Extraction and fermentation of galactomannon from fenugreek for the production of bio active secondary metabolites

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Abstract

Galactomannon extracted from Fenugreek was characterised by FTIR and NMR. The galactomannaon incorporated fermentation medium was used for secondary metabolite production. S.lactis, Yeast and Streptomyces sp were selected as fermenting microbes. The compound was extracted from fermentation by solvent extraction with ethylacetate and was used for in vitro studies. The FTIR reveals representative peaks of polysaccharide in region of 3436, 2924, 2854 and 1644 cm^{-1}. Ethanol production was fond only on yeast fermentation. Anti diabetic data reveals that the percentage of glycation inhibition by Streptomyces was 70 and by S. lactis was 45 with 76 and 26 % of amylase inhibition respectively. Streptomyces sp showed 75 % antioxidant and 66% anti-inflammatory response. The compound extracted from S. lactis also showed 64 % free radical scavenging and 51% anti-inflammatory response. The antiulcer study revealing 27, 62, 82% of ATPase inhibition was recorded respectively among yeast, Streptomyces sp and S. lactis. Further antibacterial effect toward E. coli, S. aureus and C. albicans was registered only by Streptomyces metabolite.

The GC–MS profile of extract of Streptomyces fermented fenugreek medium indicated 15 separated peaks. 1,2,3-propanetricarboxylic acid, 2-Hydroxy-, triethyl ester compounds were selected as ligands for the receptor ATPas and α-amylase in the molecular docking studies. Compound propanetricarboxylic acid was identified as the potential -amylase and ATPase antagonist based on hydrogen bonding affinities with the receptor and for drug-likeness. The molecular docking analyses discussed in this study may help in the creation of powerful inhibitors that will be useful in the management of ulcer and diabetes.

Keywords: Galactomannon, Fermentation, Antidiaetic, Antibacterial, ATPase.

Introduction

More than 80% of people worldwide, according to the WHO, rely on herbs made from plants. Currently, there is a greater interest than ever in finding antioxidant chemicals that are medicinally beneficial or possess minimal to no negative consequences for application on food and also in preventative medicine[11]. Fenugreek is known by the Latin name Trigonella and this translates to "little triangle" due to its yellowish-white colour triangle blooms. In Hindi, Urdu, Punjabi and Marathi, it is known as Methi[10]. Fenugreek seeds have therapeutic effects that include hypocholesterolemic, lactation aid, antimicrobial, anorexia gastric stimulant, anti-diabetic agent, galactagogue, hepatoprotective action and anti-cancer characteristics[7].

Fenugreek has nutritional, healing effects and is frequently used as a spice and in food preparations. Galactomannan, diosgenin, quercetin, trigonelline, 4-hydroxy isoleucine and phenolic mixtures are the principal chemical components[15]. Galactomannans are polysaccharides that have been extracted for use in industry from the seeds of the tara, guar, carob and fenugreek plants. After cellulose and starch, galactomannans (GM) are the most frequently utilised polysaccharides. The heteropolysaccharide fenugreek galactomannan is easily soluble in water, highly effective at lowering blood sugar levels and normalises surface activities in the small intestine.

The galactose:mannose (G:M) ratios in the galactomannans and the placement of galactopyranosyl units along the mannan chains are both varied. Compared to guar gum, galactomannan has a lesser capacity to store water[16]. (Fenugreek seeds mostly consist of galactomannan, which structurally consists of one galactose unit that is separated through the C-6 oxygen and a 1->4 betaD-mannosyl backbone. With an increase in galactose content, galactomannans become more soluble[3]. Higher molecular dock scores, steady molecular dynamics (MD) simulation results and decreased binding energy calculations all point to galactomannan as a possible treatment for type 2 diabetes and breast cancer[8]. Because it offers a variety of possible properties and traits, fenugreek galactomannan may one day be exploited as medicinal agents in biotechnology and bioprocess-based technologies[5].

Material and Methods

Sample Collection: Fenugreek seeds purchased commercially from local market were washed twice with distilled water and soaked for 2 days under cold conditions.

