INTERNATIONAL JOURNAL OF RESEARCH CULTURE SOCIETY
Monthly Peer-Reviewed, Refereed, Indexed Journal
Volume - 9, Issue - 12, December- 2025
ISSN(O): 2456-6683
[Impact Factor: 9.241]
IJRCS

DOIs:10.2017/IJRCS/202512014 --:-- Research Paper / Article / Review

Phytochemical screening and anti-bacterial activity of E*chinochloa frumentacea* (Barnyard millet)

[1]K.N.V.S.N. Eswari, [2]M. Madhavi Latha, [3]G. Venkatarao, [4]K. Mallikarjuna*

[1]Lecturer, Department of Botany, ASD Government Degree College for Women(A), Kakinada, Andhra Pradesh, India.
[2]Research scholar, Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar-522510, Guntur, Andhra Pradesh, India.
[3]Research scholar, Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar-522510, Guntur, Andhra Pradesh, India.
[4*]Professor, Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar-522510, Guntur, Andhra Pradesh, India.

Email - [1]knvsneswari.3@gmail.com, [2]madhavi.maragani@gmail.com, [3]venkatgemmeli94@gmail.com,
[4*]Corresponding author: mallikarjunaanu@gmail.com

Abstract: *This study aimed to analyse the phytochemical composition and antibacterial activity of barnyard millet. After collection, the millet sample was tray-dried and powdered. Methanol and aqueous extracts were prepared. Preliminary phytochemical screening revealed the presence of alkaloids, amino acids, carbohydrates, cardiac glycosides, flavonoids, terpenoids and tannins in all samples, while fixed oils and fats were absent. The antibacterial activity of the extracts was investigated against Bacillus megaterium, Klebsiella pneumoniae, and Pseudomonas aeruginosa strains using the well-diffusion method. A significant zone of inhibition was observed, with a maximum of 6 ± 0.5 mm against Gram-positive and Gram-negative bacteria, respectively. These findings suggest that barnyard millet serves as a valuable source of active compounds for pharmacological investigations.*

Key Words: *Barnyard millet, Phytochemicals, anti-bacterial.*

1. INTRODUCTION :

Minor millets are gaining attention for their impressive nutritional profile and functional properties due to their high fiber content, micronutrients and bioactive compounds. Particularly, barnyard millet (*Echinochloa frumentacea*) boasts high protein content (11.2%), high iron levels (15.5 mg/100g), and high polyphenol levels, making it a valuable grain (Chandraprabha and Sharon, 2021). The phenolic acids and flavonoids in small millets exhibit strong antioxidant activity that reduces oxidative stress (Banerjee and Maitra, 2020). With a low glycemic index and resistant starch, it is effective in managing type 2 diabetes (Dutta *et al.,* 2023). Millet seeds are packed with nutraceuticals and phytochemicals that help prevent health issues. Whole grains like millets are getting attention for their fibre and bioactive goodies like antioxidants and phytochemicals. Millets boast an array of phytochemicals, including phenolic acids, tannins, phytosterols, phytates, sterols, carotenoids, flavonoids, and alkaloids, which support immune function (Deepa and Shanmugam, 2024).

Phytochemicals are bioactive compounds produced by plants to protect themselves from oxidative stress, UV radiation, and pathogens. In humans, they help regulate oxidative balance, inflammation, and apoptosis, and reduce disease risk. The dietary phytochemicals have been linked to a lower risk of non-communicable diseases like cancer, cardiovascular diseases, type 2 diabetes, and neurodegenerative conditions, which are increasingly prevalent (Arulselvan *et al*., 2016).

2. AIM :

This study aims to expand the understanding of barnyard millet bioactive potential and its applicability in functional food development by evaluating the chemical profile and examining its antibacterial properties.

3. METHODOLOGY :

Barnyard millet samples were obtained from the organic store, Vijayawada, Andhra Pradesh, India. The millet sample was finely powdered by using an electric mixer, and the powder was preserved in an airtight container to prepare solvent extractions.

▪ Preparation of Solvent Extracts

Extraction was carried out using 20g of each powdered sample with 100 ml of solvent and kept for 48 hours with occasional stirring. Here, methanol (HPLC grade) and aqueous were used for extraction. The extraction was done at normal room temperature conditions. The extraction was filtered through Whatman paper to get the filtrate as the extract. The phytochemical screening was performed with crude extracts according to formerly reported chemical tests (Bhattacharya and Roy, 2018; Deepa and Shanmugam, 2024).

