Comparative nutritional and phytochemical profiles of some exotic and local berries for health boosting effects

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Abstract

This study investigated the comparative nutritional and phytochemical profiles of exotic berry species like strawberries, blueberry, and local ones such as *phalsa*, and *rasbhari*. The study included qualitative and quantitative analyses of sugars, proteins, vitamin C, phytochemicals, and protein profiling using SDS-PAGE. All four berries exhibited a rich array of nutrients, including significant levels of sugars, proteins, and vitamin C. Phytochemical analysis revealed the presence of flavonoids, terpenoids, glycosides, and saponins in all the berries. Strawberry and phalsa demonstrated high levels of vitamin C (0.102 mg mL and 0.208 mg mL), while phalsa exhibited the highest protein content (6 mg mL). All berries exhibited strong antioxidant activity. Protein profiling using SDS-PAGE revealed distinct protein band patterns for each species, suggesting unique protein compositions. These findings suggest that these berries possess valuable nutritional and functional properties, and their incorporation into a balanced diet may contribute to improved human health. Further research is warranted to investigate the bioavailability and potential health benefits of the identified bioactive compounds in humans.

Keywords: exotic fruits, traditional fruits, total phenolic content, phytochemistry, diet and well-being.

Perfis nutricionais e fitoquímicos comparativos de algumas frutas exóticas e locais para efeitos de melhoria da saúde

Resumo

Este estudo investigou os perfis nutricionais e fitoquímicos comparativos de espécies exóticas de frutas vermelhas, como morango, mirtilo e espécies locais, como *phalsa* e *rasbhari*. O estudo incluiu análises qualitativas e quantitativas de açúcares, proteínas, vitamina C, fitoquímicos e perfil de proteínas usando SDS-PAGE. Todas as quatro frutas vermelhas exibiram uma rica variedade de nutrientes, incluindo níveis significativos de açúcares, proteínas e vitamina C. A análise fitoquímica revelou a presença de flavonoides, terpenoides, glicosídeos e saponinas em todas as frutas vermelhas. Morango e phalsa demonstraram altos níveis de vitamina C (0,102 mg mL e 0,208 mg mL), enquanto phalsa exibiu o maior teor de proteína (6 mg mL). Todas as frutas vermelhas exibiram forte atividade antioxidante. O perfil de proteínas usando SDS-PAGE revelou padrões distintos de bandas de proteínas para cada espécie, sugerindo composições proteicas únicas. Essas descobertas sugerem que essas frutas vermelhas possuem propriedades nutricionais e funcionais valiosas, e sua incorporação em uma dieta balanceada pode contribuir para melhorar a saúde humana. Mais pesquisas são necessárias para investigar a biodisponibilidade e os potenciais benefícios à saúde dos compostos bioativos identificados em humanos.

Palavras-chave: frutas exóticas, frutas tradicionais, conteúdos de fenólicos totais, fitoquímica, dieta e bem estar.

1. Introduction

Berries, renowned for their vibrant colors and delectable flavor, have been extensively studied for their nutritional and antioxidant properties. While exotic berries such as strawberries (*Fragaria ananassa*), and blueberries (*Vaccinium corymbosum*) have garnered significant attention, the comparative nutritional composition and antioxidant potential of local berries like phalsa (*Grewia asiatica*), and rasbhari (*Physalis peruviana*) remain under-investigated. Cellular oxidative stress is primarily triggered through reactive oxygen species (ROS) that are generated due to metabolic processes (Arifandi et al., 2022; Oliveiro et al., 2022).

Plant-based antioxidants, also known as phytoantioxidants, are essential for managing and preventing an array of disorders, including metabolic syndrome, caused by oxidative stress (Dhiman et al., 2023). Through their ability to neutralize the adverse effects of reactive oxygen species (ROS) and maintain the body's redox equilibrium, antioxidants are essential in combating against oxidative stress. An imbalance between pro-oxidants and antioxidants induces oxidative stress, which is linked to a variety of chronic illnesses, including cancer, diabetes, cardiovascular disease, and neurological disorders (Yu-Jie et al., 2015).

Antioxidants might assist with the medical management of COVID-19 patients by potentially reducing the oxidative damage produced by reactive oxygen species, which are more prevalent in these individuals (Nile; Park, 2014; Diaconeasa et al., 2015; Gümdeşli et al., 2019; Golovinskaia et al., 2021). While there is a growing body of research on the health benefits of berries, comparative studies focusing on the phytochemical and nutritional profiles of native and exotic varieties, particularly in India, are still relatively scarce. These berries are characterized by a high concentration of antioxidants, including flavonoids and phenolic compounds (Häkkinen; Törrönen, 2000; Huang et al., 2012; Aaby et al., 2012; Koley et al., 2019).

These antioxidants effectively scavenge free radicals, thereby reducing the risk of various chronic health conditions. They are rich in dietary fiber, a component that promotes digestive health, improves satiety, and contributes to overall gut well-being (Drewnowski; Fulgoni, 2008; Pretorius et al., 2021). These berries serve as excellent dietary sources of vitamin C, a micronutrient vital for collagen production, immune system maintenance, and iron bioavailability (Kalt et al., 2020; Kishimoto et al., 2023). Berries typically exhibit a low caloric density, making them a nutritious and satiating snack choice.

This study seeks to address the existing knowledge gap by conducting a comprehensive comparison of sugar, protein, vitamin C, and phytochemical antioxidant parameters in strawberries, blueberries, phalsa, and rasbhari. By elucidating the differences between these berries, we aim to provide valuable insights for consumers, agricultural practices, and the development of nutrient-rich food products. The findings of this research could contribute to dietary recommendations, promoting the consumption of locally produced fruits with high nutritional value. Furthermore, the findings may contribute to agricultural practices designed to enhance the yield and quality of berries. Moreover, the food industry may leverage these findings to create novel products that capitalize on the health benefits of these fruits. This study aimed to assess the comparative nutritional and phytochemical profiles of extracts from exotic and local fruits for their effects on the human diet.