Extraction and Purification of Polysaccharide (Gm-PS): Independently guar and seeds of fenugreek were steeped in water for an entire night at 25 °C. Smashed entire seeds that had been washed, were placed in a five percent sodium chloride solution that had its pH altered by acetic acid for 24

hours at 50°C. By using muslin fabric, the polysaccharides were separated individually. The crude gums were extracted by adding IPA spirit, a 3:1 volume ratio of 10% isopropanol and 90% ethanol, while continuously stirring. This was accompanied by centrifugation at a rate of 6000 rpm for 7 minutes. Purified polysaccharides were dried in an oven at 50°C for 24 hours after being retrieved from the white precipitate that was produced by the seed and rinsed using acetone. For future use and assessment, the dehydrated polysaccharide was measured and kept in tightly sealed containers in an area that was dry and cool.

Fermentation medium: In 100 ml of water, 5 g of extracted galactomannon was dissolved and enriched with 0.1% yeast extract as nitrogen source. The medium was autoclaved and inoculated with 10% (V/V) fermenter strains. The entire flask was kept under incubation at 37°C under 150rpm for 72h. The total sugar was etimated by DNS method using glucose as standard. The total protein was estimated by Lowers method by using BAS as standard.

Ethanol detection: In a one-liter measuring container, 34 grams of potassium dichromate are dissolved in 500 ml of water distilled. In order to produce as little heat as possible, the volumetric flask is set in an ice container and 325 ml of concentrated H_2SO_4 are added drop by drop. The mixture is carefully blended, chilled and diluted with distilled water to a volume of 1 litre. 10 ml of the distilled sample and 10 ml of the dichromate reagent were added to the volumetric flask. After 20 minutes of incubation in a water bath at 60 °C, the mixture was chilled. The blank solution was made using distilled water and was used to plot the linearity curve with concentrations ranging from 1 to 10% ethanol (v/v). UV analyses a straight curve at 620 nm to figure out the amount of ethanol in the test sample.

DPPH radical scavenging assay: The DPPH radical scavenging assay was used to evaluate the extracts' capacity to scavenge free radicals. The ability of extractives to donate hydrogen atoms was demonstrated through the decolorization of a methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The ability of the samples to scavenge the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical was used to assess their antioxidant properties. In chemical studies, the steady free radical DPPH is typically used to identify the action of radical scavengers. It has an unusual electron in its molecular makeup. As antioxidants are present, DPPH turns a methanol solvent violet or purple before fading to yellow hues.

2.4 mL of a 0.1 mM DPPH in methanol solution was produced and mixed with 1.6 mL of GmPS in methanol at various concentrations. After fully vortexing, the reaction mixture was kept at RT for 30 minutes in the dark. Ascorbic acid was utilised as the standard to quantify the mixture's absorbance at 517 nm. The following equation was used to compute the percentage of DPPH radical scavenging activity:

% of DPPH radical scavenging activity={(C− T)/C}×100

Amylase inhibition assay iodine test: The pancreatic alpha amylase enzyme, 500 l of 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride) and extracts at concentrations of 25–100 g/ml (w/v) were added to the assay mixture, which was then incubated at 37°C for 10 min. Following pre-incubation, each tube received 250 l of a 1% (v/v) starch solution in the aforementioned buffer, which was then incubated at 37°C for 15 min. The process was stopped by adding iodine solution, diluting it and placing the mixture in a boiling water bath for 5 minutes. The absorbance was then measured at 540 nm. There was no substance present in the control reaction, which represented 100% enzyme activity.

% of Relative enzyme activity = (enzyme activity of test/enzyme activity of control) *100

Optimization of the Protein Aggregation Process: 200 M HSA was newly produced as a stock solution by dissolving it in a 20 mMTris-HCl buffer (pH-7.0). In tests involving HSA aggregation, the base solution was diluted with an equivalent volume of 50% (v/v) ethanol before being incubated at 37 °C for a day while being shaken at 200 rpm in the absence or presence (abbreviated as HSA + G-fibril) of 0.1 M d-glucose with a final protein concentration of 40 M. The resulting substance test investigations were only done with HSA + G-fibril since it was thought that aggregation would be larger with 0.1 M d-glucose.

Inhibition of Protein Aggregation in presence of metabolite: A characteristic of proteins' polypeptide chains known as amyloid-like aggregation is what causes many pathogenic illnesses and diseases like Huntington's, Parkinson's, Alzheimer's and diabetes mellitus type II. In order to evaluate the compound's ability to prevent aggregation, 100 L of sample was added to the protein solution after one day and the solutions were then incubated for an additional day under similar circumstances.

