Identification of α-Glucosidase Inhibitors from *Cyperus articulatus* L. Rhizome Extract Using HRLC-MS/MS and Molecular Docking

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Cyperus articulatus is widely distributed in Indian subcontinent and its rhizome has been used as folk medicine in different geographical regions for treatment of diseases like malaria, epilepsy and dysentery. In present study the rhizome extracts were studied to identify the natural inhibitor compounds for α-amylase and α-glucosidase. Among six different solvent extracts of the rhizome, the acetone extract which showed to have highest phenolics and flavonoids exhibited α-glucosidase inhibition (IC$_{50}$ 9.1 µg/mL). Different fractions were collected using column chromatography and flash chromatography to analyze the active fractions. Two major fractions significantly showed high enzyme inhibitory activity. In HRLC-MS/MS phenolic as well as non-phenolic class of compounds were identified such as quercetrin, dihydroquercetin, mycophenolic acid, koparin-2-methylether, C16-sphinganine, embelin, phytosphingosine, colforsin, 7,8-dihydroxystearic acid and palmitic acid derivative. The two active fractions having IC$_{50}$ 8.38 and 7.65 µg/mL, were shown to exhibit competitive and mixed type inhibition, respectively. Molecular docking analysis of the compounds with the α-glucosidase active site showed that the phenolic class of compounds have efficient binding with one of the aspartate (Asp66, Asp349 and Asp212) residues whereas there was non-polar contacts with other residues in case of non-phenolic compounds such as long chain hydroxyl acids. The results suggest that *Cyperus articulatus* rhizome is a potential source of drug ingredients for the management of type-II diabetes.

Keywords: *Cyperus articulatus*, Secondary metabolites, α-Glucosidase, Enzyme inhibition. Molecular docking.

INTRODUCTION

Type I diabetes occurs due to the lack of synthesis of insulin by β-cells of the pancreas, while type II diabetes is characterized by insulin resistance (peripheral cells do not respond to insulin) or less secretion of insulin from β-cell [1]. α-Amylase in the pancreatic juice and saliva primarily breaks down large insoluble starch into absorbable monosaccharides and other small molecules [2]. Whereas mammalian α-glucosidase in small intestine catalyzes the end step of digestion of starch and oligosaccharides in the diet. It hydrolyzes α-(1-4)-linked D-glucose residues of non-reducing end of sugars [3]. Inhibitors of α-amylase and α-glucosidase delay the breaking down of sugars in the small intestine and controls the postprandial hyperglycemia [4,5]. The management of blood glucose level (glycemic index) is the hallmark in the treatment of type II diabetes. The use of oral hypoglycemic drugs is considered one of the less invasive way for management of diabetes mellitus (type II). Many inhibitory compounds have been isolated from

different medicinal plants to serve as an alternative drug which can retard the postprandial blood glucose level in a person suffering from diabetes. With time modern researches find drugs from natural sources with increased potential activity and lesser adverse effects than existing synthetic drugs [5,6].

Inhibition of α-glucosidase has been earning more attention in current research [2]. Many synthetic, as well as natural inhibitors reported in several literatures, are sugar-like or derivatives of sugar moieties. Acarbose (approved for treatment of type II diabetes) is a commercially available inhibitor of α-glucosidase for the management of type II diabetes. Long term administration of the synthetic drugs (like sulfabenzamide, acarbose and miglitol) to control hyperglycemia have been reported to affect adversely and results in abdominal pain, diarrhea and flatulence [7,8]. The drugs which have resemblance with non-hydrolyzable sugar moieties were reported to have high inhibitory activity against pancreatic α-amylase but low inhibitory activity against intestinal α-glucosidase. Under such circumstances, starch gets fermented by intestinal bacteria under anae-

robic conditions causing many side effects [8,9]. Thus it is challenging to study on natural products of non-sugar derivatives, such as polyphenols/flavonoids which have high natural abundance and potential antioxidant capacity [9,10]. The present study is focused on finding new α-glucosidase inhibitors and/or exploring the chosen plant as an economically better source of drug ingredients.

The natural sources like rhizomes along with other plant products are endowed with variety of properties which have been derived from the Atharva Veda [11,12]. Many noble compounds which in one way or the other are proved to be life-saving and have significant biological functions are reported to be structural analogues to a plant metabolite or its derivative product. The importance of plants and its polyphenols and flavonoid class of compounds were earlier reported to inhibit α-glucosidase [13-15]. Also, the influence of many other unidentified compounds in the crude extract present at various concentrations cannot be ignored. Researches on α-glucosidase inhibitors have been challenging these days as the available drugs for the said purpose are associated with many adverse effects when they are consumed in long term. In this work, a chromatographic study along with other instrumental techniques was employed to identify the bioactive compounds which may explore the arena of research on new class of natural inhibitors for α-glucosidase.

Plants belong to Cyperaceae family are widely distributed in the tropical and subtropical of the world (http://powo. science.kew.org/). The rhizome of the matured plant appears like bulb (dark black to brown outside and light brownish-white inside). The plant rhizome has been used as folk medicine. The rhizome of Cyperus from various geographical regions (distributed in regions) have been reported to be used as folk medicine many Cyperus species are reported to possess varieties of phenolics, flavonoids, alkaloids and terpenoids and have different biological activities like antioxidant, antidiabetic, antimicrobial, anti-inflammatory and many other health beneficial properties [16-19].

Though several species of Cyperus have been studied for their biological importance and scientifically validated, *Cyperus articulatus* is least explored. The *Cyperus articulatus* plant rhizome has been used as folk medicine for the treatment of several ailments like malaria, epilepsy and dysentery in different parts of the world [20]. The essential oil was reported to have antimicrobial, anticonvulsant and antimalaria properties [20-23]. The purpose of the present work is to study the usefulness of different bioactive metabolites (other than essential oils) from rhizome of *Cyperus articulatus* through enzyme (α-amylase and α-glucosidase) inhibition properties of various solvent extracts and to explore its pharmaceutical and medicinal applications.

