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EVALUATION OF PHYTOCHEMICALS AND ANTIOXIDANT POTENTIAL IN MILLETS

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ABSTRACT: Millets are small-seeded grasses that have nutraceutical properties due to high content of proteins, dietary fibre, vitamins, antioxidants, and other valuable phytochemicals, important for health and wellbeing. The objective of the research was to analyse the phytochemical compounds and antioxidant properties of minor millet varieties *viz.*, Kodo, Proso, Barnyard, Finger, Foxtail, and Little Millets. A quantitative phytochemical examination revealed the presence of phenols, steroids, alkaloids, saponins, flavonoids, and tannins irrespective of the extraction method used. Antioxidant assay was done by the phosphomolybdate method. These findings indicate that different millet types possess distinct phytochemical profiles. Finger Millets and Foxtail Millets were rich in phenols and flavonoids, while Barnyard Millets had increased levels of alkaloids, saponins, and tannins. The antioxidant evaluation of Kodo, Proso, Barnyard, Finger, Foxtail, and Little Millets extracts showed that the Finger millet exhibited the highest antioxidant activity (4.12 µg AAE/ml in DW and 5.34 µg AAE/ml in Me). Methanol extracts generally showed higher antioxidant values than distilled water extracts, indicating that methanol is a more effective solvent for extracting antioxidants. These findings highlight millets' potential as a functional food with significant health and economic benefits.

INTRODUCTION: Millets belong to the Poaceae family (or Grass Family) and offer rich fibre, protein, vitamins, and mineral content [1, 2]. These phytonutrients and dietary fibre play an important role in reducing colon cancer risk, suppressing food cravings, and promoting satiety [3]. Furthermore, these are especially beneficial to diabetes or celiac disease due to their low glycemic index and gluten-free nutritional content [3]. Research suggests that millets aid in managing hyperlipidemia and reducing the risk of cardiovascular diseases.

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The high magnesium content in millets supports menstrual health by alleviating cramps. They are highly adaptable to arid and rain-fed regions and serve as a dual-purpose crop for both food and fodder, improving farm efficiency. Certain millet extracts also possess antimicrobial properties, inhibiting bacterial pathogens such as *Macrophomina phaseolina, Rhizoctonia solani,* and *Fusarium oxysporum* [3].

They are resistant to harsh environmental conditions, e.g., drought [2]. Indian millets are primarily cultivated in dry and semi-arid tropical regions, and they serve as a vital food and fuel source, which contributes to both ecological and economic stability [4]. Additionally, the antioxidant, antimicrobial, and antifungal properties of millets enhance food safety and preservation [5].

Millets were among the earliest cultivated cereals and continue to be a sustainable crop due to their minimal water requirement and require less effort than other grains during cultivation. Some major millet varieties include Kodo (*Panicum sumatrense*), Proso (*Panicum miliaceum*), Barnyard (*Echinochloa esculenta*), Finger (*Eleusine coracana*), Foxtail (*Setaria italica*), and little (*Panicum sumatrense*).

According to Food and Agriculture Organization (FAO) statistics, the global millet production in 2009 was 26.7 million mt, which was cultivated across 33.6 million hectares of land [6]. Africa emerged as the largest producer with 20.6 million MT, Asia with 12.4 million MT, and India with 10.5 million MT [6]. Sorghum secured fifth rank among major cereal groups, e.g., wheat, rice, maize, and barley [7]. India has historically been the leading producer of millets, followed by Nigeria [8]. Indian millet cultivation consists of eight major species: Sorghum, Pearl millet, Kodo millet, Foxtail millet, Proso millet, Finger millet, little millet, and Barnyard millet [8]. The Kanker, Kondagaon, Bastar, Dantewada, Bijapur, Sukma, Narayanpur, Rajnandgaon, Kawardha, Gaurela-Pendra-Marwahi, Balrampur, Koriya, Surajpur, and Jashpurregions of Chhattisgarh are actively engaged in millets production [9]. Kodo, little (Kutki), and Finger (Ragi) millets are the dominant millet varieties in the state. In line, the Chhattisgarh Millet Mission has been launched to enhance farmer's income, particularly for those who belong to tribal and forested regions under Agricultural and Processed Food Products Export Development Authority (APEDA). The Bastar region depends on millets, which it serve as a nutritional and economic lifeline for tribal populations. These small-seeded grains require minimal water, fertilizers, and pesticides, making them an eco-friendly crop. Finger millet covers the largest area, followed by Kodo, Little, and Proso millets, making them vital for food security and livelihood [10].

