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Research Article

PHYTOCHEMICAL CONTENT AND ANTIOXIDANT DEFENSE IN EMERGING MICROGREENS: FENUGREEK, HORSE GRAM AND MUSTARD

Dharmasoth Rama Devi, *Aruna Kumari Dokkada, Gajula Niharika, Veenela Varma Nadimpalli, Niharika Poltireddy, Lavanya Guntreddy

Department of Pharmacognosy, Vignan Institute of Pharmaceutical Technology, Visakhapatnam, Andhra Pradesh, India.

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ABSTRACT

Microgreens, the young seedlings of vegetables and legumes harvested at the first true-leaf stage, have emerged as functional foods with enhanced nutritional and phytochemical profiles. This study aims to comparatively assess the phytochemical content and antioxidant defense potential of fenugreek (*Trigonella foenum-graecum*), horse gram (*Macrotyloma uniflorum*), and mustard (*Brassica juncea*) microgreens. Using both qualitative and quantitative assays, the presence and levels of polyphenols, flavonoids, carotenoids, and other antioxidant compounds were evaluated. Extracts of all three microgreen types demonstrated substantial antioxidant activities, measured through DPPH, ABTS, and ferric reducing antioxidant power (FRAP) assays, reflecting strong radical scavenging and redox properties. Notably, microgreens exhibited higher concentrations of total phenolics and flavonoids compared to their respective mature leaves, correlating significantly with enhanced antioxidant capacity. The results confirm that fenugreek, horse gram, and mustard microgreens are promising dietary sources of natural antioxidants, suitable for nutritional interventions aimed at combating oxidative stress and improving metabolic health.

Keywords: Horse Gram microgreen, Mustard microgreen, Fenugreek microgreen Phytochemical content.

INTRODUCTION

Microgreens are young, tender seedlings of vegetables, herbs, and pulses harvested at the early stage of growth, typically within 7 to 21 days after germination, once the first true leaves develop. They are positioned nutritionally and developmentally between sprouts and baby greens (Sravishta *et al*, 2023). This early stage of harvest concentrates nutrients and phytochemicals, making microgreens a potent and convenient source of dietary bioactive compounds. Unlike mature plants, microgreens generally contain higher levels of vitamins, minerals, polyphenols, flavonoids, antioxidants, and other phytochemicals due to their dense cellular composition and rapid growth. The emerging popularity of microgreens stems from their rich nutritional profile and potential health benefits, including antioxidant defense, inflammation modulation, and disease risk reduction. They have been identified as functional foods, capable of delivering concentrated micronutrients and phytochemicals that may alleviate oxidative stress and contribute to metabolic and cardiovascular health (Singh A, *et al*., 2024). However, phytochemical composition and antioxidant capacity vary significantly among species, environmental growth conditions, and postharvest treatment.

Fenugreek (*Trigonella foenum-graecum*), horse gram (*Macrotyloma uniflorum*), and mustard (*Brassica juncea*) microgreens represent promising candidates for nutritional studies because of their traditional use in medicine and diets and preliminary evidence suggesting high antioxidant contents (Arya KS, *et al*., 2023). This study investigates and compares the phytochemical profiles and in vitro antioxidant activities of these three microgreens, to provide

*Corresponding Author: Aruna Kumari Dokkada, Associate Professor, Vignan Institute of Pharmaceutical Technology, Duvvada, Visakhapatnam, Email: arunampharma@gmail.com.

scientific insights into their functional potential and suitability for dietary interventions aimed at oxidative stress management.

MATERIALS AND METHODS

Sample Collection and Cultivation

Fenugreek (*Trigonella foenum-graecum*), horse gram (*Macrotyloma uniflorum*), and mustard (*Brassica juncea*) seeds were sourced from certified agricultural suppliers. Seeds were sterilized and grown under controlled environmental conditions (temperature 22–25°C, relative humidity 60–70%, photoperiod 16 h light:8 h dark) in a growth chamber. Microgreens were harvested at 10–14 days post-germination when the first true leaves developed.

Preparation of Extracts

Freshly harvested microgreens were washed, air-dried, and then homogenized. Aqueous and methanolic extracts were prepared by macerating 5 g of plant material in 100 ml solvent for 24 hours under agitation at room temperature. Extracts were filtered using Whatman No.1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40°C. The concentrated extracts were stored at 4°C for subsequent analysis.

Qualitative Phytochemical Screening

Standard qualitative tests were performed on extracts to detect the presence of major phytochemical classes including alkaloids, flavonoids, tannins, saponins, terpenoids, phenolics, glycosides, and steroids using protocols adapted from established phytochemical methods (Dereje B. *et al*., 2023).

Quantitative Phytochemical Analysis

Total Phenolic Content (TPC)

Measured by Folin-Ciocalteu reagent method. Extract aliquots (100 µl) were mixed with 1 ml of Folin-Ciocalteu reagent (diluted 1:10), incubated for 5 minutes, then 1 mL of 7.5% sodium carbonate was added. The reaction mixture was incubated for 30 minutes in the dark, and absorbance was recorded at 765 nm using a UV-Visible spectrophotometer. Gallic acid was used as the standard, and results expressed as mg gallic acid equivalent (GAE)/g extract (Das M. Nutritional, 2023).

Total Flavonoid Content (TFC)

Assayed by aluminum chloride colorimetric method. Extracts were mixed with aluminium chloride solution, incubated, and absorbance measured at 415 nm. Quercetin was used as standard and flavonoid content expressed as mg quercetin equivalent (QE)/g extract (Research Trend article, 2023).

