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α-Glucosidase Inhibition Kinetics and Molecular Docking Studies with the Bioactive Constituents from *Canna indica* L. Rhizome Extract

AYUSMAN SWAIN and HARIPRASAD PUTTASWAMY*

Centre for Rural Development and Technology, Indian Institute of Technology Delhi, New Delhi-110016, India

*Corresponding author: E-mail: phari@rdat.iitd.ac.in; ayusman.iitd@gmail.com

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The present study investigated the phytochemical constituents from *Canna indica* rhizome acetone extract, which was earlier reported to possess α-glucosidase inhibiting potential. Different fractions were collected from column chromatography of the acetone extract and the *in vitro* enzyme inhibition and the kinetic study was performed with the active fraction. The active fraction exhibited competitive inhibition of α-glucosidase. HRLC-MS/MS technique was used to identify the lead compounds from the active fraction. The major compounds were psoromic acid, usnic acid and rosmarinic acid. Molecular docking study of the compounds with the crystal structure of α-glucosidase was performed using ParDOCK. Psoromic acid and usnic acid exhibited strong binding affinity with the active site nucleophiles Asp349 and Asp212, respectively. Usnic acid also stabilized the catalytic residue Glu274. Rosmarinic acid formed multiple hydrogen bonds with the catalytic residue Glu274 and also bonded to non-catalytic residues Gln276, Arg312 and Glu408. The study illustrated informative data on the phytochemical constituents from *Canna indica* rhizome as α-glucosidase inhibitor and as potential candidates for the development of antidiabetic drugs.

Keywords: *Canna indica*, α-Glucosidase inhibitor, Rosmarinic acid, Psoromic acid, Usnic acid, Molecular docking.

INTRODUCTION

Diabetes mellitus or type-II diabetes is considered as a complex metabolic disorder having acute as well as chronic consequences [1]. It is reported that about 25% of the world population is suffered from diabetes mellitus [2]. Deficient action of insulin to regulate blood glucose leads to high sugar levels in the blood along with other byproducts and is attributed as hyperglycemia that causes severe damage or dysfunction of various organ systems [3]. Though the exact reason for deficient action of insulin has not been established, genetic and environmental factors are reported in certain cases [4]. Several classes of oral hypoglycemic drugs have been reported to exert antidiabetic effects such as thiazolidinediones, sulfonylureas, α-glucosidase inhibitors and biguanides [5,6]. Although significant progress has been made to control hyperglycemia, but the use of synthetic oral hypoglycemic drugs leads to various side effects such as drug resistance and toxicity [7,8]. Due to the several limitations associated with the use of existing synthetic antidiabetic drugs, the search for newer antidiabetic drugs from natural sources has become challenging in present-day research [9]. Among all antidiabetic drugs, α-glucosidase inhibitors are the kind of drugs (such as acarbose and miglitol), which controls the expression of certain enzymes responsible for the breakdown of carbohydrates into monosaccharides in the small intestine and thus reduces the absorption rate of sugars in the body. This class of drugs causes reduction of postprandial hypoglycemia [10,11]. Natural products or bioactive compounds from plant sources have been the thrust area for drug development [12,13]. Several drug ingredients from natural sources have been tested to have increased potential activity and lesser adverse effects than existing synthetic drugs [14,15].

Canna indica L. (family: Cannaceae), commonly known as Indian shot or Sarvajaya is a tropical perennial rhizomatous herb, grows in almost all agro-climatic zones of India [16,17]. It has been used as a source of starch/food in different regions of the world [18-20]. Canna rhizome has also been used in folk medicine to treat fever and dropsy, suppuration, malaria, diarrhea, rheumatism, dysentery, bursitis and cut [21,22]. The methanolic extract of the plant rhizome has been reported to possess antioxidant properties [18,23]. Moreover the rhizome was reported to be a very good source of vitamins, minerals,

starch, fiber and a wide range of phenolic compounds [23]. So far the works focusing on the antidiabetic study of *Canna indica* rhizome metabolite, structure activity relation of metabolites with various enzyme and biomolecules are very minimal. In an earlier study, plant rhizome extracts (acetone and water) were shown to possess high α-glucosidase inhibitory activities [23]. The objective of the present work was to identify the lead bioactive compounds from acetone extract responsible for inhibitory activities and to study the enzyme-inhibition kinetics and molecular interactions.

