



Quantitative analysis of phytoconstituents and *in-vitro* biological activities of nine edible microgreens from West Bengal

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Microgreens are promising sources of nutrition in the daily diet, which have gained attention for their rich phytochemical profile and associated health benefits. The current research aims to quantify the total phytoconstituent content and determine the *in vitro* anti-oxidant, anti-diabetic, and anti-inflammatory activities of nine different microgreens: chia, coriander, carrot, fenugreek, radish, spinach, sunflower, turnip, and beetroot. The quantification of phytoconstituents in cultivated microgreens, followed by *in vitro* biological activity profiling, was conducted. Significant differences in total phenolic, tannin, flavonoid and pigment content among the samples ($P < 0.001$) were found. Sunflower microgreen proved to be the most potent anti-oxidant agent ($IC_{50} = 48.19 \mu\text{g/mL}$). Chia microgreens recorded the highest chlorophyll content at $21.27 \mu\text{g/g FW}$. Fenugreek microgreens demonstrated potent α -amylase inhibitory activity with an IC_{50} of $11.01 \mu\text{g/mL}$. Chia and coriander microgreens were promising anti-inflammatory agents with IC_{50} values of 23.09 and $28.40 \mu\text{g/mL}$. Principal Component Analysis elaborates the correlation between morphological characters, phytoconstituent content and pharmacological activities of the microgreens. The findings suggest that these microgreens contain health-promoting phytochemicals and may serve as functional foods for managing oxidative stress, inflammation, and hyperglycemia. Further, microgreens can be examined for their *in vivo* biological activities, followed by their recommendation for daily dietary intake.

Keywords: Alpha-amylase inhibition assay, DPPH scavenging assay, Egg albumin denaturation, Microgreens, Pharmacological assay, Phytoconstituent

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Introduction

Plants are one of the most important sources of modern drug substances. According to reports of the World Health Organization, 80% of the world's total population relies on plants and natural sources for their daily nutrition and maintenance of well-being. Different parts of plants are widely used by human beings to ameliorate several diseases^{1,2}. Plants mostly act as food substances due to their different parts like rhizome, tubers, leaves and fruits. For example, turmeric and ginger (rhizomes), spinach (leaves), mango, jackfruit (fruits), sweet potato (tuber), and

saffron (stamen) have been well explored by human beings³. Even small saplings from plants are also edible and are nutritious. Microgreens are freshly germinated plant saplings with the first pair of cotyledon leaves and a small epicotyl. They have gained tremendous importance in modern days due to their rich Pharmacological attributes and hence are called 'superfood'⁴. Generally, these microgreens, along with several sprouts, are consumed for their health benefits, including anti-oxidant effects, anti-inflammatory effects, immunomodulatory effects, anti-diabetic effects, anti-hyperlipidemic effects and hepatoprotective effects⁵⁻⁸.

These properties of boosting and managing health are attributed to the presence of several important phytoconstituents in them. These phytoconstituents include a wide group of polyphenolic compounds like flavonoids, phenolic acids, tannins, and anthocyanins. Other important bioactive compounds include alkaloids, terpenoids, glycosides, carotenoids, phytosterols and volatile oil^{5,7}.

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Polyphenolic compounds have a hydroxyl group in their parent moiety. The hydroxyl group donates a proton to the electron-rich free radicals produced in our body by different cellular metabolic pathways. This stabilises and scavenges Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). In addition, they also mediate different pro-inflammatory cytokines like IL-6, IL-3, TNF- α and reduce the overexpression of immunogenic cells. Thus, these polyphenols contribute to anti-oxidative and anti-inflammatory effects^{9,10}.

Similarly, alongside the management of oxidative stress in our body, several alkaloids obtained from plants modulate different carbohydrate-metabolising enzymes, such as α -amylase, α -glucosidase, glucose-6-phosphatase, hexose kinase, IRS-1, and GLUT-4. Thus, stimulating the increased uptake of blood glucose in the cells of skeletal muscle and adipose tissue, and inhibiting the uptake of glucose from the intestine during carbohydrate digestion^{11,12}.

Therefore, the present study investigates the morphological and physical characteristics of nine different microgreens, and quantification of key phytochemicals such as Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Total Tannin Content (TTC) and Total Pigment Content including estimation of Chlorophyll a, Chlorophyll b, Total Chlorophyll, Lycopene, Beta Carotene, Total Carotenoid, Anthocyanin content of Nine different edible microgreens. Thereby correlating their content with the in vitro Pharmacological activities of these microgreens.

Although quite a few studies have highlighted the nutritional and pharmacological richness of microgreens, the studies are confined to a smaller number of species. Studies on microgreens were conducted under varied global conditions, lacking uniformity^{8,13,14}. This study bridges that gap by quantifying the bioactive components and pharmacological profile of nine different microgreens grown in Bengal under similar conditions.

Materials and Methods

Chemicals required

All chemicals used were of analytical grade and were procured from authorised suppliers. The chemicals were procured from Emplura and Emparta (Chennai). The standard chemicals were procured from Sigma-Aldrich, including quercetin, gallic acid, tannic acid, aspirin and ascorbic acid.

Instruments used

The instruments used included a UV-Visible Spectrophotometer (JASCO Corporation, V-630, Tokyo, Japan); Digital Weighing Balance (Wensar Weighing Scales Ltd., PGB-200, Maharashtra, India); pH meter (SYSTRONICS, 335, Ahmedabad, India); Incubator (Remi Elektrotechnik Ltd., Mumbai, India); Digital Thermohygrometer (HTC-1, HTC Instruments, India) and Hot Water Bath (SSFW, LWB-12H/D, Kolkata, India).

Seed sample collection

About 150 seeds of respective microgreens, including chia (*Salvia hispanica* L.), coriander (*Coriandrum sativum* L.), carrot (*Daucus carota* L.), fenugreek (*Trigonella foenum-graecum* L.), radish (*Raphanus sativus* L.), spinach (*Spinacia oleracea* L.), sunflower (*Helianthus annuus* L.), turnip (*Brassica rapa* L.), and beetroot (*Beta vulgaris* L.) were procured from the local market of Sodepur, West Bengal, India in November 2024. The physical purity of the seeds was checked from the details mentioned on the company labels. Seeds were stored in air-tight containers under dry, cool conditions ($\sim 15 \pm 2^\circ\text{C}$) before sowing in December.

Cultivation of microgreens

The different microgreens were grown in soil of Sodepur, North 24 Parganas, West Bengal, between December and January for 7-14 days in an open environment at a temperature of $20\text{--}25^\circ\text{C}$ and a relative humidity of 65–70% as measured by a digital thermohygrometer. Plants, when germinated to the two-leaf condition, were harvested and dried. The number of plants that germinated was also calculated. Harvesting was done during the early hours of the day (8:00–9:00 AM) to minimise moisture loss due to transpiration by using aseptic gloves to prevent external microbial contamination. The heights of leaves, shoots, and roots, as well as the leaf surface area, of 25 mature microgreens were measured. After harvesting, the microgreens were shade-dried under ambient room conditions ($25 \pm 2^\circ\text{C}$, protected from direct sunlight) in a well-ventilated, dust-free environment for 14 days. No artificial heat source was provided for the drying process. Drying was continued until a constant weight was achieved. The moisture content was measured by measuring the weight of the fresh and dried sample using the given formula¹⁵:

$$\text{Moisture content(\%)} = \frac{\text{Fresh Weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100\%$$

Plant extract preparation

The dried plant material was pulverised into coarse powder using a pestle and mortar. About 2 g of the powdered sample of each microgreen was taken and macerated in 20 mL of methanol for 7 days at a controlled temperature of 30±1°C in the laboratory incubator with periodic shaking. The sample was sonicated daily for 30 minutes at 30°C for 7 days. The macerated product was filtered and dried to a solid residue at room temperature and stored.

