ORIGINAL ARTICLE

Analysis of Little Millets by High-resolution Mass Spectroscopy Focusing the Components of Little Millets Effective in the Management of Diabetes Mellitus

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Abstract

Diabetes mellitus, a metabolic disorder, poses significant health risks. For diabetics, weight loss, exercise, oral glucose-lowering medicines, effective diet can help them to achieve adequate glycemic control. Millets contain many types of antioxidant elements such as phenolic flavonoids, which are important in controlling glucose levels in the body. Taking the millet diet physiologically helps to control the gluconeogenesis process. Little Millet is possessing rich antioxidant properties which helps to prevent metabolic and lifestyle disorders such as obesity, diabetes, and retinopathy. Through UHPLC-Q-TOF-MS/MS system, major antidiabetic compounds are revealed such as hispidin, caffeine, aicar, ketotifen, maltitol, tacrolimus, and pipemidic acid. These compounds indirectly control body metabolism to cope-up with diabetes mellitus.

Key words: Ayurveda, diabetes mellitus, diet, high-resolution mass spectroscopy, Little Millets

INTRODUCTION

Diabetes mellitus is a term used to describe a collection of metabolic disorders with various etiologies that are primarily characterized by the presence of chronic hyperglycemia. Chronic hyperglycemia is a symptom of the metabolic disorder diabetes mellitus (DM), which may also include etiology conditions that affect insulin secretion and/or action.[1] In 2017, there were 425 million diabetics worldwide, and by 2045, there are projected to be 693 million.[2] According to reports, India has the second-highest number of persons with type 2 diabetes mellitus (T2DM) in the world in 2017. The largest percentage of people with type 2 diabetes.[3] Many scientists who study the topic have discussed the contribution that dietary changes and modifications to lifestyles make to the reduction of disease, and the majority of them concur that these two aspects assist lower the elements that impact the start of the disease.[4] Along with healthy diet, the polyherbal formulated Madhumehantak churna like Ayurveda medicines is also been used for controlling the hyperglycemic effect.[5]

To maintain human health holistically, the quality of diet should be taken into account so that complex problems such as malnutrition can be easily diagnosed.[6] Regarding nutritional value, millets are equivalent to other cereals. Millets perform better in terms of dietary fiber, minerals, and trace elements such as iron, zinc, calcium, phosphorus, and potassium and also contain vitamin B, vital amino acids, and phytochemicals than other cereals such as rice and wheat. Millets are powerful sources of nutrients and phytochemicals, can tolerate extreme climatic conditions, and help people achieve food and nutritional security.[7] Minor millets have drawn interest in recent years because of their nutritional value, capacity to grow in challenging environments, and ability to withstand harsh weather.[7] Millet comes in various varieties, including Little Millet, Proso Millet, Finger Millet, and others. The

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following names for several millets are used in Ayurveda with the following Sanskrit, namely Kangu, Shyamaka, Nivara, Koradusa, and Makustaka. Out of these, Shyamaka (Little Millet) is one of the varieties. Little Millet is called Kutki in Hindi, Samai in Tamil, and Samalu in Telugu. It is grown as a traditional crop in India; however, the Little Millets are commonly grown in south India, i.e., Andhra Pradesh and Karnataka states.

Little Millet is nutritionally healthier, more versatile than cereals and suitable for diet[7] and is rich in antioxidant properties. It is well known among those who are having health awareness because these tiny grains, i.e., Little Millets are possessing with many micronutrients which are essential for maintaining health. Little Millets are a great complement to the diet for diabetes patients because they are free of gluten and do not create acids. Its great nutritional value also makes it essential to include in a balanced diet based on this information; in the present study, it is tried to reveal the therapeutic utility of Little Millets for diabetes. As per the previous study, LC-MS has been widely used for plant phytochemical (Novel compound) analysis. Here, the present work focuses on natural compounds (e.g., alkaloids, flavonoids, phenolics, carbohydrates, proteins, fibers, and fatty acids) of Little Millets (Panicum sumatrense) grains that have been studied by the LC-MS method. Little Millets by the name of shyamaka have been mentioned in the Ayurveda System of Medicine as a diet for the management of diabetes mellitus and obesity, etc., disease conditions. Based on these references in the present study, step has been made to find the natural compounds present in Little Millets and their therapeutic uses for the management of diabetes mellitus.

