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Research Article

Environmental influence on phytochemical composition of Brussels sprouts (*Brassica oleracea* var. *gemmifera*)

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This study aimed to assess the phytochemical profile and quantify the total phenolic and flavonoid content of Brussels sprouts under two environmental conditions. The phytochemical screening of methanol, aqueous and hydro alcohol extracts of different Brussels sprouts genotypes under open and protected environments was conducted as per standard methods. The total phenolic content was measured by the Folin–Ciocalteu assay and total flavonoid content was measured using the Aluminum chloride method. Phytochemical screening was performed to confirm the occurrence of essential bioactive compounds and results revealed significant variations in phytochemical compositions, phenolic and flavonoid content between the two environments, indicating the influence of environmental factors on secondary metabolites accumulation. Genotype Hild's Ideal under open environment had the highest phenolic content (26.32 mg GAE/g) with methanol extract, while genotype Urja had the lowest value (6.87 mg GAE/g) with aqueous extracts. The highest total flavonoid content was recorded in genotype Hild's Ideal (13.92 mg QE/g) using methanol extracts under a protected environment; whereas, the lowest flavonoid content 4.29 mg QE/g was observed in genotype Urja with aqueous extracts under an open environment. Methanol was identified as the most effective solvent for the extraction of the phenolic and flavonoid compounds. The presence of bioactive compounds suggests potential health benefits, supporting the use of Brussels sprouts as an important medicinal and functional vegetable.

Keywords: *Brussels sprouts, phytochemical screening, environment, phenolic, flavonoid content*

Introduction

Brassica oleracea var. *gemmifera*, commonly known as Brussels sprouts, is a cold-season vegetable cultivated for its edible green "buds or sprouts," which resemble mini-cabbages. This hardy and slow-growing plant belongs to the genus *Brassica* and the family Brassicaceae sharing a close phylogenetic relationship with other crops like cauliflower, broccoli, kale and collards. These crops are widely recognised for their high nutritional value and versatility in culinary applications. In Brussels sprouts the buds form in the leaf axils, the angles between the leaves and the stem, with the development initiating at the base of the stem and progressing upward. Each sprout/bud develops in the axil of a leaf, resulting in the characteristic arrangement of multiple small, round buds along the stem (Tewari et al., 2020). *Brassica* species are a significant source of essential minerals, which plays a critical role in maintaining vital physicochemical processes necessary for life. These inorganic compounds are present in all body fluids and tissues (Ayadi et al., 2022). In recent years, these vegetables have received a lot of attention for their pivotal role in human health. Epidemiological research suggests that high consumption of plant-based foods is correlated with reduced risk of chronic diseases, including atherosclerosis and cancer (Zhang et al., 2025). The health promoting effects are partly attributed to the antioxidant components found in plant. Key antioxidants present in vegetables include vitamins C, E and phenolic compounds, particularly flavonoids. These compounds help to neutralize free radicals and either prevent the initiation of oxidative reactions or disrupt their progress (Zhang et al., 2025). Medicinal plant serves as abundant natural reservoirs of bioactive compounds, playing a crucial role in contemporary pharmaceutical research and therapeutic applications

(Saifulazmi et al., 2022). Medicinal plants comprise a wide range of organic compounds that influence physiological processes in the body. The bioactive molecules include tannins, alkaloids, carbohydrates, flavonoids, steroids and other phytochemicals (Fathimath & Laveena, 2023). These compounds are produced through primary metabolism or more precisely, secondary metabolic processes in living organisms. These secondary metabolites exhibit remarkable chemical and taxonomical diversity with functions that remain largely enigmatic. However, they play a pivotal role in various applications, including human medicine, veterinary science, agriculture and scientific research, among numerous other fields. A diverse spectrum of phytochemicals, encompassing multiple chemical classes, has demonstrated inhibitory activity against various microorganisms *in vitro* (Zimowska, 2021). Brussels sprouts are valuable for phytochemical screening due to their abundant bioactive compounds, including glucosinolates, flavonoids, phenolic acids as well as carotenoids. Phytochemical screening of Brussels sprouts can aid in identifying novel compounds with therapeutic potential, contributing to drug discovery, functional food development and nutraceutical applications. The present study assessed the effect of environmental factors on the phytochemical composition, phenolic as well as flavonoid content, in four genotypes of Brussels sprouts.

Materials and Methods

Plant material

The different Brussels sprouts genotypes Hild's Ideal, Long Island Improved, Urja and Franklin F_1 were grown at the Agricultural Research Farm of the Krishi Vigyan Kendra, Kangra, District Kangra Himachal Pradesh, India (Figure 1).



