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In vitro α-amylase inhibition effect of ethanol extract of *Alpinia nigra* (Gaertn.) leaves and molecular docking, ADME/T property studies of some of its isolated compounds.

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ABSTRACT

The present study aims to investigate the α-amylase inhibition of ethanol extract of *Alpinia nigra* (EEAN) (Gaertn.) leaves by modified enzyme inhibitory action and *in silico* molecular docking used for five phytoconstituents namely α-fenchyl acetate, α-pinene, α-terpineol, camphene, camphor isolated from *A. nigra*, to identify whether these compounds interact with the responsible protein (α-amylase enzyme). And also ADME/T properties of the phytoconstituents were analyzed using Qikprop 3.2 module. EEAN had good α-amylase inhibitory activity (IC_{50} = 1.803±0.032 mg/ml) as compared to Acarbose. A wide range of docking score found during molecular docking by Schrodinger. α-fenchyl acetate, α-pinene, α-terpineol, camphene, camphor showed the docking score -3.938, -3.344, -3.291, -3.463, -3.547, respectively. Among all the compounds, α-fenchyl acetate showed highest docking score. So, α-fenchyl acetate is the best compounds for α-amylase inhibition, as it possessed higher value in Molecular docking. From the ADME profiles of all the tested compounds, it cleared that they might safe for human. Further *in vivo* investigation need to identify whether isolated compounds from *A. nigra* have α-amylase inhibitory activity or not.

Key words: *A. nigra*, α-amylase, molecular docking, ADME/T properties, α-fenchyl acetate.

INTRODUCTION

Diabetes is considered to be a severe complicated disorder characterised by symptom and abnormal sugar metabolisms and related to many complications like hyperlipidemia, retinopathy and cardiovascular diseases which are major causes of morbidity and death [1, 2]. Now, treatment of diabetes involves reduction in hyperglycaemia by various groups of drugs like biguanides, thiazolidinediones, sulphonylureas, meglitinides and α-glucosidase inhibitors in addition to insulin [1, 3-5]. As a result of unwanted severe side effects that are a serious limitation, there is associate increased the demand for new antidiabetic agents [1].

Amylases are important enzymes widely used in sugar, animal nutrition, food, fermentation, textile, paper and pulp, and bioenergy industries [6, 7] and make up 25% to 33% of the world's enzyme market [8, 9]. Amylases are a category of hydrolases widely distributed in microbes, plants and animals and specifically cleave the O-glycosidic

bonds in starch, polysaccharide and a number of other oligosaccharides [10]. The α-amylase family is the most extensively investigated class of enzymes that hydrolyze starch to yield numerous product, as well as dextrins and increasingly smaller polymers composed of glucose units [11].

Traditionally healthful plants have served to be efficient antidiabetic agents for ages because of their wealthy diversity of phytochemicals [1]. Although the progression of synthetic drugs, to certain extend, has raised the health care of people, hitherto the use and importance of phytomedicines for the same has never been neglected and a large number of plants are screened for their efficacy against diabetic and hyperglycemic diseases[12, 13]. *Alpinia nigra* (*A. nigra*) (Gaertn.) Burtt (Zingiberaceae) is that kind of plant, because it is used as traditional medicine for diabetic. Diabetic patients use it in various forms eg. juice of *A. nigra* as home remedy against diabetes mellitus. *A. nigra* commonly known as galangal, black-fruited, kala is a herb, which is widely cultivated in Asia, Africa and South America, is a diverse medicinal plant which has been therapeutically used in the treatment of various diseases. Various therapeutic activities of this plant which has been reported are anti-inflammatory [14], analgesic, antibacterial, cytotoxic [15], anthelmintic [16], anxiolytic-sedative [17] etc. Research showed that, isolated compounds from *Alpinia nigra* had well inhibition of α-glucosidase activity [18]. Diabetic patients use it in various forms eg. juice of *A. nigra* as home remedy against diabetes mellitus. The hypoglycemic effect of *A. nigra* was also evaluated by the established methods [19].

Molecular docking is a computational chemistry method which has become essential for the rational drug design process [20, 21]. Molecular docking has become a major computational method for the prediction of ligand–receptor interactions [22] . Over the previous couple of years the amount of latest molecular targets has increased because of the completion of the human genome project, also because the protein and protein–ligand complex structures isolated by high-throughput protein purification [23] and solved by crystallography and nuclear magnetic resonance spectroscopy techniques [24, 25]. At an equivalent time, the advance of computational techniques for finding out interactions of ligands with the biological targets at the atomic scale have increased and developed.