By measuring absorbance at 530 nm, the amount of congo red binding to amyloid aggregation in the samples was calculated. As a result, 100 l of Congo red (100 Mm solution in phosphate-buffered saline, 10% ethanol) and 0.5 ml of glycated albumin were combined and incubated for 20 minutes at room temperature. At 530 nm, the absorbance was measured. The percentage of inhibition was determined by the formula:

% of inhibition = [1 - (A0 - A1)/A0] × 100

Inhibition of protein denaturation method: The test extract was present in the reaction mixture in varying quantities, along with 1% BSA (aqueous solution). To change the pH of the reaction mixture, 1 N HCl was utilised. The samples were heated for 20 minutes at 37 °C, followed by 20 minutes at 57 °C, before cooling. At 660 nm, the

samples' turbidity was determined. The percentage inhibition of protein denaturation was calculated:

% of inhibition = (AC of control–A of test sample) × 100/AC)

Determination of H^+ -K^+ ATPase activity: Reyes-Chilpa method was modified to calculate the H^+K^+ ATPase activity of the extract. The parietal cell extract was preincubated with various concentrations of the test materials and standards (chloroform and methanol extract) for 30 minutes. Following the addition of 2mM adenosine-5'-triphosphate (ATP), 20mM tris-HCl (pH 7.4), 2mM magnesium chloride ($MgCl_2$) and 2mM potassium chloride (KCl), the reaction began by the addition of 2mM ATP and then incubated for 30 minutes at 27°C before being stopped with the addition of 10% trichloroacetic acid. At 2000 rpm for 10 minutes, centrifugation was finally completed. Using spectral analysis at 640 nm, the amount of inorganic phosphorous produced from adenosine 5'-triphosphate (ATP) was measured.

Antibacterial activity by Disc diffusion method: Three Gram-positive bacteria species including *S. aureus*, *E.coli* and *C.albicans* were selected for this study. Antimicrobial property of the mushroom extract has been established by the disc diffusion method. Each microbial isolate's overnight culture was emulsified with nutrient broth to a turbidity of 0.5 McFarland (105cfu/mL). A 100 L aliquot of test culture was equally distributed over the surface of the solidified agar to ascertain the extracts' antibacterial effectiveness. Sterile disc loaded with extract was placed over agar surface. Antibiotics such as ofloxacin is used for positive controls for determining inhibition zone. The formerly loaded plates with the appropriate extracts and test microorganisms were cultured for one day at 37°C for bacteria and 48°C for parasites. The inhibitory zone was measured in millimetres following incubation.

GCMS Analysis: On a GC clarus 500 Perkin Elmer system with an AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS), GC-MS analysis was performed. Helium was used as the carrier gas at a continuous flow rate of 1 ml/min, with an injection volume of 0.5 EI (split ratio: 10:1) and temperatures of 250 °C for the injector and 280 °C for the ion source. The oven temperature was set to start at 110°C (isothermal for 2 minutes), rising at a rate of 10°C/min to 200°C and then decreasing at a rate of 5°C/min to 280°C and then remaining at that temperature for 9 minutes.

Molecular docking: Compounds from extract matched with Pubchem and Smiles were retrieved for ADME. The receptor macromolecule for the gastric H+/K+-ATPase (PDB code: 2YN9) and pancreatic amylas (2QV4) makeup was downloaded from the Protein Data Bank at http://www.rcsb.org/pdb and converted to.pdb format. To locate the residues in the receptor, the cavity must be identified. The offline Autodock programme was downloaded from https://www.cgl.ucsf.edu/chimera/ and was used to determine the cavity. It was possible to isolate receptor macromolecules from ligands, solvents and nonstandard residues. The Discovery Studio 4.0 programme was used to separate macromolecules from unneeded molecules. The separation's output was saved in.pdb format. The PubChem website (http://pubchem.ncbi.nlm.nih.gov) provides a download for the ligand structure of the generated molecule.

Using Autodock Tools, optimised by adjusting the number of action torsion and changing the format to.pdbqt, the docking file preparation was carried out. The grid box was set up to know the location of the binding site when the receptor preparation was being done by adding hydrogen polar and the format was changed to.pdbqt. This file was saved on the computer's C: disc in a single folder. Utilising AutodockVina, the molecular docking process was carried out. AutodockVina was run using the command prompt programme and the ligands and receptor that were already on drive C: copied and converted in the form of notepad were stored with the name conf.txt. The findings of the binding docking study were examined using the output in log.txt format, which provided the free energy value of the binding docking results.

