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Original Article

Stress-Induced Phytochemical Enhancement and *in vitro* Antidiabetic and Anti-Inflammatory Activities of Soil-Grown Urad Microgreens: A Comparative Study

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ABSTRACT

Objectives: Microgreens are tender edible seedlings harvested 7-21 days after germination at a height of 2.5-7.5 cm. They are rich in bioactive compounds and possess potential health benefits. The present study aimed to quantify major phytochemicals, including phenolics and flavonoids, in urad (*Vigna mungo*) microgreens grown under different stress conditions and to evaluate their *in vitro* antidiabetic and anti-inflammatory activities. **Materials and Methods:** Urad microgreens were cultivated under normal conditions, 25%, 50%, and 100% salinity stress, and 24-hr dark stress. Ethanolic extracts (70-80%) were prepared for phytochemical analysis. Total Phenolic Content (TPC), Total Flavonoid Content (TFC), anthocyanin, chlorophyll, and carotenoid levels were estimated. Antidiabetic activity was assessed using the α-amylase inhibition assay, while anti-inflammatory activity was evaluated through the egg albumin denaturation assay. **Results:** Phytochemical screening confirmed the presence of alkaloids, carbohydrates, phenolics, flavonoids, and steroids. TPC was highest at 100% salinity (0.1422±0.0012 mg GAE/g), whereas TFC and anthocyanin peaked at 50% salinity (0.5969±0.2238 mg QE/g and 0.3197±0.0011 µg/g FW, respectively). Pigment content was maximum under normal conditions. Dark-grown microgreens showed elevated flavonoids but reduced phenolics, anthocyanins, and bioactivity. Microgreens grown under normal photoperiod exhibited the strongest α-amylase inhibition (IC_{50} 55.98 µg/mL) and anti-inflammatory activity (IC_{50} 9.24 µg/mL). **Conclusion:** Moderate salinity (50%) enhanced flavonoid accumulation, while normal conditions produced superior bioactivity, highlighting stress-induced urad microgreens as promising functional foods.

Keywords: Antidiabetic, Enzyme inhibition, Phytochemicals, Salinity stress, *Vigna mungo*.

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INTRODUCTION

The global burden of chronic metabolic diseases, particularly Type 2 Diabetes Mellitus (T2DM) and inflammatory disorders, has reached epidemic proportions, affecting millions worldwide (Huang *et al.*, 2025) and imposing substantial economic and healthcare challenges. Concurrently, chronic low-grade inflammation has been recognized as a pivotal pathophysiological mechanism underlying numerous diseases, including diabetes, cardiovascular disorders, and metabolic syndrome. The intricate interplay between hyperglycemia and inflammatory pathways has prompted researchers to explore multifunctional therapeutic approaches that can simultaneously target both conditions (Huang *et al.*, 2025; Van Dijk *et al.*, 2021).

Conventional pharmacological interventions, while effective, are often associated with adverse effects, high costs, and limited accessibility, particularly in developing nations. This has catalyzed growing interest in plant-based functional foods and nutraceuticals as complementary or alternative strategies for disease prevention and management (Gruda *et al.*, 2024). Among the emerging class of functional foods, microgreens have garnered substantial scientific attention due to their exceptional nutritional density, bioactive phytochemical content, and potential health-promoting properties.

Microgreens are young, tender greens harvested at the cotyledon or first true-leaf stage, typically 7-21 days after germination. Despite their diminutive size, microgreens are nutritional powerhouses, containing significantly higher concentrations of vitamins, minerals, antioxidants, and bioactive compounds compared to their mature counterparts (Bhaswant *et al.*, 2023).