MATERIALS AND METHODS

Collection and authentication of Little Millets.

The raw material of Little Millet for the analysis by liquid chromatography-mass spectrometry was collected from the Eco ventures, 3-196, Industrial Estate, Kummaravandla Palli, Kadiri, Anantapur, Karnataka. The same Little Millets is later authenticated in the Department of Dravyaguna (related to Ayurvedic Pharmacognosy) and Accession No - DG/22-23/562.

About instrument

The high-resolution accurate mass spectrometry system instrument was used with the model name orbitrap eclipse tribrid mass spectrometer developed by Thermo Fisher Scientific. For small molecules, Dionex UltiMate 3000 RSUHPLC System was used different solvent compound was used for phytochemical analysis.

Materials

Little Millet, methanol, distilled water, Whatman filter paper (100 mm pore size), Rotatory evaporator, Dry oven, Petri plate, and Measuring cylinder, The High-Resolution Accurate Mass Spectrometry System instrument was used with the Model name Orbitrap Eclipse Tribrid Mass Spectrometer developed by Thermo Fischer Scientific. For small molecules, Dionex UltiMate 3000 RSUHPLC System used different solvent compounds for phytochemical analysis.

For grain extract

The unprocessed 500 g of Little Millets grains have been made into coarse powder form for research analysis. The 40 g of Little Millet powder was taken and mixed with 400 ml of methanol in a conical flask, at every 10 min of the interval, the flask was shaken, and the mixture solution was rested for 2 days. After 2 days, the supernatant was filtered with Whatman filter paper (100 mm pore size) and collected in a beaker, 350 ml of methanolic extract grain solution was poured into a rota-evaporator, the Rota-evaporator set at a boiling temp is 450°C, and chiller temp 50°C, the extract solution was evaporated till 50 ml extract remains in the flask. The 50 ml grain extract was collected in a conical flask. Then, 50 ml extract was put on a Petri plate and evaporated at 60°C in the oven. The dried extract was formed in Petri plates and stored for analysis.

For HRMS analysis

The dried sample was collected in an Eppendorf tube and 1 ml methanol was added to a tube. This methanol sample was passed from a syringe filter (0.02 mm) and collected in an Eppendorf tube. The sample collected in the Eppendorf tube was used for HRMS analysis.

Solvent preparation of HPLC column

Solvent A: 100% water + 0.1% formic acid, solvent B: 80% acetonitrile + 0.1% formic acid, solvent C: 100% methanol + 0.1% formic acid. The column detail is the hypersil GOLD™ C18 selectivity HPLC column, particle size 1.9µm with diameter 2.1 mm, length 100 mm. All the analyses were performed by the default parameters of "compound discoverer 3.2.0.421" using online databases.

UHPLC-Q-TOF-MS/MS was used to examine the Panicum Sumatrense grain metabolite profile. Thermo compound discoverer 3.3.2.31 was used for all of the analysis, with default settings and online databases. Untargeted Metabolomics Workflow Using Molecular Networks, Online Databases, and mzLogic. The chemicals were identified based on fragment patterns produced by ChemSpider (formula or precise mass) and mzCloud (ddMS2).

RESULTS

Identified metabolites by UHPLC-MS

The total ions chromatogram and extracted ion chromatograms (EICs) for some of the significantly identified different antidiabetic drugs together with the base peak chromatogram of the sample acquired by negative and positive ion mode along their molecular structure and ion chromatogram [Figures 1-3].

A summary of all the compounds that have been discovered as antidiabetic which includes the chemical name, constituent group, molecular formula, computed molecular weight (m/z), mass error (ppm), retention period, and peak areas in the negative (NEG) and positive (POS) ion modes. The list of antidiabetic compounds is hispidin, caffeine, aicar, ketotifen, maltitol, tacrolimus, and pipemidic acid [Table 1].

