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GCMS ANALYSIS OF A PATHOGENIC *DRECHSLERA SETARIAE* OF THE CAUSAL PATHOGEN IN LEAF SPOT/ LEAF BLIGHT OF BROWN TOP MILLET

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Abstract

Millets are coarse cereals gaining popularity because of their nutritional benefits. Of all the varieties of millets, Brown top millet (*Urochloa ramose*) causes leaf spot/ leaf blight, having a huge demand in the Indian market due to its nutritional content and ability to adapt to climate change. Current study involves, the GCMS analysis of brown top millet using a pathogenic fungal extract of *Drechslera setariae.* The GC-MS analysis has shown the presence of different major compounds in the methanolic extract of *Drechslera setariae.* A total of 31 compounds were identified in which around some of the compounds have followed Lipinski rule which has antidiabetic property and has been verified through *in silico* analysis.

***Keywords*:** Brown top millet, GCMS, millet, Docking analysis.

Introduction

Challenges in 21st century like climate changes, water scarcity, increasing world population, rising food prices, and other socioeconomic impacts are main threat to agriculture and food security worldwide, especially for the poor. Hence there is need of alternative nutritive food source. Millets refers to small seeded grasses that are cultivated as grain crops grown on dry regions of temperate, tropical and subtropical situations (Baker. 2014). Small millets grown in Asia and Africa. Indian subcontinent and the regions from Southern margin of Sahara to the Ethiopian high lands of Africa (Seetharam *et al.,* 2007).

Brown top millet can accumulate toxic/lethal levels of nitrate and should not be fed to livestock if the plant has been stressed by droughty or cold conditions. Grains from taller non- shattering varieties are used as a boiled whole grain, porridge or unleavened bread.

Millet is small greenish grain, when compared to rice, wheat, jowar it has high nutritional value, rich in fiber, iron, calcium, potassium, magnesium, zinc, phosphorus, protein, and B complex Vitamin (Sarita and Ekta Singh, 2016). So, it is considered as positive grain with low glycemic index it releases the sugar slowly into blood stream and maintains the sugar level in normal person. So, it is very good for diabetic patients and for normal persons it prevents the onset of Type-2 diabetes mellitus (Lawes *et al.,* 2004). Antioxidants in Brown top millet prevent gastric ulcers and colon cancers (Hegde *et al.,* 2004).

Brown top millet has good nutritional value. Farmers are reviving cultivation of Brown top millet, can be grown on degraded soils with very little water. Brown top millet is not only nutritious but also very delicious. The millet is gluten free and rich in essential nutrients. It is a rich source of natural fiber, when compared to other grains (Wisker *et al.,* 1985). Korale contains about 12.5% fiber due to which it serves as medicine for dealing with life style diseases. Lower incidence of cardiovascular diseases, duodenal ulcer and hyperglycemia (diabetes) are reported among those who regularly consume millets. It is known for its rapid forage production. It is grown for several other purposes as well as cover crop in plantation crop groves, for soil erosion control and for high straw production. This millet can be recommended in daily diet, there is a need to encourage the farming community to grow this crop thus contributing in achieving nutrition security. Being a largely agricultural country, India has one solution, and this could be adopted in any country with minor changes. It seems that our local millets could yet provide the best answer to the global problem

Protein-ligand interaction is nothing but lock-and-key model, in which the lock binds the protein target and the key is grouped with the ligand. This is due to hydrophobic interaction (Lawes *et al.,* 2004). *In silico* techniques helps us in identifying drug target using bioinformatics tools. They are also used to explore the protein target structures for possible active sites, generate candidate molecules, dock these molecules with the target, rank them according to their binding affinities, as well as to optimize the molecules to improve binding characteristics (Miller, 2007). Nowadays, Diabetes mellitus (DM) is a leading noncommunicable disease which are affecting more than 100 million people across the world. Hence it is considered as one of the fine leading diseases which causes severe death (Sarita and Ekta Singh. 2016). Type-2 diabetes mellitus is a chronic metabolic disorder which defects in both insulin secretion and insulin action. Currently, there are few drugs that are able to counteract the development of the associated pathologies. Therefore, the need to search for new drug candidates in this field appears to be critical.

Materials and Methods

Collection of millet

The brown top millets were collected from different locations of Karnataka during 2019.