Preliminary phytoconstituent testing

The presence or absence of phytoconstituents was determined using different *in vitro* chemical testing procedures¹⁵. Alkaloid was estimated by Dragendroff's test and Mayer's test, flavonoids by Shindona Test and Alkaline reagent test. The ferric chloride test was used to determine phenolic compounds and tannins.

Glycoside was estimated by the Keller-Killiani Test¹⁶. A foam test was used to determine saponins. Terpenoid was tested using chloroform and conc. H₂SO₄¹⁷.

Total phenolic content determination

The total phenolic content (TPC) was determined using the established procedure¹⁸. Different concentrations of gallic acid were used to form a calibration curve (20, 40, 60, and 100 mg/mL) using 5 mL of 10% Folin-Ciocalteu and 4 mL of 7% NaCO₃ (Sodium carbonate) and measuring absorbance at 760 nm. Similarly, plant extract of 0.01, 0.05, and 0.1 mg/mL was used to determine TPC.

Total tannin content calculation

The established procedure of determination of Total Tannin Content (TTC) was performed¹⁸. Tannic acid was used to form a calibration curve (20, 40, 60, 80, 100 mg/mL), and absorbance was measured at 725 nm. The TTC of plant extract 0.01, 0.05, and 0.1 mg/mL were determined.

Total flavonoid content calculation

The established procedure using quercetin as a standard was used to determine the calibration curve¹⁸. Plant extract of 0.01, 0.05, and 0.1 mg/mL concentrations was used to determine TFC at 510 nm.

Total pigment content

Chlorophyll a, Chlorophyll b, Total chlorophyll content and Total carotenoid content were determined by acetone (80%) as a solvent system¹³. Similarly, anthocyanin was estimated using a methanol, HCl, and water (90:1:9 v/v) mixture, as described in the following equation in µg/g FW¹³. The readings were taken in triplicate.

$$\text{Anthocyanin Content: } ((0.0821A_{534}) - (0.00687A_{643}) - (0.002426A_{661}) \times 5)$$

$$\text{Chlorophyll a content: } (12.25A_{663.6}) - (2.79A_{646.6})$$

$$\text{Chlorophyll b content: } (21.3A_{646.6}) - (5.1 \times A_{663.6})$$

$$\text{Total chlorophyll content: } (7.15 \times A_{663.6}) + (18.71 A_{646.6})$$

$$\text{Total carotenoid content: } [(1000 \times A_{470}) - (1.82 \times \text{Chlorophyll a}) - (85.02 \times \text{Chlorophyll b})]/198$$

β-carotene and lycopene were calculated using two established procedure^{18,19} in mg/L by recording their absorbance at three different wavelengths in acetone-hexane (4:6v/v) solution. The readings were taken in triplicate.

$$\beta\text{-carotene: } 4.367A_{450} - 2.947A_{503}$$

$$\text{Lycopene: } 3.521A_{503} - 0.587A_{450}$$

DPPH free radical scavenging assay

Anti-oxidant activity of plant extract was estimated using an established procedure²⁰. 0.1 mM, 0.01 mM and 0.05 mM DPPH solutions were used to determine the standard curve. 3 mL of 0.1 mM DPPH solution, 100 µL of plant extract solution (test - of conc. 0.01, 0.05, and 0.1 mg/mL) or methanol (control); ascorbic acid (positive control) was used to determine the absorbance at 517 nm. The percentage scavenging was calculated by

$$\% \text{ Scavenging} = \frac{A_C - A_T}{A_C} \times 100\%$$

Where, A_C = Absorbance of control solution (0.1 mM DPPH) and A_T = Absorbance of Test solution.

Anti-diabetic potential

An established procedure to determine α-amylase inhibition activity was performed²¹. A sample solution was prepared at a concentration of 0.1 mg/mL and was diluted to 0.05 and 0.01 mg/mL. To the sample

solution (different concentration of plant extract – Test; Quercetin – positive control, Methanol – Control), 0.5 mg/mL α -amylase solution, 1% starch solution and 0.2 M phosphate buffer (pH=6.9) were incubated for 5 minutes, followed by the addition of 1% 3,5-Dinitrosalicylic acid to stop the reaction. The absorbance of this solution was measured at 540 nm. The anti-diabetic Percentage is calculated by

$$\% \text{ Anti - diabetic potential} = \frac{A_C - A_T}{A_C} \times 100\%$$

Where, AC = Absorbance of control solution and AT = Absorbance of Test solution

Anti-inflammatory potential

The anti-inflammatory potential was recorded by conducting an egg albumin denaturation assay with an established procedure²². 0.2 mL egg albumin, 2.8 mL phosphate buffer (pH=6.4), 0.2 mL sample (Positive control: Aspirin, Test: Plant sample, Control) at concentrations 0.1, 0.05, 0.01 mg/mL were incubated for 10 minutes at 27°C followed by heating in a water bath at 70°C for 10 minutes. The absorbance was determined at 660 nm. Anti-inflammatory potential was estimated using the formula:

$$\% \text{ Anti - inflammatory} = \left(\frac{A_T}{A_C} - 1 \right) \times 100\%$$

Where, A_C = Absorbance of control solution and A_T = Absorbance of Test solution

Statistical analysis

GraphPad Prism 10.4.1 was employed to conduct the two-way ANOVA studies, plot graphs, and perform the Principal Component Analysis (PCA). PCA was performed to analyse the correlation between morphological traits, phytoconstituent concentrations, and biological activities among the nine microgreens and to determine how the effect of phytoconstituents influences the biological activity of the microgreens. Microsoft Excel 2019 was employed for all calculations and plotting the calibration curve.

Results

The microgreens were grown in a controlled laboratory environment with periodic watering.

Germination details

The germination rate and details of germination for different microgreens are presented in Table 1. A

Table 1 — Botanical and germination details of different microgreens

S. No.	Microgreens	Scientific name	Family	Germination (%)	Growing time (days)	Colour of microgreen	Fresh weight (g) [n=25]	Dried weight (g) [n=25]	Moisture content (%)	Physical purity (Label claim) (%)
1	Chia	<i>Salvia hispanica</i> L.	Lamiaceae	97.00	7	Green	3.12±0.65	2.21±0.31	29.167±2.691	99
2	Coriander	<i>Coriandrum sativum</i> L.	Apiaceae	82.11	10	Green	4.57±0.24	2.24±0.25	50.985±4.212	98
3	Carrot	<i>Daucus carota</i> L.		61.43	10	Green	3.14±0.16	1.27±0.21	59.554±3.08	98
4	Fenugreek	<i>Trigonella foenum-graecum</i> L.	Fabaceae	80.50	8	Green	2.91±0.55	1.51±0.19	48.110±2.301	99
5	Radish	<i>Raphanus sativus</i> L.	Brassicaceae	52.31	7	Green	1.78±0.32	1.07±0.15	39.888±1.254	99
6	Turnip	<i>Brassica rapa</i> L.		52.84	7	Green	1.54±0.17	0.98±0.18	36.364±3.135	98
7	Sunflower	<i>Helianthus annuus</i> L.	Asteraceae	49.71	14	Yellowish green	2.04±0.62	1.12±0.24	45.098±2.876	99
8	Beetroot	<i>Beta vulgaris</i> L.	Amaranthaceae	49.73	8	Reddish-green	2.51±0.19	2.07±0.36	17.530±2.950	98
9	Spinach	<i>Spinacia oleracea</i> L.		78.93	8	Green	3.11±0.33	2.15±0.27	30.868±3.459	98

Unpaired t-test between the Fresh Weight and Dry weight shows $P < 0.01$ and hence shows significant differences (***) between them.

pictorial representation of different microgreens cultivated in soil is shown in Fig. 1.

