ORIGINAL CONTRIBUTION   Open Access

Identification of bioactive compounds by GC-MS and α-amylase and α-glucosidase inhibitory activity of *Rauvolfia tetraphylla* L. and *Oroxylum indicum* (L.) Kurz: an in vitro and in silico approach

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Abstract

Background: The practice of ethnomedicine remains to be the primary source of healthcare in many parts of the world, especially among the tribal communities. However, there is a lack of scientific outlook and investigation to authenticate and validate their medicinal values.

Objective: The present study investigated the trace and heavy metal content, bioactive compounds, α-amylase, and α-glucosidase inhibitory activity of *Rauvolfia tetraphylla* and *Oroxylum indicum* using in vitro and in silico methods.

Methods: Trace and heavy metal content of *Rauvolfia tetraphylla* and *Oroxylum indicum* were detected using Atomic Absorption Spectroscopy. Bioactive compounds were analyzed and identified by the GC-MS technique. α-Amylase and α-glucosidase inhibitory activity of the plants were studied using the spectrophotometric method using UV/VIS-Spectrophotometer. In silico molecular docking was carried out in AutoDock vina and the structures visualized using PyMol and Biovia Discovery Studio software. Statistical and graphical representations were performed using Excel and OriginPro.

Results: The trace and heavy metallic content such as Zn, Ni, Pb, Cr, Cu, and Mn were reported from both the plant. No Cd was detected in both the plants. GC-MS analysis revealed four major compounds in *R. tetraphylla* and seven in *O. indicum*. Biochemical studies showed that the leaf extract of *O. indicum* posses the strongest α-amylase and α-glucosidase inhibitory activity. *R. tetraphylla* showed weaker enzyme inhibition. Molecular docking study revealed that three compounds from *O. indicum* (O2, O3, and O6) and two from *R. tetraphylla* (R1 and R2) showed strong binding affinity to α-amylase and α-glucosidase. However, leaf extract of *O. indicum* showed better binding affinity with the enzymes compared to *R. tetraphylla*.

Conclusion: Inhibition of α-amylase and α-glucosidase in an important strategy of diabetes control. The present study revealed the in vitro α-amylase and α-glucosidase inhibitory activity of *Rauvolfia tetraphylla* and *Oroxylum indicum*. In conclusion, the study identified that the leaf extract of *O. indicum* as a potential inhibitor of glucose metabolizing enzymes and could be a source of antidiabetic agents.

Keywords: α-Amylase, α-Glucosidase, *Rauvolfia tetraphylla*, *Oroxylum indicum*, GC-MS, Docking

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Introduction

Diabetes is an important metabolic disorder characterized by elevated blood glucose levels leading to serious complications in the heart, blood vessels, eyes, kidneys, and nerves. It is one of the most prevalent non-communicable diseases in the world associated with carbohydrate metabolism and derangement of insulin function either due to insufficiency, defective receptors, or both [1]. There is a rapid increase in the prevalence of diabetes around the world, especially in low- and middle-income countries. The total number of diabetic people rose from 108 million in 1980 to 422 till 2014, an increase of about 300%. In 2016, an estimated 1.6 million deaths were directly caused by diabetes making it the seventh leading cause of death in 2016 [2]. Along with many other lifestyle management processes, medication is an important aspect of controlling diabetes. The major classes of oral anti-diabetic medications currently available include biguanides, sulfonylureas, meglitinide, thiazolidinedione, dipeptidyl peptidase-4 inhibitors, sodium-glucose cotransporter inhibitors, and α-glucosidase inhibitors. However, medications are also attached to many side effects such as cardiovascular complications, weight gain, nausea, vomiting, dehydration, and many other gastrointestinal disturbances [3]. α-amylase and α-glucosidase are important enzymes responsible for the catabolism of starch, glycogen, and disaccharides. Inhibition of these enzymes can control blood glucose levels and also the physiological disorder created out of this by inhibiting carbohydrate metabolism. Because of this, plenty of researchers have investigated the α-amylase and α-glucosidase inhibitory activity of plants [4, 5].

Rauvolfia tetraphylla L. (locally known as Kharwkha, Bodo language) is an important medicinal plant belonging to the family Apocynaceae. It is a small, evergreen, much-branched woody-shrub native to West-Indies, Northern and Southern America, and naturalized in many countries such as India, Pakistan, Sri Lanka, China, Bhutan, Bangladesh, Indonesia, and Myanmar. In India, it is found in the plains of many Indian states, Tamil Nadu, Andhra Pradesh, Karnataka, Kerala, Bihar, Orissa, West Bengal, Madhya Pradesh, Jammu and Kashmir, and Assam [6, 7]. *R. tetraphylla* is known to possess important medicinal properties such as cholera, eye disease, fever, antihypertensive, as well as in intestinal disorders, diarrhea, and dysentery [8, 9]. In many parts of India, the root extract is used as a medicine against snake bite, high blood pressure, stomach pain, mental disorder, as well as antidiabetic agents [10, 11]. Recent studies have revealed the antibacterial, anti-inflammatory, insecticidal and cytotoxic properties of *R. tetraphylla* [12, 13]. It is also a good source of phytochemical contents and bioactive molecules. Alkaloids such as 10-methoxytetrahydroalstonine, isoreserpiline, an isomeric mixture of 11-demethoxyreserpiline and 10-demethoxyreserpiline, α-yohimbine, reserpiline, curan-17-oic acid, and 18, 19-Secoyohimban are reported from leaves [14]. A comparative study found that the root part of *R. tetraphylla* contains a higher concentration of reserpine compared to others [15]. Indole alkaloids such as ajmalcine, yohimbine, demethyl serpentine, and mitoridine are reported from the seed coat of the plant [16]. Several phytochemical contents such as steroids, reducing sugars, sugars, alkaloids, phenols, flavonoids, saponins, tannins, etc. are reported from *R. tetraphylla* [17]. UV, IR, MS, and NMR (H, C, and HMQC) studies revealed the presence of labdane diterpene - 3βhydroxy-labda-8(17) and 13(14)-dien-12(15)-olide from the stem, and branches of the plant [18]. Five new hybrid monoterpenoid indole alkaloids bearing an unusual 2,2-dimethyl-4-oxopiperidin-6-yl moiety, namely rauvotetraphyllines F–H, 17-epi-rauvotetraphylline F, and 21-epi-rauvotetraphylline-H are isolated from the aerial parts of *R. tetraphylla* [19].