2. Materials and Methods

2.1 Sample extraction

The extraction method of Xu & Chang (2007) served as the basis for the present study, with minor adjustments [17]. Fresh samples of the fruits (strawberry, blueberry, phalsa, and rasbhari) were procured from a local market in Indore (Figure 1). Following a thorough rinse with deionized water, the fruits were allowed to air dry at ambient temperature. Fruit samples (40 g) were homogenized using a pestle and mortar, then diluted with approximately 40 mL of distilled water. The homogenate was incubated at room temperature for 30 min, subsequently filtered, and centrifuged at 5000 rpm for 10 min. The resulting supernatant, designated as the crude extract, was stored at 4 °C for subsequent experimental analyses. The materials, equipment, and methodologies used to develop the study must be systematically described. These aspects must be presented in such a way that other researchers who consult the article can reproduce it based solely on what was described in the article.

Figure 1. Berries: Strawberry, Blueberry, Phalsa, and Rasbhari, respectively. Source: Authors, 2024.

3.2 Qualitative analysis

The primary objective of this study was to characterize the principal nutrient composition of the berries. To achieve this, crude extracts of the samples were subjected to qualitative chemical tests. Labeled test tubes were prepared for each nutrient, and 1 mL of the crude extract was added to each tube. Subsequently, 1 mL of Biuret reagent (0.5% $CuSO_4$ and 10% NaOH) was added for protein detection, 1 mL of pre-heated and cooled Benedict's reagent for reducing sugars, 0.5 mL of concentrated HCl followed by 0.5 mL of 10% NaOH for anthocyanin determination, and 0.5 mL of concentrated H_2SO_4 for anthocyanin detection. The tubes were then observed for any color changes indicative of the presence of the respective nutrients.

3.2.1 Thin layer chromatography (TLC)

Thin-layer chromatography (TLC) is a valuable technique for separating and identifying various compounds. It utilizes a TLC plate consisting of a thin layer of silica gel deposited on a glass or aluminum support, which acts as the stationary phase. The mobile phase, a solvent mixture, migrates through the stationary phase carrying the sample along. The separation of compounds is based on their differing affinities for the two phases. TLC slides coated with silica gel G were used. The phytochemical analysis involved spotting the extract (5 drops) onto the TLC slides and developing them with a chloroform: methanol (15:1) solvent system. The R*f* values (retardation factors) of the observed spots were then measured. A separate TLC plate with a butanol: acetic acid: water (4:1:5) mobile phase was employed for anthocyanin separation. Visualization of phytochemicals was achieved under a UV lamp, while anthocyanin pigments were identified directly by the naked eye due to their characteristic colors.

3.2.2 Biochemical tests for different phytochemicals

The standard qualitative methods for identification of alkaloids, polyphenols, saponins, glycosides, flavonoids and terpenoids were done for strawberry extract based on color development (Patel et al., 2023).

a) Flavonoids

Flavonoids produce yellow color in alkaline conditions, thus 1ml of the extracts were taken in separate tubes and 1ml of 40% NaOH solution was added in the tubes and the color is observed.

b) Polyphenols

A general confirmatory test used for phenols is that when a reagent containing ferric ion is added to a phenolic solution, the ferric ion is reduced to ferrous ions, resulting in a change of color to a deep blue colored complex when heated. The extract was taken in separate tubes and an equal amount of 5% ferric chloride solution was added to both tubes. The blue coloration is taken as a positive test.

c) Terpenoids and Steroids - Salkowski test

This is performed for terpenoids, where 1 mL of extract was taken in tubes and mixed with 0.5 mL of chloroform and a few drops of concentrated sulfuric acid were added. The result is positive when a reddish brown color is obtained at the interface of the liquid. The sulfuric acid dehydrates the terpenoids in the solution.

d) Glycosides

1ml of extract was taken in a tube containing 2 mL glacial acetic acid and 1 mL 5% $FeCl_3$. Heat carefully, then cool, and then transfer it to a tube containing 2 mL conc. H_2SO_4.The result is positive when a reddish brown ring is obtained at the interface.

e) Alkaloids - Wagner’s test

1ml of extract was treated with a few drops of Wagner's reagent (2 g I_2 and 6 g KI in 100 mL water). Formation of brown precipitate indicates presence of alkaloids.

f) Saponins

Saponins in nature form foam with water when shaken vigorously. Hence, a couple of ml of extract was taken in separate tubes with 5 mL of distilled water and were shaken vigorously. Foam so formed if it stays for 30 s or more, then only the test is accepted as positive.

3.3 Quantitative analysis

3.3.1 Estimation of reducing sugar

The reducing sugar content of the extract was quantified using the Nelson-Somogyi method (1944) (Nelson, 1944). Briefly, 100 µL of the extract was diluted to 1 mL with distilled water and incubated with 1 mL of alkaline copper reagent in a boiling water bath for 10 min. After cooling to room temperature (25 °C), 1 mL of arsenomolybdate reagent was added, resulting in a blue color development. The solution was further incubated for 10 min at room temperature, and the absorbance was subsequently measured at 670 nm using a colorimeter. For quantification, a standard curve was constructed using a series of glucose solutions ranging from 200 to 1000 $\mu g/mL^{-1}$.

