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PHYTOCHEMICAL PROFILING, IN SILICO AND IN VITRO ANTI-DIABETIC ACTIVITY ON METHANOLIC EXTRACT OF HELICTERES ISORA

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ABSTRACT

This study investigated the *in vitro* and *in silico* anti-diabetic activity of *Helicteres isora*. GC-MS technique identified 14 compounds from the methanol extract, 1-Butanol, 3-methyl-, formate, d-Mannose, β-Acorenol, 3-O-Methyl-d-glucose, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, Phytol, E-8- Methyl-9-tetradecen-1-ol acetate, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, Campesterol, Stigmasterol, γ-Sitosterol and Lupeol. The antidiabetic potential was assessed through *α*-amylase and *α*-glucosidase enzyme inhibition assays. The crude extract demonstrated the most potent inhibition (IC_{50} = 179.72 And 143.84µg/mL for *α*-glucosidase and *α*-amylase respectively) suggesting its potential for managing postprandial hyperglycaemia. *In silico* studies employed molecular docking and dynamics simulations to elucidate the interactions between identified compounds and *α*-amylase/*α*-glucosidase enzymes. The results revealed promising binding affinities between the compounds and target enzymes, with γ-Sitosterol demonstrating the highest predicted inhibitory activity with −8.1 kcal/mol. This study highlights the presence of diverse bioactive compounds in methanolic extract of *Helicteres isora* (MEHI). The extract exhibits its potential as a complementary therapeutic approach for managing hyperglycaemia associated with type 2 diabetes.

KEYWORDS: anti-diabetic activity, *Helicteres isora*, *α*-amylase, *α*-glucosidase.

INTRODUCTION

Diabetes mellitus is affecting around 25% of the world population of both developed and developing countries.[1,2] China was the country with the highest number of diabetics worldwide, with some estimated 110 million persons, followed by India (70 million) and USA (30 million) suffering from diabetes. WHO projects that diabetes will be the 7th leading cause of death in 2030. Diabetes with cardiovascular complications imposes a major threat on human health claiming death in every 10s. Diabetes cases are exponentially increasing in India as a result of societal influence and life styles.[3] Diabetes mellitus is considered as the group of metabolic disorders with different causes, which are characterized by imbalancing in carbohydrates, proteins and fat metabolism that lead to the effect on insulin action or secretion. In modern medicine there is still no reasonable effective therapy or drug to cure diabetes.

The currently accessible anti-diabetic agents include sulfonylureas, thiazolidinedione, α-glycosidase inhibitors such as miglitol and acarbose widely used to control hyperglycemia. Nevertheless, these drugs fail to cure the disease in addition, causes several diabetic complications and side effects such as abdominal pain, diarrhea and soft feces in colon.[4,5]

Plant families are considered to be a source for the most potent hypoglycemic properties. The drugs from plant sources are usually considered to be non-toxic with lesser side effects than synthetic drugs. Traditional medicinal plants having anti-diabetic properties can provide useful sources for the discovery of safer hypoglycemic agents.[6,7] These plants are the major source for discovering new compounds with therapeutic value for drug development against most common and very prevalent disease, diabetes mellitus. The plants which have therapeutic application possess bioactive composites viz., alkaloids, glycosides, tannins, flavonoids, saponins, phenolics and vitamins.

Different parts of plants vary in their composition of bioactive compounds and also medicinal properties.[8] Chemical compounds that are often referred as secondary metabolites are the phytochemicals formed in the plants during normal metabolic processes. These secondary metabolites are an important source with a variety of structural arrangements and properties. In ancient Indian literature medicinal properties of several herbal plants

have been documented and the preparations have been found to be effective in treatment of diseases. Therefore, to come across the demand of manufacturing modern medicines and export, the need of the medicinal plants have enormously amplified.