The aim of the study to investigate the α-amylase inhibition of ethanol extract of *Alpinia nigra* (EEAN) leaves by modified enzyme inhibitory action. Furthermore, the mechanism of action of the isolated compounds from *A. nigra* was explored by molecular docking analysis and ADME/T property studies.

MATERIAL AND METHOD

Collection and Identification

Leaves of *A. nigra* were collected from Bangladesh Centre for Scientific and Industrial Research (BCSIR), Chittagong, Bangladesh, in the month of April 2014. It was identified and authenticated by standard taxonomical method at BCSIR.

Preparation of plant extract

The collected leaves (5 kg) were washed with fresh water and dried in shade at room temperature (25^0C). The dried leaves were grounded into fine powder by an electrical grinder (Wiley mill) and mesh (mesh number 50) was used to sieve the sample. Then the powder of leaves of *A. nigra* was pasted by homogenizing with mortar and was suspended with water for preparing the ethanol extract. About 900g of the leaves were dissolved in absolute ethanol (99% ethanol, source) for 7 days and then filtered. Collected supernatant was dried by using a rotary vacuum evaporator (BUCHI Rota vapor R-114). Semisolid crude extracts were again dried with water bath at 80°C. The dried extracts (yield, 12%) were kept in the freezer (4°C) and utilized for biological screening.

Chemicals and equipment

All chemicals used were of analytical reagent grade. Ethanol was purchased from Merck, Germany. α-amylase was purchased from Sigma-Aldrich (Sigma-Aldrich Co., USA). Starch, iodine was purchased from Fluka (Fluka chemie GmbH, CH-9471 Buchs). Shimatdzu Biospec 1601 UV visible spectrophotometer (Shimadzu, Japan) was used to measure the absorbance.

In vitro α-amylase inhibitory activity

This study was performed by a modified starch iodine protocol as Hossain et al [26] with minor modification. In short, 1 mL of plant extract or standard of different concentration (2, 1, 0.5 mg/mL) was taken in relabeled test tubes. The reaction mixture contained 20 µl of α-amylase solution (10 mg/ml), phosphate buffer (0.02 M, pH 7.0) with 0.006 M NaCl (0.4 ml) was added to each test tube and incubated for 10 min at 37°C. After the incubation 200 µl of 1% starch solution was added to each test tube and the mixture was re-incubated for 1 h at 37 °C. Then 200 µl of 1% iodine solution was added to each test tube and after that, 10 mL distilled water was added. Absorbance of the mixture was taken at 565 nm. Sample, substrate and α-amylase

blank were undertaken under the same conditions. Each experiment was done in triplicate. IC50 value was calculated by using regression analysis.

% α-amylase inhibition= [1 - ((SA-SBB)-SMB)/AAB] × 100
SA=Sample absorbance, SMB=Sample blank, SBB=Substrate blank, AAB=α-amylase blank

Statistical analysis
The data on *in vitro* studies were reported as mean ± S.E.M. (n = 3). Regression analysis was performed to calculate IC50 values by using Microsoft Excel 2007. For graphical presentation, GRAPHPAD PRISM® (version 6.00; GraphPad Software Inc., San Diego, CA, USA) was used.

***In silico* analysis**
Molecular docking analysis of isolated compounds from *Alpinia nigra*
Preparation of protein structure
The 3D coordinates of crystal structure of α-amylase (PDB: 1PPI) was downloaded from the RCSB protein data bank (http://www.rcsb.org/pdb) set up at Brookhaven National Laboratory in 1971. It is a worldwide repository of information about the 3D structures of large biological molecules, including proteins and nucleic acids. Water molecules were removed from the protein 1PPI before the instigation of molecular docking. The protein structure was corrected by the utilization of alternate conformations and valence monitor options as some crystallographic disorders as well as some unfilled valance atoms were present in the protein file. The resultant protein file was subjected to energy minimization by applying Chemistry at HARvard Macromolecular Mechanics (CHARMm) force fields. CHARm is a program which provides a large suite of computational tools that encompass numerous conformational and path sampling methods, free energy estimates, molecular minimization, dynamics, and analysis techniques, and mode l- building capabilities (http://www.charmm.org/). After the energy minimization the protein fie was subjected to define and edit binding site option available on tools panel to explore the plausible binding site within the protein (1PPI).

