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Original article

Functional and Antioxidant Potential of Beetroot, Mustard and Radish Microgreens Using Spectroscopic Techniques

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Microgreens are tiny plants with a pair of cotyledon leaves, a short stem, and roots. These are considered as sustainable superfoods that are easy to grow and rich in bioactive compounds. Among functional foods, microgreens are particularly noteworthy because they have enticing health-promoting properties due to their rich biochemical profiles which contribute to antioxidant activities. In this study, three varieties of microgreens, *Beta vulgaris*, *Raphanus sativus* and *Brassica juncea*, were studied to estimate phytochemicals such as total chlorophyll, carotenoids, flavonoids, and phenols. Additionally, the antioxidant potentials of methanolic extracts of these microgreens were determined by various assays such as 2, 2-diphenyl, 1-picrylhydrazyl and H_2O_2 scavenging assay, total antioxidant capacity and reducing power assay. Moreover, Fourier transform infrared spectroscopic fingerprinting was conducted to determine the functional groups associated with bioactive phytochemicals present in all microgreens. Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopic studies were also conducted to explore the morphological and elemental profiling of each microgreen. The results revealed that the studied microgreens have rich phytochemical compositions and great antioxidant potential. Furthermore, the functional groups of bioactive compounds identified in each were extensively associated with antioxidant activities. Therefore, microgreens can be recommended as promising superfoods that can be incorporated into the mainstream diet to improve human health.

Key words: microgreens, antioxidants, phytocompounds, nutraceutical

The increasing cognizance about the health benefits of microgreens has introduced them as a new class of nutrient-rich superfoods (Choe *et al.* 2018; Rizvi *et al.* 2023). Microgreens are immature seedlings of herbs, spices and other vegetable seeds that are harvested at the earliest stage, carrying a pair of cotyledonous leaves with a short delicate stem (Xiao *et al.* 2019; Rizvi *et al*. 2024). A range of vegetables, herbs and even flowers are widely cultivated as microgreens (Treadwell *et al.* 2020). They are usually ready for harvesting between 7 and 15 days from sowing (Xiao *et al.* 2012; Mir *et al.* 2017). Microgreens are different from baby greens, and they are super rich in concentrated flavours, attractive colours, tender texture, and imperative bioactive nutrients as compared to their mature

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counterparts (Xiao *et al.* 2012; Xiao *et al.* 2014; Di Gioia & Petropoulos 2019). Microgreens are highly concentrated in vitamins, minerals and numerous bioactive components. Previous studies have shown that microgreens are substantially rich in micronutrients than mature counterparts of the same crop. Instantly, the vitamins and their precursors, like carotenoids, L-ascorbic acid, phylloquinone, tocopherol, tocotrienols, folate, etc. are densely found in microgreens. Bioactive phytochemicals associated with antioxidant activities, including polyphenols, anthocyanin, chlorophyll, glucosinolates, and other secondary metabolites, are also found in higher amounts in microgreens (Xiao *et al.* 2012; Paradiso *et al.* 2018; Zhang *et al.* 2021; Rizvi *et al.* 2023). The prominent profile of microgreens plays an essential role in conditions like haemolytic anaemia, thalassemia, and other chronic diseases like cardiovascular ailments, skin and age-related diseases, and cancers (Choe *et al.* 2018; Niroula *et al.* 2019a; Rizvi *et al.* 2023). The antioxidant activities possessed by ascorbic acid, polyphenols and pigments act against diabetes, neurodegenerative diseases, and other pathways like carcinogenesis and mutagenesis (Niroula *et al.* 2019a; Niroula *et al.* 2019b). Broccoli microgreens have been reported to contain an abundant substance called N1, N10-di-feruloyl spermidine, a potent antioxidant (Liu *et al.* 2022). Another substance in broccoli microgreens is sulforaphane, a compound with robust antioxidant properties that can help prevent cancer (Zhang *et al.* 1992; Ninh Le *et al.* 2019). Sulphoraphane is an isothiocyanate molecule with anti-inflammatory and antioxidant properties (Bai *et al.* 2015). Red cabbage contains a phytocompound called sinapine, which inhibits the mitochondrial oxidative stress in cardiomyocytes and protects the heart by reducing oxidative stress in heart cells (Boulghobra *et al.* 2020).

Microgreens are becoming increasingly popular due to their nutritional benefits, ease of cultivation and rising culinary demands, particularly after the COVID pandemic. This has led to a surge in interest from young entrepreneurs, farmers, urban and peri-urban dwellers, and even inexperienced gardeners (Paraschivu *et al.* 2021). Additionally, the commercial adoption of microgreens presents an opportunity for skilled entrepreneurs to meet the rising demand for functional food and address nutritional deficiencies worldwide. Several studies have explored the health benefits of microgreens such as, red cabbage microgreens reduced weight gain and LDL levels in high-fat diet-fed mice while lowering the expression of hepatic inflammatory cytokines (Huang *et al.* 2016). This was attributed to the polyphenols in red cabbage microgreens, which have antioxidant and anti-inflammatory properties. In the present study, we cultivated beetroot, mustard and radish microgreens and analysed their nutritional parameters, i.e., total chlorophyll, carotenoids, phenols and flavonoid contents. We have also assessed 2, 2-diphenyl, 1-picrylhydrazyl (DPPH) radical scavenging activity, H_2O_2 scavenging activity, reducing power and total antioxidant capacity of the microgreen extracts. Furthermore, we used Fourier transform infrared (FT-IR) spectroscopy to fingerprint the phytocompounds by determining the types of secondary metabolites present in each microgreen. Finally, the scanning electron microscopy and energy dispersive X-ray spectroscopy (SEM-EDS) studies were done for the morphological characterization and elemental analysis of microgreens. The three microgreens were specifically chosen for the study as they apt to grow easily in almost all geographical locations and climatic patterns, particularly in India (Agic *et al.* 2018; Singh *et al.* 2020). Moreover, the year-round availability of seeds and relatively lower seed cost are yet another aspects for budding entrepreneurs, fitness enthusiasts and even farmers to cultivate these microgreens compared to other commercially available microgreens. Also, the low maintenance with a good yield and that too with higher nutritional density is another reason for conducting the study on commonly available seeds to provide the superfood in disguise of microgreens to all masses particularly of the developing nations and third world countries.

MATERIAL AND METHODS

Cultivation and growth of microgreens

The seeds of beetroot (*Beta vulgaris*), mustard (*Brassica juncea*) and radish (*Raphanus sativus*) were obtained from the National Seeds Corporation Limited, a regional office in Lucknow, India. The seeds were sterilized by soaking them in aqueous

sodium hypochlorite (2%) for 15–20 minutes, followed by washing with double-distilled water. The rinsed seeds wereuniformly sowed in perforated pots in four replicates containing 3 cm thick mixture of cocopeat and vermicompost (2:1 ratio). During cultivation, each pot was sprayed with deionized water. The pots were initially kept in dark for 24 hours to ensure proper germination and upright shooting of seedlings. Afterward, they were transferred to growing racks equipped with LED lights (Phillips) emitting approximately 1,424.11 lux light intensity with 12 hours of photoperiod (Ghoora *et al.* 2020a). The air temperature was maintained at an average of 16±5°C, and the relative humidity was at 70±5% throughout the germination phase of the three microgreens. All the growing conditions were maintained in the Plant Biotechnology Lab, Department of Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India (26°45'53.2''N, 80°55'34.4''E). The radish and mustard microgreens were harvested after 10 days of sowing, while the beetroot microgreens required a longer period of about 14 days (Figure 1). The harvesting was done with a sharp pair of ethanol-sterilized scissors by cutting closest to the growing substrate. The harvested microgreens were properly cleaned by removing the seed coat and then rinsing with deionized water to remove any leftover substrate.

Extract preparation of microgreens

The microgreens were cleaned and dried with a fan for 10–15 minutes before being frozen at –80°C. They were then freeze-dried for approximately 20 hours until completely dried. The dried microgreens were crushed to make fine powder and stored in ultra-deep freezer (–80°C) for further studies (Ghoora *et al.* 2020b). To prepare the microgreen extract, the protocol described by More and Makola was followed (More & Makola 2020). About 10 grams of each powdered microgreen sample was mixed with 80% methanol and kept for 72 hours in an incubator shaker. Each microgreen extract was filtered through the filter paper and the filtrates were evaporated in a rotatory vacuum evaporator (UTS, 1.53, Thermo-scientific). The samples collected were further dried for 1–2 hours at 50°C.

