AATCC Review

08 June 2025: Received
27 July 2025: Revised
05 August 2025: Accepted
03 September 2025: Available Online

https://aatcc.peerjournals.net/

Original Research Article

Open Access

Preliminary screening of phytochemicals in processed barnyard millet and pumpkin seeds

K. B. Nirmala[1], Jessie Suneetha W*[2], B. Anila Kumari[1], J. Hemantha Kumar[3] and K. Supriya[4]

[1]*PJTAU, Rajendranagar, Hyderabad – 500030, India*
[2]*AICRP- Women in Agriculture, PJTAU, Rajendranagar, Hyderabad – 500030, India*
[3]*Agricultural College, PJTAU, Aswaraopet, Bhadradri Kothagudem – 507301, India*
[4]*Department of Statistics and Mathematics, PJTAU, Rajendranagar, Hyderabad – 500030, India*

ABSTRACT

This study aimed to analyse the phytochemical composition of germinated barnyard millet and pumpkin seed meal. After processing, the materials were tray-dried, and methanol and aqueous extracts were prepared. Preliminary phytochemical screenings revealed the presence of carbohydrates, alkaloids, proteins, amino acids, flavonoids, terpenoids, saponins and tannins in all samples, while sterols and phenols were absent in both extracts of pumpkin seed meal, owing to the removal of lipid components. The cardiac glycosides, fixed oil and fats, phlobatinins, and quinones were absent in all samples. These findings suggested that germinated barnyard millet and pumpkin seed meal could serve as valuable sources of active compounds for pharmacological and medicinal purposes, warranting further investigation.

Keywords: *Barnyard millet, Pumpkin seed, Germination, Defatting, and Phytochemicals.*

I. Introduction

Phytochemicals were bioactive, non-nutritive compounds produced by plants to protect against oxidative stress, UV radiation, and pathogens. In humans, these substances help regulate oxidative balance, inflammation, and apoptosis to reduce the incidence of diseases. Studies have linked dietary phytochemicals to lowered risk of non-communicable diseases like cancer, cardiovascular issues, type 2 diabetes and neurodegenerative conditions, which were on the rise [1][2].

Phytochemicals were categorized into major classes like polyphenols, terpenoids, alkaloids, glucosinolates and saponins. Polyphenols have strong antioxidant and anti-inflammatory effects by targeting NF-κB and Nrf2 pathways [3]. Carotenoids played an important role in reducing oxidative stress and supporting immune health. Glucosinolates abundant in Brassicaceae vegetables broke down into isothiocyanates with anticancer properties. Saponins helped lower cholesterol levels and boost immune function by influencing gut bacteria and bile acid metabolism [4].

Minor millets were underutilized yet nutritionally rich grains gaining interest for their functional properties due to their high fiber content, micronutrients and bioactive compounds. Among them, barnyard millet (*Echinochloa frumentacea*) was notable for its high protein of 11.2%, iron of 15.5 mg/100 g and polyphenol levels [5]. It contained phenolic acids and flavonoids with strong antioxidant properties that reduce oxidative stress [6]. Its low glycemic index and resistant starch made it effective for managing type 2 diabetes [7].

*Corresponding Author: ***Jessie Suneetha W***

DOI: https://doi.org/10.21276/AATCCReview.2025.13.04.256

© 2025 by the authors. The license of AATCC Review. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

Phytates and tannins though once considered antinutrients helped reduce cholesterol and enhanced antioxidant capacity. Germination further improved its phytochemical profile by increasing. phenolics, flavonoids, and antioxidants due to enzymatic activation, macromolecule breakdown, and release of bound compounds [8].

Pumpkin seeds (*Cucurbita moschata* Poir) were nutrient-dense, containing high-quality protein (30.0%), lipids (30.0 – 35.0%), minerals and diverse phytochemicals such as phenolic acids, flavonoids, tocopherols and sterols [9]. These compounds contributed to antioxidant, anti-inflammatory and cardioprotective effects. Defatting enhanced the concentration and bio accessibility of phytochemicals by removing the lipid matrix, which otherwise dilute or binds polar compounds. Defatted pumpkin seed flour has shown significantly higher total phenolic content and antioxidant activity compared to whole seeds [10]. This process improved the extractability of bound phenolics and supported the use of defatted residues in functional food formulations promoting both nutritional and industrial utilization.

