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Evaluation of Phytochemical Analysis and Total Phenol Content of Proso Millet and Barnyard Millet

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Abstract: Whole grains of proso and barnyard millets were sequentially extracted using different solvents (hexane, chloroform, ethyl acetate, and methanol). Phytochemical analysis was performed qualitatively, and the total phenolic content in the extracts of proso and barnyard millets was quantified. Alkaloids and cardiac glycosides were identified in all solvent extracts of both millets. Anthraquinone and glycosides yielded negative results in all solvent extracts of both millets. Among all the solvent extracts, methanol extracts of proso and barnyard millets showed the presence of major compounds such as flavonoids, terpenoids, amino acids, tannins, and phenolics compounds. The maximum amount of phenols was found in methanolic extracts of proso and barnyard millets (0.669 ± 0.003 and 0.625 ± 0.003), followed by the chloroform extract of proso and barnyard millets (0.284 ± 0.002 and 0.257 ± 0.003). The minimum amount of phenolics was found in the acetone extract of proso and barnyard millets. The methanol extract of both millets showed the presence of major compounds with high phenolic content.

Keywords: Proso millet; Barnyard millet; Sequential solvent extraction; Phytochemical analysis; Total phenolic content

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1. Introduction

For thousands of years, humans have relied on natural products as valuable sources of medicines and inspiration for drug development [1]. In several countries, particularly in India, China, and Egypt, traditional medicinal systems have been based on plant-derived natural products. Food-derived natural products have been shown to be potential candidates for medicinal use [2]. More than 1 million Americans and more than 10 million people worldwide are expected to be diagnosed with cancer, a disease commonly believed to be preventable. Only 5 to 10% of all cancer cases are attributed to genetic defects, whereas the remaining 90 to 95% are caused by environmental and lifestyle factors. The lifestyle factors include cigarette smoking, diet (fried foods, red meat. Millet grains, compared to other grains like wheat, rice, and sorghum, are known for their health benefits and medicinal properties. The types of millets include finger millet, foxtail millet, proso millet, pearl millet, and little millet.

Millet seeds are rich sources of nutraceuticals and phytochemicals, which are used to prevent various health issues [3-5]. In recent years, whole grain cereals have garnered significant attention due to their fiber content and the presence of a diverse array of bioactive compounds such as antioxidants and phytochemicals [6]. Millets contain a variety of phytochemicals including polyphenols, phytosterols, phytates, sterols, carotenoids, flavonoids, and alkaloids. Among these, phenolic acids and tannins are the main polyphenols, while flavonoids play important roles in the body's immune system and act as antioxidants [7].

The health benefits of consuming whole grains, which are rich in phytochemicals, are well-documented[8] the phytochemical contents in grains have been commonly underestimated in the literature, because bound phytochemicals were not included. This study was designed to investigate the complete phytochemical profiles in free, soluble conjugated, and insoluble bound forms, as well as their antioxidant activities in uncooked whole grains. Corn had the highest total phenolic content (15.55 ± 0.60 μmol of gallic acid equivalent to per gram of grain. However, the presence of antioxidants and phytochemicals in whole grains has not been given as much attention as those in vegetables and fruits. It has been suggested that the health benefits of consuming whole grains are due to the presence of unique bioactive compounds [9]. Based on these observations, this study aims to quantify the presence of phytochemicals and total phenolic content in proso and barnyard millets.

2. Materials and methods

2.1. Plant materials

Whole grains of proso millet (Panivaragu, CO(PV)5, *Panicum miliaceum* L.) and barnyard millet (Kudiraivali, CO(KV)2, *Echinochloa frumentacea (Roxb.) Link*) were purchased and authenticated from the Department of Millet, Tamil Nadu Agriculture University, Coimbatore, Tamil Nadu, India. The seeds were thoroughly washed with running water, rinsed twice with sterile distilled water, and then left to dry at room temperature. Subsequently, the dried seeds were powdered using a blender before proceeding to the extraction process.

2.2. Sequential solvent extraction or preparation of plant extract

Fifty grams of powdered whole grains of proso millet (PM) and barnyard millet (BM) were macerated with 50 mL of hexane (1:5 v/w) at room temperature for 72 hours with intermittent stirring or shaking. After 72 hours, the solvent was collected and filtered through Whatman filter paper No. 1, and the residues were dried. The residues were then re-macerated with chloroform, ethyl acetate, acetone, and methanol after 72 hours each. Finally, the filtrates were evaporated to remove excess solvent residue in the extracts using a rotor vacuum evaporator. The extracts were dried for further investigation.

2.3. Qualitative phytochemical analysis of proso and barnyard millet solvent extracts

All the solvent extracts obtained from PM and BM were subjected to identify the presence of various bioactive constituents in both millets using several phytochemical analyzing tests (**Table 1**) [10-12].