Estimation of functional bioactive contents

Estimation of total chlorophyll content

The total chlorophyll content (TCC) of microgreen samples was determined by the direct spectrophotometric method (Tan *et al.* 2020). Firstly, about 1 gram of each microgreen sample was weighed and pulverized thoroughly in mortar and pestle. Further, 10 mL of 80% aqueous acetone solution (v/v) was added to the pulverized microgreens. The mixture was homogenized for one minute at a speed of 15,000 rpm using a homogenizer (Hi-speed homogenizer D-500, Unigenetics Instruments Pvt. Ltd.) and then sonicated (LABMAN Probe Sonicator PRO650). The homogenate was then filtered into a volumetric flask using qualitative filter paper (Grade 413, VWR International, Radnor, PA, USA). The microgreen residues were again rinsed with 80% acetone solution until they were colourless, and the volume up

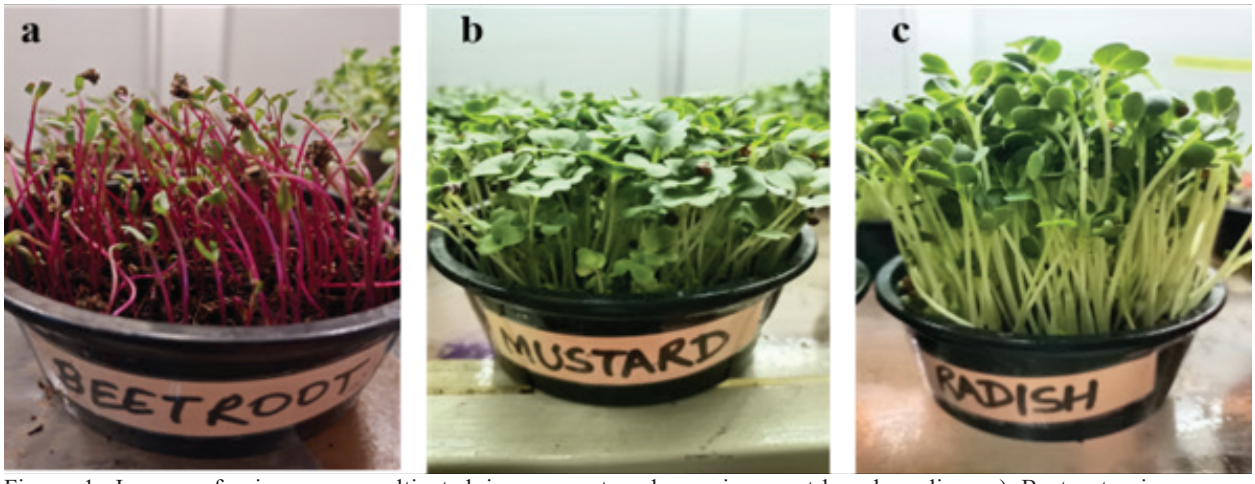


Figure 1. Images of microgreens cultivated in cocopeat and vermicompost-based medium a) Beetroot microgreens; b) Mustard microgreens and c) Radish microgreens.

to 25 mL was made up with 80% acetone (v/v). The absorbance was obtained at the wavelength of 646, 663 and 710 nm using UV-visible spectrophotometer (Eppendorf, Model: Biophotometer D30). 80% acetone (v/v) was used as a blank for the study. The formula given below was used to estimate the TCC.

$$TCC\ [mg/100g] = [(Ab_{646} - Ab_{710}) \times 0.01732 + (Ab_{663} - Ab_{710}) \times 0.00718] \times \text{dilution volume/ fresh weight (FW)}$$

Estimation of total carotenoid content

The total carotenoid content in microgreens was measured using the protocol explained by Kirk and Allen (Kirk & Allen 1965). To determine the total carotenoid content, the extraction method explained in section 2.3.1 was followed. The absorbance was observed at 480 nm wavelength. The following formula was used to calculate the total carotenoid content in each microgreen extract.

$$\text{Carotenoid content } [\mu g/g\ FW] = Ab_{480}\ (0.114 \times Ab_{663}) - (0.638 \times Ab_{645}) \times V/M$$

Where: Ab – absorbance at various wavelengths; V – volume of microgreen extract (mL) and M – weight of extract (grams).

Estimation of total flavonoid content

The study followed the protocol by Chang *et al.* (2002) to estimate the total flavonoid content (TFC) in each microgreen (Chang *et al.* 2002). For this, 1 mL aliquot of each microgreen extract was added with 0.1 mL of aluminium chloride (10%) and potassium acetate (1 M). The mixture was further added with 2.8 mL of methanol and left for 30 minutes at room temperature. Eventually, the absorbance of mixture was read at 415 nm wavelength using a spectrophotometer. The TFC of microgreens was measured as milligrams of quercetin equivalent per 100 grams [mg QE/100 g] using quercetin as standard (standard curve equation: $y = 0.0442 + 0.0069x$, $R^2 = 0.9969$). The equation given below was used to calculate the TFC.

$$[TFC = C \times V \times 100 / M]$$

Where: C, V and M are the concentration of quercetin (mg/mL), volume of microgreen extract (mL) and weight of microgreen extract (grams) respectively.

Estimation of total phenol content

The total phenolic content (TPC) of microgreens was evaluated using Folin-Ciocalteu protocol (Mohy El-Din & El-Ahwany 2016). For this, 100 µL of each microgreen extract was added with sodium carbonate (2%) and kept for about two minutes. Further, 100 µL of Folin-Ciocalteu reagent (50%) was added to the prepared mixture and kept for incubation in the dark for about 30 minutes at ambient room temperature. After the incubation, the absorbance was read at 720 nm wavelength. Various concentrations of Gallic acid were used to plot the standard curve to estimate the TPC in microgreens (standard curve equation: $y = 0.0023 + 0.0094x$, $R^2 = 0.9965$). The estimated TPC was expressed as 'milligrams of Gallic acid equivalent per 100 grams' [mg GAE/100g]. The equation given below was used to calculate the TPC of microgreen extracts.

$$[TPC = C \times V \times 100 / M]$$

Where: C, V and M are the concentration of Gallic acid [mg/mL], volume of microgreen extract (mL) and weight of extract (in grams) respectively.

Estimation of antioxidant potential in microgreens

2, 2-diphenyl, 1-picrylhydrazyl (DPPH) scavenging activity

DPPH radical scavenging assay was performed to assess the antioxidant potential present in microgreen extracts. The test was conducted on a 96-well Corning plate. Various microgreen extracts ranging in concentration from 25 – 500 µg/mL were added in 5 µL quantities. The reaction was performed in triplicates and the blank was prepared in duplicates from 0.2 mL DMSO/methanol solution. After 30 minutes of incubation in darkness, the decolourization in the reaction was read at 517 nm using a multimode plate reader (Tecan, Infinite M200 plate reader) (Bobo-García *et al.* 2015). The reaction mixture with 20 µl of deionized water served as a control. The L-ascorbic acid was used as the standard (0 – 100 µg/mL) for the analysis. The percentage inhibition was measured and presented as the DPPH scavenging activity. The calculation given below was used in calculating the percentage of DPPH scavenging.

$$\%\text{DPPH Scavenging activity} = [(Ab_{Control} - Ab_{Sample})/ Ab_{Control}] \times 100$$

Estimation of hydrogen peroxide scavenging

An experiment was conducted using the hydrogen peroxide assay to analyse the scavenging activity of H_2O_2 free radicals by microgreen extracts. Briefly, 10 mM H_2O_2 solution was freshly prepared in 0.1 M phosphate buffer (pH 7.4). Then, 1 mL of each concentration of microgreen extracts (25 – 500 µg/mL) was mixed with 2 mL of prepared H_2O_2 solution and the final mixture was incubated for 10 minutes in the dark. Subsequently, the absorbance was taken at 560 nm against a blank of microgreen extract without H_2O_2 (Oktay *et al.* 2003). The formula given below was used to compute the percentage scavenging of hydrogen peroxide free radicals.

$$\text{Percentage of } H_2O_2 \text{ scavenging} = [(Ab_0 - Ab_i) \div Ab_0] \times 100$$

Where: Ab_0 represents the absorbance of the control and Ab_i is the absorbance of the extract.

Estimation of reducing power

The reducing power of microgreen extracts was estimated using the protocol by Yamaguchi *et al.* (1998) with minimal modifications (Yamaguchi *et al.* 1998; Mohy El-Din & El-Ahwany 2016). Firstly, 0.75 mL of microgreen extract (100 – 500 µg/mL) was mixed with 0.75 mL each of the potassium ferricyanide (1%) and phosphate buffer (pH 6.6), followed by an incubation of 20 minutes at 50°C. Subsequently, 0.75 mL of trichloroacetic acid (10%) was added and the solution was centrifuged for 10 minutes at 3,000 rpm. The aliquots of 1.5 mL of the supernatant, ultrapure water and ferric chloride (0.1%) were mixed, and absorbance was taken at a wavelength of 700 nm after incubating for 10 minutes. The L-ascorbic acid served as the standard while evaluating the reducing power of microgreens in this study.

