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NUTRITIONAL AND NUTRACEUTICALS ANALYSIS OF GRAPE SEED POWDER

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ABSTRACT
Grape seeds (Vitis-Vinifera L.) are good sources of phyto-chemicals and are suitable raw materials for the production of antioxidant rich dietary supplements. Grape seeds are rich in pro-anthocyanidins which possess potent free radical scavenging activity. The proximate composition of grape seed and micro nutrients were 38.2% fiber, 15.8% total lipids, 10.7% proteins, 2.58% ash, 22.37% carbohydrates and 10.4% moisture, micronutrients and phytochemical analysis of grape seed extract revealed the presence of steroids, terpenoids, anthocyanins, emodins, glycosides, flavonoids and phenols in acetone (70%), ethanol (70%) and methanol respectively Both steroids and terpenoids were absent in water extract. Saponins were absent in methanol and water extracts. DPPH assay was used to estimate the antioxidant activity and the results revealed that acetone (70 %) is the most efficient solvent to extract the total phenolic compounds and flavonoids from grape seed when compared to the other selected solvents for the study.

KEYWORDS: Grape seed, Nutraceuticals, Phenolic, Flavonoids, Antioxidants and Phytochemicals.

INTRODUCTION
Grape (Vitis spp.) is one of the most economically important plant species due to its diverse uses in production of wine, juice, resins and other food products. Grapes are one of the most widely grown fruits and the total production of grapes is approximately 60 million tons worldwide. Grape seeds are complex matrix containing approximately 40% fiber, 10 to 20% oil 11% proteins, 26.43% of total carbohydrates and 7% complex phenols including tannins, in addition to minerals. Grape seeds are rich in antioxidants, including phenolic acids, anthocyanin, flavonoids and oligo-mericproanthocyanidin complexes (OPCs) (Yavor Ivanov, 2024, Lenka Sochorova et al., 2025).

A real inhibitory assessment of the extracts was made by implementing an in vitro digestion simulation method. It was found that the percentage of inhibition of the enzymes with the digested extract was higher compared to those with the undigested extract in buffer and salt solution. Our study proves that the high content of flavonoids and procyanidins in the two extracts determines their high inhibitory capacity against the three enzymes and their potential for managing diabetes and obesity. Grape seed extract (GSE) has different medicinal properties including anti-inflammatory anti-carcinogenic, platelet aggregation inhibiting, and metal chelating properties etc., Grape Seed Extract (GSE) is one of the best-known sources of pro-anthocyanidins (ZvonkoAntunovi´c.2024).Due to its high antioxidant content, GSE can help protect against oxidative stress, tissue damage and inflammation and prevent disease. Grape seed oil contains nutritionally essential fatty acids and tocopherols (OvidiuTit, 2021).Grape seed extracts are also included in the formulations of cosmetic products for anti-aging reasons. This is because grape seed extracts are rich in pro-anthocyanin's which have strong free radical scavenging properties.

Grape seed powder extracts were tested for antibacterial activity by pour plate method against Bacillus cereus,Bacillus coagulants, Bacillus subtitles, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. It was found that, Gram-positive bacteria were completely inhibited at 850–1000 ppm, while Gram-negative bacteria were inhibited at 1250–1500 ppm concentration (Dimitrina Krasteva 2023).

MATERIALS AND METHODS
Grape seeds were purchased from Pune Grape processing industry (Maharashtra). These seeds were ground into fine powder using an electric grinding machine. The chemical composition (moisture, proteins, lipids, ash and fibre) of sample was determined according as per the standard methods of AOAC (1990). The protein content was calculated by using conversation factor 6.25. The minerals were analysed by using wet ash.

