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Original Article

Experimental analysis of isolated compounds of Mango (*Mangifera indica L.*)Seed Kernel Extract for promoting insulin against diabetes mellitus by using computational analysis

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ABSTRACT

Mangifera indica Linn (Family: Anacardiaceae), commonly known as mango widely used in the traditional medicinal system. This study is performed to gain information by molecular docking of seven isolated compounds of M. indica that are responsible for anti-diabetic activity by promoting insulin. Molecular docking was done by Schrodinger, whereas the SwissADME and admetSAR were used for ADME and toxicological prediction. In molecular docking study, Gallic acid (-6.345kcal/mol) showed the highest docking scores where all compounds satisfied Lipinski's rule of five while without one, all other compounds showed non-toxicological properties. This study evident that isolated compounds possess significant anti-diabetic properties, which might be beneficial in the treatment of diabetes mellitus.

Keywords: Anti-diabetic, Mangifera indica, Mango, Medicinal plants

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INTRODUCTION

Mango (Mangifera indica L.) is a juicy stone fruit, also known as the king of all fruits. Mangos are produced from numerical topical trees. It is the national fruit of India and Pakistan, and the national tree of Bangladesh[1]. It is the unofficial national fruit of the Philippines [2]. However, after consumption or industrial processing of the fruits, considerable amounts of mango seeds are discarded as waste. Nevertheless, Recently, Mango seed kernel extract has shown different activities like Antioxidant and antimicrobial [3], antidiarrhoeal efficacy [4], hepatoprotective [5], and antibacterial [6]. Molecular Docking Studies has shown on Anti-Tyrosine[7] and Anti-Snake Venom[8]. It was also inferred that there was a significant increase in the shelf-life of ghee, and it is also a good source of natural antioxidants due to various types of phospholipid and phenolic compounds present in mango seed kernels.[3]

In the early 1920s, Canadian surgeon Dr. Frederick Banting and medical student Charles Best discovered that insulin could help normalize blood sugar levels. Generally, after eating food, cells in our pancreas (known as beta cells) are signaled to release insulin into the bloodstream. Insulin attaches and signals cells to absorb excess sugar from the bloodstream. Insulin often described as a "key," which unlocks the cell to allow sugar to enter the cell for energy. When there is a lack of insulin, the blood glucose level goes high. This phenomenon is known as diabetes. Diabetes is a disease that occurs when there is a lack of insulin hormone or an excess amount of glucose level in our body. Based on the requirement of insulin, diabetes is generally divided into two types. Type-1 (Insulin-dependent diabetes mellitus) and Type-2 (non-insulin-dependent diabetes mellitus). Nowadays, Insulin is of vital importance in controlling blood sugar, protecting β cells, preventing and delaying the occurrence of other diseases related to diabetes. There are lots of therapeutic ways to administer insulin for clinical practice like subcutaneous injection, oral preparations, transdermal patches, pulmonary inhalation administration, and nasal mucosal administration. However, there still exist many problems in some non-injection routes, because of a large amount of peptide hydrolase and proteolytic enzyme in the gastrointestinal tract, oral administration is efficiently catalyzed by decomposition, some drugs with relatively high molecular mass have poor permeability and low bioavailability [9]. According to a new report, more than 100 million U.S. adults are now living with diabetes or pre-diabetes and approximately 7.4 million Americans with diabetes use one or more formulations of insulin[10][11]. Our aim to predict, a possible isolated compounds of M. indica as potential promoter of insulin to reduce and maintain diabetes mellitus by using molecular docking and ADME/T analysis.

MATERIALS AND METHODS

Selection of the compounds: From the literature review, we have seen that the seed kernel of Mangifera indica L has several isolated compounds. For our study, we have selected: caffeic acid, cinammic acid, gallic acid, valine, phenylalanine [12], arbutin[13] and methyl gallate[7]. All chemical structures were downloaded from the PubChem database.