Estimation of total antioxidant capacity

The total antioxidant capacity (TAC) of microgreen extracts was evaluated using the protocol followed by Mohy El-Din and El-Ahwany (2016). To prepare the TAC solution, 7.45 mL of 0.6 M sulphuric acid, 1.23 mL of ammonium molybdate (4 mM) and 0.99 g of sodium sulphate (28 mM) were added to make up the final volume to 250 mL with autoclaved distilled water. Further, 0.1 mL of each microgreen extract and 1 mL of TAC solution were mixed and absorbance was recorded after 15 minutes at a wavelength of 695 nm. The L-ascorbic acid served as the standard for calculating the TAC of microgreen extracts, which was determined using the given equation.

$$[\text{Percentage Inhibition} = (Ab_0 - Ab_i) / Ab_0 \times 100]$$

Where: Ab_0 and Ab_i represent the absorbance of control and extract respectively.

FT-IR analysis of microgreen extracts

The possible functional groups associated with the secondary metabolites present in methanolic extracts of microgreens were identified using Fourier-transform infrared spectroscopy (FT-IR). The procedure involved mixing powdered samples of each microgreen with potassium bromide (KBr, analytical grade) in a 1:30 ratio, then macerating the mixture in a hydraulic press operating under vacuum to create thin pallets. The absorbance spectra were recorded using FT-IR equipment (Nicolet TM 6700, Thermo Fisher Scientific, USA) between 4,000 and 450/cm in the mid-infrared spectrum (Kustiati *et al.* 2022). To measure ambient air, a background spectrum potassium bromide (KBr) was used during the scanning process while searching for spectrum peaks. The results were processed using OriginPro2023 and compared against earlier works of literature to evaluate the outcomes.

SEM and EDS studies of microgreens

The microgreen samples, including beetroot, mustard, and radish were freshly harvested and gently cleaned with autoclaved distilled water for scanning electron microscopy (SEM) and elemental analysis procedures by energy dispersive X-ray spectroscopy (EDS). All the microgreens were initially fixed using a 2.5% glutaraldehyde fixative for at least 6 hours at 4°C. Primary fixation was followed by washing the samples thrice with phosphate buffer (0.1 M), with a 15-minute gap between each wash. Post-fixation was done with 1% Osmium tetrachloride for at least 2 hours, followed by three additional washing steps with 0.1 M phosphate buffer, each after a 15-minute interval. After washing, the samples were dehydrated using 30%, 50%, 70%, 90%, and 95% acetone, respectively, each for 30 minutes. The final dehydration was done in a 100% acetone solution for 30 minutes (Mishra *et al.* 2018).

The samples were then mounted on aluminium stubs with double-sided tape before being placed in the platinum sputter-coater (Auto Fine Coater, JFC 1600, JEOL, Japan) to make them conductive. Each sample was observed through a scanning electron microscope (JEOL JSM 6490 LV, Tokyo, Japan) for SEM-EDS analysis at different magnifications and accelerating voltages. The images were obtained at 500X and 2000X magnifications to determine the morphological details, including the size of guard cells (length and width). Additionally, each microgreen's leaf lamina was studied for the identification of stomatal density and the aperture (pore size) of stomata. All the measurements were executed using Image-J software.

Statistical analysis

All experiments were run in triplicates, and the results were shown as mean and standard deviation (Mean ± SD). To perform a comparative analysis and to identify significant differences among the microgreens, the findings were subjected to ANOVA using GraphPad Prism5 software (GraphPad Software, San Diego, CA, USA). To determine the significant differences among the microgreens, Tukey's test was performed where the significance level was determined at $p \leq 0.05$.

RESULTS AND DISCUSSION

Total chlorophyll content

Chlorophylls are green-coloured pigments found in plants that are crucial for photosynthesis. Plants produce higher nutritional energy when their chlorophyll content is higher (Mandal & Dutta 2020). Additionally, it improves the sensory attributes such as the freshness and appearance of plants. The total chlorophyll content of all three microgreens i.e. radish, mustard and beetroot, is presented in Figure 2a. The maximum chlorophyll content was found in radish microgreen extract (RME), followed by mustard microgreen extract (MME), and beetroot microgreen extract (BME), which were found to be 62.36, 55.38 and 27.24 mg/100 g, respectively. The results suggested that BME had the lowest content of chlorophyll as compared to RME and MME. The previous research reported chlorophyll levels as high as 64.7 and 71.5 mg/100 g in mustard and radish microgreens, respectively (Kyriacou *et al.* 2018). Additionally, another study observed 59.21 mg/100 g of chlorophyll content in radish (Kowitcharoen *et al.* 2021). The data in the present study is comparable and coincides with previous findings except for beetroot microgreens, which were around tenfold higher in the last investigation (Samuolienė *et al.* 2017) (Table 1). Besides, a significantly higher chlorophyll concentration was studied both in mustard and beetroot microgreens (up to 3.59 and 2.78 mg/g, respectively) grown in the nutritional substrate under differential LED light conditions (Samuolienė *et al.* 2017). The irradiation with a blue LED with 447 nm wavelength and 100 μmol/m^2/s photosynthetic photon flux density (PPFD) may have significantly increased the chlorophyll content and chlorophyll index in the reported microgreens. Blue LED in combination with red light or alone has been studied to increase the chlorophyll ratio, chlorophyll index, total chlorophyll content and photosynthetic metabolites in microgreens (Samuolienė *et al.* 2013; 2017). Additionally, the irradiance levels expressed in PPFD also have a key role in deciphering the chlorophyll index of the germinating seedlings. The PPFD levels between 100 – 300 μmol/m^2/s have led to increased chlorophyll content and improved biometric parameters (Samuolienė *et al.* 2013). Many studies have revealed the importance of a chlorophyll-rich diet in regulating diseases like cancers, as they play a crucial role in forming complexes with specific carcinogens (de Vogel *et al.* 2005). Besides, chlorophyll is known to play a remarkable role in promoting human health owing to its antioxidant and anti-mutagenic properties (Ferruzzi *et al.* 2002). Moreover, photosynthetic pigments are also considered to have preventive and therapeutic attributes, as they have been reported to stimulate the immune system, help in liver detoxification, and regulate blood pressure, thereby refining liver and heart functions (Chernomorsky & Segelman 1988; Yun *et al.* 1995; İnanç 2011).

Total carotenoid content

Carotenoids are a type of phytochemical found in microgreens alongside other classes (Xiao *et al.* 2012; Zhang *et al.* 2021). Among all the microgreens tested, BME had the highest carotenoid content at

59.39 µg/g FW. RME and MME followed with total carotenoid contents of 54.12 and 35.31 µg/g FW, respectively (Figure 2b). These are yellow, orange-red pigments that are grouped as carotenes (β-carotene and lycopene) and xanthophylls (lutein and zeaxanthin) (Mesquita *et al.* 2017; Namitha & Negi 2010). Carotenoids have antioxidant potential and play a crucial role in promoting human health (Anand *et al.* 2022; Hegde *et al.* 2022). β-carotene (precursor of Vitamin A) is a potent free radical scavenger and fat-soluble antioxidant that protects the cell membrane (Regnault *et al.* 1993). Bright-coloured vegetables are the primary dietary source of carotenoids. Microgreens are also identified with exceptionally rich phytochemicals, including carotenoids (Zhang *et al.* 2021). The previous findings studied a comparatively higher carotenoid content, i.e. 56.0, 156.1 and 160.0 µg/g in mustard, radish and beetroot microgreens respectively (Samuolienė *et al.* 2017; Kowitcharoen *et al.* 2021; Min Allah *et al.* 2023). Mustard microgreens were investigated with 105 – 108 µg/g of carotenoid content; the different varieties of radish microgreens reported total carotenoids between 88 and 138 µg/g (Xiao *et al.* 2019). Furthermore, exceptionally high carotenoid concentrations were observed in four genotypes of Brassica microgreens when grown in a hydroponic system provided with a nutrient solution (De la Fuente *et al.* 2019) (Table 2). The variations in carotenoid levels across the different studies can be persistently seen, which can be associated with varying factors, such as crop variety, climatic, geographical, harvesting and post-harvest conditions (Xiao *et al.*2019). The exposure of higher intensity blue light (33%) to the growing microgreens enriched the carotenoid content by 1.2 – 4.3 times in mustard and beetroot