3.3.2 Estimation of total protein

Total protein content in the extract was determined using the method described by Lowry et al. (1951) (Lowry et al., 1951). 1 mL of the extract was diluted to 5 mL with 0.1 M phosphate buffer (pH 7.4). Subsequently, 2 mL of 20% trichloroacetic acid (TCA) was added to the diluted extract and incubated for 30 min and centrifuged. The protein pellet was washed twice with chilled acetone and recovered by centrifugation. The final protein pellet was dissolved in 100 µL of 0.1 N NaOH. An aliquot of 50 µL of the protein solution was diluted to 1 mL with distilled water and mixed with 2.5 mL of alkaline copper sulfate reagent. After a 10-minute incubation, 0.25 mL of diluted (1:2) *Folin's* reagent was added, and the reaction mixture was allowed to stand at room temperature for 15 min. For quantification, a standard curve was constructed using a series of bovine serum albumin (BSA) solutions ranging from 20 to 100 $\mu g/mL^{-1}$.

3.3.3 Estimation of anthocyanin

Anthocyanin content in the extract was determined using a pH differential method described by Lako et al. (2007). Briefly, 3.3 g of phalsa was extracted with 2 ml of ethanol. The sample was then centrifuged at 5000 rpm for 5 minutes, and the supernatant was collected for anthocyanin analysis. The total anthocyanin content was estimated using two buffer solutions: 0.4 M potassium chloride buffer (pH 4.5) and 0.025 M potassium acetate buffer (pH 1.0). Aliquots of 0.4 ml extract solution were mixed with 3.6 mL of each respective buffer. The absorbance of each solution was measured at 510 nm and 700 nm using a colorimeter against a blank containing the corresponding buffer only. The net absorbance and anthocyanin content were calculated using the following formula:

$$A = (A_{510\ nm, pH1} - A_{700\ nm, pH1}) - (A_{510\ nm, pH4.5} - A_{700\ nm, pH4.5})$$

$$\text{Anthocyanin content (mg/mL)} = A \times MW \times DF \times 1000 / (\varepsilon \times L)$$

Where: A = Absorbance; MW = molecular weight of cyaniding-*3*-glucoside (449.2 g/mol); Dilution factor = 10; ε = Molar extinction coefficient (26,900 L/cm mol); L = path length = 1 cm

3.3.4 Detection and estimation of vitamin C

3.3.4.1 Sample Preparation

Vitamin C content in fruit juice was analyzed following extraction with 3% metaphosphoric acid. A 1.5 mL aliquot of the juice was mixed with 15 ml of 3% metaphosphoric acid solution and centrifuged. The resulting supernatant was collected for subsequent detection and quantification of vitamin C.

3.3.4.2 Vitamin C detection using $FeCl_3$ test

This method leverages the ability of vitamin C (ascorbic acid) to reduce ferric chloride ($FeCl_3$). When $FeCl_3$ is reduced, its characteristic yellow color fades due to the conversion of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). Conversely, vitamin C is oxidized to dehydroascorbic acid during this reaction. The test involved mixing 1 ml of 1% $FeCl_3$ solution with 1 ml of the extract. A color change was then observed to determine the presence of vitamin C. As a positive control, a 1% ascorbic acid solution was used as the standard in this test.

3.3.4.3 Vitamin C detection using methylene blue test

Ascorbic acid can reduce methylene blue dye, converting its blue form (oxidized) to the colorless leuco form (reduced). The experiment involved mixing 1 mL of 0.5% methylene blue solution with 1 mL of the extract. A color change, from blue to colorless, would indicate the presence of vitamin C in the extract. As a positive control for this test, 1% ascorbic acid solution served as the standard.

3.3.4.4 Vitamin C estimation using DCPIP method

Using a standard solution of 1 mg/mL ascorbic acid diluted in 3% metaphosphoric acid, the concentration of vitamin C in the extract was estimated. Ascorbic acid standards in the range of 20–100 µg/mL were developed and each standard was mixed with 3 mL of 0.2 mM DCPIP. At 520 nm, the absorbance of the resultant solutions was measured right away.

3 mL of DCPIP and 100 µL of the extract were used to react for the test sample. The findings were presented as ascorbic acid milligrams per milliliter of extract.

3.3.5 Estimation of total phenolic content (TPC)

Total polyphenol content in the extract was quantified using the *Folin-Ciocalteu* method with gallic acid (GA) as the standard (Patel et al., 2024). A calibration curve was constructed using a series of GA standard solutions ranging from 25 to 125 µg/mL. The *Folin-Ciocalteu* reagent reacts with phenolic compounds under alkaline conditions, resulting in the reduction of tungstate and molybdate ions to their blue-colored counterparts. The absorbance of this blue color was measured at 750 nm. For the analysis, 100 µL of the extract was mixed with 0.5 mL of deionized water (DW) followed by the addition of 5 mL of *Folin-Ciocalteu* reagent. The mixture was then incubated under alkaline conditions by adding 4 mL of sodium carbonate solution. All measurements were performed in duplicate.

3.3.6 Estimation of total flavonoid content (TFC)

A calibration curve was first prepared using a series of quercetin standard solutions ranging from 100 to 500 µg/mL. The experiment leveraged the complexation of aluminum chloride ($AlCl_3$) with flavonoid compounds, which alters their light absorption properties (Patel et al., 2024). For the analysis, a 100 µL aliquot of the extract was mixed with 0.1 mL of potassium acetate solution and 0.1 mL of 10% $AlCl_3$ solution in a test tube. The reaction mixture was then diluted to a final volume of 3.2 ml with distilled water. To ensure consistent complexation, the tubes were incubated for 30 min at 37 °C. Following incubation, the absorbance of the solutions was measured at 415 nm using a spectrophotometer.