Desiccated seed Examination

Dry seeds can be detected using seed-borne pathogens which may cause discoloration of seed coat or changes in the seed size and shape. Four hundred seeds were analysed under stereo binocular microscope after incubation. Seeds with mechanical damage, abnormalities, discoloration, smut balls and other fungal bodies were observed under a stereoscopic microscope and percentage was recorded. Millets were separated as per [ISTA 2006] rules these impurities are considered as inert matter.

Surface sterilization of Millet

Millets were collected and washed thoroughly under running tap water followed by sterile distilled water to remove the adhered debris. These millets were surface sterilized under aseptic condition in sequential steps by immersing in mercuric chloride (1mg/1ml) for 10 min and 70 % ethanol for another min followed by washing finally with distilled water.

Inoculation of implants

Infected leaves were surface sterilization, and placed 5-6 pieces on each of the solidified sterile Potato Dextrose Agar (PDA) media. The inoculated plant implants were incubated for seven days.

Identification of pathogenic fungi

Identification was done based on morphological characteristics such as growth pattern, hyphae, the colour of the colony, surface texture, margin character, aerial mycelium, mechanism of spore production and conidia characteristics using standard manuals (Barnett, 1972).

Mass production of identified pathogenic fungi

Identified pathogenic fungal species were cultured on Potato dextrose broth for mass cultivation. The inoculated flasks were incubated at room temperature (26±2ºC) for 8-15 days and allowed to grow the fungal mats. Further these mats are used as a fungal extract for the GCMS analysis.

GCMS analysis

GC-MS analysis of pathogenic fungal methanol extract were performed using a Perkin - Elmer GC Clarus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite-1, fused silica capillary column (30 mm x 0.25 mm 10 x 1µMdF, composed of 100% dimethylpolysiloxane). For GC-MS detection an electron ionization system with ionizing energy of 70ev was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1ml/min and an injection volume of 2µ1 was employed (split ratio of 10:1); injector temperature 2500^{o}C; ion-source temperature 2800^{o}C. The oven temperature programmed from 1100^{o}C (isothermal for 2 min) with an increase of 100^{o}C/min to 2000^{o}C, then 50 C/min to 2800^{o}C, ending with a 9 min isothermal at 2800^{o}C, mass spectra were taken at 70ev; a scan interval of 0.5 seconds and fragments from 45 to 450Da, total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo mass.

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name molecular weight and structure of the components of the test materials were ascertained.

Molecular Docking

Selection of a target (Protein)

The 3D structure of 1V4S (glucokinase(hexokinase4)) is PDB ID: 1V4S (Protein coding gene) is downloaded using website https://www.rcsb.org/structure/1V4S.

Preparation of ligand:

Based on GCMS analysis a total number of 31 compounds have been identified, six compounds viz. 2,2-Bis[4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]phenyl]-1,1,1,3,3,3-hexafluoropropane; Benzoic acid, 2-fluoro-, 2-oxo-2-phenylethyl ester, 2-Oxo-2-phenylethyl 2-fluorobenzoate # (antidia); Milbemycin b, 13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1-methylethyl)-, (6R,13R,25R); Hematoporphyrin ix, Hematoporphyrin (antidia); Anodendroside E 2, monoacetate, o- Acetylanodendroside E2 (anti dia) and Lycoxanthin .psi.,.psi.-Caroten-16-ol,. was selected. The SDF files of these compounds were obtained from Pubchem database and converted to PDB format using OPEN BABEL tools.

Properties of Lead-likeness

SWISS ADME, free tool which is used to generate medicinal properties of a compound which acts as a drug likeness and physicochemical of all these six compounds. Lipinski's rule (Lipinski, 2004 and Lipinski *et al.,* 2001) which is also called as rule of 5 (RO5) to determine whether the chemical compound along with biological or pharmacological activities.

Toxicity

The toxicity of the compounds was detected with admestSAR, a free online web server. This server provides the possible toxicity profile of the compounds with the values suggesting the safety.

Results and Discussion

A total 28 samples of brown top millets were collected from standing crops of Karnataka (table 1) and were stored in cloth bags at room temperature for subsequent studies.

Table: 1. Details of seed collection during 2019.

SL. No.	Name of District	Variety	Source	Number of samples collected	% of *Drechslera setariae*
1	Bangalore	Local	Field	03	7. 0
2	Chitradurga	Local	Field	04	4.0
3	Davanagere	Local	Field	03	14.80
4	Dharwad	Local	Field	05	8.0
5	Haveri	Local	Field	07	16.20
6	Mysore	Local	Field	04	8.40
7	Tumkur	Local	Field	02	9.10
	Total			28	67.50

GCMS chromatograms profile of the methanol extract of brown top millet using a pathogenic fungal extract of *Drechslera setariae.* showed the number of major compounds. The GC-MS spectrum confirmed the presence of various compounds with different retention times [Figure 1 and table 2]. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library.