Oroxylum indicum (L.) Kurz belonging to the family Bignoniaceae (locally known as Kharong khandai in Bodo language) is an important plant having several medicinal values. It is a deciduous tree growing throughout India, Sri Lanka, Malaysia, Bhutan, and many other South Asian Countries [20]. Almost all the parts of the plant such as root, leaves, seeds, flowers, and barks are used in many ethnomedicinal practices. Roots are known to medicinal properties such as anti-inflammatory, anthelmintic, antiarthritis, antidiabetic, anticancer, etc. [21]. Similarly, several studies have reported the antidiabetic effects of bark extract of *O. indicum* [22, 23]. Leaves are used as medicine for rheumatic pain, spleen problems, ulcers, cough, and bronchitis [24]. Tribal communities of India use the bark and seeds of *O. indicum* to treat fever, pneumonia, stomach problems, and respiratory troubles [25]. Pharmacological studies have revealed the anti-inflammatory, antimicrobial, anthelmintic, anticancer, cytotoxic, immunostimulant, hepatoprotective, antiproliferative, and anti-adipogenesis properties of *O. indicum* [26]. Several studies have reported the phytochemical constituents of *O. indicum*. Tran et al. [27] reported rich flavonoid contents such as Oroxylin A, chrysin, baicalein, hispidulin, orxylin-A-7O-β-D-glucuronide, and many others from the stem extract of *O. indicum*. Similarly, Peng et al. [28] reported a total of 42 components including 23 flavonoid glycosides, 13 flavonoids, and six other types of compounds. Wu et al. [29] reported another two new flavonoid glycosides, named oroxin C, and oroxin D (scutellarein 4′-methyl ether 7-O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside), together with eight known flavonoids from the seeds of *O. indicum*. Though a large number of literatures revealed the antidiabetic activity of root, bark, and seed extract of *O. indicum*, no such studies have been conducted to investigate the antidiabetic property of leaf extracts. In our earlier publication, we have reported that the roots of *R. tetraphylla* and leaves of *O. indicum*

are among the several medicinal plants that are used to control blood glucose levels [11]. Because of its ethno-medicinal value, the present study has been designed to investigate the phytochemical content and α-amylase and α-glucosidase inhibitory activity of *Rauvolfia tetraphylla* and *Oroxylum indicum* using in vitro and in silico methods.

Materials and methods
Plant material
Fresh plant parts of *Rauvolfia tetraphylla* L. (root) and *Oroxylum indicum* (L.) Kurz (leaves) were collected from villages of Kokrajhar district with the help of local people and authenticated in the Department of Botany, Bodoland University, where voucher samples were deposited with specimen voucher number BUBH2018013 and BUBH2018012, respectively. The plant parts were washed properly, cut into small pieces, and completely dried in the hot-air oven below 50 °C.

Preparation of crude extract
The dried plant parts were ground into powder form using a mechanical grinder. Plant powders were soaked in 80% methanol for 72 h and filtered using whatman filter paper-1. The process was repeated three times and the filtrate obtained was evaporated in a rotary evaporator. After complete drying, dry, solid *R. tetraphylla* (RTME) and *O. indicum* methanolic extract (OIME) obtained were kept at − 20 °C till further use. The process was followed as per the method described in our earlier publication [30]. The percent yield of dry crude extracts of plants is calculated.

Percent yield of crude extract and heavy metal analysis
After three rounds of extraction process, the percent yield of crude extract was found to be 13.07 ± 2.02% and 16.57 ± 3.11% from roots of *R. tetraphylla* and leaves of *O. indicum*, respectively. Seven elements such as lead (Pb), chromium (Cr) Nickel (Ni), cadmium (Cd), copper (Cu), zinc (Zn) and manganese (Mn) were analyzed using atomic absorption spectrophotometer (AAS, Shimadzu AA-7000) following the method of Zheljazkov and Nielson [31] with slight modification. Briefly, 1 g dry powder of plant sample was digested with conc. HNO_3, at 90 °C for 45 min. The temperature is then increased up to 100 °C and boiled for 6–7 h by addition of 5 ml HNO_3 till complete digestion of the plant. The process was continued until the extract is colourless. The solution was filtered by whatman filter No.1 and diluted to 100 ml of distilled water. Samples analysis was carried out in triplicate and values expressed as mean ± S.D.

GC-MS analysis
The phytochemical components of *R. tetraphylla* and *O. indicum* were analyzed by GC-MS system (TQ-8030 Shimadzu Corporation Kyoto, Japan) [32]. GC was run on an EB-5MS capillary column (30 m × 0.25 mm i.d.; 0.25 μm) at 57.4 kPa pressure with an initial temperature of 50 °C and maintained at the same temperature for 2.5 min. Next, the oven temperature was raised to 300 °C, at the rate of 15 °C/min, and maintained for 8 min. Injection port temperature was ensured at 300 °C and Helium flow rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 20:1. The Mass spectral scan range was set at 0-700 (m/z). Compound identification was carried out by the comparison of the spectra with the databases (NIST-11) using a probability-based algorithm.

Enzyme inhibition assays
Inhibition of α-amylase activity
The inhibition of α-amylase enzyme activity was carried out following Kwon et al. [33] with little modification. The crude extract of *R. tetraphylla* and *O. indicum* was dissolved in 5% DMSO. Different concentrations of RTME and OIME, and reference chemical (acarbose) (1, 1.5, and 2 mg/ml) were mixed with 200 μl of amylase enzyme (0.5 mg/ml). The assay mixture was incubated at 25 °C for 10 min. Next, 0.5 ml 1% starch solution was added and re-incubated for another 20 min at 37 °C. After the incubation is over, 0.5 ml DNS reagent was added to stop the reaction and the assay mixture was boiled for 5 min. The reaction mixture was then diluted after adding 5 ml distilled water, and the absorbance (Abs) was measured at 540 nm in UV/VIS double beam spectrophotometer. The control samples were prepared without any plant extracts/compounds.

The percent inhibition of amylase activity was calculated using the following formula:

$$Inhibition\ (\%) = \frac{(Abs\ control - Abs\ sample)}{Abs\ control} x\ 100$$
(1)

Abs control means absorbance of assay mixture without extract and acarbose.

Abs sample means absorbance of assay mixture with extract or acarbose.