Results and Discussion

Galactomannan from fenugreek seed is extracted by carring out the selective extraction using isopropanol and sodium chloride mediated ultrasonification. The seeds are fractionated and polymer is separated and the net yield of polysaccharide is 12.8 mg/G. Further the Fehling test on extracted polysaccharide shows negative reveals absence of glucose residues and nature of polysaccharide confirmed by TLC by spraying acid alcohol shows black spot with Rf 0.72. The functional group of the isolated galactomannan was studied by FT-IR spectrum. The FT-IR spectrum of EHI showed characteristic band at 3436 cm^{-1}. It can be attributed to ν(OH) of poly alcoholic OH group. Two bands appear at 2924 and 2854 cm^{-1} due to aromatic and aliphatic CH stretching frequency νCH(OH) and ν(CH2) respectively (Fig. 1).

^{1}H NMR spectrum of the compound EHI was recorded in DMSO-d6 (500 MHz) and the ^{1}H-NMR spectrum of isolated galactomannan is shown in fig. 2. The galactomannanpolyer is consisting of two monomeric six-member ring system, one is galactose (α-D-galactopyranose) ring and another one is mannose ring (β-D-mannopyranoses).

The existence of terminal -D-galactopyranose and -D-mannopyranoses in the main chain is compatible with two chemical shifts of the anomeric signals of 1H-NMR at 4.99 ppm and 4.69 ppm correspondingly. The 1,6-linked -D-galactopyranose residue's ring proton signals may overlap with those of the terminal sample.

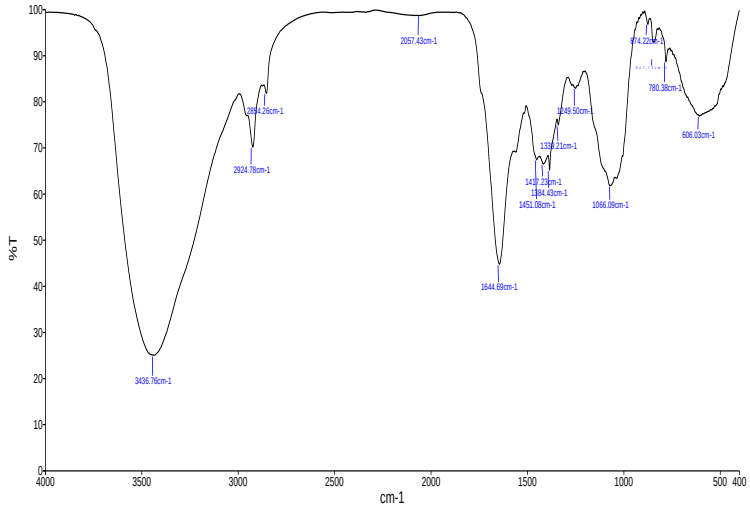

Figure 1: FTIR analysis of galactomannon

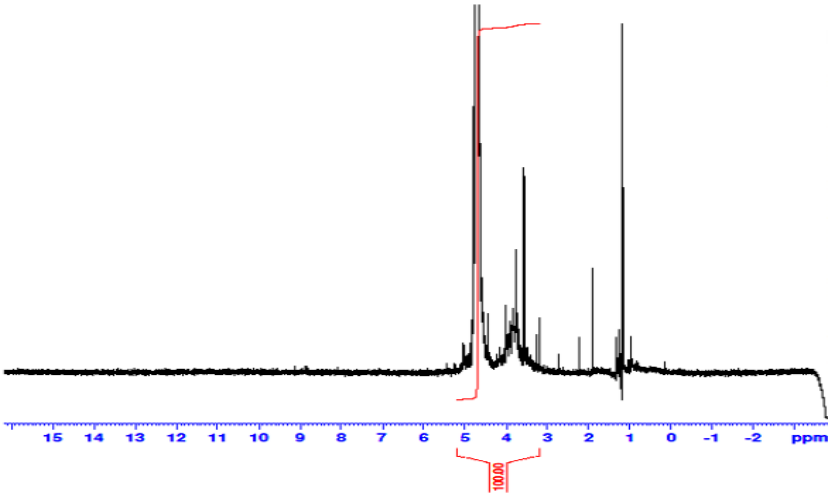

Figure 2: ^{1}H NMR of galactomannon extracted

Table 1
End product analysis of fermented medium

S.N.	Fermentation by	pH	Ethanol %	Sugar mg/L
1	*Saccharomyces cereviciae*	6	42	4.1
2	*Streptomyces sp*	7	0	2.3
3	*Streptococcus lactis*	5	13	5.2

