in absorbance of sample from 0 to 4 minutes}}{\text{change in absorbance of standard from 0 to 4 minutes}} \times \text{FRAP value of standard}$$

FOLIN CIOCALTEU'S assay

Amount of total Phenolics content of grape seed extract was assessed by Folin-Ciocalteu's reagent. It is done by mixing 0.5ml of methanolic solution of grape seed extract with 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5ml of 7.5% of NaHCO3. Blank was concomitantly prepared, containing 0.5ml methanol, 2.5ml 10% Folin-Ciocalteu's reagent dissolved in distilled water and 2.5 ml of 7.5% of NaHCO3. Different concentration of Gallic acid solutions was prepared as: 0.02, 0.04, 0.06, 0.08 and 0.1 g/l with distilled water . The samples were incubated at 45°C in water bath for 30 minutes, the absorbance volumes of different concentration of Gallic acid (as standard) and grape seed extract were determined at 765 nm (table 4) and plotted as a standard graph (graph no.7),then phenolic content in extracts was expressed in terms of Gallic acid equivalent (mg GA/g of extract) by using this formula: C (GA equivalent) = c × V/M (where c = concentration determined from standard curve (mg/ml), V = volume used during the assay (ml), and M = mass of the extract used during the assay (g) and it will give GAE in mg/g extract.

Agarose gel electrophoresis

For evaluating the oxidative DNA damage protecting capacity of grape seed extract against oxidative agent like H2O2 , produced hydroxyl radicals (·OH), 4 µl

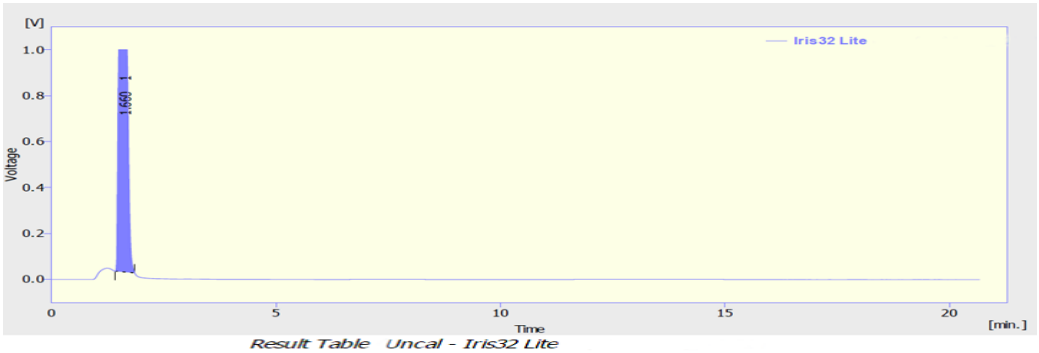
of pure Plasmid DNA was incubated with 5 µl of H2O2 (250µM: 11.3µl of 30% v/v) in well C to observe the damage of DNA. Also 4µl $FeSo_4$ (10µM: 0.278g $FeSo_4$ dissolved in 10ml of D/W) as reducing agent was added in each well. In the well D, E and F Plasmid DNA was incubated with H2O2, $FeSo_4$ and sample extract prepared in various solvent (Table 1). For preparing 1.8% gel, 0.9 g of agarose powder was dissolved in 50ml of 1x TAE buffer by using microwave oven and 2 µl of Ethium Bromide dye was added for indicating DNA under UV light. After solidification of gel and setting of electrophoresis apparatus, total 15 µl of samples were exactly added to the wells according to the table no.1 and let the gel run for about 2 hrs. When the run was about 2/3 of the length, Electrophoresis apparatus was switched off and the gel was observed under UV light.

	Wells					
Mixture	A Ladder DNA	B Control	C	D	E	F
DNA(µl)	4	4	4	4	4	4
H2O2 250µM (µl)	-----	-------	5	5	5	5
FeSO4 10µM (µl)	------	-------	4	4	4	4
Loading dye(µl)	2.5	2.5	5	5	5	5
Methanolic extract(µl)	------	------	------	------	5	------
Acetonic Extract(µl)	------	-------	------	5	------	------
Ethanolic extract(µl)	-----	------	-----	------	--------	5
Incubate at room temperature for 45 minutes then load into the wells						

Table 1. Contents of different wells in agarose gel electrophoresis.