The ethnobotanical statistics reports about 1200 odd plants that may possess anti-diabetic potential worldwide.[9,10] In the present study *Helicteres isora*, sometimes called the Indian screw tree, is a small tree or large shrub found in southern Asia and northern Oceania. It is usually assigned to the family *Malvaceae*,[11] but it is sometimes assigned to the family Sterculiaceae.[12] The red flowers are pollinated mainly by sunbirds, butterflies, and Hymenoptera. In the 19th century fibers from the bark were used to make rope and sacks, although nowadays the tree is harvested for the fruits and roots which are used in folk medicine. Henceforth, present study was aimed to explore the phytochemical constituents and in vitro and in silico docking studies for anti-diabetic activity of *Helicteres isora.*

MATERIALS AND METHODS

Plant authentication

The whole plant material has been identified taxonomically and authenticated by Dr. S. Madhava Chetty, Associate Professor, Department of Botany, Sri Venkateshwara University, Tirupati, and Andhra Pradesh.

Preparation of the extract

The collected plant was washed thoroughly with water and dried in the shade. The dried leaves were ground well to coarse powder (500gms). Methanolic extract was obtained by extracting powder with methanol by soxhlet extraction method for 72hr. After completion of the extraction the solvent was removed by rotary evaporator method. The methanolic extract was used for further study. [13]

Figure 1: Soxhlet extraction.

Organoleptic and phytochemical evaluation

Organoleptic evaluation refers to the evaluation of the formulation by the colour, odour, taste and texture. The method adopted for the organoleptic evaluation was as described in Wallis. Various physicochemical parameters like moisture content, pH, ash value were determined. PHP was also subjected to preliminary phytochemical screening to detect the presence of organic constituents using standard methods.[14-18]

Ash content

The ash values usually represent the inorganic residues such as phosphates, carbonates and silicates present in herbal drugs. These are important indices to illustrate the quality as well as purity of herbal medicine. The objective to evaluate is to remove all traces of organic matter, which may otherwise interfere in an analytical determination.

Total ash

Empty silica crucible was weighed (W1). About 3 g (W2) of the air-dried PHP was added to the previously weighed crucible. The sample was ignited gradually in an electrical muffle furnace increasing the heat to 500-600° until it is white, indicating the absence of carbon. Then it was cooled in a desiccator and reweighed (W3). Total ash content was calculated as % total ash = ((W3-W1)/ (W2-W1)) ×100.

Acid-insoluble ash

25ml of dilute HCl was added to the total ash containing crucible. It was then covered with watch-glass and boiled gently for 5 min. With 5 ml of hot water, the watch glass was washed and the washings were added to the crucible. Then, ash less filter paper was used to filter the insoluble matter and washed with hot water until the neutral filtrate was obtained. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hotplate and ignited to constant weight (W4). The residue was allowed to cool in a desiccator for 30 min and then reweighed. W1 is weight of empty silica crucible, W2 is the weight of sample including crucible for ignition, W3 is the final weight of sample including crucible weight after ignition and W4 is the constant weight after addition of HCl. Acid-insoluble ash content was calculated as, % acid-insoluble ash = (W4–W1)/(W2–W1)×100.

Water-soluble ash

In the total ash containing crucible, 25 ml of water was

added, boiled for 5 min and filtered through an ash less filter-paper. The insoluble matter collected on the filter paper was washed with hot water and then the filter paper ignited in a crucible for 15 min at a temperature not exceeding 500°. The residue was allowed to cool in a desiccator for 30 min and re-weighed (W5). % waters insoluble ash was calculated as, (W7–W6)×100, where, W1 is the weight of empty silica crucible, W2 is the weight of sample including crucible weight for ignition, W3 is the final weight of sample including crucible weight after ignition, W6 is the weight of residue, which is W5–W1, W7 is the weight of ash, which is W3–W1 and water-soluble ash is W7–W6 mg/g.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS evaluation become completed in Agilent 8890 gas chromatograph device and mass spectrophotometer, equipped with a HP-five ms fused silica column (five% phenyl methyl siloxane 30.0m ×250µm, film thickness 0.25µm), interfaced with MSD detector. Helium fuel was used as carrier gas and adjusted to column pace go with the flow of 1.2ml/min. Different GC-MS specifications are ion-source temperature, 250°C; interface temperature, 300°C; pressure, sixteen.11.367 psi; holdup time, 1.2376 min; and with split ratio 15:1 with injection temperature of 250 °C. The column temperature began at 75°C for five min and changed to a hundred and 50°C on the rate of 4°C/min. The temperature was raised to 250°C on the price of 20°C/min and held for five min. The entire elution became 53.5 min. The relative percentage quantity of each thing was calculated by means of comparing its average height location to general regions.