Fermentation analysis: All the three strains were capable to grow on fermentation medium and the pH of fermentation medium during end of the day was acidic by *S.lactis* and 6-7 in case of yeast and mould. Table 1 showed the data of fermented medium component analysis. Colorimetric quantification was performed for ethanol followed by distillation. At the end of fermentation, no alcohol was produced by both bacteria but 42% produced by yeast. During fermentation, the breakdown of galactomanaon leads to produce reducing sugars estimated at 4.1 mg in yeast fermentation, 2.3 mg in *Streptomyces sp* and 5.2 mg by *S.lactis*. The total protein in fermentation medium was 65 mg/L in yeast, 89 mg/L in *Streptomyces sp* and 34 mg/L by *S.lactis* fermentation denoting that the organisms produce extracellular enzymes during the growth (Fig. 3).

Antioxidant assay: At various concentrations, the property against oxidant towards DPPH and metal chelation was investigated. In investigations on antioxidants, the DPPH radical-scavenging technique is a frequently used method. The hydrogen-donating properties of extracted metabolites, which convert the stable violet DPPH radical to the yellow DPPH-H, may be the cause of their antioxidant actions on DPPH radical scavenging. The percentages of sample radical inhibition were 12, 75 and 64 %, respectively, for the yeast *Streptomyces sp and S.lactis*, can be used to infer the

potential of free radical scavenging (Table 2). *Streptomyces sp* was the best in scavenge free radicals out of the three and it was discovered to be a productive antioxidant. The typical ascorbic acid had a 91% antioxidant effect.

Additionally, fenugreek seed constituents have demonstrated certain molecules with antioxidant and anti-inflammatory properties. Consuming probiotics demonstrates that variant-specific living microorganism can offer antioxidant property and prevent oxidative damage, which is one of the numerous positive health impacts of probiotics.

Anti-inflammatory: The *in vitro* anti-inflammatory experiment, *Saccharomyces cereviciae* demonstrated minimum of 28 and maximum by S*treptomyces sp* 61% activity for denaturation inhibition on albumin protein which can be used to make nutraceuticals product. The extracted metabolite from *S. lactis* showed 51% activity for denaturing albumin protein, which is less superior than paracetamol at 62% activity. In comparison to *S.lactis and* yeast metabolites Streptomces demonstrated promising anti-inflammatory response.

According to research by Amdekar et al[1], *L. Acidophilus* and *L. Casei* demonstrated extremely consistent property against inflammatory and significantly reduced the thickness of rats' paws. Ganji-Arjenaki and Rafieian-Kopaei's[4] research also revealed that numerous lactobacillus strains have the ability to treat inflammatory bowel disease. According to Shashikant et al[12], mushroom extract has demonstrated effective anti-inflammatory properties.

Anti Ulcer Activity: Colorimetric analysis was used to measure the release of phosphate to measure the antiulcer effect. Calculations based on standards were made to determine how much phosphate from treatments and control groups was emitted. Figure 4 shows the calculated phosphate quantity and the percentage of ATPase blocking respectively. Phosphate release in the saline control H+/K+-ATPase was measured at 47.54 (mol Pi/mg prot/hour) considered as 100% enzyme activity. In regard to the saline category, the concentration for posphate from the *S.lactis* category was much lower recorded as 8.19 (mol Pi/mg prot/hour), indicating an 82% inhibition. In the course of treatment group by metabolite of yeast, ATPase expression was 27% suppressed. Treatment with the *Streptomyces sp*metabolite in group 3 resulted in a 62% ATPase inhibition and a much lower 80% omeprazole inhibition.

Antimicrobial activity of fermented compound: The antibacterial assays using disc diffusion method showed the efficacy for the *Strptomyces* metabolites to halt the development of the bacterial pathogens *Escherichia coli*, *Staphylococcus aureus* and *C.albicans*. The antibacterial outcomes demonstrated a strong and distinct inhibition of all of the examined bacterial pathogens' development. *E.coli* showed maximum inhibitory activity 17mm followed by 16 mm by *S.aureus* and moderately by *C.albicans*(14 mm). As compared to standard, ofloxacin and amphotericin showed similar bacterial inhibition.

