Phytochemical and antioxidant Profile screening of Perlette and King Ruby Grape leaves

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Abstract:

Objective: The primary goal of this study is to conduct a detailed analysis of both qualitative and quantitative phytochemical compounds while simultaneously assessing the antioxidant properties of different alcoholic and aqueous extracts derived from Vitis vinifera leaves.

Methods: Conduct a comprehensive assessment of the antioxidant potential of plant extracts through various methods such as total phenolic and flavonoid content analysis, DPPH analysis, linoleic acid system, and peroxidation inhibition. Emphasize the importance of preliminary phytochemical analysis and in vitro antioxidant assays.

Results: Results indicate that leaves are a noteworthy reservoir of antioxidant potential, suggesting their potential as a source for phenolic chemicals and antioxidants.

Conclusions: Based on the findings of this study, it can be inferred that the species exhibits efficacy in neutralizing free radicals and possesses the capacity to function as a potent antioxidant.

1. Introduction:

A free radical is an atom or molecule with unpaired electrons. The reactive oxygen species are oxygen free radicals like hydroperoxyl (OOH•), superoxide anion (O_2), peroxyl (ROO•), hydroxyl (OH•), and alkoxyl (RO•) radicals, as well as non-free radicals like singlet oxygen (O_2), ozone (O_3), hydrogen peroxide (H_2O_2), and hypochorous acid (HOCl) (Halliwell & Gutteridge, 2015). Reactive oxygen species are oxygen derived radicals and non-radicals (Irshad & Chaudhuri, 2002). They can be formed by endogenous and exogenous sources (Sen, Chakraborty, Sridhar, Reddy, & De, 2010). These radicals are produced by the body to stabilize natural function, but excess can cause damage. This damage can lead to chronic diseases in humans (Aiyegoro & Okoh, 2010).

Antioxidants protect against lipid peroxidation, protecting proteins, DNA, and small molecules at low concentrations. Different antioxidants are used based on the type of ROS and the target. Notable antioxidants include vitamin E, ascorbic acid, superoxide dismutase, glutathione peroxidase, catalase, and ceruloplasmin. Other proposed antioxidants are beta-carotene, carnosine, phytate, bilirubin, estrogens, dihydrolipoic acid, polyamines, and melatonin. Understanding their mode of action is crucial for evaluating effectiveness (Halliwell, 1995).

Grapes are a well-known species that belong to the Vitis genus. They originate from western Asia and southern Europe. The main compounds in grapes are phenolic compounds, proanthocyanidins, aromatic acids, stilbenoid, and flavonoids. Grapes contain nutritional constituents such as minerals, lipids, vitamins, carbohydrates, and proteins. Different parts of the grapevines have various biological activities. The content of phytochemical compounds varies in each part of the grapevines. The pharmacological activities depend on the phytochemical compounds, components used, and extraction type. (Insanu, Karimah, Pramastya, & Fidrianny, 2021).

2. Experimental Work:

2.1 Collection of Sample:

In Pakistan, grapes are grown, especially in Balochistan, but currently, Pothohar in the Punjab region is known for its diverse grape cultivation. Several well-known areas in Pakistan where grapes are grown include Pishin, Quetta, Kalat, Kot Abdullah, Loralai, Charsada, Sawat, Panjgor, Kharan, Mastung, Bahawalpur, Chitral, Abbottabad, Chakwal, and Nowshera(Akram, Khan Qadri, Jaskani, & Awan, 2019). The leaves of the grape plant were collected from Bahawalpur.

2.2 Plant materials extract:

The process of extracting plant material involved the drying of grape plant leaves at ambient temperature. Subsequently, a grinding mill from Sweden was utilized to convert all grape plant leaves into powder. In order to extract pulverized materials, a sample weighing 20g of perlette and king ruby grape leaves powder was employed. This extraction process involved the use of different solvents, namely 100% methanol, 80% methanol (with a ratio of 80%

methanol to 20% water), 100% ethanol, 80% ethanol (with a ratio of 80% ethanol to 20% water), decoction, and distilled water. Each extraction took place for a duration of 7.5 to 8.5 hours. To separate the extracts from the solids, Whatman filter paper 1 was utilized, and this process was repeated using the same solutions and extracts to ensure a thorough extraction. The collected extracts were concentrated through the application of a rotary evaporator at a temperature of 45 °C and reduced pressure. The resulting raw and concentrated extracts were stored at a temperature of -4 °C until further analysis.