PASS prediction: Major compounds of Mangifera indica L. seed kernel are selected for the PASS Prediction with the help of PASS (prediction of activity spectra for substances) online program. This server provides a quantitative structure relationship based on structure followed by the generation of models obtained from the bioactive ligand. This server estimates the predicted activity spectrum as of a compound as Probable activity (Pa) & Probable inactivity (Pi). The value of Pa and Pi varies from 0.000 to 1.000. Only activities with Pa> Pi are considered as possible predicts for a particular compound.

Molecular Docking

Ligand Preparation: As mentioned before, the structure of all the compounds is downloaded from the PubChem database as a 2d (two-dimension) SDF format. By using Ligprep in Maestro 11.1 under the force field OPLC3, all the 2D structures are converted to 3D (three-dimension) energy minimized structure for further investigation.

Preparation of protein: Three dimensional X-ray crystal structure of insulin R6 hexamer (PDB:1EV3) was downloaded from protein data bank RCSB at a resolution of 1.78 Å [14]. Protein preparation Wizard of Maestro version 11.1 was used for the preparation of protein for further docking experiments.

Receptor grid and molecular docking: Receptor grid generation was used to generate a receptor grid for ligand-protein interaction in 1EV3 using the OPLC3 force field, and all other parameters are in the default format. The standard precaution was followed to prepare flexible ligand sampling during ligand sampling. Only the best scoring pose with a docking score was noted down for each ligand.[15]

Prediction of the pharmacokinetic parameter (ADME): In-silico prediction of ADME (absorption, distribution, metabolism, excretion) properties is significant for a pharmacokinetic

parameter. In our study, the SwissADME free online server was used to predict ADME. According to Lipinski's rule of five a compound could show drug-likeliness properties if it does not fail more than one of the following principles: (i) molecular weight not more than 500; (ii) H-bond acceptors ≤10; (iii) H-bond donors ≤5; (iv) Lipophilicity < 5; and (v) Molar refractivity between 40 and 130 [16].

Prediction of toxicological properties: Toxicological properties of the isolated compounds was determined by using the admetSAR1 free online server. In our study, Ames toxicity, carcinogenic properties, acute oral toxicity, and rat acute toxicity were predicted.

RESULTS

PASS Prediction: Seven major isolated compounds of mango seed kernel, i.e., caffeic acid, cinammic acid, gallic acid, valine, phenylalanine, arbutin, and methyl gallate were invested by PASS Online for anti-diabetic activity. All the selected compounds showed higher Pa value than Pi. The result is shown in table 1

Molecular docking study: Our result shows that gallic acid has the highest binding affinity with docking score -6.345 (kcal/mol) by interacting with CYS A: 11, CYS A: 6 and HIS B: 10 (hydrogen bonding) at the same time cinammic acid showed the lowest binding affinity with docking score -4.118(kcal/mol) by interacting with CYS A: 11(hydrogen bonding).

Other compounds caffeic acid, arbutin, methyl gallate interact with the enzyme by forming three hydrogen bonds to CYS A: 11 and CYS A: 6 with docking score -5.704(kcal/mol), -5.422(kcal/mol), -6.085(kcal/mol). Valine and phenylalanine interact in the same enzymatic pocket by forming two hydrogen bonds with docking score -4.706(kcal/mol), -4.642(kcal/mol), respectively. The docking result of the compounds is shown in table 2 and the figure in figure1.

Pharmacokinetic parameter (ADME): In our study, ADME was determined by Swiss ADME free online server and the result is shown in table3, all compounds satisfied Lipinski's rule of five, which indicates that this compounds can use further for the new drug development process.