Figure 1. Brussels sprouts at the experimental site under an open and protected environment

Preparation of crude extracts

Fresh Brussels sprouts were washed under running tap water and subsequently shade dried at room temperature (26 ± 2°C) for 4 to 5 weeks. The dried samples were then finely ground and stored in airtight containers for further investigation. 5 grams of dried Brussels sprouts powder was used for extraction was carried out using aqueous, methanol as well as hydro alcohol solvents in a 1:10 (weight/volume) ratio, with continuous agitation on an orbital

shaker for 48 hours. The extracts were then filtered at room temperature using Whatman's No. 1 filter paper. The filtrates were evaporated using a rotary evaporator to obtain a semisolid crude extract, which was then transferred into airtight glass vials. All obtained extracts were stored in a refrigerator below 4° C for subsequent experiments (Figure 2).

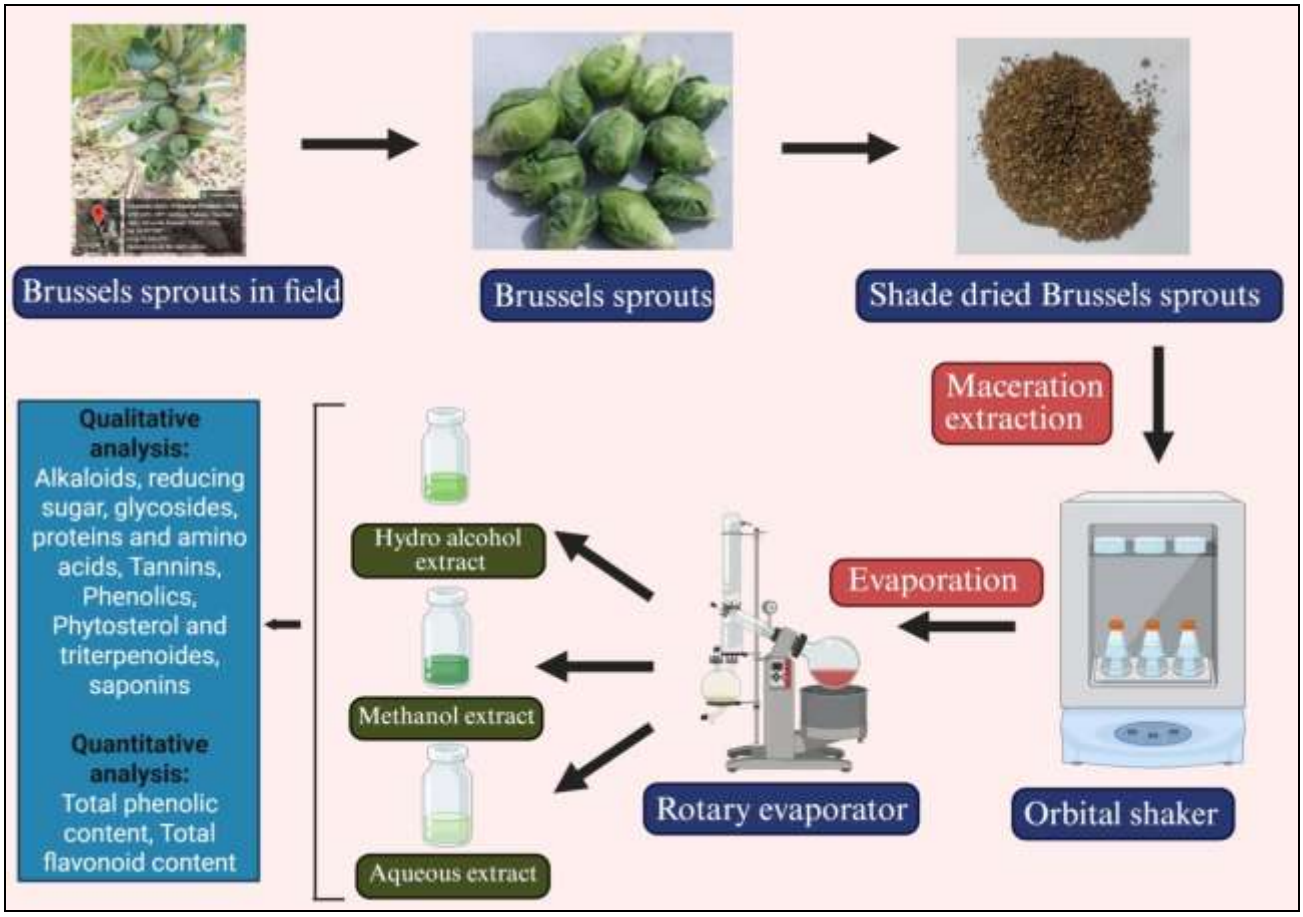


Figure 2. Schematic representation for crude extraction processing and phytochemical analysis of Brussels sprouts

Extraction yield

The extraction yield was determined following the standard method (Zhang et al. 2007). The obtained filtrate was dried using a rotary evaporator and dried extract was accurately weighed. The extraction yield was calculated as a percentage using the following formula:

$$\text{Extraction yield (\%)} = \text{weight of dried extract (g)} / \text{weight of sample (g)} \times 100$$