Preparation of ligand
The structures of compounds α-fenchyl acetate, α-pinene, α-terpineol, camphene, camphor [27-29] were drawn using ChemBioDraw software. ChemBioDraw™ is a software from PerkinElmer for development of chemical structures of bioactive compounds. The prepared ligand was then subjected to add the hydrogen bonds and the energy has been minimized using CHARm force field.

Docking analysis
To find out the accurate binding model for the active site of α-amylase enzyme, molecular docking analysis was performed using ligand fit of GLIDE software from Schrodinger (http:/ /www.schrodinger.com /) . Molecular docking analysis was performed using crystal structure of α-amylase (PDB: 1PPI). The structure of crystal structure of α-amylase (PDB: 1PPI) were obtained from Protein Data Bank (http://www.rcsb.org). The mechanism of ligand position is based on the fitting points. Fitting points are incorporated into the hydrogen bonding groups on the ligand and the proteins. The ligand fit module [30] from GLIDE software was utilized to execute the molecular docking analysis, based on shape-based searching and Monte Carlo methods. At the time of docking, variable trials Monte Carlo conformation was applied where the number of steps depends on the n umber of rotatable bonds present in the compounds/ligands. By default the torsion number is 2, the maximum minimizations steps is 300 and maximum successive failure is 110. During the docking process the top ten conformations were engendered for each o f the compound after the minimization of the energy [31].

ADME/T property analysis
Ligand based ADME/Toxicity prediction
The QikProp module of Schrodinger (Maestro, version 10.1) is a quick, accurate, easy-to-use absorption, distribution, metabolism, and excretion (ADME) prediction program design to produce certain descriptors related to ADME. It predicts both physicochemical significant descriptors and pharmacokinetically relevant properties. ADME properties determine drug-like activity of ligand molecules based on Lipinski's rule of five. ADME/T properties of the compound (DIM) was analyzed using Qikprop 3.2 module [32].

RESULTS AND DISCUSSIONS

***In vitro* α-amylase inhibitory activity**
Ethanol extract showed good α-amylase inhibition. EEAN showed IC50 value (1.803±0.032) mg/mL; $P<0.05$, where as standard Acarbose showed (0.912±0.015) mg/mL; $P<0.05$. Ethanol extract soundly inhibited α-amylase in a dose dependent manner like Acarbose. Therefore we can conclude that this leaves extract have good α-amylase inhibitory activity. All results are shown in Table 1 and Figure 1.

***In silico* analysis**

Molecular docking analysis

In this study, the binding mode of α-amylase enzyme was investigated by doing computational analysis, glide docking. Both glide standard (SP) and extra precision (XP) mode had been introduced, where extra precision mode used for cross validation purpose. The results of docking analysis were described in Table 2 and the docking figure showed in Figure 2. Among all the compounds, α-fenchyl acetate showed highest docking score.

ADME and Toxicity analysis

Ligand based ADME/Toxicity prediction

The drug-like activity of the ligand molecule was categorized using ADME properties by QikProp module of Schrodinger. The ADME properties of the α-fenchyl acetate, α-pinene, α-terpineol, camphene, camphor were evaluated with QikProp module of Schrodinger, shown in Table 3. The selected properties are known to influence metabolism, cell permeation, and bioavailability. All the predicted properties of the isolated compounds were in the range for satisfying the Lipinski's rule of five to be considered as drug like potential.

CONCLUSION

From the study it was found that, *Alpinia nigra* could be great source of new α-amylase inhibitor. Both *in vitro* and *in silico* models support that it has potential α-amylase inhibition activity and all the isolated compounds from *A. nigra* could be α-amylase inhibitor. Among all the compounds, α-fenchyl acetate showed highest docking score. So, α-fenchyl acetate is the best compounds for α-amylase inhibition, as it possessed higher value in Molecular docking. From the ADME profiles of all the tested compounds, it cleared that all compounds are safe for human. Further *in vivo* investigation need to identify whether isolated compounds from *A. nigra* have α-amylase inhibitory activity or not.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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Table 1: IC_{50} values (mg/mL) for ethanol extract of leaves of *Alpinia nigra* (EEAN) and Acarbose in α-amylase inhibitory assay.