EXPERIMENTAL

Plant material and extraction of metabolites: *Cyperus articulatus* was collected at their full grown maturity stage from the riverbanks of Cauvery river, Mysore, India. The identification (of the herbarium) was done at Botanical Survey of India (BSI), Kolkata, India. The rhizomes, collected were washed and dried at 45 ºC for 3-4 days and was grinded to make course

powder. Soxhlet apparatus was used for solvent extraction. Solvents with increasing polarity order (hexane < chloroform < ethyl acetate < acetone < methanol < water) were used to extract the metabolites. The rhizome extracts were concentrated using rota-evaporator (Buchi R-205, Switzerland) dissolved in a common solvent, methanol except water extract which was dissolved in water to prepare stock solutions in mg/mL. For enzyme inhibition assays, the dilutions were made in Millipore water.

Total phenol and flavonoids: The rhizome extract (25 µL) was added to diluted (1:9) Folin-Ciocalteu reagent (100 µL) in a 96 well micro plate (96-WMP) and incubated for 5 min. Then 1% Na_2CO_3 solution (75 µL) was added and incubated at 30 ºC for 2 h. Gallic acid was used as standard. Absorbance was read at 760 nm [24]. The results were expressed as µg GAE/mg crude extract. Total flavonoid content was determined spectrophotometrically by recording the absorbance of the mixture of the sample solution with $AlCl_3$ (2%) in methanol in 1:1 ratio at 415 nm after 15 min incubation at 30 ºC [25]. A blank was maintained without $AlCl_3$. Using quercetin (QCT) as standard the results were expressed as µg QCTE/mg crude extract.

Enzyme inhibitory assays

α-Amylase inhibition: The rhizome extract (100 µL) was mixed with 100 µL of α-amylase (2 U/mL in phosphate buffer, pH 6.9) and incubated for 10 min. Then 100 µL 1% starch solution was added and incubated for 30 min at 30 ºC. Freshly prepared DNS reagent (1 mL) was added to each test samples and heated in boiling water bath for 5-10 min. A sample was maintained adding all reagents except the rhizome extract. Samples were cooled and the absorbance was read at 540 nm. The result was reported as percentage inhibition data [24,26].

α-Glucosidase inhibition: The rhizome extract (50 µL) was added to 50 µL α-glucosidase (1 U/mL in phosphate buffer pH 6.8) in a 96-WMP and incubated for 10 min. Then 3 mM reduced glutathione (50 µL) and 50 µL 10 mM 4-nitrophenyl α-D-glucopyranoside (PNPG) were added and incubated for 20 min at 35 ºC. The reaction was terminated by adding 0.2 M sodium carbonate (50 µL). The sample and blank absorbance were read at 400 nm [24,27]. The inhibitory potential of rhizome extract was expressed as percentage data.

HRLC-MS/MS analysis of *Cyperus articulatus* rhizome extract: The experiment was performed using RP-C18 column (LCMS-Waters SYNAPT G2 with 2D nano ACQUITY System) mass spectrometer coupled to UV-Vis detector. 0.5 mL/min flow rate was used with an injection volume of 5 µL. The mobile phase was solvent A (methanol) and solvent B (1% formic acid in water). Gradient phase was maintained for 55 min. The analysis was done in both positive and negative mode. The mobile phases were 0.1% formic acid in water (A) and 90% acetonitrile in water with 0.1% formic acid (B) [28]. The LC conditions were 5% B during 0-3 min, a linear increase from 5% to 20% B during 3-25 min, from 20% to 40% B during 25-40 min and from 40% to 50% B during 40-55 min, finally from 50% to 95% B during 55-63 min followed by 15 min of maintenance. Injection volume 5 µL and flow rate 0.4 mL/min. For ESI, the scan was collected in the Orbitrap at a resolution of 30,000 in an *m/z* range of 50-1,500.

Quercitrin (m.w.: 448.1): m/z 448.1 corresponding to m.f.: $C_{21}H_{20}O_{11}$, HRLC-MS/MS major peaks (positive mode): m/z 449.1 $[M+H]^+$, 450.1 $[M+2H]^+$, 287.05 (100%), 288.05.

Dihydroquercetin (m.w.: 304.05): m/z 304.05 corresponding to m.f.: $C_{15}H_{12}O_7$, HRLC-MS/MS major peaks (positive mode): m/z 287.05 (100%) $[M+H-H_2O]^+$, 288.07, 124.08.

Koparin 2'-methyl ether (m.w.: 314.07): m/z 314.075 corresponding to m.f.: $C_{17}H_{14}O_6$, HRLC-MS/MS major peaks (positive mode): m/z 315.075 (100%) $[M+H]^+$, 316.08 $[M+2H]^+$, 124.08.

Mycophenolic acid (m.w.: 320.12): m/z m.w.: 320.12 corresponding to m.f.: $C_{17}H_{26}O_4$, HRLC-MS/MS major peaks (positive mode): m/z 325.1 (100%) $[M+Na-H_2O]^+$, 326.1, 124.08.

Phloionolic acid (m.w.: 332.25): m/z m.w.: 332.25 corresponding to m.f.: $C_{18}H_{36}O_5$, HRLC-MS/MS major peaks (negative mode): m/z 313.23 (100%) $[M-H-H_2O]^-$, 325.18, 293.2, 123.1.

7,8-Dihydroxystearic acid (m.w.: 316.25): m/z m.w.: 316.25 corresponding to m.f.: $C_{18}H_{36}O_4$, HRLC-MS/MS major peaks (negative mode): m/z 297.24 (100%) $[M-H-H_2O]^-$, 298.24, 183.13.