Inhibition of α-glucosidase activity
α-Glucosidase inhibition assay was carried out by following the method of Elya et al. [34]. RTME and OIME was dissolved in 5% DMSO. Glucosidase enzyme was dissolved in 100 mM sodium phosphate buffer, pH 6.9. Different concentrations of plant extract (1, 1.5 and 2 mg/ml) and acarbose (125, 250 and 500 μg/ml) were mixed

with 50 μl glucosidase (0.5 μg/ml buffer) and incubated for 10 min at 37 °C. Next, 100 μl of 5 mM *p*-Nitrophenyl-α-D-glucopyranoside was added and incubated for another 20 min at 37 °C. The reaction was stopped by adding 2 ml of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the absorbance at 405 nm using a UV/VIS spectrophotometer. Inhibition (%) of α-glucosidase activity was calculated using eq. (1).

Analysis of drug-likeness properties of phytochemicals
The phytochemicals of *R. tetraphylla* and *O. indicum* identified by GC-MS was verified for its drug-likeness properties using SwissADME tool [35] and PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The compounds were evaluated based on the Lipinski rule, which states that an active oral drug should qualify the following criteria: molecular weight should be in the range from 0 to 500 Da, LogP should be < 5, H-bond donor should be 0–5, and number H-bond acceptor should be < 10 [36]. Along with the drug-likeness test, bioavailability score (BS) [37], and topological polar surface area (TPSA) [38] were also analyzed from SwissADME tool.

Molecular docking
Preparation of ligands and enzymes
All the phytochemicals reported from both the plants, four from *R. tetraphylla*, and seven from *O. indicum* by GC-MS analysis were retrieved from the PubChem database in 3D SDF file format. The SDF files were converted into *.pdb* format using OpenBabel-2.3 [39] and the *.pdb* file was then converted into *.pdbqt* file format using AutoDock Tools software (version 1.5.6). Acarbose was used as a reference inhibitor for both the enzymes. Similarly, the crystal structure of both α-amylase (PDB: 2QV4) and α-glucosidase (maltase) (PDB: 2QMJ) was downloaded from the PDB database (http://www.rcsb.org/pdb). PDB files were converted into *.pdbqt* file format after removing the attached ligands in the crystal structure of the enzymes using AutoDock Tools. The information of active site amino acids of both the enzymes was obtained from ligand-interaction data of PDBs. Out of many ligands attached to the crystal structure of enzymes only the major ligand-interaction (chemical-protein) active site amino acids were collected and used for docking analysis with the phytochemicals.

Docking
After the ligands (phytochemicals and acarbose) and the target proteins (amylase and glucosidase) were prepared, docking was performed in AutoDock Vina [40]. The grid parameters used for docking of each of the ligands are given in Table 1. The individual ligand was docked with the enzymes separately and the final energy (binding affinity in kcal/mol) of a given ligand-enzyme interaction

Table 1 Grid box parameters for docking for α-amylase and α-glucosidase

Enzymes	Grid box size (Å)			Grid center coordinate		
	X	Y	Z	X	Y	Z
α-amylase	56	66	60	10.749	48.629	21.111
α-glucosidase	40	50	42	−23.755	−5.190	−11.162

was obtained after docking. The docking interaction profile was studied using PyMol (v1.3) (Schrödinger, LLC) and Biovia Discovery Studio software (v20.1.0.19295, San Diego: Dassault Systemes, 2020).

Statistical analysis
All the results were expressed as means of three experiments ± standard deviation (S.D.). Statistical difference between the extract and reference compound was tested by one-way analysis of variance (ANOVA) using SPSS. Other statistical calculations and graphical presentations were prepared in Excel and OriginPro. The significance test was calculated at $P < 0.05$.

Results
Heavy metal analysis
The present study investigates the content of seven important heavy metals Cd, Mn, Cr, Zn, Pb, Ni, and Cu in the extracts of *R. tetraphylla* and *O. indicum*. Figure 1 shows the heavy metal contents of both the plants. Heavy metal, cadmium (Cd) was not detected in both plants. Similarly, Nickel (Ni) was not detected in the leaf extract of *O. indicum*. Among the heavy metals analysed, Zn was found to be the highest in both the plants (0.2567 ppm and 0.3125 ppm), while Mn showed the lowest, 0.0137 ppm and 0.0095 ppm in RTME and OIME, respectively. The heavy metal contents in *R. tetraphylla* may be arranged in decreasing order as $Zn > Ni > Pb > Cr > Cu > Mn$ while in *O. indicum*, the sequence is $Zn > Pb > Cu > Cr > Mn$.

