ISSN 2063-5346

GC-MS ANALYSIS, INVITRO PHARMACOLOGICAL EVALUATION AND IN-SILICO DOCKING STUDIES OF METHANOLIC EXTRACTS OF *PENNISETUM GLAUCUM* AND *PASPALUM SCROBICULATUM*

Gandi Sony Pears*, B. Bhagyasri, K. Hema, V. Girija Sastry

Article History: Received: 01.02.2023 **Revised:** 07.03.2023 **Accepted:** 10.04.2023

Abstract

In this study, a comparitive phytochemical investigation for the antioxidant, antidiabetic, antimicrobial and antifungal potentials of Pearl millet and Kodo millet were performed. Methanolic extract of grains showed high antioxidant and antidiabetic activities. GC-MS analysis of methanolc extracts of Pearl millet and Kodo millet revealed the presence of many bioactive compounds with various therapeutic uses and mass spectra of the compounds obtained were compared with the National Institute of Standards and Technology (NIST) library. In-silico docking studies of the identified compounds in GC-MS was performed to validate the activity of the compounds against active site of alpha glucosidase and alpha amylase. The results of this current study revealed that kodo millet exhibited high antioxidant and antidiabetic activity and can be used as a good source of nutrition with additional health benefits.

Department of Pharmaceutical Chemistry, AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh-530003, India

DOI: 10.31838/ecb/2023.12.s1-B.142

INTRODUCTION

Millet introduction

Millets are one of the most important crops consumed worldwide, particularly in the semi-arid and arid regions of Asia (China and India) and Africa. These are the first cereal grains used by humans, with a round shape and small seeds. They are members of the Poaceae family. Millets are the sixth most important crop, feeding one-third of the world's population.(**Samuel and Peerkhan 2020; Singh et al. 2017**). Millets are high in essential fatty acids, dietary fibre, fats, protein, and other nutrients, but they are low in major micronutrients like iron, calcium, magnesium, zinc, and potassium (**Saini et al. 2021**). Millet is also being researched for its therapeutic potential as a nutraceutical for chronic illnesses such as cancer, diabetes and obesity, cardiovascular disease(**Majid and Priyadarshini 2020)**There are at least nine millet species worldwide, with a total production of 28.38 million tonnes, 11.36 million tonnes (40%) produced in Africa (**FAO, 1995**). Nutritional insecurity is a major global threat that must be addressed as soon as possible. It is estimated that at least 50% of children aged 6 months to 5 years and 2 billion people worldwide are deficient in essential micronutrients and vitamins((**Oh et al. 2020**). Percentage of millet production in different countries and production of various millets in India is represented in Figure 1 and 2, respectively.

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) and Kodo millet (*Paspalum scrobiculatum* Linn.) are cereal crops belonging to the Poacaea family , which are native to Africa and India and were domesticated between 3000 and 5000 years ago(**de morais 2017**). These millets are under-utilised crops, however, their immense nutritional potential has not been tapped. In comparison to maize or wheat that are uncultivatable in harsh conditions, pearl millet and kodo millet are cultivatable in areas with drought, low soil fertility, high salinity, low pH or high temperature. Due to the richness of millets in polyphenols and other biological active compounds, they are also considered to impart role in lowering rate of fat absorption, slow release of sugars (low glycemic index) and thus decreasing the risk of several chronic diseases, such as cardiovascular disorders, diabetes, high blood pressure and impaired vision (**yang2012,shen2015,manwarning2016,Falcinelli B 2018**).

MATERIALS AND METHODS

Materials and chemicals

6kgs pearl millet and kodo millet were procured from Araku valley, Alluri Sitharama Raju District, Andhra Pradesh, India. These millet grains were authenticated by the Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India. Grains were carefully cleaned, by removing broken seeds and dust. Finally cleaned grains were then milled into coarse powder. Solvents like Hexane, Ethyl acetate and Methanol were used according to polarity for extraction and isolation. Silica gel of mesh size 200-400 was used as adsorbent for column packing and for adsorbing extract, silica gel of mesh size 100-200 was used. Millet flour was stored in air tight container for further use.

Extraction:

Coarse powder of pearl millet and kodo millet were transferred into conical flask and solvent was poured until powder was completely submerged. Successive extraction was done using solvents like Hexane, Chloroform and Methanol according to polarity. During extraction process the extracts were filtered using Whattman filter paper No:41 by using 2grams of Sodium sulphate for removing traces and sediments of water in the filtrate. Before filtering the extract, the filter paper was made wet by using sodium sulphate and absolute alcohol.

Preliminary phytochemical analysis:

Various preliminary phytochemical tests have been done to identify various secondary phytochemicals such as alkaloids, steroids, coumarins, tannins, flavonoids, phlabotannins, saponins,fats and oils and starch etc.