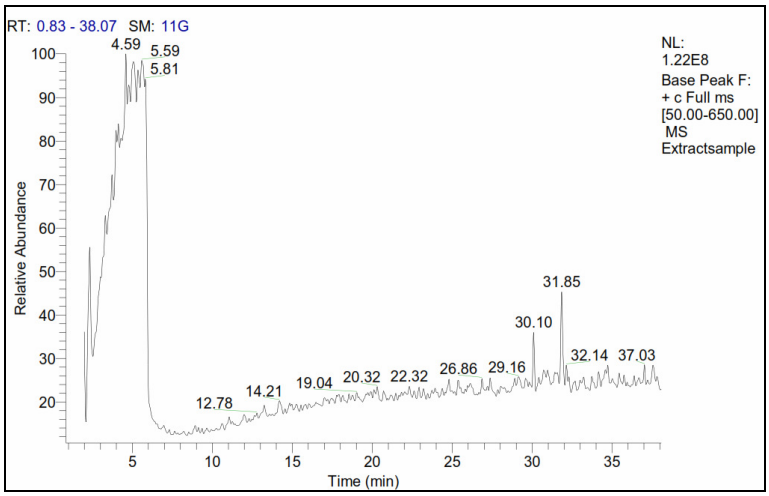
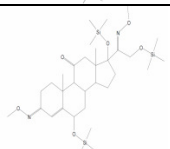
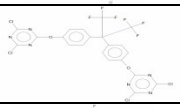
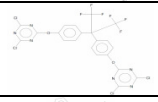
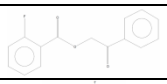
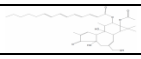
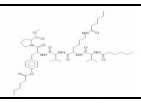
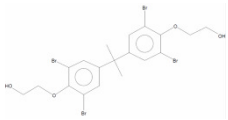
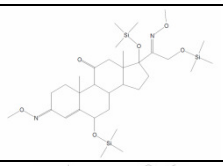
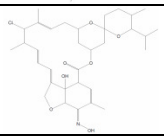
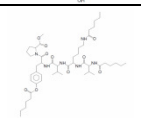
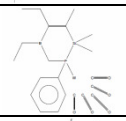
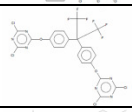
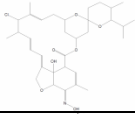
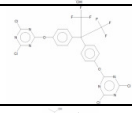
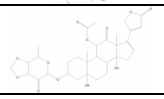
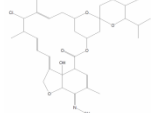
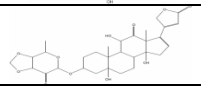
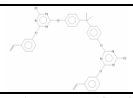
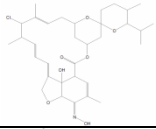
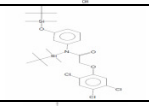
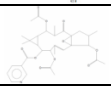


Fig. 1 : GCMS chromatogram of brown top millet

Table 2: GC-MS Analysis of methanol extract from brown top millet seed obtained from *Drechslera setariae.*

Sl. NO.	RT	Name of the compound	Molecular formula	MW	Structure
1	2.33	Di-tungsten, tris(cyclooctatetraene)	$C_{24}H_{24}W_2$	680	
2	3.29	Molybdenum, bis[(1,2,3,4,5-.eta.)-1,3-bis(1,1-dimethylethyl)-2,4-cyclopentadien-1-yl]di-	$C_{30}H_{42}Mo_2O_4$	662	
3	3.71	Pregn-4-ene-3,11,20-trione,6,17,21-tris[(trimethylsilyl)oxy]-,3,20-bis(O-methyloxime), (6á)-6,17,21-Tris[(trimethylsilyl)oxy]pregn-4-ene-3,11,20-trione 3,20-bis(O-methyloxime)	$C_{32}H_{58}N_2O_6Si_3$	650	
4	3.96	2,2-Bis[4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]phenyl]-1,1,1,3,3,3-hexafluoropropane	$C_{21}H_8Cl_4F_6N_6O_2$	630	
5	4.10	2,2-Bis[4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]phenyl]-1,1,1,3,3,3-hexafluoropropane	$C_{21}H_8Cl_4F_6N_6O_2$	630	
6	4.59	2,2-Bis[4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]phenyl]-1,1,1,3,3,3-hexafluoropropane	$C_{21}H_8Cl_4F_6N_6O_2$	630	
7	6.97	2-p-Tolyl-4,5-diphenyl-3-(3-p-tolyl-4,5-diphenylpyrrol-2-yl)-3H-pyrrol-3-ol	$C_{46}H_{36}N_2O$	632	