Morphological Evaluation

Although the study primarily focuses on phytochemical properties, morphological characteristics such as shoot height, root length, and leaf area are essential parameters of plant biomass. This may influence phytochemical accumulation.

Fig. 2 represents principal component analysis of the Moisture Content, morphological parameters, dry weight and fresh weight of 25 microgreens of each type. PC1 and PC2 account for 46.23 and 22.13% of the total variability.

Preliminary phytochemical analysis

The phytochemical tests were conducted to determine the presence or absence of different classes of phytoconstituents. The results are shown in Table 2.

Phytochemical quantification of microgreens

Standard curves of quercetin, gallic acid, tannic acid and atropine were prepared at different concentrations. Calibration curves of standard quercetin, gallic acid and tannic acid demonstrated

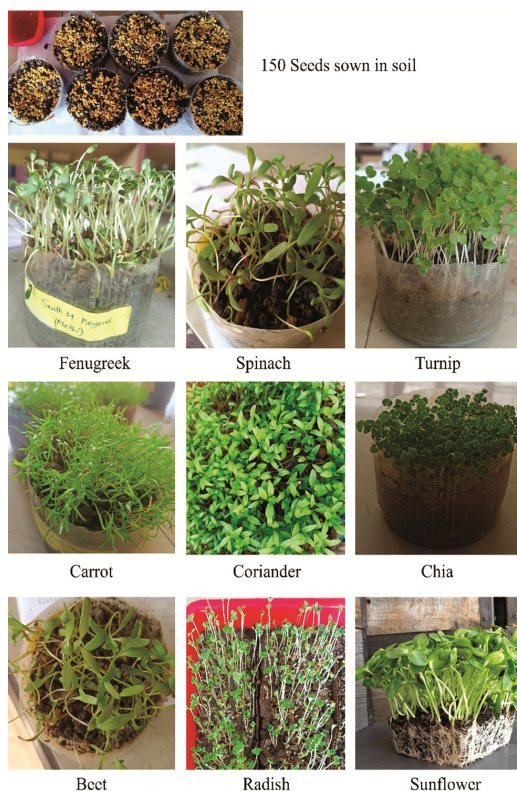


Fig. 1 — Nine different microgreen grown in soil of North 24 Parganas, West Bengal.

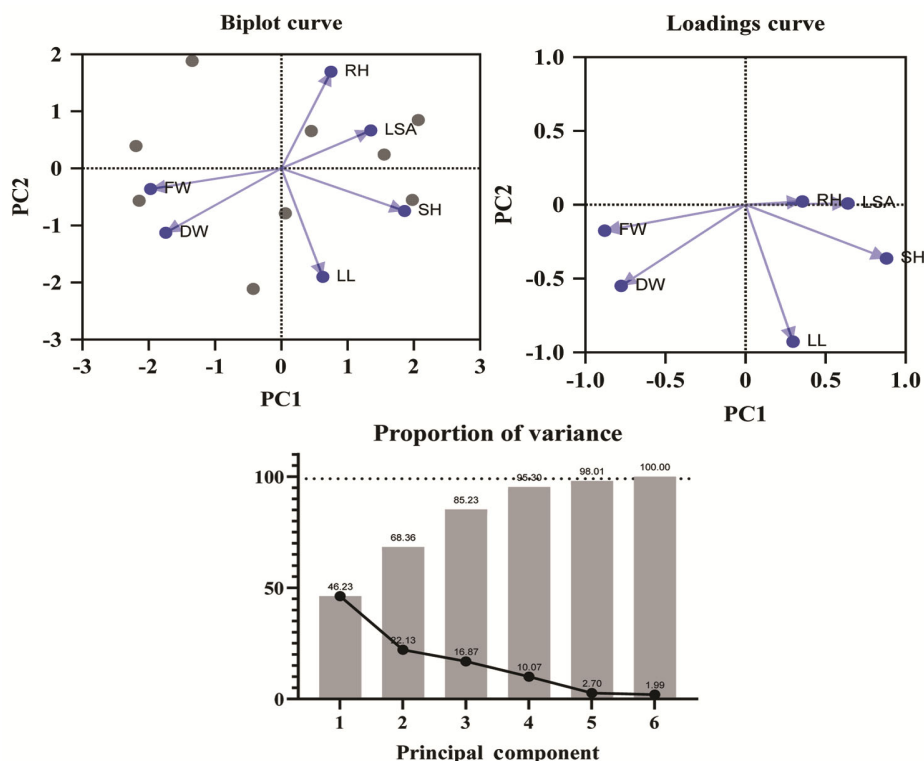


Fig. 2 — Principal component analysis of Shoot height (SH), Root Height (RH), Dry weight of 25 microgreens (DW), Fresh Weight of 25 microgreens (FW), Leaf Surface Area (LSA), Leaf length (LL) showing biplot curve, Loading Curve and Scree plot.

Table 2 — Estimation of the presence of different phytoconstituents in microgreens

S. No.	Microgreens	Phytochemical analysis					
		Alkaloids	Flavonoids	Glycoside	Saponins	Tannins	Terpenoids
1	Chia	+	+	-	-	+	-
2	Coriander	+	+	+	-	+	+
3	Carrot	+	+	-	-	+	+
4	Fenugreek	+	+	-	-	+	-
5	Radish	+	+	-	-	+	-
6	Spinach	+	+	+	-	+	-
7	Sunflower	+	+	-	-	+	-
8	Turnip	+	+	-	-	+	-
9	Beetroot	+	+	-	-	+	-

'+' indicates presence of specific phytoconstituent while '-' indicates absence of the phytoconstituent

high regression correlation (R^2) of 0.9976, 9986, and 9970 with linear equations $y = 0.0024x + 0.1651$ and $y = 0.0123x + 0.0944$, respectively, as represented in Figs. 3a-c.

The estimated value of TFC, TPC and TTC content of the microgreens is represented in Table 3. Significant differences in the phytochemical composition of different microgreens were observed, with $P < 0.01$ using a two-way ANOVA study.

Estimation of pigment content

Table 4 represents the quantification of different pigments like anthocyanin, lycopene and beta carotene. Two-way ANOVA shows that the pigment (chlorophyll and carotenoids) is significantly different with $P < 0.001$

Principal component analysis for phytoconstituent composition

Fig. 4 represents the Principal Component Analysis for different phytoconstituents to represent the correlation between each phytoconstituent. PC1 represents 54.64% variability, while PC2 accounts for 27.32% variability.

DPPH Scavenging anti-oxidant assay

The anti-oxidant potential was determined using the DPPH scavenging assay method, and the absorbance was taken at 517 nm. The standard curve of different DPPH concentrations was plotted as shown in Fig. 5. A High regression correlation of approximately 0.9989 indicates linearity of the UV-Visible spectrophotometer. The standard curve showed a linear equation of $y = 8.5554x - 0.0419$.

