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Screening of Antioxidant Capacity of Grape Extract (Vitis Vinifera) and Assessment of Its Phenolic and Flavonoid Content

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Abstract – *This study aims at the screening of the flesh extract from locally available grape fruit with respect to its antioxidant capacity, reducing activity, oxidative stability and total phenol content. The grape fruit from nearby locality around the laboratory was collected and the extracted using ethanol. The antioxidant capacity of the grape extracts was estimated by their ability to scavenge free radicals such as DPPH and superoxide. The reducing activity of the grape extract was measured by performing the phosphomolybdenum reduction assay and the potassium ferric cyanide assay. The total phenolic content and flavonoid content of the grape extract were assayed. The results of the DPPH and superoxide assays proves the radical scavenging capacity of the grape extract prepared for the study.*

Key Words: **Grape extract, radical scavenge, reducing activity, phenol, flavonoid, antioxidant capacity.**

1. INTRODUCTION

Oxidation is a chemical reaction that produce free radicals (unstable molecules) in the food products which in turn leads to the chain reactions causing the deterioration of the food. Synthetic food preservatives counteract the food deterioration but imparts undesirable effects on human health. Antioxidants obtained from bio products desirably counteract the food degradation and preserve stored food product without causing human health issues.

Aromatic fruits such as grapes, oranges are rich in their phenolic substances, also referred as the polyphenols, and are hence possess the antioxidant capacity. A great number of aromatic plants have been reported as having anti-inflammatory, antiallergic, antimutagenic, antiviral, antithrombotic and vasodilatory actions. The main objectives of the study are the characterization and quantification of the phenolic fraction of grape extract; the evaluation of the antioxidant activity of extract by using specific assays.

2. EXPERIMENTAL PROCEDURE

2.1 Materials and Reagents

Fresh grape fruit (Vitis vinifera) was purchased from a local market. Reagents such as ethanol, methanol, DPPH (1, 1-diphenyl -2-picrylhydrazyl or 2, 2-diphenyl -1--picrylhydrazyl), 4 mM ammonium molybdate, 28 mM sodium phosphate, 600 mM concentrated sulphuric acid, Potassium ferricyanide, TCA (Trichloroacetic acid), 0.2 M phosphate buffer at pH 6.6, Ferric chloride solution, Riboflavin, EDTA (Ethylene diamine tetraacetic acid), NBT (Nitro blue tetrazolium chloride), Folin Ciocalteu reagent of 1:10 dilution, 20% sodium carbonate, 5% sodium nitrite, 10% aluminum chloride, 1 M sodium hydroxide were purchased and prepared with respect to the mentioned conditions.

2.3 Extraction Procedure

The grapes were washed thoroughly and dried in aseptic conditions. Using a clean and methanol rinsed cutlery the peels of the grapes were removed carefully without piercing the flesh of the fruits. After removing the skin of the fruits, the seeds inside the flesh were removed and discarded. The flesh of the fruits were taken in a clean conical flask and suspended in 50 mL of ethanol. The ethanol extract of the grape fruit was incubated overnight at the room temperature.

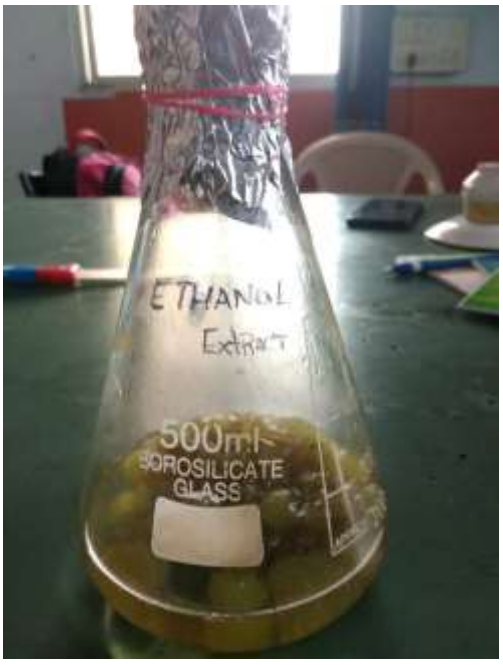

Fig -1: Grape flesh extraction in ethanol

2.3 Determination of Antioxidant activity by DPPH radical scavenging assay

DPPH (1, 1-diphenyl -2-picrylhydrazyl or 2, 2-diphenyl -1-picrylhydrazyl) is a synthetic stable radical. Various concentrations of the fruit extract in the range of 20 to 120