In India, millets are classified into three, viz., major millets, minor millets, and pseudo millets, based on their significance as food crops [3]. Major millets (e.g., sorghum, pearl millet, and finger millet) are widely cultivated and consumed as staple foods due to their nutritional value and adaptability to semi-arid and arid environments [6]. These grains thrive in diverse agro-climatic conditions and contribute significantly to food security. Next to major millets, the minor millets (e.g., proso millet, barnyard millet, kodo millet, little millet, and foxtail millet) serve as crucial crops for food and livelihood security in villages and remote areas [9].

Pseudo millets (e.g., buckwheat Millet) are nutritionally similar to true millets. These gluten-free grains are considered an important alternative food source. Kodo millet aids in digestion, strengthens the nervous system, helps regulate blood sugar levels, and is particularly beneficial for postmenopausal women with metabolic disorders[6]. Proso millet supports bone growth and maintenance, ensures the smooth functioning of the nervous system, and promotes healthy skin and strong bones [6]. Barnyard millet is a rich source of fiber, possessing antimutagenic, anti-inflammatory, and antioxidant properties and helps prevent constipation and high blood pressure, and aids in regulating blood glucose and lipid levels, making it valuable for metabolic health [6].

Besides, the aforementioned beneficial aspects of millets have also been scientifically validated by researchers and published. The methanol extracts of barnyard and proso millets exhibited the presence of major compounds like tannins, terpenoids, amino acids, flavonoids, and phenolic compounds among all the solvent extracts [11]. The higher phenolic content was observed in the methanol extracts of barnyard and proso millets, followed by the chloroform extracts. In contrast, the acetone extracts of both millets contained the lowest amount of phenolics. Millets are resilient crops and are rich in vital nutrients such as proteins, fats, carbohydrates, minerals (iron, calcium, zinc, phosphorus, and magnesium), vitamins (niacin, riboflavin, folate, thiamine, and vitamin E), and bioactive compounds [12]. They are also a valuable source of medicinal and nutraceutical properties, providing antioxidants that help in preventing health issues like high blood pressure, heart disease, obesity, cancer, cardiovascular diseases, and diabetes. The millets are known as super foods [13]. They assessed qualitative and quantitative phytochemical profiles (phenols, tannins, flavonoids, alkaloids, and terpenoids) of the aqueous extract of eight different

millets. Their findings revealed that the foxtail millets had the highest flavonoid content at 7.808 mg/g, while barnyard millet had high alkaloid levels at 2.149 mg/g, and finger millet had the lowest at 0.058 mg/g. These food products help in the prevention of diseases such as diabetes, cardiovascular diseases, cancer, inflammation, gastric issues, and other disorders. The nutritional content, antioxidant activity, and antimicrobial effects of different millet varieties, including pearl, kodo, little, fox tail, and finger millet [2]. They concluded that the millets have naturally high levels of nutrients and possess antioxidant properties. Therefore, the present research work was conducted to evaluate the quantitative Phytochemical Screening and Antioxidant Potential of Kodo, Proso, and Barnyard Millets. As per the Indian Institute of Millets Research (ICAR) published document "The Story of Millets", the proso millet has the highest protein content (12.5 g), while Foxtail millet has the highest fat content (4.3 g), finger millet stands out for its exceptionally high calcium (364 mg), making it ideal for bone health [8]. Additionally, barnyard millet is notable for its high iron content (15.2 mg), while Foxtail millet contains the most iron (81 mg), which is essential for blood health. Further, Little millet provides the highest calories (346 Kcal). Moreover, Kodo millet is rich in folic acid (39.5 mg), beneficial for prenatal nutrition.

