Phytoligands analysis of aqueous seed extract of *Momordica charantia* Linn. via GC-MS for its antidiabetic activity

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Abstract

In India, Momordica charantia Linn. is utilized in juices, health supplements and colouring agents. The aim of the current study is to analyze phytoligands by utilizing gas chromatography mass spectroscopy of aqueous seed extract of Momordica charantia Linn (MCA). The MCA was prepared by macerating the powdered seeds for 72 hrs followed by filtration and lyophilization followed by an in vitro α-glucosidase inhibitory activity. MCA was the potent inhibitor of α-glucosidase with IC_{50} value 73.75± 0.05 µg/ml. The analysis through GC-MS investigated 60 phytoconstituents which were validated using Schrodinger suite version 2020-3 against α-glucosidase (PDB ID- 5NN5).

As per docking results, the existing phytochemicals azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl)-, alpha-l-rhamnopyranose, piperidine, 3-methyl-, 1,4-Dioxane-2,5-dione,3,6-dimethyl and citronellol epoxide (R or S) from MCA, got the highest d-score of -7.230, -5.918, -5.643, -5.312 and -5.185 kcal/mol and could be promising antidiabetic drug candidates.

Keywords: Aqueous seed extract, α-glucosidase, Gas chromatograph mass spectrometer, Molecular docking, *Momordica charantia*.

Introduction

Diabetes mellitus (DM) is a frequently seen chronic illness that significantly increases mortality on a global scale. The hallmark of DM is impaired glucose homeostasis which exacerbates blood sugar. It is divided into a number of subcategories including type 1 DM, type 2 DM, neonatal DM, gestational DM, maturity-onset DM of the young ones and steroid-induced DM. In just 34 years, the number of people with DM has quadruplicated, [422 million (2014) from 108 million (1980)]. The prevalence of diabetes among individuals over 18 has increased globally, rising from 4.7% in 1980 to 8.5% in 2014[1]. Incidence rate of diabetes is thought to be between 1% and 5% in India. If immediate preventive measures are not followed, the estimated 50.8 million diabetes cases in India now may increase to 87 million by the year 2030[2].

Long-lasting diabetic symptoms (hyperglycemia, polydipsia, polyphagia and reduced sensitivity to insulin) provoke a variety of disorders including cardiovascular disorder, coronary artery disease, kidney failure, brain abnormalities, early mortality and amputation of the limb. Since ancient system, medicinal plants are used as medicine and have become a prominent part of the health care system. These medicinal plants are prescribed as herbal drugs due to low cost and less side effect. Even their biological constituents are unknown. India is named as 'largest hub for medicinal plants' as well as 'botanical garden of the world'[3].

One of the highly demanded medicinal or vegetable plant is *Momordica charantia* Linn. with family Cucurbitaceae, traditionally consumed as antidiabetic, antimalarial, anti-inflammatory, laxative, hepatoprotective, anticancer etc. It is broadly grown in India, Malaysia, China and tropical Africa[4]. Saponins especially cucurbitane glycosides are responsible for its bitterness. Climatic and geographical conditions decide the medicinal value of any medicinal plant[5].

Gas chromatography mass spectroscopy i.e. GC-MS is a hybrid analytical technology with selectivity, reproducibility, sensitivity and excellent capability of analyzing different phytoconstituents with their molecular structure in herbal extract. It is useful in identification of pure compounds even in minute quantity i.e. less than 1 mg[6].

The enzyme i.e. α-glucosidase executes a crucial role in regulation of p53 signalling which is essential for carbohydrate metabolism. As a consequence, eating foods and vegetables, high in α-glucosidase inhibitors can help to lower the chance of developing type-2 DM. Several commercially accessible enzyme inhibitors like acarbose, miglitol and voglibose are associated with bowel disturbance, diarrhoea and stomach discomfort, not prescribed for people suffering from gastrointestinal diseases.

As an substitute to currently prevailing, enzyme inhibitors, novel α-glucosidase inhibitors isolated from natural resources can be virtually screened with less adverse effects by using *in silico* approach. However, there is no information available on GC-MS analysis of MCA.

Hence, recent investigation focuses on the discovery of phytoligands showing *in vitro* α-glucosidase inhibitory activity from MCA by utilizing GC-MS and identifying lead molecules by molecular interaction with diabetes target protein (PDB ID-5NN5) using an *in silico* method.