II. Materials and Methods

Barnyard millet (***Echinochloa frumentacea***) procured from local farmers and pumpkin seeds (*Cucurbita moschata* Poir) were purchased from local markets of Hyderabad. The glassware and equipment were from the Department of Foods & Nutrition, PGRC, PJTAU, Rajendranagar, Hyderabad.

Preparation of germinated barnyard millet (GBM) and pumpkin seed meal (PSM) extracts:

Germination of barnyard millet: The millets were cleaned to remove broken pieces, inert materials, seeds of other varieties to ensure purity and stored at ambient temperature in airtight containers until further analysis.

© 2025 AATCC Review. All Rights Reserved.

The raw barnyard millet was soaked in distilled water in the ratio of 1:2 for 12 hrs followed by incubation at 75–80% RH and ambient temperature for 24 hrs. After germination, the samples were tray dried, powdered, and stored in air tight container [11].

Preparation of pumpkin seed meal: After removing foreign materials, the seeds were milled into flour and passed through a sieve with 30 meshes. The flour was defatted using hexane in the ratio of 1:3 for 16 hrs. Subsequently, it was kept at room temperature for 48 hours to remove solvent residues completely, ground in a grinding machine until the flour particles passed through 70-mesh sieve. The samples were then stored in polyethylene bags under refrigeration [12]

Preparation of methanol or aqueous extracts

About 2.0 g of germinated barnyard millet and pumpkin seed meal each were used to extract by cold steeping in 100.0 ml of methanol for 24 hrs, centrifuged at 3000 rpm for 10 min and filtered with Whatman No. 41 filter paper to obtain clear extracts. The clear filtrates were pooled, combined and stored at 4°C until further use. The procedure is repeated with distilled water instead of methanol for aqueous extract [13].

Preliminary phytochemical analysis of methanol and aqueous extracts of germinated barnyard millet and pumpkin seed meal

The initial phytochemical screening was performed following the protocol for carbohydrates, alkaloids, proteins, amino acids, flavonoids, fixed oils, terpenoids, cardiac glycosides, steroids, tannins, phlobatinins, phenols and quinones [13].

Test for carbohydrates: To 2.0 ml of sample extracts, two drops of Molisch reagent was added and shaken vigorously. To this 2.0 ml of concentrated H2 SO4 was added from the walls of the test tube. A reddish violet ring formed at the juncture of two layers immediately, which indicated the presence of carbohydrates.

Test for alkaloids: The presence or absence of alkaloids was carried out using Mayer's, Wagner's and Hager's tests.

Mayer's test: A portion of the extracts were added with 1.0% HCl and 6 drops of Mayer's reagent (1.36 g of mercuric chloride and 5.0 g of potassium iodide in 100.0 ml of water). The organic precipitate indicated the presence of alkaloids in the samples.

Wagner's test: A fraction of extracts was treated with Wagner's reagent which has 0.28 g of iodine and 0.20 g of potassium iodide in 10.0 ml water. The formation of cream-colored precipitate indicated the presence of alkaloids.

Hager's test: The extract samples were treated with Hager's reagent, containing a saturated aqueous solution of picric acid and the formation of a prominent yellow coloured precipitate indicated the presence of alkaloids.

Test for proteins: To 2.0 ml of sample extracts, 1.0 ml of 40.0% sodium hydroxide and 1 to 2 drops of 1.0% copper sulphate solution were added. The formation of violet colour indicated the presence of peptide linkages found in the proteins of leaf samples.

Test for amino acids: To 2.0 ml of sample extracts, 2.0 ml of ninhydrin reagent was added and kept in a water bath at 60°C for 20 minutes. The appearance of purple colour indicated the presence of amino acids in the sample.

Test for flavonoids: Each fraction of the sample extracts was added with 5.0 ml ammonia followed by few drops of concentrated H2SO4. The development of a yellow colour confirmed the presence of flavonoids, which disappeared on standing for a few minutes.

Test for fixed oils and fats: A few drops of 0.5 N alcoholic KOH and a drop of phenolphthalein indicator were added to the extract fractions. The mixtures were heated at 60°C in a water bath for 1½– 2 hrs. The formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

Test for terpenoids: To 5.0 ml of extracts, 2.0 ml of chloroform and 3.0 ml of concentrated sulphuric acid were added and the formation of a single layer of reddish-brown colour at the juncture was due to the presence of terpenoids.