The antioxidant potential of different microgreens at varying concentrations of methanolic extract

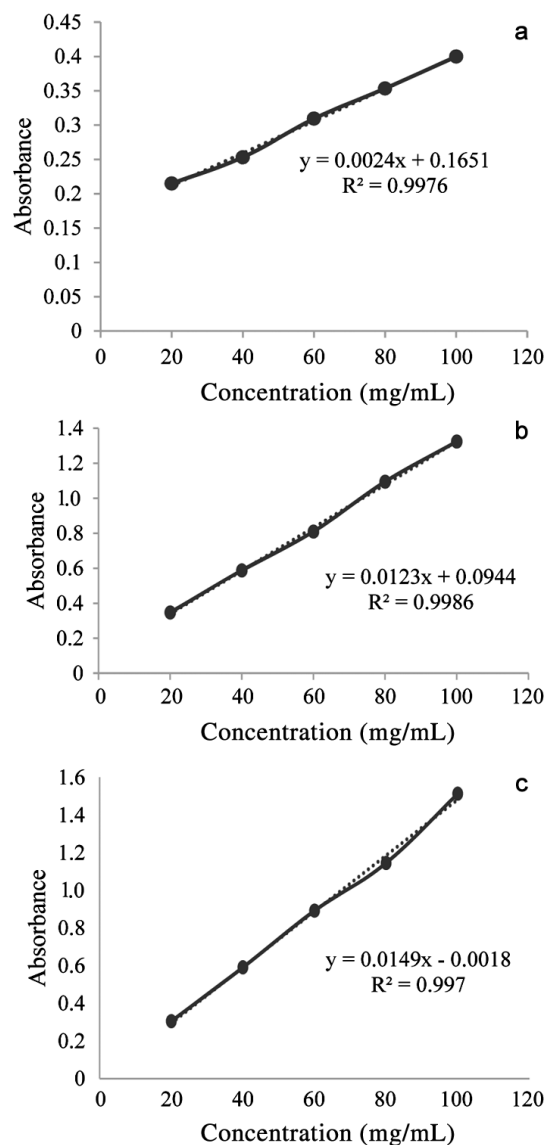


Fig. 3 — Standard curve of a) quercetin, b) gallic acid and, c) tannic acid.

Table 3 — Phytochemical quantification of different microgreens

S. No.	Microgreen	Quantification of phytoconstituents [n=3]; Mean±SD		
		Total Flavonoid Content (TFC) (mg Quercetin/ g)	Total Phenolic Content (TPC) (mg Gallic Acid/g)	Total Tannin Content (TTC) (mg Tannic acid/g)
1	Chia	115.204±5.767bcd	68.826±1.917ce	63.608±1.582de
2	Coriander	85.491±7.844bcde	63.517±0.382ce	59.225±0.315ef
3	Carrot	71.857±1.502bcde	60.311±3.450ef	56.579±2.848ef
4	Fenugreek	51.789±1.092bcd	77.420±0.183ef	70.702±0.151ef
5	Radish	43.073±0.591bcd	81.035±0.089ef	73.686±0.073ef
6	Spinach	64.986±1.031be	61.612±1.119ef	57.653±0.924ef
7	Sunflower	179.193±1.068a	138.875±0.382abd	121.434±0.315abc
8	Turnip	89.289±2.928bcde	51.468±0.126ef	49.279±0.104ef
9	Beetroot	104.193±0.225cbdf	76.074±0.081ce	69.588±0.067de

Values followed by same alphabet in each column represents that they are not significantly different at 5% probability level.

Table 4 — Pigment Quantification of different microgreens

Sl. No.	Microgreen	Quantification of Pigments [n=3]; Mean±SD						
		Total Chlorophyll a content (µg/g, FW)	Total Chlorophyll b content (µg/g, FW)	Total Carotenoid content (µg/g, FW)	Total chlorophyll content (µg/g, FW)	Total Lycopene content (µg/g, FW)	Total β-carotene content (µg/g, FW)	Total Anthocyanin content (µg/g, FW)
1	Chia	16.8553±0.2584 acdfg	21.2735±0.5365 acdfg	0.30949±0.00564 bcdghijklmn	38.4094±1.6842 a	0.01633±0.0057 bcdghijklmn	0.08749±0.00356 bcdghijklmn	3.1771±0.014 bcdghijklmn
2	Coriander	10.3714±0.4532 abcdghijklmn	13.1325±0.1375 abcdghijklmn	0.11836±0.00475 ce	23.6768±1.7568 ad	0.00677±0.00057 ef	0.03460±0.00255 ef	1.8950±0.087 ce
3	Carrot	11.3965±1.1865 abcdghijklmn	10.425±0.2845 abcdghijklmn	0.68168±0.0032 ef	21.9728±1.4721 ac	0.00899±0.00011 ef	0.03547±0.00471 ef	1.9372±0.0924 de
4	Fenugreek	2.84569±0.0578 bcdghijklmn	1.9629±0.4312 bcdghijklmn	0.41886±0.00175 en	4.8398±0.00357 acdghijklm	0.00453±0.00019 be	0.00146±0.00022 be	0.1532±0.011 el
5	Radish	2.45078±0.1635 bcdghijklmn	1.2553±0.0257 bcdghijklmn	0.46066±0.00175 be	3.7287±0.0985 acdghijkln	0.00573±0.00022 be	0.00379±0.00031 be	0.1987±0.075 em
6	Spinach	4.09711±0.8654 bcdghijklmn	4.0276±0.0854 bcdghijklmn	0.90914±0.00325 ej	8.1817±0.1175 acdfgi	0.00414±0.00031 ej	0.01130±0.0058 ej	0.8239±0.0359 eh
7	Sunflower	4.12248±0.0457 bcdghijklmn	2.1804±0.0451 bcdghijklmn	0.39298±0.0145 ek	6.3416±0.3247 acdghijl	0.00718±0.00011 en	0.00074±0.00004 en	0.2845±0.0098 ek
8	Turnip	4.58618±0.0894 bcdghijklmn	3.3709±0.1421 bcdghijklmn	1.00936±0.0554 ej	8.0095±0.0347 acdfgh	0.00930±0.00032 ej	0.00291±0.00013 ej	1.0458±0.0368 ei
9	Beetroot	9.58110±0.0975 abcdghijklmn	7.4235±0.1141 abcdghijklmn	0.59701±0.0351 eg	17.1180±0.584 abcdf	0.02249±0.00258 eg	0.01065±0.00428 eg	1.4218±0.0685 eg

Values followed by same alphabet in each column represents that they are not significantly different at 5% probability level.

(0.1, 0.05, 0.01 mg/mL) showed significant differences ($P < 0.0001$) in two-way ANOVA studies. The data shown in Table 5 is organised in ascending order of IC₅₀ values for the anti-oxidant potential of different microgreens.

Anti-diabetic activity

The anti-diabetic activity of different microgreens was calculated and compared against the standard compound (quercetin). The microgreens, with their anti-diabetic potential, are represented in Table 6 in ascending order of their anti-diabetic potential. Two-

way ANOVA showed significant differences between the anti-diabetic activity of the microgreens with $P < 0.0001$.

