IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
 Journal UGC CARE Listed (Group-I) Volume 11, Issue -12 2022

"Exploring The Antioxidant Capacity Of Millets Present In Local Market"

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1. Introduction

Millet is a major drought-resistant crop that serves as a nutrient rich food staple in Africa and Asia. In addition, millet contains an abundance of bioactive compounds with antioxidant activity. The intake of antioxidants through the diet is essential for improving human health. Millets are the most commonly consumed food items in India.milletsareone major cereal grains consumed worldwide.especisallyinaridandsemiarid area of Africa and asia (india and china) they are great intrest because of their high nutritive value and agro-industrial importance (saleh et al.,2013, Zhu et al., 2018). They contain a wide range of phenolics which are good sources of natural antioxidants. Studies report that methanolic extracts from red sorghum showed higher antioxidant activity and contain higher polyphenolic levels compared to rice, foxtail millet, prosomillet and barley (Choi, 2007). Bran, a byproduct of milling has antioxidant potential due to phenolic acids such as *p*-coumaric acid and vanillic acids that are concentrated in the bran portion of cereal kernels. Antioxidant activity of five bran extracts exhibited appreciable levels of total phenolics, flavonoids and DPPH radical scavenging activities (Iqbal, et. al., 2007).

Millets contain phytic acid, tannin and phenols which can contribute to antioxidant activity, important in health, ageing and metabolic diseases. Pearl millet (*Pennisetumtyphoideum*) is the most widely grown type of millet. Nutritionally, pearl millet is superior to major cereals with reference to energy value, high quality proteins, fat and minerals such as calcium, iron, zinc. Besides, it is also a rich source of dietary fiber and micro nutrients (Anu, 2006). While, extensive information is available on proximate composition and mineral accessibility, information on antioxidant activity and its influence on processing in pearl millet is scanty. Research on the effect of processing on retention of bioactive components with potential antioxidant activity is very important. The objective of this investigation was to evaluate the effect of various processing methods (milling, heat treatments and germination respectively) on antioxidant components as well as antioxidant activities of pearl millet extracts. The alcoholic extracts of raw and processed pearl millet and selected varieties will be analyzed for free radical scavenging activity.

The term “phytochemicals” or “plant chemicals” refers to every naturally occurring chemical substance present in plants, which also has a potential for antioxidant activity. Antioxidants

play an important role in the body's defense system against reactive oxygen species (ROS), which are the harmful byproducts generated during normal cell aerobic respiration (Ou et al., 2002). In foods, antioxidants prevent undesirable changes in flavor and nutritional quality of a product 9Zielinski, et al., 2016). Several methods have been developed to measure "antioxidant activity". Commonly used assays are reducing power assay (RPA), ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity. There are two basic categories of antioxidants, namely, natural and synthetic. Natural antioxidants are phenolic compounds (flavonoids, phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), carotenoids and ascorbic acid. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commonly used synthetic antioxidants that have been in use since the beginning of this century. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity (Velioglu, et al., 1998). Thus, natural antioxidants have gained considerable interest in recent years.

Antioxidants are compounds that inhibit oxidation (usually occurring as autoxidation), a chemical reaction that can produce free radicals. Autoxidation leads to degradation of organic compounds, including living matter. Antioxidants are frequently added to industrial products, such as polymers, fuels, and lubricants, to extend their usable lifetimes (Klemchuk, 2000). Foods are also treated with antioxidants to forestall spoilage, in particular the rancidification of oils and fats. In cells, antioxidants such as glutathione, mycothiol, or bacillithiol, and enzyme systems like superoxide dismutase, can prevent damage from oxidative stress (Helberg, 2021).

Known dietary antioxidants are vitamins A, C, and E, but the term antioxidant has also been applied to numerous other dietary compounds that only have antioxidant properties in vitro, with little evidence for antioxidant properties in vivo. Dietary supplements marketed as antioxidants have not been shown to maintain health or prevent disease in humans (Fang, 2002).

Antioxidants are used as food additives to help guard against food deterioration. Exposure to oxygen and sunlight are the two main factors in the oxidation of food, so food is preserved by keeping in the dark and sealing it in containers or even coating it in wax, as with cucumbers. However, as oxygen is also important for plant respiration, storing plant materials in anaerobic conditions produces unpleasant flavors and unappealing colors (Kader, 1989). Consequently, packaging of fresh fruits and vegetables contains an ≈8% oxygen atmosphere. Antioxidants are an especially important class of preservatives as, unlike bacterial or fungal spoilage, oxidation reactions still occur relatively rapidly in frozen or refrigerated food (Zallen, 1975). These preservatives include natural antioxidants such as ascorbic acid (AA, E300) and tocopherols (E306), as well as synthetic antioxidants such as propyl gallate (PG, E310), tertiary butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA, E320) and butylated hydroxytoluene (BHT, E321) (Iverson, 1995).