Test for cardiac glycosides: About 5.0 ml of extracts was added with 2.0 ml of glacial acetic acid containing one drop of FeCl3 solution and 1.0 ml of concentrated sulphuric acid. The formation of a brown ring at the interface indicated the deoxy sugar which was distinctive for cardenolides. A violet ring can appear below the brown ring in the acetic acid layer, whereas a green layer can also form just gradually, into a thin layer.

Test for steroids (Liebermann – Burchard test): 2.0 ml each of acetic anhydride and concentrated sulphuric acid were added to 0.5 ml of sample extracts in triplicates. The appearance of blue or green colour from initial violet colour indicated the presence of steroids.

Test for saponins: The extracts were added with 20.0 ml of distilled water and agitated vigorously in a graduated cylinder for 15 min. The formation of a centimetre layer of foam indicated the presence of saponins.

Test for tannins: A few drops of 1.0% lead acetate was added 5.0 ml of extracts and the formation of yellow precipitate indicated the presence of tannins.

Test for phlobatinins: Extracts were boiled with a few drops of 1.0% hydrochloric acid and the deposition of red precipitate indicated the presence of phlobatinins.

Test for phenols: Ferric chloride and Liebermann's tests were used to determine the presence or absence of phenols in germinated barnyard millet and pumpkin seed meal extracts.

Ferric chloride test: A fraction of each of the extracts was added with a few drops of 5.0% Ferric chloride, and the formation of a deep blue or black colour indicated the presence of phenols.

Liebermann's test: The extracts were heated with sodium nitrite and concentrated sulphuric acid solution, diluted in water, cooled and added with an excess of dilute sodium hydroxide. The formation of deep red or green or blue colours indicated the presence of phenols.

© *2025 AATCC Review.* All Rights Reserved.

Test for quinones: A small amount of each extract was treated with concentrated HCl and appearance of yellow coloured precipitate indicated the presence of quinones. The absence of yellow coloured precipitate showed that the extracts have not undergone any form of oxidation.

III. Results and Discussion: Phytochemicals or phytonutrients found naturally in plants have been found to possess antioxidant and anti-inflammatory properties that benefit human health. These compounds play a vital role in the body's cleansing process by removing harmful substances that could otherwise lead to the formation of free radicals [14]. Phytochemical screening is a simple method for determining the presence or absence of phytochemicals. It can also be utilized to assess the composition of meals and develop foods with unique functional properties. Antioxidant-rich foods were essential in today's society as they help to counteract free radicals generated by stress, pollution, aging and changing dietary patterns.

The results of phytochemical screening of both methanol and aqueous extracts were presented in Table 1. Barnyard millet phytochemical content fluctuated and was influenced by germination time. The initial phytochemical screening of these extracts revealed the strong presence of carbohydrates, flavonoids and saponins. Although total starch may decrease slightly during germination, soluble carbohydrate content (reducing sugars) increases total carbohydrate content [15]. Saponin and flavonoids content tends to increase in many cereals and legumes during germination due to metabolic shifts favouring plant secondary metabolites [16].

Both extracts showed the presence of alkaloids, proteins, amino acids, terpenoids, steroids, tannins, and phenols, while cardiac glycosides, fixed oils and fats, phlobatinins and quinones were absent. Similar studies showed that the phytochemical screening of germinated foxtail millet showed the presence of carbohydrates, alkaloids, proteins, amino acids and terpenoids [17]. The quantitative analysis revealed that optimal germination of barnyard millet for 36 hours hrs resulted in high antioxidant activity and phenolic content [18]. A review suggested that barnyard millet phytochemicals exhibited strong antioxidant properties with high levels of phenols, flavonoids, and tannins [19].

Both aqueous and methanol extraction of PSM exhibited strong presence of proteins, amino acids, flavonoids and terpenoids because defatting removes a significant portion of lipids from the pumpkin seed. Since oil is a major component (40–50%) of pumpkin seeds, its removal increases the relative concentration of non-lipid components, especially proteins, amino acids and phytochemicals per unit weight of the flour [20].