3.3.7 Estimation of total antioxidant capacity using phospho-molybdate assay (TAC)

This method relies on the ability of reducing agents in the extract to convert Mo(VI) (molybdenum in its hexavalent state) to Mo(V) (molybdenum in its pentavalent state) under acidic conditions (Patel et al., 2024). This reduction is accompanied by a color change of the solution from colorless to blue-green. The TAC value is then quantified by comparing the sample's reducing capacity to a standard curve generated with known concentrations of ascorbic acid. The assay involved mixing 100 µL of the extract with 1 ml of deionized water (DW) and 2 mL of phosphomolybdate reagent. The reaction mixture was then incubated at 95 °C for 15 min to facilitate the reduction process. Following incubation, the absorbance of the solution was measured at 765 nm using a spectrophotometer. The obtained absorbance value from the extract was compared to the calibration curve established with the ascorbic acid standards, allowing for the calculation of the TAC of the extract expressed as ascorbic acid equivalents per milligram of extract (mg AAE/mg $extract^{-1}$).

3.4 Protein profiling using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The protocol adheres to the method established by Laemmli (1970). SDS-PAGE separates proteins based on their molecular weight and electrophoretic mobility. The technique utilizes a polyacrylamide gel matrix to create a sieving effect, allowing smaller proteins to migrate faster through the gel pores compared to larger ones. Separating Gel (13%) is prepared by combining water (3.35 mL), acrylamide mix 30% (4 mL), Tris-HCl buffer (0.5 M, pH 8.8) (2.5 mL), SDS 10% (100 µL), APS 10% *w/v* (50 µL), and TEMED (5 µL). Pour the mixture into the casting unit, and allow polymerization for at least 45 min at room temperature. Prepare the stacking gel (4%) solution by combining water (3 mL), acrylamide solution (670 µL), stacking gel buffer (0.5 M Tris-HCl, pH 6.8) (1.25 mL), SDS 10% (50 µL), APS 10% *w/v* (25 µL), and TEMED (5 µL). Place the comb between the gel plates, and pour the stacking gel solution on top of the polymerized separating gel, and allow polymerization. Prepare the samples for loading by mixing them with gel loading dye in a 1:1 ratio. Heat the mixture in a water bath for 5-10 min. Load protein samples (20 µL) into the wells of the gel. Load the protein marker into a designated well and note the sequence of proteins. Fill both the upper and lower chambers of the running unit with 1X Tris-glycine buffer. Set the power supply parameters: constant voltage 100 V, maximum current 200 mA. Following electrophoresis, dismantle the casting unit and carefully transfer the gel to a staining tray. Stain the gel with Coomassie Brilliant Blue R-250 for 1 h. Destain the gel using a solution of 30% methanol and 10% acetic acid until protein bands become clearly visible.

3. Results

3.1 Sample Extraction

Table 1 provides the exact volume of the derived crude extracts (Figure 2). The difference in volume of extraction obtained may be due to the variation in water, fiber content, and other components in fruits.

Table 1. Yield and color of crude extracts

Extract	Yield	Colour	Percentage Yield
Strawberry	10 mL	Pink	50%
Blueberry	24 mL	Brown	60%
Phalsa	36 mL	Turbid white	90%
Rasbhari	18 mL	White	45%

Source: Authors, 2024.

Figure 2. Crude extracts of strawberry, blueberry, phalsa and rasbhari respectively. Source: Authors, 2024.

3.2 Qualitative analysis

The research on the berry extract demonstrated several key findings through various biochemical tests. Benedict's test revealed the presence of reducing sugars, evidenced by a yellow-orange precipitate. Additionally, the Biuret test revealed a blue-violet hue indicating the presence of protein. Vitamin C content was confirmed by the disappearance of the yellow color in reactions with $FeCl_3$ and methylene blue. Anthocyanins were identified through a red coloration with HCl that shifted to blue-green upon the addition of NaOH, as well as an orange coloration with concentrated H_2SO_4. These findings are summarized in (Table 2).

Table 2. Nutrient analysis in crude extracts.

Test	Sample	Color	Presence
Benedict's Test	Strawberry	Orange precipitate	Reducing sugar
	Blueberry	Yellow precipitate	Reducing sugar
	Phalsa	Yellowish-orange precipitate	Reducing sugar
	Rasbhari	Yellowish-orange precipitate	Reducing sugar
Biuret Test	Strawberry	Blue-violet	Protein
	Blueberry	Blue-violet	Protein
	Phalsa	Blue-violet	Protein
	Rasbhari	Blue-violet	Protein
$FeCl_3$ Test	Strawberry	Disappearance of yellow color of $FeCl_3$	Vitamin C
	Blueberry	Disappearance of yellow color of $FeCl_3$	Vitamin C
	Phalsa	Disappearance of yellow color of $FeCl_3$	Vitamin C
	Rasbhari	Disappearance of yellow color of $FeCl_3$	Vitamin C
Acid-Alkali Test	Strawberry	Pinkish red with HCl and followed by blue-green color with NaOH	Anthocyanin
		Orange color with con H_2SO_4	Anthocyanin
	Blueberry	Red with HCl and followed by blue-green color with NaOH	Anthocyanin
		Orange color with con H_2SO_4	
		Red with HCl and followed by blue-green color with NaOH	Anthocyanin
	Phalsa	Orange color with con H_2SO_4	Anthocyanin
		Orange color with con H_2SO_4	
			Anthocyanin
	Rasbhari		Anthocyanin

Source: Authors, 2024.

4.3 Biochemical tests for phytochemicals

Several phytochemicals, including flavonoids, terpenoids, glycosides, and steroids, have been identified in the berries extract according to the phytochemical analysis, as displayed in (Table 3). Flavonoids are among the dominant phytoconstituents in berries that are renowned for their wide range of biological activities, especially their anti-inflammatory and antioxidant properties.

Table 3. Phytochemical screening.