Alkaloid (Mayer's test): The formation of cream colour precipitate in the test tube after adding the Potassium mercuric iodide solution (Mayer's reagent) to the extract suggests the presence of alkaloids in the sample.

Amino acid (Ninhydrin test): The extracts were treated with Ninhydrin (α-amino acid + NH_4). The appearance of violet colour on gentle heat specifies the existence of amino acids in the extract.

Anthraquinones (Borntrager's test): The crude extract (0.5 g) was mixed with $CHCl_3$ and shaken (5 min), and to the filtrate, addition of 10% ammonia solution in the same volume, the ammoniacal layer turns into pink violet or red colour, suggesting positive results.

Carbohydrates (Molish's test): 2ml of extract was mixed with 2 ml of Molish's reagent and shaken vigorously. 2 ml of concentrated H_2SO_4 was added carefully by means of a pipette along the wall of the test tube. Formation of a reddish ring at the junction of two liquids indicated the presence of carbohydrates.

Cardiac Glycosides (Keller-Killani test): The extract dissolved in chloroform (2 ml), on addition of sulphuric acid, forms a brown ring at the interphase, confirming the existence of cardiac glycosides.

Flavonoids (Alkaline reagent test): Extracts were hydrolysed, and the residue was treated with dilute NaOH along with dilute HCl; solubility and colouration were noted. A yellow-coloured precipitation appeared, and it turned colourless with the addition of dilute HCl, confirming the flavonoids.

Glycosides: 2 ml of glacial acetic acid containing 2% $FeCl_3$ solution was added to 5 ml of extract. 1 ml of concentrated H_2SO_4 was added slowly along the wall of the test tube. Formation of a brown ring indicates the presence of glycosides.

Saponins (Foam test): 1 ml extract with 10 ml distilled water is vortexed in a graduated cylinder for 15 min; the occurrence of a foam layer indicates saponins.

Tannins (Ferric chloride test): The extracts, on treatment with $FeCl_3$ solution, form of blue colour precipitate, indicating tannins.

Terpenoids: To the 5 ml of extract, 2 ml of chloroform was added and mixed, forming a reddish-brown colour interface on the addition of conc.H_2SO_4, indicating the terpenoids.

▪ Anti-bacterial activity

The activity was conducted using 500 µl of microorganism suspension in nutrient agar medium by the agar well diffusion method. The two extracts screened for antibacterial activities against three bacterial strains, *Bacillus megatarium* (NCIM-2187) (Gram-positive), *Klebsiella pneumoniae* (MTCC-452) and *Pseudomonas aeruginosa* (MTCC-424) (Gram-negative).

Media preparation: Nutrient Agar Media (NAM) of 13g was mixed in sterile distilled water and autoclaved at 121°C/15 lbs pressure for 20 minutes. The 30 ml of sterile liquid nutrient agar below 50 °C was inoculated with thoroughly mixed 500 µl of selected microorganism and poured into sterile petri dishes, and allowed to solidify. After complete solidification, wells (8 mm) were made into nutrient agar plates using the sterile cork borer. The crude extractions (150 µl) of different concentrations (25, 50, 100 µg/ml) were prepared (w/v) from the solvents and stored in

a refrigerator to avoid evaporation. The extracts at selected concentrations of 150 µl were added to each well. After incubation for 24 hours at 37 °C, the zone of inhibition was measured. The well diameter and the zone diameter were recorded then the zone of inhibition was calculated.

4. RESULTS :

▪ Phytochemical analysis

The primary phytochemical analysis of the methanol and aqueous extracts (Table 1) demonstrated the presence of alkaloids, amino acids, carbohydrates, proteins, flavonoids, cardiac glycosides, saponins, tannins, and terpenoids. Whereas other compounds like anthraquinones, phytosterols, fixed oils and fats were absent.

Table 1. Qualitative analysis of phytochemicals

Name of the phytocomponent	Methanol	Aqueous
Anthraquinones	-	-
Amino acids	+	+
Alkaloids	+	+
Carbohydrates	+	++
Cardiac Glycoside	+	+
Flavonoids	+	-
Fixed oils and fats	-	-
Phenolic Compounds	-	-
Saponins	+	+
Tannins	+	-
Terpenoids	+	-

+ indicates the presence of the compound, and – indicates the absence of the compound