RESULTS:

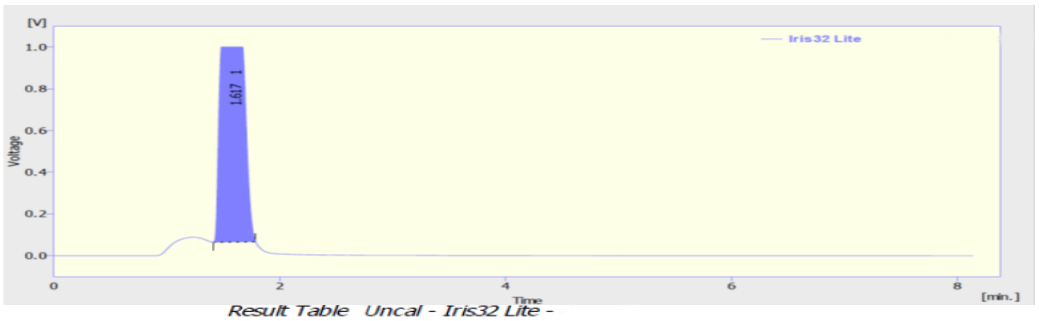
As indicated in the sample graph (Graph 1) there is only one peak with Retention time of 1.66 minute and in standard graph(Graph 2) only one peak with very near Retention Time of 1.617 minute that indicate the presence of Gallic acid as one of the major phenol compounds in grape seed extract.



Result Table Uncal - Iris32 Lite

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.660	14887.921	970.105	100.0	100.0	0.25
	Total	14887.921	970.105	100.0	100.0	

Graph 1. Sample HPLC graph, Retention Time at 1.66 minute.



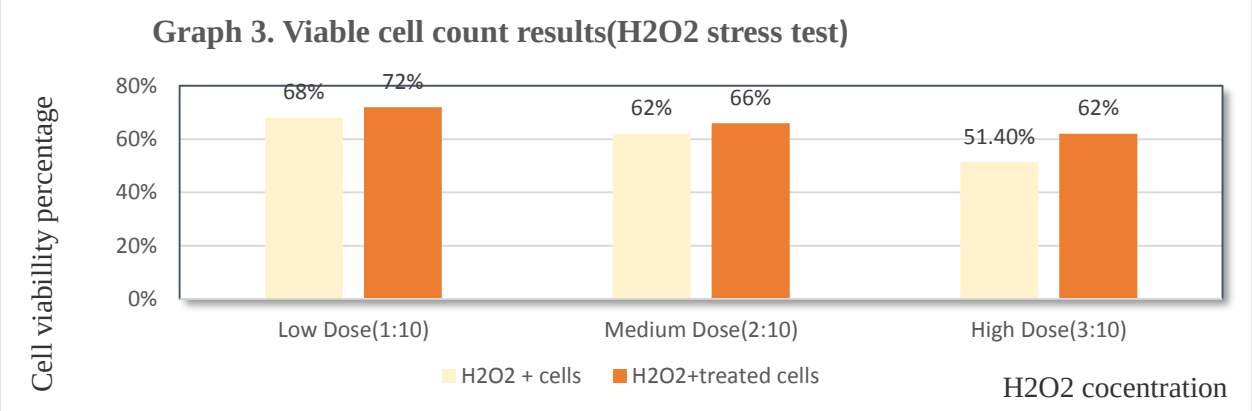
Result Table Uncal - Iris32 Lite -

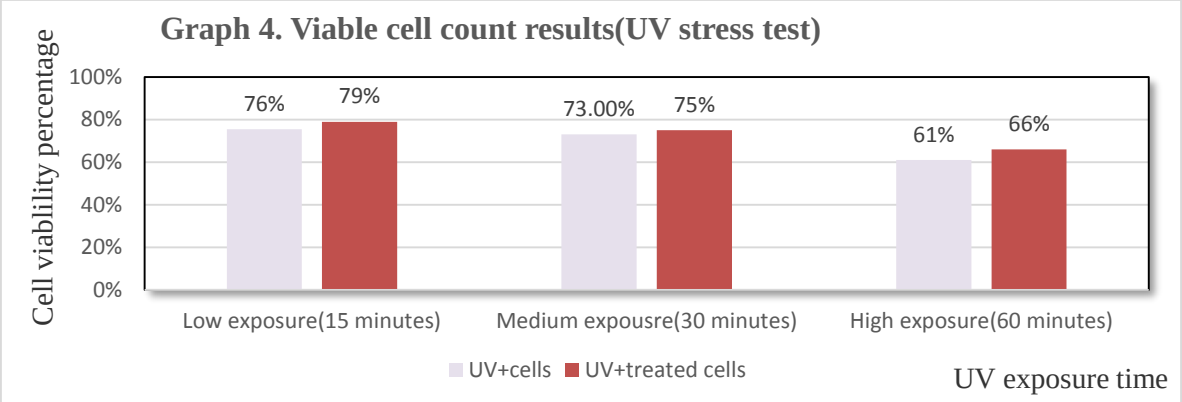
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.617	14494.907	936.059	100.0	100.0	0.26
	Total	14494.907	936.059	100.0	100.0	