Toxicological properties: On the other hand, toxicity was determined by the admetSAR1 online server, and the result is shown in table 4. According to the predicted acute oral toxicity values, cinammic acid, gallic acid, valine, phenylalanine, arbutin, methyl gallate compounds showed ''class III"; but caffeic acid showed ''class IV", and all compounds showed weak rat acute toxicity with an LD50 (median lethal dose) value of1.4041 to 2.0814mol/kg. Here ''class III" consisted of compounds with LD50 values greater than 500 mg/kg but less than 5000 mg/kg, and ''class IV" consisted of compounds with LD50 values greater than 5000mg/kg. Compounds in ''class III" are generally considered suitable from a druggable point of view. So from the discussed result, we can say that without caffeic acid, all other compounds are qualified for use as promising drugs with good oral bioavailability and safety features.

DISCUSSION

Many drug candidates fail to reach the market despite spending a huge amount of money and time because of their toxic profile and failing in critical trials[17]. This problem can be optimized by computational analysis. It has accelerated drug discovery regarding reducing a total number of repetition, time, and money[18]. From this point of view, we performed our study.

PASS prediction is a computer-aided drug discovery program to predict biological activity to accelerate for potent natural products. Our selected compounds predicted significant activity for insulin promotion for anti-diabetic activity with the appropriate Pa and Pi ratio. The compound valine showed the best insulin promoter activity with Pa value 0.810. According to the Pa value, the prediction result showed as following: Valine>phenylalanine>cinammic acid>gallic acid>caffeic acid> methyl gallate>arbutin

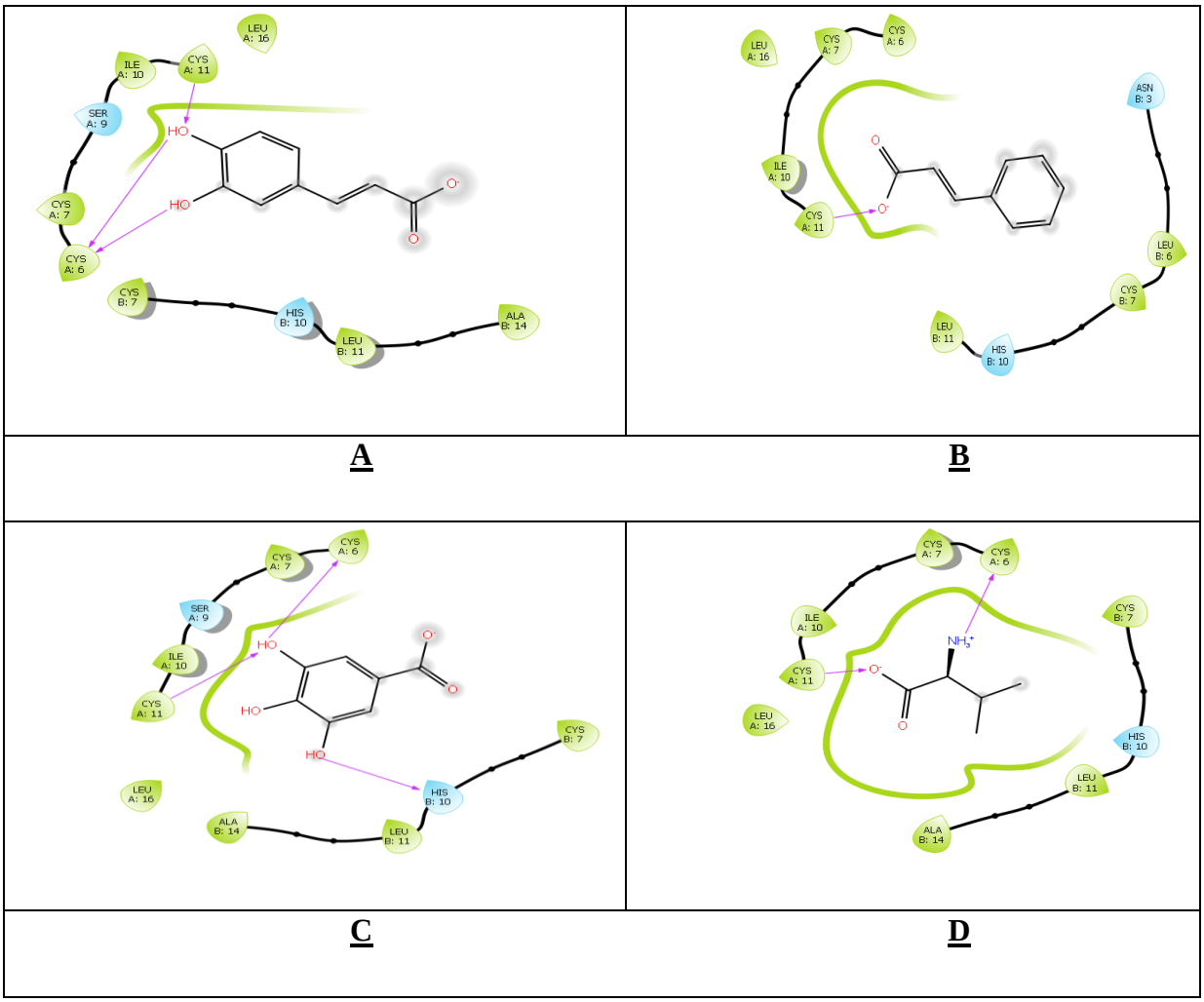
In silico molecular docking, a study is the most powerful tool to investigate the active site of the protein, and also to understand and elucidate the binding interactions between ligands and target enzymes [19] Virtual docking also requires ligand ¬protein docking to evaluate a large library of compounds to identify structures that are most likely bound to a protein receptor or enzyme[20]. Regarding this point of view, we performed our study. To determine the potential of insulin promotion against diabetes mellitus, we have subjected the docking analysis of the active compounds of M. indica to the active site insulin R6 hexamer. In order to study the interaction of the compounds cinammic acid, gallic acid, valine, phenylalanine, arbutin, methyl gallate, caffeic acid with 1EV3, we performed Glide docking analysis by Schrodinger suite v11.1, where among of these compounds, in molecular docking the best pose found for gallic acid with docking score -6.345 (kcal/mol) by interacting three hydrogen bond with CYS A: 11, CYS A: 6 and HIS B: 10. The negative