Unsaturated fats can be highly susceptible to oxidation, causing rancidification (Robards, 1988) Oxidized lipids are often discolored and can impart unpleasant tastes and flavors. Thus, these foods are rarely preserved by drying; instead, they are preserved by smoking, salting, or fermenting. Even less fatty foods such as fruits are sprayed with sulfurous antioxidants prior to

air drying. Metals catalyse oxidation. Some fatty foods such as olive oil are partially protected from oxidation by their natural content of antioxidants. Fatty foods are sensitive to photooxidation (Del Carlo, 2004) which forms hydroperoxides by oxidizing unsaturated fatty acids and ester. Exposure to ultraviolet (UV) radiation can cause direct photooxidation and decompose peroxides and carbonyl molecules. These molecules undergo free radical chain reactions, but antioxidants inhibit them by preventing the oxidation processes (Frankel, 2012).

Antimicrobial resistance (AMR) has been seriously threatened human public health and global economic development, and new antimicrobial agents are desperately needed (Laxminarayan, et al., 2016). Antibiotics, as the secondary metabolites produced by many bacteria, actinomycetes and fungi, showed remarkably antimicrobial activities, while they also bring some toxic side effects to human body, and are unavoidable to lead to the resistance (Xu, X. et al. 2018). Many plant ingredients present weaker antimicrobial activities, while some of them can reverse the resistance of antimicrobial agents4. Simultaneously, most of them are considered nontoxic to human body because of their ubiquity in all sorts of plant derived foods and beverages.

As one of the largest classes of plant secondary metabolite, flavonoids can be widely found in various parts of the plants, such as fruit, vegetables, nuts and tea (Górniak, 2019). These compounds have a wide range of pharmacological activities including antibiosis, antioxidation, and coronary heart disease prevention, etc. It is worth noting that some flavonoids can enhance the sensitivity of bacteria to antibiotics, and even reverse the AMR. Thereout, the antibacterial activities of flavonoids have been paid more and more attention to. Recently, several investigations were performed for the antimicrobial activities of flavonoids, and the probable relationships between their chemical structures and antimicrobial activities were also summarized (Cushnie, 2008) . However, the regularity conclusions on the structure–activity relationships of flavonoids against bacteria still need to be further explored.

However, no research has evaluated the antibacterial effects of aqueous extracts of the selected millets antibacterial activity. Additionally, chemical compositions and the activities of anti-oxidants will be evaluated.

The main objectives of the plan study are as follows:

- To determine the antioxidant activity of Pearl millet (Pennisetumglaucum), finger millet (Eleusinecoracana), foxtail millet (Setariaitalica), kodo millet (Paspalumsetaceum), and barnyard millet (Echinochlosutilis) extract.
- To evaluate the antimicrobial activity of Pearl millet (Pennisetumglaucum), finger millet (Eleusinecoracana), foxtail millet (Setariaitalica), kodo millet (Paspalumsetaceum), and barnyard millet (Echinochlosutilis) extract , against some human pathogenic bacterias.

2. Review of Literature

Millets, which are members of the Poaceae family (Gramineae), are regarded as the first cereal crop cultivated at the dawn of human civilization thousands of years ago (Lu et al., 2005). Pearl millet (Pennisetumglaucum), finger millet (Eleusinecoracana), foxtail millet (Setariaitalica), kodo millet (Paspalumsetaceum), little millet (Panicum sumatrense), barnyard millet

(Echinochlosutilis). Millets are gluten-free and resistant to biotic stress conditions by nature (Das and Padmaja, 2016). Pearl millet is a C4 crop (Srivastava et al., 2022) that is able to grow in semiarid and arid regions due to its capacity for adaptive evolution in drought and heat stress (Serba and Yadav, 2016). Red-pigmented finger millet testa is rich in tannin phytochemicals. This provides insect resistance to finger millets. (Chandrashekar andSathyanarayana, 2006). It possesses anti-diabetic and anti-oxidant properties (Sooriya et al., 2022).