▪ Anti-bacterial activity

The antibacterial activity of the barnyard millet aqueous and methanol crude extracts (Table 2) was evaluated against both Gram-positive (*Bacillus megatarium*) and Gram-negative (*Klebsiella pneumoniae, Pseudomonas aeruginosa*) bacteria using the well diffusion assay. For *B. megatarium*, varying concentrations of the methanol extract and the zones observed were 2 mm for 25 µg, 3 mm for 50 µg, and 6 mm for 100µg and no zones were observed for the aqueous extract. Whereas, *K. pneumoniae* exhibited inhibition zones of 1 mm (25 µg), 3.5 mm (50 µg) and 5.5 mm (100 µg) for the methanol extract and no zones for the aqueous extract. Similarly, *P. aeruginosa* exhibited inhibition zones of 2 mm (25 µg), 3.5 mm (50 µg) and 6 mm (100 µg) for the methanol extract and no zones for the aqueous extract. The results indicated that the methanol extract exhibits antimicrobial activity against both Gram-positive and Gram-negative bacterial strains; however, no significant inhibition was observed for the aqueous extract.

Table 2. Antibacterial activity of barnyard millet extracts against bacterial strains

S. No.	Name of Bacterial strain	Zone of inhibition observed			
			25 µg/ml	50 µg/ml	100 µg/ml
1	*Bacillus megatarium*	Methanol	2 mm	3 mm	6 mm
		Aqueous	-	-	-
2	*Klebsiella pneumoniae*	Methanol	1 mm	3.5 mm	5.5 mm
		Aqueous	-	-	-
3	*Pseudomonas aeruginosa*	Methanol	2 mm	3.5 mm	6 mm
		Aqueous	-	-	-

ISSN(O): 2456-6683
[Impact Factor: 9.241]

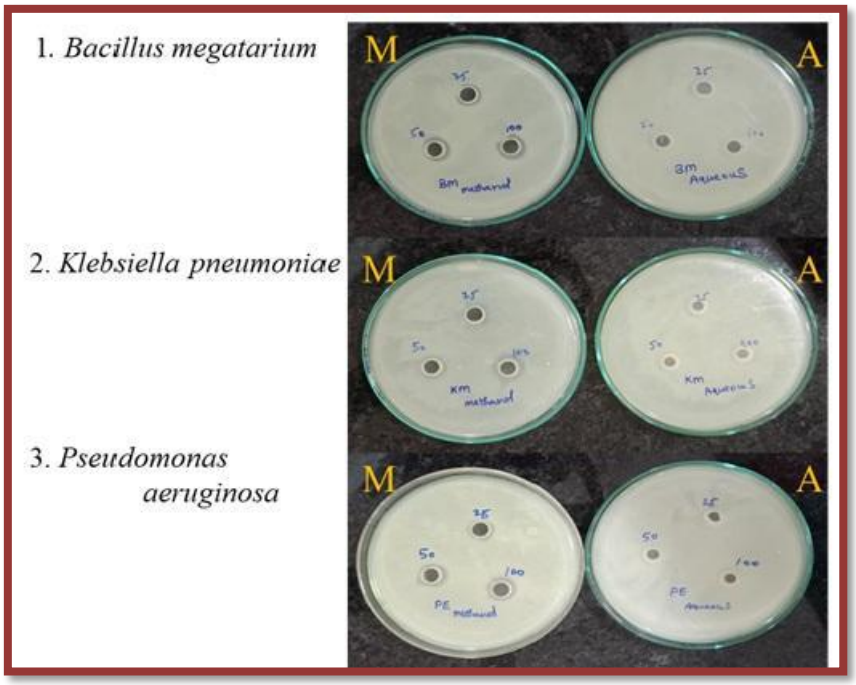

Figure 1. Antibacterial activity of methanol (M) and aqueous (A) extracts of barnyard millet

5. DISCUSSION :

Previous literature suggests that extracts prepared with different solvents have potent healing effects. Phytochemical screening of sequential solvent extracts of whole grains of barnyard millet showed that the methanolic extract contained flavonoids, terpenoids, alkaloids, steroids, tannins, and phenolic compounds. Similar compounds were found in other small millets, such as proso millet, finger millet, foxtail millet, and kodo millet (Rao *et al.,* 2011). The methanolic and aqueous extracts of foxtail millet also contained flavonoids, alkaloids, phenolics, and reducing sugars (Suma and Urooj, 2012). Differences in compound profiles may be attributed to the polarity of the extracting solvents, accounting for the observed variation in phytochemical identification across extracts (Deepa and Shanmugam, 2024). This study suggests that the phytochemical constituents in barnyard millet extracts possess various properties.

