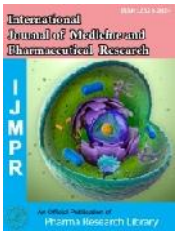
International Journal of Medicine and Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijmpr



Research Article **Open Access**

Antioxidant and Antimicrobial Activity of Grape Seed Extract

Ms. Sneha V & Dr. V. Judia Harriet Sumathy*

Postgraduate & Research Department of Biotechnology, Women's Christian College, Chennai – 600 006.

A B S T R A C T

Recent studies have shown that procyanidins in grape seeds possess anti-inflammatory, anti-arthritic, anti-allergic and anti-cancer activities. It is also reported that it prevents heart disease and skin aging besides inhibiting carrageen in dextran-induced hindpawedema which stabilizes the capillary wall and improves visual performance in humans. The concentration of phenolic compounds in grapes depends on the variety of grapevine and is influenced by viticultural and environmental factors. The synthesis of flavonoid and non-flavonoid plant polyphenols such as stilbenes is increased in plant tissues following wounding or infection by pathogenic organisms. The present study is aimed at extracting red grapes seed with methanol and perform the Phytochemical tests using qualitative analysis. Furthermore Total Phenolic content by Quantitative analysis, Column Chromatography by GC-MS to confirm the secondary metabolites for seed, Antioxidant activity by DPPH, Hydrogen Peroxide and Total antioxidant capacity and Antibacterial activity for grape seed extract is also assessed.

Keywords: Grape seed, Procyanidins, Phytochemical Analysis, Antioxidant and Antimicrobial Property

A R T I C L E I N F O

CONTENTS

Article History: Received 05August 2016, **Accepted** 15 September 2016, **Available Online** 10 October 2016

***Corresponding Author**
Dr. V. Judia Harriet Sumathy
Postgraduate & Research Department of Biotechnology, Women's Christian College, Chennai–600 006.
Manuscript ID: IJMPR3169

PAPER-QR CODE

Citation: V. Judia Harriet Sumathy. Antioxidant and Antimicrobial Activity of Grape Seed Extract. *Int. J. Med. Pharm. Res.*, 2016, 4(5): 287-292.

1. Introduction

Red grape is a fruiting berry of the deciduous woody vines of the botanical genus *Vitis* **(Figure 1)**. Grapes can be eaten raw or they can be used for making wine, jam, juice, jelly, grape seed extract, raisins, vinegar, and grape seed

oil (**Patrice *et. al.*, 2006)**. A study conducted to investigate the antibacterial activity of *Vitis vinifera* seed extracts against Gram positive bacteria *Staphylococcus aureus* and *Bacillus substilis* and Gram negative bacteria such as *Pseudomonas aeroginosa* and *Escherichia coli* revealed the assay against the bacterial strains **(E.Q. Xia *et. al*., 2010).** Polyphenols can also reduce damage to DNA and production of free radicals in the body **(BUB *et. al*., 2003)**. Many of the flavonoids found in grape juice, such as catechin, epicatechin, quercetin, and anthocyanins are known to have antioxidant, anti-inflammatory, and platelet inhibitory effects, as well as for being able to reduce LDL oxidation and oxidative damage to DNA, both in vitro and in animal studies (**Frankel *et. al*., 1998 and Singletory *et. al*., 2003**).

Figure 1: *Vitis vinifera* (Red grapes)

The antimicrobial properties of plant extracts have shown promise for development of new drugs (**Hakkinen, S, 2000)**. A study was conducted to measure the antibacterial activity of grape (*Vitis vinifera*) seed extract against *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* **(Mahkameh Mirkarimi, *et. al*., 2012).** *Vitis vinifera* is used in prescriptions for cough, respiratory tract catarrh, subacute cases of enlarged liver and spleen, as well as in alcohol-based tonics **(Wang, L, *et. al*., 2014).** Using the sap of grapevines, European folk healers sought to cure skin and eye diseases (**Monagas M *et. al*., 2003)**. Other historical uses include the leaves being used to stop bleeding, pain and inflammation of hemorrhoids (**R. Carpenter, M. N, *et. al*., 2007)**. Unripe grapes aere used for treating sore throats, and raisins given as treatments for consumption (tuberculosis), constipation and thirst. Ripe grapes aere used for the treatment of cancer, cholera, smallpox, nausea, skin and eye infections as well as kidney and liver diseases **(Shi J, Yu J, *et. al*., 2003).**