Foxtail millet is an excellent source of the amino acid proline and the alcohol-soluble proteins setarins (Sachdev et al., 2021). Potentially, the antioxidant phenolic compounds mitigate degenerative syndromes such as diabetes and hypertension (Florence and Asna, 2014). It contains antimicrobial phytochemicals, including tannins that prevent food spoilage. Millet species are widespread in tropical regions and have been used for centuries in various folk remedies. Millets are abundant in essential amino acids, micronutrients (carotenoids and tocopherols), vitamins, and minerals (such as magnesium, manganese, and phosphorus). In addition to minerals and vitamins, the edible portion of millet is composed of carbohydrates (60–70%), proteins (7–11%), and fat (1.5–5%). According to the findings of this study, pearl millet, Kodo millet, little millet, Fox tail millet, and finger millet all contain high levels of phytochemical activity. Foxtail millet aqueous extract has antihyperglycemic properties and reduces serum triacylglycerol and C-reactive protein levels. Millets possess phenolic acids, flavonoids, tannins, xylo-oligosaccharides, insoluble fibers, carotenoids, and vitamin E (Shan and Kehong, 2019).

Free radicals are compounds or tiny particles with unpaired electrons in their molecular or atomic orbitals, or simply ROS, which also include a variety of oxygen species, such as hydrogen peroxide, a strong oxidizing functional group produced by cellsduring respiration and cell-mediated immunity. Antioxidants are substances that neutralize free radicals and render them harmless, so that they cause less harm to biological processes. Compared to other cereals, pearl millet has higher levels of calcium, iron, zinc, lipids, and high-quality proteins with a high concentration of threonine and tryptophan and adequate leucine (Balasubramaniam et al., 2012). Foxtail millet, also known as Italian millet (Verma et al., 2014), is an excellent source of protein (12.3 g/100g) and dietary fiber (14 g/100g), while its carbohydrate content (60.9 g/100g) is low. In addition, it is abundant in minerals (3 g per 100 g) and phytochemicals. Foxtail millet is an excellent source of -carotene (126-191 µg/100g, according to Goudar et al., 2011).

Pearl millet (Pennisetumglaucum) is the sixth most important cereal crop in the world and is cultivated by subsistence farmers in the semi-arid regions of sub-Saharan Africa and the Indian subcontinent (Haussmann et al., 2012). It is the primary source of nutrition for 500 million of the world's poorest people, primarily in Asia and Africa. It is the most tolerant cereal crop to a wide range of harsh environmental conditions, including those with high mean temperatures, frequent droughts, and/or poor soil fertility.

Foxtail millet (Setariaitalica, formerly Panicum italicum L.) is the most important species of millet in East Asia and the second most widely planted species of millet worldwide. Among Setaria, Green foxtail, Italian millet, German millet, Chinese millet, and Hungarian millet, it has the longest history of cultivation. Millets have been cultivated in China since roughly the

sixth millennium BCE. Dwarf Setaria, foxtail bristle grass, giant setaria, green foxtail, Italian millet, German millet, Chinese millet, and Hungarian millet are alternative names for this species (Krishna Kumari et al., 1997).

Little millet is a cereal species that resembles proso millet in appearance but is smaller. It is an annual herbaceous plant that grows 30 cm to 1 m tall with straight or folded blades. The leaves are linear with occasionally hairy laminae and hairy, membranous ligules. The panicles range in length from 4 to 15 cm and have a 2 to 3.5 mm long awn. The grain is round and smooth, measuring between 1.8 and 1.9mm in length. Asia's temperate regions include the Caucasus, China, and East Asia, while the continent's tropical regions include India, Indochina, and Malaysia. It is resistant to both drought and flooding. It can be grown up to 2000 meters above sea level (Jones et al., 2010).

These crops are hardy and quite resistant to diverse agro-climatic adversities, and they play a significant role in marginal agriculture, which is more prevalent in hilly and semi-arid regions, as an important source of food grain and highly valued fodder. In various regions, these grains are used to make a variety of traditional foods and beverages, and therefore play an important role in the local food culture. Unfortunately, millets have come to be perceived as crops of the poor, which they are, and are therefore to be avoided. Continued neglect accelerates the loss of genetic diversity and traditional knowledge regarding the production, processing, and application of millets. Due to a lack of suitable, higher-yielding varieties, poor seed quality, and inefficient cultivation methods, production is inefficient. In addition, there is an absence of appealing recipes for adding value, a lack of knowledge regarding the nutritional value of millets, a poorly organized integration with markets, and a generally unfavorable environmental policy (Stefano Padulosi et al., 2015).

Finger millet (Eleusinecoracana L.) is an important millet that is widely cultivated in India and Africa, serving as a staple food for a significant portion of the population in these countries. It ranks sixth in terms of production in India, behind wheat, rice, maize, sorghum, and bajra (Malleshi et al., 1995). However, no research has evaluated the antibacterial effects of aqueous extracts of the aforementioned millets against Gram-positive bacteria *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus,* and gram-negative bacteria Escherichia coli, *Pseudomonas*, and *Klebsiella*. Additionally, chemical compositions and the activities of anti-oxidants are evaluated.

