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ASSESSMENT OF ANTIFUNGAL ACTIVITY AND ANTI-NUTRITIONAL FACTORS OR PHYTOCHEMICAL PROFILING OF HIMALAYAN ELEUSINE CORACANA L

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ABSTRACT

Finger millet (Eluesine coracana L.) or ragi occupies significant position in India in terms of production and utilization and in entire world. It is one of the most stable food crops. Finger millet is superior to rice and wheat with respect to mineral, fiber and micronutritient contents. Is a staple food in Africa and Asia, particularly in semi-arid regions. It's an annual, self-pollinated tetraploid cereal crop cultivated for its nutritious seeds. Finger millet is considered a drought-tolerant crop, though it prefers moderate rainfall. The emerging significance of natural antimicrobial agents creates an imperative need to identify novel plant sources with antimicrobial activities. Its utilization in the daily dietary at present is very limited in rural areas only. Generally it is prepared from refined wheat flour and is a rich source of protein, fat and carbohydrates but limiting in minerals and dietary fibers. Finger millet (mandua) flour is rich in minerals like iron, calcium, phosphorus, fiber and vitamin contents. Finger millet is a good source of protein, calcium, iron, and fiber, and is also a source of essential amino acids like thiamine and riboflavin. In summary, Eleusine coracana, or finger millet, is a nutritious and climate-resilient cereal crop that plays a vital role in food security and nutrition in many parts of the world May help manage blood sugar levels, improve nerve growth factor production, and aid in wound healing. It's also a source of antioxidants and phenolics.

KEYWORDS: Antifungal activity, Finger millet, Nutritional, Phytochemical, Health benefits, Value addition.

INTRODUCTION

Finger millet (Eluesine coracana L.) is one of the species of family Fabaceae, commonly known as Mandua or ragi occupies significant position in India in terms of production and utilization and in entire world. It is one of the most stable food crops. Finger millet is superior to rice and wheat with respect to mineral, fiber and micro nutritient contents. Nutritional and healthcare potential of forest foods explored sporadically is insufficient looking to the wealth and traditional knowledge that exists. Millets are often referred to as "ancient coarse grains turned into modern nutri-cereals." These grains are anticipated to be crucial in the future due to their resilience against pests and diseases, thriving in the challenging conditions of arid and semi-arid regions in Asia and Africa. Millets, known for their high protein content, are a popular choice among vegans.[1] They are recognized as a superior plant-based protein option, containing minimal saturated fats in comparison to animal proteins. However, the presence of antinutrients hampers protein digestibility, emphasizing the significance of minimizing antinutrient levels.[1] Culinary Uses: Used as a staple food, and can be used to make various dishes, including breads, porridges, and fermented beverages Climate Resilience.[2] Finger millet is known for its resilience to drought, salinity, and low fertility, making it an important food security crop in semi-arid regions and used as a traditional food of the Himalayan region as well as other parts of the world. The species is a good source of phenolic compounds.[3] minerals, and nutrients apart from this it is a rich source of amino acids which is why known as the powerhouse of health-benefit nutrients.[2] The presence of ferulic acid, quercetin, caffeic acid, gallic acid, and coumarins like compounds make it more valuable due to which it shows therapeutic effects like antioxidant, anti-diabetic, anticancer, anti-inflammatory, antimicrobial, etc.[3,4] It is also used for the preparation of value-added products like biscuits, baby food, pancakes, roti, papad bread, etc.[5,6] Finger millet is a potentially rich but underexplored crop for the source of nutrients; hence farmers should be encouraged for the cultivation of such types of nutrient-rich crops. Finger millet is associated with number of health benefits such as antioxidant, anti-ageing, anti- diabetic, hyperactivity, healing property, anticancer property,[7] Antifungal Compounds: Studies have isolated and identified several antifungal compounds from finger millet and its endophytes,

including 5-hydroxy 2(3H)-benzofuranone, Dehydrocostus lactone, and Harpagoside.[8] The current study to demonstrate the antioxidant potential of Himalayan millets, namely Finger millet and analyzing their phytochemical composition in highly antifungal activity, Cardio-protective and Anti-hyperlipidemic, Anti-diabetic properties and presence in nutritional activity, most important phytochemical compounds.