Identification of compounds

Identification of components was achieved based on their retention indices and interpretation of mass spectrum was conducted using the database of National Institute of Standards and Technology (NSIT). The database consists of more than 62,000 patterns of known compounds. The spectra of the unknown components of *Helicteres isora* extract L fraction obtained were compared with the standard mass spectra of known components stored in NIST library (NISTII).

***In Silico* Studies Preparation of Protein**

The crystal structure of human intestinal α-glucosidase in complex with acarbose PDB (protein data bank) ID: 3TOP was retrieved in pdb file format from the RCSB PDB (https://www.rcsb.org/). Water molecules and heteroatoms, including the co-crystallized acarbose, were because these can affect the docking accuracy[19] since the- glucosidase (3TOP) is a symmetrical dimer, chain B of the enzyme was removed, and only chain A was used in the docking process.[20] The incomplete side chains were also replaced using Dunbrack 2010 rotamer library.[21] The enzyme file was saved in pdbqt format.

Ligand Preparation

The ligands were downloaded in SDF format from the PubChem database (pubchem.ncbi.nlm.nih.gov) and formatted in PDB and PDBQT using software (BIOVIA Discovery Studio Visualizer 2021). The software was then used to process the ligands for molecular docking analysis using Auto Dock by adjusting ionization, torsion, degree of freedom, and stereochemical variation.

Protein Structure for Docking

Docking simulations were used to perform molecular docking using the AutoDock Vina and BIOVIA Discovery Studio Visualizer programs. Additionally, the creation of the ligand and enzyme used in the docking analysis was carried out using the Discovery Studio program, as was the execution of the docking analysis utilizing the command prompt procedure.

Docking Simulations

Molecular docking studies were carried out for the selected molecules in the binding site of target proteins using AutoDock Vina [136] and AutoDock Tools.

The grid box size was set for each receptor, and the exhaustiveness was set to 24. The results with the best conformation and energy were selected for further analysis. Discovery Studio Visualizer V19 was used for visualization and analysis of the protein- ligand complexes.

In vitro anti-diabetic assay

α - Amylase inhibition assay Procedure

0.5ml of extract was mixed with 0.5 ml of α-amylase solution (0.5mg/ml) with 0.02M of sodium phosphate buffer (pH – 6.9) with 0.006M NaCl. The mixture was incubated at room temperature for 10 minutes & 0.5 ml of starch solution (1%) in 0.02 molar sodium buffers was added. Resultant mixture was incubated at room temperature for 10 minutes and the reaction was terminated using 1 ml of di nitro salicylic acid colour agent. At this time, the test was placed in water bath (100o for 5mins) and cooled until room temperature was obtained. The mixture was then diluted with 10 ml of deionized water and absorbance was determined at 540 nm. The adsorbent of blank (buffer instead of extract and amylase solution) and control (buffer instead of extract) sample was also determined. Acarbose was used as standard drug. The inhibition of α-amylase was calculated using the following equation.[22]

$$\%\text{Inhibition of } \alpha\text{-amylase} = 1 - \frac{\text{Abs Sample}}{\text{Abs control}} \times 100$$

Where

Abs controls correspond to the absorbance of the solution without extract (buffer instead of extract) and with α-amylase solutions and abs sample corresponds to the solution with extract and α-amylase solution.

Glucose uptake in yeast cells

The yeast suspended in distilled water was subjected to

repeat centrifugation (3000G, 5min) until clear supernatant fluid were obtained and 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of plant extract (50 to 250µg/ml) were added to 1 ml of glucose solution (5mm) and incubated together for 10 minutes at 37oC Reaction was started by adding 100µl of yeast suspension followed by vertexing and further incubating at 37oC for 60 minutes. After 60 minutes the tubes were centrifuged (2500rpm, 5minutes) and amount of glucose was estimated in supernatant Metronidazole was used as standard drug.[23] The percentage increase in glucose uptake by yeast cells was calculated using following formula.

$$\text{Increase in glucose uptake} = \frac{\text{Abs sample - Abs control}}{\text{Abs sample}} \times 100$$

Where

Abs sample is the absorbance of test samples and abs control is the absorbance of control reaction (containing all regions except the test sample). All the experiments were carried out in triplicates.