2. Materials and Methods

Preparation of Extract

The Seeds were collected and dried in shade for over two weeks. The dried seeds were then ground into powder. 30grams of the dried seed powder was weighed and immersed in 300 ml of the solvents – methanol for 48hrs. After 48 hours, the extract was filtered and the filtrates were used for further phytochemical analysis.

Phytochemical Test

Preparation of Reagent

1. **20% Ethyl Alcohol** -20ml of Ethyl alcohol in 80ml of distilled water.

2. **4% Sodium hydroxide** - 4ml of NaOH in 96ml of distilled water.

3. **1% Copper sulphate** - 1g of $CuSO_4$ in 100ml of distilled water.

4. **1% Ninhydrin Reagent** -1g of Ninhydrin in 100 ml of distilled water.

5. **5% Ferric Chloride** - 5g of ferric chloride in 100ml of distilled water.

6. **Hager's Reagent** – 1g of picric acid in 100ml of distilled water.

7.1% Lead acetate solution – 1g of lead acetate in 100ml of distilled water.

Test for Carbohydrates, Proteins, Alkaloids, Flavonoids, Terponoids, Saponins and Renin were conducted using standard protocols.

Antioxidant Activity of the Extracts

Redox properties of antioxidants play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. In doing so, the antioxidants themselves become oxidised. This urges the constant need of antioxidants of replenishing them. The antioxidant properties of the skin and seed extracts of grapes are evaluated using DPPH free radical scavenging activity, Hydrogen peroxide scavenging activity and Total antioxidant capacity method.

Antimicrobial Activity of the Extracts

The antimicrobial present in the grape seed extract were allowed to diffuse out into the medium and interact in the plate freshly seeded with test organisms. The resulting zones of inhibition will be uniformly circular as there will be confluent lawn of growth. The diameter of zone of inhibition can be measured in millimetres. Column Chromatography was carried out and the purified extracts which were obtained were further analysed by Gas Chromatography –Mass Spectrometry analysis.

3. Results and Discussions

Isolation of Carotenoid Pigments by Column Preparation of Extracts

The seeds were collected and dried. The dried seeds were ground into powder and dissolved in methanol solvent and incubated for 48 hours and the extract were filtered and the filtrates were used for further phytochemical analysis **(Figure 2).**

Figure 2: Grape Seed Filtrate

Phytochemical test

Test for carbohydrates - reduction of fehling's solution

Brick red precipitate indicates the presence of carbohydrates **(Figure 3)**

Figure 3: Grape seed

Test for Proteins-Ninhydrin Test
Appearance of violet colour indicates the presence of proteins (**Figure 4)**

Figure 4: Grape seed

Test for Glycosides - Keller –Killani Test
A reddish brown colour is formed at the junction of two layers and the upper layer turns bluish green indicating the presence of glycosides **(Figure 5).**

Figure 5: Grape seed

Test for Tannins: A dark blue or green black colour appears which indicates the presence of tannins **(Figure 6)**

Figure 6: Grape seed

Test for Alkaloids: A yellow precipitate or yellow solution indicates the presence of alkaloids **(Figure 7).**

Figure 7: Grape seed

Test for Flavonoids: Appearance of white or yellow precipitate indicates the presence of flavonoid **(Figure 8).**

Figure 8: Grape seed

Test for Terponoids
A reddish brown colouration formed in the interface shows positive results for the presence of Terpenoids **(Figure 9).**

Figure 9: Grape seed

Test for Saponins: Formation of stable foam indicates the presence of saponins **(Figure 10).**

