International Journal of Current Trends in Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijctpr

Research Article **Open Access**

Comparative Analysis of Antioxidant and Antimicrobial Activity of Grape Seed and Grape Skin

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

Grape (*Vitis vinifera*) skin and seed are considered to be rich sources of poly-phenolic compounds, mainly monomeric catechin and epicatechin, gallic acid, polymeric and oligomeric procyanidins**.** Resveratrol is found in widely varying amounts among grape varieties, primarily in their skins and seeds, which, in muscadine grapes, have about one hundred times higher concentration than pulp. Fresh grape skin contains about 50 to 100 micrograms of resveratrol per gram. These grape seed and skin extract compounds act as anti-mutagenic and antiviral agents. Beneficial aspects include inhibition of carrageen in or dextran-induced edema which stabilizes the capillary wall and improvement of visual performance in humans. The present study is aimed at extracting seed and skin of red grapes with methanol and perform the Phytochemical tests for both using qualitative analysis. Furthermore Total Phenolic content by Quantitative analysis, Column Chromatography by GC-MS to confirm the secondary metabolites for skin and seed, Antioxidant activity by DPPH, Hydrogen Peroxide and Total antioxidant capacity and Antibacterial activity for grape skin and seed extract is also assessed and compared for its efficiency.

Keywords: Grape skin and seed, Resveratrol, Procyanidins, Antioxidant and Antimicrobial Property.

A R T I C L E I N F O

CONTENTS

Article History: Received 19 September 2016, **Accepted** 24 October 2016, **Available Online** 15 November 2016

***Corresponding Author**
Dr. V. Judia Harriet Sumathy
Postgraduate & Research Department of Biotechnology, Women's Christian College, Chennai – 600 006.
Manuscript ID: IJCTPR3160

PAPER-QR CODE

Citation: V. Judia Harriet Sumathy. Comparative Analysis of Antioxidant and Antimicrobial Activity of Grape Seed and Grape Skin. *Int. J. Curnt. Tren. Pharm, Res.*, 2016, 4(6): 338-344.

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).

Figure 1: *Vitis vinifera* (Red grapes)

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).** Unripe grapes are being used for treating sore throats, and raisins are given as treatments for consumption for tuberculosis, constipation and thirst. Ripe grapes are 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).** Research study indicates the extracts of *Vitis vinifera* seed to exhibit antimicrobial activity to some pathogens such as *Staphylococcus aureus, Bacillus substilis, Pseudomonas aeroginosa and Escherichia coli* **(E.Q. Xia *et. al*., 2010).** A study conducted to investigate the antibacterial activity of *Vitis vinifera* skin and seed extracts against Gram positive bacteria *Staphylococcus aureus, Bacillus substilis* strains and Gram negative bacteria is *Pseudomonas aeroginosa* and *Escherichia coli* revealed the assay against the bacterial strains. 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**). The antimicrobial properties of plant extracts have shown promise for development of new drugs in a study conducted which measured the antibacterial activity of grape (*Vitis vinifera*) seed extract against *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans*. **(Mahkameh Mirkarimi, *et. al*., 2012).** A study assessed the antimicrobial potential and chemical composition of agro-industrial wastes against pathogenic microorganisms of importance in foods. Beet stalk, peanut peel, Pinot Noir grape marc, Petit Verdot grape seed and marc, red grapes fermentation lees and guava bagasse wastes showed antimicrobial activity against *Staphylococcus aureus* and *Listeria monocytogenes*. The minimum inhibitory concentrations ranged from 0.78 to 25mg/ml. Wastes with antimicrobial activity showed the highest total phenolic compounds among the wastes studied (37.3 to 400.2g GAE/kg).

Analyses by GC-MS allowed the identification of caffeic, gallic, ferulic and ρ-coumaric acids, besides flavonoids quercetin, myricetin and epicatechin on wastes that exhibited antimicrobial activity (**José Guilherme, *et. al*., 2011).** 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 stilbene is increased in plant tissues following wounding or infection by pathogenic organisms **(Hakkinen, *et. al*., 2000; Montealegre. R.R, *et. al*, 2006 and R. Carpenter, *et. al*., 2007).** Thus the role of natural phenolic compounds extracted from plant reaches its paroxysm and the addition of these natural compounds to food products has therefore become popular as a means of increasing shelf life and to reduce wastage and nutritional losses by inhibiting microbial growth and delaying oxidation **(A.T. Serra *et. al*., 2008).**

2. Materials and Methods

Preparation of Extract

The Skin and seed were collected and dried in shade for over two weeks. The dried skin and seed were ground into powder. 30grams of the dried skin and 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 oxidized. 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 and skin 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.

