

Phytochemical Screening for Antioxidant Properties of Germinated Foxtail Millet

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Authors' contributions

Author JJDS carried out the proposed research work as part of Post graduate thesis and performed the statistical analysis. Author WJS designed the research work and wrote the draft of manuscript. Author BAK helped in compilation of data and managed the literature search. Author KBSD provided the grain and helped in statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Phytochemicals are a complex group of bioactive non-nutrient compounds of the plant kingdom and an integral part of the human diet. The present research was carried out to screen the phytochemicals in raw and germinated foxtail millets. The screening showed the presence of carbohydrates, alkaloids, fixed oils and fats, terpenoids and cardiac glycosides in both raw and germinated foxtail millets. Amino acids were not detected in raw but present in germinated, indicating in prominent in the bio-availability of amino-acids due to germination. The absence of quinones indicated that extracts have not undergone any oxidation during storage.

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1. INTRODUCTION

Foxtail millet (*Setaria italica*) is cultivated in around 23 countries of Asia, Africa and America subcontinent. It is also commonly known as Italian or German or Chinese or Hungarian millet. Drought tolerance makes it an important millet in India due to its vast rainfed and semi-arid regions [1]. Foxtail millet is believed to be originated from China and about as early as 2700 BC was domesticated in the highlands of central Asia [2].

It was a famous crop in Persia and traditionally used as cereal in Iran [3]. It is the second most widely planted species of millet, temperate, subtropical and tropical Asia and in parts of Southern Europe and essentially used as a forage in North America, South America, Australia and North Africa [4].

Phytochemicals of nutraceutical importance are non-nutritive plant bioactive constituents that promote health and mainly produced by plants to provide them protection. These substances are isolated as nutrients, dietary supplements and specific diets to genetically engineer designer foods, herbal products and beverages. Phytochemicals are broadly described as polyphenols, flavonoids, isoflavonoids, anthocyanidins, phytoestrogens, terpenoids, carotenoids, limonoids, phytosterols, glucosinolates and fiber [5].

Millet is usually dehulled and hulls can be a potential source of antioxidants. The total phenolic compounds ranged from 2 to 112 μmol ferulic acid equivalent/g defatted meal of different varieties. All of them exhibited effective inhibition of DPPH and superoxide radicals and was in the order of hull > whole grain > dehulled grain > cooked dehulled grain. Results showed that dehulling of grains affected the phenolic content and antioxidant potential for these grains [6].

Free and bound phenolic acids were isolated from native and malt finger millets for its antioxidant properties. It was found that there was a three-fold decrease in protocatechuic acid content, whereas the decrease in caffeic acid was marginal upon 96 hours of malting. The other free phenolic acids such as gallic, vanillic, coumaric and ferulic acids increased resulting in an increase in antioxidant activity as bound phenolic acid released due to malt [7].

Phytochemicals like alkaloids, reducing sugars and flavonoids were found only in methanol and aqueous extracts, whereas tannins and terpenoids were present in all solvent extract of whole grains and bran enriched fractions. Methanol extracts from whole flour and bran rich fraction exhibited a significantly higher radical scavenge activity of 44.62 and 51.80% respectively by DPPH system with reducing power of 0.381 and 0.455 respectively at 2mg in ethanol than water [8].

The finger millet polyphenols in starches hydrolysed by amylase's enzyme kinetics was studied using Michaelis–Menten and Lineweaver–Burk equations showed that K_m remained constant but V_{max} decreased in the crude extract of millet polyphenols indicating mixed non-competitive inhibition. The gallic acid, vanillic acid, quercetin and trans-cinnamic acid isolated from the polyphenol extract of millets showed uncompetitive inhibition. These results provide a scientific rationale that finger millet as low-cost nutrient is useful in reducing the chronic pathologies developing due to lifestyle changes [9].

The total phenolic content (TPC) of foxtail millet. The foxtail millet was dehulled three times to remove 90% outer layers. Later the grain was washed cooked at 100°C for 30 minutes and steamed at 100°C for 10 minutes respectively. The sample was ethanol: water: acetic acid in 70: 29.5: 0.5 v/v/v for 4 hours at 45°C. It was found that the order of TPC was whole > dehulled > cooked > steamed. The retention rate for steamed foxtail millet ranged from 47 to 55% and cooked foxtail millet 55 to 79%. TPC and cinnamic acid were rich in cooked millet than other varieties and hence cooking proved to be the most suitable method of processing [10].