RESULTS AND DISCUSSION

Table 1: Phytochemical screening of Methanolic extract of *Helicteres isora.*

S. No	Name of the Phytochemical	Methanol
1.	Carbohydrates	+
2.	Amino acids	+
3.	Proteins	+
4.	Alkaloids	+
5.	Cardiac glycosides	+
6.	Triterpenoids	+
7.	Saponins	+
8.	Flavonoids	+
9.	Phenolic compounds	+
10.	Tannins	+
11.	Steroids	+
12.	Gums	-

Table 2: Physico-chemical constants of *Helicteres isora* Powder.

Parameters	Results
Organoleptic characters	
Appearance	Powder
Colour	White
Smell	Aromatic
Taste	Slight pungent
Loss in weight on drying at 1050C	10±0.52%
Ash Values	
Total ash	4.7±0.34%
Water soluble ash	2.7±0.04%
Acid insoluble ash	0.4±0.03%

***In silico* docking studies of Methanolic extract of *Helicteres isora* (MEHI).**

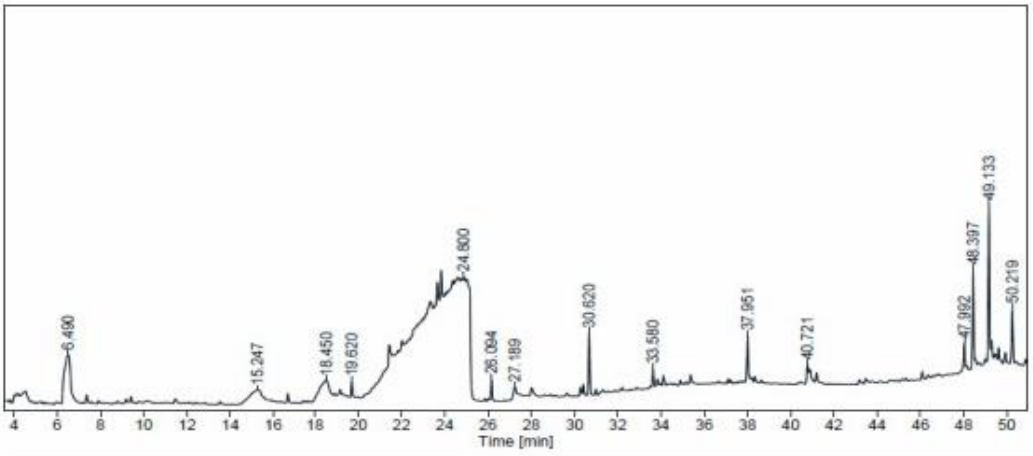

Figure 2: GC-MS chromatogram of Methanolic extract of *Helicteres isora* (MEHI).

Table 3: Bioactive compounds found in Methanolic extract of *Helicteres isora* (MEHI).

S. No	R. Time (min)	Area %	Compound name	Molecular Formula	M.W g/mol
1.	6.494	19.34	1-Butanol, 3- methyl-formate	$C_6H_{12}O_2$	116.16
2.	15.24	8.06	d-Mannose	$C_6H_{12}O_6$	180.156
3.	19.621	0.59	β-Acorenol	$C_{15}H_2O_6$	222.37
4.	24.797	7.55	3-O-Methyl-d- glucose	$C_7H_{14}O_6$	194.18
5.	26.091	1.56	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5
6.	27.191	6.49	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
7.	30.623	4.70	Phytol	$C_{20}H_{40}O$	296.5
8.	33.580	0.79	E-8-Methyl-9- tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268.4
9.	37.950	4.11	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)et hyl ester	$C_{19}H_{38}O_4$	330.5
10.	40.719	5.43	9,12-Octadecadienoic acid (Z, Z)-, 2- hydroxy-1-(hydroxymethyl)et hyl ester	$C_{21}H_{38}O_4$	354.5
11.	47.989	1.21	Campesterol	$C_{28}H_{48}O$	400.7
12.	48.395	6.42	Stigmasterol	$C_{29}H_{48}O$	412.7
13.	49.133	12.59	γ-Sitosterol	$C_{29}H_{50}O$	414.7
14.	50.214	8.35	Lupeol	$C_{30}H_{50}O$	426.7