Anti-inflammatory assay

Egg Albumin Denaturation assay was conducted to demonstrate the potential of the plant extract to prevent protein denaturation, thus to reduce inflammation. The percentage of anti-inflammatory activity at three different concentrations (0.1, 0.05, 0.01 mg/mL) of plant samples and aspirin (positive control) showed a significant difference between them

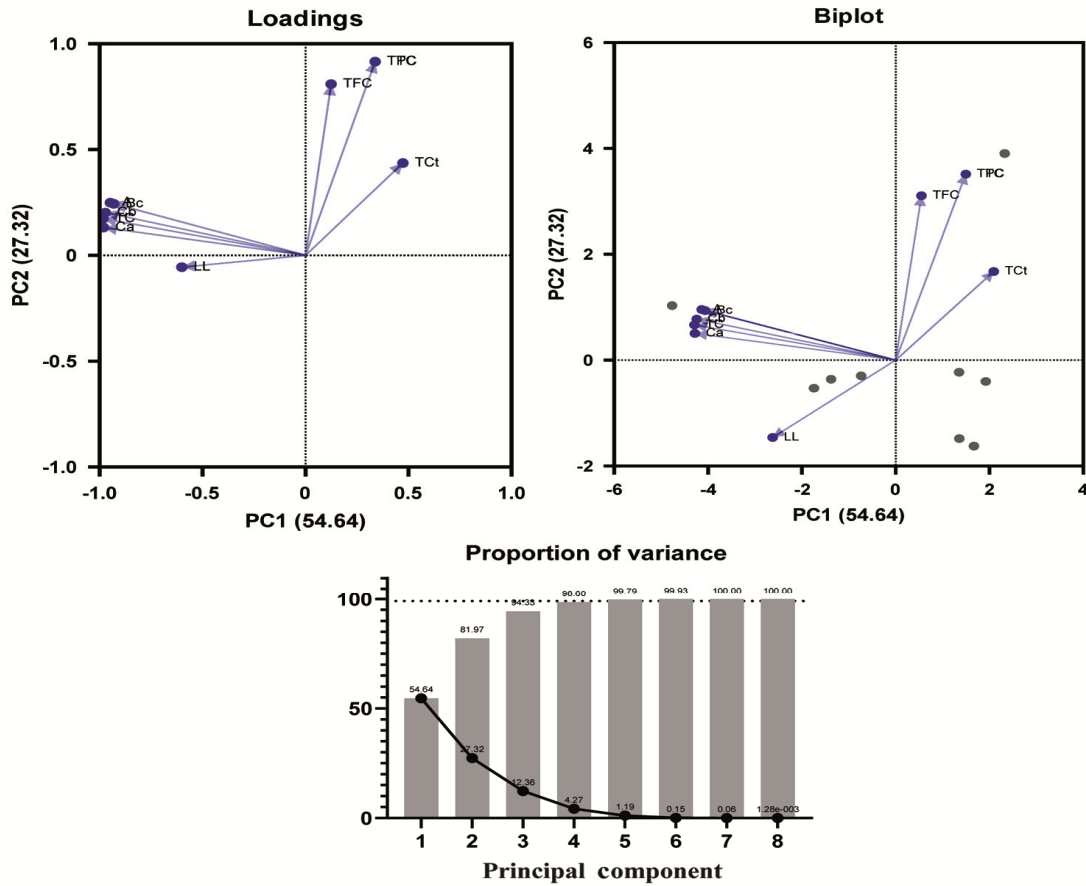


Fig. 4 — Principal Component Analysis of different Phytoconstituent present in nine different microgreens where Ca: Chlorophyll a, Cb: Chlorophyll b; TCt: Total Carotenoid Content; TC: Total Chlorophyll Content; A: Anthocyanin Content; LL: Lycopene Content; BC: β -Carotene Content; TPC: Total Phenolic Content; TFC: Total Flavonoid Content; TTC: Total Tannin Content.

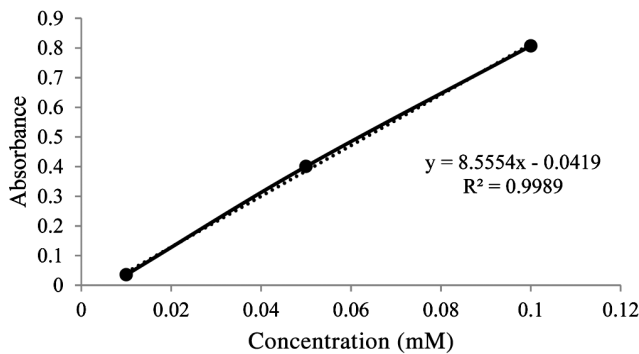


Fig. 5 — Calibration curve of DPPH

($P < 0.0001$). Table 7 shows the anti-inflammatory activity of different microgreens arranged in ascending order of their IC_{50} .

PCA for correlation between phytocomponents and pharmacological activity

Principal Component Analysis to derive the relation between different phytoconstituents like TFC,

TPC and TTC to the different Pharmacological activity is represented in Fig. 6

Discussion

Microgreens have gained popularity in recent times. These microgreens are two-leaf staged germinated sprouts or saplings from young plants, which are assumed to be highly nutritious⁶. This study evaluated the nutritional potential and *in vitro* pharmacological activities of selected microgreens to determine their possible role in the management of risks of chronic disease.

Seeds of nine plants were taken, which belong to six different families, namely Chia (Lamiaceae), Coriander, Carrot (Apiaceae), Fenugreek (Fabaceae), Turnip, Radish (Brassicaceae), Sunflower (Asteraceae), Beetroot and Spinach (Amaranthaceae). As shown in Table 1, germination of these seeds in soil showed that the species of the Asteraceae family (sunflower) took the longest time to germinate

Table 5 — Anti-oxidant activity and IC₅₀ of different microgreens

S. No.	Microgreen	Anti-oxidant potential (%)			IC ₅₀ (µg/mL)
		0.01 mg/mL	0.05 mg/mL	0.1 mg/mL	
	Positive Control	13.1226±0.19236 ⁱ	73.8248±0.1539 ^b	76.0553±0.7364 ^a	43.686
1	Sunflower	38.4799±0.05159 ^j	57.2036±0.0521 ^d	63.6720±0.03118 ^c	48.19284
2	Spinach	36.4684±0.05679 ^k	49.9876±0.08674 ^f	55.8075±0.15981 ^e	64.56429
3	Turnip	21.9289±0.12035 ^{tu}	25.6959±0.07046 ^{qr}	45.2003±0.06359 ^{sg}	118.8918
4	Radish	22.4039±0.04468 ^l	32.5898±0.02478 ^m	44.8740±0.12474 ^{sg}	120.3021
5	Beetroot	25.9562±0.05588 ^o	35.2870±0.13706 ^l	41.8670±0.03778 ^h	140.3117
6	Fenugreek	29.1780±0.04691 ^o	36.5843±0.06359 ⁿ	42.3007±0.05008 ^h	148.000
7	Coriander	5.93143±0.09301 ^{xy}	21.4167±0.03118 ^{uv}	25.1631±0.06237 ^r	185.5496
8	Chia	11.1152±0.05679 ^y	21.1152±0.18752 ^{vw}	28.1453±0.08062 ^p	208.6197
9	Carrot	11.7348±0.04468 ^x	20.5782±0.41198 ^w	23.4531±0.05856 ^s	271.1907

Values followed by same alphabet in each column represents that they are not significantly different at 5% probability level.