2. MATERIALS AND METHODS

Foxtail millet was procured from Agricultural College, PJTSAU, Polasa, Jagtial. The glassware and equipment were by Post Graduate & Research Centre, PJTSAU, Rajendranagar, Hyderabad.

2.1 Preparation of Foxtail Extracts

Firstly 2.0 g of foxtail millet samples were subjected to extraction by cold maceration in 100.0 ml of methanol for 24 hours followed by

centrifugation at 3000 rpm for 10 min and filtered through Whatman No. 41 filter paper to obtain clear extracts. The clear filtrates were collected and preserved at 4°C until further use.

2.2 Preliminary Phytochemical Screening for Foxtail Millet

The preliminary tests of carbohydrate, alkaloids, proteins, amino acids, flavonoids, fixed oils, terpenoids, cardiac glycosides, steroids, tannins, phlobatins, phenols and quinones were carried out [11].

Test for carbohydrates: To 2.0 ml of sample extracts, 2 drops of Molisch reagent were added and shaken vigorously. To this 2.0 ml of conc. H₂SO₄ was added from the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately to indicate the presence of carbohydrates.

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

Mayer's test: To a fraction of the extract, 1% 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) was added. An organic precipitate indicates the presence of alkaloids in the sample.

Wagner's test: A fraction of extract was treated with Wagner's reagent (1.27 g of iodine and 2.0 g of potassium iodide in 100.0 ml water) and observed for the formation of cream-colored precipitate.

Hager's test: A few ml of extract was treated with Hager's reagent (saturated aqueous solutions of picric acid) and observed for the formation of a prominent yellow coloured precipitate.

Test for proteins: To 2.0 ml of sample extract, 1.0 ml of 40% NaOH and 1 to 2 drops of 1% CuSO₄ solution were added. The formation of violet color indicated the presence of peptide linkage of the molecule.

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

Test for flavonoids: Each portion of sample extracts were added with 5.0 ml ammonia followed by few drops of conc. H₂SO₄. The development of a yellow colouration confirmed the presence of flavonoids and it disappeared on standing.

Test for fixed oils and fats: A few drops of 0.5 N alcoholic KOH were added to a small quantity of extract along with a drop of phenolphthalein indicator. The mixture was heated on a water bath at 60°C for 1 - 2 hrs. Formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

Test for terpenoids: To 5.0 ml of each extract, 2.0 ml of chloroform and 3.0 ml of conc. H₂SO₄ were added to form a monolayer of reddish-brown colouration at the interface. This confirmed the presence of terpenoids.

Test for cardiac glycosides: About 5.0 ml of each extract was treated with 2.0 ml of glacial acetic acid containing 1 drop of ferric chloride solution and was underplayed with 1.0 ml of conc. H₂SO₄. A brown ring at the interface indicated the deoxy sugar characteristic of cardenolides. A violet ring might appear below the brown ring in the acetic acid layer whereas a greenish ring might form just gradually into a thin layer.

Test for steroids (Liebermann - Burchard test): Acetic anhydride (2.0 ml) was added to 0.5 ml of each of the extracts along with 2.0 ml of conc. H₂SO₄. The colour changed from violet to blue or green indicating the presence of steroids.

Test for saponins: Each extract was added with 20.0 ml of distilled water and agitated for a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam indicated the presence of saponins.

Test for tannins: To 5.0 ml of extract, few drops of 1.0% Lead acetate was added and the formation of yellow precipitate indicated the presence of tannins.

Test for phlobatins: Each extract was boiled with a few drops of 1.0% HCl and the deposition of red precipitate indicated the presence of phlobatins.

Test for phenols: Ferric chloride and Liebermann's tests were used to determine the

presence or absence of phenols in these extracts.

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

Liebermann's test: The extracts were heated with sodium nitrite and conc. H_2SO_4 solution, diluted in water, cool and added with an excess of dilute NaOH. The formation of deep red or green or blue colour indicated the presence of phenols.