Literature reports have documented that these compounds show antidiabetic effects. Hispidin may be useful in defending MIN6N -cells in diabetes against ROS damage. Hispidin greatly reduced intracellular ROS and prevented caspase-3 and apoptosis that was brought on by hydrogen peroxide.[8] By intercalating between the two aromatic rings of Phe285 and Tyr613, caffeine binds at the purine inhibitor site and stabilizes the position of the 280s loop in the T state conformation.[9] AICAR reduces IL-6 and IL-8 synthesis in skeletal muscle cells and human adipose tissue. The increased insulin sensitivity brought on by AICAR may be related to lower cytokine production.[10] The ER stress sensor inositol-requiring enzyme 1 (IRE1)'s autophosphorylation was blocked by AICAR, but its endoribonuclease activity was stimulated. The anti-inflammatory and anti-diabetic actions of AICAR are influenced by ER stress responses that are inhibited by AMPK-independent mechanisms.[11] HDL and SOD levels dramatically increased, while the Ketotifen group's levels declined more noticeably.[12] Maltitol boosted glucose uptake in isolated rat muscle and decreased glucose absorption in isolated rat jejunum when insulin was present but not the other way around.[13] Post-transplantation diabetes can be reversed or at least improved by switching from tacrolimus to cyclosporine, which is a straightforward, safe, and effective treatment.[14] Dose-dependent pipemidic acid slightly raised insulin release by blocking ATP-sensitive K+ channels, which triggered hypoglycemia.[15]

DISCUSSION

Diabetes is a metabolic disorder, having significant health risks. For diabetes patient, apart from oral glucose-lowering medicines, the effective diet can help them to achieve adequate glycemic control. Millets contain many types of antioxidant elements, such as phenolic flavonoids, which are important in maintaining glucose levels in the body. Taking the millet diet physiologically helps to control the gluconeogenesis process. Little Millet is rich in antioxidant properties and helps to prevent metabolic and lifestyle disorders like diabetes. Therefore, in the present study to find through UHPLC-Q-TOF-MS/MS system, major antidiabetic compounds are studied and it is found that these compounds indirectly control body metabolism to cope-up with diabetes mellitus.

Dietary modifications have been successful in reducing the risk of pre-DM progression to T2DM in addition to medical therapies.[16] Minor millets have generated interest recently due to their nutritional content, ability to thrive in difficult conditions, and resistance to extreme weather.[7] Millets are more nutrient dense than wheat and rice because they are high in proteins, dietary fibers, iron, zinc, calcium, phosphorus, potassium, vitamin B, and essential amino acids, Little Millet is one of these. Little Millet is more adaptable than grains, has better nutritional value, and is ideal for diets.[7] Little Millets are an excellent addition to the diet of anyone who practices yoga, works out, does cardio, etc. because they are free of gluten and do not create acids. Its