8	8.22	Benzoic acid, 2-fluoro-, 2-oxo-2-phenylethyl ester 2-Oxo-2-phenylethyl 2-fluorobenzoate #	$C_{15}H_{11}FO_3$	258	
9	8.91	2,4-Difluorobenzoic acid, 2-formyl-4,6-dichlorophenyl ester	$C_{14}H_6Cl2F2O3$	330	
10	10.60	CHEMBL4289168	$C_{32}H_{41}NO_{10}$	599	
11	11.05	3-Hydroxy-1-(4-{13-[4-(3-hydroxy-3-phenylacryloyl)phenyl]tridecyl}-phenyl)-3-phenylprop-2-en-1-one	$C_{43}H_{48}O_4$	628	
12	11.99	SCHEMBL21621480	$C_{36}H_{48}O_8$	608	
13	13.25	3-Hydroxy-1-(4-{13-[4-(3-hydroxy-3-phenylacryloyl)phenyl]tridecyl}-phenyl)-3-phenylprop-2-en-1-one	$C_{43}H_{48}O_4$	628	
14	14.53	L-Proline, 1-[O-(1-oxohexyl)-N-[N-[N6-(1-oxohexyl)-N2-[N-(1-oxohexyl)-L-valyl]-L-lysyl]-L-valyl]-L-tyrosyl]-, methyl ester	$C_{49}H_{80}N_6O_{10}$	912	
15	14.82	4,4'-Isopropylidenebis(2-[2,6-dibromophenoxy]ethanol) Ethanol,2,2'-[(1-methylethylidene)bis[2,6-dibromo-4,1-phenylene)oxy]]bis-	$C_{19}H_{20}Br_4O_4$	628	
16	14.96	Pregn-4-ene-3,11,20-trione, 6,17,21-tris[(trimethylsilyl)oxy]-, 3,20-bis(O-methyloxime), (6á)- 6,17,21-Tris[(trimethylsilyl)oxy]pregn-4-ene-3,11,20-trione 3,20-bis(O-methyloxime	$C_{32}H_{58}N_2O_6Si_3$	650	
17	15.25	Milbemycin b, 13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1-methylethyl)-, (6R,13R,25R)-	$C_{33}H_{46}ClNO_7$	603	
18	16.04	L-Proline, 1-[O-(1-oxohexyl)-N-[N-[N6-(1-oxohexyl)-N2-[N-(1-oxohexyl)-L-valyl]-L-lysyl]-L-valyl]-L-tyrosyl]-, methyl ester	$C_{49}H_{80}N_6O_{10}$	912	
19	17.02	Pentacarbonyl (4,5-diethyl-2,2,3-trimethyl-1-phenyl-1- phospha-2-sila-5-boracyclohex-2-ene-P1) tungsten.	$C_{21}H_{26}BO_5PSiW$	612	
20	17.87	2,2-Bis[4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]phenyl]-1,1,1,3,3,3-hexafluoropropane	$C_{21}H_8C_{l4}F_6N_6O_2$	630	
21	22.93	Milbemycin b, 13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1-methylethyl)-, (6R,13R,25R)-	$C_{33}H_{46}ClNO_7$	603	
22	24.76	2,2-Bis[4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]phenyl]-1,1,1,3,3,3-hexafluoropropane	$C_{21}H_8C_{l4}F_6N_6O_2$	630	
23	25.39	Hematoporphyrin ix Hematoporphyrin	$C_{34}H_{38}N_4O_6$	598	
24	26.86	Anodendroside E 2, monoacetate o- Acetylanodendroside E2	$C_{32}H_{40}O_{12}$	616	
25	30.71	Lycoxanthin .psi.,.psi.-Caroten-16-ol	$C_{40}H_{56}O$	552	
26	30.98	Milbemycin b, 13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1-methylethyl)-, (6R,13R,25R)-	$C_{33}H_{46}ClNO_7$	603	
27	32.14	3,20-bis(O-methy	$C_3H_{38}O_{11}$	574	