Determination of phytochemicals

The phytochemical screening of methanol, aqueous and hydro alcohol extracts from different Brussels sprouts genotypes under open and protected environments was conducted to detect various phytoconstituents using protocols with slight modifications (Table 1. (Raaman, 2006; Sadasivam & Manickam, 2023))

Table 1. Specific phytochemical test and procedure for various phytoconstituents

Test	Procedure	Observations
Alkaloids		
Mayer's test	1 mL plant extract + 2 to 3 drops of Mayer's reagent	Creamy white precipitates
Wagner's test	1 mL plant extract + 3 to 4 drops of Wagner's reagent	Brown/reddish precipitates
Flavonoids		
Alkaline reagent test	1 mL plant extract + 1 mL of 2% NaOH + 4-5 drops of dilute HCl	Yellow colour changed to colourless after adding dilute HCl

Shinoda's test	2 mL plant extract +5 mL alcohol + magnesium ribbon fragments + 2 to 3 drops of conc. HCl	Pink colour appear
Phenolics		
Ferric chloride test	2 mL plant extract +few drops of 5% ferric chloride solution	Green precipitates
Lead acetate test	2 mL plant extract + 5 mL of distilled water + 3 mL of 10% lead acetate solution	White precipitate
Proteins and amino acids		
Millon's test	1 mL of plant extract + 4 to 5 drops of Millon's reagent	White precipitate
Tannins		
Braymer's test	1 mL plant extract + 3 mL distilled water + 4 to 5 drops of 10% ferric chloride solution	Blue green colour appear
Sodium hydroxide test	1 mL plant extract + 3 mL of 10% NaOH	Formation of emulsion
Reducing sugar		
Benedict's test	1 mL plant extract + 1 mL Benedict's reagent + boiled for 5 minutes	Green or red colour
Fehling's test	1 mL plant extract + 1 mL of each Fehling's solution A & B + boiled for 5 to 10 minutes in water bath	Red precipitation appears
Glycosides		
Keller-Killani test	1 mL plant extract + 1 ml glacial acetic acid + few drops of 5% ferric chloride + conc. H_2SO_4	A blue-coloured solution
Phytosterols and triterpenoides		
Hesse's response	2 mL plant extract + 1 mL chloroform + 1 mL conc. H_2SO_4	Pink ring (in lower chloroform layer)
Salkowski's test	1 mL plant extract + 4-5 drops of conc. H_2SO_4	A golden yellow layer appears
Saponins		
Foam test	1 mL plant extract + 5 ml of distilled water	Stable foam formation

(Raaman, 2006 ; Sadasivam & Manickam, 2023)

Total phenolic content

The total phenolic content of various extracts was measured by the Folin–Ciocalteu method (Wolfe et al., 2003), incorporating minor modifications and subsequently quantified through a spectrophotometer. Plant extract (50 µL) was mixed with 1 mL of 10% Folin-Ciocalteu reagent and 1 mL 2% sodium carbonate solution and then incubated in the dark for 1 hour. The absorbance was measured at a wavelength 765 nm.

The standard calibration curve was constructed using different concentrations of gallic acid as the reference standard and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extract. All determinations were performed in triplicate.

Total flavonoid content

The total flavonoid content was measured using the Aluminum chloride ($AlCl_3$) method (Meda et al., 2005) with minor modifications. 0.1 mL of 2% aluminium chloride solution and 0.1 mL of potassium acetate solution (1M) were mixed with plant extract (50 µL). The test solution was thoroughly mixed and the absorbance was measured at 415 nm after10 minutes, using a blank sample as the reference. A standard curve was generated using different concentrations of quercetin as the standard. The average of three readings was calculated and the results were expressed as milligrams of quercetin equivalents (QE) per gram of extract.

Statistical analysis

All experiments were conducted in triplicate. One-way analysis of variance (ANOVA) was performed using IBM-SPSS (Version-25) and differences among sample means were assessed using Tukey's (HSD) test at a $p<0.05$ significance level.

Results

Environment conditions

Environmental conditions varied notably between the open and protected environments. The average temperature inside the naturally ventilated polyhouse remained consistently higher (20°C) than the open environment (17ºC) due to the greenhouse effect. Relative humidity was also elevated in the protected environment (75%) compared to the open field (71%). These differences in microclimatic conditions are likely to influence plant growth, biochemical composition and yield parameters.