and low value of free energy of binding exhibits a strong auspicious bond between 1EV3 and gallic acid in most favorable conformations. The results of docking analysis were described in Table 2 and the docking figure showed in Figure 1.

For further confirmation, we performed the pharmacokinetic and toxicological properties of those compounds. This parameter is considered as crucial in the drug development process because it determines absorption to elimination and toxicological properties of a drug. Besides, having poor pharmacokinetic properties means fail to commercialize a drug, so it is more important to check its proprieties initially, which depends on chemical descriptors of the molecules [21]. From this point of view, we used SwissADME (free online server) to calculate ADME(absorption, distribution, metabolism, excoriation) based on Lipinski rule of five and none of the compounds violate the Lipinski rule of five which indicates that all these compounds are suitable for drugs and can pass the membrane. On the other hand, the admetSAR1 online server was used to determine toxicological properties. Our study showed that without caffeic acid, none of the compounds posed a risk of ames toxicity, carcinogenicity, acute oral toxicity, and rat acute toxicity. Therefore, all these compounds are considered to be safe and orally bioavailable from a druggable point of view.

CONCLUSION

It can be concluded that the results of the present study demonstrated bioactive compounds from the seed kernel of Mangifera indica L. has potential affectivity as an anti-diabetic agent. Moreover, ADME/toxicology analysis showed that six bioactive compounds are safe and orally bioavailable from a druggable point of view. Therefore it can be concluded that these compounds could be a good source for new anti-diabetic drug development. Further studies are recommended to revel there an in-depth mechanism in an animal model.



