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Phytochemical Screening and Antibacterial Potential of Millets

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Abstract: Plants have many constituents and serve as significant sources of novel and biologically active molecules with antimicrobial properties essential for drug development against diseases. Antibiotic resistance has emerged as a worldwide issue, and the clinical effectiveness of numerous current antibiotics is jeopardized by the rise of multidrug-resistant pathogens. Thus, there is a pressing requirement to create new antimicrobial agents that are more effective against emerging and re-emerging infectious diseases.Millets are primarily small-seeded grasses that are resilient and thrive in arid regions with poor soil fertility and moisture availability. Millet is among the oldest foods recognized by humans and likely the earliest cereal grains utilized for household purposes. The semi-arid tropics in Asia and Africa account for 97% of millet production in developing nations. The plant is preferred in arid, hot conditions because of its brief growing period and yield. India stands as the top millet producer globally, yielding approximately 11 million tons annually, which constitutes 40% of the total world production. Millets contain 2% crude fiber. 60-70% carbohydrates, 15-25% fat, and 7-11% proteins. They provide great amounts of vitamin B, antioxidants, and magnesium. Millets are also a beneficial source of various dietary minerals such as manganese, iron, and phosphorus. Phytochemical screening of the sequential whole grains showed the presence of flavonoids, terpenoids, alkaloids, steroids, tannins, and phenolic compounds.Qualitative screening of Inorganic elements of different varieties of millets were also performed.Importantly, this approach allows us to detect antibacterial activities in Millet seeds protein. Altogether, the results indicate that millet proteins are rich sources for the production of bioactive peptides with antibacterial properties.The results of Antibacterial activity of six varieties of millet seeds extracts was tested against gram positive and gram negative bacteria. All millet extracted with acidified water shows antibacterial activity while there is no result with hot water extract. The highest activity has shown against *Bacillus cereus* with kutki (24.6 mm) followed Ragi (23 mm), kodo (22 mm), Bajra (21.6 mm), sama (19 mm) and jowar (16 mm)with a zone of inhibition respectively. Similarly, *Bacillus subtilis, Pseudomonas aeruginosa* and *fluorescens* showed less antibacterial activity**.** Current data will be useful in previewing all the that are present in millets and also provides insight into all the potential health benefits exhibited by the compounds. A detailed account on the ability of millets helps in further research for identifying new phytochemicals that have the ability to manage proteins by inhibiting specific target proteins.
Keywords: Millets, Phytochemicals screening, Antibacterial activity, Zone of inhibition, Bioactive peptides, Inorganic elements.

Introduction - Plants have many constituents and serve as significant sources of novel and biologically active molecules with antimicrobial properties essential for drug development against diseases. Antibiotic resistance has emerged as a worldwide issue, and the clinical effectiveness of numerous current antibiotics is jeopardized by the rise of multidrug-resistant pathogens. Thus, there is a pressing requirement to create new antimicrobial agents that are more effective against emerging and re-emerging infectious diseases. Natural products from food sources have demonstrated potential as candidates for medicinal applications. Over 1 million Americans and over 10 million individuals globally are anticipated to be diagnosed with cancer, an illness frequently thought to be preventable. Just 5 to 10% of cancer instances are linked to genetic mutations, while the other 90 to 95% stem from environmental and lifestyle influences (Anand et.al, 2008). Millet seeds, when compared to grains such as wheat, rice, and sorghum, are recognized for their health advantages and therapeutic qualities. Millet varieties consist of finger millet, foxtail millet, proso millet, pearl millet, and little millet. Millets are primarily small-seeded grasses that are resilient and thrive in arid regions with poor soil fertility and moisture availability. Millet is among the oldest foods recognized by humans. Millets are likely the earliest cereal grains utilized for household purposes. The semi-arid tropics in Asia and Africa account for 97% of millet production in developing nations. The plant is preferred in arid, hot conditions because of its brief growing period and yield (Wypij et.al, 2021). India stands as the top millet producer globally, yielding approximately 11 million tons annually, which constitutes 40% of the total world production. Millets contain 2% crude fiber. 60-70% carbohydrates, 15-25% fat, and 7-11% proteins. They provide great amounts of vitamin B, antioxidants, and magnesium. Millets are also a beneficial source of various dietary minerals such as manganese, iron, and phosphorus.