2.4. Quantitative estimation of total phenolic content

The total phenolic content of sequential solvent extraction of PM and BM was determined by the Folin-Ciocalteu reagent method as described earlier by Singleton *et al.* [13]. Each solvent extract of PM and BM was dissolved in methanol (10 mg/mL). To 1 mL of each extract, 2 mL of Folin-Ciocalteu reagent and 2.5 mL of 20% Na_2CO_3 solution were added, and the mixture was incubated at room temperature for 30 minutes. After the incubation period, the absorbance of each sample was measured at 725 nm using a UV-spectrophotometer.

Table 1. Preliminary phytochemical analysis of proso and barnyard millet extracts

Phytochemical constituents	Methods and observation
Test for alkaloids (Mayer's test)	6 drops of Mayer's reagents + 1% HCl + steam to 1 mL of the extract Cream cloud precipitate indicated the presence of alkaloids
Test for flavonoids (Alkaline reagent test)	2–3 drops of dilute NaOH to 1 mL of the extract A deep yellow color appeared but gradually became colorless by adding a few drops of dilute HCl indicating the presence of flavonoids
Test for steroids (Liebermann-Burchard test)	2 mL of acetic anhydride + concentrated H_2SO_4 to 1 mL of the extract A green or green-blue color formation indicated the presence of steroids
Test for tannins and phenolics (Ferric chloride test)	2 mL of 5% neutral ferric chloride solution to 1 mL of the extract A dark blue or greenish-black precipitate indicated the presence of tannins
Test for terpenoids	2 mL of chloroform and concentrated H_2SO_4 to 1 mL of the extract Observed for reddish-brown color interface
Test for cardiac glycosides (Keller-Kiliani test)	1 mL of glacial acetic acid containing one drop of $FeCl_3$ + concentrated H_2SO_4 to 1 mL of the extract Brown ring formation at the junction indicated the presence of cardiac glycosides
Test for glycosides (Legal test)	Sodium nitroprusside in pyridine and sodium hydroxide to 1 mL of the extract The formation of pink to blood-red coloration indicated the presence of glycoside
Test for saponins (Froth test)	5 mL distilled water to 0.5 mL of extract, shaken well for 15 min Frothing persistence indicated the presence of saponins
Test for amino acids (Ninhydrin test)	A few drops of Ninhydrin reagent to 1 mL of the extract The formation of blue or violet coloration indicated the presence of amino acids.
Test for anthraquinone	Benzene was added to 1 mL of the extract and shaken, followed by the addition of 1 mL of diluted ammonia The formation of pink, red, or violet coloration indicated the presence of anthraquinones in the ammonia phase

3. Results

3.1. Preliminary phytochemical analysis of sequential solvent extraction of PM and BM

PM and BM whole grains were sequentially extracted using different solvents ranging from non-polar to polar (hexane, chloroform, ethyl acetate, acetone, and methanol). The yield percentage of crude extracts of PM and BM ranged from 1 to 4%.

Qualitative phytochemical analysis of different solvent extracts of PM and BM is presented in **Tables 2** and **3**. All solvent extracts of PM and BM indicated the presence of alkaloids and cardiac glycosides. Anthraquinone and glycosides yielded negative results in all solvent extracts of both millets. Steroids and saponins were detected in methanol and chloroform extracts of PM. Tannins and phenolic compounds were identified in methanol and ethyl acetate extracts of PM. Methanol extracts of PM showed the presence of flavonoids, terpenoids, amino acids, tannins, and phenolic compounds, except in other solvent extracts. Similar results were detected with minor variations in extracts of BM.

It was found that phytochemicals content varies widely due to differences in the polarity of the solvents used for extraction. When compared with other solvent extracts, methanol, a moderately polar organic solvent, yielded the most phytoconstituents. This indicates the presence of the polar nature of the phytochemicals in methanol extracts, all of which possess different medicinal properties.

Table 2. Phytochemical analysis of sequential extraction of proso millet

Phytochemical	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol
Alkaloids	+	+	+	+	+
Flavonoids	-	-	-	-	+
Steroids	-	-	-	+	+
Tannins and phenolics	-	-	-	-	+
Terpenoids	-	-	+	-	+
Cardiac glycosides	+	+	+	+	+
Glycosides	-	-	-	-	-
Saponins	-	+	-	-	+
Amino acids	+	-	-	-	+
Anthraquinone	-	-	-	-	-

Note: + indicated the presence of the compound, and - indicated the absence of the compound.

Table 3. Phytochemical analysis of sequential extraction of barnyard millet

Phytochemical	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol
Alkaloids	+	+	+	+	+
Flavonoids	-	-	+	-	+
Steroids	+	-	-	+	+
Tannins and phenolics	-	-	-	-	+
Terpenoids	-	+	+	-	+
Cardiac glycosides	+	+	+	+	+
Glycosides	-	-	-	-	-
Saponins	-	+	-	-	+
Amino acids	+	-	-	-	+
Anthraquinone	-	-	-	-	-

Note: + indicated the presence of the compound, and - indicated the absence of the compound.