EXPERIMENTAL

Metabolite extraction: The rhizome of naturally growing *C. indica* was collected from the river banks of the Cauvery, Mysore, India. The plants were cultivated and maintained at a research field (Micromodel campus), IIT Delhi, India. The identification of the plant (herbarium) was done at the Botanical Survey of India (BSI), Kolkata, India. The matured plant rhizomes were collected, washed in tap water, blot-dried and kept at 4 ºC. These rhizomes were used throughout the experiment for the extraction of metabolites and phytochemical analysis.

Soxhlet extraction of crude metabolites was performed using dried rhizome powder in solvents with increasing polarity (hexane < chloroform < ethyl acetate < acetone < methanol < water). The extracts were concentrated under low pressure using Rota Evaporator (Buchi R-205, Switzerland). Stock solutions (mg/mL) of rhizome extract was prepared by dissolving the dried samples in a common solvent (DMSO).

Active fraction collection: The acetone extract which had shown high α-glucosidase inhibiting potential was tested again for the inhibitory activity. Then it was subjected to exhaustive column chromatography with silica gel (mesh size: 60-120). Chloroform and acetonitrile were used as a gradient mobile phase (acetonitrile 0% to100%) to collect 5 different fractions. All the fractions were tested for their α-glucosidase inhibitory activities. The fraction showing significantly high α-glucosidase inhibition was further subjected to enzyme kinetic study and HRLC-MS/MS analysis..

α-Glucosidase inhibition: Rhizome extract (40 µL) was mixed with α-glucosidase (1 U/ml, 40 µL) in phosphate buffer (pH 6.8) and incubated for 10 min in a 96 WMP. Then glutathione (reduced) (3 mM, 40 µL) and PNPG (10 mM, 40 µL) were added and incubated for 15 min at 35 ºC. One control was maintained by mixing all reagents except the rhizome extract to check the maximum released product. Another blank was maintained by adding all other reagents to the rhizome extract except the enzyme. The reaction was terminated by adding 40 µL 0.2 M sodium carbonate solution. The sample and blank absorbance were read at 400 nm. The inhibitory activity was expressed in percentage [23].

Kinetic study: The active fraction collected in chromatographic separation was studied for the kinetics of enzyme (α-glucosidase) inhibition. The α-glucosidase inhibition kinetics was studied for 30 min. The substrate PNPG(α) in the concentration range of 0.5-3.5 mM was used for monitoring enzyme hydrolysis. α-Glucosidase (1U/mL) was tested in the absence and presence of different concentrations of rhizome extract active fraction. The spectrophotometric measurement was performed at 400 nm for 30 min with the measurement at every 0.5 min interval. Michaelis-Menten plot and Lineweaver-Burk plot was used to determine the inhibition type [24]. Inhibitor (15.6 µg/mL) was mixed with enzyme before performing kinetics experiment and the result was expressed in Lineweaver-Burk plot.

HRLC-MS/MS analysis of rhizome extracts of *C. indica*: HRLC-MS/MS analysis of acetone extract active fraction was performed using 6200series Q-TOF (Q-Exactive Plus Biopharma High Resolution MS) mass spectrometer coupled to HPLC equipped with UV-Vis detector (Facility; SAIF, IIT Bombay). 0.2 mL/min flow rate was maintained with injection volume 5 µL; ESI parameters: both negative and positive ion mode; mass range 100-1200 *m/z*. The solvent system: (A) formic acid (0.1%, v/v) and 10 mM ammonium acetate and (B) acetonitrile + 0.1% formic acid. Gradient mobile phase (solvent A:B): (i) 65:35, from 0 to 0.5 min, (ii) 45:55, from 10 min (iii) 5:95, from 25 to 33 min (iv) 65:35, at 35-40 min of total run time [23].

Molecular docking: The crystal structure of yeast extracted α-glucosidase (RCSB PDB id: 3A4A) was downloaded and the active site coordinates were assigned [25]. Water molecules were removed from the whole structure. PDB structures of the selected molecules (analyzed from HR-LCMS/MS) and a standard antidiabetic drug, acarbose were drawn with the help of Marvin sketch and saved as the 3D structure with all explicit H-atom. The docking study was performed using ParDOCK software (http://www.scfbio-iitd.res.in/dock/pardock.jsp) in which the binding energy of each partner is obtained based on Monte Carlo docking principle and was reported in kcal/mol [26].