The various types of millets planned for the research include finger millet, proso millet, kodo millet, barnyard millet, little millet, pearl millet, foxtail millet, and sorghum (Urnukhsaikhan et al., 2021). Millet grains, in contrast to other grains such as wheat, rice, and sorghum, are recognized for their health advantages and therapeutic characteristics. Millet seeds are abundant in nutraceuticals and phytochemicals, utilized to avert multiple health problems (Yao et.al, 2004). In recent years, whole grain cereals have received considerable interest because of their fibre levels and the presence of various bioactive compounds, including antioxidants and phytochemicals. Millets have a range of phytochemicals such as polyphenols, phytosterols, phytates, sterols, carotenoids, flavonoids, and alkaloids. Among these, tannins and phenolic acids are the primary polyphenols, whereas flavonoids are significant for the immune system and function as antioxidants.

Foxtail Millet: *Setaria italica* is the scientific name known as Foxtail millet and is grown in Tamil Nadu and Andhra Pradesh. It is typically cultivated for its seeds and also raised as feed for livestock. Featuring a thick root system and slender adventitious roots, it reaches heights of up to 220m. Research on Setaria italica indicates that it possesses antihyperglycemic, hypolipidemic, cytotoxic, antioxidant, hypoglycemic, anti-lipase, hepatoprotective, and anti-inflammatory properties. It is also a stimulant for appetite and is utilized for treating sexual disorders (Ahmad et al., 2019).

Kodomillet: *Paspalum scrobiculatum* is widely referred to as Kodomillet. It is drought tolerant and can thrive in any low-quality soil, primarily cultivated in the Deccan Plateau. It contains a significant amount of high-quality protein and has elevated antioxidant properties relative to other millets. It is high in fiber, making it potentially advantageous for individuals with diabetes (Galatage et al., 2021).

Barnyard Millet: This type of millet is categorized into two species within the genus *Echinochloa*. Echinochloa esculenta is grown in Japan, northeastern China, while Echinochloa jrumentacea occurs in Pakistan, central Africa, India, and Nepal. This millet thrives in poor soil and has quick maturity and excellent storability. It does not include gluten. Indian barnyard millet, typically cultivated in the Himalayan area, has higher anti-feed ant sat levels than rice (Galatage et al., 2021).

Little Millet: *Panicum sumatrense* is often referred to as little millet and is grown in India, Sri Lanka, Myanmar, Pakistan, and various other Southeast Asian nations. Little millet is abundant in phenolic acids, tannins, flavonoids, and phytate. It is tolerant to pests, salt, and drought (Shadang et al., 2014).

Proso-Millet: The scientific designation for Proso-Millet is *Panicum miliaceum*. Originally domesticated in China, it has expanded to European nations, Pakistan, and India. Research indicates that Prosomillet exhibits antiproliferative effects on human breast cancer (Hassan et al., 2021).

Pearl Millet: *Pennisetum glaucum*, often referred to as Bajra or Pearl millet, is grown mainly in Asia and Africa. It contains a lot of iron and zinc. It also has a significant level of antioxidant agents. Pearl millet offers potential health advantages in addressing conditions and diseases such as diabetes, cancer, anemia, and constipation (Vijayshanthi et.al, 2015).

Finger millet: Scientifically referred to as *Eleusine coracana*, is commonly called Ragi. It is widely eaten in Karnataka and Andhra Pradesh. It ranks among the top sources of calcium and iron. Finger millet shows multiple microbial and antioxidant characteristics. Furthermore, research indicates that it contains phenolic compounds that inhibit aldose reductase and phospholipases found in snake venom. It includes protein glycation inhibitors that aid in managing diabetes. Finger mill and others thus demonstrate properties related to wound healing and reducing cholesterol levels (Vetriventhan et al., 2020).