Ribitol (m.w.: 152.06): m/z m.w.: 152.06 corresponding to m.f.: $C_5H_{12}O_5$, HRLC-MS/MS major peaks (positive mode): m/z 175.05 $[M+Na]^+$, 176.05, 173.07 $[M+Na-2H]^+$, 140.06, 59.05.

C16-Sphinganine (m.w.: 273.26): m/z m.w.: 273.26 corresponding to m.f.: $C_{16}H_{35}NO_2$ HRLC-MS/MS major peaks (positive mode): m/z 274.27 (100%) $[M+H]^+$, 275.27 $[M+2H]^+$, 535.25, 124.08.

Phytosphingosine (m.w.: 317.29): m/z m.w.: 317.29 corresponding to m.f.: $C_{18}H_{39}NO_3$, HRLC-MS/MS major peaks (positive mode): m/z 318.3 (100%) $[M+H]^+$, 319.3 $[M+2H]^+$, 274.27, 124.08.

Lagochilin (m.w.: 356.25): m/z m.w.: 356.25 corresponding to m.f.: $C_{20}H_{36}O_5$, HRLC-MS/MS major peaks (positive mode): m/z 357.26 (100%) $[M+H]^+$, 358.26 $[M+2H]^+$, 342.2, 293.2, 124.08.

N-(2-hydroxyethyl)palmitamide (m.w.: 355.34): m/z m.w.: 355.34 corresponding to m.f.: $C_{22}H_{45}NO_2$, HRLC-MS/MS major peaks (positive mode): m/z 356.23, 338.34 (100%) $[M+H-H_2O]^+$, 339.34, 343.12, 326.37.

Embellin (m.w.: 294.18): m/z m.w.: 320.12 corresponding to m.f.: $C_{17}H_{20}O_6$, HRLC-MS/MS major peaks (negative mode): m/z 293.17 (100%) $[M-H]^-$, 280.94, 179.9.

Kinetics study of α-glucosidase and rhizome extract active fractions: The active fractions collected in chromatographic separation were studied for the kinetics of enzyme (α-glucosidase) inhibition. The substrate PNPG(α) in the concentration range of 0.5-3.5 mM was used for monitoring enzyme hydrolysis. About 1U/mL of α-glucosidase was tested in the absence and presence of different concentrations of active fraction. The spectrophotometric study was performed at 400 nm for 30 min with measurement at every 0.2 min interval. The enzyme inhibition Michaelis-Menten plots along with Lineweaver-Burk plots were used to determine the type of inhibition by the active fractions of different extracts. For making Lineweaver-Burk plot 15.6 µg/mL inhibitor (active fraction) was mixed with the enzyme before performing kinetics experiment [15].

Molecular docking: Crystal structure of yeast α-glucosidase (RCSB PDB id: 3A4A) [29] was downloaded and the active site coordinate was assigned using the active site prediction facility, (http://www.scfbio-iitd.res.in/dock/ActiveSite.jsp). Structure of the selected molecules was drawn with the help of Marvin sketch and saved as the 3D structure with all explicit H-atom. In the experimental structure, water molecules and other ligands were removed. The docking study was performed with ParDOCK software (SCFBio, IIT Delhi, India) (http://www.scfbio-iitd.res.in/dock/pardock.jsp) in which the binding energy of each partner is based on Monte Carlo docking procedure [30] and was reported in kcal/mol.

RESULTS AND DISCUSSION

Biological activity and bioactive metabolite analysis: The acetone extract of *Cyperus articulatus* was found to have highest total phenolic (207.5 µg GAE/mg extract) and total flavonoid (105.6 µg QCTE/mg extract) contents among all solvent extracts (Fig. 1) followed by ethyl acetate extract. The hexane, chloroform and water extracts were found to contain less amount of phenolics/flavonoids in the quantification experiment. With successive steps of Soxhlet extraction, the ethyl acetate and acetone solvents may have been the suitable for the extraction of all the major phenolic metabolites. The
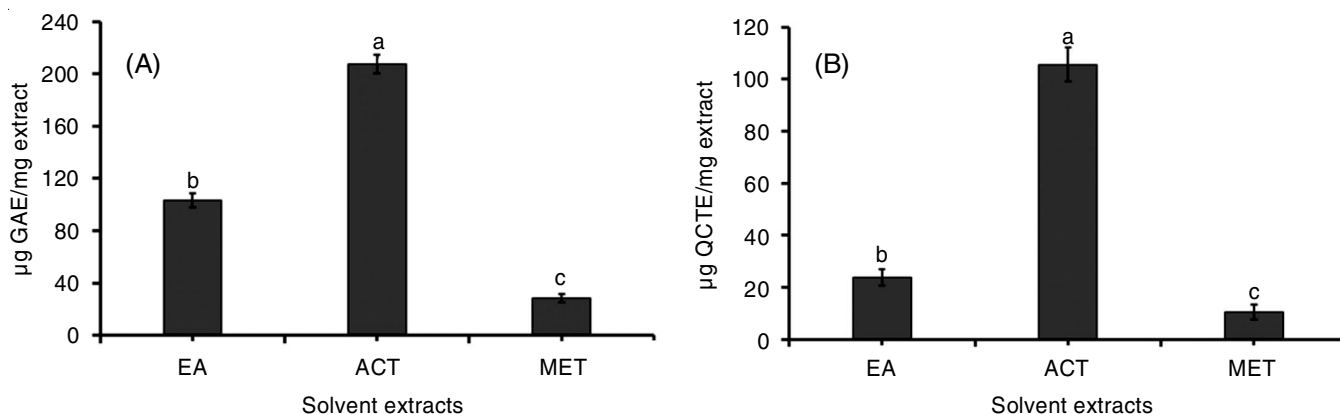

