International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 22s, 2025
https://theaspd.com/index.php

Pharmacognostical Characterization, Total Phenolic and Flavonoid Content, And Antioxidant Activity of Celery Leaf and Raspberry Fruit Extracts

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Abstract
The present study aimed to evaluate and compare the phytochemical composition and antioxidant activity of extracts obtained from celery leaves and raspberry fruits at different stages of ripeness using various solvents. Standard phytochemical screening revealed the presence of multiple bioactive compounds, including alkaloids, flavonoids, phenols, phytosterols, saponins, and glycosides. Total phenolic content (TPC) and total flavonoid content (TFC) were significantly influenced by both the solvent used and the maturity of the fruit. Hydroalcoholic extracts consistently exhibited the highest TPC and TFC values, with unripe raspberry fruit extract showing the greatest concentrations (TPC: 102.3 mg/g; TFC: 95.14 mg/g). Antioxidant activity, assessed through DPPH, ABTS, nitric oxide, hydrogen peroxide, hydroxyl radical, superoxide, and metal chelating assays, revealed that celery leaf extract (CLE) demonstrated superior activity in most assays, particularly ABTS and hydroxyl radical scavenging. RUFE showed strong DPPH activity but lower performance in other assays, while RRFE had comparatively weaker antioxidant potential. The IC_{50} values confirmed these findings, with CLE and RUFE showing better efficacy than RRFE. Although quercetin and ascorbic acid exhibited superior antioxidant effects, the extracts demonstrated promising natural antioxidant properties. These findings support the potential application of these plant extracts in nutraceutical and therapeutic formulations targeting oxidative stress-related disorders.

Keywords: *Celery Leaf Extract, Raspberry Fruit Extract, Antioxidant Activity, Phytochemical Screening, Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Radical Scavenging Assays, IC_{50} Value, Hydroalcoholic Extract, Oxidative Stress.*

1. INTRODUCTION

Oxidative stress, caused by an imbalance between reactive oxygen species (ROS) and antioxidant defences, is a central factor in the progression of numerous chronic diseases, including cardiovascular disorders, neurodegenerative conditions, diabetes, cancer, and skin aging. Natural antioxidants derived from plants have garnered significant attention in recent years due to their capacity to neutralize free radicals, inhibit oxidative damage, and enhance cellular protection (Aboody, 2021; Gu et al., 2020; Umbayev et al., 2020). Among plant sources, leafy vegetables and fruits stand out for their rich phytochemical content and diverse therapeutic properties. Celery (Apium graveolens), a commonly consumed green vegetable, and raspberry (Rubus idaeus), a widely popular fruit, both possess an array of biologically active compounds that may offer considerable health benefits (Ispiryan et al., 2021; Khattab et al., 2020; Misic et al., 2020; Prakoso et al., 2020; Tao et al., 2023).

Celery leaves, often discarded in culinary use, are an underexplored part of the plant rich in vitamins, minerals, and secondary metabolites such as flavonoids, phenols, alkaloids, and terpenes. These compounds are known to contribute to the plant's pharmacological properties, including anti-inflammatory, antioxidant, and antimicrobial activities. Similarly, raspberry fruits are valued not only for their flavour and nutritional content but also for their abundance of phenolic acids, anthocyanins, flavonols, and ellagitannins. Studies have reported that the antioxidant potential of raspberry fruits varies significantly with ripeness, with unripe fruits often retaining higher levels of bioactive compounds. The extraction solvent plays a critical role in determining the efficiency and yield of phytochemicals from plant materials. Polar solvents such as methanol, ethanol, water, and their hydroalcoholic mixtures are frequently employed in the extraction of plant-based antioxidants due to their ability to dissolve a wide range of phenolic and flavonoid compounds. Selecting an appropriate solvent is crucial for maximizing the recovery of these bioactives and evaluating the full antioxidant capacity of the extracts (Aboody, 2021; Ispiryan et al., 2021; Tao et al., 2023; Zorga et al., 2020).

Phytochemical screening remains an essential preliminary step in evaluating the medicinal potential of plant extracts. The presence of key secondary metabolites such as flavonoids, alkaloids, glycosides, saponins, phytosterols, and phenolic compounds provides a strong indication of therapeutic relevance. Further quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) offers deeper insight into the antioxidant potential of the extracts. These phytochemicals act as hydrogen donors, metal chelators, and singlet oxygen quenchers, helping to protect biological systems from oxidative damage (Arsenov et al., 2021; Lopez-Corona et al., 2022; Pan et al., 2024).

This study aims to perform a comparative evaluation of the phytochemical profile and antioxidant activities of celery leaf extract (CLE), raspberry unripe fruit extract (RUFE), and raspberry ripe fruit extract (RRFE) using various extraction solvents. The antioxidant potential was assessed through multiple radical scavenging assays, including DPPH, ABTS, nitric oxide, hydrogen peroxide, hydroxyl, superoxide, and metal chelating activities. The determination of IC_{50} values for each assay provided a quantitative measure of efficacy. By identifying optimal extraction conditions and profiling the antioxidant properties of these extracts, the study provides valuable data for future development of plant-based antioxidant formulations. The findings may support the application of celery leaves and raspberry fruits as natural sources of antioxidants in the formulation of functional foods, nutraceuticals, or therapeutic agents aimed at preventing oxidative stress-related diseases.

2. MATERIAL AND METHODS

2.1 Assortment, identification, and extraction of the plants

Celery (Apium graveolens) and raspberries (Rubus idaeus L.), the plants used in this study, were gathered locally and purchased commercially from a reputable Ayurvedic and Herbal store in Bhopal (Madhya Pradesh Province). A botanist verified the authenticity of the plant material, which was gathered from the surrounding area between February and April of 2022. The herbariums and voucher specimens were kept for future use (SS/RI/2022/384 and SS/AG/2022/347).

2.2 Extraction of Plant material

The method outlined by Oktay et al. (2003) was used for manufacturing the extract (Oktay et al., 2003). In short, the celery leaves and raspberries were collected, let to dry in the shade for five days, and then ground into a powder. About 0.95 kg of powdered drug material was extracted using 99% pure ethanol in a 1:2 (w/v) ratio for each medication in an airtight container. The dried bulk was weighed and recorded after the resulting extract was dried in a steam bath. The yield percentage was computed. Secondly, hydroalcoholic extracts of celery leaves and Raspberries unripe and ripe fruits were prepared by traditional maceration method. 500gm powder of each was socked in 2.5 liters of 80% hydroalcoholic solvent (Ethanol: Distilled water) for 72 hours and kept for extraction (Houghton & Raman, 2012). The alcohol was evaporated under low pressure till a paste of solvent free extract was obtained. The extract was collected in a glass bottle and stored at 4oC until it was time for further examination.

2.3 Drugs and chemicals

High-purity reagents were used to ensure experimental accuracy. Collagenase and FALGPA were obtained from Sigma-Aldrich (USA), along with HEPES, N-Methoxysuccinyl-Ala-Ala-Pro-Val p-nitroanilide, L-leucyl-glycyl-L-prolyl-L-alanine, DMAB, ABTS, and DPPH. Quercetin was received as a gift sample. Additional reagents and solvents of analytical grade were procured from SRL Mumbai and E. Merck India. All chemicals supported enzymatic, antioxidant, and formulation studies, ensuring reliable and reproducible results.

2.4 Pharmacognostical Study

Pharmacognostical analysis included total ash, acid-insoluble ash, and water-soluble ash values. Two grams of powdered sample were incinerated at 500–600°C to obtain total ash. Acid-insoluble ash was determined by boiling ash with dilute HCl, filtering, and igniting the residue. Water-soluble ash was assessed by boiling ash with water, filtering, and weighing the ignited residue. Moisture content was estimated by drying two grams of sample at 100–105°C until constant weight. Extractive values were calculated by macerating powdered fruits in water, methanol, and hydroalcoholic solvents, followed by evaporation and weighing of the dried extracts.