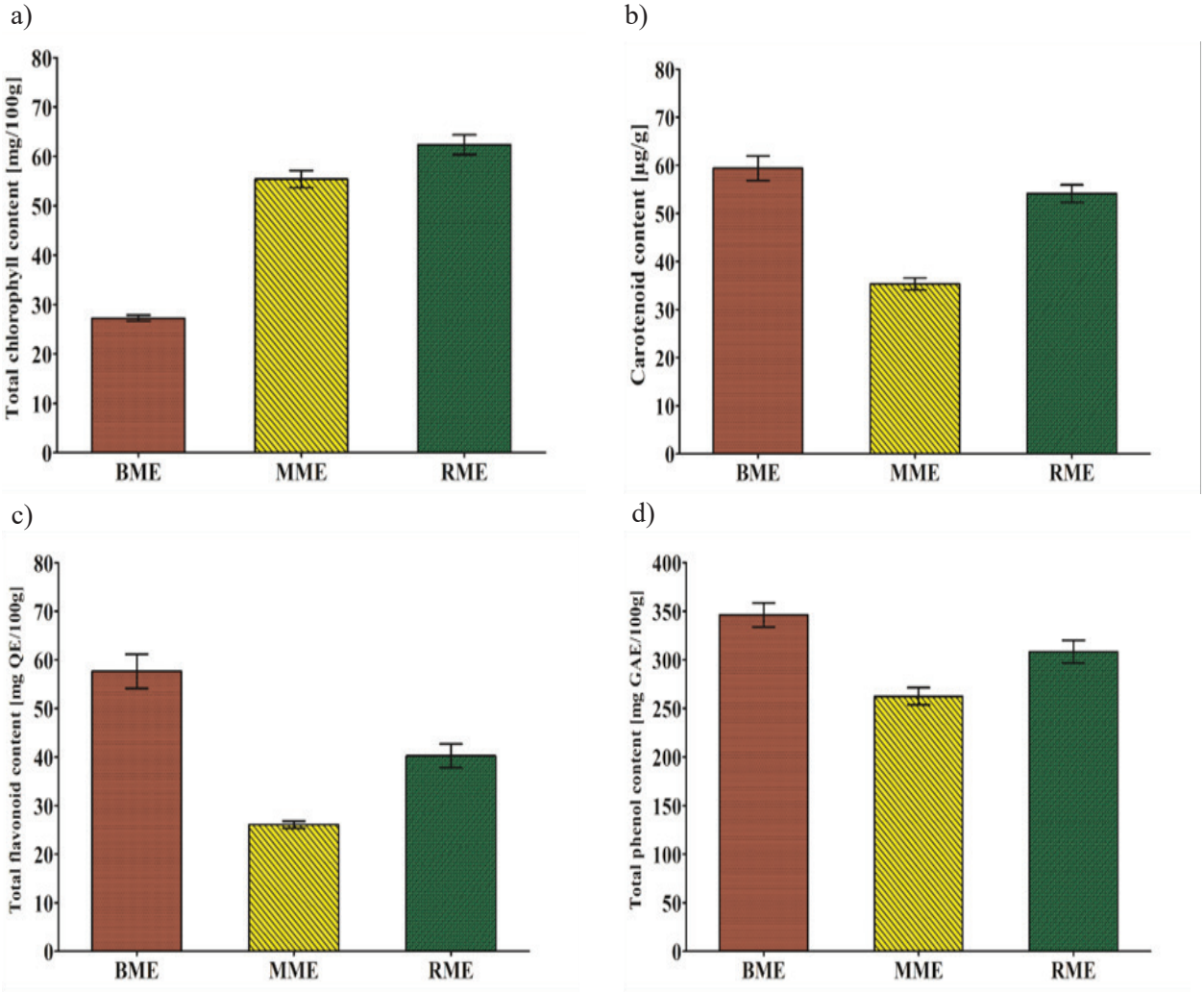


Figure 2. a) Total chlorophyll content, b) Total carotenoid content, c) Total flavonoid content, d) Total phenol content of beetroot microgreen extract (BME), mustard microgreen extract (MME) and radish microgreen extract (RME) (n = 3 and $p \leq 0.05$).

T a b l e 1

Comparative table of the bioactive contents estimated in this study and other previous studies

Micro-greens	Nutrients	Reported concentrations	Growth conditions/ Requirements	References
Beetroot	Phenols [mg/100 gm]	346.10 ± 12.21	***	Present study
		313.80	Cultivated by Lal Microfarm (Istanbul, Turkey)	Rocchetti *et al.* 2020
		248.56 ± 1.27	Growth conditions[1]	Gunjal *et al.* 2024
		639.85 ± 17.56	Purchased from local market	Stajčić *et al*. 2024
	Flavonoids [mg/100 gm]	57.64 ± 3.514	***	Present study
		17.84 ± 0.14	Growth conditions[1]	Gunjal *et al.* 2024
	Carotenoids [µg/g]	59.39 ± 2.577	***	Present study
		160.00 ± 2.00	Growth conditions[2]	Samuolienė *et al.* 2017
Mustard	Phenols [mg/100 gm]	262.4 ± 8.852	***	Present study
		208.60 ± 3.80	Growth conditions[3]	Xiao *et al.* 2019
		1,889.76 ± 64.8	Growth conditions[4]	De la Fuente *et al.* 2019
	Flavonoids [mg/100 gm]	26.08 ± 0.743	***	Present study
		1.10 ± 0.20	Growth conditions[5]	Ghoora *et al.* 2020b
		89.00 ± 3.00	Wild mustard grown on wet cotton at 25 – 30°C.	Salah *et al.* 2024
	Carotenoids [µg/g]	35.31 ± 1.240	***	Present study
		56.00 ± 3.00	Peat substrate was used for seed germination, which was further grown hydroponically in Hoagland nutrient solution by sub-irrigation method under 32°C temperature and 85% relative humidity.	Min Allah *et al.* 2023
		105.00 ± 0.40	Growth conditions[3]	Xiao *et al.* 2019
		110.00 ± 2.00	Growth conditions[2]	Samuolienė *et al.* 2017
		2242.70 ± 9.35	Growth conditions[4]	De la Fuente *et al.* 2019
	Chlorophylls [mg/100 gm]	55.38 ± 1.742	***	Present study
		359.00 ± 0.25	Growth conditions[2]	Samuolienė *et al.* 2017
		64.70 ± 3.90	Growth conditions[6]	Kyriacou *et al.* 2018
Radish	Phenols [mg/100 gm]	308.30 ± 11.74	***	Present study
		264.80 – 811.2	Growth conditions[3]	Xiao *et al.* 2019
		135.74 ± 0.0	Cultivated on the mixture of coco pith, perlite and vermiculite (2:1:1 ratio) in greenhouse with up to 27°C temperature.	Yadav *et al.* 2019
		145.04 ± 0.0	Growth conditions[7]	Kowitcharoen *et al.* 2021
		298.70 ± 0.0	Microgreens were cultivated in peat moss media in unheated greenhouse under ambient temperature and light.	Dereje *et al.* 2023
		260.27 ± 1.27	Growth conditions[1]	Gunjal *et al.* 2024
	Flavonoids [mg/100 gm]	40.21 ± 2.462	***	Present study
		2.10 ± 0.2	Growth conditions[5]	Ghoora *et al.* 2020b
		39.83 ± 0.0	Cultivated on the mixture of coco pith, perlite and vermiculite (2:1:1 ratio) in greenhouse with up to 27°C temperature.	Bhaswant *et al.* 2023
		57.34	'Koregon red variety' of radish grown on soilless trays having meshes, 600 µmol/m²/s photon flux density, 16/8 hours photoperiod and 20 ± 2°C temperature.	Tilahun *et al.*2023
	Carotenoids [µg/g]	54.12 ± 1.813	***	Present study
		88.00 – 138.00	Growth conditions[3]	Xiao *et al.* 2019
		1,622.89 ± 5.50	Growth conditions[4]	De la Fuente *et al.* 2019
		156.10	Growth conditions[7]	Kowitcharoen *et al.* 2021
	Chlorophylls [mg/100 gm]	62.36 ± 1.992	***	Present study
		71.50 ± 1.90	Growth conditions[6]	Kyriacou *et al.* 2018
		50.90 ± 0.40	Growth conditions[5]	Ghoora *et al.* 2020b
		59.21	Growth conditions[7]	Kowitcharoen *et al.* 2021