Extract/Standard	Concentrations in mg/mL with (% Inhibition)			IC_{50} value mg/mL
EEAN	0.50(35.61±0.421)	1.00(43.48±0.486)	2.00(51.47±0.575)	1.803
Acarbose	0.25(15.89±1.117)	0.50(26.05±0.438)	1.00(55.38±0.888)	0.912

Values are the mean of triplicate experiments and represented as mean±SEM (n=3).

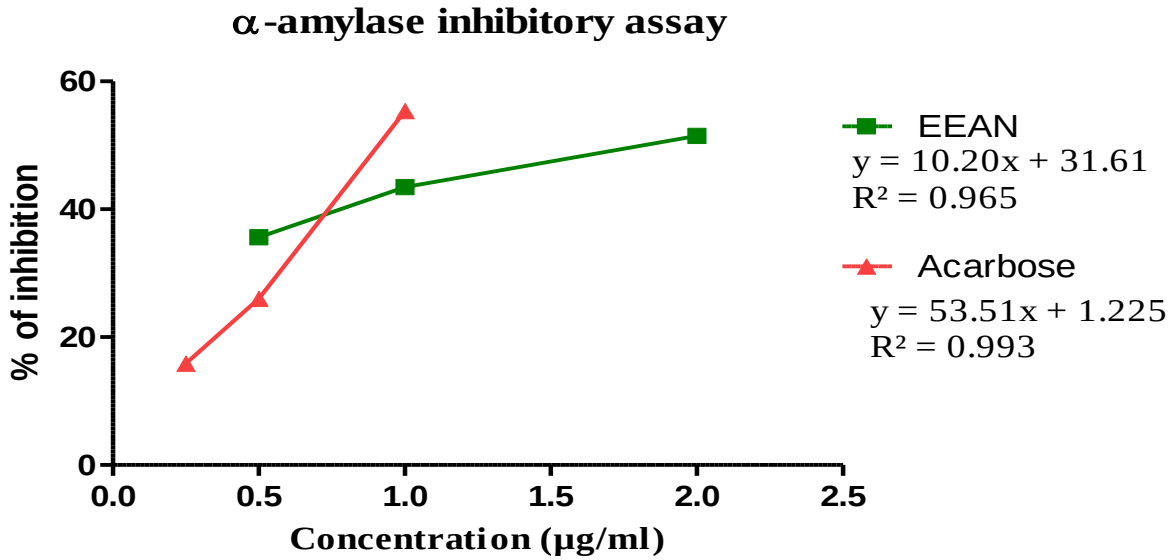


Figure 1: IC_{50} values (mg/mL) for ethanol extract of leaves of *Alpinia nigra* (EEAN) and Acarbose in α-amylase inhibitory assay.

Table 2: Docking results with α-fenchyl acetate, α-pinene, α-terpineol, camphene, camphor in the α-amylase enzyme (PDB: 1PPI).

Compound Name	Docking Score	Glide Emodel	Glide Energy
α-fenchyl acetate	-3.938	-30.816	-24.717
α-pinene	-3.344	-18.186	-14.439
α-terpineol	-3.291	-24.613	-20.07
camphene	-3.463	-22.081	-17.774
camphor	-3.547	-23.151	-17.19