µg/mL were taken in a set of 6 test tubes. A control sample was taken in a separate tube which had no fruit extract. 1 mL each of methanol solution and 0.1 mM DPPH solution were added in all the tubes using a micropipette. The samples were incubated at room temperature for 30 minutes in the dark. After incubation, the absorbance of the samples having different concentrations of fruit extract were read at 517 nm using a spectrophotometer. Percentage of inhibition of the grape extract is calculated for each sample concentrations using the formula, % of inhibition = (control - sample)/control × 100.

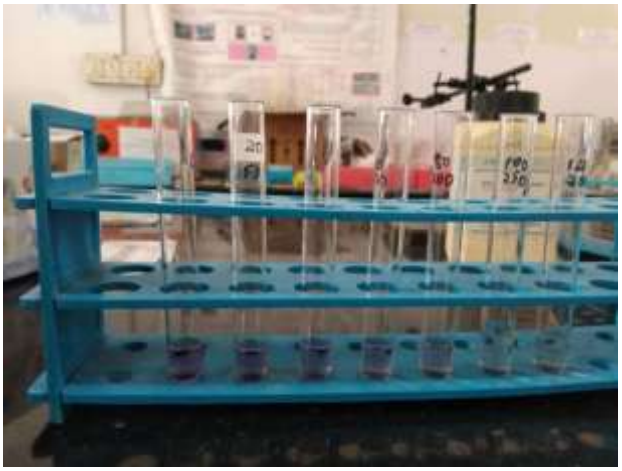

Fig -2: DPPH radical scavenging assay samples after incubation

2.4 Determination of Antioxidant activity by Phosphomolybdenum reduction assay

Various concentrations of the fruit extract in the range of 20 to 120 µg/mL were taken in a set of 6 test tubes. A control sample was taken in a separate tube which had no fruit extract. 1 mL of methanol solution was added in all the tubes using a micropipette. 1 mL of the reagent, containing 4 mM ammonium molybdate, 28 mM sodium phosphate, 600 mM concentrated sulphuric acid, was added in each test tubes. The samples were incubated in a water bath at 95˚c for 90 minutes. After incubation, the absorbance of the samples having different concentrations of fruit extract were read at 695 nm using a spectrophotometer. Percentage of reduction of the grape extract is calculated for each sample concentrations using the formula, % of reduction = (sample - control)/sample × 100.

2.5 Determination of Antioxidant activity by Potassium ferricyanide assay

Potassium ferricyanide solution was prepared by dissolving 0.3 g of potassium ferricyanide in 30 mL of dissolved water. TCA solution and 0.2 M phosphate buffer at pH 6.6 were prepared. Ferric chloride solution is prepared by dissolving 100 µL $FeCl_3$ in 10 mL distilled water. Various concentrations of the fruit extract in the range of 20 to 120 µg/mL were taken in a set of 6 test tubes. A control sample was taken in a separate tube which had no fruit extract. 1 mL each of methanol solution, potassium ferricyanide solution, phosphate buffer were added in all the tubes using a micropipette. The samples are then incubated in a water bath for 30 minutes. 500 µL of prepared TCA solution and 300 µL of $FeCl_3$ solution were added in each test tubes. The absorbance of the samples having different concentrations of fruit extract were read at 700 nm using a spectrophotometer. Percentage of reduction of the grape extract is calculated for each sample concentrations using the formula, % of reduction = (sample - control)/sample × 100.