2.5 Preliminary phytochemical screening: Qualitative phytochemical analysis

To determine which chemical components were present in the extracts, a series of standard phytochemical tests and routine chemical assays were performed (Harborne, 1998). Alkaloids were detected via Mayer's,

Wagner's, Dragendorff's, and Hager's tests, showing characteristic precipitates. Carbohydrates were confirmed through Molisch's, Benedict's, and Fehling's tests. Glycosides were identified by Modified Borntrager's and Legal's tests. Saponins showed persistent foaming in the foam test. Phytosterols were confirmed using Salkowski, Libermann-Burchard, and Tshugajeu tests. Phenols gave a bluish-black colour with ferric chloride. Flavonoids were identified by Alkaline Reagent, Lead Acetate, and Shinoda tests. These findings indicate strong antioxidant, anti-inflammatory, and therapeutic potential of the extracts (Harborne, 1998).

2.6 Estimation of total phenolic compounds

The Folin-Ciocalteu method, which was previously described (Lamuela-Raventós, 2018), was used to calculate the total phenolic content of celery leaves, ripe and unripe raspberries, and other fruits. Total phenolic content was estimated using the Folin-Ciocalteu method, based on a redox reaction between phenolics and the Folin-Ciocalteu reagent under alkaline conditions, producing a blue chromophore measurable at 650 nm. Extracts of powdered raspberries were prepared using water, acetone, ethanol, methanol, and hydroalcoholic solvents. For analysis, 0.2 mL of each extract was mixed with 3 mL distilled water, 0.5 mL Folin-Ciocalteu reagent, and 2 mL of 20% sodium carbonate. The mixture was briefly heated, cooled, and its absorbance measured at 650 nm. A gallic acid standard curve was used to calculate phenolic content, expressed as mg GAE/g extract using the formula:

$C = cV/m,$

where C is total phenolics, c is gallic acid concentration from the standard curve, V is extract volume, and m is extract mass. The regression equation from the gallic acid calibration curve was:

$Y = mx + c$, where x = concentration and y = absorbance.

2.7 In vitro antioxidant activity

2.7.1 Reducing power assay

The reducing power of the extracts was measured using the previously described technique (Mitsuda et al., 1996; Oyaizu, 1986). Extracts at different concentrations (50–250 µg/mL) were made using one millilitre of distilled water, 2.5 millilitres of potassium ferricyanide [K3Fe(CN)6] (1 percent), and 2.5 millilitres of phosphate buffer (2.5 millilitres, 0.2 millilitres, pH 6.6). The mixture was incubated at 50 °C for 20 minutes. After adding 2.5 mL of 10% trichloroacetic acid, the mixture was centrifuged at 3000 rpm for 10 minutes. A spectrophotometer (UV-1601 Shimadzu, Japan) was used to measure the absorbance at 700 nm after the superficial solution layer had been treated with 0.5 mL of FeCl3 (0.1 percent) and 2.5 mL of distilled water. The reaction mix's reducing potency is increased by ingestion. Quercetin, vitamin C, and sodium metabisulphite were used as common antioxidants.

2.7.2 Total antioxidant activity

The thiocyanate method was used to calculate the total antioxidant activity (Mitsuda et al., 1996). Ten millilitres of water were mixed with ten milligrammes of the extract. 2.5 ml of linoleic acid emulsion in potassium phosphate buffer (0.04 M, pH 7.0) was mixed with 50–250 µg/mL of extract or standard samples. The components of a five-milliliter linoleic acid emulsion are as follows: 15.5 µl linoleic acid, 0.04M potassium phosphate buffer (pH 7.0), and 17.5g Tween-20. However, 5.0 ml of the control solution contains 2.5 ml of a potassium phosphate buffer and 2.5 ml of a linoleic acid emulsion (0.04 M, pH 7.0). In total darkness, the mixture was incubated at 37°C. A spectrophotometer (UV-1601 Shimadzu, Japan) was used to measure the absorbance at 500 nm at various points during the incubation period after three minutes of agitation in order to calculate the amount of peroxide. Peroxides were created when linoleic acid was oxidised. Fe2+ was changed into Fe3+ by these peroxides. SCN creates a compound with the latter Fe3+ ions that shows absorbance at 500 nm. Thus, higher absorbance suggested more oxidation of linoleic acid. The blank samples lacked the extract and standards. The levels of total antioxidant activity were determined using the triplicate analytical findings. The reported percentage of lipid peroxidation inhibition was determined using the following formula:

Inhibition (%) = $(A_0 - A_t / A_0) \times 100$

Where A_0 = Absorbance (Control reaction)

A_t = Absorbance in the presence of the sample.

The antioxidant molecule α-tocopherol was used as the benchmark in each of the three trials. The mean and standard deviation data were shown on a graph.

2.7.3 Appraisal of DPPH (1 – 1 – diphenyl – 2 – picryl hydrazyl) radical scavenging activity

The previously established technique was used to assess the extracts' ability to scavenge free radicals (Shimada et al., 1992). 1–3 milliliters of extract solution at varying concentrations (50–250 µg/ml) was added to a 0.1 mM DPPH• solution in ethanol. After a violent vortex, the liquid was left to stand at room temperature for 30 minutes. Thus, we used a spectrophotometer (UV-1601 Shimadzu, Japan) to measure the absorbance at 517 nm. Higher free radical scavenging activity was shown by the reaction blend's lower absorbance. As reference materials, quercetin and ascorbic acid were used. The following formula was used to get the %DPPH• scavenging function:

DPPH• scavenging effect (%) = $[(A_0 – A_t / A_0) \times 100]$

Where A_0 = Absorbance of the control reaction and A_t = Absorbance in the presence of the standard or extract

2.7.4 ABTS (2, 2′ – azinobis – 3 – ethylbenzothiazoline – 6 – sulfonic acid) radical decolorization assay

A previously published technique was used to assess the ABTS radical scavenging activity (Re et al., 1998). The solution was made by adding ABTS to water at a concentration of 7 mmol/l. The ABTS radical was created by mixing the ABTS stock solution with 2.45 mM potassium persulfate (final concentration). Twelve to sixteen hours before usage, the mixture was allowed to come to room temperature in a dark place. The ABTS stock solution was diluted with 5 mM of phosphate-buffered saline (pH 7.4) to test the samples, and the blend's absorbance at 734 nm was set to 0.70. The absorbance was measured shortly after the first five minutes of mixing (20 µL of sample + 1.0 mL of diluted ABTS). Combining quercetin with ascorbic acid.

% ABTS• – scavenging activity = [Control absorbance – Sample absorbance] / [Control absorbance] × 100

2.7.5 Evaluation of superoxide radical ($O_2^{\bullet -}$) scavenging activity

Tests for scavenging superoxide radicals (O2•) have been carried out based on the extracts' capacity to prevent the formation of blue formazon. The combination of nitroblue tetrazolium (NBT), light, and riboflavin produced a superoxide radical (Beauchamp and Fridovich, 1971). The total volume of the reactant mixture was three millilitres. 0.1 mg NBT, 12 mM EDTA, 50 mM sodium phosphate buffer, 1 ml of sample solution, and 3 ml of riboflavin (20 µg) were all included in the reaction mixture. The reaction was initiated by shining extract concentrations ranging from 50 to 250 µg/ml on the reaction mixture for 90 seconds. The absorbance at 590 nm was measured right after illumination. The box containing the reaction system was sealed with aluminium foil and maintained in a controlled environment. The blank consisted of identical, non-illuminated tubes containing the reaction mixture. The standard compound was ascorbic acid. The following formula was used to determine the percentage of inhibition of superoxide anion generation:

% Inhibition = $(A_0 – A_t / A_0) \times 100$

Where, A_0 = Absorbance of the control (without extract) and A_t = absorbance in the presence of the extract or standard.