The chromatogram of GC-MS displayed in Figure 2. Whereas the chemical constituents with their Retention Time (RT), atomic equation, Molecular weight (MW) and Area (%) within the MEIP is displayed in Table 3. The following bioactive compounds were present in the GC-MS analysis carried on methanolic fraction of *Helicteres isora* was found the following bio active compounds 1-Butanol, 3-methyl-, formate, d-Mannose, β-Acorenol, 3-O-Methyl-d-glucose, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, Phytol, E-8- Methyl-9-tetradecen-1-ol acetate, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, Campesterol, Stigmasterol, γ-Sitosterol and Lupeol.

Table 4: Docking Scores of Compounds with Alpha Glucosidase (PDB: 2QMJ).

S. No	Name of the compound	Docking Score (KCal/mol)
1.	3-O-Methyl-d-glucose	-5.5
2.	Hexadecanoic acid, methyl ester	-5.8
3.	n-Hexadecanoic acid	-7.3
4.	Phytol	-7.6
5.	E-8-Methyl-9-tetradecen-1-ol acetate	-6.5
6.	Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl ester	-6.2
7.	9,12-Octadecadienoic acid (Z, Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	-6.8
8.	Campesterol	-7.6
9.	Stigmasterol	-8.0
10.	γ-Sitosterol	-8.1
11.	Acarbose	-8.5

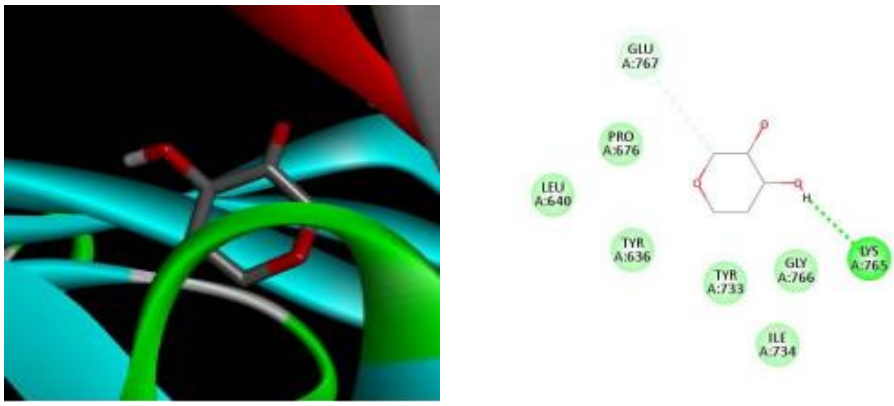

Figure 3A: Standard-acarbose (-8.5).

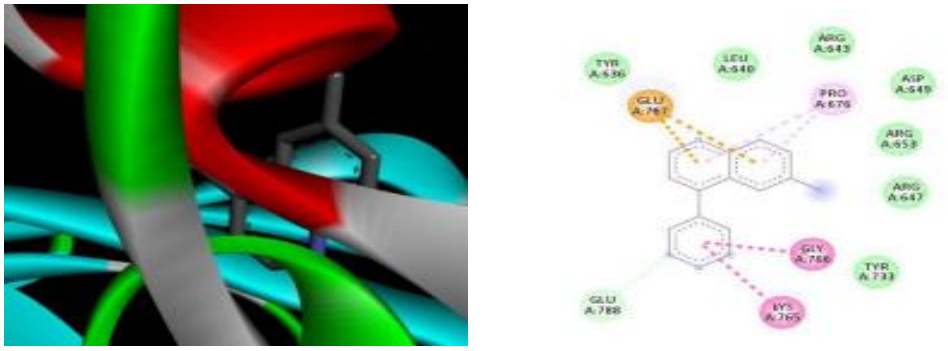

Figure 3B: γ-Sitosterol docking score (-8.1).