***Combination of cocopeat and vermicompost (2:1 ratio), LED of ~1,424.11 lux intensity, 12 hours photoperiod, 16 ± 5°C temperature and 70 ± 5% relative humidity. [1]Grown on soilless medium (burlap), 20 to 22°C temperature, 65 – 67% relative humidity, 0.45 g/L carbon dioxide, 12 hours (light/dark) and 55 µmol/m²/s light intensity. [2]Peat substrate with nutrients and microelements irradiation blue LED light (447 nm wavelength, 100 µmol/m²/s photon flux density 21/17 °C day/night temperature and 50 – 60% relative humidity. [3]Microgreens were cultivated in peat moss media in unheated greenhouses in ambient temperature and light. [4]Grown hydroponically in nutrient solution in an unheated greenhouse with 18°C average daily temperature and no artificial light and 61% relative humidity. [5]Cultivated in vermicompost-enriched soil. [6]Grown on commercial peat based media with Hoagland and Arnon formulations under LED (400 – 700 nm emission wavelength), 300 ± 10 µmol/m²/s photon flux density and 12 h photoperiod. Day/night temperatures of 22/18°C were established with a 12 h photoperiod and 65 – 75% relative air humidity. [7]Cultivated under controlled conditions on sponge under 23°C temperature, 65 ± 5% relative humidity, and up to 1,000 mg/L carbon dioxide concentration.

T a b l e 2

Table represents the Fourier-transform infrared spectroscopy(FT-IR) spectral data of microgreen extracts with corresponding major peaks assignment and the probable phytochemicals present

Microgreen Extract	Frequencies observed [cm]	Tentative functional groups	Propable phytochemicals
Beetroot microgreen extract (BME)	3,377.7 (OH or N–H stretching) 3,010.8 (= C–H stretching) 2,925.2 (CH stretching) 2,854.7 (CH stretching) 1,741.1 (C=O stretching) 1,651.8 (C=N or C = O stretching) 1,460.1 (N=N–O or Ring stretch 1,374.1 (CH_3 deformation) 1,321.5 (N=N–O or CF_3 stretch) 1,158.8 (C=S stretching) 1,075.4 (C–N or sulphonic acids) 720.0 (– $(CH_2)_n$ –) 618.8 (S–C≡N or S–C stretch) 522.1 (C_nH_{2n+1} or NO_2 deformation)	Alcohols/ phenols/aromatic amines/amides Aromatic unsaturated hydrocarbons Alkanes Alkanes Ester/ethers Oximes, Imines, benzophenones, amines, β-ketone esters Azoxy compounds/aromatic compounds Isopropyl group Azoxy compounds/aromatic compounds Thiocarbonyl compound Primary aliphatic amines/Sulphonic acids Aliphatic compounds Thiocyanates Alkyl group/Aromatic nitro compounds	Alkaloids, flavonoids, glucoside, glucosinolate, thiocyanates, polyphenols, carboxylic acid containing phytocompounds, etc.
Mustard microgreen extract (MME)	3,383.0 (O–H or N–H stretch) 2,928.7 (C–H stretching) 1,618.3 (C=C stretching) 1,408.3 (C–N stretching) 1,077.0 (SO_3 stretching) 617.1 (S–C≡N or S–C stretching)	Alcohols/ phenols/ aromatic amines Alkane Vinyl ethers Primary amide Sulphonic acid Thiocyanates	Alkaloid, flavonoid, polyphenols, glucosinolates, thiocyanates, sulphonic acid containing phytocompounds, etc.
Radish microgreen extract (RME)	3,370.2 (O–H stretching) 2,923.0 (O–H or N–H stretching) 1,637.8 (C=O stretch or NH_3 deformation) 1,412.5 (S=O stretching) 1,241.8 (C–O stretching) 1,103.9 (C–N stretching) 1,065.7 (C–O or S=O or SO_3 or C–N stretching) 765.3 (C–S stretch) 618.6 (Plane ring deformation or S–C stretching) 537.0 (C–C–CN bend/ ring deformation)	Alcohol or phenol Carboxylic acid or amine salt Amides/ amino acids/ ketone esters Sulphate Epoxide Primary amines Sulphoxide/ sulphonic acid/ primary aliphatic amine Sulphonyl chloride Nephthalene/ pyridines/ thiocyanates Nitriles/ Benzene derivatives	Alkaloid, flavonoid, polyphenols, thiocyanates, carboxylic acid containing phytochemicals.

microgreens (Samuolienė *et al.* 2017). The illumination of 330–440 µmol/m²/s enhanced the carotenoid and antioxidant components in Brassicaceae microgreens (Samuoliene *et al.* 2013; Brazaityte *et al.* 2015). Some studies suggest that microgreens have carotenoid concentrations that are many times higher than those found in mature vegetables (Sinha & Dua 2015; Rizvi *et al.* 2023). Therefore, microgreens are great dietary supplements for individuals looking to add carotenoids to their diet.

Total flavonoid content

Flavonoids fall into the category of plant secondary metabolites, which are synthesised in sunlight. These have with a polyphenolic structure (Panche *et al.* 2016). The total flavonoid content (TFC) of selected microgreen extracts is shown in Figure 2c. BME had the highest flavonoid content, with 57.64 mg/100g quercetin equivalent, followed by RME and MME, which had 40.21 and 26.08 mg QE/100g flavonoid content, respectively. Various concentrations of quercetin were used to plot the standard curve with $y = 0.0442 + 0.0069x$ and $R^2 = 0.9969$ to estimate the TFC in microgreens (Suppl. Figure 1). Flavonoids are powerful antioxidants that can combat reactive oxygen species and reduce the peroxidation of low-density lipids (Kwon *et al.* 2020). Due to their potent therapeutic effects against various diseases and anti-inflammatory responses, flavonoid-based diets are gaining global interest for their bioactive efficacy (Sharifi-Rad *et al.* 2022). In our study, we found that BME had the highest flavonoid content, which was also comparatively higher than 20 mg/100g as reported previously in mature beetroot (Takács-Hájos & Vargas-Rubóczki 2022). The radish microgreens were previously reported to have 39.83 mg/100 g of total flavonoids, comparable to our findings (Bhaswant *et al.* 2023). Another study found 1.1 and 2.1 mg/100 g of TFC in mustard and radish microgreens, which were significantly higher in our findings (Ghoora *et al.* 2020b). Interestingly, the TFC in our study was approximately 20–25 folds higher than the previously reported flavonoid contents of radish and mustard microgreens, as discussed in Table 1. Several other microgreens have been studied only to find the TFC of about 3.71 and 1.86 mg/100 g in mung bean and lentil microgreens genotypes, respectively (Priti *et al.* 2021). Light provides a crucial environment for plants that affect growth and morphogenesis and promotes flavonoid synthesis by modulating the enzymes involved in flavonoid metabolism (Qin *et al.* 2024). Several studies have proved that different intensities of light and varying photoperiods indirectly control flavonoid accumulation by regulating the genes involved in flavonoid synthesis (Jaakola 2013).

Total phenol content

Phenols are a diverse group of plant secondary metabolites containing aromatic ring/s and one or more hydroxyl groups. Polyphenols are responsible for the sensory attributes, such as the flavour, taste, astringency and bitterness in plants (Cheynier 2012; Ulla *et al.* 2016; Tomas *et al.* 2021). The phenylpropanoid metabolic pathway produces phenolic compounds, starting with trans-cinnamic acid derived from L-phenylalanine. Under oxidative stress, phenolic compounds are oxidized to form quinone, which is a potent antioxidant (Xiao *et al.* 2014). Vegetables owe their unique taste, flavour and astringency due to the presence of tannins and phenolic acids (Ulla *et al.* 2016). Phenols have bioactive properties, including antioxidant and anti-inflammatory activities, and help in improving glucose homeostasis and other metabolic conditions (de Sales *et al.* 2012; Rodríguez-Pérez *et al.* 2019). The total phenol content (TPC) found in methanolic extracts of microgreens (BME, MME and RME) were 346.11, 262.4 and 308.3 mg GAE/100 g respectively (Figure 2d). The standard curve used for estimating the TPC is shown in Suppl. Figure 2. These phenolic profiles are substantially higher than those of mature vegetables, such as broccoli, kale, and Brussels sprouts, and even their mature equivalents (Li *et al.* 2018). The TPC identified in beetroot microgreens were comparatively higher than in the previous study (Bhaswant *et al.* 2023). However, for mustard and radish microgreens, different amounts of TPC were reported in literature, as given in Table 1. The data reported about total phenols by Dereje *et al.* 2023 agreed with 262.4 and 308.3 mg/100 g of mustard and radish microgreens, respectively, from our findings. The mustard microgreens were previously studied as 180 mg GAE/100 g (Min Allah *et al.* 2023) and 150 mg GAE/100 g (Xiao *et al.*