Vigna mungo (L.) Hepper, commonly known as black gram or urad bean, is a vital leguminous crop extensively cultivated in South Asia, particularly in India, where it serves as a major source of dietary protein and essential nutrients. While mature

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urad beans have been well-characterized for their nutritional composition and health benefits, the microgreen stage of this legume remains relatively unexplored. Preliminary investigations suggest that urad microgreens possess substantial amounts of proteins, phenolic compounds, and antioxidant capacity, warranting comprehensive evaluation of their bioactive potential and therapeutic applications such as anti -inflammatory (Seth *et al*., 2025), anti-oxidant (Hadi *et al*., 2025), anti-diabetic (Partap *et al*., 2023), cardioprotective (Rizvi *et al*., 2023).

The biosynthesis and accumulation of phytochemicals in plants are significantly influenced by environmental conditions and stress factors. salinity, and light variations, trigger complex physiological and biochemical responses in plants as adaptive mechanisms. These stress-induced responses often result in the enhanced production of secondary metabolites, particularly phenolic compounds, flavonoids, and other antioxidants, as part of the plant's defense system against oxidative damage.

MATERIALS AND METHODS

Chemicals required

All chemicals used were of analytical grade and were procured from authorized 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.

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) and Hot Water Bath (SSFW, LWB-12H/D, Kolkata, India).

Seed sample collection

About 150 seeds of Uradwhole procured from the local market of Sodepur, West Bengal, India in September 2025. 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 (~15±2°C) before sowing in October.

Cultivation of microgreens

The microgreen is grown in soil of Sodepur, North 24 Parganas, West Bengal, under different stress condition (25%, 50%, 100% salinity, normal condition, 24 hr dark) in the month of October for 1-6 days in an open environment at a temperature of 20-25°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±2°C, protected from direct sunlight) in a well-ventilated, dust-free environment for 6 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 formula (Balik *et al*., 2025).

Moisture content=(Fresh Weight - Dry weight) / Fresh Weight * 100%

Plant extract preparation

The dried plant material was crushed into a coarse powder using a pestle and mortar. About 2 g of the powdered sample of microgreen was taken and macerated in 10 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 min 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 procedures. Alkaloid was estimated by Dragendroff's test and Mayer test, flavonoids by Lead acetate Test. Carbohydrate was estimated by Molisch test. The ferric chloride test was used to determine phenolic compounds and tannins. Glycoside was estimated by the Modified Brontrager Test. A foam test was used to determine saponins. Terpenoid was tested using chloroform and conc. image2. Identification of proteins was done by performing Ninhydrin test (Charoensiddhi *et al*., 2022).

Total phenolic content determination

The Total Phenolic Content (TPC) was determined using the established procedure. Different concentrations of gallic acid were used to form a calibration curve (20, 40, 60, 80 and 100 mg/mL) using 5 mL of 10% Folin-Ciocalteu and 4 mL of 7% Na_2CO_3 (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 (Chakraborty *et al*., 2025; Dimita *et al*., 2022) (Figure 1).

Total flavonoid content calculation

The established procedure using quercetin as a standard was used to determine the calibration curve. Plant extract of 0.01, 0.05, and 0.1 mg/mL concentrations was used to determine TFC at 510 nm (Martins *et al*., 2021).

Total tannin content calculation

The established procedure of determination of Total Tannin Content (TTC) was performed. 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 (Galvão *et al.*, 2018).

Total pigment content

Chlorophyll *a*, chlorophyll *b*, total chlorophyll content, and total carotenoid content were determined using 80% acetone as the solvent system. Anthocyanin content was estimated using a methanol-HCl-water mixture (90:1:9, v/v/v) according to the reported method and expressed as µg/g Fresh Weight (FW). All measurements were performed in triplicate (Gunjal *et al.*, 2024).