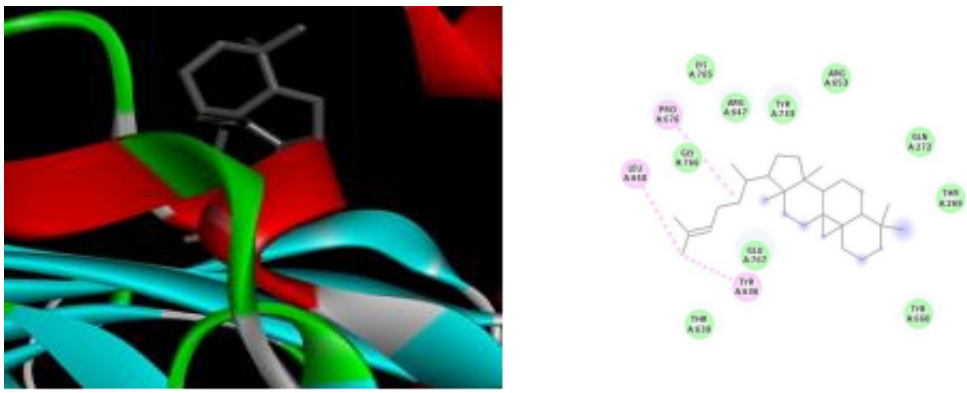

Figure 3C: Stigmasterol docking score (-8.0).

Figure 3: 2D and 3D interactions of α-glucosidase with ligands.

Molecular docking study is also done by using Auto dock vina to identify the significant binding interactions at the active pocket of targeted protein Alpha Glucosidase with PDB ID: 2QMJ. The standard drug showed a binding affinity of -8.5 kcal/mol and was found stabilized by hydrogen bonding with LYS-A:765, TYR-A:605 and vander waals bond with GLU- A:767, PRO-A:676, LEU-A:640, TYR-A:636, TYR-A:733, GLY-A:766, ILE-A:734 Here 11 compounds were selected and subjected to molecular docking with targeted protein PDB ID: 2QMJ. Compound γ-Sitosterol was found with highest docking score of -8.1 kcal/mol and found stabilized by hydrogen bonding with GLU-A:767, PRO-A:676, GLU- A:788, vander waals bond with TYR-A:733, TYR -A:636, ARG-A:643, LEU-A:640, ARG-A:647, ARG-A:653, ASP-A:649. Compound has formed similar interactions in comparison with standard interactions with TYR-A: 733, TYR -A: 636, LEU-A: 640, PRO-A: 676, GLU- A: 767 might be significant and contributed for docking score. Further experimental investigation might help us to understand significant interactions and correlate docking results.

***In vitro* anti-diabetic assay**

Table 6: Glucose uptake by yeast cells.

Concentration (µg/ml)	Increase in Glucose uptake %			
	Extract (MEHI)	IC50 Value	Standard (Metronidazole)	IC50 Value
50	37.51	179.72	47.54	149.88
100	43.24		53.64	
150	47.12		59.65	
200	52.24		61.54	
250	58.14		67.51	

MEHI of different concentrations were assessed for in vitro Anti-diabetic activity by glucose uptake assay using a yeast model. MEHI exhibited potent activity (68.1% at 250µg/mL). A dose-dependent rise in the % of glucose uptake with increasing concentration (100–500µg/mL) of MEHI extract was observed.

Table 7: Alpha amylase inhibition assay.

Concentration (µg/ml)	Increase in Glucose uptake %			
	Extract (MEHI)	IC50 Value	Standard (Acarbose)	IC50 Value
50	30.42	143.84	54.31	114.36
100	38.78		66.54	
150	57.47		73.76	
200	70.51		85.35	
250	78.67		90.42	