3. Analyzing the Antioxidant Capacity of Plant Extraction:

The antioxidant capacity of various grape leaf varieties was assessed through the following tests:

3.1 Total Phenolic Components Determination (TP):

The investigation of phenol concentration in grape leaves was conducted using the Folin-Ciocalteu reagent method (Clarke, Ting, Wiart, & Fry, 2013) . In this method, a mixture of 1 mL of crude and 7.5 mL of deionized water with a concentration of 1 mg/mL, along with 0.5 mL of Folin-Ciocalteu reagent, was utilized. Subsequently, the sample solution was combined with 1.5 mL of sodium carbonate (20% w/v), incubated at room temperature for 10 minutes, heated at 40°C for 20 minutes, and then cooled using an ice bath. The absorbance was measured at 630 nm (Khan et al., 2021).To establish a standard curve, gallic acid ranging from 5 to 100 ppm was employed, and the total phenolic content (TF) was expressed as CE per air-dried substance. Ensuring consistency in the findings, the results from three studies of each sample were averaged.

3.2 Total Flavonoids (TF) Determination:

Ingredients were introduced into a volumetric flask in the following manner: 1 mL of product material, which contained 100 mg of dried material per mL, was added, followed by 5 mL of distilled water, 0.3 mL of $NaNO_2$, and the desired amount of TF. Subsequently, 0.6 mL of 10% aluminum chloride and 2 mL of 1 M NaOH were included, and an additional 5 minutes were provided for the reaction to occur. The remaining space in the flask was filled with an adequate quantity of distilled water. Following thorough mixing, the absorbance of the solution

was measured at a wavelength of 510 nm. The concentration of TF was determined by employing a catechin standardization curve within the range of 5-100 ppm (Fatima et al., 2015). The content of TF was expressed as CE per air-dried substance, and each individual experiment underwent three separate analyses.

3.3 DPPH Radical Analysis:

The primary aim of this experiment was to determine the IC50 value for the DPPH radical scavenging ability of CCE/PRFs obtained from grape leaves, specifically from the leaves of Perlette and King Ruby grape varieties. The procedure involved the combination of 5 mL of methanol with DPPH at a concentration range of 0.004%, with methanol concentrations varying from 0.10 to 5.0 mg/mL. The resulting mixture was allowed to remain at room temperature for approximately 20 minutes, and the optical density (OD) was measured at 517 nm (Clarke, Ting, Wiart, & Fry, 2013).

The percentage of inhibition (I) was determined using the following formula:

$$I\ (\%) = [A_b \div A_s] \times 10$$

In this equation, A_s represents the absorbance of the sample, and A_b represents the absorbance of the blank. The term "inhibition" is also represented as (I).

3.4 Determination of Antioxidant Activity in the Linoleic Acid System:

The assessment of the antioxidant activity of the extracts was conducted through the evaluation of the percentage of peroxidation inhibition in a linoleic acid system. This evaluation was done with slight modifications based on the method previously described by researchers. The dried extracts, in amounts of 5 mg each, from each treatment were combined with 0.13 mL of linoleic acid, 10 mg of ethanol (99.8%), and 0.2 M sodium phosphate buffer (pH 7), resulting in a 10 mL solution. Following this, 25 cc of distilled water was added to the mixture. The solution was then subjected to incubation at a temperature of 40°C, and the degree of oxidation was determined utilizing the thiocyanate method.