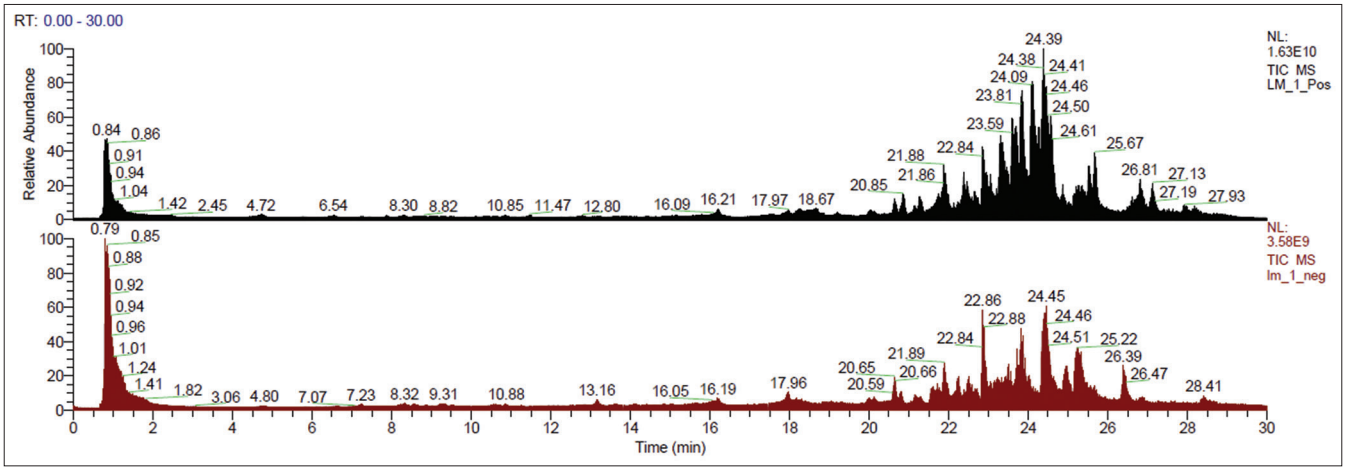

Figure 1: Total ion chromatogram obtained by UHPLC-TOF-MS. Figure 1 shows total ion chromatogram obtained by UHPLC-TOF-MS analysis of the Panicum sumatrense grain sample in positive and negative ion mode

Table 1: Major compounds identified by UHPLC-TOF-MS; major compounds identified by UHPLC-TOF-MS analysis through positive and negative ion mode, showing: RT (retention time) values, calculated air mass error ppm), the peak area of Panicum sumatrense grain sample in negative (NEG) and positive (POS) ion mode

Chemical compound	Formula	Cal MW	RT (min)	Delta mass (ppm)	Area NEG	Area POS
Hispidin	$C_{13}H_{10}O_5$	246.05332	1.332	2.04	40097564.75	_
Caffeine	$C_8H_{10}N_4O_2$	194.08113	1.382	3.88	13421717.27	_
Aicar	$C_9H_{14}N_4O_5$	258.09612	1.418	-1.17	_	3275770.398
Ketotifen	$C_{19}H_{19}NOS$	309.11794	3.169	-2.58	_	40502164.98
Maltitol	$C_{12}H_{24}O_{11}$	344.13341	12.669	4.5	_	9979629.812
Tacrolimus	$C_{44}H_{69}NO_{12}$	803.48469	23.814	3.38	7431738.385	_
Pipemidic Acid	$C_{14}H_{17}N_5O_3$	303.1339	10.298	2.5	_	22682397.13