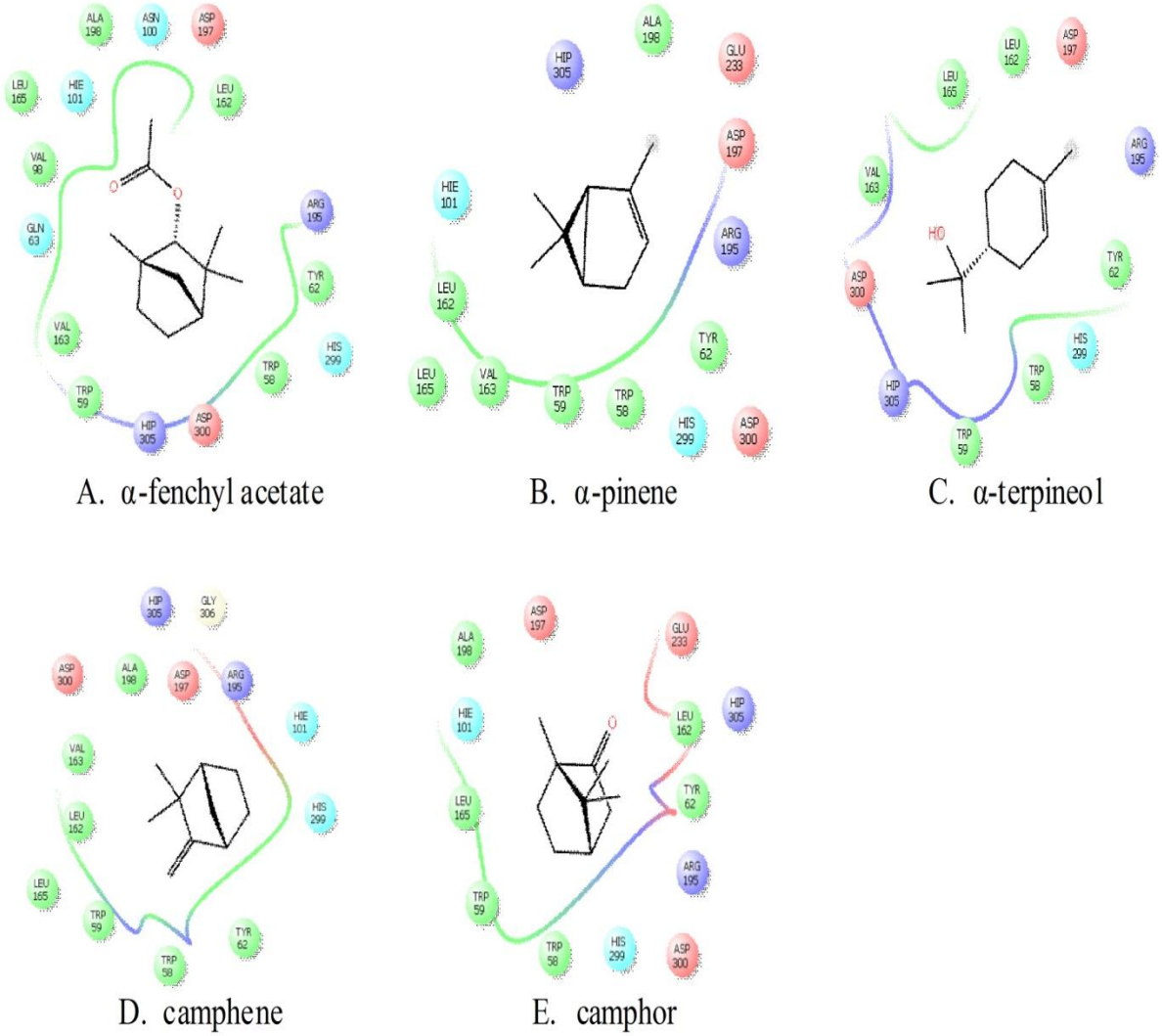
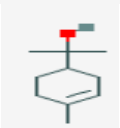


Figure 2: Molecular docking analysis of A. α-fenchyl acetate, B. α-pinene, C. α-terpineol, D. camphene, E. camphor with α-amylase enzyme (PDB: 1PPI) receptor complex obtained from Glide docking.

Table 3: ADME/T properties of α-fenchyl acetate, α-pinene, α-terpineol, camphene, camphor by QikProp.

Name of Molecules	PubChem CID	Structure	MW[α]	HB donor[β]	HB acceptor[€]	LogP[¥]	Molar Refractivity[µ]
α-fenchyl acetate	6427102		196.28	0	2	3.659129	55.34
α-pinene	6654		136.23	0	0	2.586490	43.96
α-terpineol	17100		154.25	0	1	3.949099	47.07
camphene	6616		136.23	0	0	2.697560	43.76
camphor	2537		152.23	0	1	2.950680	44.39

[α]Molecular weight (acceptable range: <500).
[β]Hydrogen bond donor (acceptable range: ≤5).
[€]Hydrogen bond acceptor (acceptable range: ≤10).
[¥]High lipophilicity (expressed as LogP, acceptable range: <5).
[µ]Molar refractivity should be between 40-130.

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