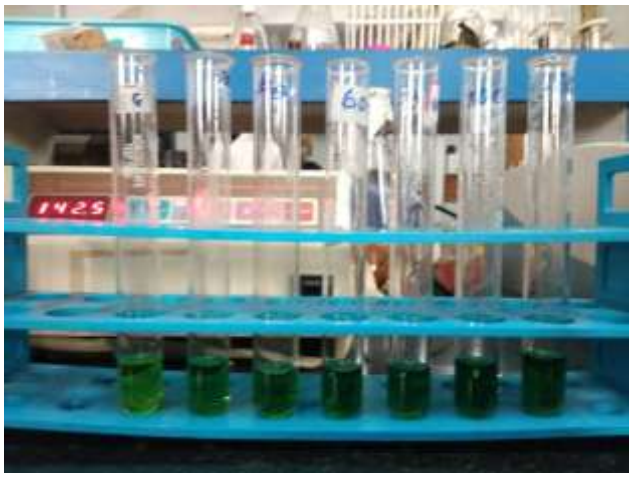

Fig -3: Potassium ferricyanide assay samples after the addition of TCA and $FeCl_3$ solutions

2.6 Determination of Antioxidant activity by Superoxide radical scavenging assay

Various concentrations of the fruit extract in the range of 20 to 120 µg/mL were taken in a set of 6 test tubes. A control sample was taken in a separate tube which had no fruit extract. 1 mL of methanol solution, 200 µL of riboflavin, 200 µL of EDTA, 100 µL of NBT were added in all the tubes using a micropipette. The samples are then illuminated in a UV lamp for 15 minutes. The absorbance of the samples having different concentrations of fruit extract were read at 590 nm using a spectrophotometer. Percentage of inhibition of the grape extract is calculated for each sample concentrations using the formula, % of inhibition = (control - sample)/control × 100.

2.7 Estimation of Phenolic content

100 µL of the fruit extract was taken in a test tube with 900 µL of methanol solution. 1 mL of the Folin Ciocalteu reagent of 1:10 dilution was added to the tube. 20% of sodium carbonate solution was prepared by dissolving 20 g of Na_2CO_3 in 100 mL of distilled water. 1 mL of the prepared 20% Na_2CO_3 solution was added to the test tube. The sample was then incubated at room temperature for 30 minutes in the dark. After incubation, the absorbance of the supernatant was read at 765 nm using a spectrophotometer.

2.8 Estimation of Flavonoid content

500 µL of the fruit extract was taken in a test tube with 500 µL of methanol solution. 5% of sodium nitrite solution was prepared by dissolving 5 g of $NaNO_2$ in 100 mL of distilled water. 500 µL of the prepared 5% $NaNO_2$ solution was added

to the test tube. 10% of aluminum chloride solution was prepared by dissolving 10 g of $AlCl_3$ in 100 mL of distilled water. 500 µL of the prepared 10% $AlCl_3$ solution was added to the test tube. 100 µL of the prepared 1 M NaOH solution was added to the test tube. The sample was then incubated at room temperature for 30 minutes. After incubation, the absorbance of the supernatant was read at 510 nm using a spectrophotometer.

3. RESULTS AND DISCUSSION

3.1 Determination of Antioxidant activity by DPPH radical scavenging assay

The DPPH method is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant capacity. The absorbance of the samples, having different concentrations of the fruit extract which were read and the percentage of inhibition of the grape extract, which were calculated for each sample concentrations, were recorded and indicated in the following tabulation.

Table -1: DPPH radical scavenging assay

S. No	Sample Concentration (µg/mL)	Optical Density at 517 nm	% of Inhibition
1	Control	0.236	-
2	20	0.212	10.17%
3	40	0.180	23.73%
4	60	0.149	36.86%
5	80	0.116	50.85%
6	100	0.066	72.03%
7	120	0.064	72.88%

From the tabulation, it was found that the absorbance value of the samples having different concentrations of fruit extract, decreases as the sample concentration increases. The calculated percentages of inhibition for each sample concentrations shows that the radical scavenging activity of the grape flesh increases with the increase in sample concentrations. It can be concluded that the antioxidant activity increases with the increase in the concentration of the grape extract.