To summarize, a solution consisting of 10 mL of ethanol (75%), 0.2 mL of an aqueous ammonium thiocyanate solution (30%), 0.2 mL of the sample solution, and 0.2 mL of a ferrous chloride solution (20 mM in 3.5% HCl) was prepared. The absorbance was subsequently measured at a wavelength of 500 nm after 3 minutes of agitation. In the control experiment, linoleic acid was utilized without any extracts. The positive controls encompassed the artificial antioxidants butylated hydroxytoluene (BHT) and ascorbic acid (200 ppm). A test point devoid of an antioxidant component was employed, and the highest recorded level of peroxidation was 432 hours (18 days). The quantification of the antioxidant activity was achieved by calculating the percentage inhibition of linoleic acid peroxidation.

Linoleic acid peroxidation inhibition percentage = 100 – [(Abs. increase of sample at 360 h/Abs. increase of control at 432 h) – 100]

3.5 Peroxidation inhibition by CCEs/PRFs:

The efficacy of different CCEs/PRFs in suppressing the oxidation of linoleic acid was evaluated by homogenizing 5 milligrams of CCEs/PRFs with 0.1 milliliter of linoleic acid, 10 milliliters of a buffer solution, and 10 milliliters of highly pure ethanol. Once homogenized, 5 milliliters of distilled water were added, and the resulting mixture was incubated at a temperature of 40 degrees Celsius. The sample solution was prepared by combining 0.2 milliliters of ferrous chloride, 10 milliliters of a 75% ethanol solution, and 0.2 milliliters of a 30% ammonium thiocyanate solution.

The optical density (OD) value was then determined at a wavelength of 500 nanometers using the following equation:

$$I(\%)= 100-[A_s \div A_c]-100$$

Where A_s represents the absorbance value of the sample at 350 hours, A_c represents the absorbance value of the control (without CCE/PRF), and (I) represents the percentage of inhibition.

For comparison purposes, positive controls such as butylated hydroxytoluene (BHT) and ascorbic acid were utilized. The evaluation aimed to ascertain the capacity of the CCEs/PRFs to impede the oxidation of linoleic acid.

3.6 CCE/PRF power reduction:

The evaluation of the reducing capability of CCEs/PRFs entailed the usage of a solution composed of 1.0% potassium ferricyanide. Furthermore, the examination encompassed the inclusion of CCEs/PRFs at various quantities (ranging from 5 to 20 milligrams) along with sodium phosphate at a concentration of 0.2 M and a pH of 6.6. The incubation period for these compounds lasted for 20 minutes while maintaining a temperature of 50 degrees Celsius. Subsequently, the resulting solution, which weighed 980 grams, underwent treatment with trichloroacetic acid at a concentration of 10% and was subjected to centrifugation for a duration of 5 minutes. The supernatant that was recovered then underwent mixing with 0.5 mL of ferric chloride at a concentration of 0.1% and distilled water.

The spectrophotometer was employed to determine the optical density (OD) of the final reaction solution at a wavelength of 700 nm. This measurement was conducted in accordance with the methodology delineated by Mannan, Ahmed, Hussain, Jamil, and Miza in the year 2012. This process facilitated the assessment of the reducing power exhibited by CCEs/PRFs under the specified experimental conditions.

4. Statistical Analysis:

Mean percentage ± SD for each experiment. Correlation coefficient calculated to assess differences within groups. ANOVA used to detect significant variations among groups ($p < 0.05$).

5. Results and Discussions:

5.1 Extraction Yield

Perlette and King Ruby grape leaves produced extracts ranging from 7.14 to 15.58 g/100g. 80% ethanol had the highest yield at 36%, while distilled water had the lowest at 23%. King's Ruby

grape extracts ranged from 8.47 to 14.84 g/100g, with aqueous ethanol having the highest yield at 35% and distilled water having the lowest at 24.44% (Ghafoor & Choi, 2009)..

Solvent extraction capacities were in the order of aqueous ethanol, aqueous methanol, absolute ethanol, absolute methanol, decoction, and distilled water. 80% ethanol was the most effective in recovering antioxidant components.