Table 6 — Anti-diabetic activity of different microgreens

S. No.	Microgreen	Anti-diabetic potential (%)			IC ₅₀ (µg/mL)
		0.01 mg/mL	0.05 mg/mL	0.1 mg/mL	
	Positive control	50.35842±0.3185 ^{mmn}	71.72043±0.4301 ^f	96.02151±0.2584 ^a	8.547549
1	Fenugreek	51.88769±0.4944 ^m	57.83751±0.2542 ^l	75.26882±0.2933 ^e	11.01766
2	Beetroot	48.25568±0.2897 ^{no}	64.71924±0.7365 ^{ij}	77.50299±0.2517 ^e	12.22412
3	Sunflower	47.29988±0.8057 ^o	68.94863±0.7597 ^{gh}	83.30944±0.4538 ^d	12.90746
4	Radish	47.06093±0.0716 ^o	61.76822±2.3273 ^k	64.71924±0.2690 ^{ij}	19.33606
5	Turnip	39.78495±0.8352 ^p	79.65352±0.6314 ^{hi}	90.39427±0.4979 ^b	22.07355
6	Coriander	36.7264±1.2520 ^q	66.28435±0.4839 ⁱ	90.77658±0.9328 ^b	29.18469
7	Chia	29.60573±0.2867 ^r	83.51254±0.5056 ^{fg}	87.34767±0.58676 ^c	33.0419
8	Spinach	26.21266±1.4701 ^s	62.44922±0.3090 ^{jk}	75.57945±0.25174 ^c	45.40772
9	Carrot	37.00119±0.9512 ^q	47.58662±1.1107 ^o	64.54002±0.3252 ^{ij}	54.2748

Values followed by same alphabet in each column represents that they are not significantly different at 5% probability level.

Table 7 — Anti-inflammatory effects of different concentration of microgreens

S. No.	Microgreen	Anti-inflammatory potential (%)			IC ₅₀ (µg/mL)
		0.01 mg/mL	0.05 mg/mL	0.1 mg/mL	
	POSITIVE CONTROL	52.7692±3.4832 ^{hi}	73.1420±1.6878 ^c	97.8119±1.6167 ^a	4.206584454
1	Chia	41.1312±1.0699 ^{kl}	70.3527±0.2255 ^{cc}	84.7820±0.3508 ^b	23.09263823
2	Coriander	44.3737±0.6592 ^{jk}	57.9684±1.1942 ^{fg}	66.5741±1.9433 ^e	28.40352068
3	Carrot	29.2670±1.1346 ^{no}	66.4815±0.3208 ^e	72.1820±3.3457 ^{cd}	42.99753093
4	Radish	18.0112±1.6096 ^r	55.3158±0.1676 ^{gh}	68.2435±0.2284 ^{de}	57.91717814
5	Beetroot	32.6316±0.2037 ^{mo}	44.5838±0.1013 ^{jk}	61.5869±0.2709 ^f	64.89257789
6	Spinach	28.5755±2.7309 ^{op}	45.8253±1.1749 ^j	55.8845±0.2455 ^{gh}	74.29484498
7	Sunflower	6.31927±0.2071 ^s	39.5330±0.4050 ^l	50.8569±0.3881 ⁱ	85.9495544
8	Fenugreek	18.1790±0.3936 ^r	24.4963±0.1527 ^{pq}	55.4797±0.1080 ^{gh}	90.61748996
9	Turnip	23.6076±0.3014 ^q	33.2812±0.2024 ^{mn}	35.1647±0.0524 ^m	180.7051155

Values followed by same alphabet in each column represents that they are not significantly different at 5% probability level.

(14 days), while Turnip and *Raphanus sativus* (radish), the members of the Brassicaceae family, germinated within only 7 days to form mature microgreens. The delayed germination of sunflower seeds and the formation of mature microgreens can be attributed to the presence of a thick seed coat. Thus, the plumule and radical took a longer time to emerge

from the seed²³. Similarly, it was found that *Salvia hispanica* L. (chia) seeds germinated to form a two-leaf stage microgreen in only 7 days after sowing and showed the highest percentage of germination (97%).

Some microgreens, such as sunflower, turnip, and beetroot, showed quite low germination rates (<50%). This could be attributed to factors such as a thicker

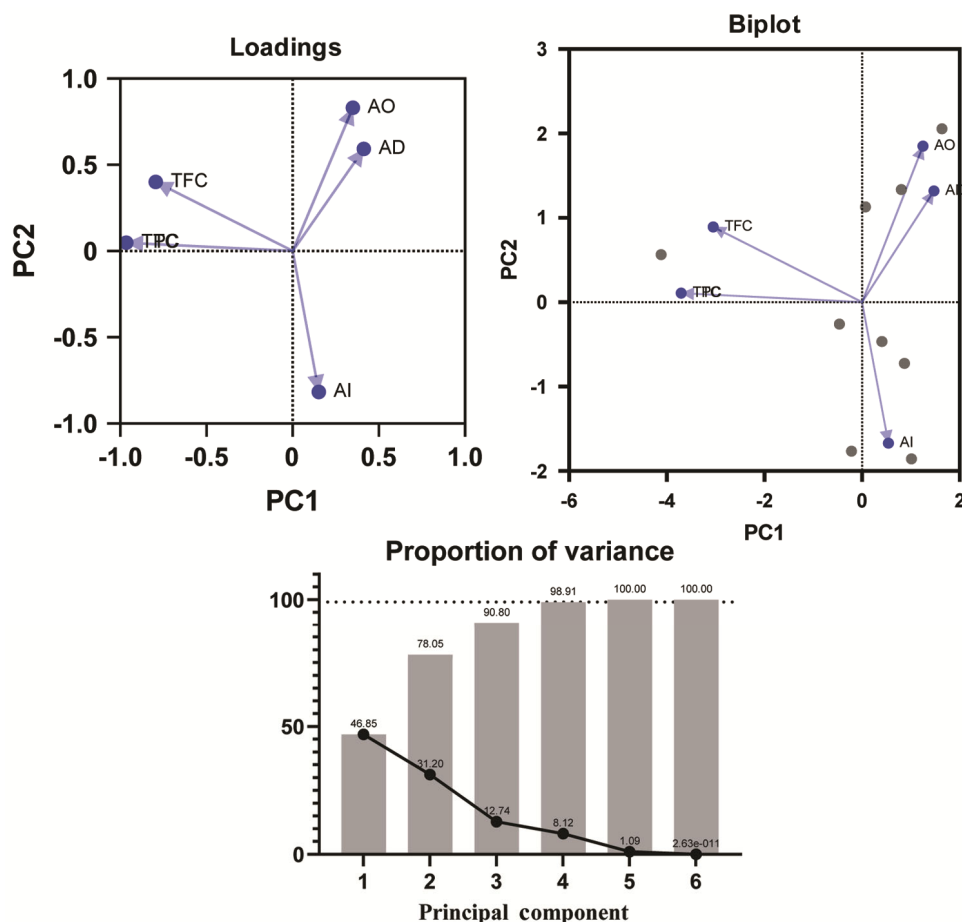


Fig. 6 — Principal Component Analysis of different Phytoconstituent present in nine different microgreens and the Pharmacological activities elicited by them, where TPC: Total Phenolic Content; TFC: Total Flavonoid Content; TTC: Total Tannin Content, AI: IC₅₀ of Anti-inflammatory activity, AD: IC₅₀ of Anti-diabetic activity, AO: IC₅₀ of Anti-oxidant activity.

testa layer, reduced viability due to storage time, or natural species-specific germination characteristics.

Morphological characteristics of different microgreens suggest that the Apiaceae family (Coriander and Carrot) has the highest moisture content and loss on drying, resulting in a lower dried weight reading. This trend was also observed in earlier studies on the water retention capacity of microgreens. On the other hand, freshly germinated microgreens of the Brassicaceae family (radish and turnip) had the lowest moisture content among them. The shoot height of the sunflower was comparatively the highest. Principal Component Analysis of different morphological characters (Fig. 2) reports that the Pearson correlation (r) between shoot and root length is almost 0.224, which means that a two times increase in shoot length contributes to only a 0.224 times increase in root length. Still, it overall reduces the fresh weight of the samples by 0.725 times.