Graph 2. Standard graph (Gallic acid), Retention Time at 1.617 minute as reference peak.

Viable cell count using yeast model

Baker's yeast (Saccharmoycescerevisie) is one of the simplest eukaryotic organisms but many essential cellular processes are conserved between yeast and humans, about 20% of human disease genes have counterparts in yeast (Roger Schneiter, January 2004)[12]. This suggests that much diseases result from the disruption of very basic cellular processes such as DNA repair, cell division or the control of gene expression. Viable cells of the yeast stained with Methylene blue were counted using haemocytometer under the microscope. Control yeast cells, stressed yeast cells (oxidative stress with H_2O_2) and treated yeast cells (stressed but incubated with grape seed extract for 15 minutes) were observed. Results are depicted in graphs 3-4 as Bar diagram.



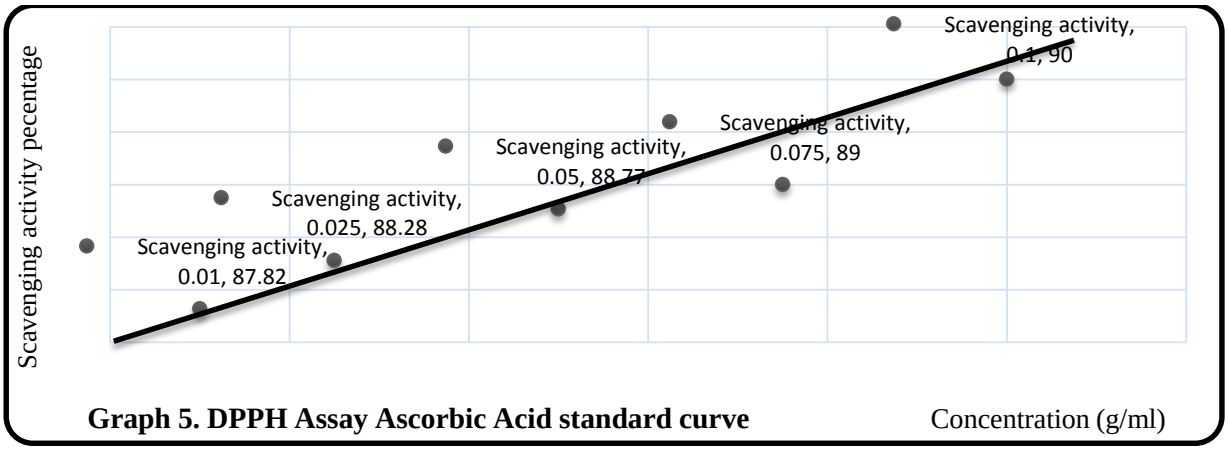


The above result revealed that oxidative stresses by H_2O_2 and UV cause cell damage in yeast model. Hydrogen peroxide is supposed to produce single stranded breaks in DNA and intracellular iron acts as mediator was reported by Hoffmann & Meneghini in 1979 [6]. Ultraviolet radiation can alter the normal state of life by inducing a variety of mutagenic and cytotoxic DNA lesions such as pyrimidine dimers (CPDs) and DNA strand breaks by interfering the genome integrity (Rajesh P. Rastogi et al, 2010)[23]. But the Polyphenolic Components including Gallic acid as an antioxidant, present in grape seed extract, was found to inhibit cell damage by increasing the number of viable cells. Data in graphs 3 and 4 indicating that free radicals produced by different concentration of H_2O_2 reagent and exposure to UV light resulted in decrease in cell number due to cell death .Treatment with grape seed extract showing prevention of cell damage by increasing the percentage of viable cell count i.e.10-11% in case of H_2O_2 stress and 5% in case of UV stress in case of high dose or exposure.