2015) of TPC, which is comparatively lesser than our findings. However, the soluble polyphenols content was higher by around 7-fold in radish and mustard microgreens when cultivated hydroponically in the presence of a nutrient solution (De la Fuente *et al.* 2019). These variations in phenolic content could be attributed to various intrinsic and extrinsic factors, including growth conditions, crop species, harvest phases and postharvest modules (Kowitcharoen *et al.* 2021). Interestingly, the TPC of radish microgreens is many times higher than that of mature radish. The TPC investigated in both the root and leaf of 85-day-old beetroot has previously been found to be less than 200 mg GAE/100 g, which is significantly lower than the phenolic profiles observed in beetroot microgreens in this work, which are greater than 250 mg GAE/100 g (Takács-Hájos & Vargas-Rubóczki 2022). A diet high in phenolic content has been found to demonstrate analgesic and anti-inflammatory effects, as well as contribute to glucose homeostasis in individuals who consume microgreens (Alrifai *et al.* 2019; Tan *et al.* 2020).

DPPH scavenging activity

An antioxidant is a substance that noticeably suppresses or delays the oxidation process of a compound when present in lower concentrations, as compared to the oxidisable substrate (Halliwell & Gutteridge 1995). According to Halliwell & Gutteridge (1995), these substances are responsible for scavenging the free radicals and reducing the harmful effects produced by oxidants before they cause

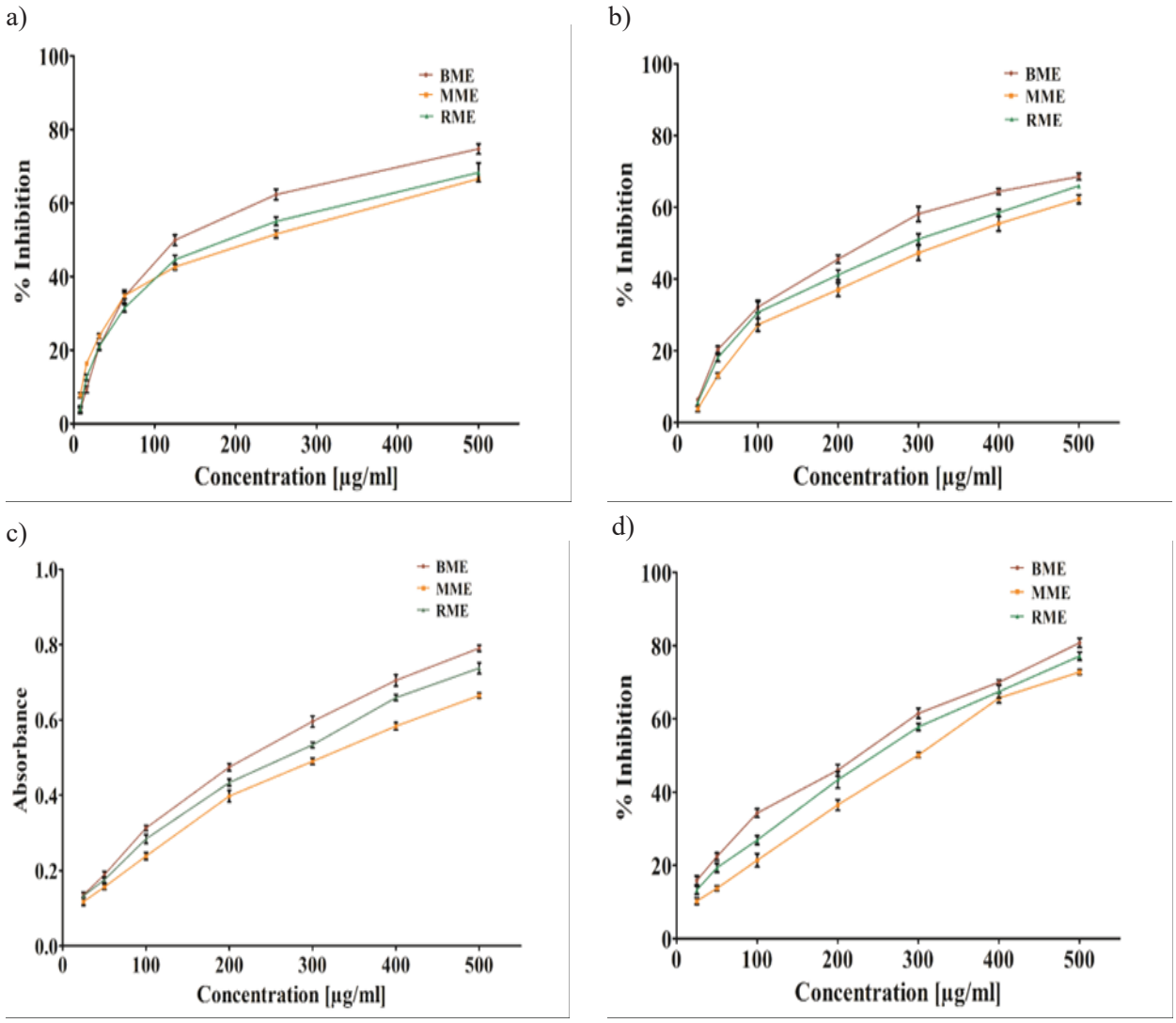


Figure 3. a) 2,2-diphenyl, 1-picrylhydrazyl (DPPH) scavenging activity, b) Hydrogen peroxide (H2O2) scavenging activity, c) Reducing power assay, d) Total antioxidant capacities of beetroot microgreen extract (BME), mustard microgreen extract (MME) and radish microgreen extract (RME) (n = 3 and $p \leq 0.05$).

damage to the cells and lead to conditions like cancer (Shebis *et al.* 2013). Based on the 2, 2-diphenyl, 1-picrylhydrazyl (DPPH) assay conducted on the methanolic extract of microgreens (BME, MME and RME), BME showed the highest antioxidant capacity against DPPH free radicals compared to the other microgreens (Figure 3a). At 500 µg/mL concentration, the scavenging activities of BME, MME and RME were found to be 74.67, 66.53 and 68.26%, respectively. The percentage of DPPH scavenging by L-ascorbic acid was found to be 80.67% (Suppl. Figure 3). However, at lower concentrations, MME showed better scavenging activity than BME and RME. The IC_{50} values of BME, MME and RME were 137.9, 192.8 and 180.5 µg/mL, respectively. The IC_{50} of the L-ascorbic acid standard was determined to be 22.09 µg/mL. Hence, the radical scavenging properties of microgreen extracts observed in BME> MME> RME order. The antioxidants (polyphenols and flavonoids) in microgreens donate hydrogen or electrons, which scavenge the radicals produced by DPPH (Bobo-García *et al.* 2015). The IC_{50} values of microgreen extracts indicate that they possess promising antioxidant activities. Moreover, the highest scavenging activity possessed by BME can be attributed to betalains. Betalains have exceptional antioxidant capacities, up to 2 times that of anthocyanin, up to 7.5 times that of ascorbic acid, and many folds higher than catechin and rutin, as explained by *in-vitro* studies (Cai *et al.* 2003; Gliszczyńska-Świgło *et al.* 2006; Gengatharan *et al.* 2015).

Estimation of hydrogen peroxide scavenging

Hydroxyl radicals (OH•) are produced when hydrogen peroxide dissociates, leading to cellular and nuclear damage that ultimately causes cell death (Jakubczyk *et al.* 2020). Antioxidants can donate hydrogen, which converts hydroxyl radicals (OH•) to water molecules and thus prevents the detrimental health effects induced by free radicals (Pleh *et al.* 2021). Microgreens are rich sources of antioxidants, including polyphenols, flavonoids and carotenoids. In this study, the H_2O_2 percentage scavenging activity was found to be highest in BME, followed by RME and MME, with values of 68.55, 65.96 and 62.26%, respectively, at the maximum concentration of 500 µg/mL (Figure 3b). The IC_{50} values of BME, MME and RME were 223.7, 318.5 and 271.1, respectively. However, at 100 µg/mL, the L-ascorbic acid had an H_2O_2 scavenging activity of 83.3% (Suppl. Figure 4). The IC_{50} value of L-ascorbic acid was 36.18 µg/mL. The great antioxidant potential of microgreens must be attributed to the significant amounts of phenolics, flavonoids and carotenoid contents they contain (Ghoora *et al.* 2020b).