Material and Methods

The Khari Baoli market in Old Delhi was visited to procure dried seeds of *M. charantia* Linn. Mr. R.S. Jayasomu Senior Principal Scientist, Head and Dr. Sunita Garg as Emeritus Scientist, Raw Material Herbarium and Museum, Council of Scientific and Industrial Research - National Institute of Science Communication and Information Resources in New Delhi verified the authenticity of the *M. charantia* Linn. dried seeds. The number for a voucher specimen is NISCAIR/RHMD/Consult/2021/3745-46. All chemicals used were of AR grade.

Preparation of MCA for GC-MS analysis: The harvested seeds were first thoroughly cleaned with double distilled water, then shade dried. A mechanical blender was used to pulverize the dried seeds. The ground seeds were stirred and occasionally shaken while being left to macerate for 72 hours. 460mm x 510mm Whatmann filter paper was used to filter the entire combination. At a temperature of 20°C, a rotary evaporator was used to concentrate the crude extract. For continuing use, the extract was lyophilized and stored in a refrigerator.

α-glucosidase inhibitory activity: The inhibition assay of α-glucosidase inhibitory activity of MCA was assessed by standard methodology[7]. A reaction mixture including 10 µl of α-glucosidase (1µ/ml), 50 µl of phosphate buffer (50 mM) with pH 6.8 and 20 µl of varying extract concentrations (25 to 100 µg/ml) was allowed to pre-incubate at 37°C for fifteen minutes. After that, 20 µl of p-nitrophenyl-α-D-glucopyranoside (1 mM) was mixed as a substrate followed by incubation for another 30 minutes at 37 °C. The addition of 0.1 M sodium carbonate (50 µl) caused the reaction to stop and the absorbance was assessed at 405 nm. Acarbose was used as a standard. The result is expressed as percentage inhibition.

Detection of Phytoconstituents by GC-MS: The MCA was analyzed by GC-MS i.e. "Thermo scientific TSQ 8000 high resolution Gas Chromatograph Mass spectrometer" equipped with Elite-5 MS fused silica capillary column having length 30 m, thickness 0.25 mm and diameter 0.25 mm. The flow of helium as carrier gas (99.999 %) was maintained at 1ml/min. The volume of injection was 10 µL. Electron capture detector was used for detection purpose. Mass range was set between 40-650 m/z. Total run time was 27.09. GC-MS was set up with NIST i.e. National Institute Standard and Technology Library. NIST database was used for the interpretation of mass spectra obtained by GC-MS[8]. The phytocomponents were quantified by calculating their relative peak area.

Molecular docking: All the 60 phytoligands analyzed by GC-MS were investigated for potential antidiabetic properties through *in silico* molecular docking. PubChem and PDB database were used for retrieval of the 3D structure of all metabolites and target protein (PDB ID:5NN5). Using Maestro v12.8 of Schrodinger suite including various tools like Protein Preparation Wizard (assign bond orders, adding missing side chain, removal of water molecules, hydrogen bonding optimization and energy minimization), LigPrep (generation of 3D geometries at force field OPLS3e and ionization state by Epik at 7.0+3.0.), Sitemap tool (Validation of binding site in protein), Grid generation, Ligand docking, Glide (binding affinity and their interaction with protein) and Ligand interaction, molecular docking was conducted. The relative potency of the binding interactions of the best-docked phytoligands was evaluated on the basis of their binding energy and best binding pose[9].

Pharmacokinetics properties: ADMET properties were also evaluated for best interacted structure form of the MCA by Quikprob.

Statistical analysis: Software Graph pad prism version 9.5.1 was used for analyzing the data. The data is displayed as mean ± standard error.

Results and Discussion

α-glucosidase inhibitory activity: The MCA possesses powerful antidiabetic characteristics with an IC_{50} value of 73.75± 0.05 µg/ml than standard (89.13 ±0.05 µg/ml) as evidenced by its *in vitro* antidiabetic action. In figure 1, the percentage inhibition with regard to concentration is represented graphically.