The fermented barnyard millet protein hydrolysate (PRJ-1) shows notable antimicrobial activity against both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria. In a well diffusion assay, the hydrolysate produced inhibition zones of 10 mm, 11 mm, and 12 mm against *E. coli* at concentrations of 40 µL, 80 µL, and 160 µL, respectively. Similarly, against *S. aureus*, the zones were 10 mm, 12 mm, and 14 mm for the same concentrations. However, no significant antifungal activity was observed against *Candida albicans*. The antimicrobial activity is likely due to proteolytic processes during fermentation, which break down proteins into bioactive peptides that disrupt bacterial cell membranes. This makes fermented barnyard millet protein hydrolysate a promising natural antimicrobial agent for functional food development and preservation (Kamboj *et al.,* 2025). Other millets, such as finger millet, soybean, foxtail millet (PRM-I), and proso millet, have shown substantial antibacterial activity. Barnyard millet's crude protein, however, wasn't initially known for significant antimicrobial activity. Fermentation is a game-changer; studies show that fermented foxtail millet flour proteins (FFM and FFMp) are more effective at fighting bacteria post-fermentation. Specifically, using Lactobacillus paracasei with enzyme enhancement boosts the millet's antioxidant activity, antimicrobial powers, and nutritional profile (Amadou *et al.,* 2013).

7. CONCLUSION :

The study of preliminary phytochemical analysis of the presence of essential phytoconstituents plays an important role in the pharmacological activities. Some non-nutritive phytochemicals may have disease-preventive properties. The preliminary phytochemical screening showed the alkaloids, amino acids, carbohydrates, cardiac glycosides, flavonoids, proteins, saponins, terpenoids, and tannins. The bacterial strains of *Bacillus megaterium, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* have shown some sensitivity to the methanolic extract of barnyard millet. The pure compounds of the extracts needed to be assessed for their biological activities.

REFERENCES:

1. Chandraprabha S, Sharon CL. Optimisation of conditions for barnyard millet germination. Plant Archives (09725210). 2021 Apr 1;21(1).
2. Banerjee, Pradipta & Maitra, Sagar. (2020). The Role of Small Millets as Functional Food to Combat Malnutrition in Developing Countries. Indian Journal of Natural Sciences. 10(60):20412-7.
3. Dutta, Anuradha & Joshi, Deepa & Suri, Shweta & Trivedi, Nalini & Acharya, Ranjana & Malik, Rushda Anam & Bose, Shayani & Dobhal, Ankita. (2023). Bioactive and Techno-Functional Properties of Barnyard Millet (Echinochloa frumentacea). 10.1201/9781003251279-9.
4. 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.
5. Deepa Priya Ramadoss, Kirubanandan Shanmugam, (2024). Evaluation of Phytochemical Analysis and Total Phenol Content of Proso Millet and Barnyard Millet. Vol. 8 (2): 13-19.
6. Kamboj, N., Kumar, R., Kumar, N., Pal, M., & Gautam, P. (2025). Antibacterial and Antiprotease activities of fermented barnyard millet (*Echinochloa frumentacea*) protein hydrolysate. *Food chemistry: X*, *27*,102511. https://doi.org/10.1016/j.fochx.2025.102511
7. Bhattacharyya, S., & Roy, S. (2018). "Qualitative and quantitative assessment of bioactive phytochemicals in gobindobhog and black Rice, cultivated in West Bengal, India" *International Journal of Pharmaceutical Sciences and Research*, Vol.9(9): 3845-3851.
8. Sandra M. Oses., Ana Pascual-Mate., Daniel de la Fuente., Ana de Pablo., Miguel A. Fernández-Muiño., and M Teresa Sancho., (2016). "Comparison of methods to determine antibacterial activity of honeys against Staphylococcus aureus" *NJAS-Wageningen Journal of Life Sciences*, Vol.78(1):29-33.
9. https://aatcc.peerjournals.net/wp-content/uploads/2025/11/Preliminary-screening-of-phytochemicals-in-processed-barnyard-millet-and-pumpkin-seeds.pdf
10. Rao BR, Nagasampige MH, Ravikiran M, 2011, Evaluation of Nutraceutical Properties of Selected Small Millets. J Pharm Bioallied Sci, 3(2): 277–279. https://doi.org/10.4103/0975-7406.80775
11. Suma PF, Urooj A, 2012, Antioxidant Activity of Extracts from Foxtail Millet (Setaria italica). J Food Sci Technol, 49(4): 500–504. https://doi.org/10.1007/s13197-011-0300-9
12. Amadou, I., Le, G. W., Amza, T., Sun, J., & Shi, Y. H. (2013). Purification and characterization of foxtail millet-derived peptides with antioxidant and antimicrobial activities. Food Research International, 51(1), 422–428. https://doi. org/10.1016/j.foodres.2012.12.045