Compound	Strawberry	Blueberry	Phalsa	Rasbhari
Flavonoids	+	+	+	+
Polyphenols	-	-	-	-
Tannins	-	-	-	-
Saponins	+	-	+	+
Terpenoids	+	+	+	+
Steroids	+	+	+	+
Glycosides	+	+	+	+
Alkaloids	-	-	-	-

Note: (+) positive test. (-) negative test. Source: Authors, 2024.

4.4 TLC separation

TLC profiling of berries provided information regarding the existence of different colored pigments and phytochemicals. Under visible light, colored patches representing anthocyanins were seen, as shown in Figure 3 A. Table 4 provides a breakdown of each anthocyanin with R*f* value. Furthermore, spots seen at short UV wavelengths (Figure 3B) suggested the presence of phytochemicals essential to this fruit's antioxidant action.

Table 4. Thin layer chromatography separation.

Sample	Chemical group	Mode of observation	No. of spots	Colour and distance travelled (cm)	Distance by mobile phase (cm)	R_f
Strawberry	Anthocyanin (Mobile phase: Butanol:acetic acid: water)	Visible light	1	Pinkish red (3.2)	3.5	0.914
	Phytochemical (Mobile phase: $CHCl_3$: CH_3OH)	Uv light	4	Orange (2.5) Orange (3.0) Blue (3.5) Yellow(2.7)	4.0	0.625 0.75 0.875 0.675
Blueberry	Anthocyanin (Mobile phase: Butanol:acetic acid: water)	Visible light	3	Brown (1.8) Purple (2.1) Yellow (3.2)	3.5	0.51 0.6 0.91
	Phytochemical (Mobile phase: $CHCl_3$: CH_3OH)	Uv light	2	Orange (0.6) Orange (2.0)	3.9	0.15 0.51

Phalsa	Anthocyanin (Mobile phase: Butanol:acetic acid: water)	Visible light	2	Pink (2.2)	3.0	0.73
				Purple (2.5)		0.83
	Phytochemical (Mobile phase: $CHCl_3$: CH_3OH)	Uv light	2	Orange (0.3)	2.0	0.15
				Orange (0.6)		0.30
Rasbhari	Anthocyanin (Mobile phase: Butanol:acetic acid: water)	Visible light	2	Yellow (2.5)	2.5	1.0
				Yellow (2.2)		0.88
	Phytochemical (Mobile phase: $CHCl_3$: CH_3OH)	Uv light	2	Blue (0.9)	4.0	0.225
				Blue (3.0)		0.75

Source: Authors, 2024.

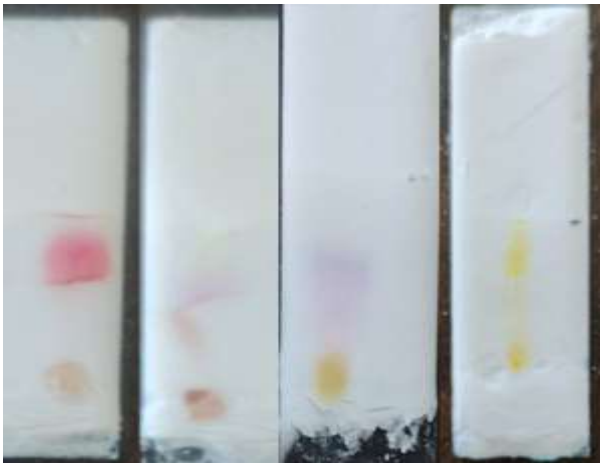

Figure 3A. Thin layer chromatography profile of extracts for anthocyanin under visible light for Strawberry, Blueberry, Phalsa and Rasbhari, respectively. Source: Authors, 2024.

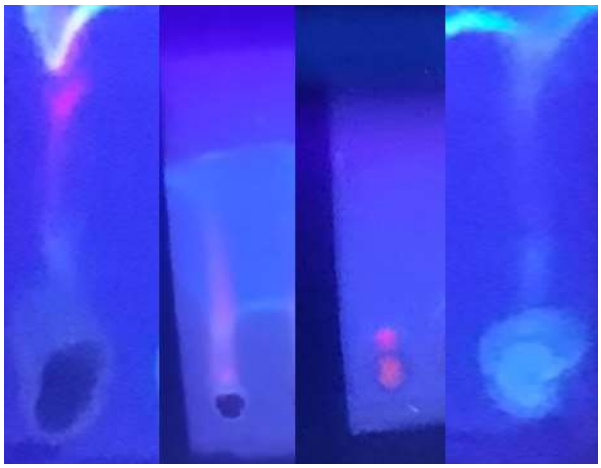

Figure 3B. Thin layer chromatography profile of extracts for phytochemicals under UV light for Strawberry, Blueberry, Phalsa and Rasbhari, respectively. Source: Authors, 2024.

4.5 Quantitative analysis of nutrients

The results demonstrate that the four berries exhibit varying nutrient profiles (Table 5). The nutritional analysis of berries revealed a substantial presence of reducing sugars, proteins, and vitamin C, underscoring their nutritional richness. Strawberry stands out as a rich source of reducing sugars and anthocyanins, while Phalsa is a good source of protein and vitamin C. These findings highlight the nutritional diversity of the berries and their potential health benefits.

Table 5. Nutrient composition of extracts.

Nutrient	Concentration (mg/mL^{-1})			
	Strawberry	Blueberry	Phalsa	Rasbhari
Reducing sugar	109	1.4	1.86	1.22
Total Protein	1.2	0.021	6	4.3
Vitamin C	0.102	0.09	0.208	0.08
Anthocyanin	78.48	43.417	20	5

Source: Authors, 2024.