RESULTS AND DISCUSSION

Enzyme inhibitory activity and kinetic study: Among the 5 different fractions collected from the column chromatography, the 3rd fraction showed high α-glucosidase inhibitory potential (Fig. 1) having IC_{50} 19.8 µg/mL. Earlier literature had confirmed the inhibition by the crude acetone extract with IC_{50} 27 µg/mL [23]. The 3rd fraction was the active fraction which competitively inhibited the α-glucosidase enzyme (Fig. 2). It was observed from the experiments that the inhibitor(s) hardly have any effect on V_{max} but increases the value of K_m. As it is evident that competitive inhibition can prevent fast break down of sugars and thus control the glycemic index [24,27], the comp-

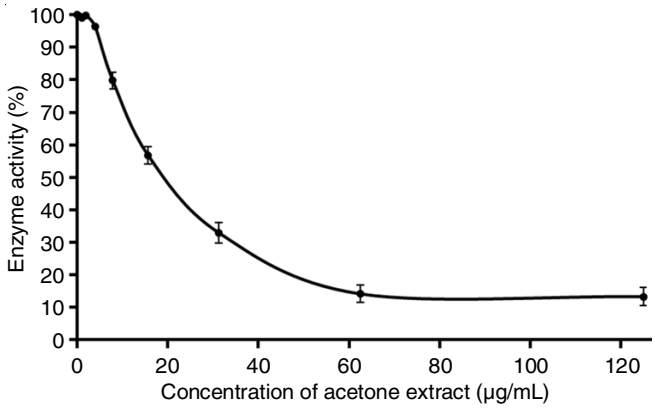

Fig. 1. α-Glucosidase activity plot in presence of increasing concentration of acetone extract active fraction of *C. indica* (IC_{50} = 19.8 µg/mL)

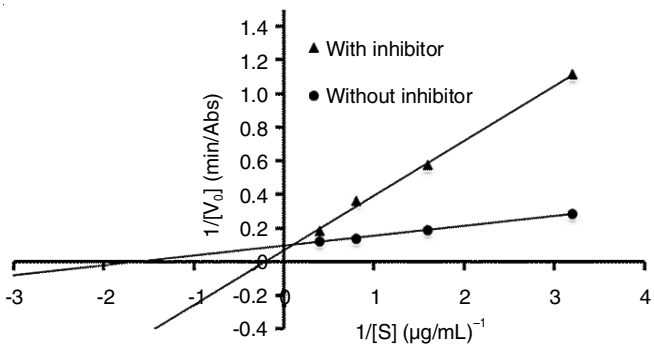

Fig. 2. The mode of α-glucosidase inhibition by inhibitors present in the active fraction of acetone extract of *C. indica*. The Lineweaver-Burk plots: Active fraction showed competitive inhibition

ounds in the active fraction may be considered as potent antidiabetic candidates and thus the rhizome can be a source for development of antidiabetic drugs. Polyphenolic compounds such as catechin gallates, quercetin, isoquercetin and rutin were well studied for their α-amylase and α-glucosidase inhibiting properties [28]. The *Canna indica* acetone extract was earlier studied to have total phenol of 334 µgGAE/mg extract [23]. So the active fraction was expected to be rich in polyphenolic constituents.

Metabolite profile: Earlier study reported that the acetone extract was rich in phenolics and thus possesses high antioxidant activities [23]. Upon subjecting the active fraction to HRLC-MS/MS, presence of rosmarinic acid, psoromic acid and usnic acid were detected (Fig. 3).

MS/MS pattern of identified compounds

Psoromic acid: m.w.: 358.06; *m/z* 358.06 corresponding to molecular formula $C_{18}H_{14}O_8$, HRLC-MS/MS major peaks (negative mode): *m/z* 358.06 (25%), *m/z* 357.05 (100%) $[M-H]^-$.

Usnic acid: m.w.: 344.08; *m/z* 344.08 corresponding to molecular formula $C_{18}H_{16}O_7$, HRLC-MS/MS major peaks (negative mode): *m/z* 343.078 (100%) $[M-H]^-$.