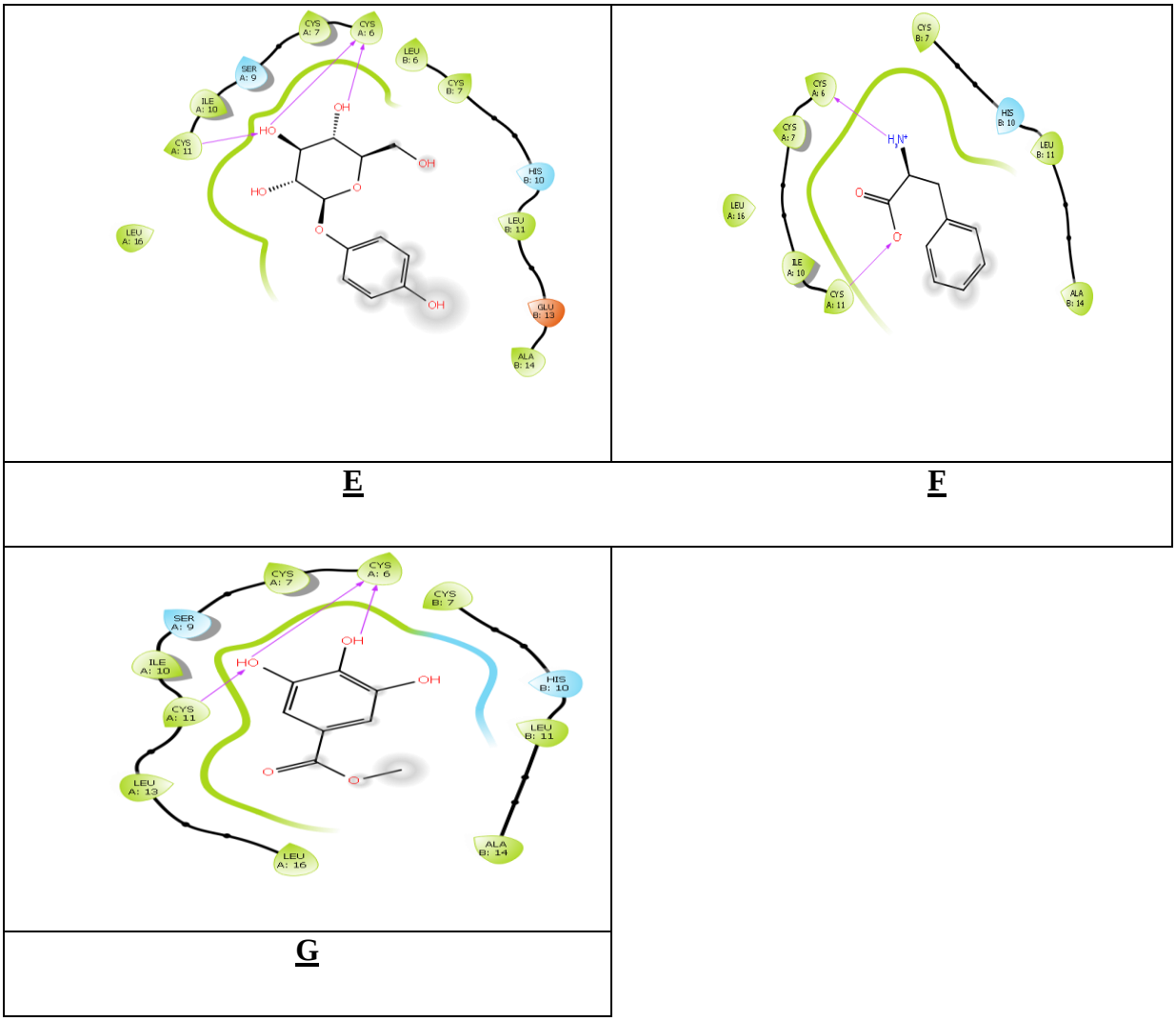


FIGURE 1: 2D interactions of the caffeic acid (A), cinammic acid (B), gallic acid (C), valine(D), phenylalanine(E), arbutin (F) and methyl gallate (G) with the active site of insulin R6 hexamer (PDB:1EV3). Colors indicate the residue (or species) type: Green-hydrophobic (Ala, Ile, Leu, Cys), Blue-polar (Ser, Asn, His), Light gray-other (Gly, water) and Darker gray-metal atoms. Interactions with the protein are marked with lines between ligand atoms and protein residues: Solid pink: H-bonds to the protein backbone. Ligand atoms exposed to solvent are marked with gray spheres. The protein "pocket" is displayed with a line around the ligand, colored with the color of the nearest protein residue. The gap in the line shows the opening of the pocket.

Table 1: Anti-diabetic activity for *Mangifera indica L.* seed kernel major compounds by PASS online.

Phytoconstituents	Biological activity (Insulin promoter)	
	Pa	Pi
Caffeic acid	0.469	0.037
Cinammic acid	0.631	0.011
Gallic acid	0.567	0.018
Valine	0.810	0.004
Phenylalanine	0.777	0.004
Arbutin	0.293	0.148
Methyl gallate	0.479	0.034

Table 2: Docking scores and binding interactions of the selected compounds against insulin R6 hexamer.

Compounds	Docking score (kcal/mol)	Glide e model (kcal/mol)	Glide energy (kcal/mol)
Insulin R6 hexamer (PDB: 1EV3)			
Caffeic acid	-5.704	-34.024	-24.446
Cinammic acid	-4.118	-23.494	-17.995
Gallic acid	-6.345	-33.923	-24.174
Valine	-4.706	-18.305	-13.11