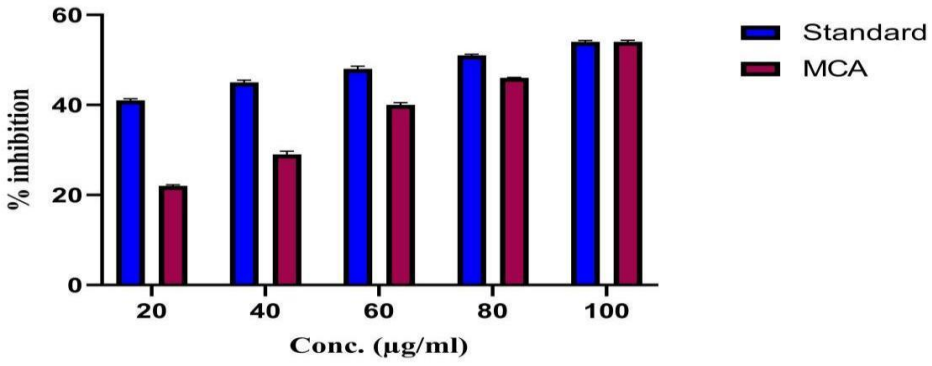


Fig. 1: α-glucosidase inhibitory activity of MCA and standard

GC-MS and Molecular docking: In recent study, the resultant extract was found dark brown in colour having percentage yield 3%. By GC-MS analysis of MCA, 60 phytocomponents along with their molecular weight, RT, molecular formula and peak area were identified by comparison with NIST library that are listed in table 1. GC-MS chromatogram is depicted in figure 2.

All these metabolites were further examined for their antidiabetic activity by carrying molecular docking using target protein of diabetes PDB ID-5NN5. Phytoligands were elucidated for their best possible binding confirmation and interaction with target protein. Among phytoligands, alpha-l-rhamnopyranose, azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl)-, piperidine, 3-methyl-, citronellol epoxide (R or S) and 1,4-Dioxane-2,5-dione,3,6-dimethyl were found to generate the most perfect ligand-protein complexes than other compounds. Their mass spectrum are depicted in figure 3. and their 2D and 3D structure are listed in figure 4 and figure 5. The analysis of protein ligand interaction is depicted in table 2.

Phytocomponents like alkaloids, flavonoids, organic acids, amino acids and other chemicals can all be identified from plant extracts using the trustworthy method known as GC-MS[10]. *In silico* predictions of pharmacological, pharmacokinetic and toxicological parmeters are made using computational prediction models[11].

A quick and inexpensive method for developing and testing medications is molecular docking. This method provides information on how drugs interact with receptors, which can be used to predict how a drug candidate would interact with the target proteins, resulting in consistent interaction with the binding sites of ligands. A computerized docking software called Schrodinger was used to examine the specific intermolecular interactions between diabetic target protein (PDB ID-5NN5). Grid-dependent ligand docking along with energies is carried out and the system looks for significant molecular interaction between a typically bigger protein molecule and ligand molecules[12]. When compared to the reference drug, acarbose, the five molecules show good binding affinity following Lipinski rule of five.

Table 1
Phytoconstituents identified in MCA by GC-MS analysis.