Rosmarinic acid: m.w.: 360.08; *m/z* 360.08 corresponding to molecular formula $C_{18}H_{16}O_8$, HRLC-MS/MS major peaks (negative mode): *m/z* 360.06 (25%), *m/z* 359.07 (100%) $[M-H]^-$.

Several literatures reported that the phenolic compounds possess α-glucosidase inhibition activity [24,29,30]. Psoromic acid was reported to be a novel compound having antioxidant and rab-prenylation inhibitory activity [31,32]. Rosmarinic acid and usnic acid were reported to possess antioxidant character and also other health beneficial effects [31,33]. Thus the active fraction is highly rich in health beneficial metabolites suggesting the rhizome to be a medicinal and pharmacological important source. In this study, psoromic acid, usnic acid and rosmarinic acid are proposed as candidate molecules for inhibition of α-glucosidase.

Docking study of bioactive metabolites: The active site of α-glucosidase (3A4A) has amino acid residues such as Asp349, Asp212, Arg439, His277, Glu274 and Asp66. The catalytic residues are Glu274, Asp349 and Asp212 (assigned number to each amino acid in our study is 3 number less than the original sequence of the crystal structure 3A4A). The binding energy

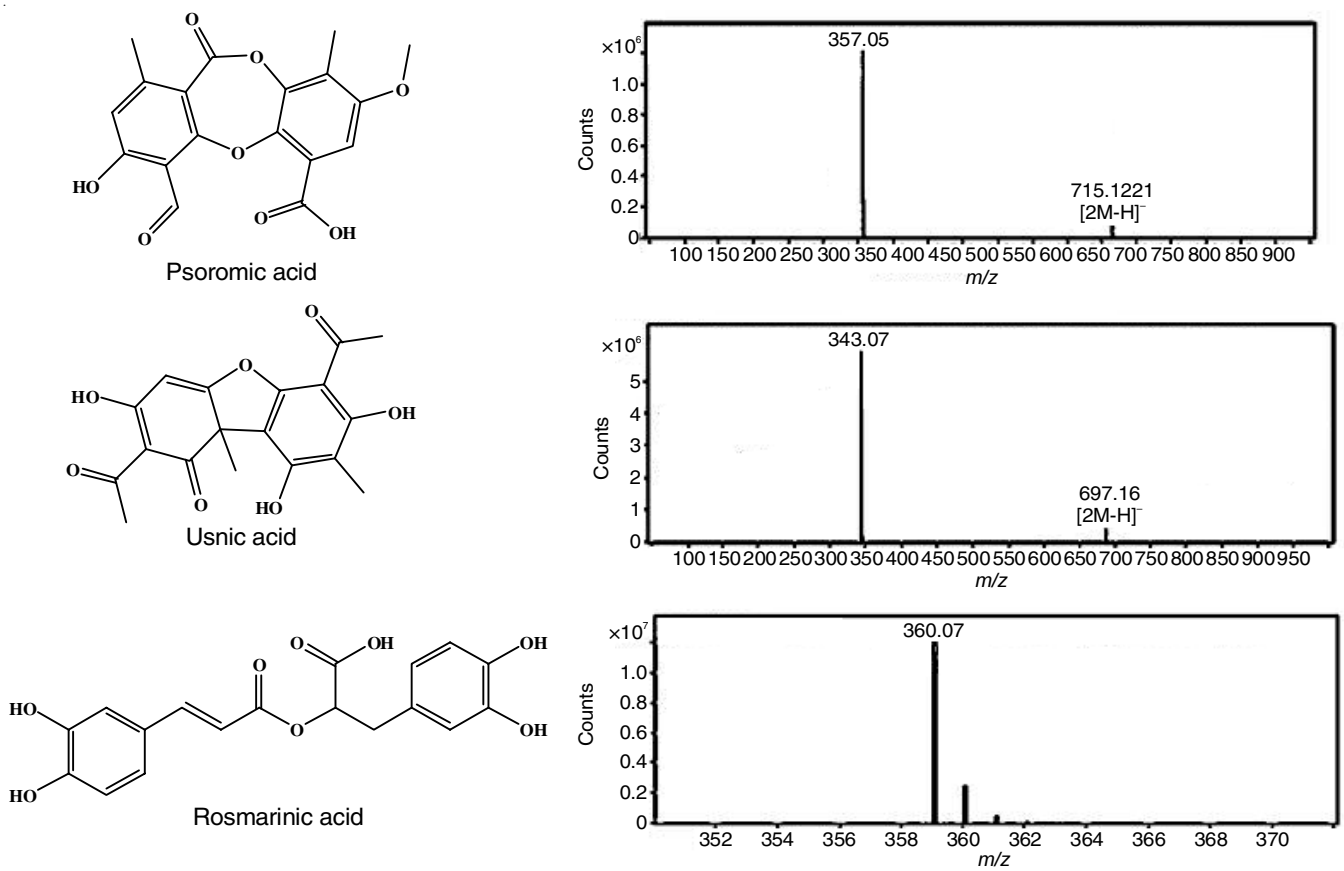

Fig. 3. Three major compounds in the acetone extract active fraction (showing high α-glucosidase inhibition) identified using HRLC-MS/MS

of ligand enzyme systems was calculated for the best fit structure in the active pocket [9,26].