Alkaloids, saponins and tannins were present in both aqueous and methanol extraction. Carbohydrates were detected in aqueous extraction but not in methanol extraction of PSM. Phenols, fixed oils and fats, cardiac glycosides, phlobatinins, and quinones were absent in both extracts of PSM. The quantitative analysis of defatted pumpkin seed flour revealed that defatted pumpkin seed flour contained phytates (16.24 mg/100g) and tannins (3.31 mg/100g) [21]. The defatted sesame seeds showed total phenols ranged from 20.2 – 24.5 mg GAE/g and flavonoids ranged from 7.3 – 8.0 mg CAE/g, showing that phytochemicals and antioxidants present in pumpkin seed meal may be effective in blocking the chain reactions by interacting with the peroxyl radicals [22].

Germinated barnyard millet and pumpkin seed meal in the present study contained high levels of tannins, organic acids and phenolic acid derivatives which were known for their strong antibacterial, anti-inflammatory antioxidative properties. Factors like temperature, canal water, humidity, soil conditions that include pH, soil fertility, acidity, moisture, microbiota and cultivar differences influenced the plant's phytochemical makeup [23].

Table 1: Phytochemical screening of Barnyard millet and pumpkin seed meal-based methanol and aqueous extracts

S. No.	Name of the test	Methanol extract		Aqueous extract	
		GBM	PSM	GBM	PSM
1	Test for carbohydrates	++	-	++	+
2	Test for alkaloids:				
	Mayer's test	+	+	+	+
	Wagner's test	+	+	-	+
	Hager's test	+	+	+	+
3	Test for proteins	+	+++	+	+++
4	Test for amino acids	+	+++	+	+++
5	Test for flavonoids	++	++	++	++
6	Test for fixed oils and fats	-	-	-	-
7	Test for terpenoids	+	++	+	++
8	Test for cardiac glycosides	-	-	-	-
9	Test for steroids	+	-	+	-
10	Test for saponins	++	+	++	+
11	Test for tannins	+	+	+	+
12	Test for phlobatinins	-	-	-	-
13	Test for phenols:				
	Ferric chloride test	+	-	+	-
	Liebermann test	+	-	+	-
14	Test for quinones	-	-	-	-

***Note**: All the tests were carried out in triplicates.*
GBM: Germinated barnyard millets
PSM: Pumpkin seed meal

Conclusion: In conclusion, the plants rich in complex bioactive compounds provided significant health benefits including anti-inflammatory, anti-cancer, antioxidant and antibacterial effects. The screening of phytochemicals was an easy way to find out the presence or absence of compounds that assist in designing special foods with desired functions. These antioxidants assist in neutralizing free radicals developed due to ageing, dietary habits, anxiety, ecological pollution and atmospheric situations.

Acknowledgement: The authors thank the honourable Vice Chancellor of Professor Jayashankar Telangana Agricultural University, Rajendranagar, Hyderabad for his encouragement. Also, authors acknowledged the facilities provided by the Post Graduate & Research Centre, AICRP on Women in Agriculture, and Central Instrumentation Cell, PJTAU, Rajendranagar, Hyderabad for the current research.

References

1. Wang L, Tu YC, Lian TW, Hung JT, Yen JH, Wu MJ. Distinctive antioxidant and anti-inflammatory effects of flavonols. Journal of agricultural and food chemistry. 2006 Dec 27;54(26):9798-804.

2. Arulselvan P, Fard MT, Tan WS, Gothai S, Fakurazi S, Norhaizan ME, Kumar SS. Role of antioxidants and natural products in inflammation. Oxidative medicine and cellular longevity. 2016; 2016:1–20.

© *2025 AATCC Review.* All Rights Reserved.

3. Scalbert A, Brennan L, Manach C, Andres-Lacueva C, Dragsted LO, Draper J, Rappaport SM, Van Der Hooft JJ, Wishart DS. The food metabolome: a window over dietary exposure. The American journal of clinical nutrition. 2014 Jun 1;99(6):1286-308.

4. Xiao MY, Li S, Pei WJ, Gu YL, Piao XL. Natural Saponins on Cholesterol-Related Diseases: Treatment and Mechanism. Phytotherapy Research. 2025 Mar;39(3):1292-318.

5. Chandraprabha S, Sharon CL. Optimisation of conditions for barnyard millet germination. Plant Archives (09725210). 2021 Apr 1;21(1).

6. Banerjee P, Maitra S, Banerjee P. The role of small millets as functional food to combat malnutrition in developing countries. Indian Journal of Natural Sciences. 2020 Jun;10(60):20412-7.