RT	Compound name	Molecular formula	Molecular weight ($gmol^{-1}$)	% of peak area	Binding affinity (Kcal/mol)
3.25	3-Hexanone,5-hydroxy-2-methyl-	$C_7H_{14}O_2$	130.18	0.69	-3.663
3.25	Butanamide,3,N-dihydroxy-	$C_4H_9NO_3$	119.12	0.69	-3.429
3.25	1,4-Dioxane-2,5-dione,3,6-dimethyl	C_6H8O4	144.12	0.69	-5.312
3.25	Hydroperoxide,1-methylbutyl	$C_5H_{12}O_2$	104.1476	0.69	-2.923
3.68	Oxirane,(ethoxymethyl)-	$C_5H_{10}O_2$	102.1317	1.49	-3.164
3.68	Oxirane,[(1-methylethoxy)methyl]-	$C_6H_{12}O_2$	116.1583	1.49	-2.749
3.68	Ethanol, 2-[2-(ethenyloxy)ethoxy]-	$C_6H_{12}O_3$	132.1577	1.49	-3.102
3.68	(3-Methyl-oxiran-2-yl)-methanol	$C_4H_8O_2$	88.11	1.49	-4.437
3.68	12-Crown-4	$C_8H_{16}O_4$	176.2102	1.49	-4.088
4.97	Hydroperoxide, 1-methylhexyl	$C_7H_{16}O_2$	132.2007	1.94	-1.997
4.97	5-Aminovaleric acid	$C_5H_{11}NO_2$	117.1463	1.94	-4.635
4.97	Piperidine, 3-methyl-	$C_6H_{13}N$	99.1741	1.94	-5.643
4.97	Azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl)-	$C_7H_{14}N_2O$	142.20	1.94	-7.230
4.97	N-Isopentyl-N-nitroso-pentylamine	$C_{10}H_{22}N_2O$	186.29	1.94	-1.822
5.19	2-Heptanol, 3-methyl-/ 2-Heptanol, 4-methyl-	$C_8H_{18}O$	130.2279	0.85	-2.843
5.19	2-Ethyl-1-butanol, methyl ether	$C_7H_{16}O$	116.2013	0.85	-3.397
5.19	Hydrazine, 1-methyl-1-(2-propynyl)-	$C_4H_8N_2$	86.14	0.85	-2.142
6.48	1-Butanamine, 3-methyl-N-(3-methylbutylidene)-	$C_{10}H_{21}N$	155.2804	1.60	-1.822
6.48	N-Isopentyl-N-nitroso-pentylamine	$C_{10}H_{22}N_2O$	186.29	1.60	-1.822
6.48	3-Ethyl-2-heptanol	$C_9H_{20}O$	144.2545	1.60	-3.486
6.48	2-Octanol, 3-methyl-	$C_9H_{20}O$	144.2545	1.60	-2.749
6.48	N-methylene-n-octylimine	$C_9H_{19}N$	141.25	1.60	-2.516
13.05	2-(2-Butoxyethoxy)ethyl2,2,3,3,3-pentafluoropropanoate	$C_{11}H_{17}F_5O_4$	308.2423	1.79	-2.142
13.05	1,2-Propanediol, 3-methoxy-	$C_4H_{10}O_3$	106.1204	1.79	-2.807
13.05	Oxirane, (butoxymethyl)-	$C_7H_{14}O_2$	130.1849	1.79	-2.646
13.05	1-Deoxy-d-arabitol	$C_5H_{12}O_4$	136.15	1.79	-4.650

13.05	Ethanol, 2-[2-(2-propenyloxy)ethoxy]-	$C_7H_{14}O_3$	146.1843	1.79	4.075
16.17	Tridecanoic acid, thiophen-2-ylmethylenehydrazide	$C_{18}H_{30}N_2OS$	214.34	19.87	-1.623
16.17	Cyclohexane, 1,4-diethoxy-, trans-	$C_{10}H_{20}O_2$	144.21	19.87	-3.054
16.41	Dodecanamide	$C_{12}H_{25}NO$	199.3330	19.81	0.844
16.61	7-Nonenamide	$C_9H_{17}NO$	155.24	32.12	-2.790
16.61	8-Methyl-6-nonenamide	$C_{10}H_{19}NO$	169.2640	32.12	-3.269
16.61	3-Cyclohexylpropionamide	$C_9H_{17}NO$	155.24	32.12	-4.817
16.61	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281.4766	32.12	0.207
16.61	Citronellol epoxide (R or S)	$C_{10}H_{20}O_2$	172.26	32.12	-5.185
19.13	Cyclopentadecanone, 4-methyl-	$C_{16}H_{30}O$	238.41	1.68	-3.486
19.13	cis-1,2-Cyclododecanediol	$C_{12}H_{24}O_2$	200.322	1.68	-4.627
19.13	Oleic acid	$C_{18}H_{34}O_2$	282.5	1.68	1.482
19.13	cis-Vaccenic acid /6-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	282.4614	1.68	0.700
19.58	cis-9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254.4082	0.90	1.950
19.58	2-Hexadecenoic acid, methyl ester, (E)-	$C_{17}H_{32}O_2$	268.4	0.90	1.624
19.58	ç-Thionodecalactone	$C_{10}H_{18}OS$	186.313	0.90	-2.726
19.69	Z-7-Tetradecenol,trimethylsilyl ether	$C_{17}H_{36}OSi$	284.6	1.40	0.918
19.69	12-Hydroxydodecanoic acid	$C_{12}H_{24}O_3$	216.3172	1.40	1.948
19.69	Cyclohexanone, 2-ethyl-4-methoxy-	$C_9H_{16}O_2$	156.22	1.40	-4.751
19.96	2-Hydroxyhexadecyl butanoate	$C_{20}H_{40}O_3$	328.5298	6.29	-1.585
19.96	2-Acetylamino-3-hydroxy-propionic acid	$C_5H_9NO_4$	147.13	6.29	-4.642
19.96	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	$C_{11}H_{16}O_4$	212.2423	6.29	-2.998
19.96	Alpha-l-rhamnopyranose	$C_6H_{12}O_5$	164.16	6.29	-5.918
19.96	9Z,12Z,15Z)-9,12,15-Octadecatrienoic acid-2-trimethylsilyloxy-1-[(trimethylsilyloxy)methyl]ethylester	$C_{27}H_{52}O_4Si_2$	496.8704	6.29	-0.885
20.49	7-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	268.4348	2.29	-1.969
20.49	Dodecanoic acid, 3-hydroxy-	$C_{12}H_{24}O_3$	216.32	2.29	1.020
20.90	Methyl 12-oxo-9-dodecenoate	$C_{13}H_{22}O_3$	226.3120	0.94	0.639
23.88	Oxirane, [(hexadecyloxy)methyl]-	$C_{19}H_{38}O_2$	298.511	3.31	1.103
23.88	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$	296.5310	3.31	-3.027
23.88	Carbonic acid, allyl pentadecyl ester	$C_{19}H_{36}O_3$	312.5	3.31	-1.438
23.88	Carbonic acid, allyl hexadecyl ester	$C_{20}H_{38}O_3$	354.5	3.31	-1.623
23.88	Carbonic acid, allyl octadecyl ester	$C_{22}H_{42}O_3$	354.6	3.31	-1.331
25.32	9-Octadecenoic acid (Z)-, phenylmethyl ester	$C_{25}H_{40}O_2$	372.6	1.63	-2.463
25.32	2,3-Dihydroxypropyl elaidate	$C_{21}H_{40}O_4$	356.5	1.63	-2.933