The possible small molecule inhibitors such as psoromic acid, usnic acid and rosmarinic acid showed significant binding affinities (Table-1). The molecular docking study revealed that phenolic compounds like psoromic acid and usnic acid have high binding affinity to the catalytic active site of the enzyme (Fig. 4). Psoromic acid stabilizes Asp349 residue by hydrogen bond, which is the catalytic residue in the enzyme-substrate reaction at the active site. Another -OH group was bonded to other non-catalytic amino acid residue, Gln276 (Fig. 4). Usnic acid, on the other hand, stabilized Asp212 by hydrogen bond and exerts extra stability by forming bonds with catalytic residue Glu274 and another non-catalytic residue Hie109. The mode of binding shows that both the compounds have high affinity towards the catalytic residues in the active site of the enzyme. Rosmarinic acid exerts strong hydrogen bonding with catalytic residue Glu274. The phenolic -OH of both the rings formed strong bond with Glu274 and Gln276. But the other hydrogen bonds were with the residues other than those of the active site. The ligand structure was coiled to such an extent that carboxylic group formed hydrogen bonding with nearby non-catalytic residues Arg312 and Glu408 (Fig. 4).

TABLE-1
THEORETICAL BINDING ENERGY OF THE α-GLUCOSIDASE ENZYME (3A4A) AND INHIBITOR MOLECULE INTERACTION

Compounds	Binding energy (kcal/mol)
Psoromic acid	-6.75
Usnic acid	-6.44
Rosmarinic acid	-6.12
Acarbose	-10.72

Acarbose was observed to bind extensively with the peripheral amino acid residues by H-bonding at the active site. The residues bonded to the acarbose are Asp349, Arg312, Asp304,