Gas chromatography-Mass spectroscopy:

The GC-MS analysis of the *Pennisetum glaucum* and *Paspalum sarobiculatum* methanolic extracts were performed on a Shimadzu QP2010 Plus GC-MS model. A fused silica capillary column with dimensions of 30m length, 0.25mm diameter, and 0.25m thickness is included with the instrument. At a pressure of 49.5kPa, the column oven temperature and injection temperature were regulated during equipment operation. The total flow and column flow rates were both kept constant at 13.4ml/min and 0.95ml/min, respectively. The injector was configured to split mode with a split ratio of 10.0. The ion source and interface temperatures were set to 250.00^0 C 300.00^0C, respectively, with a solvent cut time of 3.00 min. The starting oven temperature was set to 50^0C (2 minutes), then increased to 300^0C at a ramp rate of 7.50^0C per minute. With a linear velocity of 35.3cm/sec and a purge flow of 3.0ml/min, helium (>99.99%) was used as a carrier gas. The MS programme began at 3.00min and ended at 36.00min, with a scan speed of 3333l/sec and an event time of 0.30sec.

Antimicrobial activity:

Anti-bacterial and Anti-fungal activities of methanolic extracts of pearl millet (MPM) and kodo millet (MKM) were performed on bacterial strains such as *Escherichia coli, pseudomonas aeruginosa, staphylococcus aureus* and *Bacillus subtilis* and fungal strains of *Aspergillus niger* and *Candida tropicalis* . These activities were performed by following cup and plate method described by (**Cappuccino JG.,1999**).

Invitro pharmacological studies:

Antioxidant activity:

DPPH radical scavenging activity:

As previously reported, a DPPH radical scavenging assay was carried out (**Tiwari,A.K;Manasa 2013**). The absorbance at 517 nm spectrophotometrically measured the scavenging of 2,2-diphenyl-1-picryhydrazyl (DPPH) radicals by Methanolic extract of pearl millet (MPM) and kodo millet (MKM) (50µ g of 2 mg/mL solution dissolved in DMSO) were measured in 100 mM Tris-HCl buffer (pH 7.4). The standard was ascorbic acid (50 µg of 2 mg/mL solution in DMSO). The results were expressed as %-scavenging and calculated using the formula: $(A_c-A_t)/100\times A_c$ where A_c is the control absorbance and A_t is the test sample absorbance.

ABTS radical scavenging activity:

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation (ABTS•+) was scavenged using the previous method [**Deepthi,s;Anusha**]. As previously described, methanolic extract (20µg of 2 mg/mL solution dissolved in DMSO) of pearl millet and kodo millet were incubated with ABTS•+ solution in 6.8 mM phosphate buffer (pH 8.0). The discoloration of the ABTS•+ solution was determined by spectrophotometrically measuring the absorbance at 734 nm. The standard was ascorbic acid (20 µg of a 2 mg/mL solution dissolved in DMSO). The activity was expressed as a percentage of scavenging and calculated as follows:$(A_c-A_t)/(100\times$ Ac)where A_c is the control absorbance and A_t is the test sample absorbance.

Antidiabetic activity:

Alpha-glucosidase activity:

The previous method was used to perform an intestinal alpha-glucosidase enzyme inhibition assay (**Duong,T.H 2020**). A total 20µL (40µg) of methanolic extracts

of pearl millet and kodo millet were incubated for 10 minutes in 100 mM phosphate buffer (pH 6.8) with 50 µL of rat intestinal -glucosidase enzyme (89.93 mM, prepared in 0.9% NaCl). Following the incubation period, 50 µL of solution (4-nitroplenyl α-D glucopyranoside) was added. The absorbance at 405 nm spectrophotometrically recorded the release of p-nitrophenol from the substrate. The standard was acarbose (40 µg of 2 mg/mL solution dissolved in DMSO). The activity was expressed and calculated: % alpha-glucosidase inhibition=$(A_c-A_t)/100\times A_c$, where A_c was the absorbance of the control and A_t was the absorbance of the test sample.

Alpha-amalyse inhibitory activity:

The 3,5-dinitrosalicylic acid (DNSA) method was used to conduct the alpha-amylase inhibition assay [**Miller GL 1959**]. Methanolic extracts of pearl millet(MPM) and kodo millet(MKM) were dissolved in 10% DMSO and then in buffer ((Na_2HPO_4/NaH_2PO_4 (0.02 M), NaCl (0.006 M) at pH 6.9) to yield concentrations ranging from 10 to 1000 µg/ml. 200 µl of alpha-amylase solution (2 units/ml) was mixed with 200 µl of extract and incubated at 30 °C for 10 minutes. The starch solution (1% in water (w/v)) was then added to each tube and incubated for 3 minutes. The reaction was stopped by adding 200 µl DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM 3,5-dinitrosalicylic acid solution) and boiling for 10 minutes in an 85-90 °C water bath. The mixture was cooled to room temperature and diluted with 5 mL of distilled water before measuring absorbance at 540 nm with a UV-Visible spectrophotometer. By replacing the millet extract with 200 µl of buffer, a blank with 100% enzyme activity was created. In the absence of the enzyme solution, a blank reaction was prepared using the plant extract at each concentration. Acarbose (100 µg/ml-2 µg/ml) was used as a positive control sample, and the reaction was carried out in the same manner as the reaction with plant extract described above. The alpha-amylase inhibitory activity was expressed as a percentage of inhibition and calculated using the following equation: $(A_c-A_t)/100\times A_c$, where A_c was the absorbance of the control and A_t was the absorbance of the test sample.