Extraction yield

The extraction yield varied significantly among genotypes, solvents and growing environments. Methanol was the most efficient solvent, yielding the highest percentage of crude extracts across all genotypes and environmental conditions. The genotype Urja exhibited the highest extraction yield (38.6%) under the open environment using methanol, while Long Island Improved recorded the maximum yield (39.2% under the protected environment with the same solvent. In contrast, aqueous extraction produced the lowest yield, ranging from 11 to 15%. Overall, protected environment conditions enhanced the extraction efficiency in most cases, particularly with methanol and hydro alcohol solvents (Table 2).

Table 2. Influence of environment and solvent on extraction yield from genotypes of Brussels sprouts

		Open environment			Protected environment		
Genotypes	**Extraction**	**Dry weight of sample (g)**	**Crude extract weight (g)**	**% yield of crude extract (%)**	**Dry weight of sample (g)**	**Crude extract weight (g)**	**% yield of crude extract (%)**
Hild's Ideal	**Methanol**	5	1.19	23.8	5	1.73	34.6
	Aqueous	5	0.76	15.2	5	0.75	15
	Hydro alcohol	5	1.38	27.6	5	1.19	23.8
Long Island Improved	**Methanol**	5	1.45	29	5	1.96	39.2
	Aqueous	5	0.65	13	5	0.56	11.2
	Hydro alcohol	5	1.34	26.8	5	1.05	21
Urja	**Methanol**	5	1.93	38.6	5	1.43	28.6
	Aqueous	5	0.64	12.8	5	0.55	11
	Hydro alcohol	5	1.30	26	5	1.10	22
Franklin F_1	**Methanol**	5	1.34	26.8	5	1.52	30.4
	Aqueous	5	0.61	12.2	5	0.64	12.8
	Hydro alcohol	5	1.00	20	5	0.63	12.6

Phytochemicals screening

The phytochemical characteristics in methanol, aqueous and hydro alcohol extracts of different Brussels sprouts genotypes under open and protected environment data were presented in the Figure 3. Results indicated the existence of bioactive compounds in various Brussels sprouts genotypes. It was observed that, Mayer's test showed a consistent occurrence of alkaloids in methanol, aqueous as well as hydro alcohol extracts, whereas, Wagner's test confirmed the occurrence of alkaloids in methanol extracts and absence of alkaloids in aqueous as well as hydro alcohol extracts of all the genotypes across both the environments.