28	32.69	2,2-Bis[4-[[4-chloro-6-(3-ethynylphenoxy)-1,3,5-triazin-2-yl]oxy]phenyl]propane	$C_{37}H_{24}Cl_2N_6O_4$	686	
29	33.73	Milbemycin b, 13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1-methylethyl)-, (6R,13R,25R)-	$C_{33}H_{46}ClNO_7$	603	
30	37.56	N-(3-Hydroxyphenyl)-2-(2,4,5-trichlorophenoxy)acetamide ditbdms	$C_{26}H_{38}Cl_3NO_3Si_2$	573	
31	37.80	CHEMBL4205536	$C_{32}H_{39}NO_{10}$	597	

Molecular Docking:

Table 3: Results of docking performed with a software iGEMDOCK and Autodock Vina between the drug targets with ligands and 1V4S (glucokinase(hexokinase4))

Sl.No	Ligand	Binding Affinity	rmsd/ub	rmsd/lb
1	1v4s_5281245_uff_E=1558.30	-5.2	0	0
2	1v4s_540438_uff_E=1400.43	-9.1	0	0
3	1v4s_569869_uff_E=135.10	-6.4	0	0
4	1v4s_579993_uff_E=742.80	-8.3	0	0
5	1v4s_9601634_uff_E=1663.39	-3.1	0	0
6	1g83_445319_uff_E=44.26	-3.3	0	0

The energy values and the binding affinities are presented in Table 3. The energy values obtained by iGEMDOCK of the drug targets of eight compounds were -5.2, -.9.1, -6.4, -8.3, -3.1, -3.3 Kcal/mol, respectively.

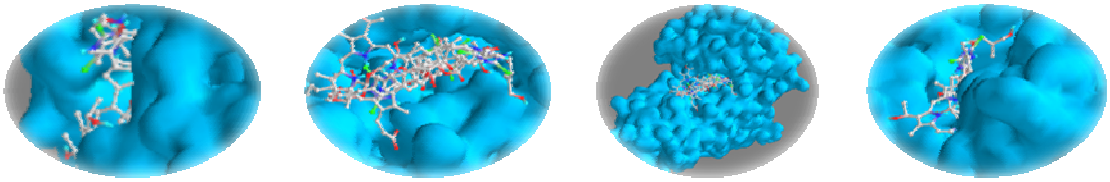
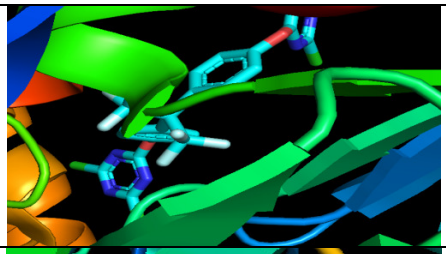
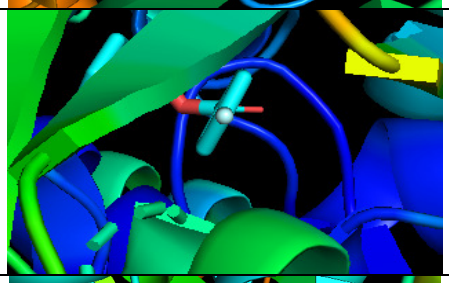
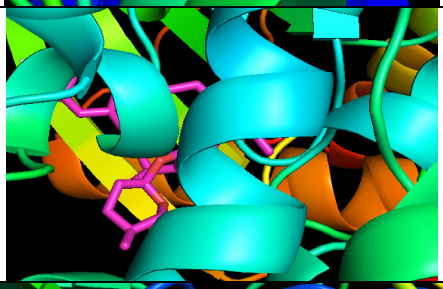
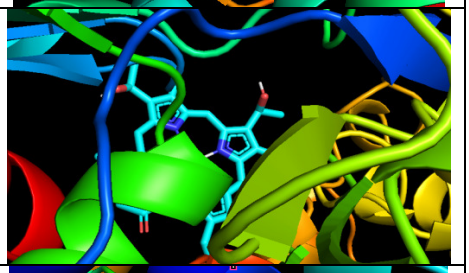
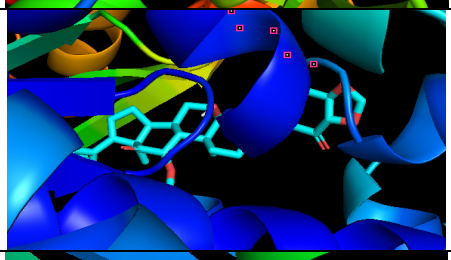
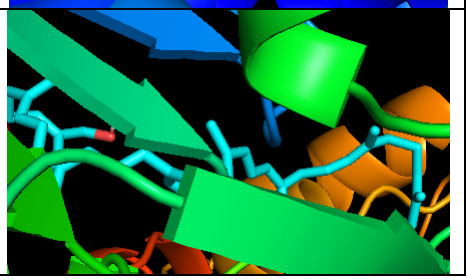