2.7.6 Evaluation of nitric oxide scavenging activity

A previously published Greis reaction was used to measure the nitric oxide scavenging activity (Kumaran and Joel Karunakaran, 2006, Sreejayan and Rao, 1997). At a pH that is consistent with the physiological system, sodium nitroprusside spontaneously produces nitric oxide. When this oxide reacts with oxygen, nitrate ions are created, which can be detected using the Greiss reagent. By fighting with oxygen, nitric oxide scavengers reduce nitrite ions. Different amounts of reconstituted extracts in methanol were combined with phosphate buffered saline supplemented with sodium nitroprusside (10 mM), and the mixture was incubated for 150 minutes at room temperature. As a control, a reaction mixture was prepared without the extracts but with the appropriate amount of methanol. After the incubation period, the reaction mixture was mixed with 0.5 mL of Greiss reagent, which included 0.1 percent N-(1-naphthyl) ethylenediamine dihydrochloride, 2 percent H3PO4, and 1 percent sulfanilamide. A chromophore was produced by diazotising nitrite with sulfanilamide and then combining the resultant material with naphthyl ethylenediamine. At 546 nm, the chromophore's absorbance was measured. Positive controls were ascorbic acid standard solutions prepared in a manner similar to Greiss reagent tests. then used the formula that follows:

% Inhibition = $(A_0 – A_t / A_0) \times 100$

Where, A_0 = absorbance of the control (without extract) and A_t = absorbance in the presence of the extract.

2.7.7 Appraisal of hydrogen peroxide scavenging activity

A previously published technique was used to evaluate hydrogen peroxide's (H2O2) capacity for scavenging (Ruch et al., 1989). Using spectrophotometry, which measures absorbance at 230 nm with a molar absorbtivity of 81 (mol•l−1) −1 cm−1, the amount of hydrogen peroxide was determined after a solution of hydrogen peroxide (2 mmol/ml; pH 7.4) was produced in phosphate buffer. The extracts, which ranged in concentration from 50 to 250 µg/ml, were combined with 0.6 ml of H2O2 solutions. The absorbance of H2O2 at 230 nm was compared ten minutes later using a blank solution that contained phosphate buffer. Ascorbic acid was used as a standard. The following formula was used to determine the % inhibition:

% Inhibition = $(A_0 - A_t / A_0) \times 100$

Where, A_0 = absorbance of the control (without extract) and A_t = absorbance in the presence of the extract or standard.

2.7.8 Hydroxyl radical scavenging activity

The previously reported 2-deoxy-d-ribose test method was used to evaluate the extracts' ability to scavenge hydroxyl radicals (Halliwell et al., 1987). As reference materials, quercetin and ascorbic acid were used. The inhibition % was calculated using the following formula.

$$\% \text{ Inhibition } = \frac{(A_0 - A_t)}{(A_0)}$$

where at is the optical density/absorbance in the presence of the extract or standard and A0 is the control optical density/absorbance (without extract).

2.7.9 Metal chelating activity

The extracts' ability to chelate ferrous ions was evaluated using a well-known method that has been previously published (Dinis et al., 1994; Kumaran & Joel Karunakaran, 2006). At varying doses (50–250 µg/ml), the extracts were mixed with 0.05 mL of a FeCl2 (2 mmol/l) solution. To start the reaction, 0.2 ml of ferrozine (5 mmol/l) was then added. After a violent vortex, the reaction mixture was let to stand at room temperature for ten minutes. At 562 nm, the absorbance of the resulting solution was then determined. In this experiment, ethylenediaminetetraacetic acid (EDTA) was commonly used as a chelating agent. The following formula was used to determine the % inhibition of ferrozine-ferrous complex formation:

% Inhibition = $(A_0 - A_t / A_0) \times 100$

Where, A_0 = absorbance of the control (without extract) and A_t = absorbance in the presence of the extract or standard.

3. RESULTS AND DISCUSSION

3.1 Assortment, identification, and extraction of the plants

Celery (Apium graveolens) and raspberries (Rubus idaeus L.), the plants used in this study, were gathered locally and purchased commercially from a reputable Ayurvedic and Herbal store in Bhopal (Madhya Pradesh Province). A botanist verified the authenticity of the plant material, which was gathered from the surrounding area between February and April of 2022. The herbariums and voucher specimens were kept for future use (SS/RI/2022/384 and SS/AG/2022/347).

Table 1. Estimation of The Ash Value and Loss on Drying of The Ripe and Unripe Raspberries and Celery Leaves.

Sr. No.	Parameters	Celery leaves and Raspberry plant parts		
		Leaves	Unripe Fruit	Ripe Fruit
1.	**Ash Value** (%)			
a.	Total Ash	4.0	3.5	2.5
b.	Acid Insoluble Ash	0.9	1.0	1.0
c.	Water Soluble Ash	1.0	2.5	1.5
2.	**Loss on Drying** (%)	4.5	2.5	4.0

The extractive value and colour of extracts of Celery leaves and Raspberry unripe and ripe fruits were investigated. The extractive values of celery leaf extracts in aqueous, methanol, ethanol, acetone, chloroform, petroleum ether, and hydroalcoholic forms were 20%, 10%, 5%, 3%, 3%, 1%, and 24.5%, respectively, according to Table 2. Compared to the other extracts, the petroleum ether extract had a

lower extractive value of 1%. Comparing hydroalcoholic extract to aqueous, methanol, ethanol, acetone, chloroform, and petroleum ether, the extractive value was higher (33%) in the former. Table 3 showed the extractive values of the unripe raspberry fruit. For aqueous, methanol, ethanol, acetone, chloroform, petroleum ether, and hydroalcoholic extracts, the corresponding extractive values were 30%, 15%, 5%, 1.5%, 2.5%, 0.5%, and 33%. The extraction value of the petroleum ether extract was the lowest of all the extracts, at 0.5%. Hydroalcoholic extract had the highest extractive value (33%) when compared to petroleum ether, aqueous, methanol, ethanol, acetone, and chloroform extracts. Table 4 represents the extractive values of Raspberry ripe fruit. In aqueous, methanol, ethanol, acetone, chloroform, petroleum ether and hydroalcoholic extract the extractive values were 15%, 10%, 10%, 10%, 10%, 5% and 29.5% respectively. Among all extracts the hydroalcoholic extract showed highest extractive value (29.5%). The extractive value in petroleum ether extract value was low (5%) as compared to all other extracts. As seen in Figure 1, the hydroalcoholic extract of celery leaves and raspberry ripe and unripe fruit had a higher comparative percent extractive value than water, methanol, ethanol, acetone, chloroform, and petroleum ether.

Table 2. Extractive Value of Celery Leaves

		Celery Leaves	
Sr. No.	**Solvents (ml)**	**Colour of Extract**	**Extractive Value** (%)
1	Water	Dark Brown	20
2	Methanol	Yellowish Green	10
3	Ethanol	Green	5
4	Acetone	Dark Green	3
5	Chloroform	Dark Green	3
6	Petroleum Ether	Light Green	1
7	Hydroalcoholic	Dark Green	24.5

Table 3. Extractive Value of Raspberry Unripe Fruits and Ripe Fruits

		Raspberry Unripe Fruits		**Raspberry Ripe Fruits**	
Sr. No.	**Solvents (ml)**	**Colour of Extract**	**Extractive Value** (%)	**Colour of Extract**	**Extractive Value** (%)
1	Water	Brown	30	Orange	15
2	Methanol	Orange	15	Orange	10
3	Ethanol	Yellowish Green	5	Light Green	10
4	Acetone	Pale Yellow	1.5	Colourless	10
5	Chloroform	Yellowish Green	2.5	Colourless	10
6	Petroleum Ether	Colourless	0.5	Colourless	5
7	Hydroalcoholic	Reddish Brown	33	Reddish Brown	29.5

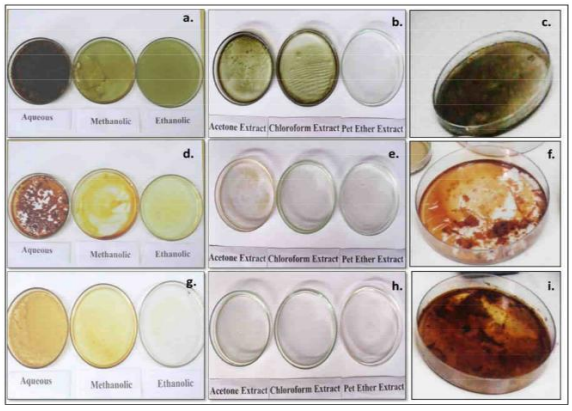

Figure 1. Extracts of Celery leaves (CLE) and Raspberry unripe (RUFE) and ripe fruit (RRFE)