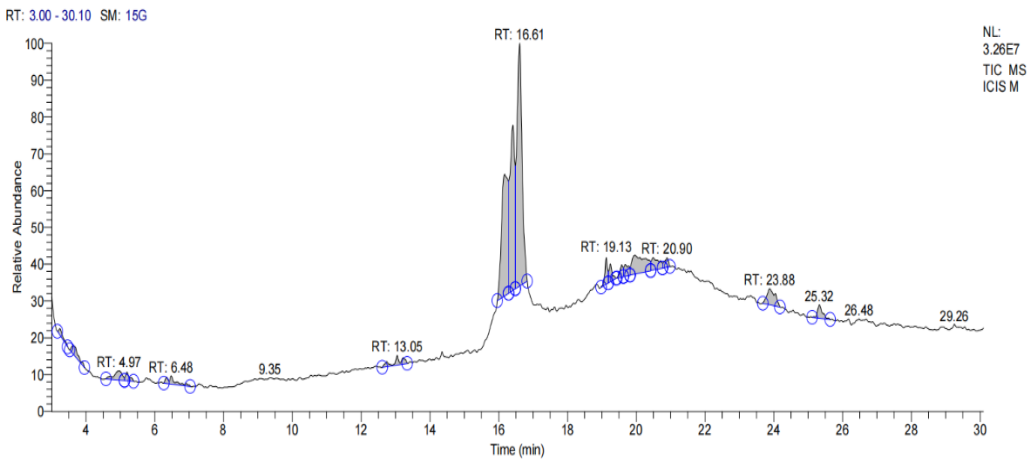
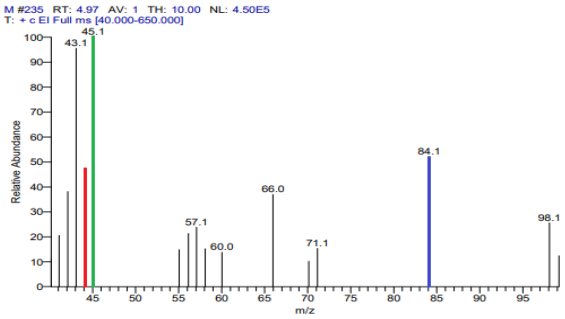
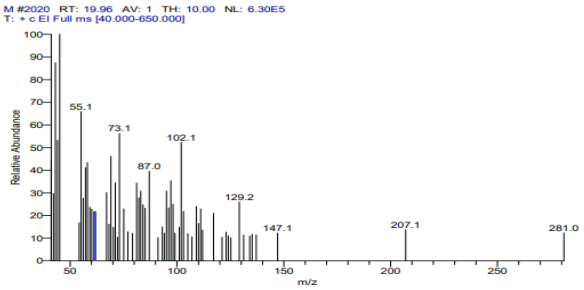


Fig. 2: Chromatogram of MCA.