GC-MS analysis
GC-MS study of methanolic extracts of the *R. tetraphylla* and *O. indicum* showed the presence of several compounds with different concentrations. The GC-MS chromatograms of both the plants were shown in Fig. 2. Four and seven phytochemicals were reported from *R. tetraphylla* and *O. indicum*, respectively. The retention time, name of the phytochemicals identified, and other GC-MS profiles are presented in the Table 2. 8-(Dimethylamino)-7-(3-(4-ethylphenoxy)-2-hydroxypropyl)-3-methyl-3,7-dihydro-1H-purine-2,6-dione tms (**R1**), Chlorflurenol (**R2**), 2-(4-Nitro-pyrazol-1-yl) propionic acid, hydrazide (**R3**), and 9-(2-Methoxyethyl) carbazole (**R4**) was reported from the root extract of *R. tetraphylla* which corresponds to 100% of the
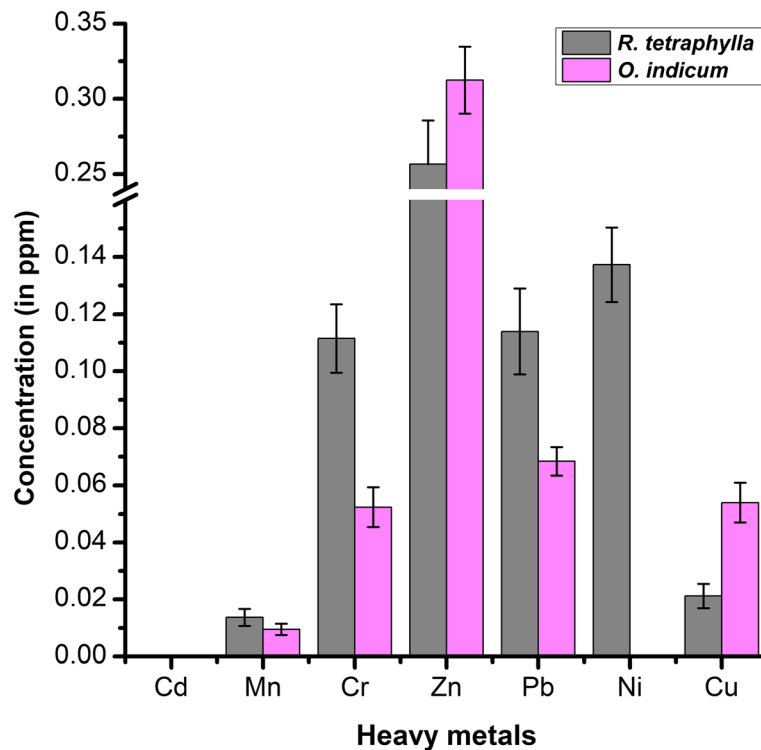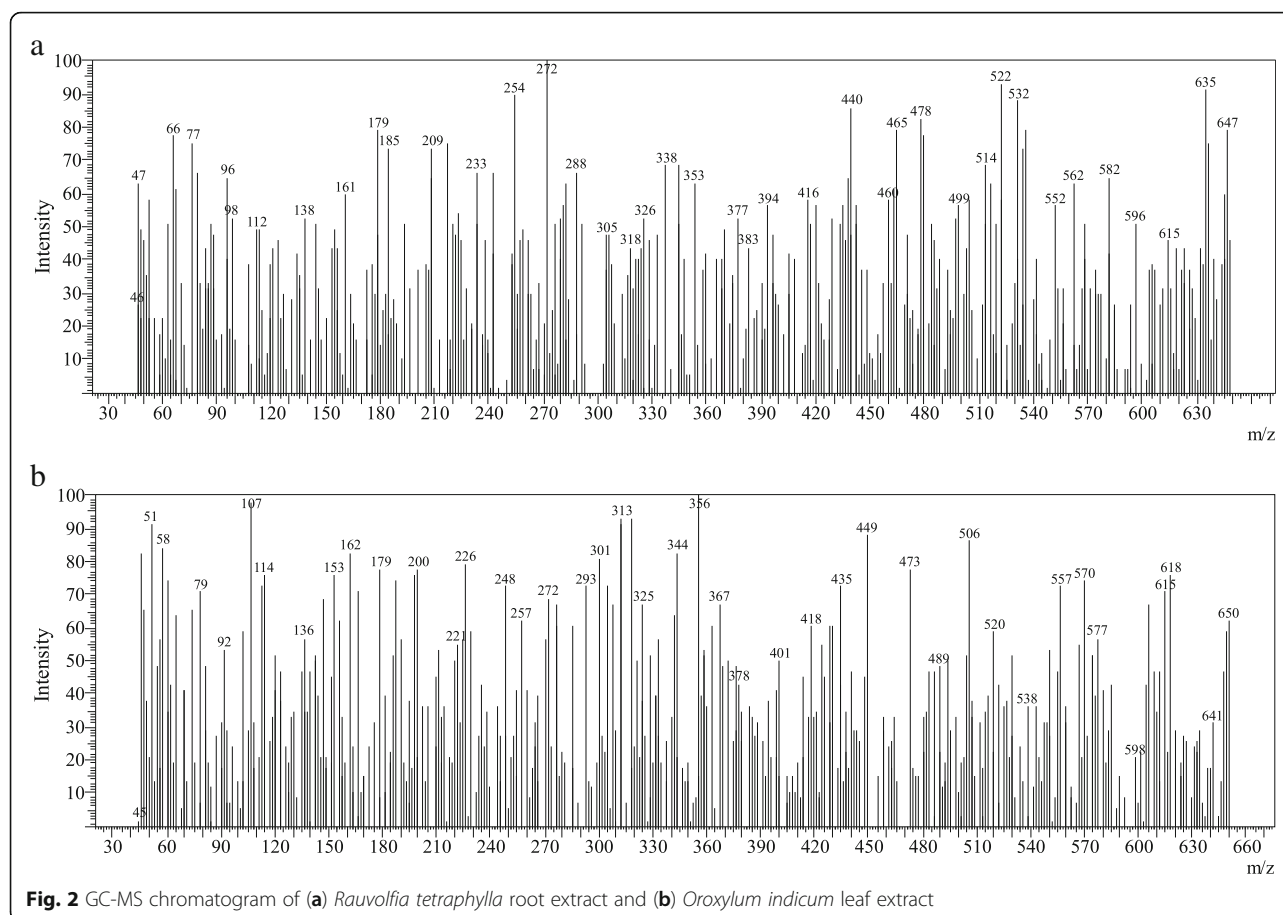
Fig. 1 Heavy metal contents of methanolic extracts of *Rauvolfia tetraphylla* and *Oroxylum indicum*. All the experiments were carried out in triplicate and values expressed as mean ± SD

chromatogram. Similarly, seven chemicals - Benzo [B] thiophene, octahydro-2-methyl- (2.alpha.; 3a.beta.; 7a.beta.)- (**O1**), 2-Benzimidazolemethanol, .alpha. -(p-bromophenyl)- (**O2**), Tricyclo [8.4.1.1(4,9)] hexadeca-4,6,8,10, 12,14-hexaene, 2,3-bis (2,6-dimethylphenylimino)-, anti- (**O3**), 1-(3′,5′-Dibromo-4′-hydroxyphenyl)-5,5-dibromo-2,4-dioxohexahydropyrimidine (**O4**), Dithiocarbamate, S-methyl-N-(2,3-dimethyl-4-oxo-2-pentyl)- (**O5**), Pregnane-3,7,20-trione, (5.alpha.)- (**O6**), and Methylenebis (ethyl thioglycolate) (**O7**) were reported from leaf extracts of *O. indicum*. The most prominent peak area was depicted by Chlorflurenol (47.97%) and Dithiocarbamate, S-methyl-, N-(2,3-dimethyl-4-oxo-2-pentyl)- (40.07%) in *R. tetraphylla* and *O. indicum*, respectively.