MATERIALS AND METHODS: This research work was carried out to assess the quantitative phytochemical screening and antioxidant potential of Kodo, Proso, Barnyard, Finger, Foxtail, and Little Millets. The samples (millets) were purchased from a Chhattisgarh Government-authorised vendor, C-Mart, Near Nutan Chowk, Seepat Road, Bilaspur (CG).

Preparation of Millets Extract: Millets were ground with the help of a mixer grinder to obtain a fine millet powder. The ground samples of millets were kept in resealable plastic bags **Fig. 1.** The Cold extraction method was used to prepare the extract. 5.0 g of ground sample of millets was mixed with 50 ml distilled water and 50 ml methanol, respectively and kept it for 24 hours at room temperature. Centrifuge the aqueous and methanol extract at 4000 RPM for 10 minutes and then filtered it through Whatman's filter paper number 1. Phytochemical analysis and antioxidant test of the filtered extract were done by following the standard protocol.

FIG. 1: FINE POWDER OF MILLETS

Quantitative Analysis of Phytochemicals Extracted from Millets: The presence of different secondary metabolites, including phenol, alkaloids, flavonoids, saponins, and tannins, in aqueous and methanol extracts of Kodo, Proso, Barnyard, finger, Foxtail, and Little Millets was quantitatively analysed [2, 14, 15, 16, 17].

Antioxidant Assays: The antioxidant activity of millet extracts was determined using the Phosphomolybdenum Method [18]. First, millet extracts were prepared in both distilled water and methanol. A phosphomolybdate reagent was then prepared by mixing ammonium molybdate (4 mM), sulfuric acid (0.6 M), and sodium phosphate (28 mM) in distilled water. In separate test tubes, 1.0 ml of millet extract was mixed with 3.0 ml of the phosphomolybdate reagent. The tubes were then incubated in a water bath at 95°C for 90 minutes. After cooling to room temperature, the absorbance of the reaction mixture was measured at 695 nm using a digital spectrophotometer. A standard curve was generated using ascorbic acid at different concentrations, and the antioxidant activity of the millet extracts was expressed in terms of ascorbic acid equivalent (AAE) per millilitre.

RESULT AND DISCUSSION: During the present research work, we have evaluated quantitative phytochemical screening and antioxidant potential of Kodo, Proso, Barnyard, Finger, Foxtail, and Little Millets purchased from the C-Mart (Chhattisgarh Government-authorised vendor), Near Nutan Chowk, Seepat Road, Bilaspur (CG).

Quantitative Analysis of Phytochemical Compounds: The comparative phytochemical analysis of Kodo, Proso, Barnyard, Finger, Foxtail, and Little Millets extracts revealed the presence of phenols, steroids, alkaloids, saponins, flavonoids, and tannins **Fig. 2.**