In-silico docking studies:

In-silico molecular docking studies are one of the computational techniques to explore the possible binding modes of a ligand with a prescribed receptor, enzyme or any other binding site (**Tomi IH.et al**). In the current research work, we used AU Docker software to evaluate the binding energies of identified compounds in GC-MS analysis, with the target enzyme. AU Docker software consists of two major programs, autogrid which precalculates grid maps of interaction energies of different atoms in a ligand with an enzyme, and docking of the ligand to the specific grids is carried out by autodock program (**Morris GM.et al**). Considering the obtained results of invitro pharmacological activities In-silico molecular docking studies were performed to validate the molecular binding interactions with the targeted enzymes and to support results of the antioxidant and antidiabetic activities. Molecular docking was performed using human alpha-amylase (PDB-1HNY) & alpha-glucosidase enzymes (PDB-3L4X) and were obtained from protein data bank.

RESULTS:

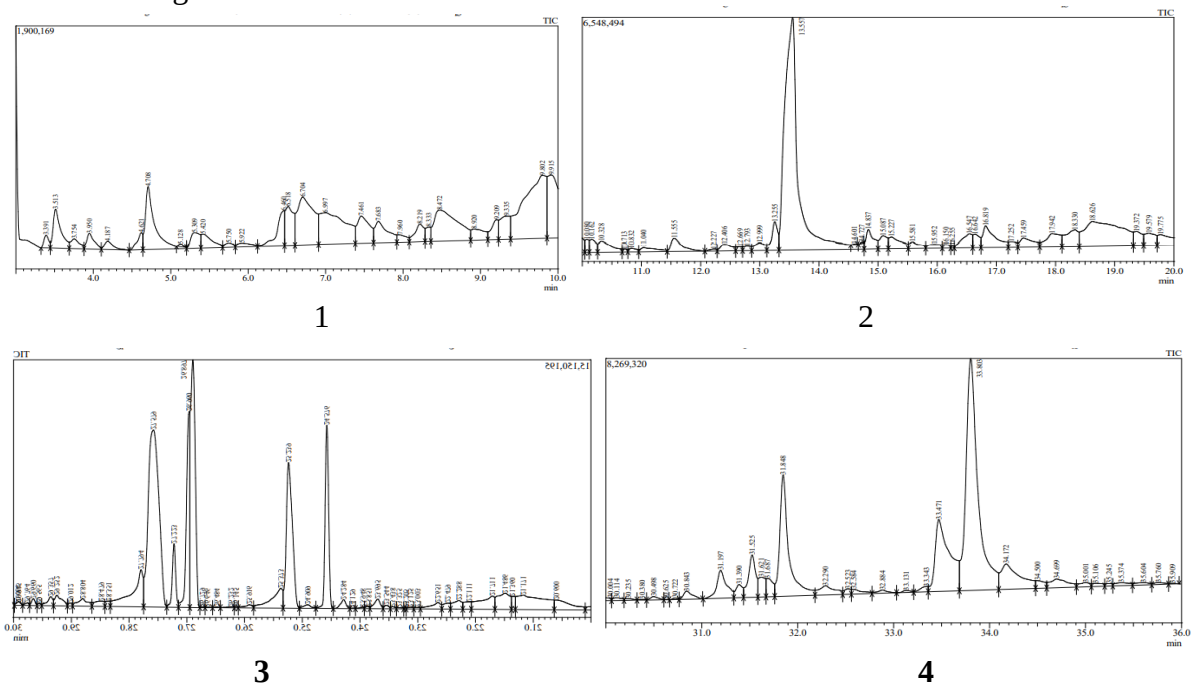
Preliminary phytochemical analysis: From the preliminary phytochemical analysis we conclude that coumarins, steroids, carbohydrates, flavanone, flavonoid, tannins, alkaloids, glycosides, phenolic acids, proteins, amino acids, saponins, terpenoids, starch, fats and oils are present in the extract.it is represented in Table1.

Table 1: Results of preliminary phytochemical analysis of Pearl millet and Kodo millet.

S.no	Name of the test	Pearl millet	Kodo millet
1	Test for coumarins	+	+
2	**Test for steroids**		
	a) Liebermann buchard	+	+
	b) Salkowski'test	+	+
3	Test for Phlabotannins	-	-
4	**Test for Carbohydrates**		
	a) Fehling's solution	+	+
	b) Molisch test	+	+
	c) Benedict's test	+	+
5	Test for flavanon	+	+
6	Test for flavonoids	+	+
7	Test for Tannins	+	+
8	Test for Anthocyanin	-	-
9	**Test for Alkaloids**		
	a) Hager's test	-	-
	b) Dragendroff test	+	+
10	**Test for Glycosides**		
	a) Legal's test	-	-
	b) Keller Killiani test	+	+
11	**Test for Phenolic acids**		
	a) $FeCl_3$ test	+	+
	b) Lead acetate test	+	+
12	**Test for proteins**		
	a) Biuret test	+	+
13	**Test for Amino acids**		
	a) Ninhydrin test	+	+
14	Test for saponins	+	+
15	**Test for Terpenoids**		
	a) Liebermann buchard test	+	+
	b) Salkowski test	+	+
16	Test for Fats and oils	+	+
17	Test for starch	+	+