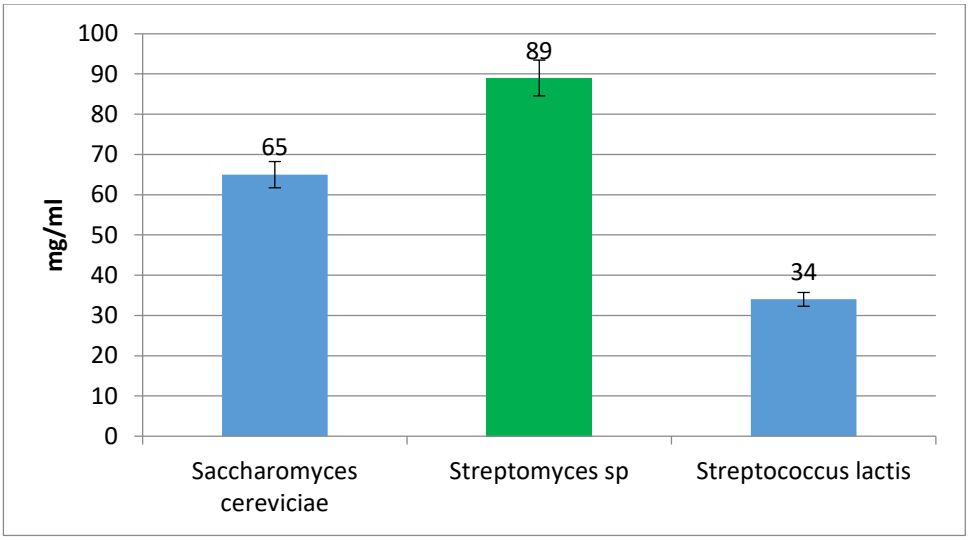

Figure 3: Estimated total protein fermented medium

Table 2
Anti oxidant activity by DPPH method

S.N.	Sample	% of radical scavenging
1	standard	91
2	*SCF*	12
3	*STF*	75
4	*SLF*	64

Table 3
Anti inflammatory activity by protein denaturation

S.N.	Test Sample	% of inhibition
1.	Paracetamol	62
2.	*Saccharomyces cereviciae*	28
3.	*Streptomyces sp*	66
4.	*Streptococcus lactis*	51

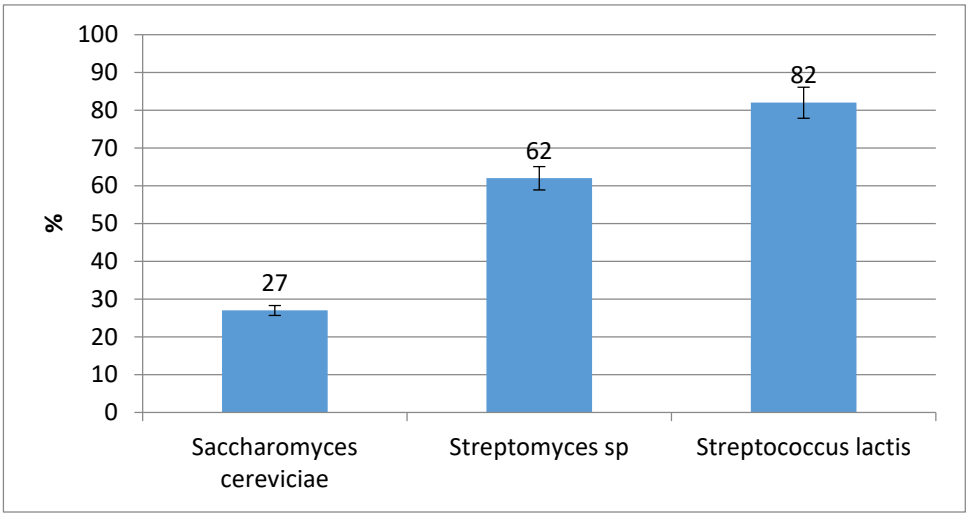

Figure 4: Percentage of ATPase inhibition

Table 4
Anti bacterial activity by disc diffusion method

Sample	*E.coli*	*S.aureus*	*C.albicans*
S.Lactis	0mm	0mm	0mm
Yeast	0mm	0mm	0mm
Strptomyces	17mm	16mm	14mm
Ofloxacin	18mm	18mm	22mm

Characterization and ADME of compounds: The GCMS spectrum of active compound given in figure 5 reveals presence of 15 peaks eluted between the retention time 6.495 to 39.075. The NIST library searched compound are listed in table 5 along with retention time. The first eluted compound was lactic acid(0.32%) and the last eluted one was 2-[(trimethylsilyl)ethynyl)heptamethyltrisilane].