Figure 1: Finger millets.

MATERIALS AND METHODS

In the Indian Himalayan region of Uttarakhand, we procured plant samples of Finger Millet from the local markets of Rudraprayag (Jakholi) and Uttarkashi (Batwari) districts. The herbarium samples were prepared for the identification of plant samples from herbarium library of Forest Research Institute, Dehradun (FRI). The matched accession number for the plants is *Eleusine coracana* (L.) Gaertn. (Acc. number-77313).

Preparation of extract

Dried and powdered (100 g) were taken in a large beaker and extracted sterile distilled water (500 ml X 3) through stirring with a mechanical stirrer for 8 h. The aqueous extracts so obtained was filtered through filter paper (Whatman No.1), concentrated using rotary flash evaporator and finally evaporated to dryness to obtain water free extracts. The extracts was finally dried over anhydrous sodium sulphate and stored in sealed glass bottle and preserved at 5^0C until further analysis.

Determination of antifungal activity in finger millets

Test fungi

For antifungal evaluation of Finger millets, some important and frequently occurring pathogenic fungi viz., Alternaria alternata (AA), Aspergillus flavus (AF), Aspergillus niger (AN), Cladosporium cladosporidies (CC), Drechslera halodes (DH) and Fusarium moniliforme (FM) were selected. These fungi were isolated from the infected Finger millets by Standard Blotter Method[9] and identified based on growth characteristic, mycelial morphology, spore morphology and other important characters using standard protocol.[10,11] Pure cultures of each of the selected fungal species were made separately and maintained at on PDA slant. These pure cultures were used as antifungal assay.

Preparation of test solutions

Test solutions of a series of concentrations viz, 5, 10, 20, 30, 40, and 50 mg/ml were prepared from the aqueous extract in dimethyl sulfoxide (DMSO). All test solutions were kept in refrigerator at 4^0C till further used.

Preparation of fungal inoculums

For antifungal assay cultured slants were used for preparing spore suspension in 0.9% saline water. The fungal spore suspension was adjusted to give a final concentration of 1.5 × 105 cfu/ml.

Preparation of media

The medium was prepared by dissolving Potato dextrose agar (PDA) media (HiMedia) in distilled water and autoclaving at 121 ^{0}C for 15 minutes. 20 ml of sterile PDA media was poured in sterilized petridishes (9 cm diameter) and allow solidifying which were used for antifungal assay.

Antifungal activity assay

Antifungal activity of aqueous extract Finger millets seed was determined using agar-well diffusion method.[12] Spore suspensions (0.2ml) were applied on the surface of the presterilized and autoclaved PDA petridishes and spread by using a sterile glass spreader. Wells of 6mm diameter were made in centre of each of the PDA petriplates with the help of sterilized cork borer. The wells were filled with test solutions of bark extract as prepared above with three replications for each treatment. Carbendazim (2mg/ml) and DMSO were served as positive and negative control respectively for each of the three extracts. All the petridishes including treatments and controls were allowed to diffuse at room temperature for 2 hours and then incubated at room temperature (28 ± 2^0C) for 72 hours. After incubation, the antifungal activity of extracts was determined by measuring the diameter (mm) of inhibition zones.

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined through the broth dilution method.[13] Fungi were first grown in the potato dextrose broth for 24 hrs and then the inoculums were diluted for five times (10-5dilution) to control its vigorous growth. Then each test tube was added with 1.8 ml of potato dextrose broth and different concentrations (1-10 mg/ ml) leaf extract followed by inoculation of 0.2 ml of respective fungi and kept at 28°C for 48 hrs. The tubes were examined for visual turbidity. Lowest concentrations of the extracts showing no turbidity (without microbial growth) were considered as the minimal inhibitory concentration.