Fig. 1. Total phenolics (µg GAE/mg extract) (A) and total flavonoid (µg QCTE/mg extract) (B) quantified in different solvent extracts of C. *articulatus* rhizome (EA: Ethyl acetate, ACT: Acetone, MET: Methanol). The hexane, chloroform and water extracts showed little presence of such class of compounds. Results are mean values of three determinations ± SD (with significance P < 0.05)

acetone extract has shown α-glucosidase inhibitory activity (IC$_{50}$ 9.1 μg/mL) where 62.5 μg/mL crude extract was sufficient to inhibit 95% of enzyme activity (Fig. 2). No rhizome extracts shown α-amylase inhibition in the *in vitro* study. The result revealed that the metabolites present in acetone extract have high α-glucosidase and low/no α-amylase inhibition. As discussed earlier, controlling the excessive expression of enzyme the glycemic index can be managed in a less invasive way. For such purpose a non-sugar type structure, preferably the phenolic molecules may be chosen. Hence the metabolites of acetone extract could be the potential inhibitors of α-glucosidase envisaging the rhizome as a source of ingredients for antidiabetic drug formulation.

The acetone extract was subjected to exhaustive column chromatography (silica gel: mesh size 60-120) using chloroform
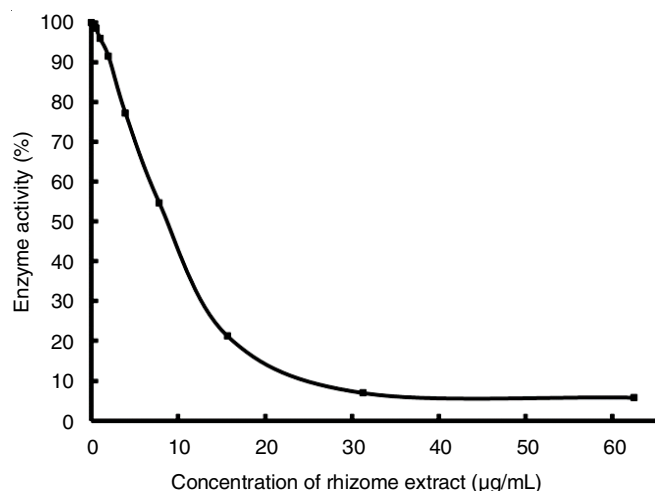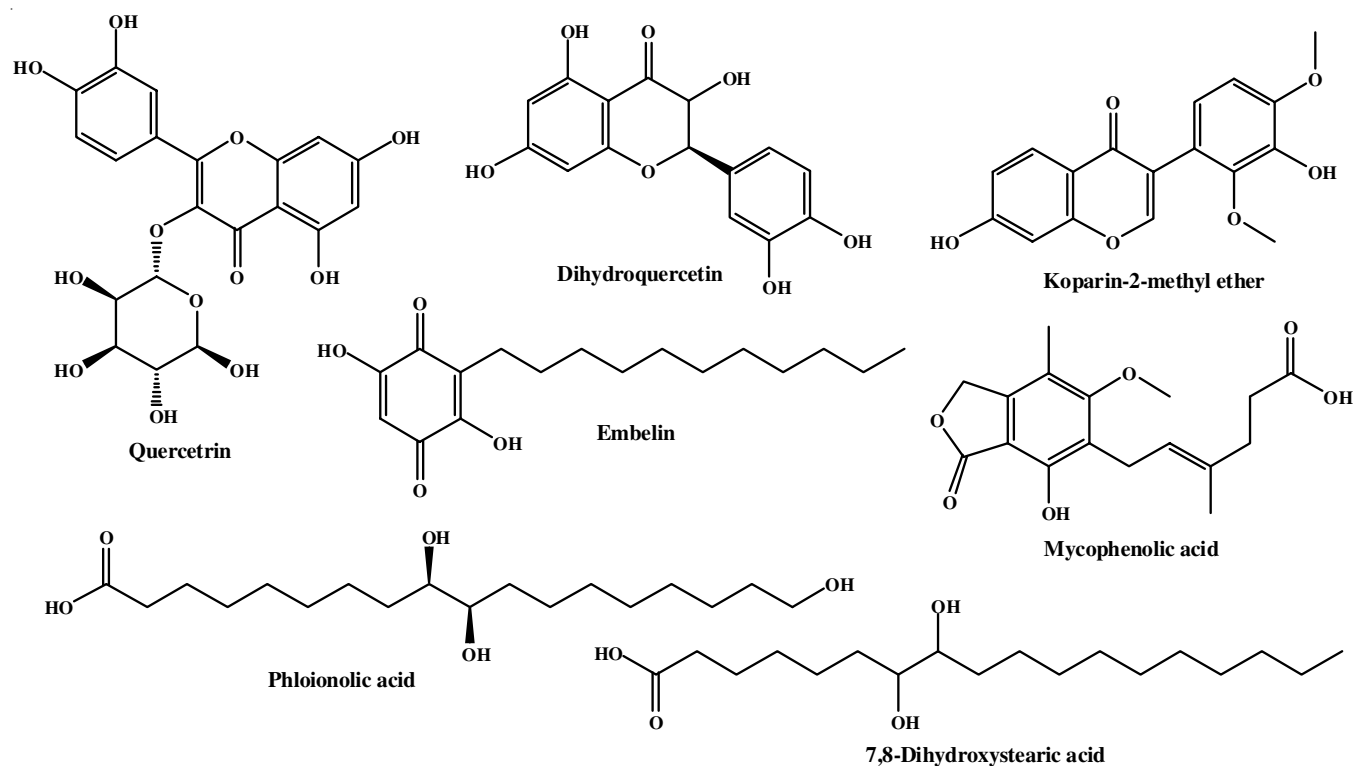

Fig. 2. α-Glucosidase activity plot in presence of increasing concentration of acetone extract *C. articulatus*. (IC$_{50}$ = 9.1 μg/mL)

gradually enriched with methanol to obtain eight different fractions. The fractions were then concentrated and tested for enzyme inhibition potential. The 5th and 8th fraction showed high inhibition (IC$_{50}$ 8.38 and 7.65 μg/mL, respectively) against α-glucosidase. Then the active fractions were further separated using flash chromatography. Elution of different fractions was performed using gradient mobile phase, chloroform and methanol (0% methanol to 100% methanol with increasing polarity). Each fraction of 20 mL was collected and the active fractions (showing α-glucosidase inhibition) were subjected to HRLC-MS/MS analysis.