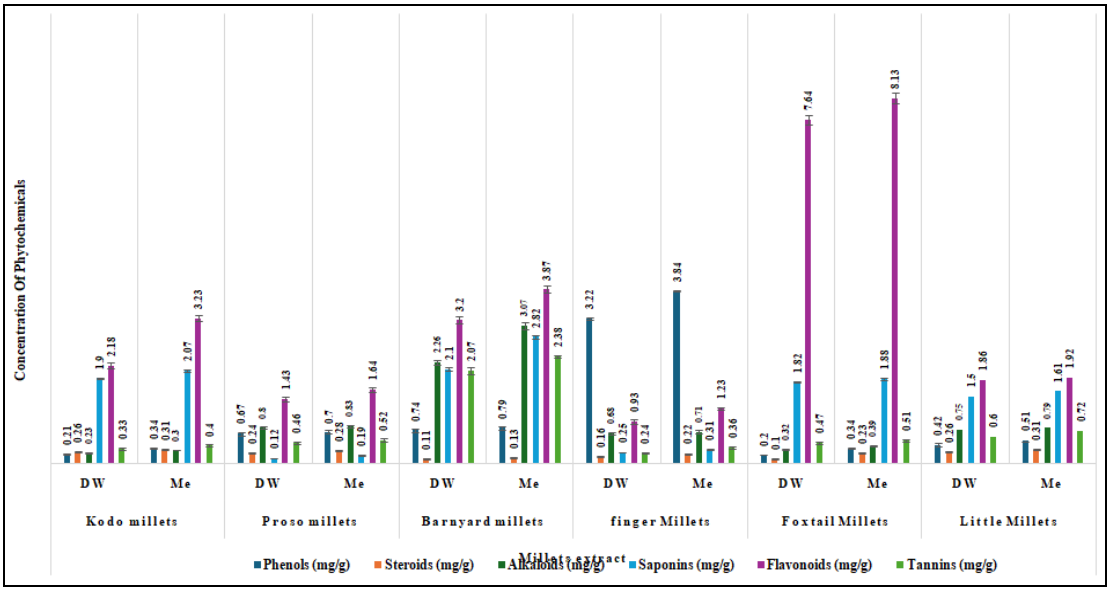

FIG. 2: QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF MILLETS

The phytochemical analysis of millet extracts revealed significant variations in their composition. Finger Millets exhibited the highest phenol content of 3.84 mg/g with Me, which was substantially higher than in other millet varieties. Similarly, observations divulged the highest flavonoid content of 8.13 mg/g in the Methanol (Me) extract of Foxtail Millets followed by Barnyard Millets (3.87 mg/g). Barnyard Millets showed the highest concentration of saponins (2.82 mg/g) with Me extract, whereas Proso Millets had the lowest at 0.12 mg/g in the distilled water (DW) extract. Tannin content was also notably higher in Barnyard Millets (2.38 mg/g in Me extract). Steroid content remained relatively low across all millet types. Additionally, Barnyard Millets exhibited the highest alkaloid content of 3.07 mg/gwith Me extract and significantly surpassing other millet varieties. These findings indicate that different millet types possess distinct phytochemical profiles, e.g., Finger Millets and Foxtail Millets were rich in phenols and flavonoids, while

Barnyard Millets displayed elevated levels of alkaloids, saponins, and tannins **Fig. 2.**

Antioxidant Assay: Antioxidant activity was determined by the phosphomolybdate method. The antioxidant evaluation of Kodo, Proso, Barnyard, Finger, Foxtail, and Little Millets extracts showed that the Finger millet exhibited the highest antioxidant activity (4.12 µg AAE/ml in DW and 5.34 µg AAE/ml in Me), followed by Little millet (3.11 µg AAE/ml in DW) and Barnyard millet (3.78 µg AAE/ml in Me). Methanol extracts showed higher antioxidant values than distilled water extracts **Table 1** and **Fig. 3.** The result exhibited that methanol was a more effective solvent for extracting antioxidants. Methanol and water differ significantly in their extraction efficiency due to their polarity and ability to dissolve bioactive compounds. Methanol has been reported for its intermediate polarity and more effective extraction of semi-polar compounds, e.g., phenolics, flavonoids, and alkaloids, at a higher extraction yield of 33.2% [19]. In contrast, water, being highly polar, is limited to extracting highly polar metabolites and typically produces a lower extraction yield. Additionally, methanol strongly disrupts cell structures and facilitates the release of bioactive compounds and promotes protein denaturation as compared to water [20]. These proteins act as cell degradation interfering compounds [21, 22]. Henceforth, they need to be degraded for effective extraction of metabolites from cells.