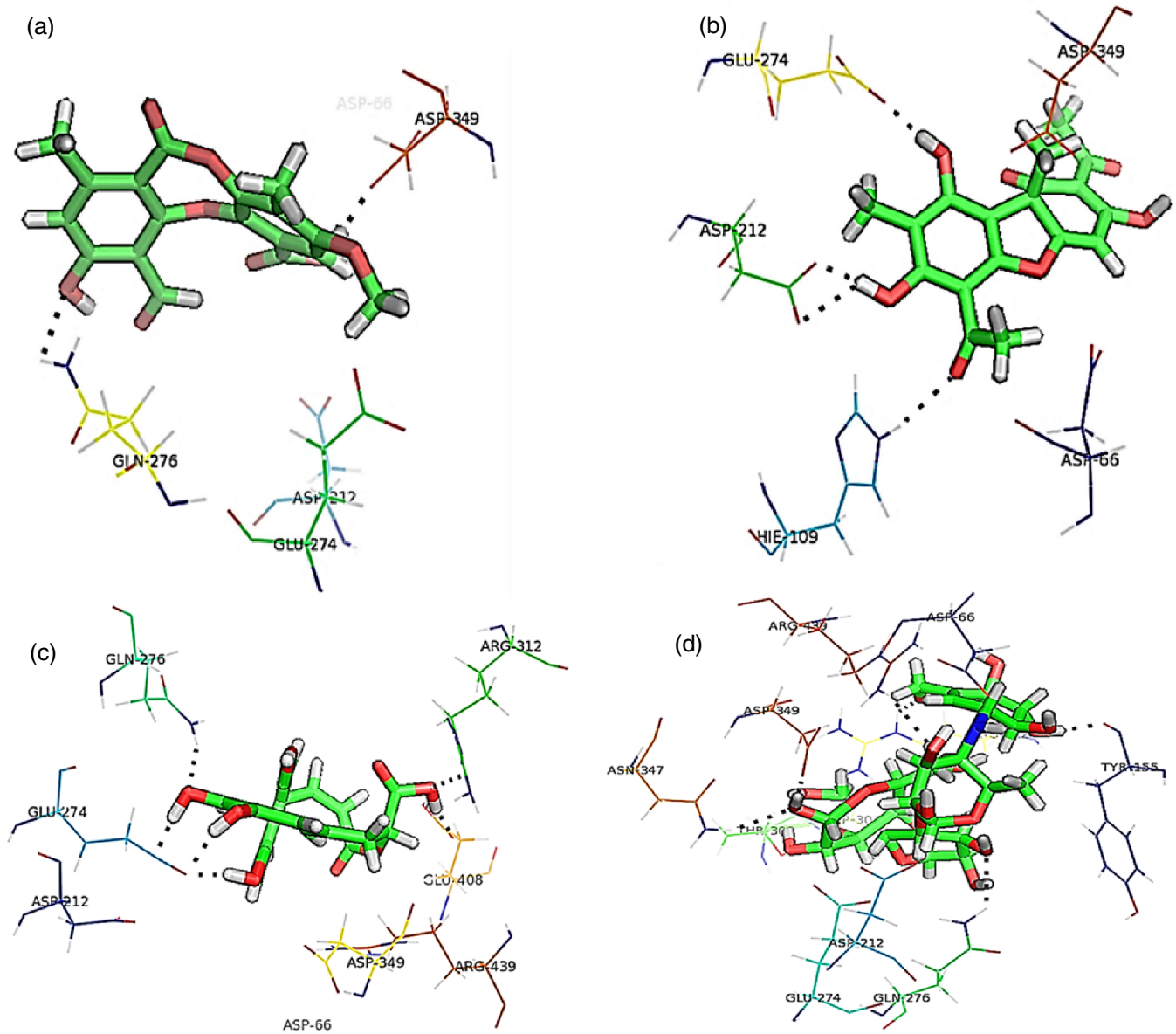

Fig. 4. Docking model of α-glucosidase active site with ligands (a) psoromic acid, (b) usnic acid, (c) rosmarinic acid and (d) acarbose

Gln276, Tyr155, Arg439 and Tyr303. In the best fit structure (binding energy -10.72 kcal/mol) the other aspartate residues (Asp212 and Asp66) were at a distance beyond the reach of the ligand atoms. The high binding energy was expected as there were extensive polar contacts of hydroxyl groups along with the bonding to catalytic nucleophile Asp349 (H-bond length 1.6 Å theoretically).

It was observed that at the active site, psoromic acid stabilized Asp349, usnic acid stabilized Asp 212 and Glu274. Whereas rosmarinic acid had interaction with only catalytic residue Glu274. Thus, all the residues of the active site were not blocked in any of the docking model. In addition to the catalytic interactions, the non-catalytic interactions with the residues other than the active site may have contributed to the overall stability and high binding energy.

Conclusion

The biological activity shown by acetone extract revealed the potential of rhizome as a source of α-glucosidase inhibitors. Competitive nature of inhibition by the phenolic compounds recorded by the active fraction of acetone extracts suggested these metabolites as alternative drug ingredients for managing glycemic index or type II diabetes. Phenolic class of inhibitors such as psoromic acid, usnic acid and rosmarinic acid were reported first time in this study as potential candidate molecule for α-glucosidase inhibition. The compounds analyzed for their potential enzyme inhibiting potential were supported by the molecular docking studies. Controlling the glycemic index is the basis of discovery of antidiabetic drug and hence the identified polyphenolic metabolites may lead to a new avenue of natural product research and drug design. Further *in vivo* studies with the individual bioactive compounds may explore their antidiabetic properties leading to their promising medicinal and pharmaceutical usability.

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CONFLICT OF INTEREST

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

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