Anthocyanin Content: (0.0821A534 -(0.00687A643) -(0.002426A661) *5 ----------(i)

Chlorophyll a Content: (12.25A663) -(2.79A646.6) ----------(ii)

Chlorophyll b Content: (21.3A646.6) -(5.1A663.6) ----------(iii)

Total Chlorophyll Content: (7.15A663.6) +(18.71A646.6) ----------(iv)

Total Carotenoid Content: {(1000*A470) - (1.82*Chlorophyll a) -(85.02*Chlorophyll b)}/198 ----------(v)

Anti diabetic potential

The α-amylase inhibitory activity was evaluated using an established method. The sample solution was prepared at a concentration of 0.1 mg/mL and further diluted to obtain 0.05 and 0.01 mg/mL. Reaction mixtures containing different concentrations of the plant extract (test), acarbose (positive control), or ethanol (control) were incubated with α-amylase solution (0.5 mg/mL), 1% starch solution, and 0.2 M phosphate buffer (pH 6.9) for 5 min. The reaction was terminated by the addition of 1% 3,5-dinitrosalicylic acid, and the absorbance was measured at 540 nm (Kifle *et al.*, 2021). The antidiabetic percentage was calculated by,

% Antidiabetic potential= (Ac-At/Ac) * 100 ----------(vi)

Where A_c=Absorbance of control solution and A_t=Absorbance of test Solution.

Anti-inflammatory potential

The anti-inflammatory activity was assessed using an egg albumin denaturation assay following an established method. The reaction mixture consisted of 0.2 mL egg albumin, 2.8 mL phosphate buffer (pH 6.4), and 0.2 mL of the sample at concentrations of 0.1, 0.05, and 0.01 mg/mL (aspirin as the positive control, plant extract as the test sample, and appropriate control). The mixtures were incubated at 27°C for 10 min, followed by heating in a water bath at 70°C for 10 min. The absorbance was measured at 660 nm (Vučetić *et al.*, 2025, Balik *et al.*, 2024). Anti-inflammatory potential was estimated by using the formula:

% Anti-inflammatory= (AT/AC -1) * 100 ----------(vii)

Where, A_C=Absorbance of control solution, A_T=Absorbance of test solution.