Phenylalanine	-4.642	-23.915	-16.97
Arbutin	-5.422	-38.085	-30.425
Methyl gallate	-6.085	-36.469	-27.016

Table: 3: ADME properties of the isolated compounds by the SwissADME for good oral bioavailability

	Lipinski rule of five					
COMPOUNDS	MW (g/mol)	HBA	HBD	LogP	AMR	Ro5V
Rule	< 500	≤ 10	≤ 5	< 5	40–130	≤ 1
Caffeic acid	180.16	4	3	0.93	47.16	0
Cinammic acid	148.16	2	1	1.79	43.11	0
Gallic acid	184.15	5	3	0.57	43.79	0
Valine	117.15	3	2	-0.78	30.63	0
Phenylalanine	165.19	3	2	-0.01	45.5	0
Arbutin	272.25	7	5	-0.88	62.61	0
Methyl gallate	184.15	5	3	0.57	43.79	0

MW: molecular weight, *HBA:* hydrogen bond acceptor, *HBD:* hydrogen bond donor, *Log P:* lipophilicity, *AMR:* molar refractivity; Ro5V- Rule of five violation.

TABLE 4: Toxicological properties of the isolated compounds by admetSAR -1.

Compounds	Parameters				
	AMES Toxicity	Carcinogens	Acute Oral Toxicity	Rat Acute Toxicity	Blood-Brain Partition Coefficient
Caffeic acid	Non-AMES toxic	Non-carcinogens	IV	1.4041	BBB-
Cinammic acid	Non-AMES toxic	Non-carcinogens	III	1.7416	BBB+
Gallic acid	Non-AMES toxic	Non-carcinogens	III	1.8670	BBB-
Caline	Non-AMES toxic	Non-carcinogens	III	1.4765	BBB+
Phenylalanine	Non-AMES toxic	Non-carcinogens	III	1.9053	BBB+
Arbutin	Non-AMES toxic	Non-carcinogens	III	1.9110	BBB+
Methyl gallate	Non-AMES toxic	Non-carcinogens	III	2.0814	BBB-

Category-III means (500 mg/kg> LD50< 5000 mg/kg) and Category-IV means (5000 mg/kg >LD_{50})

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