GC-MS analysis:

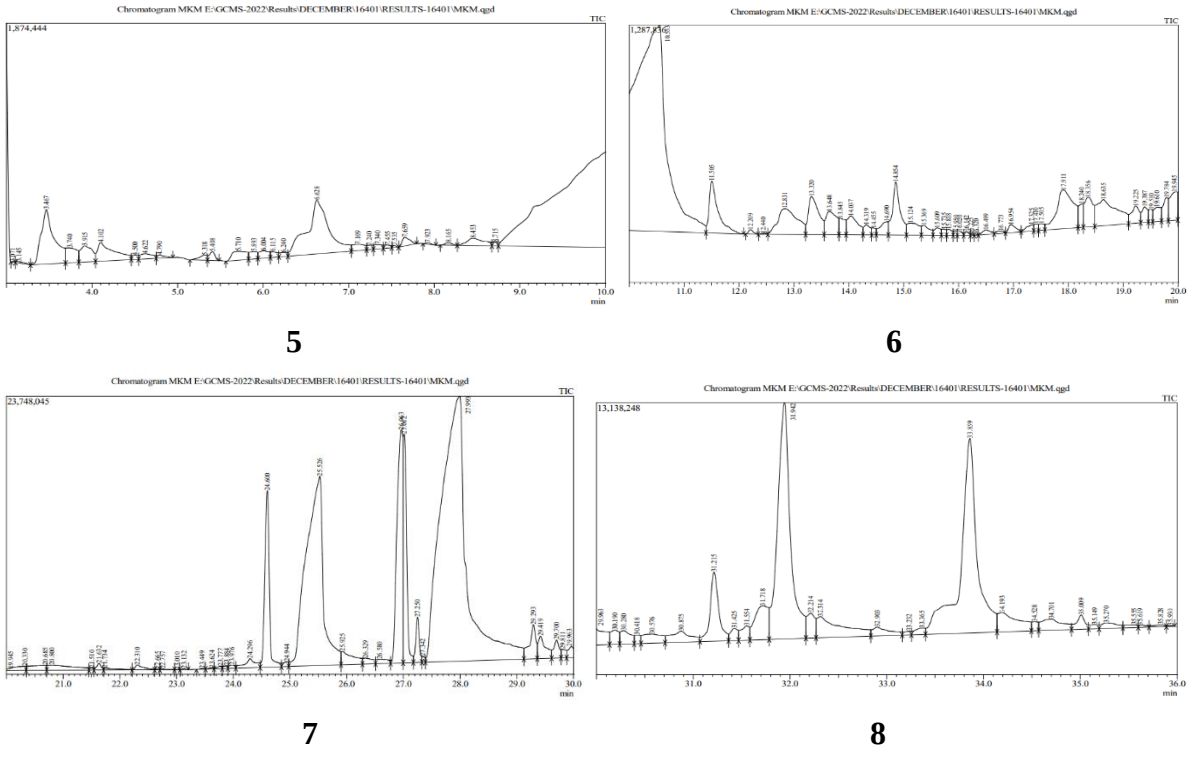
GC-MS analysis of methanolic extract of pearl millet and kodo millet discovered the presence of various phytochemicals. These compounds were identified and confirmed based on the peak area, retention time and molecular formula. GC-MS chromatograms of methanolic extract of pearl millet and kodo millet are represented in Figures 1-4 and Figures 5-8, respectively. The active phytochemicals identified along with their retention time (RT), molecular formula, molecular weight (MW) and peak area in percentage are represented in Table 2 and Table 3.



1 **2**

3 **4**

Figure 1-4: GC-MS chromatogram of pearl millet



5 **6**

7 **8**

Figure 5-8: GC-MS chromatogram of kodo millet

Table2: GC-MS analysis of Pearl millet

S.No	Retension time	Name of the compound	Molecular formula	Molecular weight
1	7.461	2-Furancarboxaldehyde	$C_6H_6O_2$	110
2	9.802	Furaneol	$C_6H_8O_3$	128
3	11.555	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144
4	14.837	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150
5	16.642	4-Ethylcatechol	$C_8H_{10}O_2$	138
6	21.711	2-Propenoic acid,3-(4-hydroxy-3-methoxyphenyl),methyl ester	$C_{11}H_{12}O_4$	208
7	25.239	N-Hexadecanoic acid	$C_{17}H_{34}O_2$	270
8	35.001	Squalene	$C_{30}H_{50}$	410