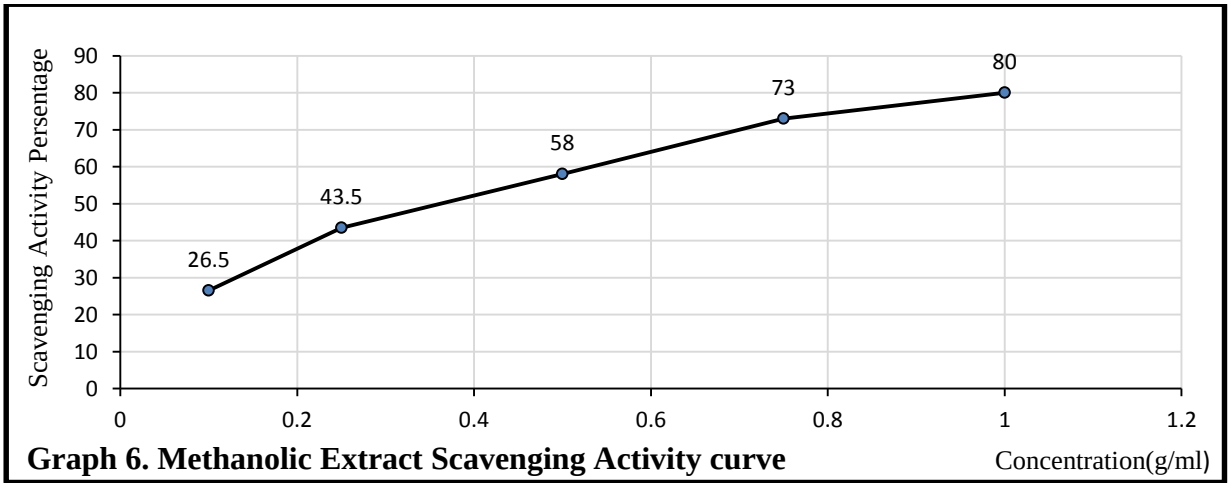
DPPH assay to show % scavenging activity of grape seed extract

Antioxidants react with DPPH and the absorbance decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability. From the Table.2 and graph 6 it can be inferred that grape seed extract seemed to have scavenging activity and the percentage of activity increased by increasing the concentration of the sample. The kinetics is same for different concentrations of ascorbic acid also as a

standard (graph 5). Spectrophotometric assay of DPPH revealed that increase in concentration of the sample (Grape seed extract) decreased the value of absorbance and vice-versa (Table2). Thus anti-oxidant potential of grape seed extract was established by DPPH assay.



Graph 5. DPPH Assay Ascorbic Acid standard curve



Graph 6. Methanolic Extract Scavenging Activity curve

	Concentration	Absorbance (at 517nm)	Scavenging activity%
Standard(g/ml)			
Standard 1	0.01	0.185	87.82
Standard 2	0.025	0.178	88.28
Standard 3	0.05	0.171	88.77
Standard 4	0.075	0.167	89
Standard 5	0.1	0.157	90
Methanolicgrape seed extract:			
Sample 1	10 %	1.116	26.5
Sample 2	25 %	0.859	43.5
Sample 3	50 %	0.643	58
Sample 4	75 %	0.411	73
Sample 5	100 %	0.300	80
Control	---------	1.52	----------

Table 2.DPPH Assay data, scavenging of DPPH (changes in absorbance) by various concentrations of grape seed extract.

FRAP assay to find out the Ferric reducing power of Grape seed extract

At low pH, reduction of ferric tripyridyltriazine (Fe III TPTZ) complex to ferrous form (which has an intense blue color) can be monitored by measuring the change in absorption at 593nm. The change in absorbance (Table 3) is directly related to the total reducing power of the electron donating antioxidant present in the reaction mixture. From the given formula Ferric reducing power of the sample (Grape seed extract) is 8.454 µM which strongly indicates its antioxidant potential (FRAP value of standard is 2).

$$\frac{\text{change in absorbance of sample from 0 to 4 minutes}}{\text{change in absorbance of standard from 0 to 4 minutes}} \times \text{FRAP value of standard}$$

Standard	0 minutes	4 minutes
0.1mM	0.069	0.096
0.2mM	0.122	0.178
0.4mM	0.166	0.219
0.6mM	0.246	0.458
0.8mM	0.340	0.620
1mM	0.499	0.886
EXTRACT	0.397	2.033