3.2 Preliminary phytochemical screening: Qualitative phytochemical analysis

The extracts were subjected to standard phytochemical and chemical assays to identify key secondary metabolites such as alkaloids, glycosides, flavonoids, polyphenols, phytosterols, saponins, and tannins. These compounds, while not essential for plant growth, offer vital protection against pathogens and environmental stress and hold significant therapeutic potential. The qualitative screening revealed the presence of diverse phytoconstituents in both plant extracts, with Borntrager's test confirming glycosides in all samples. In celery leaf extracts, the aqueous extract showed the presence of proteins, amino acids, carbohydrates, glycosides, flavonoids, alkaloids, and tannins, while steroids were absent. The methanol extract contained most secondary metabolites, except cardiac glycosides and flavonoids. Acetone extract lacked proteins, carbohydrates, and anthraquinone glycosides but showed steroids, cardiac glycosides, and flavonoids. Ethanol and hydroalcoholic extracts displayed a full range of metabolites, whereas chloroform and petroleum ether extracts showed minimal presence. Unripe raspberry fruit extracts showed all major phytochemicals in aqueous and hydroalcoholic extracts. Methanol extract included proteins, carbohydrates, amino acids, glycosides, flavonoids, and tannins. Ethanol extract lacked steroids, and acetone extract showed presence of amino acids, flavonoids, alkaloids, and tannins. Chloroform and petroleum ether extracts contained fewer compounds, mainly carbohydrates, flavonoids, and alkaloids. In ripe raspberry fruit, carbohydrates, proteins, flavonoids, and tannins were consistently present across extracts. Amino acids were detected in aqueous, methanol, ethanol, and hydroalcoholic extracts but were absent in acetone, chloroform, and petroleum ether. Cardiac glycosides were found in aqueous, ethanol, and hydroalcoholic extracts, while anthraquinone glycosides appeared only in aqueous and hydroalcoholic. Saponins were absent in chloroform and petroleum ether. All extracts showed alkaloids, except petroleum ether. A detailed summary is presented in Table 4.

Table 4. Phytochemical screening for the plant extracts

Phytoconstituents	**Qualitative Findings**		
	Celery leaves and Raspberry plant parts		
	CLE	RUFE	RRFE
Saponins	√	√	√
Sterols	√	√	√
Carbohydrates	√	√	√
Tannins	√	√	√
Flavanoids	√	√	√
Fatty acid	√	-	√
Terpenoid	√	√	√
Glycosides	√	√	√

3.3. Estimation of total phenolic compounds

Total phenolic and flavonoid content

The TPC and TFC results varied significantly with solvent type and fruit ripeness. Hydroalcoholic extracts consistently yielded the highest levels of phenolics and flavonoids. Celery leaves showed maximum TPC (58.31 mg/g) and TFC (45.35 mg/g) in hydroalcoholic extract, followed by ethanol and crude powder. Methanol performed moderately, while aqueous and acetone extracts were least effective. Unripe

raspberry fruits had notably higher TPC and TFC than ripe fruits across all solvents, with the hydroalcoholic extract of unripe fruit showing the highest TPC (102.3 mg/g) and TFC (95.14 mg/g). Ripe fruit extracts showed reduced values, indicating bioactive degradation during ripening. Overall, hydroalcoholic solvents were most efficient, and unripe raspberry fruit emerged as the richest source of bioactives, offering potential for antioxidant and therapeutic applications.

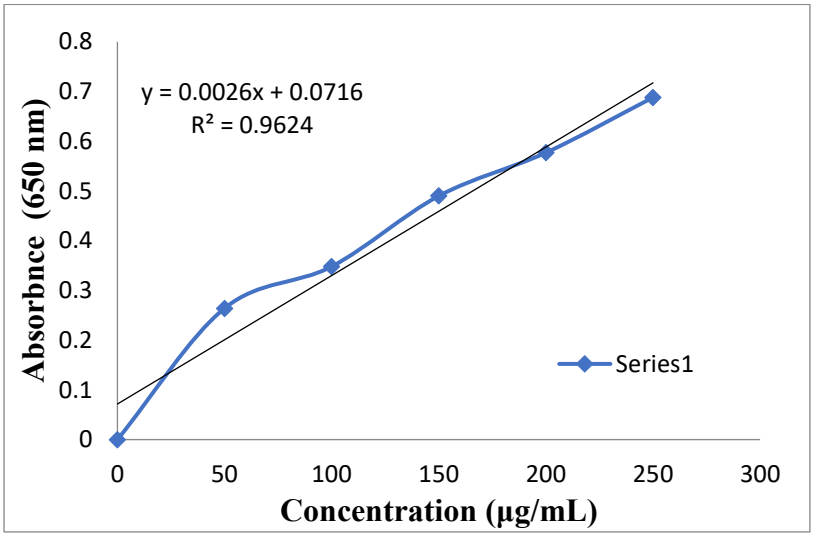

Figure 2. Standard Curve for Estimation of Total Phenolic Content

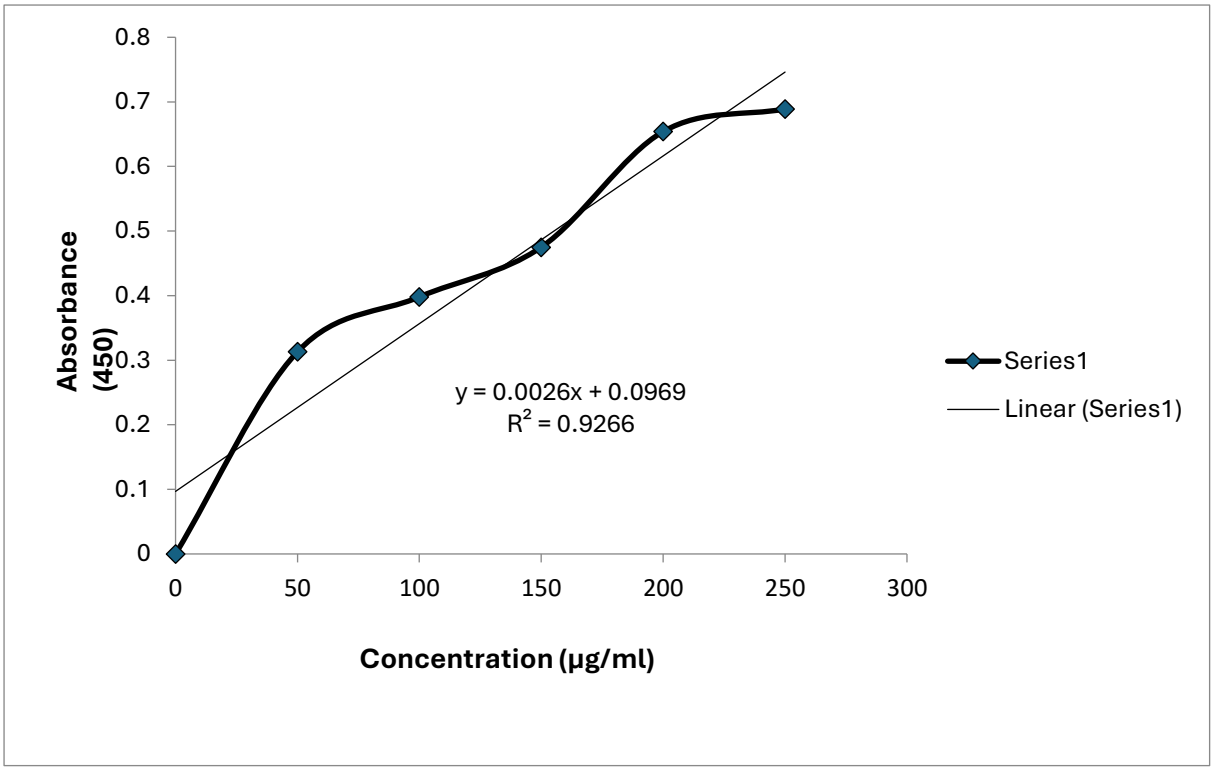

Figure 3. Standard Curve for Estimation of Total Flavanoid Content

Table 5. The Total Amount of Flavonoids and Phenols (Mg/Gm) In A Variety of Celery Leaf Extracts (CLE).