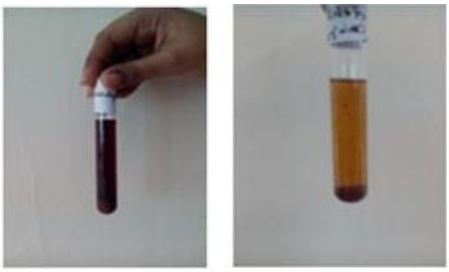
3. Results and discussions

The Skin and seed were collected and dried .The dried skin and seed 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 **(Figures 2 - 3).**

Figures 2& 3: Grape Skin and Seed Filtrate

Phytochemical Test

Test for Carbohydrates-Reduction of Fehling's solution: Brick red precipitate indicates the presence of carbohydrates **(Figures 4 & 5)**



Figures 4& 5: Grape Skin and Seed

Test for Proteins - Ninhydrin Test

Appearance of violet colour indicates the presence of proteins (**Figures 6 & 7)**

Figures 6& 7: Grape Skin and Seed

Test for glycosides - keller –killani test

A reddish brown colour is formed at the junction of two layers and the upper layers turns bluish green indicating the presence of glycosides **(figures 8 & 9).**

Figures 8& 9: Grape Skin and Seed

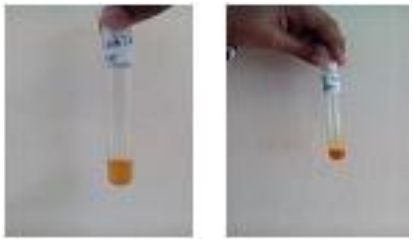
Test for tannins

A dark blue or greenish black colour appearance indicates the presence of tannins **(figures 10 & 11)**

Figures 10 & 11: Grape Skin and Seed

Test for alkaloids

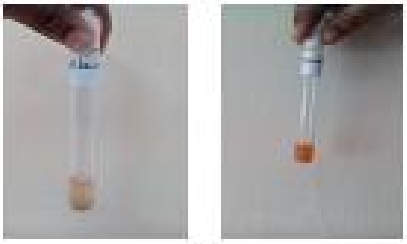
A yellow precipitate or yellow solution indicates the presence of alkaloids **(figures 12 & 13).**



Figures 12& 13: Grape Skin and Seed

Test for flavonoids

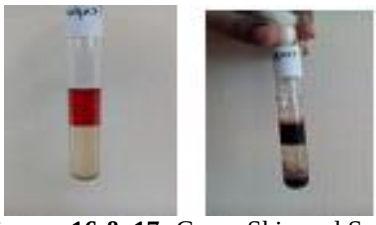
Appearance of white or yellow precipitate indicates the presence of flavonoid **(figures 14 & 15).**



Figures 14 & 15: Grape Skin and Seed

Test for terponoids

A reddish brown colouration formed in the interface shows positive results for the presence of terpenoids **(figures 16 & 17).**



Figures 16 & 17: Grape Skin and Seed

Test for saponins

Formation of stable foam indicates the presence of saponins **(figures 18 & 19).**

Figures 18 & 19: Grape Skin and Seed

Test for resins - acetone water test

Appearance of turbidity indicates the presence of resins (**figures 20 & 21, table 1)**

Figures 20 & 21: Grape Skin and 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. **(Figure 22 & Table 2)**