(A)



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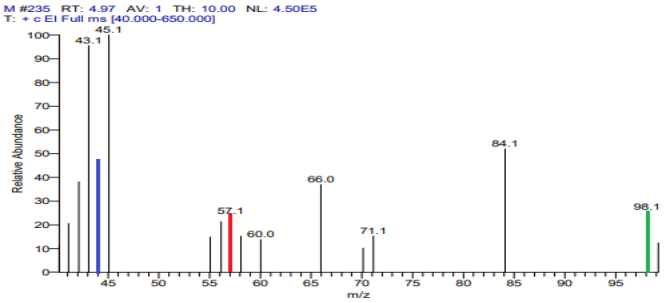

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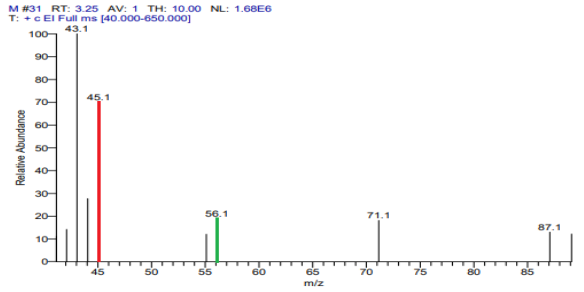

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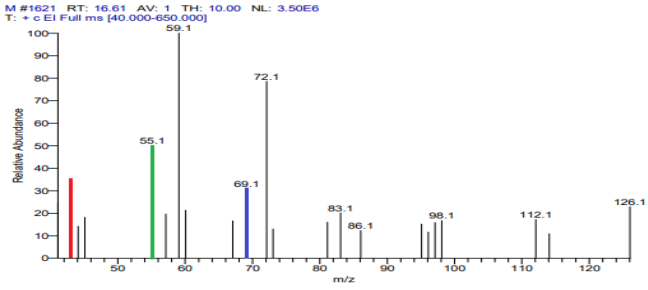

(E)

Fig. 3: Mass spectrum of (A) azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl)-, (B) alpha-l-rhamnopyranose, (C) piperidine, 3-methyl-, (D) 1,4-Dioxane-2,5-dione,3,6-dimethyl (E) citronellol epoxide (R or S)

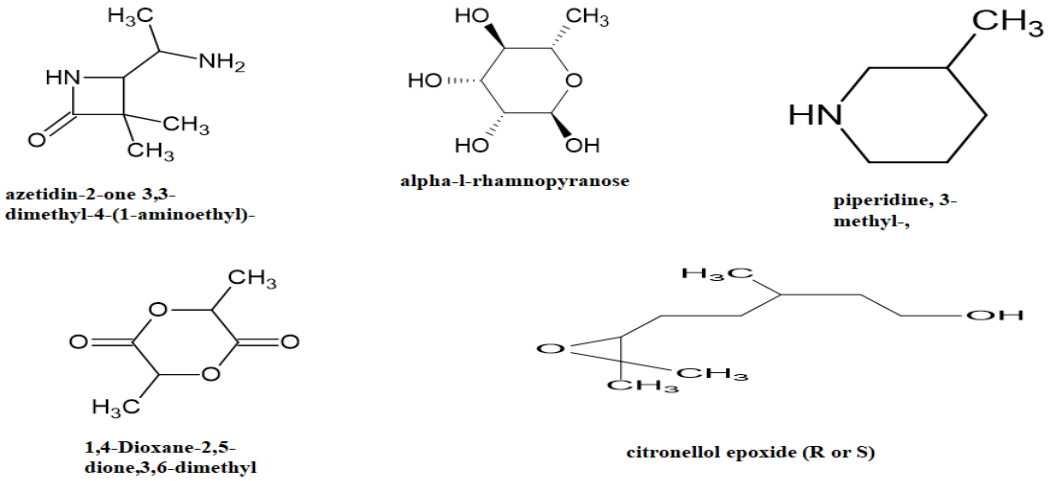

Fig. 4: Structure of selected lead compounds analysed by GC-MS

Table 2
Analysis of molecular docking of selected compounds of MCA against α-glucosidase (PDB ID:5NN5)