Table 3: GC-MS analysis of Kodo millet

S.No	Retension time	Compound	Molecular formula	Molecular weight
1	10.553	1,2,3-Propanetriol	$C_3H_8O_3$	92
2	11.505	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144
3	19.945	Vanillic acid	$C_8H_8O_4$	168
4	21.734	2-Propenoic acid,3-(4-hydroxy-3-methoxyphenyl),methyl ester	$C_{11}H_{12}O_4$	208
5	25.526	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
6	27.993	9,12-Octadecanoic acid(Z,Z)	$C_{18}H_{32}O_2$	280
7	33.859	Isopropyllinoleate	$C_{21}H_{28}O_2$	322
8	35.009	Squalene	$C_{30}H_{50}$	410
9	35.270	Stigmasterol	$C_{29}H_{48}O$	412

Antimicrobial activity:

The methanolic extract of *Paspalum scrobiculatum* and *Pennisetum glaucum* tested against 4 different bacterial organisms (*Escherichia coli, pseudomonas aeruginosa, staphylococcus aureus* and *Bacillus subtilis*). The extracts showed significant zone of inhibition against *Escherichia coli* and not on other organisms.The methanolic extract of *Paspalum scrobiculatum* and *Pennisetum glaucum* tested against 2 different fungal organisms *Aspergillus niger* and *Candida tropicalis* and produced no zones of inhibition against two organisms.

Antioxidant activity:

ABTS Radical Scavenging Activity:

Methanolic extract of pearl millet and kodo millet were scavenged ABTS•+ radicals, and the results are presented in Table 4. The results showed that methanolic extracts effectively neutralised

ABTS•+ radicals (more than 70%) and had activity comparable to the ascorbic acid standard. These results proved that kodo millet exhibited highest ABTS radical scavenging activity 82.52±0.11 than pearl millet 79.47±1.78% inhibition. .

DPPH radical scavenging activity:

Based on the results obtained from the antioxidant activities, kodo millet showed the radical scavenging activity of 83.72±0.3% inhibition. whereas pearl millet showed radical scavenging activity of 81.72±1.43% inhibition. Kodo millet showed better antioxidant activity as kodo millet is exhibited high total polyphenol content of 81.64±0.15µmol of ferulic acid equiv/g of defatted meal than peal millet as it exhibited low total polyphenol content of 9.14±0.17µmol of ferulic acid equiv/g of defatted meal (**Chandrasekara A, Shahidi F**).

Table 4: ABTS and DPPH radical scavenging activity results of Pearl and Kodo millets:

S.no	Compound name (MPM)	DPPH scavenging activity (% inhibition)	ABTS activity (% inhibition)
1	MPM(2mg/ml)	81.72±1.43	79.47±1.78
2	MKM (2mg/ml)	83.72±0.3	82.52±0.11
3	Ascorbic acid(2mg/ml)	98.21±0.47	89.63±0.64

Antidiabetic activity:

Alpha-amalyase enzyme inhibition activity results:

The α-amylase inhibitory studies revealed that methanolic extract of kodo millet had significant inhibitory potential 78.32±0.66% inhibition. These α- amylase inhibitors are also known as starch blockers because they prevent or slow starch absorption into the body by preventing the hydrolysis of 1,4-glycosidic linkages in starch and other oligosaccharides into maltose, maltriose, and other simple sugars. The amylase inhibitory activity in methanol extract is most likely due to polar compounds, which should be investigated further and pure active compounds isolated. The Table 5 shows the results for α-Amalyse inhibitory activity.

Alpha-glucosidase Enzyme Inhibition Activity results:

The α-glucosidase enzyme is a critical enzyme in disaccharide digestion. α-glucosidase inhibition in the intestine slows digestion and the overall rate of glucose absorption into the blood. This has been shown to be one of the most effective methods for lowering post-prandial blood glucose levels and, as a result, avoiding the onset of late diabetes complications. Kodo millet exhibited highest activity of 74.60±1.33.

Table 5: α-Glucocidase and α-Amalyse enzyme inhibitory activity results:

s.no	Compound name(code)	Alpha-Glucosidase activity (% inhibition)	Alpha-Amalyse activity (% inhibition)
1	MPM(2mg/ml)	72.03±1.65	73.62±0.66
2	MKM (2mg/ml)	74.60±1.33	78.32±0.12
3	Acarbose(2mg/ml)	84.74±1.2	90.21±0.58

In-silico docking studies ;

Compounds identified in GC-MS analysis of methanolic extract of kodo millet exhibited efficient binding with targeted enzymes. Dock scores of all compounds identified in GC-MS analysis of kodo millet and pearl millet are represented in Table 6 and Table 7, respectively.

Table 6: Dock scores of compounds identified in GC-MS of methanolic extract of kodo millet.

S.No	Compound name	Dock score	
		α-amylase	**α-glucosidase**
1	**ACARBOSE**	**-9.267**	**-9.015**
1	9,12-Octadecanoic acid(Z,Z)	**-5.5**	**-3.6**
2	Isopropyllinoleate	**-5.3**	**-4.6**
3	N-Hexadecanoic acid		
4	Squalene	**-6.3**	**-6.3**
5	Stigmasterol	**-7.3**	**-8.1**
6	Vanillic acid		**-6.0**
7	2-Propenoic acid,3-(4-hydroxy-3-methoxyphenyl),methyl ester	**-6.3**	**-5.0**
8	2-Methoxy-4-vinylphenol	**-5.5**	**-5.1**
9	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	**-5.0**	**-5.0**

Table 7: Dock scores of compounds identified in GC-MS of methanolic extract of pearl millet.