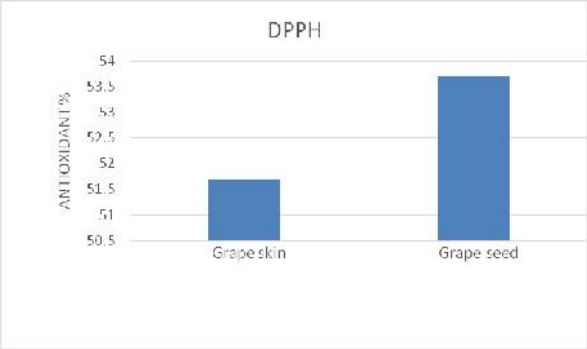


Figure 22: Antioxidant Assay by DPPH

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 **(Figure 23 & Table 3)**

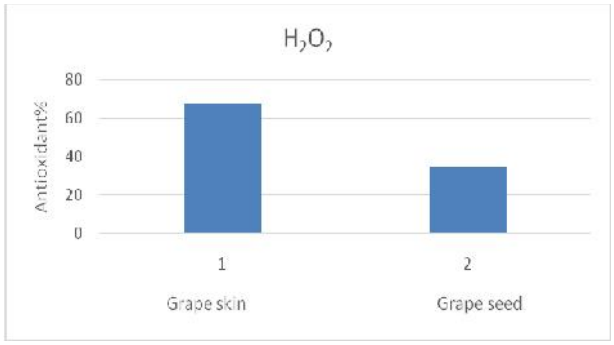


Figure 23: Hydrogen Peroxide Antioxidant Activity

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 **(Figure 24 & Table 4).**

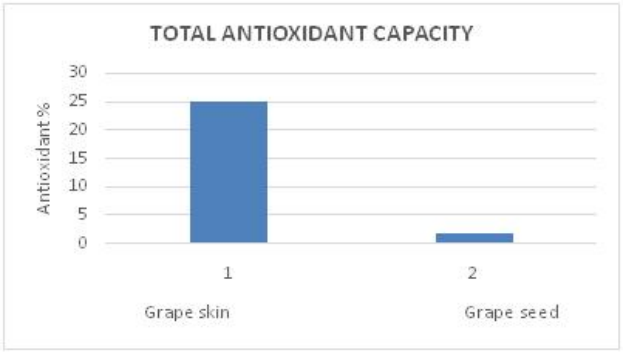


Figure 24: Total Antioxidant Capacity

Antimicrobial Activity of Grape Skin and Seed Extracts

Petriplates containing Muller Hinton Medium were seeded with 24 hours culture of bacteria. 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°C for 24 hours. The antibacterial activity was assayed with *Staphylococcus auerus, Esherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* by measuring the diameter of the inhibition zone formed around the well **(Figures 25 – 32 & Table 5).**

Staphylococcus auerus

Figures 25& 26: Grape Skin and Seed

Escherichia .coli

Figures 27& 28: Grape Skin and Seed

Bacillus subtilis

Figures 29& 30: Grape Skin and Seed

Pseudomonas aeruginosa

Figures 31& 32: Grape Skin and Seed

Column Chromatography

Samples were purified for GC-MS Analysis in order to determine compounds present in the purified sample **(Figure 33).**

Figure 33: Column Chromatography

Gas Chromatography- Mass Spectroscopy

GC-MS identified the secondary metabolites present in grape skin and seed **(Tables 6 - 7 & Figures 34 - 35).** The peak area percentage and peak area coverage of the **Grape Skin (GSK)** is given below.

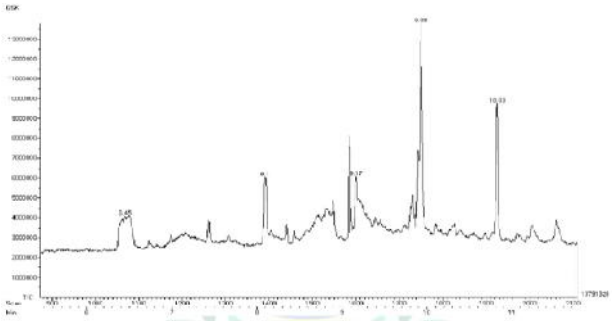


Figure 34: GCMS for Grape Skin

The peak area percentage and peak area coverage of the Grape Seed (GSD) is given below.