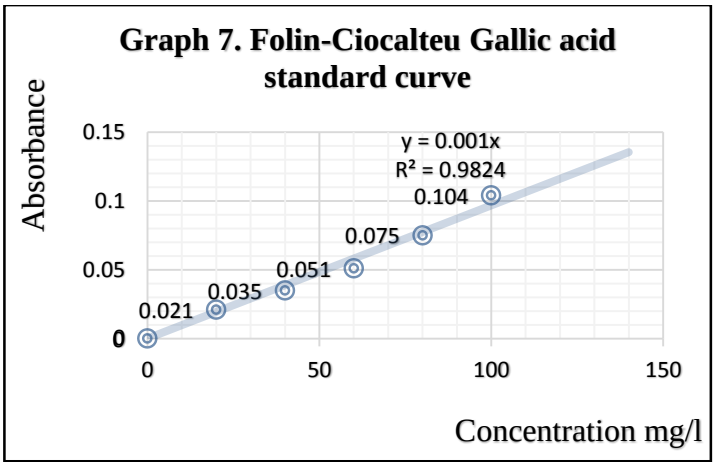
Table 3: Showing the absorbance of different concentrations of standard and sample from 0-4 minutes.

Folin-Ciocalteu assay to find out the total Phenolic content of grape seed extract

All phenolic compounds contained in extract are oxidized by Folin-Ciocalteu reagent and form blue complex. The blue coloration produced has a maximum absorption in the region of 765 nm, and is proportional to the total quantity of phenolic compounds originally present.Total Phenolic content of the extract is 136×5(dilution factor) = 680 mg/l , which correspond to 0.0680 mg GA per gram of dried grape seed, indirectly proved the antioxidant potential of grape seed extract.

Standard	Absorbance (at 765 nm)	Concentration (mg/l)
Standard 1	0.021	20
Standard 2	0.035	40
Standard 3	0.051	60
Standard 4	0.075	80
Standard 5	0.104	100
EXTARCT	0.112	136

Table.4 Concentration and absorbance of standard (Gallic acid) and sample (Grape seed extract).



Study of DNA damage and its prevention by anti-oxidant present in grape seed extract

Using Agarose Gel Electrophoresis, DNA profile was studied by giving oxidative stress to Plasmid DNA to investigate DNA – damage in comparison to the Control. Moreover, prevention of DNA damage by anti-oxidant (i.e. Gallic acid in grape seed extract) was also studied by incubating Plasmid DNA in grape seed extract and then by giving oxidative stress to observe whether Gallic acid can inhibit or prevent DNA damage. The profile of anti-oxidant treated-stressed DNA was comparable to that of Control. Under UV trans-illuminator, there was no or very faintly glowing bands for the stressed DNA, whereas Control DNA can be easily identified by the presence of glowing bands. It clearly indicated that oxidative stress can lead to DNA damage (Fig 2). The profile of grape seed extract (i.e. Gallic acid) treated stressed DNA was identified by the appearance of glowing bands which can be compared with that of control (Fig 2). In the gel, in well A, ladder bands were narrow that could not be seen clearly in the photograph. There was a strong band in well B indicating the control DNA in comparison with a weakly glowing band in well C which was indicating that H_2O_2 as an oxidative agent had caused the damage of DNA. In the well E, the strongly glowing band reappeared which clearly indicated that methanolic extract of grape seed helped prevention of DNA damage from oxidative stress given by H_2O_2. In the wells D & F, there are very weak bands that implied lower antioxidant capacity of acetonic and ethanolic extract.

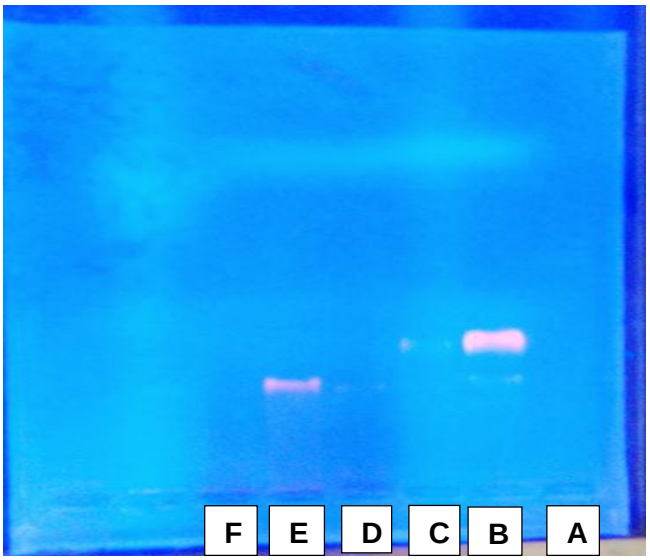


Fig 2. Agarose gel Electrophoresis: Profile of control (B), H2O2 stressed (C) and extract treated (D=Acetonic, E=methanolic & F=ethanolic) DNA.