S.no	Compound name	Dock score	
		α-amylase	**α-glucosidase**
1	**ACARBOSE**	**-9.267**	**-9.015**
1	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	**-5.0**	**-5.0**
2	2-Furancarboxaldehyde	**-3.7**	**-4.6**
3	N-Hexadecanoic acid	**-4.6**	**-4.4**
4	2-Methoxy-4-vinylphenol	**-5.5**	**-5.1**
5	2-Propenoic acid,3-(4-hydroxy-3-methoxyphenyl),methyl ester	**-6.3**	**-5.0**
6	Squalene	**-6.3**	**-6.3**
7	Furaneol	**-4.4**	**-5.4**
8	4-Ethylcatechol	**-5.5**	**-6.0**

DISCUSSION:

GC-MS analysis:

GC-MS analysis of methanolic extracts of pearl millet and kodo millet revealed the presence of various phytocompounds which possess many biological activities. This makes pearl and kodo millet a nutraceutical as they have wide range of therapeutic effects against a number of non-communicable diseases like diabetes, heart diseases, cancer, common cold, arthritis, hypertension, dyslipidemia, inflammatory bowel disease, depression, etc. for example, 9,12-octadecanoic acid, methyl ester possess anticancer, hypocholesterolemic, antiarthritic, hepatoprotective, antiandrogenic, antiacne, 5-Alpha reductase inhibitor, nematicide, antihistaminic and anticoronary properties. 5-hydroxymethylfurtural possess antioxidant and antiproliferative activities (**Sermakkani M, Thangapandian V**).

Anti-microbial activity:

Polyphenols have the ability to prevent the spread of germs, according to published research. Similar to our work, **Gutierrez, Baculi, Pastor, Puma-at, and Balangcod (2013)** investigated the antibacterial ability of a few medicinal plants from the Cordillera region of the Philippines, where results were recorded against test markers in the 12.17-28.16 mm zone. The antimicrobial study demonstrates that polyphenol derivatives offer organic defence against infections by harmful bacteria. The research has stated that millets' high polyphenol content is responsible for their excellent preservation qualities (**Devi et al., 2011**). *S. aureus, L. mesenteroides, B. cereus, and E. faecalis* were used as test bacteria for the inhibitory activity of polyphenols extracted using various solvents. Antibacterial activity was determined by the well diffusion method of Kirby Bauer. The strains used as test organisms to determine the antimicrobial activity of hexane extract of Pearl millet, were *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa, Serratia marcescens, Klebsiella pneumoniae, Shigella dysenteriae, Enterococcus sp. and Salmonella sp*. The extract produced no zones of inhibition against all the test organisms (**Singh et al., 2015**)In the present study, methanolic extracts of pearl millet and kodo millet showed antibacterial activity only on *E.coli* and has not shown any activity on *P.aeruginosa, S.aureus, B.subtilis,* and has not observed any antifungal activity on *A.niger, C.tropicalis.*

Invitro pharmacological activities:

Antioxidant activity:

The importance of antioxidant secondary metabolites is rising due to their special functions as lipid stabilisers and inhibitors of unneeded oxidation that causes ageing and cancer **(Namiki, 1990)**. Antioxidant activity can take many different forms, including the inhibition of oxidising enzymes, chelation of transition metals, radical-to-radical transfer of hydrogen or a single electron, deactivation of single oxygen atoms, and enzymatic detoxification of reactive oxygen species. Different techniques should be utilised to assess the antioxidant capacity of pure substances or extracts in order to properly define the total antioxidant activity (**Prior et al., 2005; Dykes and Rooney, 2006**). Since this type of chemical is very polar, the position and level of hydroxylation of the phenolic rings affects the effectiveness of polyphenolics' antioxidant activity. The extraction method also affects this activity (**Miyake and Shibamoto, 1997**). The pre-generated ABTS•+ radical cation and polyphenol, an antioxidant, interact to form the basis of the ABTS/PP test. Both hydrophobic and hydrophilic antioxidants in dietary components were found using the amphiphilic properties of the ABTS•+ cation.One of the most well-liked and frequently applied techniques for determining a compound's capability to function as a free radical scavenger or hydrogen donor, as well as to evaluate the

antioxidant capacity of foods, is the DPPH assay. A deep purple organic nitrogen radical with a protracted half-life is known as the DPPH radical. In contrast to other scavenging assays, it is commercially available and does not call for pre-generation. The colour of the related hydrazine changes from purple to yellow when a DPPH radical solution is combined with an antioxidant/reducing substance. The decrease in absorbance at 515-528 nm as the produced equivalent hydrazine DPPH yields a yellow solution can be used to measure an antioxidant's ability to diminish DPPH, as can electron spin resonance. The DPPH radical was used to gauge an antioxidant's reducing ability. These fundamental chemical tests demonstrate the radical scavenging and reduction capacities of possible antioxidant candidates. In the DPPH radical scavenging assay, methanolic extract of kodo millet successfully scavenged DPPH radicals by more than 50% than pearl millet, as kodo millet exhibited high content of phenolics.