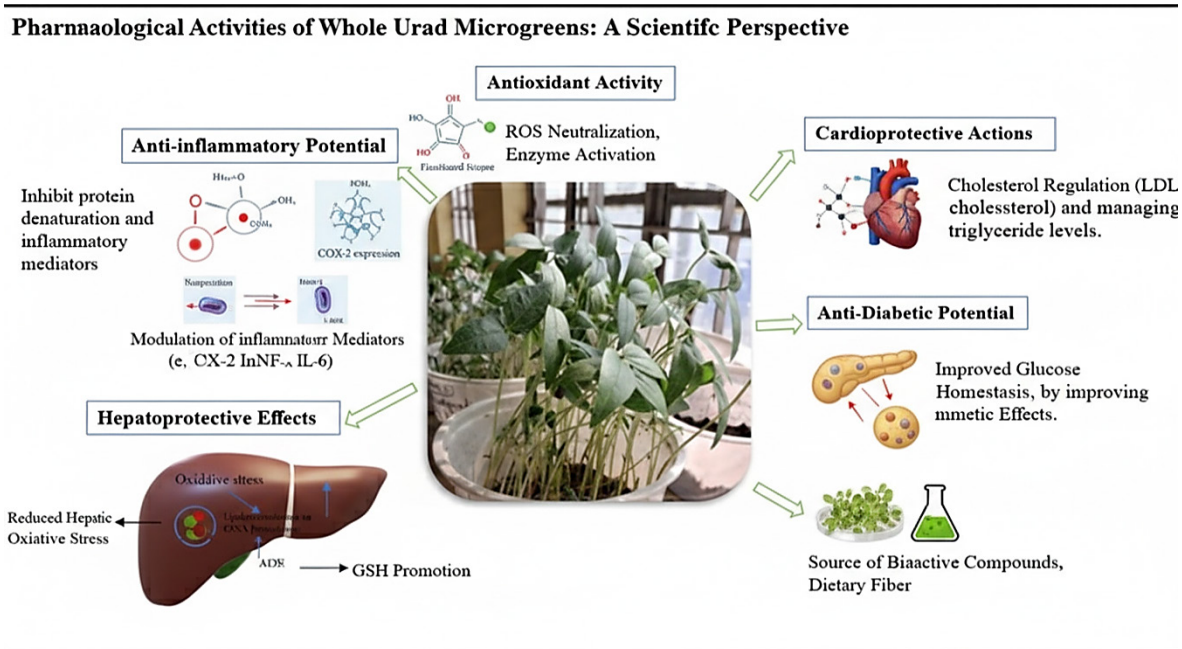

Figure 1: Common Pharmacological activities of Uradwhole Microgreen.

Ethical Statement

Ethical approval was not required for this study as it involved only plant materials and did not include human participants or experimental animals.

Statistical Analysis

All experiments were performed in triplicate, and the results are expressed as Mean±Standard Deviation (SD). The mean and standard deviation values were calculated using Microsoft Excel 2021 version. No inferential statistical tests were applied.

RESULTS

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

Germination details

The germination rate and details of germination for different stress condition microgreens are presented in Figures 2 and 3.

Preliminary Phytochemical Test

The phytochemical screening was performed to assess the presence or absence of various classes of phytoconstituents. The results are shown in Table 1.

Phytochemical quantification

Standard curve of quercetin, gallic acid and Tannic acid was prepared at different concentration. Calibration curves of standard quercetin, gallic acid and tannic acid and demonstrated high Regression Correlation (R^2) of 0.9519, 0.9253, 0.99 with linear equations

y=0.0003x+0.1941, y=0.0058x-0.0007 and y=0.0014x+0.023

Respectively, as presented in Figures 4a-c.

Phytochemical Quantification and Pigment Content Estimation

For phytochemical quantification, total Flavonoid Content increased in 50% salinity (0.5969)>24 hr Dark (0.5656)>25% salinity (0.2946)>Normal (0.2502)>100% salinity (0.0714). Total Phenolic Content increases in 100% salinity (0.1422)>50% salinity (0.1345)>25% salinity (0.0537)>Normal (0.0414)>24 hr Dark (0.0329). Total Tannin Content 25% salinity (27.9)>Normal (14.02)>100% salinity (11.59)>24 hr Dark (9.04)>50% salinity (0.80) as presented in Table 2.

Pigment content estimation identified Total Anthocyanin content increases in 50% salinity (0.3197)>Normal (0.263)>25% salinity (0.2001)>100% salinity (0.0889)>24 hr Dark (0.00017) and chlorophyll a increases 100% salinity (1.8914) ≈ 24 hr Dark (1.89)>50% salinity (1.6502)>25% salinity (1.600)>Normal (0.955) chlorophyll b increases in Normal (7.26)>50% salinity (3.5675)>24 hr Dark (0.76)>25% salinity (0.7045)>100% salinity (0.4518) and total chlorophyll content increases in Normal (8.291)>50% salinity (5.2600)>25% salinity (2.318)>100% salinity (1.4429) ≈ 24 hr Dark (1.44) and total carotenoid content increases in 24 hr Dark (23.12)>Normal (1.8014)>50% salinity (1.5366)>100% salinity (1.1416)>25% salinity (0.5937). Quantification of different pigment contents like anthocyanin, Chlorophyll, and Carotenoid is represented in Table 2.

In vitro Assay

Anti-diabetic activity

The anti-diabetic activity of different stress condition of Uradwhole microgreens was calculated and compared against the standard compound (Acarbose). The antidiabetic potential

Table 1: Estimation of the presence of different phytoconstituents in microgreen.