5.2 Total Phenolic Contents:

Phenolics are increasingly valued in the food industry due to their potential anti-carcinogenic and lipid oxidation-slowing properties. The importance of screening fresh plant material for phenolics is underscored by the significance of total phenols in the antioxidant activity of fruits and vegetables (González-Centeno et al., 2012).

Table 4.1 presents the TPC (GAE mg/100g dry sample) in the leaf extracts of Perlette Grape and King Ruby.★

Sr. No.	Extracts	Total phenolic content	
		Perlette leaves	King Ruby Leaves
1	100% Methanol	11.70 ± 0.02	9.29 ± 0.01
2	80% Methanol	13.37 ± 0.01	13.15 ± 0.02
3	100% Ethanol	12.40 ± 0.02	10.61 ± 0.02
4	80% Ethanol	15.75 ± 0.01	15.34 ± 0.01
5	Decoction	10.44 ± 0.02	8.53 ± 0.01
6	Distilled water	9.15 ± 0.02	7.98 ± 0.01

★The data, including the mean and standard deviation from three experiments, reveal significant differences ($p < 0.05$) denoted by various superscript letters within the same column.

6.3 Total Flavonoid Contents:

Flavonoids are important for the antioxidative activity of fruits and vegetables. It is necessary to examine fresh plant material for these compounds. The overall content of flavonoids in grape leaves varies depending on the solvents used for extraction(Ghafoor, Choi, Jeon, & Jo, 2009). The quantity of flavonoids extracted from grape leaves varies with different solvents. The order of solvent effectiveness for extracting flavonoids is ethanol, methanol, decoction, distilled water, and aqueous ethanol. Ethanol is the preferred solvent for extracting antioxidant

compounds from plants. Different solvents yield different concentrations of antioxidant compounds in grape leaves.

Table 4.2 presents the concentrations of TFC (CE mg/100g dry weight) and GAE (mg/100g dry sample) in the extracts of grape leaves from the Perlette and King Ruby varieties. ★

Sr. No.	Extracts	Total flavonoids	
		Perlette Leaves	King ruby
1	100 % Methanol	11.63 ± 0.02	10.84 ± 0.01
2	80 % Methanol	14.44 ± 0.01	13.48 ± 0.02
3	100 % Ethanol	12.94 ± 0.02	11.24 ± 0.01
4	80 % Ethanol	15.80 ± 0.01	15.87 ± 0.02
5	Decoction	10.55 ± 0.02	9.83 ± 0.01
6	Distilled water	8.98 ± 0.02	8.40 ± 0.01

★The results, expressed as mean ± SD from three experiments, illustrate significant differences ($p < 0.05$) indicated by various superscript letters within the same column.

6.4 Assay for Scavenging DPPH Radicals:

The absorption peaks occurring within the range of 515 to 528 nm are generated by the abundant violet hue of DPPH, which is a persistent organic radical. The appearance of a yellow color is induced when a hydrogen donor, typically phenolics, donates a proton. It is widely acknowledged that as the concentration or level of hydroxylation of phenolic components increases, the capacity of leaf extracts to scavenge DPPH radicals, and therefore function as antioxidants, also increases.

Table 4.3 provides a breakdown of the IC50 values for the scavenging activity of DPPH radicals in extracts derived from the leaves of Perlette and King Ruby grapes.★

Sr. No	Extracts	Perlette grape Leaves	King ruby grape Leaves
1	100% Methanol	0.111 ± 0.71	0.090 ± 0.72
2	80% Methanol	0.084 ± 0.39	0.069 ± 0.37
3	100% Ethanol	0.087± 0.72	0.074 ± 0.24
4	80% Ethanol	0.072 ± 0.67	0.048 ± 0.53
5	Decoction	0.208 ± 0.13	0.147 ± 0.28

6	Distilled water	0.319 ± 0.49	0.182 ± 0.21

★The results, expressed as mean ± SD from three experiments, reveal significant differences ($p < 0.05$), indicated by various superscript letters within the same column.