Sr. No.	Extracts	Total Phenolic Content (mg/gm GAE)	Total Flavonoid Content (mg/gm QE)
1	Aqueous	42.28 ± 0.015	31.72 ± 0.015
2	Acetone	34.63 ± 0.037	24.14 ± 0.032
3	Ethanol	54.51 ± 0.011	33.18 ± 0.005

4	Methanol	46.27 ± 0.020	34.72 ± 0.025
5	Hydroalcoholic	58.31 ± 0.005	45.35 ± 0.010
6	Crude Powder	54.31 ± 0.037	40.42 ± 0.025

Note: GAE = equivalent of Gallic Acid, QE = equivalent of Quercetin

Table 6. Total Phenolic and Flavonoid Content (Mg/Gm) In Different Raspberry Fruit Extracts, Both Ripe And Unripe

Sr. No.	**Extracts**	**Total Phenolic Contents** (mg/gm Gallic Acid equivalent)		**Total Flavonoid Contents** (mg/gm Quercetin equivalent)	
		Raspberry unripe fruit	Raspberry ripe fruit	Raspberry unripe fruit	Raspberry ripe fruit
1	Aqueous	87.90 ± 0.010	50.47 ± 0.032	62.80 ± 0.36	22.78 ± 0.015
2	Acetone	80.41 ± 0.009	29.21 ± 0.015	61.06 ± 0.075	20.71 ± 0.009
3	Ethanol	95.72 ± 0.020	62.37 ± 0.005	65.00 ± 0.005	33.17 ± 0.026
4	Methanol	92.38 ± 0.005	51.33 ± 0.030	70.23 ± 0.037	41.61 ± 0.010
5	Hydroalcoholic	102.3 ± 0.009	77.61 ± 0.010	95.14 ± 0.040	50.53 ± 0.010
6	Crude Powder	97.61 ± 0.015	70.54 ± 0.032	85.73 ± 0.015	44.09 ± 0.015

3.4 In vitro antioxidant activity

3.4.1 Reducing power assay

All three samples (CLE, RUFE, and RRFE) showed concentration-dependent increases in reducing power, suggesting that they have the capacity to donate electrons and may have antioxidant properties. BHA and α-tocopherol showed the strongest reducing power at every tested concentration, indicating that these standards are still more effective than the extracts. Interestingly, CLE and RUFE showed nearly identical absorbance values across all concentrations, implying they may share similar active components or mechanisms of reduction. RRFE demonstrated a slightly lower reducing power at lower concentrations (50–100 µg/ml) compared to CLE and RUFE but reached comparable levels by 250 µg/ml. Overall, these findings highlight the presence of potent reducing constituents in the test samples while also underscoring that their antioxidant efficacy, though significant, is relatively lower than that of the synthetic standard (BHA) and α-tocopherol (figure 4.3).

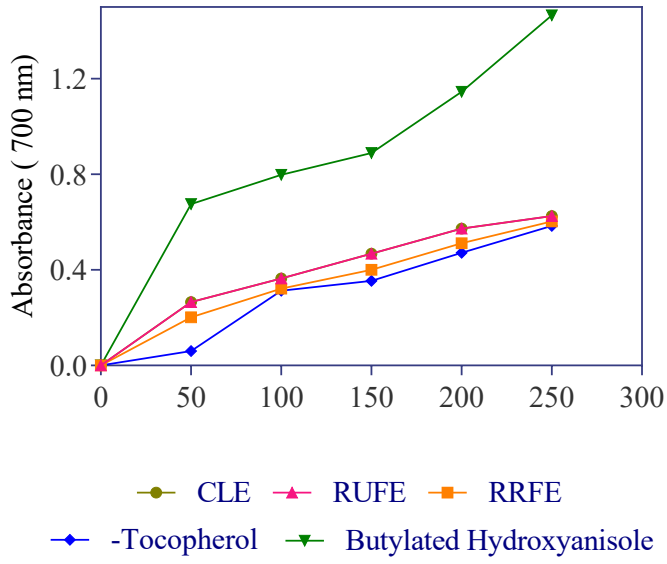

Figure 4. Showing the Reducing Power Assay of The Extracts

3.4.2 Total antioxidant activity

The extracts' total antioxidant activity (CLE, RUFE, and RRFE) was assessed using the thiocyanate procedure. In this total antioxidant activity assay, BHT emerged as the most potent standard, maintaining

consistently high percentages (above 90% for much of the test) and only gradually declining after 36 µg/ml. In contrast, α-tocopherol displayed a less predictable trend, starting at a modest 32.67%, peaking at about 74.85% by 48 µg/ml, and then dropping markedly to 34.77% by 72 µg/ml. Among the plant extracts (CLE, RUFE, and RRFE), all exhibited a noteworthy increase in total antioxidant activity through 36 µg/ml, with RUFE typically showing the highest values overall (e.g., 82.91% at 36 µg/ml), while CLE and RRFE followed closely. However, at 24 µg/ml, RRFE (74.84%) temporarily exceeded RUFE (71.87%), highlighting that antioxidant capacity among the extracts can fluctuate depending on concentration or time. Despite some fluctuations, RUFE generally maintained stronger antioxidant activity across the evaluated points compared to CLE and RRFE. All extracts, though, showed meaningful antioxidant capacity—often surpassing α-tocopherol at select concentrations. Notwithstanding, none of the extracts reached the high, stable activity levels observed with BHT. These findings suggest that while the natural extracts hold considerable potential as antioxidants, they may need optimization or combination with other antioxidant sources to match or exceed the robustness of synthetic standards like BHT (Figure 4.4).