Estimation of reducing power

The reducing power of a compound is strongly linked to its antioxidant potential. This reflects its ability to donate electrons/hydrogen and reduce compounds associated with reactive oxygen species (ROS) producing pathways. The degree of colour change of the reagent (potassium ferricyanide) from yellowish green to blue indicates the antioxidant potential of the extract (Jayanthi & Lalitha 2011). In this study, the highest reducing power was found in BME, followed by RME and MME, which were 0.79, 0.74 and 0.66 respectively at 500 µg/mL (Figure 3c). The reducing power of standard L-ascorbic acid was 0.83 at 100 µg/mL (Suppl. Figure 5). The antioxidants present in microgreen extracts reduce the ferricyanide Fe^{3+} complex to Fe^{2+} complex, which was measured by taking the absorbance at 700 nm. Every time the concentration of a microgreen extract increases so does its reducing power.

Estimation of total antioxidant capacity

A phosphomolybdate assay was done to determine the TAC of microgreen extracts. During this process, Mo (VI) was reduced to Mo (V) by the extracts' antioxidant properties, resulting in a green-colour phosphate-Mo (V) complex (Rahman *et al.* 2015). The changes in absorption resulting from this reduction were measured at 695 nm. BME, MME, and RME were assessed for their total antioxidant capacity in this study, with the results presented as a percentage inhibition. BME, RME and MME exhibited percentage inhibitions of 80.75, 77.14 and 72.76%, respectively (Figure 3d). The percentage inhibition shown by standard L-ascorbic acid was 91.66% (Suppl. Figure 6).

Characterization of phytocompounds by FT-IR

The Fourier transform infrared (FT-IR) spectra of potential metabolites were obtained using the

extracts of beetroot, mustard and radish microgreens (Figure 4). The absorbance peaks of spectra were analysed to identify the functional groups as per the study by Kustiati *et al.* (2022). The FT-IR data showed different peaks representing the functional groups, as indicated by the wavenumber [per cm] and percentage of absorbance. The functional groups found in BME, MME and RME are presented in Table 2, and their peaks represent the O-H stretch or O-H vibrations and NH stretch of aromatic primary amines and amides, which are found in all the microgreen extracts studied. However, the extracts also contained peaks representing the presence of CH stretch and OH stretch of alkanes and carboxylic acids, respectively. In addition, the peaks marked the C=O stretch corresponding to esters or saturated aliphatic metabolites, as well as the aromatic rings including the benzophenones, which are known for their antioxidant potential as a phenolic constituent. The presence of sulfoxide, which acts as a neuroprotective antioxidant (Sanmartín-Suárez *et al.* 2011; Kustiati *et al.* 2022), was also indicated by the SO stretching in RME.

The presence of thiocyanates, which act as therapeutic agents and contribute to host defence due to their antioxidant properties, is indicated by the S-C stretching (Chandler & Day 2012). The FT-IR analysis of microgreen extracts (BME, MME and RME) revealed the presence of various functional groups including alcohols, phenols, alkanes, amines, amides, esters, ethers, carboxylic acids, amino acids, benzene rings, and other groups (Kähkönen *et al.* 1999; Liu & Ng 2000). These are likely to be multiple phytochemicals and metabolites that possess antioxidant activities and may belong to different classes, such as polyphenols, flavonoids, alkaloids, glucosinolates, and their derivatives (Poojary *et al.* 2015).

SEM-EDS study to determine morphological and elemental parameters of microgreens

The study involved observing the leaf lamina of each microgreen and analysing its morphological

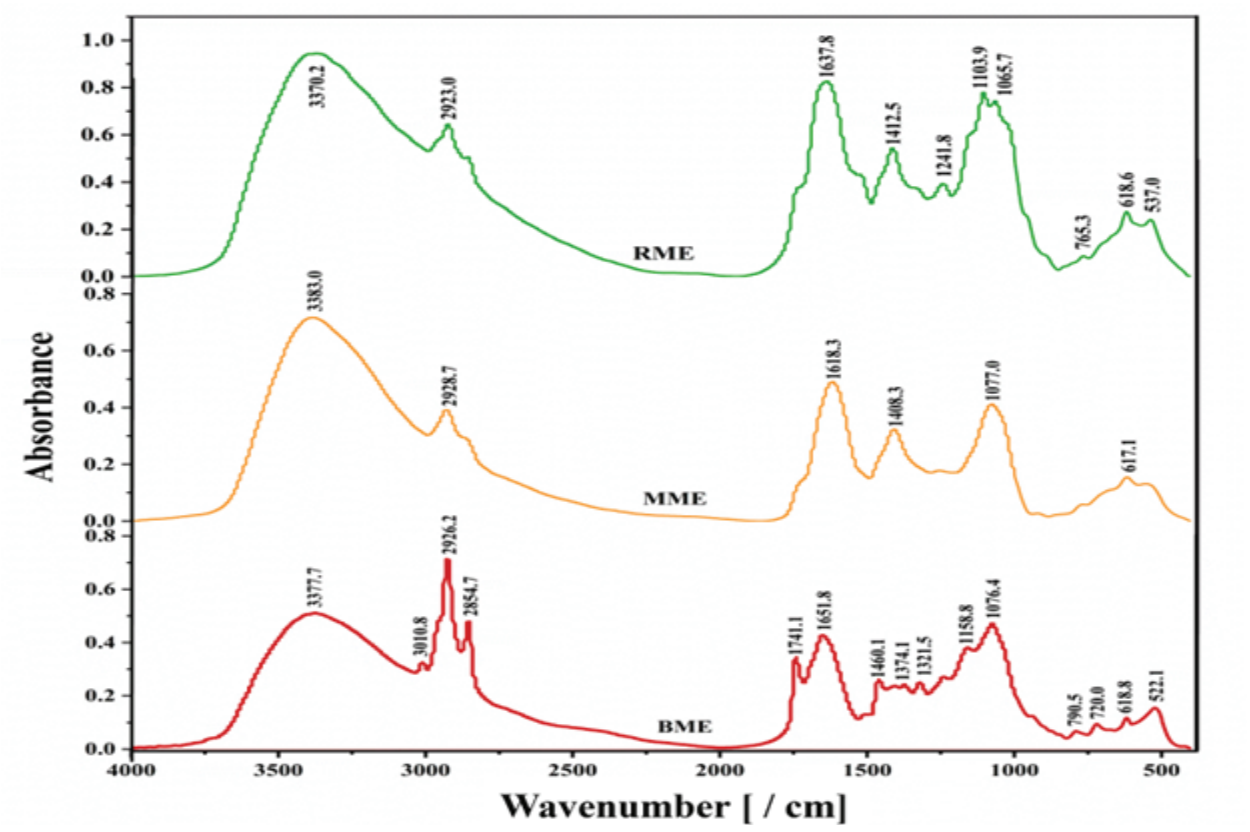


Figure 4. Fourier transform infrared spectra with peaks showing different frequencies [1/cm] corresponding to the phytochemicals present in the methanolic extract of microgreens beetroot microgreen extract (BME), mustard microgreen extract (MME) and radish microgreen extract (RME) – see Table 1.

and elemental details through Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray Spectroscopic (EDS) analysis respectively (Figure 5a-c and Figure 6). The samples for SEM analysis were prepared in triplicate, but no significant differences were observed during imaging. The SEM examination of microgreen leaves showed the epidermal cells along with the stomatal impressions, including stomatal openings, pore sizes and the length, width and turgidity of guard cells. The epidermal cells were seen slightly shrunk, which might have occurred during the fixation and dehydration processes. At 500X magnification, the stomatal density of approximately 545.0, 632.7 and 725.0/mm^2 was observed in beetroot, mustard and radish microgreens respectively (Figure 5d-f). At 2000X magnification, the margins of the guard cells were visible, allowing the precise measurement of length, width and stomatal aperture/ pore size. At 2000X magnification, a 10 µm scale was set to measure the morphological details of the stomatal apparatus using Image-J software. The stomatal pore size measured from the abaxial leaf surface of beetroot, mustard and radish microgreens were 2.31, 2.52 and 2.54 µm respectively (Figure 5g-i). Additionally, the average length and width of guard cells in beetroot were observed as 13.47 and 8.31 µmat 2000X magnification (Figure 5g). In the case of mustard, it was reported as