Jowar: *Sorghum bicolor* is referred to as Jowar. It is one of the principal cereal crops grown globally. Sorghum is utilized for animal feed and fodder, construction material, and fencing. Research has shown that sorghum possesses significant anti-inflammatory and anti-colon cancer properties. Sorghum serves as a renewable energy resource for the production of biofuels (Cedric et.al, 2017).

This research aimed to examine the phytochemical study, Qualitative elements and Antimicrobial activity in raw whole grains of different varities millets . It has been proposed that the advantages of eating whole grains stem from the existence of distinct bioactive compounds (Gani et.al, 2012)

Methodology:

1. Field Survey: Conducted a field survey regarding the understanding and usage trends of millets among respondents (Table 1).
2. Sample collection and Preparation: Six varieties of millet (Ragi, Jowar, Bajra, Sama, Kodo, and Kutki) were collected from a departmental store in Chhindwara, Madhya Pradesh during February, 2025 (Fig.1).Millets were crushed with a mortar and pestle to achieve a fine powder. The powder was then sifted through a 2mm sieve to achieve

smaller particles. The samples in powder form were kept in poly zipper bags.

Fig.1 :- Showing collection of different varieties of Millets.

3. Extract preparation:1 gm sample of powdered millets was individually mixed in 20ml of acidified water (40% acetic acid and 60% water) and Hot Water. The solution was allowed to sit at room temperature for 24 hours and was filtered using Whatmann no. 1 filter paper. The filtrate was utilized for subsequent analysis.

4. Phytochemical Screening: Qualitative phytochemical investigation of extracts of various types of *Millets* was carried out according to the established protocol Harborne (2020). Standard methods for phytochemical screening (alkaloids, flavonoids,amino acid, phenols, resins, quinones, saponins, tannins, carbohydrates, sterols and triterpenes) were employed.

5. Qualitative Assessment for In-organic Elements as per protocol by Sethi et al., 2021.

Test for magnesium: A white precipitate forms with Ammonium Carbonate but not with Ammonium Chloride solution, indicating magnesium's presence.

Potassium test: In a sample of 2-3 ml, add several drops of Sodium Cobalt Nitrite solution. A yellow precipitate of Potassium Cobalt Nitrite is seen.

Iron test: Add a few drops of 2% Potassium Ferrocyanide to a 5ml sample. A dark blue hue is noted.

Sulphate test: The addition of Lead Acetate produces a white precipitate that dissolves in NaOH.

Phosphate test: To 5ml sample prepared in HNO_3, add a few drops of Ammonium Molybdate solution. Warmed for 10 minutes then chilled. A yellow crystalline precipitate of Ammonium Phosphomolybdate is seen.

Chloride test: To approximately 5 to 7 ml of filtrate, add 3 to 5 ml of Lead Acetate solution. A white precipitate that dissolves in hot water is noted.

Test for carbonates: A white precipitate is seen with a Magnesium Sulphate solution.

Test for sodium: 10ml sample combined with 2ml of Potassium Pyroanthllollate yields a white precipitate.

Nitrate test: Produces red vapour when heated with H_2SO_4 and water.

6. Antimicrobial Activity

The Agar well diffusion method was used to assess anti-microbial activity. The Mueller Hinton Agar medium underwent sterilization in an autoclave at 121°C, 15 lbs of pressure for 15 minutes. Subsequently, it was transferred into sterilized petri dishes and allowed to solidify. Cultures of *Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa,* and *Pseudomonas fluorescens* were distributed on the solid medium. Wells were created on the medium using a cork borer, and 10 µl of sample extract were added to each well along with 10 µl of the corresponding solvent as a negative control using a micropipette. The plates were maintained at 37°C for 24 hours. Following incubation, the inhibition zone was recorded in millimeters.