TABLE 1: ANTIOXIDANTPOTENTIAL OF MILLETS

Millets	Antioxidant value (µg AAE/ml)	
	DW	Me
Kodo	2.16	3.82
Proso	1.04	2.91
Barnyard	2.91	3.78
Finger	4.12	5.34
Foxtail	2.74	2.93
Little	3.11	3.07

DW: Distilled Water; Me: Methanol

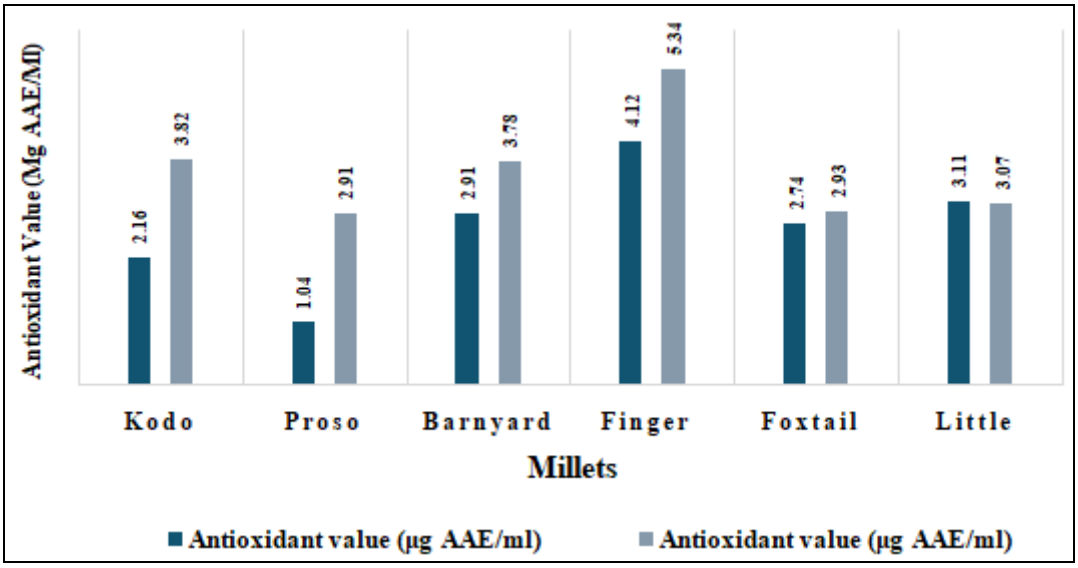

FIG. 3: COMPARATIVE ANTIOXIDANT POTENTIAL OF MILLETS

Present findings were aligned with some recent literature [2, 13, 23] but differed from literature regarding the phytochemical content of the millets [24]. Phytochemical contents have diverse pharmaceutical applications. Alkaloids are known for their medicinal properties, including antimalarial, anticancer, and antibacterial effects. Flavonoids exhibit antioxidant, anti-inflammatory, and anticancer activities, while tannins contribute to blood clotting, lipid reduction, and immune response modulation [25]. The present study revealed that the Finger millet exhibited the highest antioxidant activity (4.12 µg AAE/ml in DW and 5.34 µg AAE/ml in Me).

Previous studies reported the antioxidant value of Kodo millet as 24.64±0.38 mg AAE/ml [26], Barnyard millet at 10.125±0.03 mg GAE/g [27], and Proso millet's antioxidant value ranged from 18.0 to 26.5 mg GAE/g [28]. Kodo millet is a nutritious grain, with its macronutrient and micronutrient composition including moisture, fat, crude fiber, protein, calcium, iron, phosphorus, and zinc, making it a valuable dietary component [26]. Additionally, Barnyard millet has been recognized as a significant iron source for vegetarians [29].