Sl. No.	Identification	Test Name	Normal condition	25% salinity	50% salinity	100% salinity	24 hr Dark
1.	Alkaloid	Dragendroff's Test	-	-	-	-	-
		Mayer Test	-	-	+	+	+
2.	Carbohydrate	Molisch Test	+	+	+	+	+
3.	Glycoside	Modified Brontrager	-	-	-	-	+
4.	Flavonoids	Lead acetate Test	+	+	+	+	-
5.	Tanins and Phenolics	Ferric Chloride Test	-	-	+	+	+
6.	Protein	Ninhydrin Test	-	-	-	-	-
7.	Steroid	Salkowski Test	+	+	+	+	+
8.	Saponin	Frothing's Test	-	-	-	-	-

'+' indicates presence of phytoconstituent; '-'indicates absence of phytoconstituent.

of the Uradwhole Microgreen is represented in Table 3. In Table 3 anti-diabetic effect increases in Normal (55.98)>50% salinity (56.44)>100% salinity (62.46)>25% salinity (74.81)>24 hr Dark (92.23) (Figure 1).

Anti-inflammatory activity

The egg albumin denaturation assay was employed to evaluate the inhibitory effect of the plant extract on protein denaturation, a key event in inflammatory responses. The anti-inflammatory activity of the plant samples was analyzed at three concentrations (0.1, 0.05, and 0.01 mg/mL) and compared with aspirin as a reference standard. The findings indicated significant differences in activity both among the concentrations tested and between the Microgreen extracts and aspirin. The anti-inflammatory potential of the Uradwhole Microgreen is represented in Table 3. In this Table anti-inflammatory effect increases in Normal (9.24)>100%

Table 2: Phytochemical and Pigments Quantification of Uradwhole (*Vigna mungo)* Microgreen.

Sl. No.	Stress Condition	TFC (mg QE/g)	TPC (mg GAE/g)	TTC (mg TAE/g)	Chlorophyll a content (µg/g)	Chlorophyll b content (µg/g)	Total Chlorophyll content (µg/g)	Total anthocyanin content (µg/g)	Total carotenoid content (µg/g)
1.	Normal (*Vigna mungo)*	0.25015±0.1203	0.0414±0.0046	14.02±0.96	0.955±0.0086	7.26±0.0653	8.291±0.0703	0.263±0.0010	1.8014±0.8014
2.	25% salinity *(Vigna mungo)*	0.2946±0.0011	0.0537±0.0017	27.9±1.00	1.600±0.0096	0.7045±0.0116	2.318±0.0091	0.2001±0.0005	0.5937±0.0069
3.	50% salinity *(Vigna mungo)*	0.5969±0.2238	0.1345±0.2295	0.80±0.53	1.6502±0.0039	3.5675±0.0054	5.2600±0.0044	0.3197±0.0011	1.5366±0.0062
4.	100% salinity (*Vigna mungo)*	0.0714±0.0033	0.1422±0.0012	11.59±0.73	1.8914±0.0106	0.4518±0.0033	1.4429±0.0138	0.0889±0.0687	1.1416±0.0117
5.	24 hr Dark (*Vigna mungo)*	0.5656±0.0030	0.0329±0.0005	9.04±0.64	1.89 ±0.0104	0.76±0.0059	1.44±0.0135	0.00017±0.00001	23.12±0.1900

Values are expressed Mean±Standard deviation.

Table 3: Antidiabetic and Anti-Inflammatory activity.

Antidiabetic activity					
Sl. No.	**Stress Condition**	**0.01 mg/mL**	**0.05 mg/mL**	**0.1 mg/mL**	**IC_{50} (µg/mL)**
1.	Normal	2.07±1.0142	88.64±2.0556	77.25±0.0230	55.9866
2.	25% salinity	87.48±0.1905	41.94±0.4271	95.02±0.0152	74.8166
3.	50% salinity	6.78±0.1212	38.72±0.7825	23.84±0.5024	56.4477
4.	100% salinity	57.49±0.5164	34.77±0.2203	95.11±1.1983	62.4611
5.	24 hr Dark (*Vigna mungo*)	99.48±0.09	98.97±1.56	78.24±0.25	92.23
Anti- Inflammatory activity					
Sl. No.	Stress Condition	0.01 mg/mL	0.05 mg/mL	0.1 mg/mL	IC_{50} (µg/mL)
1.	Normal	20.3±0.6519	2.67±0.9351	4.77±0.4099	9.2488
2.	25% salinity	67.32±0.0642	67.13±56.66	6.56±0.0721	47.0077
3.	50% salinity	62.41±0.3435	55.59±0.0230	34.53±0.0360	50.8477
4.	100% salinity*)*	28.69±0.0351	3.23±0.1242	2.55±0.0230	11.4955
5.	24 hr Dark (*Vigna mungo*)	54.60±4.29	95.7±3.48	88.24±0.56	79.51