The major compounds identified from the 5th active fractions were dihydroquercetin, quercitrin, mycophenolic acid and koparin-2-methyl-ether. In 8th fraction, the metabolites detected were hydroxy long chain acids such as, embelin, lagochilin, c-16 sphinganine, phytosphingosine, ribitol, *N*-(2-hydroxyethyl)palmitamide, phloionolic acid and 7,8-dihydroxy-stearic acid (Fig. 3). The HRLC-MS/MS analysis showed that there were two different class of compounds (phenolics and non-phenolics) responsible for enzyme inhibition.

The phenolic compounds and flavonoids like quercetin, taxifolin, luteolin have been reported to have α-glucosidase inhibition properties [15,31,32]. Different lipo-soluble acids like stearic acid, oleic acid, palmitic acid, *etc*. and other fat soluble compounds were also reported to have such activities [27,33,34]. In present study, apart from the reported compounds (flavonoids and fatty acids) for similar activities we have first time proposed the role of molecules like embelin, ribitol, phytosphingosine, c-16 sphinganine, lagochilin, 7,8-dihydroxy stearic acid and phloionolic acid in influencing the potential α-glucosidase enzyme activity.

Antidiabetic properties of rhizome extracts of *Cyperus rotundus* was studied by Raut and Gaikwad [35] in alloxan

Quercetrin

Dihydroquercetin

Koparin-2-methyl ether

Embelin

Mycophenolic acid
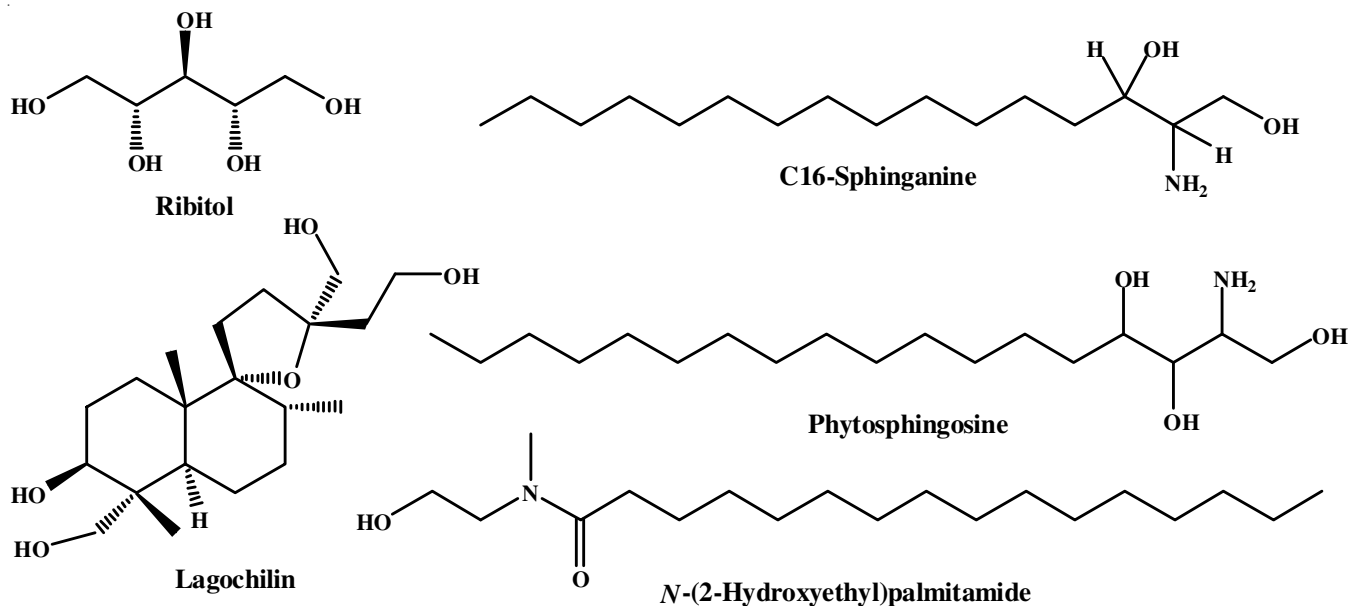
Phloionolic acid

7,8-Dihydroxystearic acid

Fig. 3. Phenolic and non-phenolic compounds detected in HRLC-MS/MS analysis of the two active fractions (showing α-glucosidase inhibition) identified from acetone extract of *C. articulatus* rhizome

monohydrate induced diabetic rats. Oral administration of acetone extract at 300 mg/kg significantly reduced blood glucose level which was comparable to metformin (450 mg/kg). Hence further experiments and animal studies may give a convincing result regarding the oral administration of extracted metabolites of *Cyperus articulatus*.