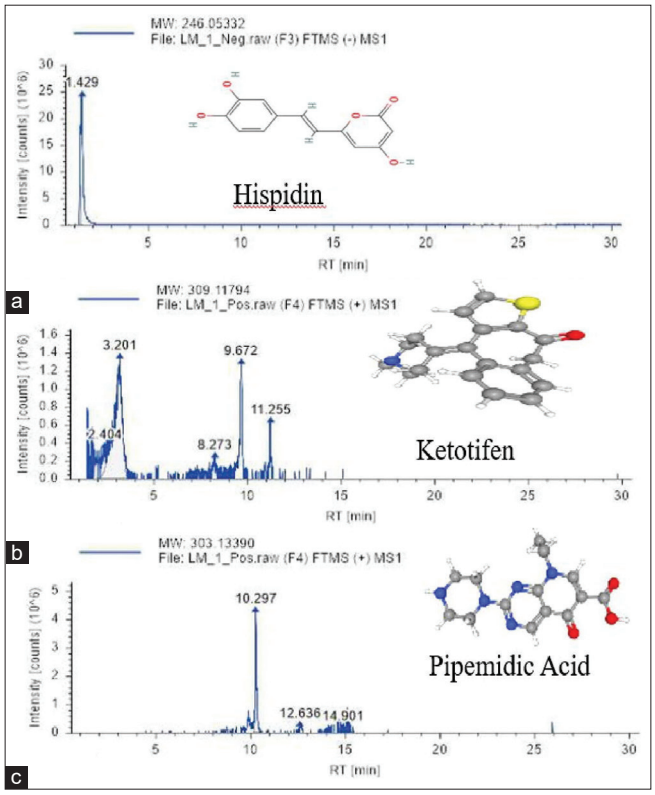

Figure 2: Separate ion chromatogram of each antidiabetic compound in positive and negative ion mode along with their molecular structure (a) ions chromatogram of Hispidin; (b) Ion's chromatogram of Ketotifen; (c) Ion's chromatogram of pipemidic acid. The 2D and 3D images of compounds used in ions chromatogram in (a-c) are taken from PubChem source

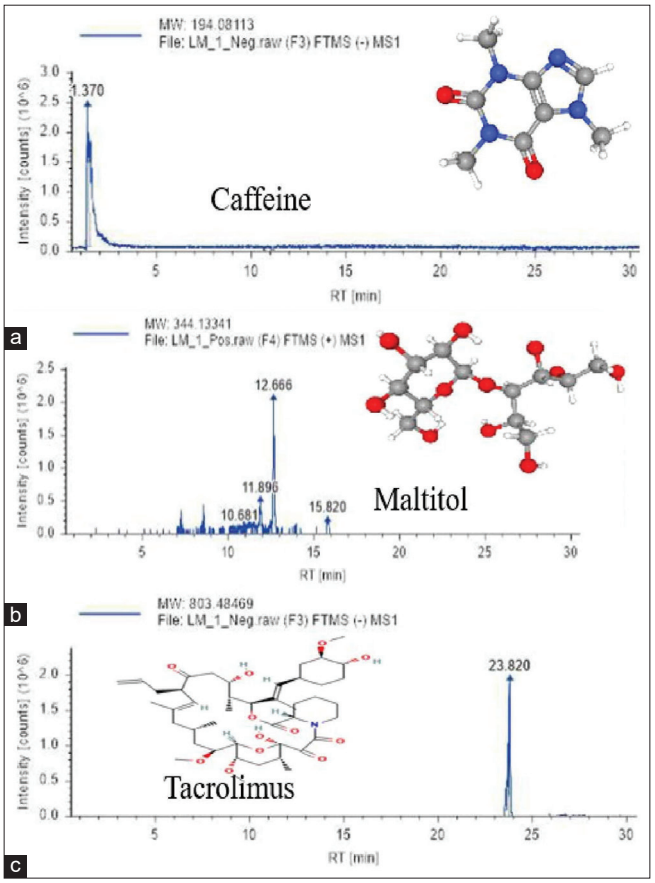

Figure 3: Separate ion chromatogram of each antidiabetic compound in positive and negative ion mode along with their molecular structure (a) ions chromatogram of Caffeine; (b) Ion's chromatogram of Maltitol; (c) Ion's chromatogram of Tacrolimus. The 2D and 3D images of compounds used in ions chromatogram in (a-c) are taken from PubChem source

great nutritional value also makes it essential to include in a balanced diet. Through HRMS analysis, we found major antidiabetic compounds which are listed as hispidin, caffeine, aicar, ketotifen, maltitol, tacrolimus, and pipemidic acid [Figure 4]. Hispidin significantly reduced the damage caused by intracellular ROS, as elevated ROS can disrupt cellular macromolecules and result in genetic instability. AICAR was implemented to improve insulin sensitivity. When insulin was present, maltitol increased glucose uptake and decreased glucose absorption in isolated rat jejunum. By inhibiting ATP-sensitive K+ channels, pipemidic acid marginally increased insulin release, which led to hypoglycemia. However, this study is having its limitations since these findings are until and unless clinically proven, it is difficult to make statement as Little Millets are more appropriate diet for diabetes mellitus patient.