Inhibition study of α-amylase and α-glucosidase

α-Amylase and α-glucosidase are two important enzymes required for carbohydrate metabolism. In an attempt to validate the ethnomedicinal knowledge, the present study also investigated the α-amylase and α-glucosidase inhibtion property of RTME and OIME. Figure 3 showed the enzymatic activities of α-amylase and α-glucosidase after the enzymes and its assay mixtures were exposed to plant extracts, and acarbose. Both the enzymes showed concentrations-dependent inhibition of enzyme activities. The leaf extracts of OIME showed

better inhibition activity against α-amylase enzyme compared to RTME as well as reference chemical, acarbose (Fig. 3a). At the highest concentration of plant extracts, and acarbose (2 mg/ml), the percent inhibitions were found to be 38.50%, 70.96%, and 59.80% for RTME, OIME, and acarbose, respectively. All the three parameters between RTME, OIME, and acarbose showed a statistically significant difference to each other at the $P < 0.05$ probability level. Similarly, α-glucosidase showed concentration-dependent sensitivity to plant extracts and the reference chemical. The leaf extracts of OIME showed much stronger α-glucosidase inhibition activity compared to RTME (Fig. 3b). The percent inhibitions were found to be 25.74% and 66.78% at the highest concentration (2 mg/ml) of RTME and OIME, respectively. The difference in the α-glucosidase inhibition potentials of plant extracts was found to be significant at $P < 0.05$ probability level. The reference chemical, acarbose, on the other hand, showed better α-glucosidase inhibitory activity at a lower concentration (0.125, 0.25, and 0.50 mg/ml) than the plant extracts. The methanolic extract of *R. tetraphylla* was found to have slightly stronger α-amylase inhibition activity compared to α-glucosidase enzyme. *O. indicum,* on the other hand, showed almost similar percent of inhibition in both α-amylase and α-glucosidase enzyme activities.

Fig. 2 GC-MS chromatogram of (**a**) *Rauvolfia tetraphylla* root extract and (**b**) *Oroxylum indicum* leaf extract
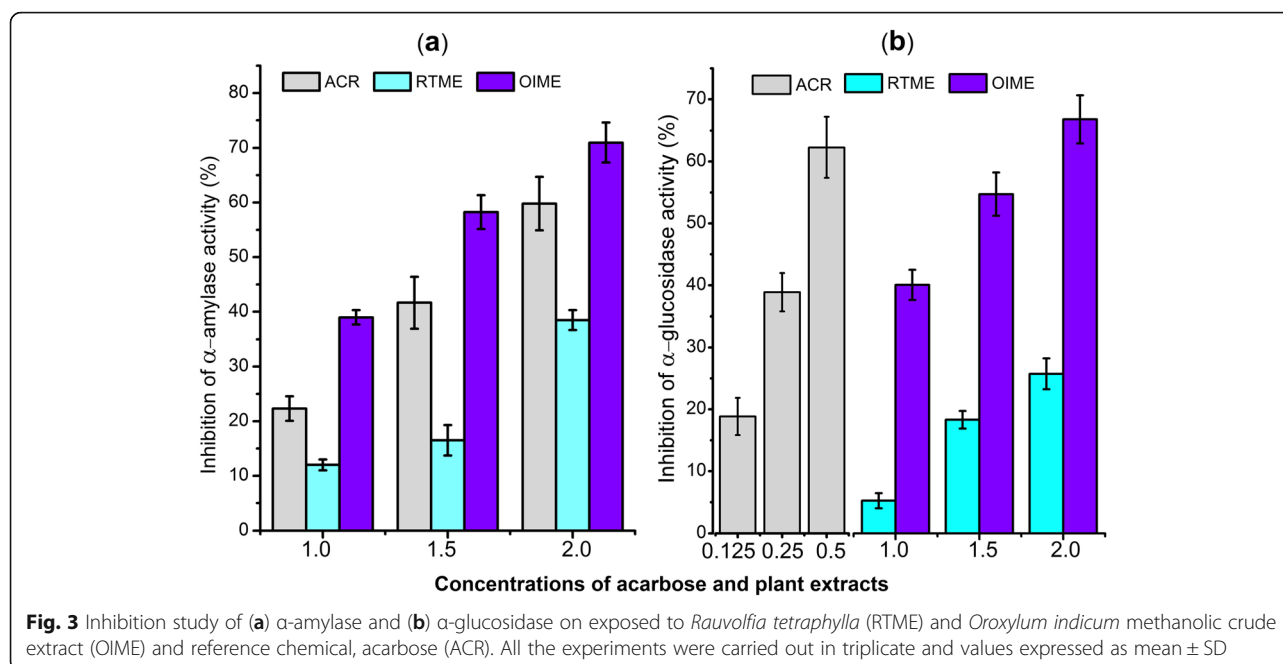
Drug-likeness properties of identified compounds

Drug-likeness is an important physico-chemical property of compounds that determine the effectiveness of target compounds for its drug-likeness. Table 3 showed the various properties of the compounds as per Lipinski's rule of 5, bioavailability score, and Topological Polar

Table 2 GC-MS profiles of the compounds identified from *Rauvolfia tetraphylla* and *Oroxylum indicum*

	Retention time	Name of the compounds	Base m/z	Area (%)	Height (%)	Mol. weight (g/ mole)	Mol. formula
Rauvolfia tetraphylla	12.228	8-(Dimethylamino)-7-(3-(4-ethylphenoxy)-2-hydroxypropyl)-3-methyl-3,7-dihydro-1H-purine-2,6-dione tms	272.00	8.71	23.61	459.6	$C_{22}H_{33}N_5O_4Si$
	19.050	Chlorflurenol	193.00	47.97	33.43	260.67	$C_{14}H_9ClO_3$
	20.831	2-(4-Nitro-pyrazol-1-yl) propionic acid, hydrazide	114.00	36.88	21.65	199.17	$C_6H_9N_5O_3$
	24.960	9-(2-Methoxyethyl) carbazole	179.75	6.43	21.31	225.28 g/mol	$C_{15}H_{15}NO$
Oroxylum indicum	5.975	Benzo [B] thiophene, octahydro-2-methyl- (2.alpha., 3a.beta., 7a.beta.)-	356.00	16.94	14.98	156.29	$C_9H_{16}S$
	6.105	2-Benzimidazolemethanol, .alpha.-(p-bromophenyl)-	600.00	6.65	9.00	303.15	$C_{14}H_{11}BrN_2O$
	6.137	Tricyclo[8.4.1.1 (4,9)]hexadeca-4,6,8,10,12,14-hexaene, 2,3-bis(2,6-dimethylphenylimino)-, anti-	442.00	5.07	10.99	156.29	$C_9H_{16}S$
	9.159	1-(3′,5′-Dibromo-4′-hydroxyphenyl)-5,5-dibromo-2,4-dioxohexahydropyrimidine	119.00	11.67	15.38	521.78	$C_{10}H_6Br_4N_2O_3$
	12.288	Dithiocarbamate, S-methyl-, N-(2,3-dimethyl-4-oxo-2-pentyl)-	480.00	40.07	19.75	219.4	$C_9H_{17}NOS_2$
	21.419	Pregnane-3,7,20-trione, (5.alpha.)-	330.00	8.06	13.33	330.5	$C_{21}H_{30}O_3$
	24.304	Methylenebis (ethyl thioglycolate)	459.00	11.54	16.56	252.4	$C_9H_{16}O_4S_2$