The extraction solvent, process and time greatly influence the characteristics of the phytoconstituent extracted to form the extract. The microgreen samples collected were macerated using methanol as a solvent. Further research was carried out using this solvent.

Phytochemical estimation (Table 2) revealed the presence of flavonoids and tannins in all the different microgreen samples, which subsequently led to the quantification of polyphenolic compounds, flavonoids, and tannins in various microgreens (Table 3). The linear equation of tannic acid (Fig. 3c) standard in methanol was consistent with values reported in previous analytical validations²⁴. Significant differences were noted in a two-way ANOVA ($P < 0.001$) between different microgreens based on their TFC, TPC, and TTC content. The studied microgreens exhibited distinct phytoconstituent profiles, indicating that species-specific factors significantly influence the

biosynthesis of secondary metabolites. Considerably, the highest amount of TFC was reported in Sunflower followed by *Salvia hispanica* L. (chia). Likely, TPC and TTC were also found to be the highest in Sunflower. The obtained TFC and TPC content is within a comparable range²⁵. The lowest TFC content amongst the nine microgreens was found in *Raphanus sativus* (radish) (43.073 ± 0.591 mg Quercetin/g) and was comparable to already reported TFC (42 mg Quercetin/g)²⁶. Turnip recorded the lowest levels of TPC and TTC. *Trigonella foenum-graecum* (fenugreek) also contains a significantly higher amount of TFC.

Analysis of the pigment content in nine different microgreens (Table 4) revealed significant differences in the content of chlorophyll a, total chlorophyll, chlorophyll b, beta-carotene, lycopene, anthocyanin, and total carotenoids, with $P < 0.001$ in a two-way ANOVA study. The order of photosynthetic pigments (Chlorophyll) in respective families is Lamiaceae > Apiaceae > Amaranthaceae > Asteraceae > Brassicaceae. *Salvia hispanica* L. (chia) microgreens with the highest germination density (97.00%) had the highest total chlorophyll content, followed by coriander, carrot and beetroot (*Beta vulgaris*) microgreens. The chlorophyll a content in chia microgreens was similar to the reported content of chlorophyll a in sprouted chia seeds²⁷. Significant differences were reported in chlorophyll content of chia microgreens and chia seeds. The reddish green colour of radish corresponds to the lowest content of photosynthetic pigment. The photosynthetic pigment content is often lesser in sprouted seeds than in mature leaves because of their ability to photosynthesise and thus increase on maturity²⁸. The chlorophyll content estimated in radish microgreen soil media and previously reported in soilless media of growth exhibits significant differences ($P < 0.05$)¹³. Carotenoid content, conversely, was found to be comparatively higher in beetroot microgreen due to the reddish colour of the sprouted plants (0.597 ± 0.0351 $\mu\text{g/g}$ FW), which emphasises its higher content of beta carotene and lycopene. These results are significantly different from the total carotenoid content in mature leaves and fruits of beetroot, as previously reported²⁹. Fig. 4 presents the Principal Component Analysis of all major phytoconstituents quantified in various microgreens. Total chlorophyll content and total carotenoid content have a negative Pearson correlation factor of -0.389.

TPC and TTC, regardless of all conditions, increase and decrease at a constant rate, with an 'r' value of 1.000, indicating that tannins are an integral part of the phenolic content and validating the derived results. A one-fold increase in Total chlorophyll content leads to an almost 0.98-fold increase in Total Anthocyanin Content, which is close to the reported value with no significant differences³⁰.

To analyse the health benefits of the nine different microgreens, different *in vitro* pharmacological assays were conducted. The free radical scavenging anti-oxidant assay was conducted by the DPPH assay, where the ability of the plant extract to convert DPPH to DPPHH was measured³¹. Here, DPPH (α , α -diphenyl- β -picrylhydrazyl) acted as a stable free radical. The anti-diabetic assay was conducted by measuring *in vitro* α -amylase inhibition activity to estimate the effect of plant extract in the inhibition of α -amylase and preventing carbohydrate digestion in the human intestine. Lastly, an *in vitro* anti-inflammatory assay was conducted by an egg albumin denaturation assay, which is based on the principle of estimating the effect of plant extract in elevating anti-inflammatory protein to prevent protein denaturation of the egg albumin. All the assays were conducted at an average temperature of 25-27°C and a Relative humidity of 52-60%.

An *in vitro* DPPH scavenging assay was conducted, taking ascorbic acid as a positive control. Literature suggests the IC₅₀ value of the methanolic extract of ascorbic acid in scavenging DPPH is about 41.25 $\mu\text{g/mL}$ ³². Our study indicated the IC₅₀ value of ascorbic acid to be 43.66 $\mu\text{g/mL}$, which is quite comparable. Three concentrations of methanolic extract were studied to determine the IC₅₀ value of the microgreens. All the readings obtained for the percentage of anti-oxidant activity for different microgreens at different concentrations were found to be significantly different with $P < 0.001$. Among all, sunflower microgreens demonstrated the highest anti-oxidant potential, likely due to their elevated phenolic and flavonoid content, which are known contributors to radical scavenging activity. This is followed by spinach and turnip microgreens, as shown in Table 5. The high content of carotenoids and lycopene in radish and beetroot microgreens also makes them potent anti-oxidant agents and has shown excellent radical scavenging in the *in vitro* assay. This reading correlates to the phytochemical composition of the plants. Sunflower microgreens showed the highest

TFC, TPC and TTC content. The phenolic constituents may contribute to anti-oxidant activity through hydrogen or proton donation mechanisms, as widely reported. Thus, it can balance oxidative stress in the human body. This might be the reason *Helianthus annuus* (Asteraceae) is the most potent anti-oxidant among nine microgreens. Carrot microgreen was the least potent anti-oxidant with an IC₅₀ value of 271.190 µg/mL. Thus, carrots as a vegetable show a significant difference in anti-oxidant properties with respect to carrot microgreens. The large content of carotenoids in carrots is responsible for its free radical scavenging activity, which is almost absent in the green microgreens of carrots. Table 8 correlates the phytoconstituent composition to the pharmacological activity. It shows that IC₅₀ of anti-oxidant activity decreases with an increase in TPC (r=-0.1508), TFC (r=-0.335), TTC (r=-0.15082) and anthocyanin content (r=-0.6767). This indicates that with an increase in phenolic content and pigment content, the IC₅₀ of anti-oxidant reduces, thereby increasing the potency of the plant extract as anti-oxidants due to the presence of hydroxyl group in the structure of these phytoconstituents³³.

To analyse the *in vitro* anti-diabetic activity of different plant extracts, α -amylase inhibition activity assay was performed. Suitable positive controls for

this assay are quercetin and acarbose, with acarbose having a higher IC₅₀ than quercetin as an anti-diabetic agent³⁴. According to the Literature, the IC₅₀ of the methanolic extract of quercetin is 0.008 mg/mL, which accounts for 8 µg/mL³⁵. This study shows the IC₅₀ of quercetin is 8.54 µg/mL, which is very close to the established IC₅₀ of quercetin. This provides internal validity to the work done. A two-way ANOVA was conducted to determine the statistical significance of anti-diabetic activity between different concentrations and microgreens. The differences were statistically significant ($P < 0.0001$). Fenugreek (*Trigonella foenum-graecum*) microgreens exhibited the strongest α -amylase inhibition activity, consistent with their traditional role in glycemic control, possibly due to the presence of tannins and other inhibitory phytoconstituents (IC₅₀ of 11.017 µg/mL). It is even potent than fenugreek seeds in terms of inhibition of α -amylase, since according to literature, the IC₅₀ of fenugreek seeds is 17.2 µg/mL³⁶. This study reveals that microgreens of beetroot (*Beta vulgaris*) and sunflower (*Helianthus annuus*) are also good sources of anti-diabetic agents. These attributes are attributed to the high phenolic content in the said microgreens, as estimated in Table 3. Fenugreek, beetroot and sunflower have excellent TPC. Carbohydrates are polyhydroxy aldehydes and