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

3. RESULTS AND DISCUSSION

Phytochemicals also known as phytonutrients is naturally occurring substances found in plants. These substances have been found to be beneficial to human health as well as possessing antioxidant activity. Phytochemicals could act as antioxidants and anti-inflammatory agents. It

plays a vital role in detoxification of harmful and deleterious chemicals of the body [12].

The phytochemicals tests were carried out using standard methods of analysis for carbohydrates, alkaloids, proteins, amino acids, flavonoids, fix oils and fats, terpenoids, cardiac glycosides, steroids, saponins, tannins, phlobatinins, phenols and quinones. The results of phytochemical screening are given in Table 1. The present study showed that carbohydrates and alkaloids are strongly detected in both raw and germinate foxtail millet. In Wagner's test the alkaloids detected in germinated foxtail millet were slightly lower may be due to the utilization of certain metabolites during germination. Proteins were present in raw and strongly detected in germinated foxtail millet. Amino acids were detected only in germinated foxtail millet, it may be due to bioavailability increases during germination and in raw it didn't indicate may be due to peptide bonds are not hydrolysed properly. Fixed oils and fats, terpenoids and cardiac glycosides are present in raw and strongly present in germinated foxtail millet. Terpenoids are large class of organic compounds and glycosides are sugar molecule bound to another functional group were strongly detected in germinated may be due to compounds were hydrolyzed properly, loose cell wall and form easily available. Flavonoids,

Table 1. Phytochemical screening of foxtail millet

Phytochemicals	Test	Raw foxtail millet	Germinated foxtail millet
Carbohydrates	Molisch test	++	++
Alkaloids	Mayer's test	+	+
	Wagner's test	++	+
Proteins	Hager's test	++	++
	NaOH and $CuSO_4$	+	++
Amino acids	Ninhydrin test	-	+
Flavonoids	With ammonia solution	-	-
Fixed oils and fats	Foam test	+	+
Terpenoids	Chloroform and H_2SO_4	+	++
Cardiac glycosides	Glacial acetic acid and Ferric chloride solution	+	++
Steroids	Liebermann's - Burchard test	-	-
Saponins	Foam test	-	-
Tannins	$FeCl_3$ test	-	-
Phlobatinins	With HCl	-	-
Phenols	Ferric chloride test	-	-
	Liebermann's test	-	-
Quinones	With conc. HCl	-	-

Note: + indicate the presence, ++ more prominent and - indicate the absence of phytochemicals.

All screening tests were carried out in triplicates

steroids, saponins, tannins, phlobatinins, phenols and quinones were not detected in both raw and germinate foxtail millet. Quinones was not detected indicate that sample not undergone oxidation.

Germinated foxtail millet increases free, bound and the total phenolic content due to cell wall-degrading enzymes, which became active during germination and modified the cell wall structure of the grain. The bound phenolic compounds get liberated by the action of cell wall degrading enzyme mainly ester bond [13,14]. Tannin content decreases on germination could be indicated due to the leaching of soluble tannin compound during soaking which further gets reduced during subsequent germination [15].

Germination has also found to decrease the level of antinutrients present in cereals and maximizes the level of some of the utilizable nutrients [16,17,18].

The process of germination of foxtail millet has been gaining much interest in researchers due to its ability to significantly increase the functional components of the grain. The germination of foxtail millet seeds enhanced the quantity of bioactive compounds such as total phenolics, antioxidants, total flavonoids, dietary fiber, proteins and minerals and decreased the anti-nutritional factors by modifying its composition [19].

Research studies demonstrated that phytochemicals like polyphenols and phenolics in whole grains are concentrated in outermost layer and removal lead to less beneficial for health [20,21].

4. CONCLUSION

Screening of phytochemicals is an easy way to find out the presence and absence of phytochemicals yet it is expensive as it requires a lot of chemicals to perform. This helps in knowing the composition and help in designing of food for special functional foods. Now in modern world antioxidant is in high demand as it plays a very important role in neutralizing free radical which is formed due to stress, environmental pollution, ageing and food habits.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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