GC-MS spectra showed 15 peaks and the major compounds 1,2,3-propanetricarboxylic acid, 2-hydroxy-, triethyl ester (72%) followed by 9.26% of ethanone, 2-(4-chlorophenyl)-1-cyclohexyl-2-(1-piperidinyl), 2.39% ethyl 1,2,3,4,5,6,7,8-octahydro-8-oxo-1-naphthalenecarboxylate, 2.91% Furo [2,3-c] pyridine, 2,3-dihydro-2,7-dimethyl, 1.8% 3-Butoxy-1, 1, 1, 7, 7, 7-hexamethyl-3,5,5-tris (trimethyl siloxy) tetrasiloxane, 3.39% (trimethylsilyl) ethynyl) heptamethyltrisilane and 2.04% 4-tert-Octylphenol, derivatives. Out of 15, 1,2,3-Propanetricarboxylic acid, 2-hydroxy-, trihexyl ester having molecular weight 444 g/mol obeyed Lipinski rule with 0 violation, moderately soluble, number of H-bond acceptors 7 and donor is 1 with maximum GI absorption and no BBB permeant and CYP1A2 inhibitor. The compound with drug property was confirmed by ADME and was selected for docking.

Anti ulcer docking: The homology model of human gastric H+/K+-ATPase has been produced based on the template provided by HUMAN gastric H+/K+-ATPase (PDB code: 2YN9). After molecular mechanics optimization, induced-fit docking simulation between gastric H+/K+-ATPase and Propanetricarboxylic acid has significantly inhibition activity of H+/K+-ATPase. The results of ligand docking showed that the binding region contains the amino acid residues LEU, LYS and THR and the docking score was -6 and -5.9 kcal/mol.

In addition to the strong hydrogen bond, the ligand also produced hydrophobic interaction (GLY, LEUALA) and weak hydrogen bond formation with LEU and THR. The inhibition of ATPase is an important strategy to control acidity of small intestine. The standard pantoprazole showed formation of hydrogen bond with docking score of -7.7 kcal.

Amylase inhibition: Comparative binding poses and interactions of ligands, namely propanetricarboxylic acid with the enzyme α-amy lase. It can be noted that these compounds, three residues GLN, ARG and PHE were found to show three hydrogen-bonded interactions, one ligand (O1, O3 and O5) showed four hydrogen-bonded interactions,

TYRGLNPHE showed hydrophobic interaction with C1, C3, C10atoms and ASP-C19 showed weak hydrogen bond formation. The interaction of ligand with amylase showed strong hydrogen bond and the predicted docking score was -5.7 (Table 7). The docking score of acabose standard was -8.1kcal by interacting with LEU, PHE, ARG, ASP and THR. The biological activity propanetricarboxylic acid by H-bond and hydrophobic interactions was reported by Madeswaran et al[6].