S.N.	Compound	Number of hydrogen bonds	Hydrogen bond interacting amino acids with hydrogen bond distance
1.	Azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl)-	3	ASP616 (1.76), HIE674 (1.93), ASP518 (1.93)
2.	Alpha-l-rhamnopyranose	4	ASP616 (2.35), HIE674 (1.84), ASP404 (1.73), ASP518 (1.77)
3.	Piperidine, 3-methyl-	1	ASP518 (1.65)
4.	1,4-Dioxane-2,5-dione,3,6-dimethyl	1	HIE674 (1.94)
5.	Citronellol epoxide (R or S)	4	ASH443 (1.76), ASP518 (1.64), TRP481 (2.14), HIE674 (1.63)

Table 3
Drug-likeness violations of chosen lead compounds

S.N.	Best hit molecules	MW	Donor HB	Accpt HB	QPlogP o/w	QPP Caco	QPlog HERG	QPlogBB	% Human Oral Absorption
1	Azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl)-	142.200	3	3	-0.686	85.485	-2.286	-0.108	57.506
2	Alpha-l-rhamnopyranose	164.158	4	8.5	-1.500	246.734	-2.502	-0.953	60.977
3	1,4-Dioxane-2,5-dione,3,6-dimethyl	144.127	0	6	-1.421	1312.992	-1.275	-0.204	69.984
4	Piperidine, 3-methyl-	99.175	1	1.5	0.839	740.642	-3.160	0.746	87.667
5	Citronellol epoxide (R or S)	172.267	1	3.7	1.676	3673.660	-2.351	-0.134	100

MW: Molecular weight; donorHB: donors of hydrogen bond; accptHB: acceptors of hydrogen bond; QPlogPo/w: octanol/water partition coefficient; QPPCaco: Cell permeability; QPlogHERG: IC_{50} value to block HERG K+ channels; QPlogBB: brain/blood barriers.

Prediction of ADME properties: The best hit molecules i.e. azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl)-, alpha-l-rhamnopyranose, piperidine, 3-methyl-, citronellol epoxide (R or S) and 1,4-Dioxane-2,5-dione,3,6-dimethyl were predicted for pharmacokinetics properties in order to get rid of undesirable drug-like candidates utilizing Qikprop. ADME features identify ligand compounds that have drug-like activity depending on Lipinski's rule of five[13]. The hit molecules adhered to Lipinski's rule of five because of their low molecular weight (less than 500 kDa), low hydrogen bond acceptors (less than 10), low hydrogen bond donors (less than 5) and estimated octanol/water partition coefficient (QPlogPo/w) (less than 5)[14]. All the characteristics were found within the range.