4.6 Phytochemical estimations

4.6.1 TPC

The total phenolic content (TPC) of the berry extracts was determined using the Folin-Ciocalteu method and expressed as gallic acid equivalents (GAE) per milliliter. The results showed significant variations in TPC among the berries (Figure 4). Strawberry extract exhibited the highest TPC (951.1 µg GAE/mL^{-1}), followed by Phalsa (900 µg GAE/mL^{-1}), Blueberry (647.9 µg GAE/mL^{-1}), and Rasbhari (316.6 µg GAE/mL^{-1}) (Table 6). These findings suggest that strawberry and Phalsa are rich sources of phenolic compounds.

Table 6. Total phenolic compounds, total flavonoid content, and total antioxidant capacity of extracts.

Sample	Total Phenolics (µg GAE/mL^{-1})	Total Flavonoids (µg QCE/mL^{-1})	TAC (µg AAE/mL^{-1})
Strawberry	951.1	2800	422.2
Blueberry	647.9	2620	533.8
Phalsa	900	771.42	1042
Rasbhari	316.6	1800	208

Source: Authors, 2024.

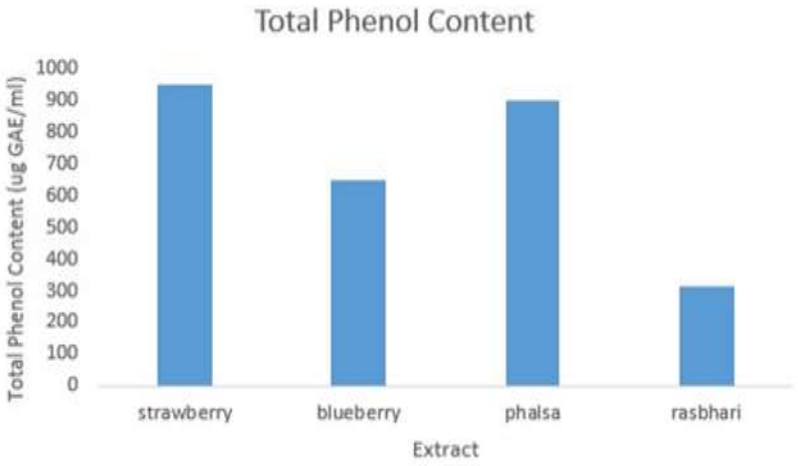

Figure 4. Total phenolic compounds of extracts. Source: Authors, 2024.

4.6.2 Total flavonoid content

The total flavonoid content (TFC) was measured using the aluminum chloride colorimetric method and expressed as quercetin equivalents (QCE) per milliliter. Strawberry extract demonstrated the highest TFC (2800 µg QCE/mL^{-1}), followed by Blueberry (2620 µg QCE/mL^{-1}), Rasbhari (1800 µg QCE/mL^{-1}), and Phalsa (771.42 µg QCE/mL^{-1}) (Table 6). These results indicate that strawberry and blueberry are abundant sources of flavonoids (Figure 5).

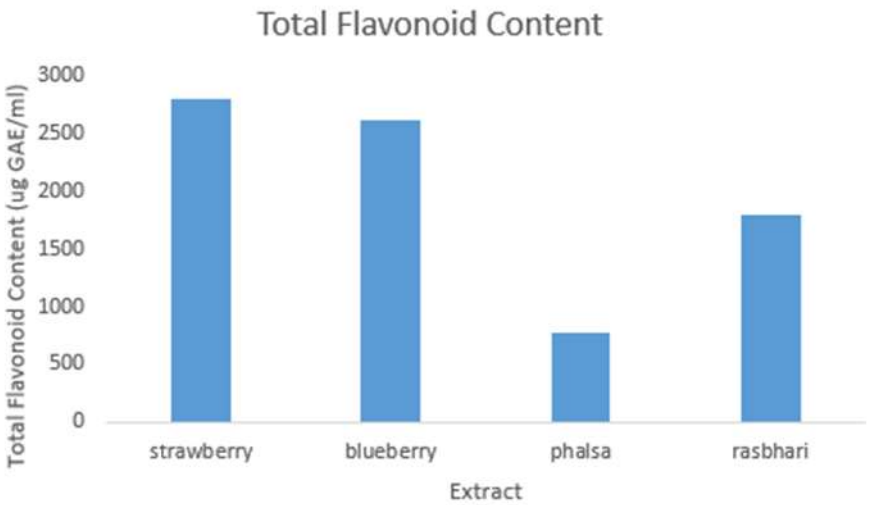

Figure 5. Total flavonoid content of extracts. Source: Authors, 2024.

4.6.3 Total antioxidant capacity

The antioxidant activity of the berry extracts was evaluated using the DPPH radical scavenging assay and expressed as ascorbic acid equivalents (AAE) per milliliter. Phalsa extract exhibited the highest antioxidant activity (1042 µg AAE/mL^{-1}), followed by Blueberry (533.8 µg AAE/mL^{-1}), Strawberry (422.2 µg AAE/mL^{-1}), and Rasbhari (208 µg AAE/mL^{-1}) (Table 6). These findings suggest that Phalsa possesses the strongest antioxidant capacity among the tested berries (Figure 6).