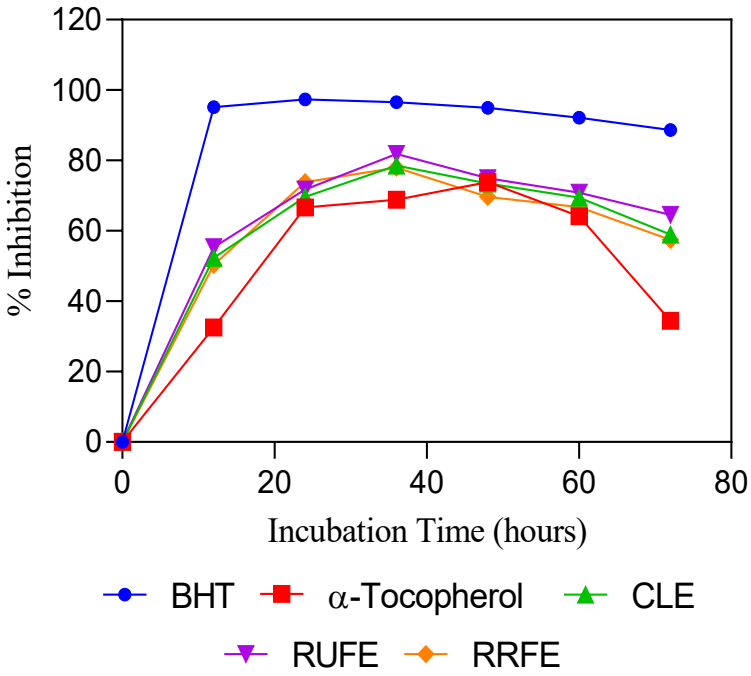

Figure 5. Depicting the Total Antioxidant Activity Of CLE, RUFE and RRFE

3.4.3 Appraisal of DPPH (1 – 1 – diphenyl – 2 – picryl hydrazyl) radical scavenging activity

The DPPH radical scavenging activity of the tested extracts (CLE, RUFE, and RRFE) exhibited a concentration-dependent increase, demonstrating their potent antioxidant potential. At lower concentrations (50 µg/ml), RUFE and RRFE displayed slightly higher scavenging activity (42.87% and 42.99%, respectively) than CLE (41.56%), though all extracts were outperformed by ascorbic acid (49.23%). However, at 100 µg/ml, the extracts matched or even slightly exceeded ascorbic acid, suggesting comparable free radical neutralization ability at mid-range concentrations. At higher concentrations (150–250 µg/ml), all extracts demonstrated near-maximal DPPH scavenging activity, closely approaching the activity of ascorbic acid and quercetin. At 200 µg/ml, RUFE exhibited the highest antioxidant activity (96.78%), surpassing ascorbic acid (96.45%) and quercetin (94.32%). Similarly, at 250 µg/ml, all extracts (CLE, RUFE, and RRFE) reached nearly 99% scavenging efficiency, equaling or closely approaching ascorbic acid (98.99%). Overall, these findings suggest that the plant extracts possess strong antioxidant activity, comparable to standard antioxidants at higher concentrations. Among them, RUFE showed the highest potency, particularly at 200 µg/ml, highlighting its potential as a natural antioxidant source. The results indicate that these extracts could serve as effective alternatives to synthetic antioxidants in therapeutic or nutraceutical applications (Figure 4.5 and Table 4.7).

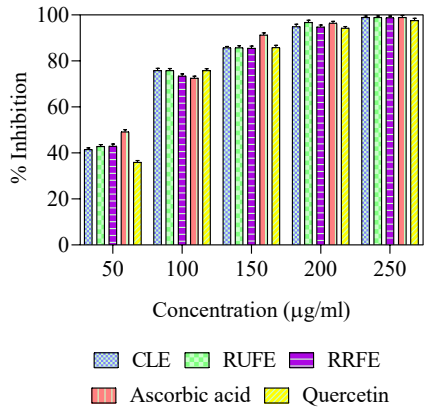

Figure 6. Depicted the antiradical activity of CLE, RUFE and RRFE (DPPH)

3.4.4 ABTS (2, 2′ – azinobis – 3 – ethylbenzothiazoline – 6 – sulfonic acid) radical decolori zation assay
The ABTS radical scavenging activity of CLE, RUFE, and RRFE demonstrated a concentration-dependent increase, suggesting their strong antioxidant potential. At lower concentrations (50–100 µg/ml), all extracts exhibited moderate activity, with CLE (36.87% at 50 µg/ml) showing slightly higher scavenging efficiency than RUFE (35.81%) and RRFE (34.67%). Ascorbic acid and quercetin displayed superior activity at these concentrations, with quercetin (43.61% at 50 µg/ml) being the most potent. At mid-range concentrations (150–200 µg/ml), a significant increase in antioxidant activity was observed across all extracts, with CLE (76.55% at 150 µg/ml) leading, followed by RUFE (75.81%) and RRFE (73.78%). Interestingly, the plant extracts outperformed ascorbic acid (60.83% at 150 µg/ml), indicating their higher radical scavenging efficiency. However, quercetin remained the strongest antioxidant, reaching 87.89% at 150 µg/ml and 94.51% at 200 µg/ml. At the highest concentration (250 µg/ml), all extracts exhibited near-complete scavenging activity, with CLE (95.01%) being the most potent among them, closely followed by RUFE (94.25%) and RRFE (93.35%). Ascorbic acid, though effective, remained lower (82.89%) compared to the extracts. Quercetin (98.98%) demonstrated the highest scavenging capacity, reinforcing its role as a powerful antioxidant. Overall, the results indicate that CLE, RUFE, and RRFE possess remarkable antioxidant properties, particularly at higher concentrations, surpassing ascorbic acid but remaining slightly less potent than quercetin. Among the three extracts, CLE consistently showed the highest activity, suggesting its potential as a strong natural antioxidant for therapeutic applications. (Table 4.7).

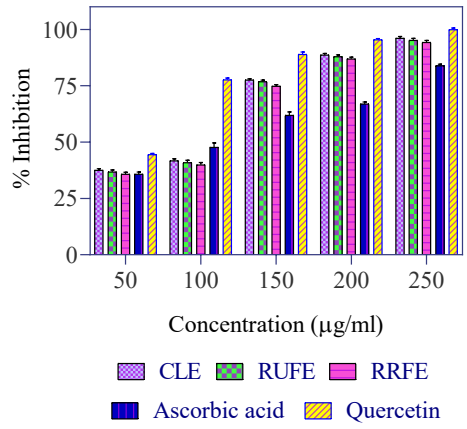

Figure 7. Depicted the antiradical activity of CLE, RUFE and RRFE (ABTS)

3.4.5 Evaluation of superoxide radical ($O_2^{\bullet -}$) scavenging activity
The efficiency of these compounds at scavenging was determined by the concentration of phenol as well as the number and distribution of hydroxyl groups in the phenolic and flavonoid compounds (Ashokkumar et al., 2008; Erasto et al., 2007). The superoxide radical scavenging activity of CLE, RUFE,

and RRFE exhibited a concentration-dependent increase, highlighting their potential as antioxidants. At 50 µg/ml, CLE demonstrated the highest scavenging activity (24.51%) among the extracts, followed by RUFE (22.56%) and RRFE (20.49%). However, ascorbic acid exhibited significantly greater scavenging ability (55.47%), indicating its superior radical neutralization efficiency at lower concentrations. As the concentration increased, all extracts showed a progressive enhancement in superoxide radical scavenging. At 100 µg/ml, CLE (42.69%) remained the most effective among the extracts, whereas RUFE (37.81%) and RRFE (32.87%) showed slightly lower activities. Ascorbic acid continued to maintain higher efficiency (64.68%). At 150 µg/ml, the scavenging activity of all extracts improved considerably, with CLE (53.78%), RUFE (50.86%), and RRFE (49.78%) approaching moderate effectiveness compared to ascorbic acid (74.89%). At higher concentrations (200–250 µg/ml), the antioxidant potential of the extracts became more pronounced. CLE, RUFE, and RRFE achieved 67.76%, 64.81%, and 62.68% scavenging at 200 µg/ml, respectively, indicating strong superoxide radical neutralization. At 250 µg/ml, CLE exhibited the highest activity (80.51%), followed by RUFE (77.62%) and RRFE (74.59%). Although these values are substantial, ascorbic acid remained the most potent antioxidant, reaching 88.89%. Overall, the results suggest that CLE, RUFE, and RRFE possess strong superoxide scavenging capabilities, with CLE consistently demonstrating the highest activity among them. However, ascorbic acid remained superior at all concentrations. These findings indicate that the extracts have promising antioxidant potential and could serve as natural alternatives, though further enhancement may be needed to match the efficiency of synthetic standards (Table 4.7).

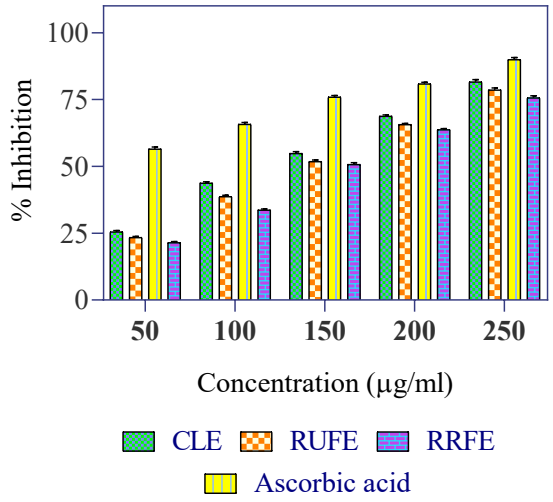

Figure 8. Depicted the Antiradical Activity Of CLE, RUFE And RRFE (Superoxide Radical)