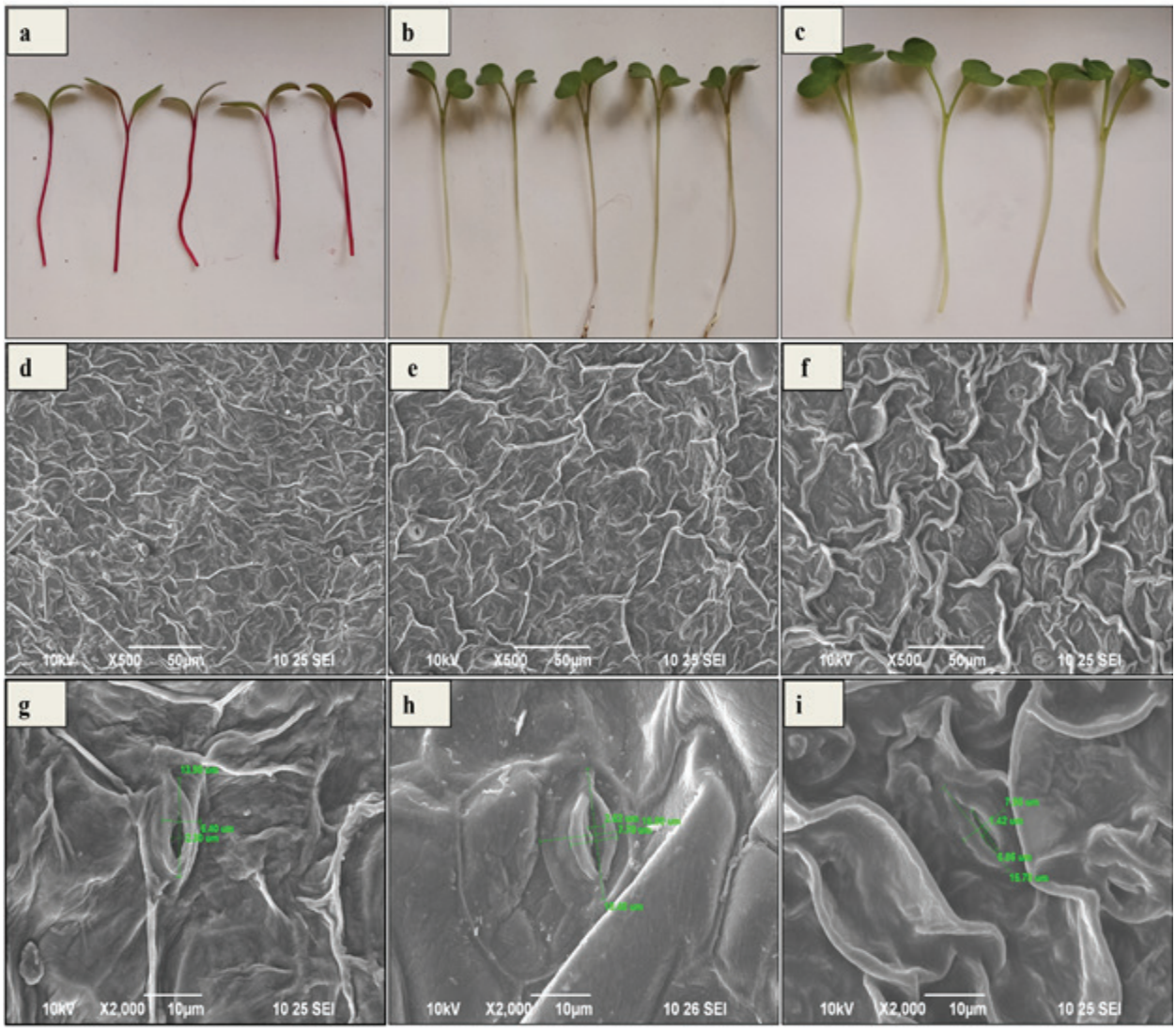
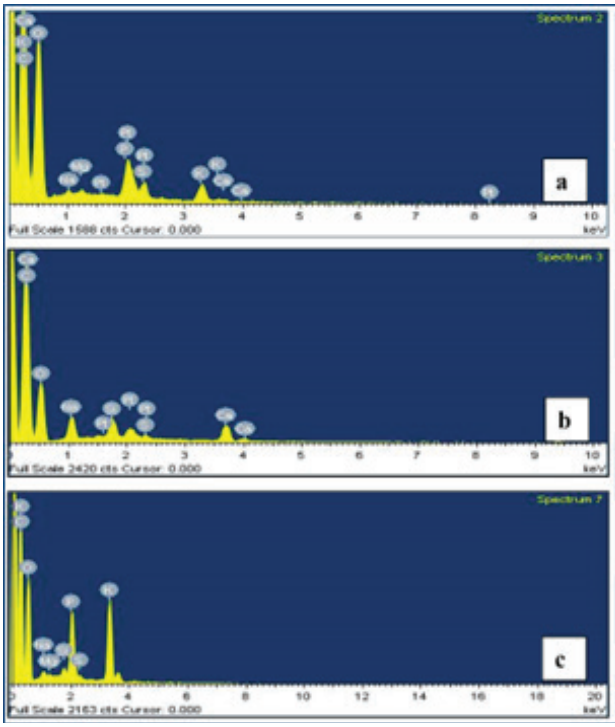


Figure 5. Scanning electron micrographs showing surface morphology of chemical fixed microgreen leaves: beetroot, mustard and radish microgreen leaf for scanning electron microscopic analysis (a, b, c); Abaxial surface of beetroot, mustard and radish microgreen leaf showing stomatal density at 500X magnification (d, e, f); Magnified view of abaxial surface showing stomatal aperture and guard cell morphology of beetroot, mustard and radish microgreen at 2000X (g, h, i).



d

Elements (weight %)	Beetroot	Mustard	Radish
Carbon	42.15	57.82	35.76
Oxygen	50.72	35.10	49.90
Sodium	0.31	2.44	0.79
Magnesium	0.28	ND	0.32
Phosphorus	0.65	ND	4.72
Sulphur	0.87	0.22	0.24
Potassium	1.15	ND	7.73
Calcium	0.12	1.66	ND

Figure 6. Energy-dispersive X-ray spectra with absorption peaks of various elements detected in a) beetroot microgreens, b) mustard microgreens; c) radish microgreens, d) weight percentage of elements in each microgreen.

18.63 and 8.33 µm whereas 16.11 and 9.02 µm was the average measurement of guard cells observed in radish microgreen leaves (Figure 5h-i).The elemental analysis by EDS spectra is illustrated in Figure 6a-c. The study found that radish microgreens had the highest potassium concentrations, making up approximately 7.73% of their total weight compared to other microgreens. This makes radish microgreens a great dietary supplement for potassium intake, which can help control blood glucose levels and improve blood pressure (He & MacGregor 2008). Beetroot microgreens were detected to have sodium, magnesium, phosphorus, sulphur, potassium and calcium, as shown in Figure 6d. Overall, the microgreens studied have a good mineral profile and can be a great addition to our daily diet to ensure adequate mineral intake.

CONCLUSIONS

Microgreens are tender seedlings of various herbs, spices and vegetables. They are used as garnishing components in fine dining and have emerged as a new class of nutritionally important crops in recent years. Microgreens are many folds higher in terms of nutrients as compared to their mature counterparts. Our study investigated the higher content of total chlorophyll, carotenoids, flavonoids, and phenolic content in all three microgreens. We found that microgreens have high antioxidant potential, as demonstrated by their radical scavenging activities in DPPH and H_2O_2 assays, as well as other antioxidant assays. Fourier transform infrared (FT-IR) fingerprinting of microgreen extracts revealed the presence of functional groups associated with bioactive secondary metabolites that exhibit antioxidant potential. Scanning electron microscopy and energy dispersive X-ray spectroscopic (SEM-EDS) analysis identified the morphological details of each microgreen, as well as the identification of various functionally important elements. Therefore, we recommend consuming these microgreens as a complement to mature leafy vegetables for a nutritionally remarkable and wholesome diet. Microgreens have potential as nutraceuticals for special clinical needs based on oxidative stress conditions, such as degenerative, inflammatory and chronic disorders. Further research and analytical studies on microgreens are essential to elucidate the bioactive compounds

present and to understand the specific mechanisms through which they contribute to medicinal and health benefits. Additionally, *in vivo* studies on their bioavailability are necessary to determine how these benefits can be effectively translated to human applications. Despite their potential, microgreens remain relatively underappreciated across various societal segments, particularly among middle and lower-income groups, resulting in a limited consumer base. The higher production costs also contribute to their elevated market prices. Moreover, challenges such as post-harvest management, limited shelf life, the need for optimal growing conditions, and susceptibility to bacterial and fungal contamination further complicate microgreen cultivation.

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