Literature comprehensively explored the nutritional profile of millet flour [13]. They divulged that pearl millet has the highest energy content (361 Kcal), followed by sorghum (346 Kcal) and little millet (342 Kcal). They also reported that Sorghum had the highest carbohydrate content (73.1 g), while Foxtail millet had the highest protein (12.5 g), making them excellent energy and protein sources. Additionally, they claimed that the Barnyard millet contained the highest fiber (9.7 g), beneficial for digestion, while pearl millet had the lowest (1.3 g). Moreover, they mentioned that the Finger millet stood out for its exceptionally high calcium content (344 mg), supporting bone health, whereas barnyard millet had the least calcium (11 mg). Lastly, they stated that the Pearl millet was richest in iron (16.9 mg) and potassium (272 mg), while barnyard millet also had high iron (15.3 mg) and potassium (296 mg). β-carotene, an important antioxidant, was observed in pearl millet (132 mg), sorghum (47 mg), and finger millet (42 mg), but was absent in Barnyard, Kodo, Proso, and Little millets. The variations in these nutrients highlight the diverse health benefits of millets and reinforcetheir nutritional and medicinal importance, particularly their high antioxidant potential when extracted using methanol. The nutritional and therapeutic potential of millets, along with their nutritional composition, has been widely reviewed in recent literatures [30, 31].

Further, the Indian government has implemented various initiatives to enhance millet production and promote economic stability among farmers due to its potential benefits. The National Food Security Mission (NFSM) improves millet productivity via seed distribution, demonstrations, and training for farmers. The Rashtriya Krishi Vikas Yojana (RKVY) facilitates the promotion of enhanced millet cultivars, offers financial support, and enhances farmer awareness. The Pradhan Mantri Krishi Sinchai Yojana (PMKSY) promotes millet cultivation in drought-prone areas, facilitating efficient water utilization. The National Mission for Sustainable Agriculture (NMSA) incorporates millets within the framework of climate-resilient agricultural practices. The National Food Processing Mission (NFPM) promotes millet-based value-added products, enhancing marketability and income prospects.

The Pradhan Mantri Annadata Aay Sanrakshan Abhiyan (PM-AASHA) guarantees a Minimum Support Price (MSP) for millets, thereby protecting farmers' incomes. State initiatives such as the Millets Promotion and Development Program (MPDP) in Karnataka emphasize awareness campaigns, educational programs, and financial incentives to promote millet cultivation[3]. Millets possess nutritional, environmental, and economic importance, positioning them as a crucial element in food security and sustainable agriculture. Government initiatives persist in enhancing millet production and consumption, contributing to a healthier and more resilient future.Still, the more systematic awareness programs among farmers and consumers at the baseline level regarding the benefits of millet, adoption of millets, their nutritional profile, and their potential for sustainable agriculture need to be addressed with scientific evidence and case studies.

CONCLUSION: Millets serve as a staple food for over 60% of the world's underprivileged population due to their cost-effective cultivation, high nutritional value, climate resilience, and health benefits. These small-seeded grains are rich in proteins, fibre, vitamins, and minerals, making them essential for preventing high blood pressure, heart disease, tumours, and diabetes. However, their low productivity still remains a challenge that could be overcome by putting efforts into developing high-yielding varieties using global germplasm. India is the largest producer of millets, and the major millets include Sorghum, Pearl, Finger, Foxtail, Kodo, Proso, Barnyard, and Little millets. Chhattisgarh is a key millet-growing region. To promote millet production, the Indian government has introduced various schemes, including the Rashtriya Krishi Vikas Yojana,

National Food Processing Mission, and Millets Mission in Chhattisgarh, where millets are procured at the Minimum Support Price (MSP). Millet-based ready-to-cook and ready-to-eat products are being developed commercially, with research support from the ICAR. The phytochemical and antioxidant properties of millets were analyzed using cold extraction and spectrophotometric methods, revealing higher antioxidant activity in methanol extracts. These findings highlight millets' potential as a functional food with significant health and economic benefits.

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