Preparation of seeds extract
The extraction was carried out using four different solvents, separately i.e. ethanol: water (70:30 v/v), acetone: water (70:30 v/v), methanol absolute and water. The seed powder (0.4 g) was mixed with 20 mL of solvent and stirred for 2 hrs. at 45°C. The extract was centrifuged at 4000 rpm for 10 min and subsequently decanted. The residue was re extracted for 2 hrs and supernatants were combined and sample extract evaporated to dr0yness (modified of Huali et al., 2008)

Quantitative analysis of phytochemicals

Determination of Total polyphenols and total flavonoids content: Total polyphenols (TP) was determined using the Folin-Ciocalteu reagent, according to Maurya and Singh, 2020. The calibration curve was made with standard of solution of Gallic acid in the range of 0.01- 0.05 mg ml-1 and measures were carried out at 760 nm using a UV-V is spectrophotometer. All analysis was performed in triplicate. Gallic acid was employed as a calibration standard and results were expressed as milligrams of equivalent Gallic acid per gram of sample. The flavonoid content of each extract was measured based on methods described by Ebrahimzadeh et al. (2018). Briefly, 0.5ml of sample (5g/L) was mixed with 1.5ml of methanol and then 0.1 ml of 10% potassium acetate and 2.8 ml of distilled water. The mixture was incubated at room temperature for 30 min. The absorbance was measured by a spectrophotometer at 415 nm. The results were expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g extract). The standard curve was prepared by quercetin in different concentrations (5-50 mg/L).

Determination of total antioxidant activity

The antioxidant activities of the acetone (70%), methanol absolute, ethanol (70%) and water extracts were assessed by measuring free radical scavenging activity via the discoloration of these solvents of the free radical 1,1 diphenyl-2- picrylhydrazyl (DPPH) as described by Brand – Williams et al. (2019) as follows: Two ml of acetone (70%), methanol, ethanol (70%) and water solution of either test material at various concentrations (1-64 µg/ml) and methanol solution used as control were added to 2 ml solution of DPPH (25mg/L) in methanol, and the reaction mixture was shaken vigorously and left in darkness for 30 min. Finally, the absorbance of the mixture was measured against pure methanol (blank) at 517 nm T80 UV/Vis sample that is required to scavenge 50% of the DPPH free radicals.

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Preliminary Phytochemical screening: Qualitative phytochemical analysis

Steroids:

An aliquot of the seed extract (1ml) was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids (Gibbs, 2015).

Terpenoids:

An aliquot of the seed extract (2ml) was added to 2ml of acetic anhydride and concentrated
H_2SO_4. The formations of blue green ring indicate the presence of terpenoids (Ayoola et al., 2018).

Tannins:

An aliquot of the seed extract (2ml) was added to few drops of 1% lead acetate, and the yellowish precipitate indicated the presence of tannins (Treare and Evans, 2024).

Saponins:

An aliquot of the seed extract (5ml) was mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of Saponins (Kumar et al., 2019).

Anthocyanins:

An aliquot of the seed extract (2ml) was added to 2ml of 2 NHCl and ammonia. The appearance of pink-red which turns to blue-violet indicates the presence of anthocyanins (Farnsworth, 2015).

Glycosides:

2ml glacial acetic acid, one drop of 5% $FeCl_3$ and conc. H_2SO_4 were added into 5ml extract, the appearance of brown ring indicates the presence of glycosides (Khandewal, 2018).

Emodins:

Two ml of NH_4OH and 3 ml of Benzene were added to the extract. Appearance of red colour indicates the presence of emodins (Rizk, 2022).

Alkaloids:

A Mayer's test: To the acidic solution, Mayer's regent (Potassium mercuric iodide solution) was added. Cream colored precipitate indicates the presence of alkaloids (Gibbs, 2014).

Phenol:

Half ml of $FeCl_3$ solution was added into 2 ml of test solution, formation of an intense color indicates the presence of phenols (Gibbs, 1974).

Flavonoids:
An aliquot of the seed extract (2-3ml) and few drops of sodium hydroxide solution were added into a test tube. Formation of intense yellow colour that became colourless on addition of few drops of dilute HCl indicates the presence of flavonoids (Khandewal, 2018).