DISCUSSION

Grape seed extracts are industrial derivatives from whole grape seeds that have a great concentration of vitamin E, flavonoids, linoleic acid and phenolic OPCs. The typical commercial opportunity of extracting grape seed constituents has been for chemicals known as polyphenols having antioxidant activity in vitro. In recent years, naturally occurring plant phenolics in grape by-products have raised a lot of attention, due to their health-promoting effects and to the antioxidant role they play in biological and food systems.(Volf, I. Mamaliga and V. I. Popa, 2006 and A. Balas and V. I. Popa 2007)[2,3].Apart from being a rich source of high value fatty oil, grape seeds have also been appreciated due to their high content of phenolic compounds, such as gallic acid ,catechin and epicatechin, and of a wide variety of procyanidins. The latter are also referred to as condensed tannins. Grape seed extracts and procyanidins have been a matter of intense investigations with respect to their potentially beneficial effects on human health. (T. Maier, A. Schieber, D. R. Kammerer and R.Carle, 2009)[4]. Human case reports and results from basic research provide preliminary evidence that grape seed extract is beneficial for heart diseases such as hypertension, high levels of blood cholesterol, platelet aggregation or inflammation. (Jha, Prabhat; Marcus Flather, Eva Lonn, Michael Farkouh, and Salim Yusuf ,1995)[5] Gallic acid seems to have anti-fungal, anti-viral properties and acts as an antioxidant also by protecting our cells against oxidative damage. Gallic acid was found to show cytotoxicity against cancer cells, without harming healthy cells. The antioxidant activity of grape seed phenolic compounds is closely associated with activity against various cancer types, cardiovascular diseases and several dermal disorders (Li, P. et al.)[22].

Clinical trials were done for assessing potential effects of grape seed extracts on human diseases, such as breast cancer, blood estrogen levels in postmenopausal women, and coronary artery disease(Vertuani, Silvia; Angusti, Angela; Manfredini, Stefano ,2004)[13]. A meta-analysis of randomized controlled trials concluded that "grape seed extract appears to significantly lower systolic blood pressure and heart rate, with no effect on lipid or C-reactive protein levels"(Valko, M; Leibfritz, D; Moncol, J; Cronin, M; Mazur, M; Telser, J ,2007)[14]. Other researches on disease models include: Ultraviolet damage – dietary proanthocyanidins are under study for mechanisms against carcinogenesis and sunscreen protection(Jacob, RA ,1996)[10]. In vitro cancer studies – grape seed proanthocyanidins decreased tumor numbers and reduced the malignancy of papillomas (German, JB,1999)[8]. Anti-viral effects was also reported by Knight, JA in 1998[10]. Seed phenolics may inhibit oral sugar metabolism and retard growth of certain bacteria that cause dental caries (Benzie, I 2003)[7]. Liver function is also promoted by the seed extract (Wolf, George 2005)[11]. OPCs induced vascular endothelial growth factor and accelerated healing of injured skin in mice (Werner Dabelstein, Arno Reglitzky, Andrea Schütze and Klaus Reders, 2007)[24].

Present study revealed that Gallic acid being present as a main bioactive compound seems to be responsible for its anti-oxidant property. Grape seed extract helps cell survival in yeast model 10-11% more in case of H_2O_2 stress and 5-6%% more in case of UV stress in comparison to the percentage of cell survival in case of oxidative stressed condition without sample extract. Also grape seed extract possesses the potential of genoprotection was evidenced by our studies. DNA profile in Agarose Gel Electrophoresis showed that glowing DNA band reappeared in methanolic extract treated oxidative stressed DNA, which was not visible properlyin stressed condition without extract. Ethanolic and Acetonic extract of grape seed did not show much potential like methanolic extract. Free radical scavenging activity, ferric reducing power and total

phenolic content of grape seed extract were determined to check its antioxidant potential.