Fig. 2: Docking of1V4S (glucokinase(hexokinase4)) with the compounds

The above figures display the docking stages of various compounds with their protein drug targets. The docking stages were analysed, and the amino acid residues involved in the various interactions were evaluated.

PDB Structures of Protein 1G83

Protein and ligand docking Visualization by PyMOL

SL.NO	*LIGANDS*	*Protein-1G83*
1	2,2-Bis[4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]phenyl]-1,1,1,3,3,3-hexafluoropropane	
2	*Benzoic acid, 2-fluoro-, 2-oxo-2-phenylethyl ester 2-Oxo-2-phenylethyl 2-fluorobenzoate # (antidia)*	
3	*Milbemycin b, 13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1-methylethyl)-, (6R,13R,25R*	
4	Hematoporphyrin ix Hematoporphyrin (antidia)	
5	Anodendroside E 2, monoacetate o- Acetylanodendroside E2 (anti dia)	
6	*Lycoxanthin .psi.,.psi.-Caroten-16-ol,.)*	

Drug likeness and other properties

Table 4: General properties of 4H-1,2,4-Triazol-3-amine-4-methyl; Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide

Molecules	Name of the Ligand/compound	Chemical formula	SMILES	IUPAC Name
1	2,2-Bis[4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]phenyl]-1,1,1,3,3,3-hexafluoropropane	$C_{21}H_8C_{l4}F_6N_6O_2$	C1=CC(=CC=C1C(C2=CC=C(C=C2)OC3=NC(=NC(=N3)Cl)Cl)(C(F)(F)F)C(F)(F)F)OC4=NC(=NC(=N4)Cl)Cl	2,4-dichloro-6-[4-[2-[4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]phenyl]-1,1,1,3,3,3-hexafluoropropan-2-yl]phenoxy]-1,3,5-triazine
2	*Benzoic acid, 2-fluoro-, 2-oxo-2-phenylethyl ester 2-Oxo-2-phenylethyl 2-fluorobenzoate # (antidia)*	$C_{15}H_{11}FO_3$	C1=CC=C(C=C1)C(=O)COC(=O)C2=CC=C(C=C2)F	phenacyl 4-fluorobenzoate
3	*Milbemycin b, 13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1-methylethyl)-, (6R,13R,25R)*	$C_{33}H_{46}ClNO_7$	CC1CCC2(CC3CC(O2)CC=C(C(C(C=CC=C4COC5C4(C(C=C(C5=NO)C)C(=O)O3)O)C)Cl)C)OC1C(C)C	(10E,14E,16E,21E)-12-chloro-24-hydroxy-21-hydroxyimino-5',11,13,22-tetramethyl-6'-propan-2-ylspiro[3,7,19-trioxatetracyclo[15.6.1.1^{4,8}.0^{20,24}]pentacosa-10,14,16,22-tetraene-6,2'-oxane]-2-one
4	Hematoporphyrin ix Hematoporphyrin (antidia)	$C_{34}H_{38}N_4O_6$	CC1=C(C2=CC3=NC(=CC4=NC(=CC5=C(C(=C(N5)C=C1N2)C(C)O)C)C(=C4CCC(=O)O)C)C(=C3C)CCC(=O)O)C(C)O	3-[18-(2-carboxyethyl)-8,13-bis(1-hydroxyethyl)-3,7,12,17-tetramethyl-22,23-dihydroporphyrin-2-yl]propanoic acid
5	Anodendroside E 2, monoacetate o-Acetylanodendroside E2 (anti dia)	$C_{32}H_{40}O_{12}$	CC1C2C(C(=O)C(O1)OC3CCC4(C5C(CCC4(C3)O)C6(CC=C(C6(C(=O)C5OC(=O)C)C)C7=CC(=O)OC7)O)C)OCO2	[5,14-dihydroxy-10,13-dimethyl-3-[(4-methyl-7-oxo-4,7a-dihydro-3aH-[1,3]dioxolo[4,5-c]pyran-6-yl)oxy]-12-oxo-17-(5-oxo-2H-furan-3-yl)-1,2,3,4,6,7,8,9,11,15-decahydrocyclopenta[a]phenanthren-11-yl] acetate
6	*Lycoxanthin .psi.,.psi.-Caroten-16-ol,.*	$C_{40}H_{56}O$	CC(=CCCC(=CC=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC=C(C)CCC=C(C)CO)C)C)C)C	(2E,6E,8E,10E,12E,14E,16E,18E,20E,22E,24E,26E)-2,6,10,14,19,23,27,31-octamethyldotriaconta-2,6,8,10,12,14,16,18,20,22,24,26,30-tridecaen-1-ol