Values are expressed Mean±Standard deviation.

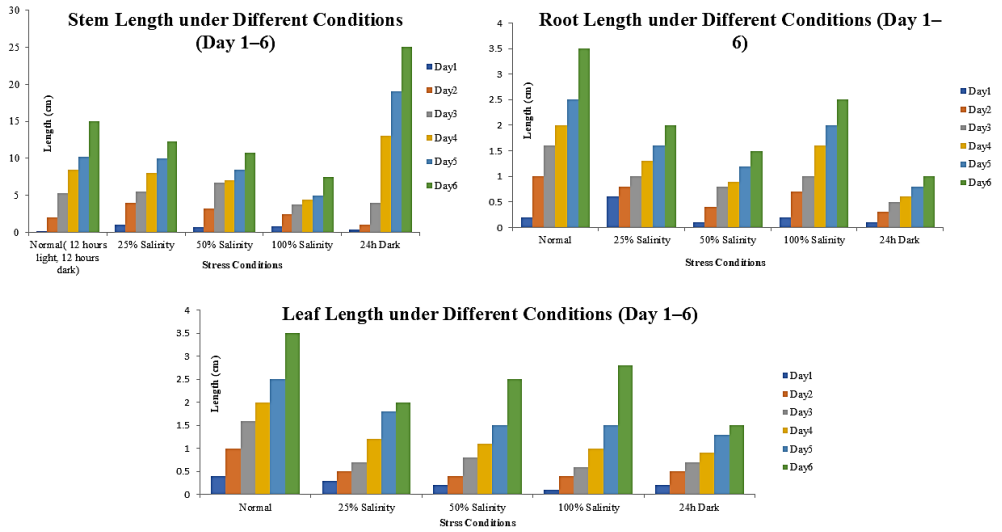

Figure 2: Germination rate of different stress condition *Vigna mungo* (Uradwhole microgreen).