3.4.6 Evaluation of nitric oxide scavenging activity

The nitric oxide (NO) scavenging activity of CLE, RUFE, and RRFE exhibited a concentration-dependent increase, demonstrating their potential antioxidant effects. At the lowest concentration (50 µg/ml), CLE showed the highest scavenging activity (31.97%), followed by RUFE (27.86%) and RRFE (26.79%), though all extracts remained lower than ascorbic acid (36.89%). As the concentration increased, all samples showed significant improvement in their NO scavenging ability. At 100 µg/ml, CLE (45.95%) remained the most effective extract, followed by RUFE (41.76%) and RRFE (40.79%), nearing the activity of ascorbic acid (47.79%). The gap between the extracts and the standard remained relatively small at this concentration. At 150 µg/ml, CLE reached 51.87%, while RUFE (48.75%) and RRFE (46.90%) followed closely, although ascorbic acid maintained a higher activity (60.89%). At higher concentrations (200–250 µg/ml), the extracts displayed more substantial scavenging activity. CLE achieved the highest value among the extracts (56.96% at 200 µg/ml and 65.89% at 250 µg/ml), while RUFE and RRFE exhibited slightly lower but comparable activity. However, ascorbic acid remained the most potent scavenger, reaching 67.91% at 200 µg/ml and 72.89% at 250 µg/ml. Overall, the data suggest that CLE, RUFE, and RRFE exhibit significant nitric oxide scavenging potential, with CLE being the most effective among them. Although the plant extracts showed promising activity, they did not surpass ascorbic acid, which remained the most efficient radical scavenger at all concentrations. These findings indicate that while the extracts

are potent natural antioxidants, further enhancement or formulation improvements may be required to achieve activity comparable to synthetic antioxidants (Table 4.7).

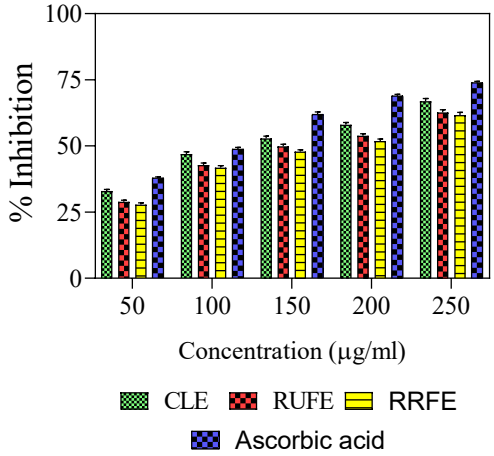

Figure 9. Antioxidant Activity Of CLE, RUFE And RRFE at 50-250 Mg/Ml Concentration (Nitric Oxide Scavenging Activity)

3.4.7 Appraisal of hydrogen peroxide scavenging activity

The hydrogen peroxide (H_2O_2) scavenging activity of CLE, RUFE, and RRFE showed a concentration-dependent increase, demonstrating their potential as antioxidants. At 50 µg/ml, CLE exhibited the highest scavenging activity (39.86%) among the extracts, followed by RUFE (36.78%) and RRFE (35.91%), though all remained slightly lower than ascorbic acid (43.83%). As the concentration increased, the extracts showed a notable enhancement in activity. At 100 µg/ml, CLE (61.62%) surpassed ascorbic acid (53.88%), indicating its strong ability to neutralize H_2O_2 at this concentration. Similarly, RUFE (58.87%) and RRFE (57.88%) also exhibited greater activity than ascorbic acid, suggesting that these extracts are effective at scavenging hydrogen peroxide. At 150 µg/ml, CLE (65.92%) continued to show strong activity, closely approaching ascorbic acid (67.78%), while RUFE (63.89%) and RRFE (62.93%) followed closely. At higher concentrations (200–250 µg/ml), the extracts demonstrated significant scavenging efficiency, nearing that of ascorbic acid. CLE (76.69%) showed the highest activity at 200 µg/ml, followed by RUFE (74.42%) and RRFE (72.72%), while ascorbic acid remained slightly higher (79.85%). At the maximum concentration of 250 µg/ml, CLE (87.43%) came very close to the activity of ascorbic acid (88.89%), with RUFE (84.88%) and RRFE (83.91%) also showing strong antioxidant effects. Overall, these results indicate that CLE, RUFE, and RRFE possess significant hydrogen peroxide scavenging abilities, with CLE consistently demonstrating the highest activity among them. Interestingly, at certain concentrations (100–150 µg/ml), the extracts even outperformed ascorbic acid, highlighting their potential as natural antioxidants. These findings suggest that the extracts could be promising candidates for mitigating oxidative stress caused by hydrogen peroxide, with CLE emerging as the most effective among the three (Table 4.7).

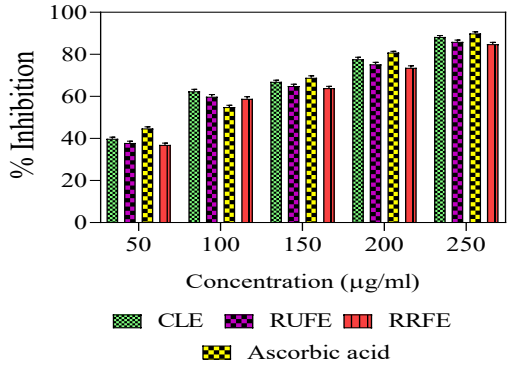

Figure 10. Antioxidant activity of CLE, RUFE and RRFE at 250 µg/ml concentration (Hydrogen peroxide scavenging activity)

3.4.8 Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of CLE, RUFE, and RRFE exhibited a concentration-dependent increase, indicating their potential antioxidant properties. At the lowest concentration (50 µg/ml), CLE showed the highest scavenging activity among the extracts (21.68%), followed by RUFE (18.91%) and RRFE (17.89%). However, both ascorbic acid (30.42%) and quercetin (41.69%) demonstrated superior activity, highlighting their stronger radical-neutralizing capacity at lower concentrations. At 100 µg/ml, all extracts showed notable improvements, with CLE reaching 36.41%, RUFE at 34.70%, and RRFE at 32.59%. However, they still remained lower than ascorbic acid (43.72%) and quercetin (56.90%), indicating that while the extracts exhibit hydroxyl radical scavenging potential, they are less effective than standard antioxidants at this concentration. At 150–200 µg/ml, the extracts demonstrated significant scavenging activity, with CLE (58.63% at 150 µg/ml, 72.81% at 200 µg/ml) maintaining its lead over RUFE (55.81%, 70.83%) and RRFE (53.65%, 69.37%). Although the extracts performed well, ascorbic acid (82.61%) and quercetin (87.78%) continued to show greater efficiency. At the highest concentration (250 µg/ml), all extracts displayed strong scavenging activity, with CLE (85.36%) showing the highest among them, followed by RUFE (81.66%) and RRFE (79.52%). Ascorbic acid (91.44%) and quercetin (96.87%) remained the most effective scavengers, reinforcing their superior radical-quenching properties. Overall, these results suggest that CLE, RUFE, and RRFE possess strong hydroxyl radical scavenging potential, with CLE consistently demonstrating the highest activity among them. However, their efficacy, while significant, remains lower than that of ascorbic acid and quercetin. This suggests that while these extracts can serve as effective natural antioxidants, they may require higher concentrations or combination strategies to achieve antioxidant performance comparable to standard compounds (Figure 4.10 and Table 4.7).