Fig. 3 Inhibition study of (**a**) α-amylase and (**b**) α-glucosidase on exposed to *Rauvolfia tetraphylla* (RTME) and *Oroxylum indicum* methanolic crude extract (OIME) and reference chemical, acarbose (ACR). All the experiments were carried out in triplicate and values expressed as mean ± SD

Surface Area retrieved from the SwissADME tool and PubChem database. The results have seen that the reference chemical acarbose violates almost all the four properties (mol. Weight, LogP, H-bond donor, H-bond acceptor) of Lipinski's rule of 5. Moreover, the bioavailability score was found to be only 0.17. Similarly, R3 and O3 violated the Lipinski's rule in terms of LogP, and O4 in terms of molecular weight. R2 showed slightly better BS value 0.56 compared to others which showed 0.55.

Molecular docking

In silico docking analysis was carried out to investigate the binding properties of the GC-MS reported phytochemicals with the enzymes under study. Table 4 showed the binding affinities of the phytochemicals and enzymes. Phytochemicals-enzymes interaction study has found that compound O6 from *O. indicum* and R2 from *R. tetraphylla* showed the strongest binding affinity with α-amylase enzyme. The binding affinities were found to be – 9.2 kcal/mol and – 7.6 kcal/mol, respectively. Compounds R1, O2, and O3 also showed a strong binding affinity with the active site of amylase enzyme. While the reference inhibitor, acarbose showed – 8.1 kcal/mol binding affinity with the α-amylase enzyme. The binding affinities of four chemicals from *R. tetraphylla* ranged from – 6.1 kcal/mol to – 7.6 kcal/mol while in *O.*

Table 3 Drug-likeness properties of phytochemicals of *R. tetraphylla* and *O. indicum* based on Lipinski's rule and SwissADME

Compounds	PubChem ID	Mol. weight (< 500 Da)	LogP (< 5)	H-bond donor (< 5)	H-bond acceptor (< 10)	BS	TPSA
Acarbose	41,774	645.6	−8.5	14	19	0.17	321 Å^2
R1	91,723,645	459.6	2.3	1	6	0.55	120 Å^2
R2	17,169	260.67	2.6	2	3	0.56	57.5 Å^2
R3	6,422,266	199.17	−0.8	2	5	0.55	119 Å^2
R4	602,121	225.28	3.1	0	1	0.55	14.2 Å^2
O1	535,099	156.29	3.1	0	1	0.55	25.3 Å^2
O2	609,707	303.15	3	2	2	0.55	48.9 Å^2
O3	634,947	442.60	7.8	0	2	0.55	24.7 Å^2
O4	635,787	521.78	3.2	2	3	0.55	69.6 Å^2
O5	5,363,717	219.40	1.9	1	3	0.55	86.5 Å^2
O6	22,213,249	330.50	2.8	0	3	0.55	51.2 Å^2
O7	521,987	252.40	2.2	0	6	0.55	103 Å^2

BS – bioavailability score, TPSA – Topological Polar Surface Area

Table 4 Binding affinities of phytochemicals with α-amylase and α-glucosidase enzymes

Name of compounds	α-amylase (kcal/mol)	α-glucosidase (kcal/mol)
Acarbose, reference inhibitor	−8.1	−7.2
8-(Dimethylamino)-7-(3-(4-ethylphenoxy)-2-hydroxypropyl)-3-methyl-3,7-dihydro-1H-purine-2,6-dione tms	−7.4	−7.2
Chlorflurenol	−7.6	−6.1
2-(4-Nitro-pyrazol-1-yl) propionic acid, hydrazide	−6.1	−6.4
9-(2-Methoxyethyl) carbazole	−6.5	−5.4
Benzo [B] thiophene, octahydro-2-methyl- (2.alpha., 3a.beta., 7a.beta.)-	−5.4	−4.5
2-Benzimidazolemethanol, .alpha.-(p-bromophenyl)-	−7.3	−7.2
Tricyclo[8.4.1.1 (4,9)]hexadeca-4,6,8,10,12,14-hexaene, 2,3-bis(2,6-dimethylphenylimino)-, anti-	−7.4	−8.5
1-(3′,5′-Dibromo-4′-hydroxyphenyl)-5,5-dibromo-2,4-dioxohexahydropyrimidine	−6.4	−5.6
Dithiocarbamate, S-methyl-, N-(2,3-dimethyl-4-oxo-2-pentyl)-	−4.6	− 4.7
Pregnane-3,7,20-trione, (5.alpha.)-	−9.2	− 7.3
Methylenebis (ethyl thioglycolate)	−4.5	−5.0

indicum the binding affinity ranged from − 4.5 kcal/mol to − 9.2 kcal/mol with the crystal structure of α-amylase enzyme. Similarly, O3 from *O. indicum* and R1 from *R. tetraphylla* showed the strongest binding affinity with α-glucosidase with binding energy − 8.5 kcal/mol and − 7.2 kcal/mol, respectively. Compounds such as O2 and O6 also showed strong binding affinities with the α-glucosidase enzyme. The binding affinity of acarbose and α-glucosidase was found to be − 7.2 kcal/mol.