Resveratrol:
Aliquots of the extracts (10 mg/mL) were filtered through a nylon filter (45 μm) prior to HPLC-DAD analysis. Quantification of -resveratrol (in μg/mL) was performed by plotting the results into a calibration curve constructed with a reference pure standard in five different concentrations (1.0; 2.5; 5.0; 10.0; and 20.0 mg/L). The identification and quantification of -resveratrol were carried out by comparison of the retention times and peak areas, with those of -resveratrol standard or by conjunction with the sample (spike test), respectively.

RESULTS AND DISCUSSION:

Table 1: Chemical composition of grape seed

Components	Values (%)
Moisture	10.5 ± 0.16
Fiber	34 ± 0.13
Fat	11 ± 1.18
Protein	10.7±1.16
Carbohydrates	26 ± 2. 22
Ash	2.58 ± 0.14
Phenols	8 mg GAE/100gram

Values are expressed as mean ± SD (n=3)

The grape seed contained 34% fiber, 11% fat, 10.7% protein, 2.58% ash, 10.5% moisture phenols 8mg GAE/100g and 26% carbohydrate. The results in the present study are consistent with the previous observations of Owon (2019) who reported that grape seeds contain 2.86% of ash and 12.69% of oil. Baydar and Akkurt (2021)

Table 2: Data on the mineral content of grape seed

Minerals	Values (mg/100 g)	Minerals	Values (mg/100 g)
Ca	212.77 ± 0.01	Phosphorous	1.7 ± 0.01
Mg	169.43 ± 0.02	Cl	0.61 ± 0.02
Na	68.93 ± 0.04	Copper	1.60 ± 0.04
K	224.00 ± 0.08	Iron	0.83 ± 0.03
Mn	4.57 ± 0.06	Selenium	0.41 ± 0.04
Zn	32.83 ± 0.04	Cobalt	0.73 ± 0.08

Values are expressed as mean ± SD (n= 3)

Exhibits the values of the composition of minerals found in grape seed. it was observed that the calcium content in grape seed was 212.77/100g, magnesium 169.43/100g, sodium 68.93/100g, potassium 224.00/100g, monobidinum 4.57/100g, zinc32.83/100g, phosphorous 1.7/100g, chlorine 0.61/100g, copper 1.60/100g, iron 0.83/100g, selenium 0.41/100g, and cobalt 0.73/100g.Have determined the profile of macro- and micro minerals mean value of 0.20% sodium, 0.18% phosphorus, 0.46% potassium, 0.63% calcium and0.09% magnesium, for macro minerals. For micro minerals, they found the mean values of 37.77 mg/kg iron, 383.27 mg/kg zinc, 57.03 manganese and 11.9 mg/kg copper. In comparison with the present investigation the results were lower shown by Akin and Çitil (2011),

Table 3: Total Phenolic and total flavonoids in grape seed powder (mg/g)

Sample	Total Phenolic	Total flavonoids
Acetone	463.25 ± 14.77c	148.52 ± 0. 61c
Ethanol	581.65 ±12.12b	152.64 ± 0.85b
Methanol	683.43 ± 4.00a	187.25 ± 0.40a
Water	241.58 ± 6.478d	137.45 ± 0.50d

Values are expressed as mean ± SD (n=3). Values in the same column with different superscript letters are significantly different ($p < 0.05$) as per the Duncan's multiple comparison test

In the present study, total phenol and flavonoid content of grape seeds were assessed and shown in Table 3. This study has demonstrated that the total phenolic compounds in various extracts of grape seeds ranged between 186 - 528 mg/ g, as GAE. In this table total phenolic contained of grape seed exactly acetone was 463.25mg/g, ethanol 581.658, methanol 683.432, water 241.589.Total flavonoids of grape seed acetone of grape seed was148.521mg/g, following ethanol 152.645mg/g, methanol 187.256mg/g, water 137.456mg/g.In our study, total flavonoids content (Table 3) decreases in the following order methanol (70%) > ethanol (70%)