3. Material and Methods

Pearl millet, finger millet, fox tail millet, kodo millet grains were purchased and washed before being sterilized with 0.1 percent Mercuric chloride ($HgCl_2$) and kept at room temperature (25°C).

3.1

3.3 Antimicrobial activity

Pearl millet, finger millet, fox tail millet, kodo millet grains powder extract will be s tested for the presence or lack of anti-bacterial and anti-fungal activities.

Component	Method	Material	Observation

Antimicrobial activity	Disk diffusion Methods	**Microorganisms:** Gram-positive bacteria *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus,* and gram-negative bacteria Escherichia coli, *Pseudomonas*, and *Klebsiella* will be used the microorganisms studied. The bacterium strains used in this study were obtained from the National Chemical Laboratory (NCL) in Pune, India. The bacteria were cultured in nutrient broth at 37 degrees Celsius and kept on nutrient agar slants at 4 degrees Celsius. **Antimicrobial Assay:** Pearl millet, finger millet, fox tail millet, kodo millet grains powder extract will be determine antimicrobial activity (Antibacterial and Antifungal) using agar disc diffusion technique (Nair, et al., 2005) at four concentrations: 100, 75, 50, and 25 mg/ml. The plates were seeded with suitable microorganisms after Muller Hinton agar was prepared according to the manufacturer's instructions (Gram positive bacteria; Bacillus subtilis, Bacillus cereus, Bacillus frimicutes and Gram negativebacteria; Escherichia coli, Entrobacter, Klebsiella, Escherichia coli). Whatmann filter paper No. 24 was used to make 6 mm diameter discs, which were sterilised. Bacteria will be utilised as a control group.	The plates will be incubated for 24 hours at 37 degrees Celsius, and the zones of inhibition were quantified using a measuring scale. The above experiment will be repeated three times to ensure accuracy.

3.4 Antioxidant Activity

Component	Method	Material	Observation
Antioxidant Activity	**Deoxyribose assay to assess OH -radical scavenging activity (Fenton Reaction):** The OH- radical scavenging activity of Pearl millet, finger millet, fox tail millet, kodo millet grains powder extract extract (10–100 ug/ml) was determined according to the deoxyribose method reported of Halliwell, et al., (1987). In the protocol the presence of 100 lM EDTA. $FeCI_3$, H_2O and ascorbic acid were prepared in degassed H_2O	Deoxyribose (3mM) - 4.023mg in 100 ml ddw. $FeCl_3$ (Fe_3 + 0.1mM freshly prepared) – 1.622mg in 100 ml ddw. EDTA (0.1mM) – 2.922mg in 100ml. Ascorbic acid (0.1mM) - 1.761mg in 100 ml ddw. H_2O_2 (1mM) – 1ml in 99ml of ddw. TBA (1%) - 1gm in 100 ml of (0.05N) NaOH	The result of lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

	prior to use. The reaction tube contained (final concentrations) 3.6 mM deoxyribose, 100 lM EDTA, 1 mM H_2O_2, 100 lM L-ascorbic acid, 100 lM $FeCl_3$, H_2O in 25 mM phosphate buffer, pH 7.4 in 1.0 ml total volume. Samples was kept in incubation at 38º C, 1 hrs, 1.0 ml 1.0% TBA in 0.05 M NaOH and 1.0 ml 10% TCA were added to the reaction mixture after that samples was heated in a boiling water bath for 15 min. After the samples were cooled, the absorbances were read at 532 nm. The IC50 value of the plant extract was compared with that of ascorbic acid, which was used as the standard.	TCA (5%) - 5gm in 100 ml ddw. PBS- pH-7.4 Solution A-2.72 gm of KH2PO4 in 100 ml of ddw. Solution B-0.80gm of NaOH in 100ml of ddw. Working solution of PBS-50ml of solution A & 39.5ml of solution B were mixed together and made upto 1000ml with ddw. DMSO- (Control) - 10µl in 99.9ml of ddw.	

The percentage of inhibition of hydroxyl radical will be calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Abs: 532 nm Control Abs. - 532 nm sample Abs.} \times 100}{\text{532 nm Control Abs}}$$

Antioxidant capacity of test compounds will be expressed as IC_{50}, the concentration necessary for 50% inhibition concentration of TBARS.

Stastiscal analysis: the statistical significance will be calculated using student's 't 'test at $p<0.05$.

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