Isolation and Identification of fungi in finger millets

The isolation of fungi from the stored *Eluesine coracana* was carried out by blotter test method (ISTA,2003). A pair of white blotter papers of 8.5cm diameter was jointly soaked in sterile distilled water and placed in pre-sterilized petridishes of 9cm diameter. Stored seeds of *Eluesine coracana* were placed at equidistance on moist blotters in autoclaved separate petridishes. All petridishes were incubated at room temperature (28±2^{o}C) for 6 days. Fungi were isolated and identified standard using standard protocol.[14,15] Antifungal activity was evaluated using the agar tube dilution method as described by.[16,17] A known amount of finger millet extract was dissolved in DMSO. Using aseptic techniques, dissolved finger millet extract (66.6 µl) was added to a glass tube containing sterilized Sabouraud dextrose agar (4 ml) and the tube was allowed to solidify in a slanting position at room temperature to prepare an agar slant. From each extract, four agar slants were prepared having four different concentrations (400, 800, 1600 and 3200 µg/ml). Each tube was inoculated with a piece of fungus having a diameter of 4 mm taken from a seven-day old culture of fungus and incubated at 27 ^{o}C for 7 days. For *Eleusine coracana* was used as the standard drug. DMSO was used as the control. Growth of the fungus was evaluated by measuring the linear growth (in mm) and growth inhibition percentage was calculated with reference to the control using the following equation.

$$\text{Inhibition \%} = \frac{\text{100 - Linear growth in test (mm)}}{\text{Linear growth in control (mm)}} \times 100$$

Anti-nutritional factors in finger millets

Iron, calcium, and phosphorus content in ragi grains are exceptionally high as compared to other cereals. However, bio availability of these minerals may be at stake, due to the presence of anti-nutritional factors like, phytic acid and tannins (Polyphenols).Tannins and phytic acid bind the mineral as well as proteins and reduce their digestible contents. These anti-nutritional factors could be reduced by conventional processing techniques like germination, fermentation and dehulling.[18]

Qualitative phytochemical screening of finger millets

Stock solution (1% w/v) of all the extracts including n-hexane, chloroform, ethyl acetate, acetone and methanol of Finger millets were prepared and subjected to phytochemical screening to detect the presence and or absence of various phytochemicals constituent.[19,20,21]

RESULTS AND DISCUSSION

Anti-diabetic properties

Diabetes also known as “Diabetes mellitus” is a major health concern that is rapidly increasing in India and several other developing as well developed countries. In a study,[22] chemical synthetic inhibitors of “glucosidase” and “pancreatic amylase” can be effectively used to treat hyperglycemia and finger millet phenolic extracts were found to be effective inhibitors of these enzymes.[23] Food formulations and preparations based on finger millet have a lower glycemic index and cause a lower glycemic response.[24] Certain anti-nutritional factors found in whole finger millet fractions (such as tannins, phenolics, and phytates) may help to reduce glycemic response by reducing starch digestibility and absorption. Independent rat studies have successfully demonstrated that a finger millet diet fastens the wound healing process and delays cataractogenesis.[25]

Cardio-protective and Anti-hyperlipidemic properties

Cardiac diseases are one of the most severe problems suffered by people worldwide. Risk factors such as high blood pressure, high cholesterol, hypertension, depression, obesity, and diabetes are associated with the problem.[26] Finger millet rich diet lowers lipid per oxidation reaction, which reduces the risk of arteriosclerosis and thus provides important protection against strokes and heart attacks. A similar recent study has found that a multigrain formulated diet containing finger millet was effective in controlling lipid and antioxidant metabolism in high cholesterol intake rat models.[27]

Antifungal compounds

The antifungal activity of Finger millets extract determined by the agar-well diffusion method is shown in Table 1. Minimum inhibitory concentrations (MIC) Finger millets extract for all the six test fungi were found in the range of 3.50 – 4.25 mg/ml. It is confirmed by the results that aqueous extract of Finger millets have very low MIC value against the test fungi.

Table 1: Zone of inhibition in test fungi with Finger millets extract.