Figure 10: Grape seed

Test for Resins - Acetone Water Test

Appearance of turbidity indicates the presence of resins (**Figure 11, Table 1)**

Figure 11: Grape seed

Antioxidant activity of the extracts

DPPH free radical scavenging activity

The percentage inhibition of the DPPH radical by the samples was calculated according to the formula

IP = [(AC (0)- AA(t)/ AC(0))] × 100

Where, AC(0) is the absorbance of the control at t = 0 min; and AA(t) is the absorbance of the antioxidants at t = 16 min. **(Table 2)**

Hydrogen Peroxide Scavenging Activity

The percentage of H_2O_2 scavenging by the extract and standard compounds was calculated as follows:

% of inhibition = (A of control – A of test)/A of control *100

Where A of control is the absorbance of the control reaction and A of test is the absorbance of the sample extracts **(Table 3)**

Total Antioxidant Capacity

The absorbance of the aqueous solution of each was measured at 695 nm against a blank. Ascorbic acid was used as the standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid **(Table 4).**

Antimicrobial Activity of Grape Skin and Seed Extracts

Petriplates containing Muller Hinton Medium were seeded with 24 hours culture of bacterial cultures. Wells were made in each of these plates using sterile cork borer. Crude extracts were added into the wells and allowed to diffuse. The plates were then incubated at 37^{o}C for 24 hours. The antibacterial activity was assayed with *Staphylococcusauerus, Esherichia coli, Bacillus subtilis, Pseudomonasaeruginosa*, by measuring the diameter of the inhibition zone formed around the well **(Figures 12 – 15 & Table 5).**

Figure 12

Figure 13

Figure 14

Figure 15

Column Chromatography

Samples were purified for GC-MS Analysis in order to determine compounds present in the purified sample **(Figure 16).**

Figure 16: Column Chromatography

Gas chromatography-MASS Spectroscopy

GC-MS identified the secondary metabolites present in grape seed **(Table 6 & Figure 17).** The peak area percentage and peak area coverage of the **Grape Seed (GSD)** is given below.

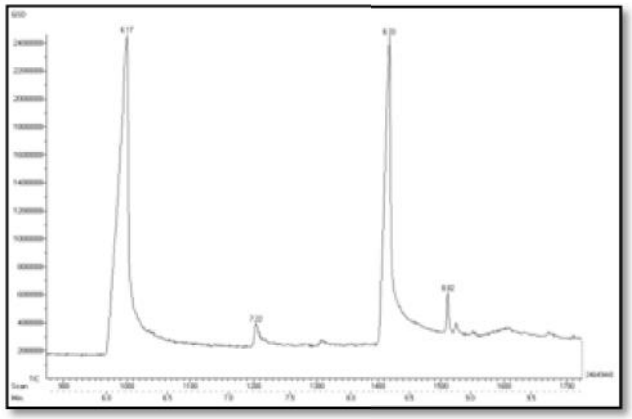


Figure 17: GCMS for Grape Seed

4. Conclusion

Extraction of samples with methanol solvent resulted in crude extract. The Phytochemical Analysis revealed minimal of phytochemical compounds for grape seed. Column chromatography was done by GC-MS to confirm the secondary metabolites from grape seed. Antioxidant activity by DPPH, Hydrogen Peroxide, Total Antioxidant and Antibacterial activity capacity were found to be more in grape seed. Recent studies have shown that procyanidins in grape seeds possess anti-inflammatory, anti-arthritic, anti-allergic, anti-cancer activities, and it prevents heart disease and skin aging besides inhibiting carrageen in dextran-induced hindpawedema which stabilizes the capillary wall and improves visual performance in humans. The concentration of phenolic compounds in grapes depends on the variety of grapevine and is influenced by viticultural and environmental factors. The synthesis of flavonoid and non-flavonoid plant polyphenols such as stilbenes is increased in plant tissues following wounding or infection by pathogenic organisms. Thus the present study emphasizes on the role of natural phenolic compounds extracted from grape seed to be a powerful tool in inhibiting microbial growth.