In Vitro Antioxidant Assays

DPPH Radical Scavenging Assay

The ability of extracts to scavenge DPPH free radicals was measured by mixing extract aliquots with DPPH solution (0.1 mM in methanol). After 30 minutes incubation in the dark, reduction in absorbance was measured at 517 nm. Percent inhibition was calculated, and IC50 values (concentration of extract to inhibit 50% of DPPH radicals) were determined (Gunjal M, *et al*., 2024).

ABTS Radical Cation Decolorization Assay

ABTS radicals were generated and mixed with extracts. The decrease in absorbance at 734 nm after 6 minutes was noted as a measure of antioxidant capacity.

Ferric Reducing Antioxidant Power (FRAP) Assay

The reduction of ferric-tripyridyltriazine complex to ferrous form by antioxidants in the extracts was monitored by absorbance increase at 593 nm after 30 minutes incubation at 37°C. Results reported as mmol Fe (II)/g extract.

RESULTS AND DISCUSSION

Fenugreek microgreens display a rich presence of phytochemicals such as alkaloids, flavonoids, tannins, and saponins, which contribute to their robust antioxidant activity. Horse Gram microgreens contain phenols, glycosides, and flavonoids but lack some compounds like tannins and saponins, but their antioxidant activity is strong and comparable to fenugreek. Mustard microgreens have the highest total phenolic content among the three but lack flavonoids and several other compounds, implying that their antioxidant properties derive mainly from phenolics. Antioxidant assays show that all three microgreens increase antioxidant effectiveness with concentration, with Fenugreek and Mustard generally showing superior antioxidant activity compared to Horse Gram. These findings correspond well with their phenolic and flavonoid content, indicating a strong correlation between these phytochemicals and antioxidant potential. From a nutritional and health standpoint, the unique phytochemical and antioxidant profiles suggest that consuming a combination of Fenugreek, Horse Gram, and Mustard microgreens could provide a complementary range of bioactive compounds beneficial for oxidative stress reduction and overall health promotion.

Table. 1 Preliminary Phytochemical Screening.

S. No	Name Of the Test	Fenugreek	Horse Gram	Mustard
1	Alkaloids	+ve	-ve	+ve
2	Amino Acids	-ve	-ve	-ve
3	Phenols	+ve	+ve	+ve

4	Glycosides	-ve	+ve	+ve
5	Flavanoids	+ve	+ve	-ve
6	Tannins	+ve	-ve	-ve
7	Saponins	+ve	-ve	-ve

Table 2. Total Phenol Content (mg GAE/g).

S. No	Extract	Total Phenol Content (mg GAE/g)
1	Fenugreek Microgreens	22.07±8.14
2	Horse Gram Microgreens	34±9.03
3	Mustard Microgreens	43.42±16.39

Table 3. Total Flavonoid Content (mg QE/g).

S. No	Extract	Total Flavonoid Content (mg QE/g)
1	Fenugreek Microgreens	42.5±35.7
2	Horse Gram Microgreens	23.82.5±5.79
3	Mustard Microgreens	36.53.5±24.5

Table: 4 *In- Vitro* Antioxidant Assays (DPPH Method).

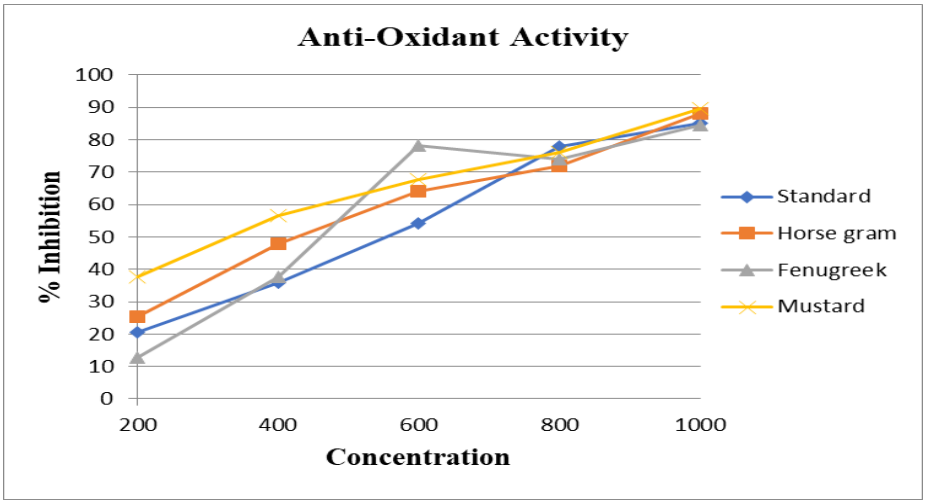

Figure 1. % Inhibition of Micro greens.

CONCLUSION

Fenugreek, Horse Gram, and Mustard microgreens each have distinct phytochemical profiles and antioxidant capacities. Fenugreek shows a broad spectrum of phytochemicals including alkaloids, flavonoids, tannins, and saponins, with moderate phenolic but high flavonoid content. Horse Gram is rich in phenols and flavonoids but lacks some compounds found in fenugreek. Mustard has the highest phenolic content but lacks flavonoids and several other phytochemicals. Antioxidant activity increases with concentration in all three, with fenugreek and mustard generally showing stronger effects than horse gram. This highlights the complementary nutritional and functional benefits of including these microgreens in a diet for antioxidant support.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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