Treatments Conc.(mg/ml)	Zone of Inhibition(mm)					
	AA	AF	AN	CC	DH	FM
5	2.33±0.19	2.03±0.56	2.66±0.16	3.16±0.19	3.66±0.43	2.96±0.26
10	5.63±0.13	5.66±0.21	4.15±0.11	6.05±0.15	6.45±0.25	5.65±0.29
20	12.16±0.33	10.33±0.31	10.59±0.21	11.09±0.21	12.09±0.23	12.45±0.13
30	20.25±0.16	18.85±0.21	19.15±0.31	21.05±0.36	22.05±0.31	22.19±0.33
40	29.16±0.21	25.63±0.56	26.23±0.35	28.23±0.33	29.33±0.23	29.13±0.16
50	30.55±0.09	29.65±0.15	30.15±0.16	31.15±0.16	30.15±0.25	31.55±0.15
+ Control	31.13±0.07	30.63±0.13	31.85±0.16	32.93±0.15	31.23±0.35	31.96±0.13
- Control	-	-	-	-	-	-

Values are given in mean ± SD for three replicates. AA: *Alternariaalternata*; AF: *Aspergillus flavus*; AN: *Aspergillus niger*; CC: *Cladosporiumcladosporidies*; DH: *Drechslerahalodes*; FM: *Fusarium moniliforme*

Fungal growth inhibition results presented in Table 1 clearly indicated that the aqueous extract of Finger millets exhibited varying degrees of antifungal activity against all the six test fungi. Of the different test concentrations, it is observed that inhibition of radial growth at concentration of 5, 10, 20 and 30 mg/ml is much less than that of positive control. However, 40 and 50 mg/ml test concentration of extract is considerably effective on growth inhibition of all the test fungi. The mean radial growth inhibition of test fungi Alternaria alternata, Aspergillus flavus, Aspergillus niger, Cladosporium cladosporidies, Drechslera halodes and Fusarium moniliforme with various concentrations of aqueous extract of Finger millets seeds ranged between 2.33- 30.55, 2.03- 29.65, 2.66-30.15, 3.16-31.15, 3.66-30.15 and 2.96-31.55 mm respectively.[28]

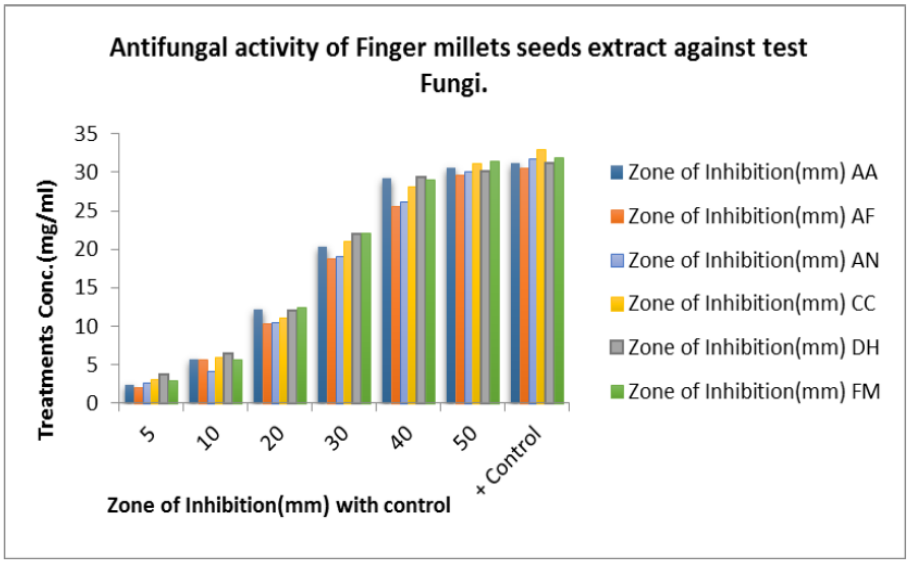

Figure 2: Antifungal activity of finger millets extract against test fungi.

Characterization of antifungal natural products isolated from endophytic fungi of finger millet

Finger millet contains various compounds, including phenolic acids, flavonoids, tannins, and saponins, which are known to disrupt fungal cell membranes, inhibit fungal enzymes, and damage fungal cell walls. The objectives of this study were to confirm the endophytic lifestyle of the three remaining anti-Fusarium isolates, to identify the major underlying antifungal compounds, and to initially characterize the mode(s) of action of each compound. The antifungal compounds produced by finger millet and its endophytic fungi can act through various mechanisms, including disrupting fungal hyphae (the thread-like structures of fungi), inhibiting fungal growth, and even killing the fungus. The antifungal properties of finger millet have implications for its use as a natural antifungal agent, particularly in food preservation and potentially in agricultural applications to reduce fungal diseases in crops.Studies have isolated and identified, purified several antifungal compounds from finger millet and its endophytes, including 5-hydroxy 2(3H)-benzofuranone, Dehydrocostus lactone, and Harpagoside (an iridoide glycoside). In the present study, methanolic extracts of finger millet varieties were evaluated for antifungal activities. Although none of the extracts of the three finger millet varieties was active against the tested fungal strains at 400, 800 and 1600 µg/ml concentrations.[29]