Anti-diabetic activity:

A chronic metabolic disease called diabetes mellitus causes hyperglycemia due to insufficient or inefficient insulin secretion as well as alterations in how proteins, carbohydrates, and lipids are broken down (**Lebovitz, 2001**). According to recent research, hyperglycemia can cause various proteins to undergo non-enzymatic glycosylation and may contribute to the emergence of serious complications related to diabetes. For the treatment of diabetes and the prevention of chronic vascular complications, managing the rise in postprandial blood glucose is crucial (**Bravo, 1998; Lebovitz, 2001**). Consuming a diet heavy in fibre, dietary polyphenols, and complex carbohydrates could all help to reduce them. The millet diet is popularly advised for diabetics and is recognised for its exceptional resistance to ailments that affect the human body. The potential advantage of phenolics is due to their considerable inhibition of α -amylase and α-glucosidase during the hydrolysis process of complex carbohydrates and their ability to delay the uptake of carbohydrates, such as glucose, which in turn modulates postprandial blood glucose concentrations (**Chethan et al., 2008; Misciagna et al., 2000**).Digestive enzymes like α -amylase, lipases, trypsin, pepsin, and α -glucosidase have all been thoroughly investigated and are known to be inhibited by polyphenols. Comparable to miglitol, voglibose, and acarbose, they can serve as α -glucosidase and α -amylase inhibitors, reducing postprandial hyperglycemia (**CJ, 2001; Rohn et al., 2002**). Over the course of 28 days, several researchers examined the protective effects of whole-grain flour prepared from the millets finger (Eleusine coracana) and kodo (Paspalum scrobiculatum) against oxidative stress and blood glucose management in type II diabetes rats (alloxan-induced diabetic rats) (**Hegde et al., 2005**). In the current study, methanolic extract of kodo millet exhibited highest antidiabetic activity than pearl millet due the presence of high content of phenolics in kodo millet.

In-silico docking studies ;

Ranking of all the compounds identified in GC-MS were given according to the binding energies obtained after interaction with targeted enzymes, alpha-amylase and alpha-glucosidase enzymes. In-silico molecular docking studies validate that kodo millet has highest antioxidant and antidiabetic potentials than pearl millet. These compounds of methonolic extract of kodo millet exhibited highest dock score of -8.1 Kcal/mol and -7.3 Kcal/mol with alpha-glucosidase and alpha-amylase, respectively.

CONCLUSION:

In recent times, pharmaceutical industry is facing challenges to discover novel drugs to improve the quality of the people’s

health with low toxicity and side effects. Millets promise a novel way of discovering the bioactive compounds as they are rich in polyphenols which have a variety of health benefits such as antimicrobial, antioxidant, antidiabetic, hypocholesterolemic effects and also safe guard against diet-related diseases. This study provides valuable insights in to antioxidant and antidiabetic potentials of pearl and kodo millet.

Methanolic extract of kodo millet exhibited high antioxidant and antidiabetic activity as kodo millet is rich in polyphenols. Antimicrobial and antifungal of the present work indicated that methanolic extracts of pearl millet and kodo millet are active against only a few strains of bacteria and fungi. The results of the present research work are important to exploit the use of millets, among all other cereals, as a nutraceutical and to promote their use in the prevention of risk of diabetes, cancer and other chronic diseases.

REFERENCES:

- Samuel KS, Peerkhan N. Pearl millet protein bar: nutritional, organoleptic, textural characterization, and in-vitro protein and starch digestibility. Journal of Food Science and Technology. 2020 Sep;57(9):3467-73.
- Saini S, Saxena S, Samtiya M, Puniya M, Dhewa T. Potential of underutilized millets as Nutri-cereal: an overview. Journal of Food Science and Technology. 2021 Feb 6:1-3.
- Majid A, Priyadarshini CG P. Millet derived bioactive peptides: A review on their functional properties and health benefits. Critical reviews in food science and nutrition. 2020 Oct 27;60(19):3342-51.
- Oh C, Keats EC, Bhutta ZA. Vitamin and mineral supplementation during pregnancy on maternal, birth, child health and development outcomes in low-and middle-income countries: a systematic review and meta-analysis. Nutrients. 2020 Feb 14;12(2):491.
- de Morais Cardoso L, Pinheiro SS, Martino HS, Pinheiro-Sant'Ana HM. Sorghum (Sorghum bicolor L.): Nutrients, bioactive compounds, and potential impact on human health. Critical reviews in food science and nutrition. 2017 Jan 22;57(2):372-90.
- .Yang X, Wan Z, Perry L, Lu H, Wang Q, Zhao C, Li J, Xie F, Yu J, Cui T, Wang T. Early millet use in northern China. Proceedings of the National Academy of Sciences. 2012 Mar 6;109(10):3726-30.
- Shen S, Wang Y, Li M, Xu F, Chai L, Bao J. The effect of anaerobic treatment on polyphenols, antioxidant properties, tocols and free amino acids in white, red, and black germinated rice (Oryza sativa L.). Journal of Functional Foods. 2015 Dec 1;19:641-8.
- Manwaring HR, Bligh HF, Yadav R. The challenges and opportunities associated with biofortification of pearl millet (Pennisetum glaucum) with elevated levels of grain iron and zinc. Frontiers in Plant Science. 2016 Dec 23;7:1944.
- Falcinelli B, Calzuola I, Gigliarelli L, Torricelli R, Polegri L, Vizioli V, Benincasa P, Marsili V. Phenolic content and antioxidant activity of wholegrain breads from modern and old wheat (Triticum aestivum L.) cultivars and ancestors enriched with wheat sprout powder. Italian Journal of Agronomy. 2018 Aug 31;13(4):297-302.
- Cappuccino JG. Microbiology: a laboratory manual/James G., Cappuccino and Natalie Sherman. 1999.