The binding pocket surface view of enzymes and phyto-compounds and its 2D display of the binding interactions are presented in Figs. 4 and 5. The interaction map of ligands with best binding affinities from plants and the active site (pocket) amino acids are depicted in different colors. Acarbose-amylase interactions showed five conventional H-bond with four amino acids (Gln63, Thr163, His299, and Asp300) and van der Waal's interactions with several other amino acids (Fig. 5a). Similarly, acarbose and glucosidase interactions showed four H-bonds with amino acids Tyr301, Gln302, Asn306, and Met331. The study also showed three other interactions, van der Waal's interactions, C-H bond, and
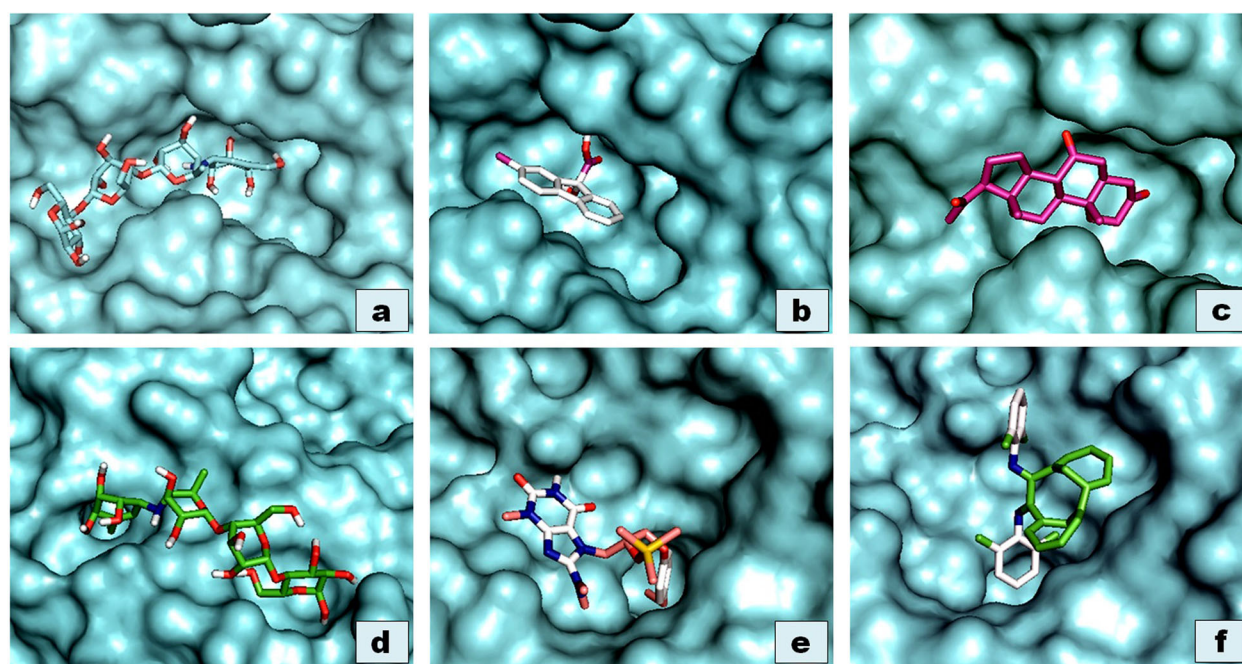

Fig. 4 Binding interaction of plant compounds and enzymes showing in surface view. **a** α-amylase and acarbose, **b** α-amylase and R2, **c** α-amylase and O6, **d** α-glucosidase and acarbose, **e** α-glucosidase and R1, and **f** α-glucosidase and O3

Fig. 5 Docking interactions between compounds and enzymes. **a** α-amylase and acarbose, **b** α-amylase and R2, **c** α-amylase and O6, **d** α-glucosidase and acarbose, **e** India α-glucosidase and R1, and **f** α-glucosidase and O3
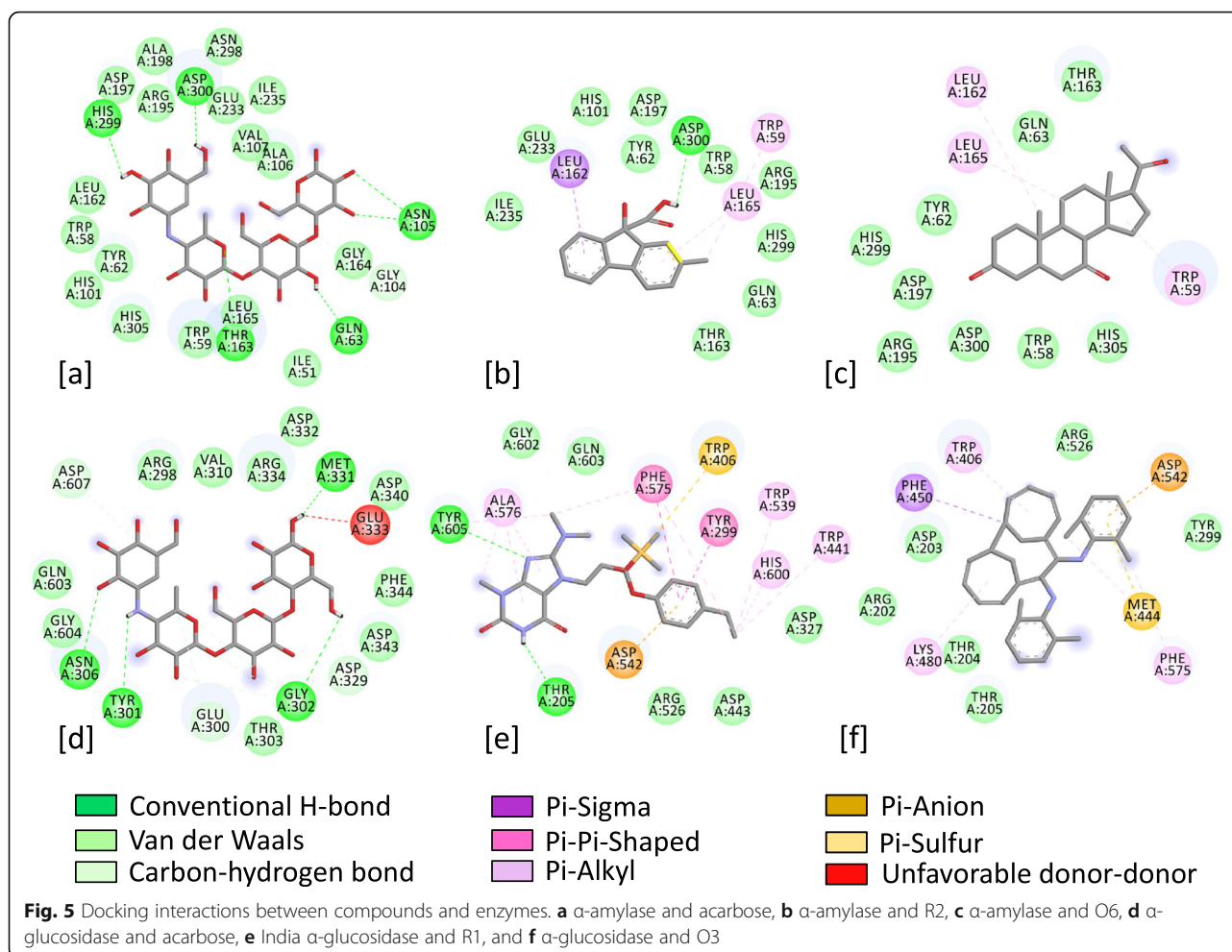
one unfavorable donor-donor bond (Fig. 5d). Amylase and R2 interactions showed one H-bond (Asp300) and 12 numbers of other types of interactions such as ten van der Waals, Pi-sigma, and Pi-alkyl interactions (Fig. 5b). Amylase and O6 did not show any conventions H-bond interactions but showed van der Waal's, and Pi-alkyl interactions. Similarly, glucosidase showed two H-bond with compound R1 and several other interactions including van der Waal's interactions (Fig. 5e). Like α-amylase, α-glucosidase did not show any H-bonding with *O. indicum* best docking score ligand O3. However, O3 showed other interactions such as van der Waals and Pi-bonds (Fig. 5f).