Characterization of antifungal natural products isolated from endophytic fungi of finger millet

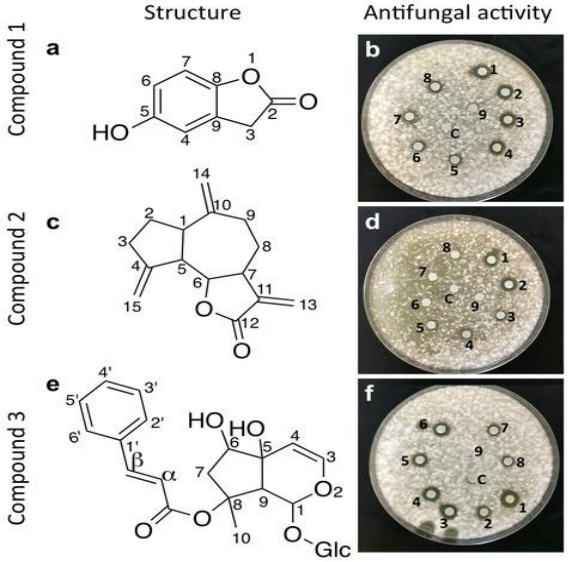

Figure 3: Finger millets.

Structures and in vitro activity of the purified anti-Fusarium compounds from fungal endophyte strains 1,2,3.(a) Structure of compound 1,5-hydroxy benzofuranone (from strain WF5); (b) Representative picture of the disc diffusion assay showing the anti-Fusarium activity of compound 1; (c) Structure of compound 2, dehydrocostus lactone (from strain WF6); (d) Representative picture of the disc diffusion assay showing the anti-Fusarium activity of compound 2; (e) Structure of compound 3, harpagoside (from strain WF7); (f) Representative picture of the disc diffusion assay showing the anti-Fusarium activity of compound 3. Numbers (1–9) denote a concentration gradient of 400, 800 and 1600 µg/ml µg/mL while C denotes the solvent control.[30]

Significance of nutritional composition

Finger Millets are nutritionally superior to other cereal crops of same variety such as rice and wheat because it serves as an excellent source of carbohydrate (80%), proteins (7–9%) with essential amino acids as well as non essential amino acids like valine, methionine, and tryptophan, minerals (calcium, phosphorus, potassium, and iron) as well as vitamins (thiamine, niacin, and riboflavin), and fats for which they are extensively been researched.[31]

General nutrient composition of finger millets per 100G
Table 2

Nutrient composition of Finger Millet	
Moisture	13.24%
Protein	7.6%
Carbohydrate	74.36%
Fiber	1.52%
Minerals	2.35%
Fat	1.35%
Energy	341.6 cal/100g

Qualitative phytochemical analysis

The results of qualitative phytochemical analysis are summarized in the Table 2. It revealed the presence of carbohydrate, protein, amino acids, steroids, terpenoids, phenolics, flavonoids, tannins, saponins, glycosides and fatty oil in the Finger millets.

Table 3: Phytochemical screening of extracts of finger millets.

Phytochemicals	Methanol	n-Hexane	Aqueous ectracts
Terpenoid	+	+	–
Fatty oil	–	+	–
Steroids	+	+	–
Terpenoids	+	+	+
Alkaloids	–	–	–
Phenolics	+	–	+

Flavonoids	+	+	+
Saponins	+	–	+
Tannins	+	–	+
Carbohydrates	+	–	+
Glycosides	+	–	+
Protein	+	+	+
Amino acids	+	–	+

(+) Present; (–) Absent

Fatty oil was found only in n-hexane extract. Phenolics and flavanoids were recorded in all extract except n-Hexane. Remarkably, Phenolics and flavonoids were present all the extracts except n-hexane whereas presence of alkaloids was not detected in any of the extracts.