Table 8 — Correlation between phytoconstituents and Pharmacological activity shown by microgreens by Principal Component Analysis

	IC ₅₀ of anti-inflammatory activity	IC ₅₀ of anti-diabetic activity	IC ₅₀ of anti-oxidant activity	Total Flavonoid Content	Total Phenolic Content	Total Tannin Content	Total Carotenoid Content	Total Chlorophyll Content	Total Anthocyanin Content
IC ₅₀ of anti-inflammatory activity	1.0000	-0.29052	-0.472	-0.33577	-0.15082	-0.15082	0.292989	-0.70362	-0.67674
IC ₅₀ of anti-diabetic activity	-0.29052	1.0000	0.490082	-0.27075	-0.20923	-0.20923	-0.3988	0.35863	0.292902
IC ₅₀ of anti-oxidant activity	-0.472	0.490082	1.0000	0.202338	-0.34292	-0.34292	-0.25612	0.617142	0.625148
Total Flavonoid Content	-0.33577	-0.27075	0.202338	1.0000	0.697797	0.697798	-0.14038	0.369694	0.352414
Total Phenolic Content	-0.15082	-0.20923	-0.34292	0.697797	1.0000	1.0000	-0.0074	0.013636	-0.02663
Total Tannin Content	-0.15082	-0.20923	-0.34292	0.697798	1.0000	1.0000	-0.0074	0.013637	-0.02663
Total Carotenoid Content	0.292989	-0.3988	-0.25612	-0.14038	-0.0074	-0.0074	1.0000	-0.38978	-0.21874
Total Chlorophyll Content	-0.70362	0.35863	0.617142	0.369694	0.013636	0.013637	-0.38978	1.0000	0.980508
Total Anthocyanin Content	-0.67674	0.292902	0.625148	0.352414	-0.02663	-0.02663	-0.21874	0.980508	1.0000

ketones. Thus, they contain hydroxyl groups. Polyphenolic compounds present in different plant extracts also contain hydroxyl groups. These might competitively inhibit the binding of the carbohydrate to the active sites of alpha amylase and prevent carbohydrate digestion. Thus, they show alpha-amylase inhibitory activities³⁷. On the contrary, carrot microgreen with only 60.311 ± 3.450 mg Gallic acid/g TPC is shown to have the least potency as an anti-diabetic agent with an IC_{50} value of 54.278 μ g/mL. Table 8 shows Principal Component Analysis to correlate the effect of phytoconstituents on the anti-diabetic activity of plant extracts. The established Pearson correlation shows a positive relation between anti-oxidant and anti-diabetic action with $r=0.49$. The correlation depicts that the IC_{50} value of anti-diabetic action increases, or anti-diabetic potency decreases with an increase in TPC content ($r=-0.209$), TFC content ($r=-0.27$); TTC content ($r=-0.209$) and total carotenoid content ($r=-0.398$). This indicates the role of polyphenolic compounds in eliciting anti-diabetic activity of the plant extracts.

In vitro anti-inflammatory activity was estimated by quantifying the ability of the plant extract to prevent the denaturation of egg albumin under heat. Two-way ANOVA revealed significant differences in the percentage of anti-inflammatory action between different microgreens, with $P < 0.001$. To test the significance, aspirin, an NSAID, was used as a positive control as an anti-inflammatory drug. Literature suggests that the IC_{50} of aspirin is 25 μ M, which is equivalent to 4.5048 μ g/mL aspirin³⁸. This study reported an IC_{50} of aspirin as 4.206 μ g/mL, which was quite close and non-significant to the study conducted by others, and thus revalidates the scientific precision and consistency of the experiment. Table 7 arranges different microgreens in ascending order of their IC_{50} , indicating the microgreens from most potent to least potent. Chia microgreens (*Salvia hispanica* L.) were the most potent anti-inflammatory agent, followed by coriander and carrot. The anti-inflammatory action of chia microgreens can be attributed to its high carotenoid content, TPC and anthocyanin content. The anti-inflammatory activity observed in coriander may be attributed to its chlorophyll and flavonoid content, both of which can downregulate pro-inflammatory mediators such as TNF- α and IL-6. The IC_{50} value of chia microgreens was found to be higher than that of chia seeds, as reported in the literature³⁹. This indicates that chia

microgreens may have higher anti-inflammatory potency than their seeds, due to greater accumulation of certain bioactive compounds during seed maturation.

Egg albumin denaturation is defined as the loss of native protein structure due to external stress (heat) application, thus exposing its hydrophobic groups. In our bodies, this stress leads to the release of pro-inflammatory cytokines, such as prostaglandins and histamines. Polyphenols present in different plant extracts form hydrogen bonds with amino acid residues of albumin and hence stabilise its structure, which prevents heat-induced unfolding and aggregation of the protein, and shows anti-inflammatory actions by inhibiting protein denaturation⁴⁰. Thus, the anti-inflammatory actions exhibited by different plant extracts are due to the polyphenolic content. Table 8 correlates different pharmacological activities and phytoconstituents present in the plant sample with anti-inflammatory activity. It is seen that IC_{50} of anti-inflammatory activity increases or potency decreases with an increase in TFC ($r=0.335$), TTC, TPC ($r=-0.15082$), Total Anthocyanin Content ($r=-0.6764$) and Total chlorophyll content ($r=-0.7032$). Moreover, it is seen that with an increase in anti-inflammatory activity potency, the potency of anti-diabetic and anti-oxidant activity also increases. Principal component and correlation analyses suggested interrelationships among phytochemical concentrations and biological activities, implying synergistic effects of multiple compounds.

Thus, this research involves the phytochemical quantification of nine different microgreens and correlating the phytoconstituent to the *in vitro* pharmacological action. All the microgreens show more or less good and potent anti-inflammatory, anti-diabetic and anti-oxidant activity and hence should be incorporated in the daily diet to maintain a normal lifestyle and prevent lifestyle-related disorders. These microgreens are easy to grow at home on a regular basis, so they can be consumed as sprouts or salads to meet the body's need for anti-oxidant and other essential nutrients. However, *in vivo* validation, standardisation, and product development studies are essential to translate these findings into practical therapeutic or dietary applications. Furthermore, dose standardisation and bioavailability assessments are necessary, followed by evaluation of shelf life and formulation stability, which are crucial steps toward commercialisation.

Conclusion

The different phytochemical content of microgreens, including phenolics, flavonoids, anthocyanins, carotenoids, and chlorophyll, indicates their ability to modulate oxidative stress, glucose metabolism, and inflammation *in vitro*. This research shows evidence to support the use of microgreens in the daily diet and the dietary intake of these microgreens for the well-being of the human body. This work pioneers the phytoconstituent quantification and the therapeutic application of different microgreens when grown in the soil of West Bengal, India. Sunflower and fenugreek microgreens are potent anti-oxidant and anti-inflammatory agents, respectively. The growth of microgreens is cost-effective, with a good germination rate and fast germination, suggesting potential for economical large-scale cultivation. Further research can be extended to examine the *in-vivo* pharmacological activities shown by these microgreens and conduct bioavailability studies, and assessment of challenges in standardisation and commercialisation.

Conflict of interest

The authors declare that there is no conflict of interest.

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