Discussions

Rauvolfia tetraphylla and *Oroxylum indicun* are among the important medicinal plants having several medicinal values. The present study investigates the trace and heavy metal content, bioactive compounds and enzyme inhibition property of *R. tetraphylla* and *O. indicum* extracts on α-amylase and α-glucosidase activity in in vitro system. Study of metallic content is an important aspect

of pharmacology to ascertain whether the plant has any toxic effect. Trace elements and metallic contents play many important physiological functions including cofactors or activators of enzyme functions [41]. On the other hand, heavy metals such as Cd, Cr, Hg, Pb, etc. do not have any biological role but known to be toxic elements leading to many health complications and diseases [42]. Higher accumulation of Cd is known to cause pulmonary complications, kidney-related diseases, unusually rapid heartbeat, liver-problem, along with many other complications [43]. Similarly, exposure to Pb may lead to health problems starting from headaches, vomiting, or abdominal pain to the severe complications such as brain damage, kidney damage and many other life-threatening diseases [44]. The present study showed that the *R. tetraphylla* (root) and *O. indicum* (leaves) possess a negligible amount of toxic heavy metals compared to the WHO permissible level [2]. The result, therefore, suggests the negligible toxic effect of plants in terms of heavy metal content. Phytochemical analysis by GC-MS revealed four major compounds from *R. tetraphylla* and seven from *O. indicum*. Several phytochemicals have

been reported from different parts of both the plants by many other researchers [14, 28]. However, none of the reported compounds from the present study were found to have any literature regarding its biological activities.

Enzymes involved in the metabolism of carbohydrates such as α-amylase and α-glucosidase can significantly reduce the postprandial hyperglycemia. Inhibition of such enzymes, therefore, can be an important strategy in the management of type-2 diabetes. Currently, plant-based medicines and phytochemicals that modulate physiological functions leading to the prevention and cure of diabetes and obesity gained much attention and research drive. Many research findings have revealed the α-amylase and α-glucosidase enzyme inhibition activity of medicinal plants [45, 46]. Based on our earlier publication revealing the ethnomedicinal and antidiabetic property of *R. tetraphylla* and *O. indicum*, an in vitro experiment was carried out to study the α-amylase and α-glucosidase inhibitory activity of the plants. In the present study methanolic crude extracts of *R. tetraphylla* and *O. indicum* showed strong inhibitory activity against α-amylase and α-glucosidase which justifies the traditional claim of antidiabetic medicinal value. The leaf extract of *O. indicum* showed stronger inhibition against α-amylase and α-glucosidase compared to the root extract of *R. tetraphylla*. However, very few literatures have been found regarding the enzyme inhibition activity of both the plants. Recently, Li et al. [47] isolated two compounds, oroxins-C and -D from the seed extracts of *O. indicum* showing strong amylase and glucosidase inhibitory activity. In another study, bioactive compound baicalein from *O. indicum* in combination with acarbose showed postprandial blood glucose level [48]. From the present finding it may be indicative that the synergistic effects of phytochemicals present in both the plants inhibited the α-amylase and α-glucosidase enzyme activity in a dose-dependent manner. Similarly, we also revealed similar pattern of binding affinity when the identified compounds (R1 - R4 and O1 - O7) were docked with α-amylase and α-glucosidase enzyme. Molecular docking is a widely used, relatively fast, and economical computational tool for predicting in silico binding modes and affinities of molecular recognition events. By using in silico method virtual screening of large number of chemicals can be screened in order to select the likely drug candidate [49]. In silico molecular docking has been used by many researchers to verify the effectiveness of phytochemicals against amylase and glucosidase enzyme [50, 51]. Ligand – enzyme binding and interaction studies have shown that the compounds from *O. indicum* showed better affinity to both the enzymes compared to *R. tetraphylla*. Mainly three compounds, O2, O3, and O6 showed strong affinity to both α-amylase and α-glucosidase. Almost similar patterns of inhibitory activities were observed in the in vitro enzyme inhibition study

which augmented the in vitro assays. However, our study did not find any significant correlation to the binding energies and drug-likeness properties of the compounds. Furthermore, the findings of present study may not be conclusive enough to suggest the same effectiveness of plant extract in in vivo system. Because of the difference in various physiological factors phytochemicals or drugs showing promising result in in vitro experiments fails to show the same in in vivo system.

Conclusion

The present study revealed the α-amylase and α-glucosidase inhibitory property of *Rauvolfia tetraphylla* and *Oroxylum indicum*. The phytochemicals identified by the GC-MS analysis and in silico molecular docking supports the enzyme assay signifying the importance of both the plants for possible candidate of antidiabetic drug discovery. However, detailed in vivo and phytochemical characterisation leading to the isolation and purification of active compounds need to be carried out to know the exact mode of action.

Authors' contributions
AS designed the main research design, manuscript writing; MD carried out the experimental work. All author(s) read and approved the final manuscript.

Availability of data and materials
Not applicable.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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