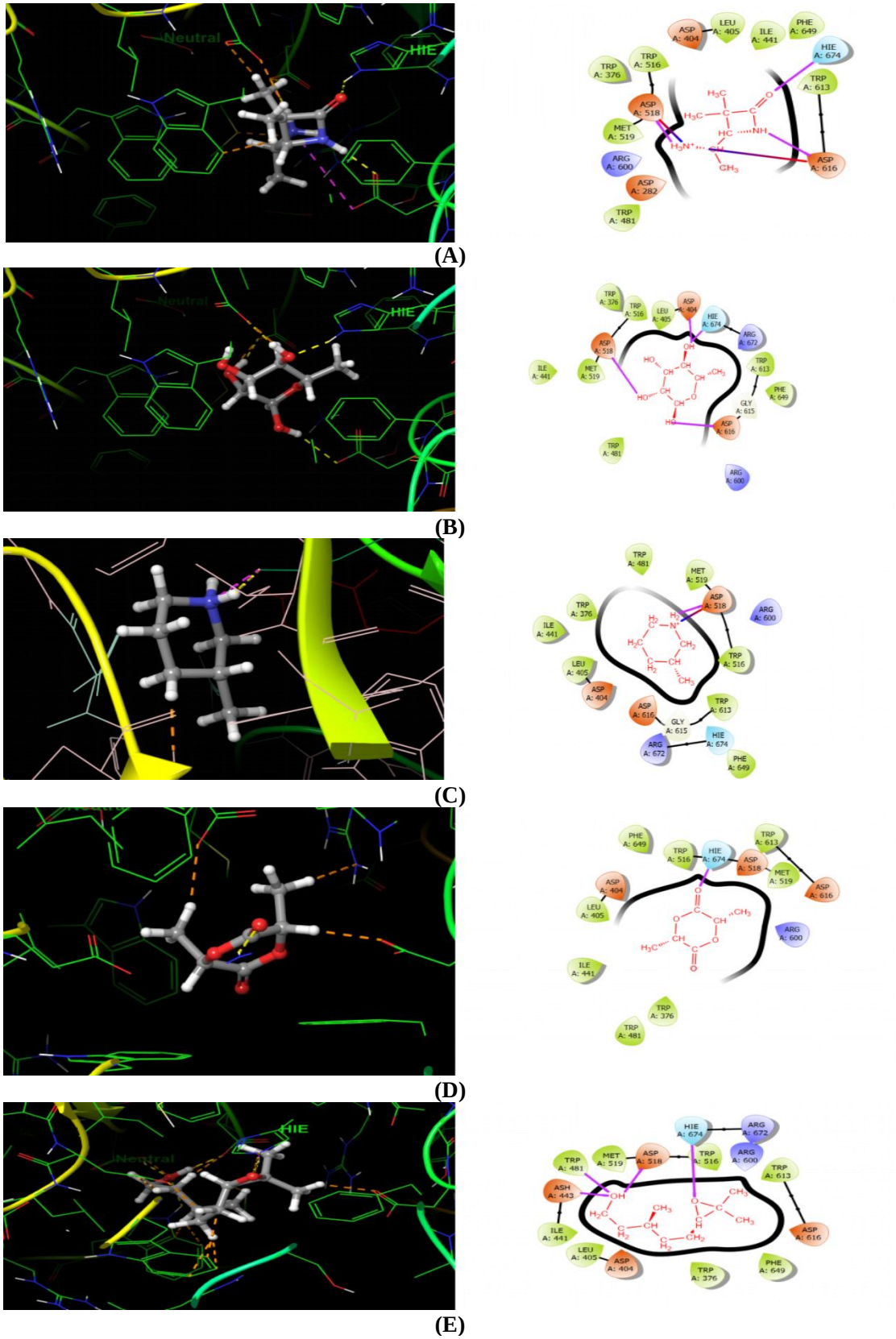

Fig. 5: 3D and 2D representation of molecular interaction of selected compounds of MCA (A) azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl)-, (B) alpha-l-rhamnopyranose, (C) piperidine, 3-methyl-, (D) citronellol epoxide (R or S) and (E) 1,4-Dioxane-2,5-dione,3,6-dimethyl with *α*-glucosidase

Along this, cell permeability (QPPcaco2), an important determinant of drug metabolism and its accessibility to biological membranes, ranged from 85.485 to 3673.660 (permitted range: 500 is great and 25 is poor). IC_{50} value to block HERG K+ channels ranged from -1.275to -3.160 (permitted range: below –5), estimated brain/blood barriers

was within the tolerable range of -2.023 to -0.573 and oral human absorption ranged from 57.506-100 (less than 25% is bad). All the pharmacokinetics properties of hit molecules, fall inside the acceptable range, making them highly recommended for drug development as depicted in table 3.

Conclusion

This study evaluated the enzyme inhibitory action, anti-diabetic properties and phytoligands contained in the MCA using GC-MS. It demonstrated greater enzyme inhibition and anti-diabetic properties. Investigations into metabolic profiling have inferred the existence of 60 phytoligands in MCA depending on the m/z molecular ions that were discovered in GC-MS. According to molecular docking studies, the phytochemicals azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl)-, alpha-l-rhamnopyranose, piperidine, 3-methyl-, citronellol epoxide (R or S) and 1,4-Dioxane-2,5-dione,3,6-dimethyl have the potential to interact with the enzymes α-glucosidase.

The present study came to the conclusion that the extract of MCA showed several different components due to the synergistic effects. The molecular screening suggests that azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl)-, alpha-l-rhamnopyranose, piperidine, 3-methyl-, 1,4-Dioxane-2,5-dione,3,6-dimethyl and citronellol epoxide (R or S) should be taken into consideration as potential compounds with anti-diabetic efficacy. However, additional research is needed to characterize and purify these compounds and pinpoint the molecular basis of their anti-diabetic action for development of forthcoming therapies.

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