Kinetics of α-glucosidase inhibition by active fractions of rhizome extracts: In enzyme inhibition study, it was found that there are broadly two class of compounds responsible for inhibitory activity. One active fraction was rich in phenolic class of compounds (quercitrin, dihydroquercetin, koparin-2-methyl ether mycophenolic acid) whereas the other active fraction had non-phenolic long chain hydroxyl acids and poly hydroxyl compounds. These two fractions were separately studied for their mode of action on the enzyme function. The kinetic study revealed that the 5th fraction was exhibiting a competitive type inhibition and the 8th fraction showed a mixed type of inhibition (Fig. 4). Quercetin was reported to competi-

tively inhibit α-glucosidase whereas dihydroquercetin works non-competitively [15] and different flavonoids were also individually studied to show competitive, non-competitive and mixed inhibition. Proenca *et al*. [15] proposed that the C-ring substitution in flavonoids and the planarity of molecule plays a crucial role in deciding the mode of inhibition. In the present study, acetone extract active fraction contained few known flavonoids and quercitrin (a glycosylated derivative of parent compound quercetin) along with other phenolics (Fig. 3). The overall effect may be due to the most potent molecule or a combination interactive forces of all the molecules on the enzyme, which leads to competitive inhibition. The next active fraction was found to be better than the flavonoid/phenolics class having lower IC_{50} (7.65 μg/mL). The molecules identified were mostly long chain fatty acid derivatives, sphingosine classes and hydroxyl compounds. This fraction also showed nearly competitive or a mixed type inhibition. So the search for suitable inhibitors having non-sugar moiety may reach these simple and small
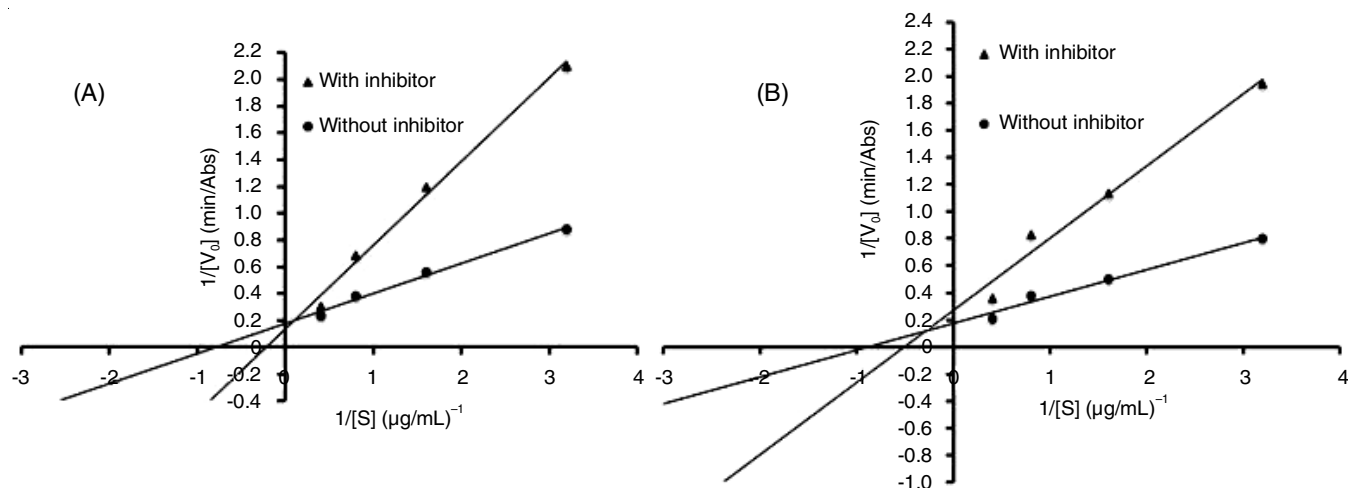

Fig. 4. Mode of α-glucosidase inhibition by inhibitors present in the active fraction of acetone extract of *C. articulatus*. Line-weaver-Burk plots: Band 5 shows competitive inhibition (A), and the band 8 shows a mixed type of inhibition (B)

molecules which will be an efficient and economical way to carry out advanced studies in drug design.

Docking study of lead bioactive metabolites: Docking approach helped to understand the interaction of enzyme (α-glucosidase) and the inhibitor compounds. The active site of the enzyme α-glucosidase (3A4A) contains amino acid residues Asp349, Asp212, Arg439, His277, Glu274 and Asp66. The catalytic residues are Glu274, Asp349, and Asp212 and the binding energy of ligand protein systems was calculated for the best fit structure in the active pocket.

The theoretical prediction of binding energy for the possible inhibitors are given in Table-1. Among the possible small molecule inhibitors, significant binding affinities were shown by *N*-(2-hydroxyethyl)palmitamide (-9.4 kcal/mol) followed by 7,8-dihydroxystearic acid, embelin, stearic acid, phloionolic acid, mycophenolic acid, quercitrin, c-16 sphinganine, dihydroquercitin and koparin-2-methylether. The long chain hydroxyl compounds were shown to have higher binding affinity than small inhibitory molecules. The commercially available drug acarbose also studied and found to have a high binding affinity (-10.72 kcal/mol) towards α-glucosidase active site. The molecular docking study showed that the phenolic compounds like quercitrin bind to the nucleophile aspartate (Asp 349 and Asp 212) in the active site of α-glucosidase and this may block the site from substrate interaction thus inhibits the enzyme-substrate reaction. However, the polyhydroxy compounds and fatty acids stabilize the active site by hydrophobic interactions with other amino acid residues other than the catalytic residues and forms hydrogen bond with glutamate residues.