3.2. Total phenol content of extracts of PM and BM

Phenolics are prominent antioxidants that play several important roles in preventing numerous diseases. It is one of the major classes of phytonutrients that have been widely studied in phytochemical research [14]. The total phenolic content was estimated from different solvent extracts (hexane, chloroform, ethyl acetate, acetone, and methanol) of PM and BM. The phenolic content of the five different solvent extracts displayed vast differences, ranging from 0.120 ± 0.002 to 0.569 ± 0.003 in both millets (**Table 4**). The maximum amount of phenols was found in methanol extracts of PM and BM (0.669 ± 0.003 and 0.625 ± 0.003), followed by chloroform extract of PM and BM, which showed 0.284 ± 0.002 and 0.257 ± 0.003. The minimum amount of phenolics was found in acetone extracts of PM and BM.

Table 4. Total phenol content of proso and barnyard millet extracts (mean ± SD of three determinations)

Extract	Proso millet (g)	Barnyard millet (g)
Hexane	0.162 ± 0.002	0.156 ± 0.003
Chloroform	0.284 ± 0.002	0.257 ± 0.003
Ethyl acetate	0.156 ± 0.002	0.144 ± 0.002
Acetone	0.122 ± 0.003	0.120 ± 0.002
Methanol	0.669 ± 0.003	0.625 ± 0.003

Based on the preliminary results of quantitative phytochemical analysis and estimation of phenolic content in different solvent extracts of PM and BM, methanol extracts of both millets showed the presence of major compounds and the highest phenolic content. Therefore, methanol was further used for the isolation of bioactive compounds by supercritical fluid extraction.

4. Discussion

Since ancient times, plants have served as a significant reservoir of drugs, bioactive compounds, and remedies for various diseases worldwide. The majority of bioactive components in plants consist of flavonoids, alkaloids, tannins, and phenolic compounds, which have been shown to be crucial sources of main compounds in the development of new anticancer drugs.

For over 3,500 years, constituents derived from plants have been used in cancer treatment. It has been reported that between 1981 and 2014, globally, 75% of new chemical entities introduced as anticancer drugs were derived from natural products or inspired by them [15]. Despite the successful utilization of synthetic chemicals as drugs for many diseases over the years, pharmaceutical industries have not achieved complete success in certain diseases (such as cancer, diabetes, cardiac diseases, and AIDS) due to the complexity of these diseases.

Previous literature suggests that preparing extracts using different solvents has a potent healing effect. Phytochemical screening of the sequential solvent extracts of PM and BM whole grains showed the presence of flavonoids, terpenoids, alkaloids, steroids, tannins, and phenolic compounds, among others, in the methanolic extract. Rao *et al.* reported the presence of phenols, tannins, alkaloids, flavonoids, and saponins in small millets (proso millet, finger millet, foxtail millet, and kodo millet) [16]. Suma and Urooj reported the presence of flavonoids, alkaloids, phenolics, and reducing sugars in methanolic and aqueous extracts of foxtail millet [17]. Differences in the presence of compounds might be attributed to the polarity of the extracting solvents, accounting for the observed variation in phytochemical identification in the extracts. This study suggests that the presence of these phytochemical constituents in PM and BM extracts possesses various medicinal properties, including metabolic activity, antitoxic, antioxidant, anti-inflammatory, anti-cancer, anti-carcinogenic properties, and cholesterol-lowering activity.

Total phenolic content (TPC) in the millets examined in this study was found to be higher than the TPC in other cereals including *Triticum turgidum* subsp. *durum* (Durum wheat) [8,18,19]. Among all the solvent extracts of PM and BM, the maximum amount of phenol was detected in the methanolic extract of PM and BM. Kim *et al.* demonstrated that PM contains 18–26.5 mg GAE/g of TPC [20]. It was reported that Khodo millet exhibited a maximum of 10.3% phenolic content in the methanolic extract [16]. Abraham *et al.* reported that among different varieties of PM, whole grains of red-coloured millet exhibited higher TPC

compared to whole grains of light-coloured millet [21]. Whole grain soluble extracts of Khodo millet contained higher TPC than other small millet varieties [22], namely kodo millet and finger millet. Zhang *et al.* stated that a higher amount was estimated in bound phenol content than free phenol content of PM, ranging between 83.44–456.95 mg GAE/g [23], as well as the antioxidant activity and anti-proliferative properties of three diverse proso millet varieties. Panwar *et al.* corroborated that BM demonstrated a greater phenolic content than finger millet [24].

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Disclosure statement

The authors declare no conflict of interest.

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