>acetic acid> water. Acetone extract exhibited the highest value of flavonoids (14 mg/g) while water extract exhibited the lowest value (9.75 mg/g). Ioana et al. (2021) reported that polyphenols and total flavonoids content in grape seeds extract were 506.25mg GAE/100g. Hassan and Nahla (2023) reported that grape seed extract contains a logical amount of phenolic compounds ethanol grape seed extract contain high amount of phenolic compounds and flavonoids (66.60 and 11.56mg/g) in comparison with water grape seed extract (31.20 and 6.85mg/g grape seed, respectively).

Table 4: Antioxidant activity of grape seed powder

Sample	%inhibition IC50(µg/ml)	IC 50 (µg/ml)
Acetone (Acetone 70%)	89.191	36.64
Ethanol (Methanol)	90.25	39.57
Methanol (Ethanol70%)	96.35	39.55
Water(water)	83.48	39.65

Table 4 shows that antioxidant activity in grape seed % inhibitionIC50 (µg/ml) method shows that extraction was followed in methanol 96.35, followed by ethanol 90.25, acetone and water. In IC50 (µg/ml) high value of antioxidant in grape seed water followed by ethanol and least one methanol and ethanol. The free radicals (DPPH) used to find antioxidant (scavenging) activity of various extracts. DPPH is stable free radical at room temperature and accepts an electron/hydrogen radical to become a stable diamagnetic molecule (David et al., 2024). The reduction capability of DPPH radical is determined by the decrease in its absorbance at 517 nm, induced by antioxidants. The decrease in absorbance DPPH radical is caused by antioxidants, because of the reaction between antioxidant molecules and radicals, progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in colour from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the antioxidant activity (Edamatsu et al., 2023).

Table 5: Phytochemical screening tests for constituents of grape seed extract

Constituent	Aceton70%	Ethanol 70%	Methanol 70%	Water
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Tannins	+	+	+	-
Saponins	+	+	-	+
Anthocyanins	+	+	+	+
Emodins	+	+	+	+
Alkaloids	+	+	+	+
Phenol	+	+	+	+
Resveratrol	+	+	+	+
Flavonoids:	+	+	+	+

+ indicate the presence of component - Indicate the absent of the component

In this table shows the screening of phytochemicals in different four methods like acetone, ethanol, methanol and water. In acetone and all the ethanol phytochemicals were present like steroids, terponoids, tannins, resveratrol, saponins, anthocyanin's, emodins, alkaloids, phenol flavonoids and in methanol except saponins all the phytochemicals are present. In water extraction except tannins all the phytochemicals are present.

CONCLUSION

Among the various solvents used in this study, the acetone extract of grape seed has been found to possess good antioxidant activity, total phenolic compounds and flavonoids content. The extract is also rich in various phytochemical components. It was conducted that the grape seed constitute a good source of healthy compounds, could be useful in the prevention of diseases. Hence it can be utilized in different concentration in food product preparation.

REFERENCES

1. Agarwal, c, singh, r.p. and agarwal, r., 2022, gse induces apoptotic death of human prostate carcinoma cells via Caspase activation and Cytochrome 'C' release. Carcinogenesis.23 (11):1869-1876.
2. ELA, E. I. AND OMARA, E.A., 2024, Genotoxic and histopathological aspects of treatment with Grape Seed Extract on CPA induced mice.CellBiology.2(3):18-27.
3. FANG, J.Y., LI, Z.H., LI,Q. AND HUANY, W.S., 2023, Resveratrol affects Protein Kinase 'C' activity and promotes apoptosis in human colon carcinoma cells. Asian Pacific Journal of Cancer Prevention.13(12): 6017-6022.
4. HALIM, A.H., FYIAD, A.A., ALI, M.A. AND SOLIMAN, S.M., 2025. Journal of Chemical Pharmaceutical Research.7 (4):913-921.