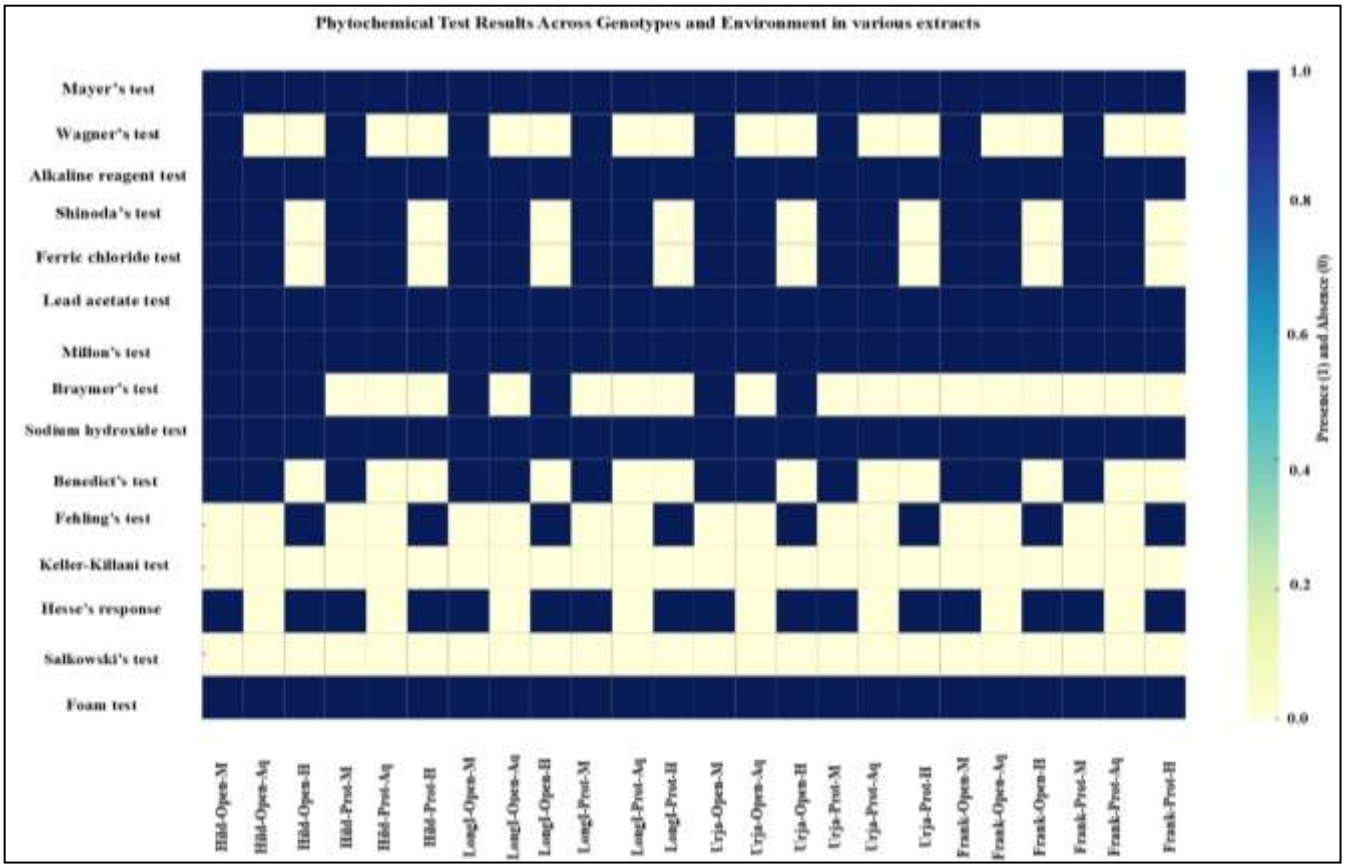


Figure 3. Heatmap presents the phytochemical screening results of Brussels genotypes under open and protected (prot) environments using different extracts-methanol extract (M), aqueous extract (Aq) and hydro alcohol extract (H). Hild-Hild's Ideal; Long I- Long Island Improved; Urja; Frank- Franklin F_1

Alkaline reagent test confirmed the appearance of flavonoids in methanol, aqueous and hydro alcohol extracts, whereas Shinoda's test confirmed the existence of flavonoids in methanol and aqueous extracts and absent in hydro alcohol extract across all the genotypes and environmental conditions. The lead acetate test was consistently positive across all genotypes and environments, confirming the presence of phenolic compounds. Ferric chloride test showed the presence of phenolics with methanol and aqueous extracts and the absence of phenolics with hydro alcohol extracts. Million's test confirms the existence of proteins and amino acids with methanol, aqueous as well as hydro alcohol extracts, whereas, Braymer's test showed the absence of tannins in methanol, aqueous and hydro alcohol extracts of all the genotype under protected environment, whereas, under open environment it is absent in aqueous extracts of genotypes Long Island Improved and Urja. The sodium hydroxide test was positive across all genotypes and conditions, confirming the presence of tannins. Benedict's test confirmed the presence of reducing sugar with methanol as well as aqueous extracts of all the genotypes under open environment, whereas, under protected environment, it confirmed the presence of reducing sugar with methanol extracts and absence of reducing sugar with aqueous and hydro alcohol extracts of all the genotypes of Brussels sprouts. Keller-killani test was consistently negative with methanol, aqueous and hydro alcohol extracts in all genotypes under both environmental conditions. Hesse's test showed the presence of phytosterols and triterpenoids with methanol and hydro alcohol extracts of different Brussels sprouts genotypes under both environments and it confirmed the absence of phytosterol and triterpenoids with aqueous extracts, whereas, salkowski's test confirmed the absence of phytosterols and triterpenoids with methanol, aqueous and hydro alcohol extracts across all the genotypes under both environments. Foam test showed a positive reaction across all genotypes of Brussels sprouts and environmental conditions. Saponins are stable across different environments and genotypes, indicating their consistent biosynthesis in Brussels sprouts.

Total phenolic content

The result of the phenolic content of various extracts of Brussels sprouts genotypes is presented in Figure 4.