SMILES: Simplified Molecular Input Line Entry Specification
IUPAC: International Union of Pure and Applied Chemistry

Table 5: Physicochemical properties of different compounds.

Molecules	Molecular Weight (g/mol)	No. heavy atoms	No. arom. heavy atoms	Fraction CSP3	No. rotatable bonds	No. H-bond acceptors	No. H-bond donors	Molar refractivity	TPSA ($^oA^2$)
1	632.13	39	24	0.14	8	14	0	125.62	95.80
2	258.24	19	12	0.07	5	4	0	67.40	43.37
3	604.17	42	0	0.70	1	8	2	163.27	106.81
4	598.69	44	10	0.35	8	8	6	178.53	171.36
5	616.65	44	0	0.75	5	12	2	149.38	164.12
6	552.87	41	0	0.35	17	1	1	189.39	20.23

Table 6: Lipophilicity and hydrophilicity of the compounds

Lipophilicity		Hydrophilicity											
Molecules	**Consensus Log P**	**ESOL Log S**	**ESOL Solubility (mg/ml)**	**ESOL Solubility (mol/l)**	**ESOL Class**	**Ali Log S**	**Ali Solubility (mg/ml)**	**Ali Solubility (mol/l)**	**Ali Class**	**Silicos-IT LogSw**	**Silicos-IT Solubility (mg/ml)**	**Silicos-IT Solubility (mol/l)**	**Silicos-IT class**
1	7.22	-9.70	1.27e-07	2.01e-10	Poorly soluble	-11.46	2.18e-09	3.45e-12	Insoluble	-11.13	4.63e-09	7.33e-12	Insoluble
2	3.17	-3.65	5.77e-02	2.23e-04	Soluble	-3.88	3.44e-02	1.33e-04	Soluble	-5.24	1.49e-03	5.75e-06	Moderately soluble
3	4.58	-6.84	8.73e-05	1.45e-07	Poorly soluble	-7.26	3.30e-05	5.46e-08	Poorly soluble	-4.43	2.23e-02	3.70e-05	Moderately soluble
4	3.07	-4.52	1.80e-02	3.01e-05	Moderately soluble	-5.34	2.74e-03	4.58e-06	Moderately soluble	-7.04	5.46e-05	9.12e-08	Poorly soluble
5	1.51	-3.67	1.31e-01	2.12e-04	Soluble	-3.56	1.71e-01	2.77e-04	Soluble	-3.11	4.79e-01	7.76e-04	Soluble
6	11.03	-11.16	3.82e-09	6.90e-12	Insoluble	-14.83	8.26e-13	1.49e-15	Insoluble	-5.74	1.00e-03	1.82e-06	Moderately soluble

o/w: octanol/water

Table 7: Pharmacokinetics properties of the compounds

Molecules	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log Kp (cm/s)
1	Low	No	Yes	Yes	No	No	No	No	-3.38
2	High	Yes	No	Yes	Yes	No	No	No	-5.54
3	Low	No	Yes	No	No	No	No	No	-6.24
4	Low	No	Yes	No	No	No	No	No	-8.45
5	Low	No	Yes	No	No	No	No	No	-9.68
6	Low	No	Yes	No	No	No	No	No	0.49

GI absorption: Gastrointestinal absorption, BBB: Blood Brain Barrier, CYP: cytochrome P