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REFERENCES:

1. Sies, Helmut (1997). "Oxidative stress: Oxidants and antioxidants". Experimental physiology **82** (2): 291–5.
2. Volf, I. Mamaliga and V. I. Popa, (2006). Cellulose Chem. Technol., **40**, 205.
3. A. Balas and V. I. Popa(2007). BioResources, **2**, 363.
4. T. Maier, A. Schieber, D. R. Kammerer and R.Carle. (2009). Food Chem., **112**, 551.
5. Jha, Prabhat; Marcus Flather, Eva Lonn, Michael Farkouh, and Salim Yusuf (1995). "The Antioxidant Vitamins and Cardiovascular Disease: A Critical Review of Epidemiologic and Clinical Trial Data". *Annals of Internal Medicine***123** (11): 860–872.
6. Hoffmann, M. E., and Meneghini, R. 1979. Action of hydrogen peroxide on human fibroblast in culture. Photochem. Photobiol. 30: 151–155.
7. Benzie, I (2003). "Evolution of dietary antioxidants". Comparative Biochemistry and Physiology 136 (1): 113–26.
8. German, JB (1999). "Food processing and lipid oxidation". Advances in experimentalmedicine and biology. Advances in Experimental Medicine and Biology **459**: 23–50.
9. Jacob, RA (1996). "Three eras of vitamin C discovery". Sub-cellular biochemistry. Subcellular Biochemistry **25**: 1–16.
10. Knight, JA (1998). "Free radicals: Their history and current status in aging and disease". Annals of clinical and laboratory science **28** (6): 331–46.
11. Wolf, George (2005). "The discovery of the antioxidant function of vitamin E: The contribution of Henry A. Mattill". The Journal of nutrition **135** (3): 363–6.
12. Roger Schneiter (Jan 2004). Genetics, Molecular and Cell Biology of Yeast. University of Fribourg.
13. Vertuani, Silvia; Angusti, Angela; Manfredini, Stefano (2004). "The Antioxidants and Pro-Antioxidants Network: An Overview". Current Pharmaceutical Design **10** (14): 1677–94.
14. Valko, M; Leibfritz, D; Moncol, J; Cronin, M; Mazur, M;Telser, J (2007). "Free radicals and antioxidants in normal physiological functions and human disease". The International Journal of Biochemistry & Cell Biology **39** (1): 44–84.
15. Halliwell B, Gutteridge JMC (1999). The chemistry of oxygen radicals and other oxygen-drived species. In: Free Radicals in Biology and Medicine. Oxford University Press, New York, pp. 20-64.
16. Pong K (2003). Oxidative stress in neurodegenerative disease: Therapeutic implication for superoxide dismutase mimetics. ExpOpin. Biol. Ther., 3: 127-139.
17. Ozsoy N, Can A, Yanardag R, Akev N (2008). Antioxidant activity of Smilax excelsa leaf extracts. Food Chem., 110: 571-583.
18. Naimiki M (1990). Antioxidant/ anti-mutagens in foods. CRC Crit. Rev.Food Sci. Nutr., 29: 273-300.
19. McClements J, Decker EA (2000). Lipid oxidation in oil-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food system. J. Food Sci., 65: 1270-1282.
20. Silva EM, Souza JNS, Rogez H, Rees JF, Larondelle Y (2007).Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. Food Chem., 101: 1012-1018.
21. Frankel EN, Huang SW, Aeschbach R, Prior E (1996). Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol, and rosmarinicacis, in bulk oil and oil-in-water emulsion. J.Agric. Food Chem., 44: 131-135.
22. Hua Li,Xiaoyu Wang, Peihong Li,Yong Li and Hua Wang. Journal of Food and Drug Analysis, 16 (6) 67-73, (2008). Comparative StudyAntioxidant Activity of Grape (Vitisvinifera) Seed Powder Assessed by Different Methods**.**
23. Rajesh P. Rastogi et al. Journal of Nucleic Acids Volume (2010), Article ID 592980, 32 pages. Molecular Mechanisms of Ultraviolet Radiation-Induced DNA Damage and Repair.
24. Werner Dabelstein, Arno Reglitzky, Andrea Schütze and Klaus Reders "Automotive Fuels" in Ullmann's Encyclopedia of Industrial Chemistry 2007, Wiley-VCH, Weinheim.

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