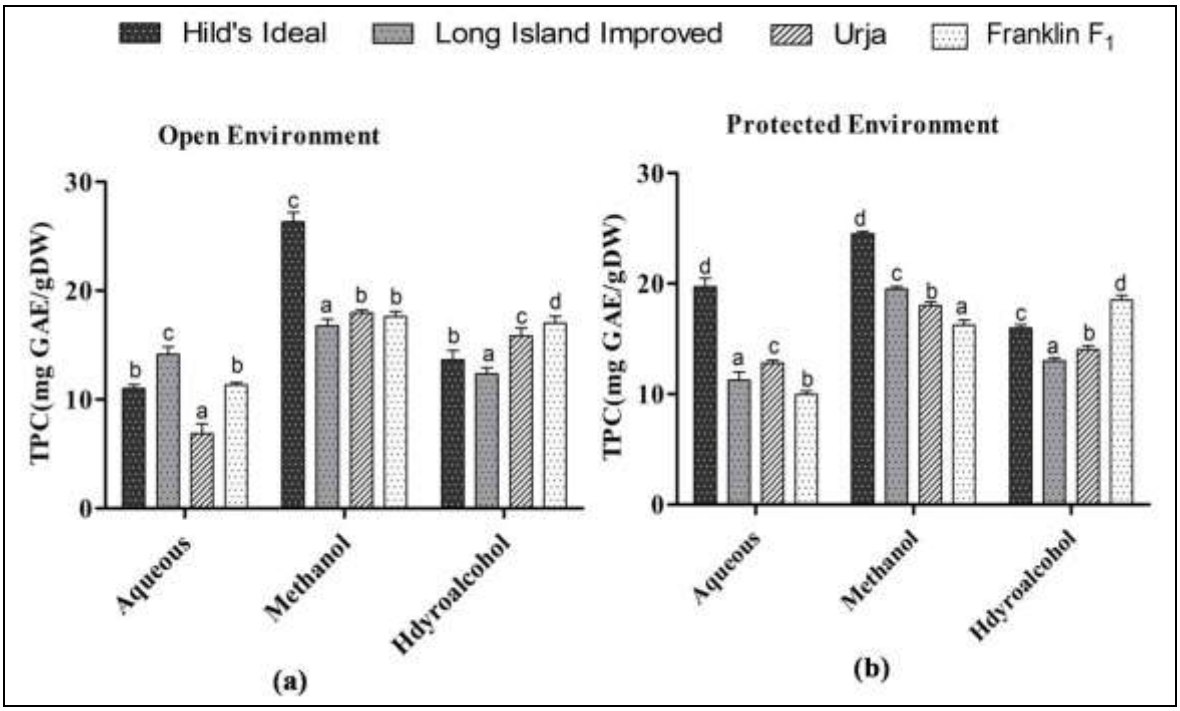


Figure 4. Total phenolic content of Brussels sprouts genotypes under (a) Open environment (b) Protected environment. Bars with different letters indicate statistically significant differences according to Tukey's (HSD) test ($p<0.05$). Bars sharing the same letter are not significantly different.

Under open environment with aqueous extracts genotype Long Island Improved showed highest phenolic content (14.14 mg GAE/g DW) and lowest phenolic content (6.87 mg GAE/g DW) was found in genotype Urja, whereas, with methanol extracts genotype Hild's Ideal (26.32 mg GAE/g DW) showed the highest phenolic content and lowest (16.76 mg GAE/g DW) in genotype Long Island Improved. In hydro alcohol extracts, genotype Franklin F_1 (17.00 mg GAE/g DW) had the highest phenolic content than the other genotypes and the lowest total phenolic content (12.36 mg GAE/g DW) was observed in genotype Long Island Improved. Under protected environment genotype Hild's Ideal showed highest total phenolic content (19.70 mg GAE/g DW) with aqueous extract while the lowest phenolic content (9.97 mg GAE/g DW) was found in genotype Franklin F_1, whereas, methanol extract the maximum phenolic content (24.52 mg GAE/g DW) also observed in genotype Hild's Ideal and minimum (16.17 mg GAE/g DW) in genotype Franklin F_1. In hydro alcohol extract, maximum phenol content was observed with genotype Franklin F_1 (18.51 mg GAE/g DW), while minimum phenolic content (13.00 mg GAE/g DW) in genotype Long Island Improved. The results confirmed that methanol extract was superior to aqueous as well as hydro alcohol extracts for the extraction of total phenol content.

Total flavonoid content

The results of the flavonoid content in Brussels sprouts genotypes are presented (Figure 5). Under open environment with aqueous extract the highest total flavonoids content (7.19 mg QE/g DW) was found in genotype Long Island Improved while the minimum flavonoid content (4.29 mg QE/g DW) was observed in genotype Urja, whereas, with methanol extract the maximum flavonoid amount (10.91 mg QE/g DW) was measured in genotype Hild's Ideal which was statistically higher than others except the genotype Franklin F_1 (10.69 mg QE/g DW) which was statistically comparable and minimum flavonoid amount (7.62 mg QE/g DW) was also recorded in genotype Urja. In hydro alcohol extract the maximum flavonoid amount (9.83 mg QE/g DW) was also recorded in genotype Hild's Ideal which was statistically higher than others and the minimum amount (6.72 mg QE/g DW) observed in genotype Urja. Under protected environment with aqueous extracts, the maximum flavonoid content (5.91 mg QE/g DW) was measured in genotype Urja which was statistically comparable with genotype Franklin F_1 (5.06 mg QE/g DW), while the minimum amount of flavonoid (4.14 mg QE/g DW) in genotype Hild's Ideal. In methanol extract genotype Hild's Ideal showed highest flavonoid content (13.92 mg QE/g DW) which was statistically higher than the others except genotype Franklin F_1 (13.43 mg QE/g DW) which was statistically at par and minimum flavonoid content (7.85 mg QE/g DW) was found in genotype Long Island Improved. In hydro alcohol extract genotype Hild's Ideal showed the highest flavonoid content (9.85 mg QE/g DW) which was statistically higher than the other genotypes and the lowest flavonoid content (5.87 mg QE/g DW) was found in genotype Long Island Improved.