TABLE-1
THEORETICAL BINDING ENERGY OF α-GLUCOSIDASE
ENZYME AND LIGANDS (LEAD MOLECULES) INTERACTION

Compounds	Binding energy (kcal/mol)
Quercitrin (quercetrin)	-6.81
Dihydroquercetin	-5.81
Mycophenolic acid	-7.13
Embelin	-7.85
Koparin-2-methyl ether	-5.55
Lagochilin	-6.57
Phloionolic acid	-7.32
7,8-Dihydroxy stearic acid	-8.99
Dihydrosphingosine	-6.54
Phytosphingosine	-6.86
N-(2-Hydroxyethyl)-palmitamide	-9.40
Acarbose	-10.72

Quercitrin binds to the main catalytic residues Asp212 with B-ring hydroxyl group and Asp349 along with C-ring carbonyl group. The ideal binding in the active site supports the quercitrin derivatives as a suitable α-glucosidase inhibitor. In dihydroquercetin all the ring OH groups formed H-bond with Asp66, Arg210, Glu274 and Asp212. The mode of binding shows that phenolics and flavonoid class of compounds have high affinity towards the catalytic residues in the active site of the enzyme (Fig. 5a-b). Similar binding modes with aspartate residues were observed in other phenolics such as mycophenolic acid and koparin-2-methylether. The side chain carboxylic acid in the structure of mycophenolic acid was bonded with the active site catalytic residue Asp349 along with other amino acids (His348 and Arg439). The high binding energy (-7.13 kcal/

mol) was theoretically predicted which may be due to both H-bond and hydrophobic interactions (Fig. 5c). The results showed that hydrophobic interactions play a major role in extent of enzyme inhibition. Koparin-2-methyl ether showed interaction with Asp349, Arg439 and Asp239 but the binding affinity was found to be lower than other phenolics (Fig. 5d).

Phloionolic acid has maximum polar contacts (Fig. 5h) and it stabilizes the catalytic residue Asp349 along with Asp66 through H-bonding and also high binding energy can be attributed to maximum stabilization by hydrophobic interactions through its long hydrocarbon chain which were not catalytic residues for substrate hydrolysis. In the binding mode of embelin molecule the carbonyl and hydroxyl oxygen atoms forms H-bond with Glu408 and Arg312, respectively and the hydrocarbon chain was expected to be stable due to hydrophobic interactions (Fig. 5e). Binding energy for 7,8-dihydroxy stearic acid at the α-glucosidase active site was predicted as -8.99 kcal/mol. Surprisingly the catalytic residues were not observed to have any polar contact with the molecule. The H-bonds formation was with Arg312, Arg356 and Thr303 (Fig. 5f). The long hydrocarbon chain was expected to be stabilized by hydrophobic interactions. The binding in case of *N*-(2-hydroxyethyl)-palmitamide (-9.40 kcal/mol) also observed at residues other than catalytic nucleophile moieties such as Gln350, Arg356 and Thr303 (Fig. 5g).

The sphinganine and sphingoshine class of compounds were present in the active fraction which showed potential inhibition. The amine (-NH$_2$) of c-16 sphinganine at the active site of α-glucosidase was bonded to Asp349 with H-bond thus stabilizes the catalytic residue. The hydroxyl groups form polar contact with Glu274 and Gln276 (Fig. 5i)

All the proposed α-glucosidase inhibitors in present study, subjected to molecular docking yielded results which were quite convincing as the interactions with the catalytic residues by the proposed active metabolites gave a theoretical idea regarding competition of compound and substrate at the active site of enzyme. Also the docking studies supported the present experimental data. Phenolics and hydroxyl acids showed significant inhibition and have proximal interaction with the catalytic residues responsible for substrate hydrolysis. The delaying of the process of sugar release to blood is the hallmark of anti-diabetic drug discovery and the present results may lead to further research on phenolics non-phenolic class of compounds of natural origin for drug design.

Conclusion

The biological activity shown by acetone extract revealed the potential of the rhizome (*Cyperus articulatus*) as a source of α-glucosidase inhibitors. Competitive nature of inhibition recorded by active fractions of acetone extracts enhanced the chances of use of these metabolites as alternative drug for managing glycemic index and thus for treatment of type II diabetes. Different classes of compounds were analyzed for their enzyme inhibiting potential and the theoretical/kinetic studies supported the experimental results. Further studies with individual metabolites and *in vivo* analysis with purified lead bioactive compounds may lead to the acceptance of *Cyperus articulatus* as a potential source of drug ingredients and of pharmaceutical importance.

Fig. 5. Docking model of α-glucosidase active site with ligands: quercetrin (a), dihydroquercetin (b), mycophenolic acid (c) koparin-2-methyl ether (d), embelin (e), 7, 8-dihydroxystearic acid (f), N-(2-hydroxyethyl)palmitamide (g), phloionolic acid (h) and c-16 sphinganine (i)

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. T. Heise, L. Nosek, B.B. Rønn, L. Endahl, L. Heinemann, C. Kapitza and E. Draeger, *Diabetes*, **53**, 1614 (2004);
    https://doi.org/10.2337/diabetes.53.6.1614

2. M. Avery, C. Mizuno, A. Chittiboyina, T. Kurtz and H. Pershadsingh, *Curr. Med. Chem.*, **15**, 61 (2008);
    https://doi.org/10.2174/092986708783330656

3. V. Manohar, N.A. Talpur, B.W. Echard, S. Lieberman and H.G. Preuss, *Diabetes Obes. Metab.*, **4**, 43 (2002);
    https://doi.org/10.1046/j.1463-1326.2002.00180.x

4. Y.-I. Kwon, E. Apostolidis and K. Shetty, *J. Food Biochem.*, **31**, 370 (2007);
    https://doi.org/10.1111/j.1745-4514.2007.00120.x

5. T. Matsui, I. Ogunwande, K. Abesundara and K. Matsumoto, *Mini Rev. Med. Chem.*, **6**, 349 (2006);
    https://doi.org/10.2174/138955706776073484

6. H. Matsuda, M. Yoshikawa, T. Morikawa, G. Tanabe and O. Muraoka, *J. Trad. Med.*, **22**, 145 (2005).

7. R. Rabasa-Lhoret and J.-L. Chiasson, eds.: R.A. DeFronzo, E. Ferrannini, H. Keen and P. Zimmet, α-Glucosidase Inhibitors, In: International Textbook of Diabetes Mellitus, John Wiley & Sons, Ltd.: Chichester, UK, p. d0612 (2004).