- Duong TH, Le Pogam P, Tran TN, Mac DH, Dinh MH, Sichaem J. α-Glucosidase inhibitory depsidones from the lichen Parmotrema tsavoense. Planta Medica. 2020 Jul;86(11):776-81.
- Tiwari AK, Manasa K, Kumar DA, Zehra A. Raw horse gram seeds possess more in vitro antihyperglycaemic activities and antioxidant properties than their sprouts. Nutrafoods. 2013 Jun;12:47-54.
- Deepthi S, Anusha K, Anand A, Manasa A, Babu KS, Mudiam MK, Tiwari AK. Micronutrients and phytochemicals content in various rice (Oryza sativa Linn.) samples control carbohydrate digestion variedly and present differential antioxidant activities: an in vitro appraisal.
- Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical chemistry. 1959 Mar 1;31(3):426-8.
- Gutierrez RM, Baculi R, Pastor N, Puma-at T, Balangcod T. Antibacterial potential of some medicinal plants of the Cordillera Region, Philippines.
- Devi PB, Vijayabharathi R, Sathyabama S, Malleshi NG, Priyadarisini VB. Health benefits of finger millet (Eleusine coracana L.) polyphenols and dietary fiber: a review. Journal of food science and technology. 2014 Jun;51:1021-40.
- Singh N, Meenu G, Sekhar A, Jayanthi A. Evaluation of antimicrobial and anticancer properties of finger millet (Eleusine coracana) and pearl millet (Pennisetum glaucum) extracts. The Pharma Innovation. 2015;3(11, Part B):82.
- Sermakkani M, Thangapandian V. GC-MS analysis of Cassia italica leaf methanol extract. Asian J Pharm Clin Res. 2012;5(2):90-4.
- Namiki M. Antioxidants/antimutagens in food. Critical Reviews in Food Science & Nutrition. 1990 Jan 1;29(4):273-300.
- Chandrasekara A, Shahidi F. Content of insoluble bound phenolics in millets and their contribution to antioxidant capacity. Journal of agricultural and food chemistry. 2010 Jun 9;58(11):6706-14.
- Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of agricultural and food chemistry. 2005 May 18;53(10):4290-302.
- Dykes L, Rooney LW. Sorghum and millet phenols and antioxidants. Journal of cereal science. 2006 Nov 1;44(3):236-51.
- Miyake T. and Shibamoto, T Antioxidative activies of Imatural compounds found in plants. J. Agirc. Food Chem. 1997;45:1819-22.
- Lebovitz HE. Effect of the postprandial state on nontraditional risk factors. The American journal of cardiology. 2001 Jul 26;88(6):20-5.
- Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutrition reviews. 1998 Nov 1;56(11):317-33.
- Chethan S, Dharmesh SM, Malleshi NG. Inhibition of aldose reductase from cataracted eye lenses by finger millet (Eleusine coracana) polyphenols. Bioorganic & medicinal chemistry. 2008 Dec 1;16(23):10085-90.
- Misciagna G, Cisternino AM, Freudenheim J. Diet and duodenal ulcer. Digestive and Liver Disease. 2000 Aug 1;32(6):468-72.

- CJ B. New approaches to the pharmacotherapy of diabetes. Text Book of Diabetes.. Blackwell Science Ltd. 2001:73-10.
- Rohn S, Rawel HM, Kroll J. Inhibitory effects of plant phenols on the activity of selected enzymes. Journal of Agricultural and Food Chemistry. 2002 Jun 5;50(12):3566-71.
- Hegde PS, Rajasekaran NS, Chandra TS. Effects of the antioxidant properties of millet species on oxidative stress and glycemic status in alloxan-induced rats. Nutrition Research. 2005 Dec 1;25(12):1109-20.
- Tomi IH, Al-Daraji AH, Abdula AM, Al-Marjani MF. Synthesis, antimicrobial and docking study of three novel 2, 4, 5-triarylimidazole derivatives. Journal of Saudi chemical society. 2016 Sep 1;20:S509-16.