Table 1: Phytochemical Tests

Phytochemical tests	Grape seed
Carbohydrates	+
Proteins	-
Glycosides	+
Tannins	+
Alkaloids	-
Flavonoids	+
Terpenoids	+
Saponins	+
Resins	+

Table 2: Antioxidant Assay by DPPH

Name of the Sample	Control OD	Sample OD	Antioxidant (%)
Grape Seed	0.612	0.283	53.70

Table 3: Hydrogen Peroxide Antioxidant Activity

Name of the sample	Control OD	Sample OD	Antioxidant (%)
Grape seed	0.86	0.56	34.8

Table 4: Total Antioxidant Capacity

	Grape seed
Control OD	0.54
Sample OD	0.53
Antioxidant (%)	1.85

Table 5: Zone of Inhibition

	S. aureus (mm)	***E. coli*** (mm)	***B. subtilis*** (mm)	***P. aeruginosa*** (mm)
Grape Seed	2mm	2mm	10mm	10mm

Table 6: GCMS for Grape Seed

Peak no.	RT (Min.)	Compound Name	Peak Area	Peak Area (%)
1	6.17	16-Hexadecanoyl hydrazide	24649440	41.51
2	7.22	d-Glucitol, 2,5-anhydro-1-O-octyl-	3976000	6.70
3	8.33	Palmitic anhydride	24546096	41.34
4	8.82	Cholestan-3-ol,2-methylene-,[3a,5a]-	6210816	10.46
		Total	59382352	100.00

5. References

[1] Bub, A. (2003) Fruit juice consumption modulates antioxidative status, immune status, and DNA damage. *Journal of Nutrition Biochemistry*, Volume. 14, p. 90-98.

[2] E.-Q. Xia, G.-F. Deng, Y.-J. Guo and H.-B. Li (2010) "Biological Activities of Polyphenols from Grapes," *International Journal of Molecular Sciences*, Vol. 11, No. 2, 2010, pp. 622-646.

[3] Frankel, E. N. (1998) Commercial grape juices inhibit the in vitro oxidation of human low-density lipoproteins. *Journal of Agriculture and Food Chemistry*, Volume 46, p. 834-838, 1998.

[4] Hakkinen, S (2000) Flavonols and phenolic acids in berries and berry products (pp. 17–21). Finland: Doctoral disertation, Kuopio University Publications D. Medical Sciences pg 221.

[5] Mahkameh Mirkarimi, S.Mahmoud Amin-Marashi, Majid Bargrizan, Amir Abtahi,Abbas Ali Imani Fooladi (2012) The Antimicrobial Activity of Grape Seed Extract against Two Important Oral Pathogens, pg 17.

[6] Patrice; Lacombe, Thierry and Thomash, Mark R. (2006). "Historical Origins and Genetic Diversity of Wine Grapes" (PDF). *Trends in Genetics* 22 (9): 511–519.

[7] R. Carpenter, M. N. O'Grady, Y. C. O'Callaghan, N. M. O'Brien and J. P. Kerry (2007) Evaluation of the Antioxidant Potential of Grape Seed and

Bearberry Extracts in Raw and Cooked Pork, Meat Science, Vol. 76, No. 4, 2007, pp. 604-610.

[8] Monagas M, Cordoves GC, Bartolome B, Laureano O and Ricardo da Silva JM (2003). Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from Vitisvinifera L. Cv. Graciano, Tempranillo, and Cabernet Sauvignon. *Agri Food Chem,* pg 51

[9] Shi J, Yu J, Pohorly JE, Kakuda Y (2003). "Polyphenolics in grape seeds-biochemistry and functionality". *J Med Food* 6 (4): 291–9.

[10] Wang, L; Waltenberger, B; Pferschy-Wenzig, EM; Blunder, M; Liu, X; Malainer, C; Blazevic, T; Schwaiger, S; *et al*. (2014). "Natural product agonists of peroxisome proliferator-activated receptor gamma (PPARγ): a review". *Biochem Pharmacol* 55 (1): 73–89.