5. IQBAL AND RAMESHWAR, 2022, Inhibitory role of GSE on cancer cell metabolism by down regulating Pyruvate Kinase M2 via inhibition of mammalian target of Rapamycin. Journal of Oncology.23: 213-219.
6. SINGH, R.P., TYAGI, A.K., DHANALAKSHMI, S., AGARWAL, R. AND AGARWAL, A., 2024, GSE inhibits advanced human prostate cancer growth.International Journal of Cancer.108: 733-740.
7. RAY, S. D., KUMAR, M.A. AND BAGCHI. D., 2023, A novel GSE prevents Acetaminophen- Induced programmed and unprogrammed cell death in mouse liver. Archives of Biochemistry and Biophysics.369: 42-58.
8. AOAC. (2005). Official method of Analysis (8th ed.). Association of Officiating Analytical Chemists, Washington DC, Method 935.14 and 992.24.
9. Arora, A., & Saini, C. S. (2016). Development of bun from wheat flour fortified with de-oiled maize germ. Cogent Food and Agriculture, 2. https://doi.org/10.1080/23311932.2016.1183252
10. Aruwa, C., Akindusoye, A., & Awala, S. (2017). Socio-demographic Characteristics and Food Hygiene Level Assessment of Food Handlers in Cafeterias around a Federal University in Nigeria. Journal of Scientific Research and Reports, 14(4), 1–9. https://doi.org/10.9734/JSRR/2017/33273
11. Chakraborty, R., Sabruna, S., Roy, R., Majumdar, S., & Roy, S. (2021). Banana pseudostem substitution in wheat flour biscuits enriches the nutritional and antioxidative properties with considerable acceptability. SN Applied Sciences, 3(1), 75. https://doi.org/10.1007/s42452-020-03988-1
12. Hassan El-Mallah, M., M. El-Shami, S., & Hassanein, M. M. (2003). Detailed studies on some lipids of Silybum marianum(L.) seed oil. Grasas y Aceites, 54(4), 397–402. https://doi.org/10.3989/gya.2003.v54.i4.227
13. Krishnamoorthy, S., Kunjithapatham, S., & Manickam, L. (2013). Traditional Indian breakfast (Idli and Dosa) with enhanced nutritional content using millets. Nutrition and Dietetics, 70, 241–246. https://doi.org/10.1111/1747-0080.12020
14. Ma, Z., & Zhang, H. (2017). Phytochemical Constituents, Health Benefits, and Industrial Applications of Grape Seeds: A Mini-Review. Antioxidants, 6(3), 71. https://doi.org/10.3390/antiox6030071
15. Vanitha, N. (2019). Pectins: Extraction, Purification, Characterization and Applications.
16. Sabir, A., Unver, A., & Kara, Z. (2012). The fatty acid and tocopherol constituents of the seed oil extracted from 21 grape varieties (Vitis spp.). Journal of the Science of Food and Agriculture, 92(9), 1982–1987. https://doi.org/10.1002/jsfa.5571
17. Terra, X., Montagut, G., Bustos, M., Llopiz, N., Ardèvol, A., Bladé, C., Fernández-Larrea, J., Pujadas, G., Salvadó, J., Arola, L., & Blay, M. (2009). Grape-seed procyanidins prevent low-grade inflammation by modulating cytokine expression in rats fed a high-fat diet. The Journal of Nutritional Biochemistry, 20(3), 210–218. https://doi.org/10.1016/j.jnutbio.2008.02.005
18. Tolve, R., Simonato, B., Rainero, G., Bianchi, F., Rizzi, C., Cervini, M., & Giuberti, G. (2021). Wheat bread fortification by grape pomace powder: Nutritional, technological, antioxidant, and sensory properties. Foods, 10. https://doi.org/10.3390/foods10010075