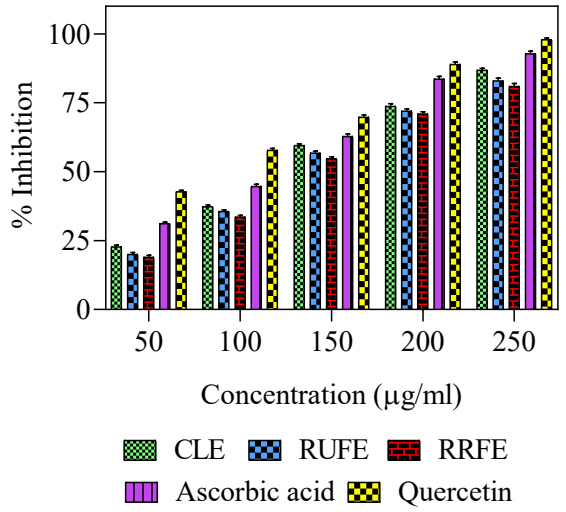

Figure 11. Antioxidant Activity Of CLE, RUFE And RRFE at 250 Mg/Ml Concentration (Hydroxyl Radical Scavenging Action)

3.4.9 Metal chelating activity

The metal chelating activity of CLE, RUFE, and RRFE exhibited a concentration-dependent increase, indicating their ability to bind and neutralize metal ions. However, their chelating potential remained significantly lower than that of EDTA, a well-established metal chelator. At the lowest concentration (50 µg/ml), CLE showed the highest chelating ability among the extracts (10.66%), followed by RUFE (9.58%) and RRFE (8.76%), while EDTA displayed a markedly superior activity (41.77%). As the concentration increased, all extracts showed a steady improvement in metal chelation. At 100 µg/ml, CLE (13.71%) maintained the highest activity among the extracts, followed by RUFE (10.85%) and RRFE (9.77%), but these values were still significantly lower than EDTA (57.65%). A similar trend was observed at 150 µg/ml, where CLE (25.81%) exhibited the strongest chelating effect, followed by RUFE (23.84%) and RRFE (22.88%), though EDTA remained far more effective (64.19%). At higher concentrations (200–250 µg/ml), the extracts continued to show improved chelating activity. CLE (30.51% at 200 µg/ml,

37.68% at 250 µg/ml) remained the most effective among them, with RUFE (27.59%, 34.88%) and RRFE (26.77%, 33.74%) following closely. However, EDTA maintained the highest efficiency, reaching 84.79% at 250 µg/ml, significantly outperforming the plant extracts. Overall, while CLE, RUFE, and RRFE demonstrated metal chelating activity, their efficacy was considerably lower than that of EDTA. Among the extracts, CLE consistently exhibited the highest chelating potential, followed by RUFE and RRFE. These findings suggest that although the extracts possess some metal chelating ability, they may require optimization or combination with stronger chelators to enhance their effectiveness for applications in oxidative stress management and metal detoxification (Figure 4.11 and Table 4.7).

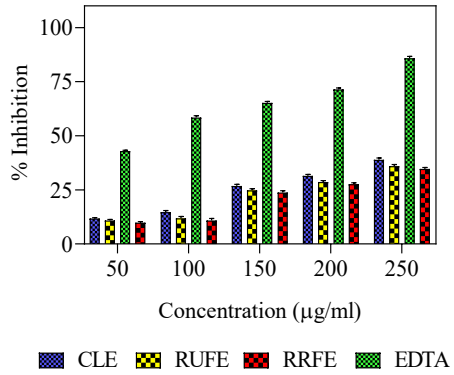

Figure 12. Antioxidant activity of CLE, RUFE and RRFE at 250 µg/ml concentration (Metal chelating activity)

3.4.10. The calculation of IC_{50} values

The antioxidant activity of CLE, RUFE, and RRFE was evaluated through multiple radical. The antioxidant potential of celery leaf extract (CLE), raspberry unripe fruit extract (RUFE), and raspberry ripe fruit extract (RRFE) was evaluated using multiple radical scavenging assays. IC_{50} values, derived from linear regression, indicated the concentration required to inhibit 50% of radical activity—lower values signifying higher antioxidant strength. RUFE showed the strongest DPPH activity (IC_{50} = 86.34 µg/mL), closely followed by RRFE and CLE, all comparable to ascorbic acid, while quercetin showed the highest activity (IC_{50} = 82.41 µg/mL). In the ABTS assay, CLE outperformed other extracts (IC_{50} = 111.76 µg/mL), though quercetin remained superior. Superoxide and nitric oxide scavenging assays showed moderate to low activity, with CLE performing best among the extracts but less effectively than ascorbic acid.

Hydrogen peroxide and hydroxyl radical assays also showed moderate activity, with CLE again leading. However, quercetin and ascorbic acid had significantly lower IC_{50} values. Metal chelating activity was weakest across all extracts, with high IC_{50} values compared to EDTA. Overall, CLE exhibited the best antioxidant performance among the extracts, particularly in ABTS, hydroxyl, and hydrogen peroxide scavenging. RUFE showed strong DPPH activity, while RRFE had the weakest antioxidant profile. The results affirm the antioxidant potential of the extracts but underscore the superior efficacy of standard antioxidants. Further in vivo and mechanistic studies are warranted to identify active compounds and enhance therapeutic relevance.

Table 7. Using Linear Regression Analysis, The Antioxidant Activity Potential Of CLE, RUFE And RRFE In Comparison to Reference Substances Was Determined Using IC50 (Mg/Ml) Values.

Sl. No.	Antioxidant Assay	IC_{50} (µg/ml)					
		CLE	RUFE	RRFE	Ascorbic acid	EDTA	Quercetin
1.	Determination of DPPH radical scavenging activity	88.69	86.34	87.65	91.22	-	82.41
2.	ABTS radical decolorization assay	111.76	113.74	114.82	132.19	-	83.23

3.	Assay of superoxide radical ($O_2^{\bullet -}$) scavenging activity	143.87	152.91	154.72	92.33	-	-
4.	Assay of nitric oxide scavenging activity	145.95	170.66	172.54	110.09	-	-
5.	Hydrogen peroxide scavenging activity	111.48	117.52	120.73	109.92	-	-
6.	Hydroxyl radical scavenging	136.62	144.45	147.83	114.85		79.45
7.	Metal chelating activity	323.53	352.82	354.77	-	116.69	-

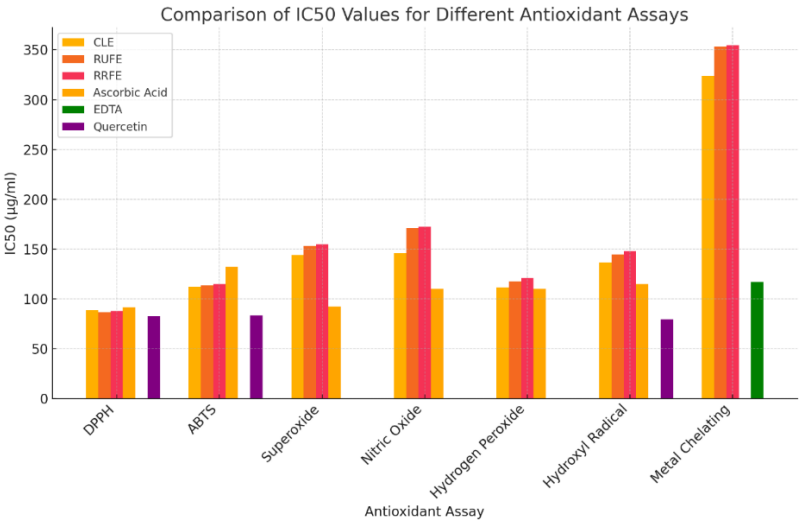

Figure 13. Comparison of IC50 Values for Different Antioxidant Assays

CONCLUSIONS

This study demonstrated that both celery leaves and raspberry fruits, particularly in their unripe form, are valuable sources of bioactive phytochemicals with significant antioxidant properties. Phytochemical screening confirmed the presence of key secondary metabolites such as flavonoids, phenols, alkaloids, and saponins, all of which contribute to the therapeutic potential of these plants. The choice of extraction solvent played a crucial role, with hydroalcoholic extracts yielding the highest concentrations of phenolic and flavonoid compounds. Among the samples tested, the hydroalcoholic extract of unripe raspberry fruit emerged as the richest source of antioxidants, while celery leaf extract exhibited strong radical scavenging activity in multiple assays. Despite the extracts being less potent than standard antioxidants like quercetin and ascorbic acid, their consistent activity across various assays highlights their potential as natural antioxidant agents. The observed reduction in antioxidant capacity with fruit ripening suggests that the timing of harvest can influence bioactivity. These findings provide a strong foundation for the future development of plant-based antioxidant supplements. Further research should focus on isolating specific active compounds, optimizing extraction techniques, and conducting in vivo studies to confirm therapeutic efficacy in oxidative stress-linked conditions, thereby enhancing their application in pharmaceuticals, nutraceuticals, and cosmeceuticals.

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