6.5 Antioxidant Action in the System of Llinoleic Acid:

Linoleic acid, a fatty acid that is polyunsaturated, undergoes reactions of oxidation with oxygen, resulting in the production of peroxides that convert Fe2+ to Fe3+. This Fe3+ forms a complex with SCN, and the concentration of this complex can be determined by measuring the absorbance of the complex at 500 nm using spectrophotometry. Higher levels of absorbance indicate higher concentrations of peroxides, which suggest lower levels of antioxidant activity.

Table 4.4 presents the activity of the antioxidants in extracts of grape leaves from the Perlette and King Ruby varieties, expressed as the percentage of inhibition.★

Sr. No.	Extracts	% Inhibition of linoleic acid oxidation	
		Perlette Grape Leaves	King Ruby Grape Leaves
1	100% Methanol	64.22 ± 0.37	85.74 ± 0.41
2	80% Methanol	79.26 ± 0.43	94.52 ± 0.52
3	100% Ethanol	70.35 ± 0.53	89.44 ± 0.30
4	80% Ethanol	84.46 ± 0.23	97.13 ± 0.57
5	Decoction	60.35 ± 0.57	81.15± 0.47
6	Distilled water	57.82 ± 0.81	77.44 ± 0.42

★The data, which are the mean ± standard deviation from three experiments, show significant differences ($p < 0.05$) indicated by different superscript letters within the same column.

6.6 Reducing Power Extract:

Measuring reducing potential assesses plant extract antioxidant activity. Ferric ions change color from yellow to blue-green when converted to ferrous ions. Extracts with higher antioxidant compounds have more intense color and absorption. Reducing power and antioxidant activity correlate. Extracts consistently showed increased reduction power. Absorbance values for Perlette and King Ruby grape leaf extracts follow a similar pattern.

Table 4.5: Reducing Power of Perlette and King Ruby Grape Leaves Extracts★

Sr. No.	Extracts	Concentration (mg/mL)	Reducing power	
			Perlette Grape Leaves	King Ruby Leaves
1	100% Methanol	10	0.454 ± 0.01	0.505 ± 0.01
		20	0.500 ± 0.01	0.537 ± 0.01
		30	0.518 ± 0.01	0.579 ± 0.02
		40	0.592 ± 0.02	0.695 ± 0.01
2	80% Methanol	10	0.518 ± 0.02	0.559 ± 0.02
		20	0.548 ± 0.02	0.589 ± 0.01
		30	0.597 ± 0.01	0.646 ± 0.01
		40	0.648 ± 0.02	0.789 ± 0.01
3	100 % Ethanol	10	0.450 ± 0.02	0.521 ± 0.01
		20	0.539 ± 0.01	0.544 ± 0.01
		30	0.573 ± 0.01	0.601 ± 0.01
		40	0.599 ± 0.01	0.755 ± 0.02
4	80 %Ethanol	10	0.522 ± 0.01	0.593 ± 0.01
		20	0.594 ± 0.02	0.625 ± 0.02
		30	0.608 ± 0.02	0.649 ± 0.01
		40	0.668 ± 0.01	0.805 ± 0.01
5	Decoction	10	0.428 ± 0.01	0.491 ± 0.02
		20	0.443 ± 0.01	0.514 ± 0.01
		30	0.460 ± 0.02	0.548 ± 0.02
		40	0.488 ± 0.01	0.671 ± 0.02
6	Distilled water	10	0.420 ± 0.02	0.446 ± 0.01
		20	0.438 ± 0.01	0.511 ± 0.01
		30	0.455 ± 0.02	0.544 ± 0.02
		40	0.482 ± 0.02	0.668 ± 0.01

★Data represent the mean ± SD from three distinct experiments. Different superscript letters within the same column indicate significant variability ($p<0.05$) in the average of various extracting solvents.

6. Future Perspectives:

Leaves provide antioxidants and phenolic chemicals, requiring further research on grape cultivars and their components to extract biochemicals and antioxidants. Advanced techniques are necessary to identify and separate bioactive compounds. The bioactivities and impact on human health of these compounds should be studied, including genotoxicity and toxicology.

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