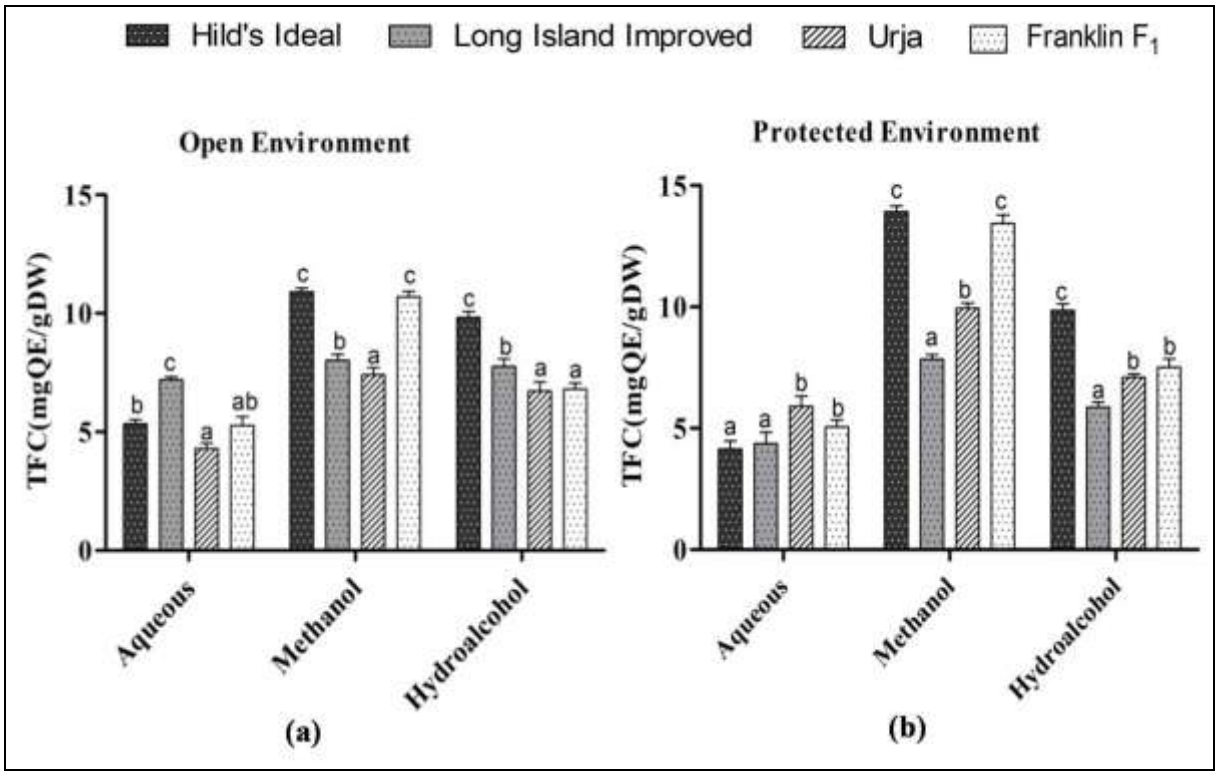


Figure 5. Total flavonoids content of Brussels sprouts genotypes under (a) Open environment (b) Protected environment. Bars with different letters indicate statistically significant differences according to Tukey's (HSD) test ($p<0.05$). Bars sharing the same letter are not significantly different