7. Dutta A, Joshi D, Suri S, Trivedi N, Acharya R, Malik R, Bose S, Dobhal A. Bioactive and Techno-Functional Properties of Barnyard Millet (Echinochloa frumentacea). In Nutri-Cereals 2023 Aug 14 (pp. 213-231). CRC Press.

8. Bhavadharani PV, Gurumoorthi P. Impact of germination on nutritional components, antinutritional, and functional properties of proso and barnyard millets. Food Chemistry Advances. 2025 Mar 1-10

9. Šamec D, Loizzo MR, Gortzi O, Çankaya İT, Tundis R, Suntar İ, Shirooie S, Zengin G, Devkota HP, Reboredo-Rodríguez P, Hassan ST. The potential of pumpkin seed oil as a functional food—A comprehensive review of chemical composition, health benefits, and safety. Comprehensive reviews in food science and food safety. 2022 Sep;21(5):4422-46.

10. Apostol L, Berca L, Mosoiu C, Badea M, Bungau S, Oprea OB, Cioca G. Partially defatted pumpkin (Cucurbita maxima) seeds-a rich source of nutrients for use in food products. Rev. Chim. 2018 Jun 1;69(6):70-5.

11. Kulla S, Hymavathi TV, Kumari BA, Reddy RG, Rani CV. Impact of germination on the nutritional, antioxidant and antinutrient characteristics of selected minor millet flours. Annals of Phytomedicine2021;10: 178-84.

12. Zakeri K, Sadeghi Mahoonak A, Ghorbani M, Moayedi A, Maghsodlou Y. Optimization of hydrolysis condition of pumpkin seeds with alcalase enzyme to Achieve maximum antioxidant and nitric oxide inhibition activity. Research and Innovation in Food Science and Technology. 2019 Feb 20;7(4):445-58.

13. Harbourne, J.B. 1993. Phytochemistry. Academic press: London, pp 89-113.

14. Meghana KJ, Suneetha WJ, Devi TS, Chaitanya V. Phytochemical screening of tray dried Amaranthus viridis L. (Chirryaku) and Alternanthera sessilis (Ponnaganti kooraaaku) methanol extracts. Journal of Community Mobilization and Sustainable Development. 2024 19(3): 1-5

15. Devi PB, Vijayabharathi R, Sathyabama S, Malleshi NG, Priyadarisini VB. Health benefits of finger millet (Eleusine coracana L.) polyphenols and dietary fiber: a review. Journal of food science and technology. 2014 Jun;51(6):1021-40.

16. Singh A, Sharma S, Singh B. Effect of germination time and temperature on the functionality and protein solubility of sorghum flour. Journal of Cereal Science. 2017 Jul 1;76: 131-9.

17. D Sangma JJ, Suneetha WJ, Kumari BA, Devi KB. Phytochemical screening for antioxidant properties of germinated Foxtail millet. Current Journal of Applied Science and Technology. 2019 Nov 30;38(6):1-6.

18. Sharma S, Saxena DC, Riar CS. Analysing the effect of germination on phenolics, dietary fibres, minerals and γ-amino butyric acid contents of barnyard millet (Echinochloa frumentaceae). Food Bioscience. 2016 Mar 1;13: 60-8.

19. Pujari N, Hoskeri JH. Minor millet phytochemicals and their pharmacological potentials. Pharmacognosy Reviews. 2022 Jul;16(32):101.

20. Habib M, Singh S, Sagar NA, Ahmad S, Qureshi I, Jan S, Jan K, Bashir K. Physicochemical and functional characterization of pumpkin seed protein isolate. Sustainable Food Technology. 2025;3(2):445-55.

21. Alozie Y, Udo A, Orisa C. Proximate, anti-nutrient and vitamin composition of full-fat and defatted Seed Flour of Telfairia occidentalis. Turkish Journal of Agriculture-Food Science and Technology. 2017 Oct 30;5(11):1256-60.

22. Rani G, Rani S, Khabiruddin M. Phytochemical study of Sesame (Sesamum indium) seed oil and defatted seed cake. International Journal of Chemical Studies. 2017; 5:1187-92.

23. Kataria, R and Surti, A., 2024. Preliminary Screening of Phytochemicals and Essential Oils in Selected Herbs and Spices. International journal of pharmaceutical sciences. 2(12): 1034-1041.

© *2025 AATCC Review.* All Rights Reserved.