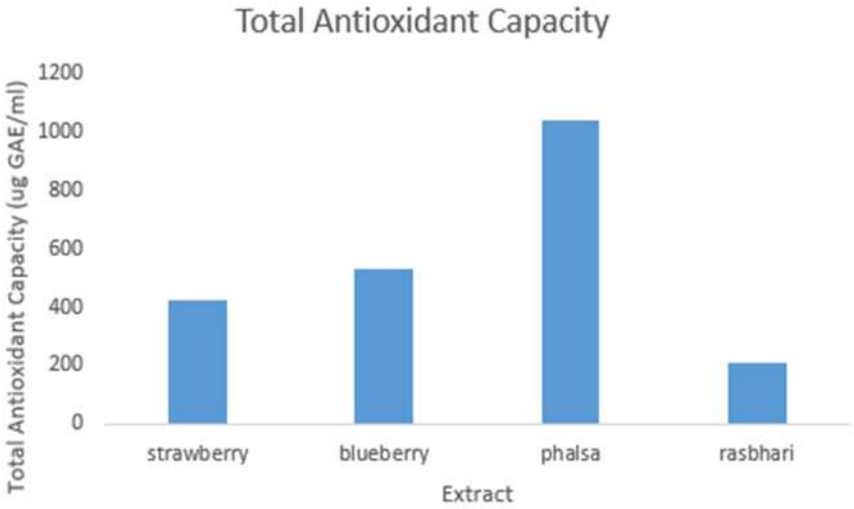

Figure 6. Total antioxidant capacity of extracts. Source: Authors, 2024.

4.7 Protein Profiling by SDS-PAGE

Protein profiling of the four berry species was conducted using SDS-PAGE (Figure 7). The results revealed distinct protein band patterns for each species, suggesting unique protein compositions.

•Blueberry: A single prominent protein band was observed in the range of 35-48 kDa.

•Strawberry: Three distinct protein bands were observed in the following approximate molecular weight ranges: 35-48 kDa, 25-35 kDa, and 25 kDa.

•Rasbhari: Two protein bands were identified. One band was observed around 40 kDa, while the other was detected in the range of 20-25 kDa.

•Phalsa: A single prominent protein band was observed in the high molecular weight range of 75-100 kDa.

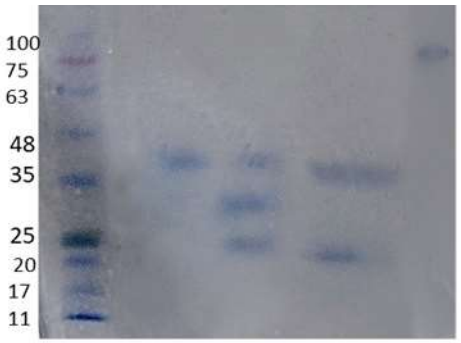

Figure 7. SDS-PAGE of isolated protein from berries with protein ladder. Lane1: Blueberry, Lane 2: Strawberry, Lane 3: Rasbhari, Lane 4: Phalsa. Source: Authors, 2024.

4. Discussion

The study aims to delve into the nutritional and biochemical profiles of four berries: strawberry, blueberry, phalsa, and rasbhari. The results show that all four berries possess a rich array of nutrients, phytochemicals, and have high antioxidant activity, which backs their potential as valuable functional foods (Sanchez-Salcedo et al., 2015).

Strawberry exhibited the lowest sugar content (0.11 g/100 g^{-1}), while phalsa was found to show the highest (1.67 g/100 g^{-1}). Phalsa displayed the highest protein content (5.4 g/100 g^{-1}), followed by rasbhari (3.2 g/100 g^{-1}). Strawberry and blueberry contained relatively lower protein content, 0.9 g/100 g^{-1} and 0.015 g/100 g^{-1}, respectively. Strawberry and phalsa demonstrated substantial vitamin C content (76.5 mg/100 g^{-1} and 34.7 mg/100 g^{-1}, respectively), capable of meeting a significant portion of day-to-day requirements. All four berries exhibited a diverse range of phytochemicals, including flavonoids, terpenoids, glycosides, and saponins. Strawberry and rasbhari demonstrated the highest flavonoid content, substantially exceeding the polyphenol content in both cases. Strawberry and blueberry displayed high anthocyanin levels, which likely contribute significantly to their antioxidant activity (Nakajima et al., 2004). All the berries exhibited strong antioxidant activity as assessed by the phosphomolybdate assay, with phalsa demonstrating the highest activity.

Phalsa emerged as a promising candidate for a functional food due to its high protein, sugar, and vitamin C content, along with its significant antioxidant activity (García-Martínez et al., 2021). Strawberry stood out for its high vitamin C content, and substantial flavonoid levels (Wang; Lewers, 2007). Rasbhari exhibited a high protein content, and significant flavonoid levels, suggesting its potential as a valuable dietary source. Blueberry demonstrated a good balance of nutrients, and a strong antioxidant profile. SDS-PAGE provides preliminary evidence for significant inter-species variations in the protein profiles of the four berry species.

5. Conclusions

This study provides comprehensive data on the nutritional and biochemical profiles of the four berries, highlighting their potential as valuable sources of nutrients and antioxidants. The findings underscore the importance of incorporating these berries into a balanced diet for promoting overall health and well-being. These findings suggest that all four berries have the potential to contribute to improved human health. However, further research is crucial to determine the bioavailability and synergistic effects of the identified bioactive compounds in the human body.

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7. Authors' Contributions

Lakshmi Pillai: project writing, laboratory analyses, article writing. *Rupali Chouhan*: laboratory analyses, article writing and revision. *Rupali Sagre*: laboratory analyses and article writing. *Simran Khanuja*: project writing, laboratory analyses, data evaluation, and article writing. *Rani Kolasiya*: article writing, final revisions. *Vishal Rathore*: final revisions and publication. *Sanjana Patel*: article organization, revisions, and publication. *Rashmi Limaye*: laboratory analyses, data evaluation, article writing, and publication. *Payal Puri*: data analysis, article writing, post-review revisions, and publication.

8. Conflicts of Interest

No conflicts of interest.

9. Ethics Approval

Not applicable.