DISCUSSION

Diabetes mellitus is a non-communicable disease often genetic in nature but can be developed due to the life style. In modern medicine there is no acceptable effective therapy or medication to treat diabetes.[24] Medicinal plants having anti-diabetic properties can provide a useful source for the unearthing of safer economic anti-diabetic drug. Recent extensive review by.[25] listed 47 species that belong to 29 plant families as a source of alpha glucosidase inhibitors, in the present research investigation MEHI was evaluated for their anti-diabetic activity. Two different in vitro assays were used to evaluate anti-diabetic activities of MEHI viz., alpha-amylase and glucose uptake assay. Alpha-amylase is type of the intestinal enzyme which play important role in carbohydrate digestion and glucose absorption. Suppression of the activity of digestive enzymes such as α-amylase, would delay the digestion of starch and oligosaccharides, which in turn decreases the absorption of glucose and consequently reduce the blood glucose.[26] This significant anti-diabetic activity was comparable to the standard drug inhibition. This technique is one of the anti-diabetic therapeutic approaches to reduce the post prandial glucose level in blood by inhibiting activity of alpha-amylase enzyme and it can be used as a strategy in management of blood glucose.

Regulation of glucose level in the blood of diabetic patient can prevent numerous complications associated with the disease. The maintenance of plasma glucose concentration for longer time under variation in dietary condition is one of the most important and closely regulated processes observed in the mammalian species[27], especially type II diabetes characterized by deficiency of insulin causing increased level in blood glucose level and it depends on the uptake of glucose by the cells. In the present study, MEHI was subjected to in vitro anti-diabetic assay by means of yeast as model. Percentage of increase in glucose uptake in yeast cells by the action of MEHI was compared with the standard drug metronidazole. The increased concentration of extracts correspondingly increased percentage of glucose uptake in yeast cells. This result indicated that high concentrations of extracts exhibited high glucose uptake. Plants are the major source for discovering new compounds with medicinal value for drug development.[28] In chromatography methods, gas chromatography (GC) is one of the most widely used techniques and has become one of the most important tools for the separation of phytocompounds. In the last few years GC-MS has become firmly established itself as a powerful technique for identification of secondary metabolites in both plant and non-plant species.[29] In the present study methanolic extract of *Helicteres isora* was subjected to GC-MS analysis. GC-MS spectrum confirms the presence of various bioactive compounds in extract with different retention time. The gas chromatogram shows presence of relative concentration of various compounds present in MEHI getting eluted at different retention time.[30] The peak height represents relative concentration of components present in MEHI. The mass spectrometer helps in the identification of compounds eluted at different retention time (Fig. 2). The major phytoconstituents were found to be 1-Butanol, 3-methyl-, formate, d-Mannose, β-Acorenol, 3-O-Methyl-d-glucose, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, Phytol, E-8- Methyl-9-tetradecen-1-ol acetate, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, Campesterol, Stigmasterol, γ-Sitosterol and Lupeol. From an extensive literature review it was observed that *Helicteres isora* is widely used as a popular substitute remedy in certain regions of the Africa The plant extracts, particularly methanolic, showed several biological activities such as antimicrobial, pesticidal, analgesic, antipyretic, anticancer and antitrypanosomal among others.

Hence, the present study also supporting and provides a clear scientific basis that *Helicteres isora* can be a potent source for the novel medicines. Anti-diabetic activities of aqueous *Helicteres isora* leaves extract reported for the first time showed its therapeutic potential to be used as a cost effective safe herbal anti diabetic agent. Accordingly, these results encourage further studies on extracts and identify particular active chemical compounds responsible for the specific biological activity in order to standardize the plant preparation for maximum therapeutic benefit to treat diabetes.

CONCLUSION

Traditional therapeutic plants are frequently used in rural parts, since the availability of extravagant amount of medicinal plants in those areas. Thus, treating diabetes mellitus with herbal derived composites that are accessible and do not necessitate laborious pharmaceutical production seems extremely attractive. This is of great importance to developing countries such

as India. The existence of phytochemcials diversities in MEHI showed broad spectrum of diverse biological activities these results of GC-MS profile can be used as pharmacognostical tool for the identification of novel drugs from MEHI. Based on the results obtained from different in vitro and in silico anti-diabetic assays, MEHI has shown significant anti-diabetic activity in both assays. The present study revealed that MEHI exhibited significant in vitro anti-diabetic activity. Further, purification of the specific active constituents needs to be carried out, that can be used for the discovery of novel drugs to treat diabetes mellitus, a worldwide epidemic disease.

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