Phytochemical composition

Finger millet is found to be rich in certain phytochemicals such as Tannins, steroids, polyphenols, alkaloids, terpenoids, cardiac glycosides, balsams, lignans, phytooestrogens and phytocyanins. Some phenolic acid derivatives (Hydroxybenzoic Gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, syringic acid), hydroxycinnamic acid derivatives (Ferulic acid, trans—cinnamic acid, p- coumaric acid, caffeic acid, sinapic acid) and some flavonoids such as Quercetin , Proanthocyanidins and condensed tannins,[32] which are important in healing, aging, prevents deterioration of human health, lowers blood pressure, lowers risk of diabetes, and helps combating the metabolic syndromes.[33]

Chemical structures of major phytochemicals compounds present in finger millet

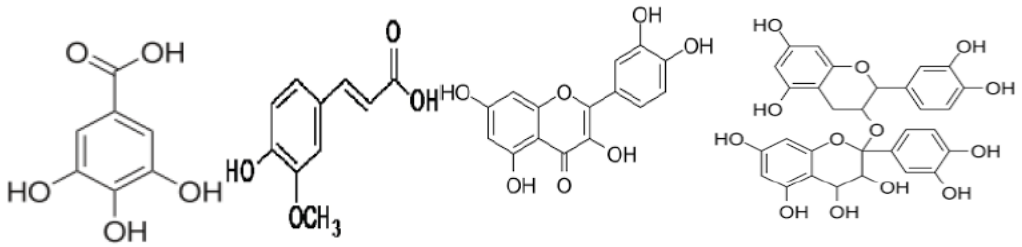

Figure 4: a) Galic acid, b) Ferulic acid, c) Quercetin d) Proanthocyanidins.

CONCLUSION

In the current study, to the best of our knowledge, this is the first report revealing, Highest Antigunal activity and Anti-nutritional factors and Cardio-protective and Anti-hyperlipidemic activity, phytochemical screening of various extracts were obtained using solvents of high polarity; methanolic extract and aqueous extract manifested greater power of extraction Finger millet for phenolic compounds. From the result it is also evident that growth inhibition of all the fungi increased with increase in test concentration of extracts. Further, growth inhibition results in all the six test fungi is the highest at 50 mg/ml concentration of extract and are found to be higher than the positive control. Test concentration of 50mg/ml showed growth inhibition almost at par with synthetic fungicide Carbendazim taken as positive control (fig-2).

Different types of Phytochemical compounds present in Finger millet extract. phytochemical screening of various extracts of Finger millet have reported the presence flavonoids, tannins, carbohydrates, proteins, amino acids, glycosides and saponins in aqueous extract.[34] In recent years, a number of studies have been conducted on the antifungal activity of phenolics compounds including flavones and related flavonoids glycosides, coumarins and derivatives, and anthraquinones.[35,36] The antifungal potency of aqueous extract of Finger millet may be due to presence of phenolic chemical constituent of complex molecular structure and diverse action mechanisms.

The results of this study, it can be concluded that Finger millet seeds exhibiting antifungal activity comparable to commercially known synthetic fungicide can be a promising source of botanical fungicide. For many years, synthetic fungicides have been used to control plant diseases. Indiscriminate of such synthetic chemicals in plant protection has caused environmental contamination and toxicity to living organisms. Synthetic fungicides though being highly effective led to their repeated use that has caused severe environmental pollution, development of resistance, and residual toxicity.[37,38]

Although new fungicides based on natural plant extracts are continually developing, more research is necessary for optimizing applications and become a safe alternative for eliminating the chemical fungicides from agriculture and plantation programs. Compared to synthetic fungicides, plant-derived herbal fungicides show relatively low or little toxicity, thus are safe and may

serves as essential tools for plant disease management. In addition, botanical fungicides will play an important role in reducing environmental pollution in agricultural ecosystems.[39]

Therefore, the plant deserves proper attention towards systematic approach for the collection, storage, processing and value addition that could be helpful in the economic development of tribal areas in the Himalayan foot hills where the species is mostly grown.

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