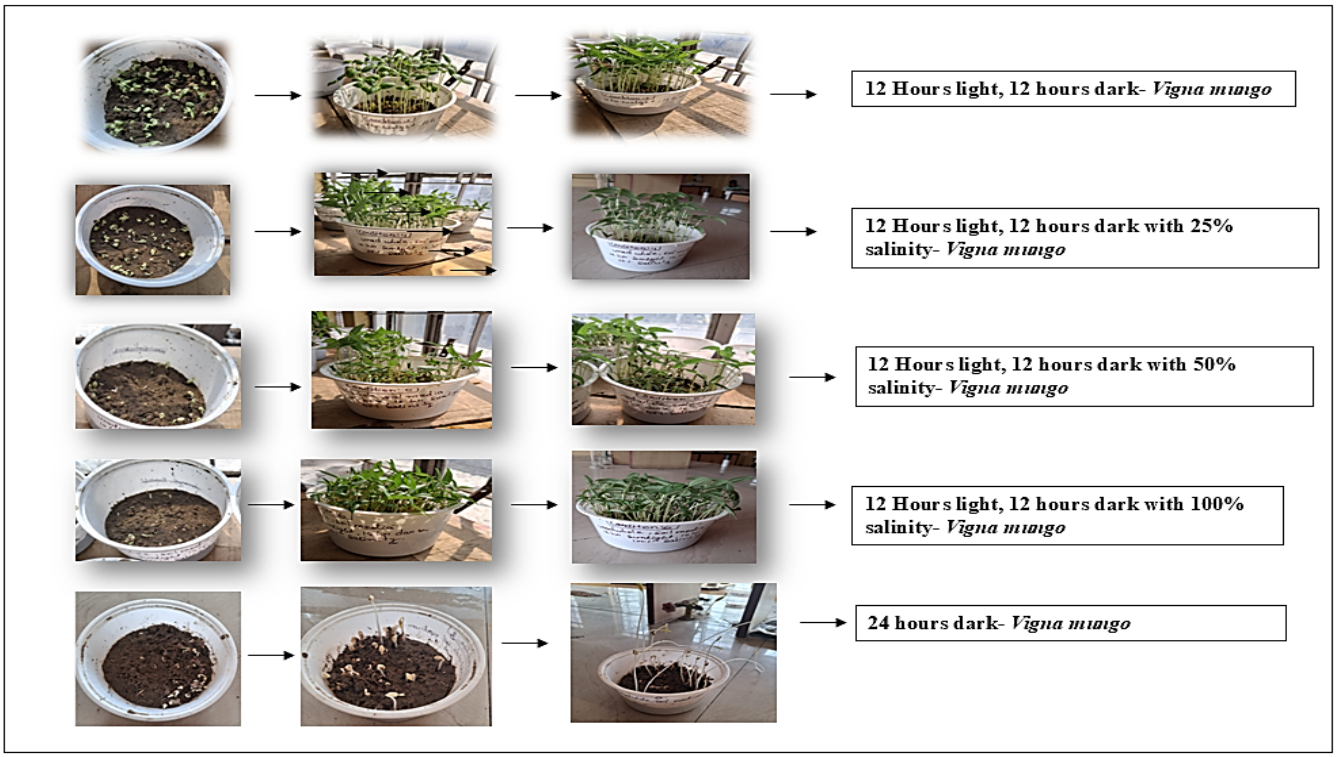

Figure 3: Germination details Uradwhole Microgreen.

salinity (11.49)>25% salinity (47.00)>50% salinity (50.84)>24 hr Dark (79.51).

DISCUSSION

The present study evaluated how different stress conditions influence the phytochemical composition and biological activities of *Vigna mungo* microgreens. The findings clearly demonstrate that environmental stress plays a crucial role in modulating secondary metabolite production and associated bioactivities.

Salinity stress significantly affected phytochemical accumulation. Moderate salinity (50%) markedly enhanced total flavonoid and anthocyanin content, suggesting that controlled stress can stimulate secondary metabolism as a defensive response. In contrast, severe salinity (100%) resulted in the highest total phenolic content, indicating activation of protective antioxidant mechanisms under higher stress intensity. Mild salinity (25%) favored tannin accumulation, showing that different stress levels selectively influence specific phytochemical pathways.