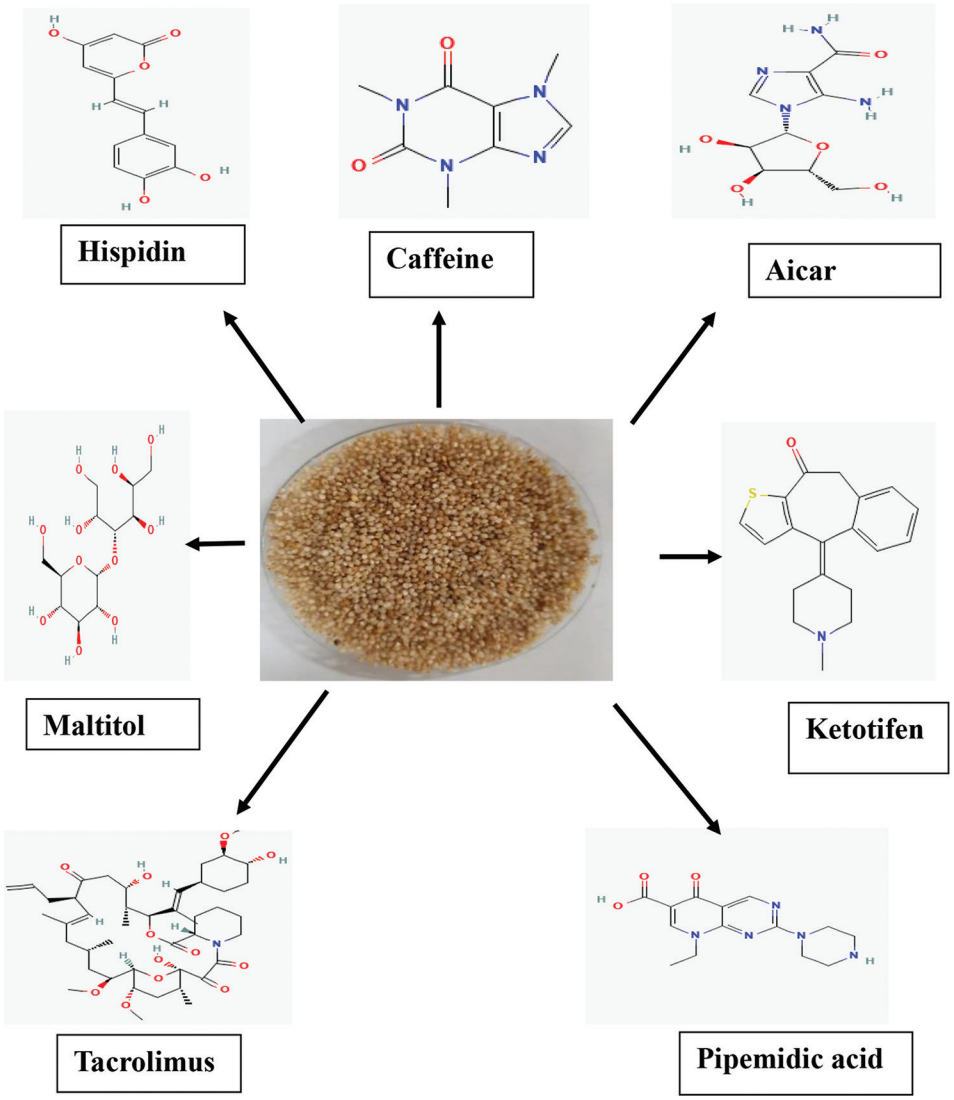

Figure 4: Antidiabetic compounds in Little Millet grains explorated from HRMS

CONCLUSIONS

Since diabetes mellitus prevailing lifestyle disorder, apart from medication, there is an urgent need to evaluate safe dietary substances which may be cost effective, safe, organic, and available easily for diabetes patients. Therefore, in the present study, components of Little Millets were identified by UHPLC-Q-TOFMS/MS system and it is found that these compounds are hispidin, caffeine, aicar, ketotifen, maltitol, tacrolimus, and pipemidic acid. These compounds indirectly control body metabolism and are therapeutically useful for the management of diabetes mellitus.

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