Table 8: Druglikeness and leadlikeness of the compounds

Molecules	Lipinski, #violations	Ghose, #violations	Veber #violations	Egan #violations	Muegge #violations	Bioavailability Score	PAINS #alerts	Brenk #alerts	Leadlikeness #violations	Synthetic Accessibility
1	No; 2	No; 2	Yes	No; 1	No; 3	0.17	0	0	No; 3	2.81
2	Yes, 0	Yes	Yes	Yes	Yes	0.55	0	0	Yes	1.96
3	Yes, 1	No, 4	Yes	Yes	No, 2	0.55	0	5	No, 2	9.07
4	No; 2	No; 3	No; 1	No; 1	No; 2	0.11	0	0	No; 2	8.41
5	No; 2	No; 3	No; 1	No; 1	No; 3	0.17	0	2	No; 1	7.34
6	No; 2	No; 4	No; 1	No; 1	No; 3	0.17	0	2	NO; 3	5.94

Table 9: Tabulates the toxicity profile of the compounds, which were non-toxic in hERG, AMES toxicity, acute oral toxicity, and Human oral bioavailability.

Name of ligand	hERG inhibition	AMES toxicity	Carcinogenicity (Class III)	Acute oral toxicity (kg/mol)	uman-oral bio availability
2,2-Bis[4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]phenyl]-1,1,1,3,3,3-hexafluoropropane	0.9217	0.8727	0.5889	0.6335	0.6571
Benzoic acid, 2-fluoro-, 2-oxo-2-phenylethyl ester 2-Oxo-2-phenylethyl 2-fluorobenzoate # (antidia)	0.9792	0.8880	0.6449	0.7118	0.7143
Milbemycin b, 13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1-methylethyl)-, (6R,13R,25R)	0.9551	0.6239	0.4232	0.6052	0.6143
Hematoporphyrin ix Hematoporphyrin (antidia)	0.9647	0.7089	0.5606	0.5920	0.5857
Anodendroside E 2, monoacetate o- Acetylanodendroside E2 (anti dia)	0.9689	0.8964	0.4648	0.8417	0.6429
Lycoxanthin .psi.,.psi.-Caroten-16-ol,.	0.7838	0.9132	0.6507	0.8552	0.6571

hERG: human Ether-a-go-go related gene

From the table 5, physicochemical properties show the number of atoms, molecular weight, fraction CSP3, topological polar surface area and number of rotatable bonds, molar refractivity.

From the table 6, Lipophilicity and hydrophilicity demonstrates the octanol- water partition coefficient values of the ligands. As indicated in this table, these values were within the permissible range of -0.4 to +5.6 which implies a good lipophilic compound.

From the table 7, the pharmacokinetic properties of the ligands were studied for all the six ligands.

From the table 8, Druglikeness and leadlikeness shows that some of the compounds which follows the Lipinski's rule of 5 and other filters, like Veber [32] and Egan [33], with four violations for Ghose filter[34] of the ligand for Milbemycin b, 13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1-methylethyl)-, (6R,13R,25R) and Lycoxanthin .psi.,.psi.-Caroten-16-ol and three vilolations for the ligands Hematoporphyrin ix and Anodendroside E 2, monoacetate aswell as two vilations of the ligand 2,2-Bis[4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy] phenyl]- 1,1,1,3,3,3-hexafluoropropane.

From the table 9, The toxicity profile of all the six compounds were studied. In which some of the compounds were non-toxic in hERG, AMES toxicity, acute oral toxicity. Regarding the carcinogenicity, values were determined using admetSAR.

Conclusion

Millets are a rich source of essential amino acids, soluble and insoluble dietary fiber, proteins, vitamins, iron, calcium, magnesium, potassium, phosphorous, and vital vitamins. Brown top millet is not only nutritious but also very delicious. The millet is gluten free and rich in essential nutrients. It is a rich source of natural fiber, when compared to other grains. Korale contains about 12.5% fiber due to which it serves as medicine for dealing with life style diseases. Lower incidence of cardiovascular diseases, duodenal ulcer and hyperglycemia (diabetes) are reported among those who regularly consume millets. Gas chromatography –Mass spectroscopy (GC-MS) is a valuable tool for reliable identification of major compounds. In the present study, 31 compounds have been identified. Docking analysis was done using autodock vina and pymol. Six major compounds are selected which undergo antidiabetic activity. These compounds were docked against 1V4S (glucokinase (hexokinase4)). All the six compounds had a good inhibitory potential. Hence these compounds can be analysed by further through *in vitro* studies and can be a lead in the designing of potential drug in the treatment of diabetes disease. This study encourages the utility of the compound for further drug discovery through advanced techniques.

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