8. F.A. van de Laar, *Vasc. Health Risk Manag.*, **4**, 1189 (2008);
    https://doi.org/10.2147/VHRM.S3119

9.   W. Benalla, S. Bellahcen and M. Bnouham, *Curr. Diabetes Rev.*, **6**, 247 (2010);
     https://doi.org/10.2174/157339910791658826

10. H. Gao, Y.-N. Huang, B. Gao, P. Li, C. Inagaki and J. Kawabata, *Food Chem.*, **108**, 965 (2008);
     https://doi.org/10.1016/j.foodchem.2007.12.002

11. M.M. Pandey, S. Rastogi and A.K.S. Rawat, *Evid. Based Complement. Alternat. Med.*, **2013**, 376327 (2013);
     https://doi.org/10.1155/2013/376327

12. B. Ravishankar and V. Shukla, *Afr. J. Tradit. Complement. Altern. Med.*, **4**, 319 (2008);
     https://doi.org/10.4314/ajtcam.v4i3.31226

13. Y.Q. Li, F.C. Zhou, F. Gao, J.S. Bian and F. Shan, *J. Agric. Food Chem.*, **57**, 11463 (2009);
     https://doi.org/10.1021/jf903083h

14. H. Matsuda, T. Morikawa and M. Yoshikawa, *Pure Appl. Chem.*, **74**, 1301 (2002);
     https://doi.org/10.1351/pac200274071301

15. C. Proença, M. Freitas, D. Ribeiro, E.F.T. Oliveira, J.L.C. Sousa, S.M. Tomé, M.J. Ramos, A.M.S. Silva, P.A. Fernandes and E. Fernandes, *J. Enzyme Inhib. Med. Chem.*, **32**, 1216 (2017);
     https://doi.org/10.1080/14756366.2017.1368503

16. P. Dhar, D.G. Dhar, A.K.S. Rawat and S. Srivastava, *Ind. Crops Prod.*, **108**, 232 (2017);
     https://doi.org/10.1016/j.indcrop.2017.05.053

17. K. Hemanth Kumar, S. Razack, I. Nallamuthu and F. Khanum, *Ind. Crops Prod.*, **52**, 815 (2014);
     https://doi.org/10.1016/j.indcrop.2013.11.040

18. Q.-P. Hu, X.-M. Cao, D.-L. Hao and L.-L. Zhang, *Sci. Rep.*, **7**, 45231 (2017);
     https://doi.org/10.1038/srep45231

19. O. Lawal, A. Ojekale, O. Oladimeji, T. Osinaike, A. Sanni, M. Simelane, R. Mosa and A. Opoku, *Br. J. Pharm. Res.*, **7**, 52 (2015);
     https://doi.org/10.9734/BJPR/2015/18631

20. N.O. Olawore, L.A. Usman, I.A. Ogunwande and K.A. Adeleke, *J. Essent. Oil Res.*, **18**, 604 (2006);
     https://doi.org/10.1080/10412905.2006.9699179

21. O.M. Ameen, L.A. Usman, I.A. Oladosu, N.O. Olawore and I.A. Ogunw, *J. Med. Plants Res.*, **5**, 1031 (2011).

22. C. Desmarchelier, E. Mongelli, J. Coussio and G. Ciccia, *J. Ethnopharmacol.*, **50**, 91 (1996);
     https://doi.org/10.1016/0378-8741(95)01334-2

23. E. Mongelli, C. Desmarchelier, J. Coussio and G. Ciccia, *Rev. Argent. Microbiol.*, **27**, 199 (1995).

24. S. Ayusman, P. Duraivadivel, H. G. Gowtham, S. Sharma and P. Hariprasad, *Food Biosci.*, **35**, 100544 (2020).
     https://doi.org/10.1016/j.fbio.2020.100544

25. S. Berk, B. Tepe, S. Arslan and C. Sarikurkcu, *Afr. J. Biotechnol.*, **10**, 8902 (2011).

26. M.R. Bhandari, N. Jong-Anurakkun, G. Hong and J. Kawabata, *Food Chem.*, **106**, 247 (2008);
     https://doi.org/10.1016/j.foodchem.2007.05.077

27. M. Miyazawa, N. Yagi and K. Taguchi, *J. Oleo Sci.*, **54**, 589 (2005);
     https://doi.org/10.5650/jos.54.589

28. A. Mari, D. Lyon, L. Fragner, P. Montoro, S. Piacente, S. Wienkoop, V. Egelhofer and W. Weckwerth, *Metabolomics*, **9**, 599 (2013);
     https://doi.org/10.1007/s11306-012-0473-x

29. K. Yamamoto, H. Miyake, M. Kusunoki and S. Osaki, *FEBS J.*, **277**, 4205 (2010);
     https://doi.org/10.1111/j.1742-4658.2010.07810.x

30. A. Gupta, P. Sharma and B. Jayaram, *Protein Pept. Lett.*, **14**, 632 (2007);
     https://doi.org/10.2174/092986607781483831

31. P. Jiang, J. Xiong, F. Wang, M.H. Grace, M.A. Lila and R. Xu, *J. Chem.*, **2017**, 8516964 (2017);
     https://doi.org/10.1155/2017/8516964

32. J.S. Kim, C.S. Kwon and K.H. Son, *Biosci. Biotechnol. Biochem.*, **64**, 2458 (2000);
     https://doi.org/10.1271/bbb.64.2458

33. S.-H. Lam, J.-M. Chen, C.-J. Kang, C.-H. Chen and S.-S. Lee, *Phytochemistry*, **69**, 1173 (2008);
     https://doi.org/10.1016/j.phytochem.2007.12.004

34. H. Teng and L. Chen, *Crit. Rev. Food Sci. Nutr.*, **57**, 3438 (2017);
     https://doi.org/10.1080/10408398.2015.1129309

35. N.A. Raut and N.J. Gaikwad, *Fitoterapia*, **77**, 585 (2006);
     https://doi.org/10.1016/j.fitote.2006.09.006