Results And Discussion: Survey were performed and reportedabout respondents, knowledge and consumption of millets.Survey reveled that 100% respondents heard about millets and 93.33% respondents consumes millets. 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.The six millets collected from Chhindwara andwere sequentially extracted using acidified water and hot water as solvents ranging from. Qualitative phytochemical analysis of extracts of presented in **Tables 1. (see in last)**

It was found that phytochemicals content varies widely due to differences in the polarity of the solvents used for extraction. This indicates the presence of the phytochemicals in extracts, all of which possess different medicinal properties. Previous literature suggests that preparing extracts using different solvents has a potent healing effect. Phytochemical screening of the sequential 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)(Liyana et.al, 2006). Suma and Urooj reported the presence of flavonoids, alkaloids, phenolics, and reducing sugars in methanolic and aqueous extracts of foxtail millet. 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 extracts possesses various medicinal properties, including metabolic activity, antitoxic, antioxidant, anti-inflammatory, anti-cancer, anticarcinogenic properties, and cholesterol-lowering activity. **Table 2** represents qualitative analysis of Inorganic elements of different varieties of millets were performed.

Importantly, this approach allows us to detect antibacterial activities in Millet seeds protein. Altogether, the results indicate that millet proteins are rich sources for the production of bioactive peptides with antibacterial properties. The results of Antibacterial activity of six varieties of millet seeds extracts was tested against gram positive and gram negative bacteria which were studied (**Table 3 and Fig. 2**). **(see in last)**

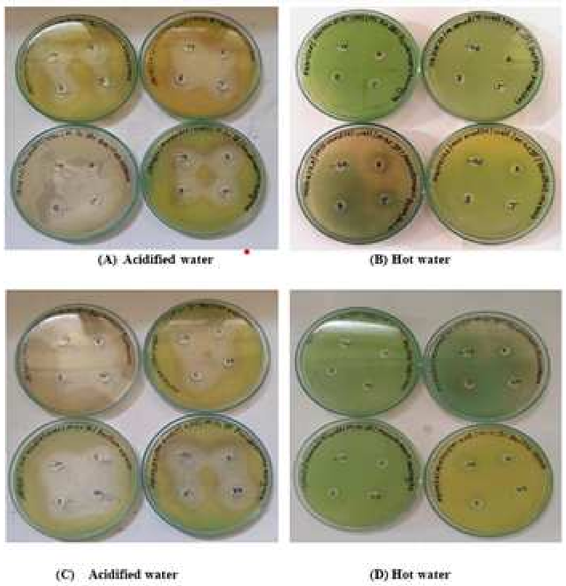

Fig. 2 : Antibacterial activity of different extract of millet (R= Ragi, J= Jawar , B= Bajra ,K= Kutki , K= Kodo and S= Sama) against *Pseudomonas fluorescens, Bacillus subtilis* , *Bacillus cereus* and *Pseudomonas aeruginosa* .

All millet extracted with acidified water shows antibacterial activity while there is no result with hot water extract. The highest activity has shown against *Bacillus cereus* with kutki (24.6 mm) followed Ragi (23 mm), kodo (22 mm), Bajra (21.6 mm), sama (19 mm) and jowar (16 mm)with a zone of inhibition respectively. Similarly, *Bacillus subtilis, Pseudomonas aeruginosa* and *fluorescens* showed less antibacterial activity**.** Current data will be useful in previewing all the that are present in millets and also provides insight into all the potential health benefits exhibited by the compounds. A detailed account on the ability of millets helps in further research for identifying new phytochemicals that have the ability to manage proteins by inhibiting specific target proteins. Thus, the data can become useful content for carrying out further research with a special focus on therapeutics of millet phytochemicals and antibacterial study. The compounds identified from such extracts can be further isolated for evaluating their activity in in-vivo conditions.