3.2 Determination of Antioxidant activity by Phosphomolybdenum reduction assay

The antioxidant activity of samples can be evaluated by the green phosphomolybdenum complex formation. The absorbance of the samples, having different concentrations of the fruit extract which were read and the percentage of reduction of the grape extract, which were calculated for each sample concentrations, were recorded and indicated in the following tabulation.

Table -2: Phosphomolybdenum reduction assay

S. No	Sample Concentration (µg/mL)	Optical Density at 695 nm	% of Reduction
1	Control	0.034	-
2	20	0.930	96.34%
3	40	1.797	98.11%
4	60	1.833	98.15%
5	80	1.718	98.02%
6	100	1.435	97.63%
7	120	2.455	98.62%

From the tabulation, it was found that the absorbance value of the samples having different concentrations of fruit extract, increases as the sample concentration increases. The calculated percentages of reduction for each sample concentrations shows that the reducing activity of the grape flesh increases with the increase in sample concentrations. It can be concluded that the antioxidant activity increases with the increase in the concentration of the grape extract.

3.3 Determination of Antioxidant activity by Potassium ferricyanide assay

Potassium ferricyanide is used to determine the ferric reducing power potential of samples for the determination of their antioxidant property. The absorbance of the samples, having different concentrations of the fruit extract which were read and the percentage of reduction of the grape extract, which were calculated for each sample concentrations, were recorded and indicated in the following tabulation.

Table -3: Potassium ferricyanide assay

S. No	Sample Concentration (µg/mL)	Optical Density at 700 nm	% of Reduction
1	Control	0.411	-
2	20	0.822	50%
3	40	0.951	56.78%
4	60	1.532	73.17%
5	80	1.532	73.17%
6	100	1.767	76.74%
7	120	2.201	81.33%

From the tabulation, it was found that the absorbance value of the samples having different concentrations of fruit extract, increases as the sample concentration increases. The calculated percentages of reduction for each sample concentrations shows that the reducing activity of the grape flesh increases with the increase in sample concentrations. It can be concluded that the antioxidant activity increases with the increase in the concentration of the grape extract.

3.4 Determination of Antioxidant activity by Superoxide radical scavenging assay

The absorbance of the samples, having different concentrations of the fruit extract which were read and the percentage of inhibition of the grape extract, which were calculated for each sample concentrations, were recorded and indicated in the following tabulation.

Table -4: Superoxide radical scavenging assay

S. No	Sample Concentration (µg/mL)	Optical Density at 590 nm	% of Inhibition
1	Control	0.109	-
2	20	0.063	42.20%
3	40	0.054	50.46%
4	60	0.048	55.96%
5	80	0.045	58.72%
6	100	0.038	65.13%
7	120	0.035	67.88%

From the tabulation, it was found that the absorbance value of the samples having different concentrations of fruit extract, decreases as the sample concentration increases. The calculated percentages of inhibition for each sample concentrations shows that the radical scavenging activity of the grape flesh increases with the increase in sample concentrations. It can be concluded that the antioxidant activity increases with the increase in the concentration of the grape extract.

3.5 Estimation of Phenolic and flavonoid content

Phenolics are the largest group of phytochemicals that account for most of the antioxidant activity in plants or plant products. Flavonoids are the largest group of naturally occurring phenolic compounds that occurs in different plant parts both in free state and as glycosides. In plants, Flavonoids act as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellants, and for light screening. In the grape extract sample, the assessment of phenolic content shows the absorbance value of 0.493 at 765 nm and the assessment of flavonoid content shows the absorbance value of 0.410 at 510 nm. From these values, it was found that there is considerable amount of phenols and flavonoids are present in the flesh extract from the grape.

4. CONCLUSION

In the study, the antioxidant capacity, reducing activity, oxidative stability, total phenol and flavonoid content of the flesh extract from the grape fruit were screened and assessed. The results of the DPPH radical scavenging assay, Phosphomolybdenum reduction assay, Potassium ferricyanide assay, Superoxide radical scavenging assay shows the high antioxidant capacity of the grape flesh. The existence of the phenols and flavonoids were confirmed by their assessments. This study concludes that the examined grape flesh contain the high antioxidant property proving them to be a good antioxidant source for natural and desirable food preservatives.

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