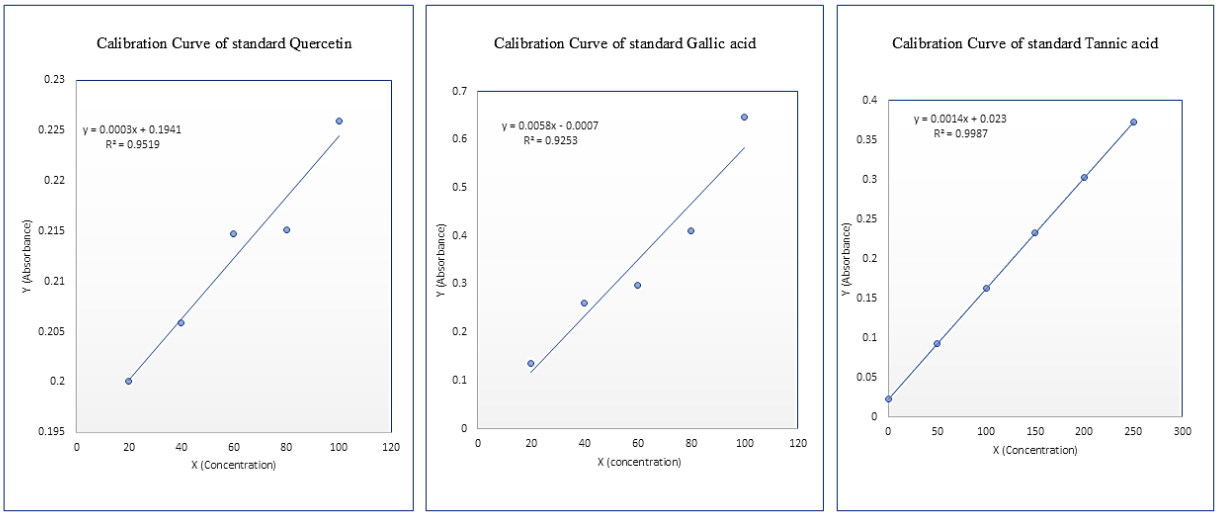

Figure 4: Calibration Curve of standard Quercetin 4a, Gallic acid 4b, Tannic acid 4c.

Pigment analysis further supported these observations. Normal growing conditions maintained the highest total chlorophyll content, reflecting optimal photosynthetic activity. However, chlorophyll a increased under severe salinity, while anthocyanin accumulation peaked at 50% salinity, indicating stress-induced pigment modulation. Interestingly, 24-hr dark treatment drastically reduced anthocyanin content but showed unusually high carotenoid levels, possibly due to altered plastid development under light deprivation.

Biological assays revealed meaningful differences among treatments. Although phytochemical enhancement was observed under stress, the strongest antidiabetic and anti-inflammatory activities were associated primarily with normal and high-salinity conditions. In contrast, microgreens grown under complete darkness showed comparatively weaker enzyme inhibition and anti-inflammatory activity despite elevated flavonoid levels. This suggests that overall bioactivity depends not merely on total flavonoid quantity but on the specific composition and interaction of phenolic compounds, which are influenced by light exposure and stress intensity.

Taken together, these results highlight the importance of optimizing environmental conditions to enhance the nutraceutical potential of microgreens.

CONCLUSION

This study demonstrates that environmental stress significantly influences the phytochemical profile and therapeutic potential of *Vigna mungo* microgreens. Moderate salinity stress effectively enhanced flavonoid and anthocyanin accumulation, while severe salinity maximized total phenolic content and showed strong biological activity. Normal conditions maintained optimal chlorophyll levels and also exhibited considerable bioactivity.

Although dark treatment stimulated flavonoid accumulation and morphological elongation, it reduced phenolic and anthocyanin synthesis and resulted in weaker antidiabetic and anti-inflammatory activities. Therefore, complete light deprivation does not enhance functional properties. Overall, controlled salinity stress-particularly moderate to high levels-appears to be a promising strategy for improving the phytochemical richness and medicinal value of urad microgreens. Further *in vivo* studies and bioavailability assessments are recommended to validate their potential as functional foods or nutraceuticals.

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ABBREVIATIONS

T2DM: Type 2 Diabetes Mellitus; **TPC:** Total Phenolic Content; **TFC:** Total Flavonoid Content; **TTC:** Total Tannin Content**; FW:** Fresh Weight; **GAE:** Gallic Acid Equivalent; **QE:** Quercetin Equivalent; **TAE:** Tannic Acid Equivalent; **IC_{50}:** Half maximal Inhibitory Concentration; **OD:** Optical Density.

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SUMMARY

This study demonstrates that salinity stress enhances phytochemical content in *Vigna mungo* microgreens, influencing their antidiabetic and anti-inflammatory potential. Moderate

salinity improved flavonoid accumulation, whereas optimal biological activity was observed under normal growth conditions.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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