Conclusion: With changing lifestyle and unhealthy food habits, human life has taken a considerable graph in emerging life-threatening diseases. Therefore, the demand for discovery of new drugs and therapeutic agents is never-ending. Even though chemically synthesized drugs are efficient in treating several medical conditions, side effects associated with them makes ways for several drawbacks. Phytochemicals has been an emerging field in finding cure for such diseases. Millets being one of the under-utilized groups of cereals, whose consumption rates are decreased over the years, are now known to avail several health benefits due to the presence of potential phytochemicals. Millet phytochemicals discussed in the current review are reported to showcase the major health benefits in crude methanol and ethanol extracts of minor millets. The compounds identified from such extracts can be further isolated for evaluating their activity in in-vivo conditions.

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Table. 1. Preliminary Phytochemical screening of different varieties of millet extracts.

Extract/test	Acidified water						Hot water					
	Ragi	Jowar	Bajra	Kutki	Kodo	Sama	Ragi	Jowar	Bajra	Kutki	Kodo	Sama
Alkaloids	+	-	-	+	-	+	-	-	+	-	-	-
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-	+	+	+	+	+	+
Amino acid	-	+	+	-	-	-	-	+	+	-	-	-
Tannis	-	-	-	-	-	-	-	-	-	+	+	+
Ressins	+	+	+	+	+	+	-	-	+	+	-	-
Quinones	-	-	-	-	-	-	-	-	-	-	-	-

+ = presence, - = Absence: Ragi –(*Eleusine coracana*) , Jowar –(*Sorghum bicolor*), Bajra – (*Pennisetum glaucum*) , Kutki– (*Picrorhizakurrooa*), Kodo– (*Paspalum scrobiculatum*), and Sama – (*Echinochloa frumentacea*)

Table 2: Qualitative analysis of Inorganic elements of different varieties of millet extracts.

Inorganic Elements	Acidified water						Hot water					
	Ragi	Jowar	Bajra	Kutki	Kodo	Sama	Ragi	Jowar	Bajra	Kutki	Kodo	Sama
Magnesium	-	-	-	-	+	-	-	-	-	-	-	+
Phosphate	+	+	+	+	+	+	-	+	-	+	-	+
Iron	-	-	-	-	-	-	-	-	-	-	-	-
Sulphate	+	-	+	-	+	-	+	+	+	+	+	+
Chloride	+	-	+	-	-	-	-	+	+	-	-	-
Carbonate	+	-	-	+	-	-	-	-	+	+	-	+
Nitrates	-	-	-	-	-	-	-	-	-	+	-	-

+ = presence, -= Absence, Ragi –(*Eleusine coracana*) , Jowar –(*Sorghum bicolor*), Bajra – (*Pennisetum glaucum*) , Kutki– (*Picrorhizakurrooa*), Kodo– (*Paspalum scrobiculatum*), and Sama – (*Echinochloa frumentacea*)

Table 3: Antimicrobial activity of different varieties of Millets extracts.

Microorganism	Zone of inhibition (mm)											
	Acidified water						Hot water					
	Ragi	Jowar	Bajra	Sama	Kodo	Kutki	Ragi	Jowar	Bajra	Sama	Kodo	Kutki
Bacillus subtilis	20.3	21.3	20	18	19	22	-	-	-	-	-	-
Bacillus cereus	23	16	21.6	19	22	24.6	-	-	-	-	-	-
Pseudomonas aeruginosa	18.3	21	17	16	21.6	18	-	-	-	-	-	-
Pseudomonas fluorescens	18.3	14	18	21.3	16.6	17.3	-	-	-	-	-	-

Ragi –(*Eleusine coracana*) , Jowar –(*Sorghum bicolor*), Bajra – (*Pennisetum glaucum*) , Kutki– (*Picrorhizakurrooa*), Kodo– (*Paspalum scrobiculatum*), and Sama – (*Echinochloa frumentacea*)

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