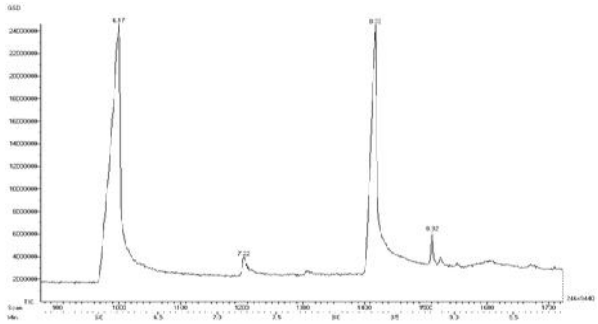


Figure 35: GCMS for Grape Seed

Table 1: Phytochemical Analysis

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

Table 2: Antioxidant Assay by DPPH

Name of the Sample	Control OD	Sample OD	Antioxidant (%)
Grape Skin	0.612	0.295	51.70
Grape Seed	0.612	0.283	53.70

Table 3: Hydrogen Peroxide Antioxidant Activity

Name of the sample	Control OD	Sample OD	Antioxidant (%)
Grape skin	0.86	0.38	67.4
Grape seed	0.86	0.56	34.8

Table 5: Total Antioxidant Capacity

	Grape skin	Grape seed
Control OD	0.44	0.54
Sample OD	0.33	0.53
Antioxidant (%)	25	1.85

Table 6: Zone of Inhibition

	S. aureus	*E. coli*	*B. subtilis*	*P. aeruginosa*
Grape skin	Mm	mm	Mm	mm
Grape seed	1mm	1mm	5mm	5mm
	2mm	2mm	10mm	10mm

Table 6: GCMS for Grape Skin

Peak No.	RT (Min.)	Compound Name	Peak Area	Peak Area (%)
1	6.45	1Amino 2-[4-chorobenoyl] -6,7,8,9 – tetrhydro 5 – methylthieno[2,3-c] isoquinoline	4071328	10.25
2	8.1	Propanoic acid, 2-[3-acetoxy-4,4-14-trimethylandrost-8-en-17yl]	6054720	15.24
3	9.17	Estra -1,3,5[10]-trien 17a-ol	6071120	15.28
4	9.33	Dasycarpidan-1-methanol, acetate [ester]	13791328	34.71
5	10.83	1 H-Pyrrolo[2,3-c]pyridine-3-propanoic acid, 5[4h]-oxo-6,7-dihydro,methyl ester	9746000	24.53
		Total	39734496	100.00

Table 7: 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

4. Conclusion

Extraction of samples with methanol solvent resulted in crude extract. The Phytochemical Analysis revealed more number of phytochemical compounds for grape skin than grape seed. Column chromatography was done by GC-MS to confirm the secondary metabolites from grape skin and grape seed. Antioxidant activity by DPPH, Hydrogen Peroxide, Total Antioxidant and Antibacterial activity capacity were found to be more in grape seed than grape skin. Nowadays bacteria, yeasts and free radicals cause real health problems because of their involvement in many diseases mainly those in which an oxidative stress is involved such as cancer and cardiovascular disease and in food borne diseases**.** Currently there is a growing scientific interest to use natural antibacterial compounds, as bio-preservatives face to conventional synthetic additives, due to consumer preferences towards more natural and healthier products**.** Thus the present study emphasizes on the role of natural phenolic compounds extracted from plants as a popular means of inhibiting microbial growth and leading to a healthy living.

5. References

[1] T. Serra, A. Matias, V. M. Nunes, M. C. Leitão, D. Brito, R. Bronze, S. Silva, A. Pires, M. T. Crespo, M. V. San Romão and C. M. Duarte (2008) "In Vitro Evaluation of Olive- and Grape-Based Natural Extracts as Potential Preservatives for Food," Innovative Food Science and Emerging Technologies, Vol. 9, No. 3, 2008, pp. 311-319.

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

[3] 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.

[4] 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.

[5] 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.

[6] José Guilherme Prado Martin1*, Ernani Porto1, Cristina Bani Corrêa1, Severino Matias de Alencar1 , Eduardo Micotti da Gloria1 , Ingridy Simone Ribeiro Cabral1 , Lígia Maria de Aquino (2011) Antimicrobial potential and chemical composition of agro-industrial wastes, pg 35-80,.

[7] 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.

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

[10] 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.

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

[12] 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.