Discussion

The result demonstrated that both environmental conditions and solvent polarity played a crucial role in determining the percentage yield of crude extracts. The protected environment favoured higher extraction yields, particularly for polar solvents like methanol, while variability among genotypes indicated genetic influence on metabolite accumulation. The results obtained in experiments performed with different extraction methods are similar to those reported by Barros et al. (2010). The preliminary phytochemical screening revealed the consistent presence of alkaloids, flavonoids, phenolics, reducing sugars, proteins, saponins and phytosterols in Brussels sprouts extracts, with variation across genotypes, solvents and environmental conditions. The comparative study showed that all the genotypes of Brussels sprouts contain the same phytochemicals in both environmental conditions, while only tannin was absent in all genotypes under the protected environment. Glycosides by Keller-Killani test were absent in all genotypes of Brussels sprouts under both environmental conditions. Rizwan & Masoodi, (2025) reported the presence of flavonoids, tannins, anthocyanosides and saponins as well as carbohydrates and proteins in kohlrabi. Khandayataray& Murthy, (2019) also reported the presence of alkaloids, carbohydrates, phenols, flavonoids, proteins, saponins and tannins in *Brassica* sp. Numerous studies have reported that these phytochemicals influence various physiological processes. Medicinal alkaloids play a crucial role in the production of potent analgesic agents and exhibit cytotoxic, antispasmodic and antibacterial agents (Faruq et al., 2024). Phenolic, flavonoids and other bioactive molecules exhibit anti-inflammatory, antioxidant and anti-carcinogenic properties. Plant steroids are recognized for their anti-viral, anti-fungal, antioxidant, anticancer, hepatoprotective, and anti-inflammatory properties (Khan et al., 2024). Saponins are natural glycosides with diverse pharmacological properties, including antifungal, anti-parasitic, anti-inflammatory, expectorant, hypoglycaemic, diabetes, obesity, among others (Faruq et al., 2024). The total phenolic content of Brussels sprouts genotypes was markedly influenced by the type of solvent used and the environmental growing conditions. Across all genotypes, methanol extracts yielded the highest total phenolic content, followed by hydro alcohol and aqueous extracts. Genotype Hild's Ideal under open environment had the highest phenolic content (26.32 mg GAE/g) with methanol extract, while genotype Urja had the lowest value (6.87 mg GAE/g) with aqueous extracts, using the standard curve of gallic acid. These findings emphasize the combined role of genotype, extraction solvent and environmental factors in enhancing the phenolic content of Brussels sprouts. Doniec et al. (2022) reported total polyphenol content (253.01 mg GAE/100g fresh material) in Brussels sprouts. Podsedek et al. (2006) reported that total phenolic content in Brussels sprouts ranged from 133.4 to 140.13 mg/100g (FW). Jaiswal et al. (2012) observed that 60% methanol extract exhibited the highest phenol content, measuring 23.6 mg GAE/g in broccoli, 20.4 mg GAE/g in Brussels sprouts and 18.7 mg GAE/g in white cabbage. These variations may be influenced by the different Brussels sprouts genotypes analyzed as well as differences

in cultivation practices and countries of origin. Total flavonoid content of Brussels sprouts genotypes was significantly influenced by the growing environment, extraction solvent and genotypes. Methanol consistently extracted the highest flavonoid content in both environments. Flavonoid levels were enhanced in the protected environment compared to the open field, likely due to the favourable microclimatic conditions such as elevated temperature and humidity. Comparative analysis indicated that the highest flavonoid content was observed in genotype Hild's Ideal (13.92 mg QE/g) with methanol extracts under the protected environment, whereas the lowest flavonoid content 4.29 mg QE/g was recorded in the aqueous extract of the genotype Urja grown under an open environment. Jaiswal et al. (2012) reported 60% methanol extract showed the highest total flavonoid content (15.4 mg QE/g) in Brussels sprouts. Lin & Tang, (2007) reported 106.2 mg per 100 g (FW) flavonoid content in Brussels sprouts. Jeon et al. (2022) reported that Brussels sprouts exhibited approximately 36% higher total phenolic content and 2.5-fold greater flavonoid content compared to cabbage. Flavonoids are one of the most diverse and widely distributed groups of natural compounds and are regarded as the most significant natural phenolics. These compounds exhibit a wide spectrum of chemical and biological activities (Kumar & Pandey, 2013). The relationship between the structural characteristics of flavonoids and their antioxidant activity has been extensively investigated. The antioxidant potential of flavonoids is influenced by the number as well as the position of hydroxyl groups and the nature of other substituents and the glycosylation patterns of the flavonoid molecules (Lewandowska et al., 2013).

Conclusion

The present study demonstrates the significant influence of environmental conditions on the extractive potential and biochemical profile of Brussels sprouts. A comparative assessment of four genotypes (Hild's Ideal, Long Island Improved, Urja and Franklin F_1) grown under open environment and protected environment revealed marked differences in the accumulation and extractability of phytochemicals. The protected environment characterized by higher average temperature and greater relative humidity created a favourable microclimate that enhanced the biosynthesis and retention of secondary metabolites, especially total phenolic content and total flavonoid content. Methanol extracts under protected conditions consistently showed higher total phenolic and total flavonoid content compared to the open environment, indicating that controlled conditions favour antioxidant compound accumulation. Preliminary phytochemical screening supported this observation, yielding more consistent and positive results for alkaloids, flavonoids, saponins and phytosterols under protected conditions; however, genotype specific responses were also evident. Regarding extraction yield, methanol proved to be the most efficient solvent, especially under protected conditions. Across the genotypes, extraction yield was highest in methanol extracts of genotype Long Island Improved and Hild's Ideal under the protected environment. The protected cultivation system enhances both the quantity and quality of phytochemicals, with genotypic variation playing an additional role in metabolite accumulation.

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Author contributions

L. Thakur – Methodology, investigation, and write original draft preparation; P. Kumar –supervision, draft reviewing, conceptualization.

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Ethics approval

Not applicable.

Competing interests

The authors declare no conflict of interest. The manuscript has not been submitted for publication in another journal.

AI tool declaration

The authors declare that no AI or related tools were used in the creation of the scientific content of this manuscript.

Availability of material and data

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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