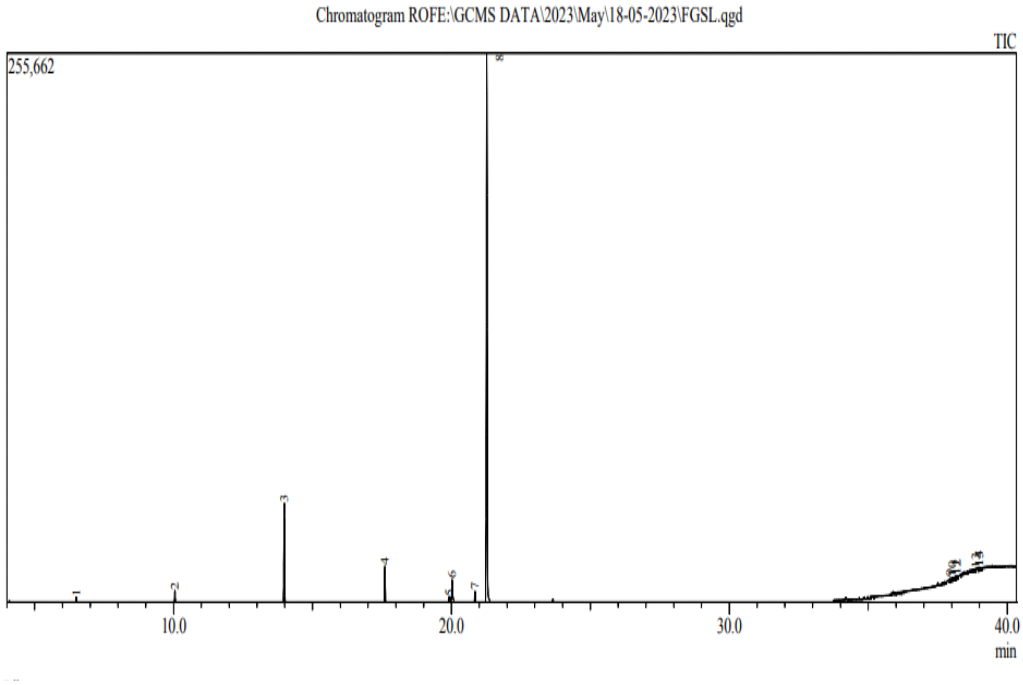

Figure 5: GCMS spectrum of extracted metabolite

Table 5
NIST library matched GCMS

Peak	Retention time	Start time	End Time	Area %	Name
1	6.495	6.48	6.51	0.32	lactic acid
2	10.047	10.025	10.07	1.19	BENZOIC ACID, 2,6-BIS(TRIMETHYLSILOXY)-, TRIMETHYLSILYL ESTER
3	13.983	13.95	14.02	12.13	Cyclohexasiloxane, dodecamethyl-
4	17.604	17.575	17.635	4.09	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane
5	19.917	19.89	19.955	1.11	Furo[2,3-c]pyridine, 2,3-dihydro-2,7-dimethyl-
6	20.025	19.985	20.095	3.55	ETHYL 1,2,3,4,5,6,7,8-OCTAHYDRO-8-OXO-1-NAPHTHALENECARBOXYLATE
7	20.849	20.825	20.87	1.31	TRI-O-TRIMETHYLSILYL, N-PENTAFLUOROPROPIONYL DERIVATIVE OF TERBUTALINE
8	21.27	21.225	21.38	72.15	1,2,3-PROPANETRICARBOXYLIC ACID, 2-HYDROXY-, TRIETHYL ESTER
9	37.94	37.915	37.985	0.68	ETHANONE, 2-(4-CHLOROPHENYL)-1-CYCLOHEXYL-2-(1-PIPERIDINYL)-
10	38.025	38.015	38.05	0.3	ETHANONE, 2-(4-CHLOROPHENYL)-1-CYCLOHEXYL-2-(1-PIPERIDINYL)-
11	38.139	38.11	38.155	0.48	ETHANONE, 2-(4-CHLOROPHENYL)-1-CYCLOHEXYL-2-(1-PIPERIDINYL)-
12	38.19	38.155	38.235	0.91	ETHANONE, 2-(4-CHLOROPHENYL)-1-CYCLOHEXYL-2-(1-PIPERIDINYL)-
13	38.86	38.845	38.875	0.35	1,3-DIPHENYL-1-((TRIMETHYLSILYL)OXY)-1(E)-HEPTENE
14	38.971	38.945	38.985	0.52	4-tert-Octylphenol, TMS derivative
15	39	38.985	39.075	0.93	2-((TRIMETHYLSILYL)ETHYNYL)HEPTAMETHYLTRISILANE

Table 6
Hydrogen bond formation and docking score with ATPase

Ligand atom	Receptor	Score affinity (kcal/mol)
O3	Y863(A) OLEU	-6
O5	S871(A) OGTHR	
O3	Y48(B) OH LYS	-5.9
O7	Y48(B) OH	

Table 7
Hydrogen bond formation and docking score with amylase

Ligand atom	Receptor	Score affinity (kcal/mol)
O5	Y62(A) OGLN	-5.7
O3	D300(A) OD2 ARG	
O1	T163(A) OG1PHE	
O3	D300(A) OD2 ARG	

Conclusion

One such promising source that provides a galactomannan with a high G:M ratio is fenugreek. Galactomannan, a prebiotic fibre made from fenugreek seeds, is also fermented by the probiotic bacteria *Streptococcus lactis, S. cerevisiae and Streptomyces.* The three fermentation's isolated metabolites had strong anti-inflammatory and antioxidant properties. However, there is strong evidence of antioxidant, antibacterial and anti-diabetic activity. The compound was a potent enzyme inhibitor and can be used as an anti-diabetic and anti-ulcer prophylaxis.

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