10. References

Aaby, K., Mazur, S., Nes, A., & Skrede, G. (2012). Phenolic compounds in strawberry (*Fragaria* x *ananassa* Duch.) fruits: Composition in 27 cultivars and changes during ripening. *Food Chemistry*, 132(1), 86-97. https://doi.org/10.1016/j.foodchem.2011.10.037

Arifandi, Y. A., Rosyid, A. N., Indiastuti, D. N., & Effendi, W. I. (2022). The role of antioxidant to counteract oxidative stress in COVID-19 : A literature review. *International Journal of Research Publications*, 115(1). https://doi.org/10.47119/IJRP100111511220224306

Dhiman, N. K., Saini, R., Mehra, H., & Goyal, T. (2023). Dietary antioxidants and their potential role in human disease management. *Current Nutrition & Food Science*, 19(3), 262-281. https://doi.org/10.2174/1573401318666220520151057

Diaconeasa, Z., Ranga, F., Rugină, D., Leopold, L., Pop, O., Vodnar, D., Cuibus, L., & Socaciu, C. (2015). Phenolic content and their antioxidant activity in various berries cultivated in Romania. *Bulletin UASVM Food Science and Technology*, 72(1), 99-103. https://doi.org/10.15835/buasvmcn-fst:11127

Drewnowski, A., & Fulgoni III, V. (2008). Nutrient profiling of foods: creating a nutrient-rich food index. *Nutrition Reviews*, 66(1), 23-39. https://doi.org/10.1111/j.1753-4887.2007.00003.x

Golovinskaia, O., & Wang, C-K. (2021). Review of functional and pharmacological activities of berries. *Molecules*, 26(13), 3904. https://doi.org/10.3390/molecules26133904

Gündeşli, M. A., Korkmaz, N., & Okatan, V. (2019). Polyphenol content and antioxidant capacity of berries: A review. *International Journal of Agriculture Forestry and Life Sciences*, 3(2), 350-361. https://dergipark.org.tr/en/pub/ijafls/issue/47015/649812

Häkkinen, S. H., & Törrönen, A. (2000). Content of flavonols and selected phenolic acids in strawberries and *Vaccinium* species: influence of cultivar, cultivation site and technique. *Food Research International*, 33(6), 517-524. https://doi.org/10.1016/S0963-9969(00)00086-7

Huang, W., Zhang, H., Liu, W., & Li, C. (2012). Survey of antioxidant capacity and phenolic composition of blueberry, blackberry, and strawberry in Nanjing. *Journal of Zhejiang University. Science B*, 13(2), 94-10. https://doi.org/10.1631/jzus.B1100137

Kalt, W., Cassidy, A., Howard, L. R., Krikorian, R., Stull, A. J., Tremblay, F., & Zamora-Ros, R. (2020). Recent research on the health benefits of blueberries and their anthocyanins. Advances in Nutrition, 11(2), 224-236. https://doi.org/10.1093/advances/nmz065

Kishimoto, Y., Taguchi, C., Iwashima, T., Kobayashi, T., Kikoku, Y., Nishiyama, H., Masuda, Y., & Kondo, K. (2023). Effects of acute strawberry consumption on serum levels of vitamin C and folic acid, the antioxidant potential of LDL and blood glucose response: a randomised cross-over controlled trial. *Journal of Nutritional Science*, 12, e39. https://doi.org/10.1017/jns.2022.117

Koley, T. K., Khan, Z., Oulkar, D., Singh, B., Bhatt, B. P., & Banerjee, K. (2019b). Profiling of polyphenols in phalsa (*Grewia asiatica* L) fruits based on liquid chromatography high resolution mass spectrometry. Journal of Food Science and Technology, 57(2), 606-616. https://doi.org/10.1007/s13197-019-04092-y

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680-685. https://doi.org/10.1038/227680a0

Lako, J., Trenerry, V. C., Wahlqvist, M., Wattanapenpaiboon, N., Sotheeswaran, S., & Premier, R. (2007). Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chemistry*, 101(4), 1727-1741. https://doi.org/10.1016/j.foodchem.2006.01.031

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry*, 193(1), 265-275.

Nelson, N. (1944). A photometric adaptation of the somogyi method for the determination of glucose. *Journal of Biological Chemistry*, 153, 375-380.

Nile, S. H., & Park, S. W. (2014). Edible berries: Bioactive components and their effect on human health. Nutrition, 30(2), 134-144.

Oliverio, M., Bulotta, S., & Duarte, N. (2022). Editorial: Nature inspired protective agents against oxidative stress. *Frontiers in Pharmacology*, 13. https://doi.org/10.3389/fphar.2022.859549

Patel, S., Sharma, P., Supat, M., Mali, S., Bafna, A., Rangwala, T., Limaye, R., & Puri, P. (2024). Investigation of antioxidant potential of black beans due to phytochemical and globulin content for nutraceutical application. *Defence Life Science Journal*, 9(4), 326-334. https://doi.org/10.14429/dlsj.9.19920.

Patel, S., Dey, R., Verma, K., Deshbhratar, R., Maru, K. K., Sharma, P., Limaye, R., & Puri, P. (2023). Comparative analysis of antioxidant and anti-inflammatory activities of red, blue, and black tea for health benefits. *Brazilian Journal of Science*, 2(4), 76-89. https://doi.org/10.14295/bjs.v2i4.299

Pretorius, B., Ambuko, J., Papargyropoulou, E., & Schönfeldt, H. C. (2021). Guiding nutritious food choices and diets along food systems. *Sustainability*, 13(17), 9501. https://doi.org/10.3390/su13179501

Xu, B. J., & Chang, S. K. (2007). A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *Journal of Food Science*, 72(2), S159-S166. https://doi.org/10.1111/j.1750-3841.2006.00260.x

Zhang, Y-J., Gan, R-Y., Li, S., Zhou, Y., Li, A-N., Xu, D-P., & Li, H-B. (2015). Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Molecules*, 20(12), 21138-21156. https://doi.org/10.3390/molecules201219753

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