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IN SILICO STUDY OF ANTIDIABETIC ACTIVITY AND TOXICITY OF TRANS-ANETHOLE, FENCHONE, AND ESTRAGOLE

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ABSTRACT

Background: An unhealthy lifestyle characterized by consuming foods with a high glycemic index can increase blood sugar levels significantly. Diabetes mellitus therapy using antidiabetic drugs in patients aims to help control blood sugar levels. **Objective:** This study aims to determine the affinity and interaction models of trans-anethole, fenchone, and estragole as test compounds against the target proteins pancreatic α-amylase and dipeptidyl peptidase-4 (DPP-4) enzyme, as well as in silico toxicity prediction of the test compounds. **Methods:** This study used AutoDock 4.2 as a molecular docking method to evaluate the affinity and interaction models of the test compounds against the target proteins pancreatic α-amylase (PDB ID: 2QV4) and DPP-4 (PDB ID: 3W2T) as a potential antidiabetic agent. In addition, the toxicity of these compounds was predicted using the Toxtree program with Cramer Rules, Benigni/Bossa, Verhaar Sceme, and Kroes TTC parameters. **Results:** The results showed that the test compounds had binding energies that showed their affinity to the target protein. The trans-anethole did not show any structural features indicating potential toxicity. Fenchone has the class III category for the Cramer Rules parameters, and estragole has a structural alert for genotoxic carcinogenicity based on the Benigni/Bossa Rulebase parameter. **Conclusion:** Trans-anethole, fenchone, and estragole have been observed to exhibit antidiabetic potential through their interactions with the pancreatic proteins α-amylase and dipeptidyl peptidase-4 (DPP-4) enzyme, although their effectiveness is not as high as that of the native ligands. Additionally, further toxicity testing is required for the three compounds.

Keywords: Trans-anethole; Fenchone; Estragole; Molecular Docking; Antidiabetic

INTRODUCTION

Hyperglycemia is a medical condition characterized by abnormally high blood glucose levels, often associated with various diseases, especially diabetes mellitus. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to abnormalities in insulin secretion, insulin action, or both[1]. Unhealthy lifestyle habits, such as consuming foods high in starch, can cause blood sugar and insulin levels to increase significantly. Consuming foods with a high glycemic index regularly over a long period has been shown to increase the risk of developing type 2 diabetes[2]. Pancreatic α-amylase is an enzyme that catalyzes the hydrolysis of starch (accounting for 70% of ingested starch), which is further degraded by α-glucosidase to glucose[3]. An increase in postprandial blood sugar levels stimulates the incretin hormone, thereby encouraging the body to produce more insulin[4].

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There are two types of incretin hormones: glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1)[5,6]. These hormones play a crucial role in inducing 50-70% of postprandial insulin secretion[7]. However, GLP-1 and GIP have a short half-life of approximately 1-2 minutes due to rapid enzymatic degradation by the DPP-4 enzyme[8]. Therefore, inhibition of the α-amylase and DPP-4 enzyme could be a mechanism for controlling postprandial blood sugar levels in diabetes.

Fennel (*Foeniculum vulgare* Mill) has been known to have α-amylase inhibitory activity of 82.26% in vitro[9]. Fennel is known to have the main chemical content of trans-anethole (65.05%), fenchone (25.56%), and estragole (3.44%)[10-11]. The inhibition mechanism of trans-anethole, fenchone, and estragole against α-amylase as well as DPP-4 enzyme has not been known yet. Therefore, it is necessary to conduct a study to determine these compound's mechanism of action through their affinity and binding mode to the target by using in silico molecular docking. In silico, the potential toxicity of compounds is also carried out to determine the safety of compounds for further development.

METHODS

The 3D structures of the test compounds trans-anethole, fenchone, and estragole were obtained from https://pubchem.ncbi.nlm.nih.gov/ (Figure 1). The 3D structure of the target proteins pancreatic α-amylase (PDB ID: 2QV4) and DPP-4 (PDB ID: 3W2T) obtained from http://www.rcsb.org/pdb/home/home.do (Figure 2).

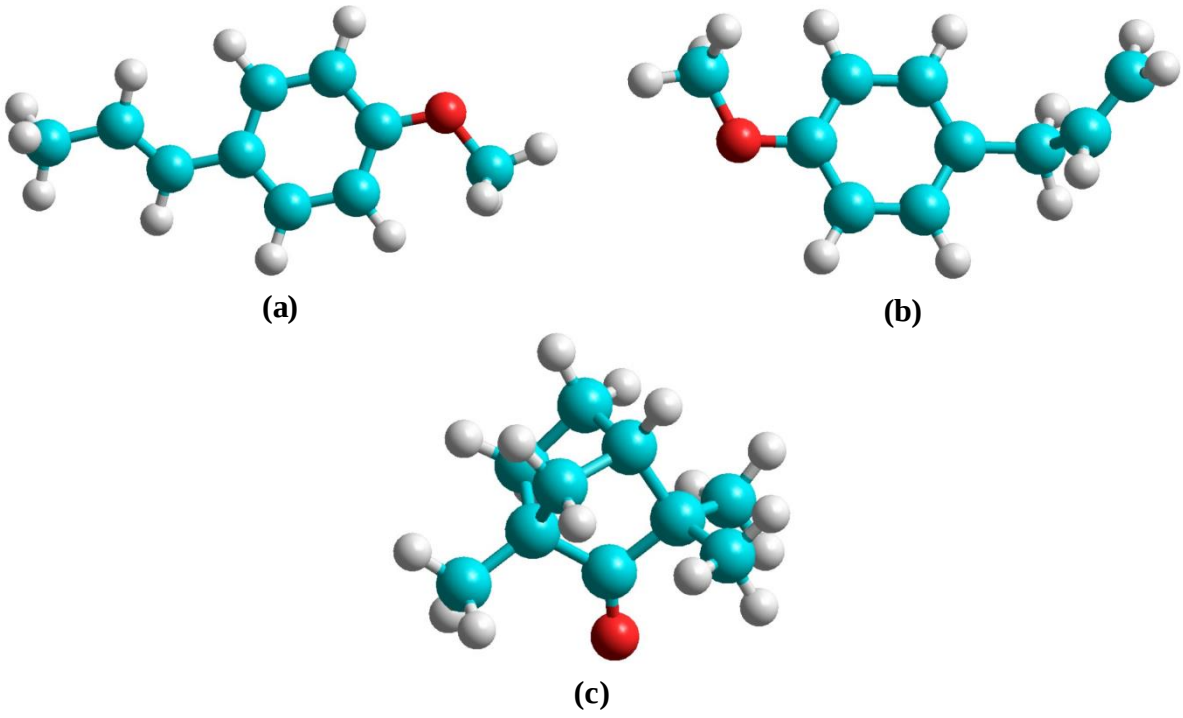

Figure 1. 3D Structure of Trans-anethole (a); Estragole (b) and Fenchone (c)

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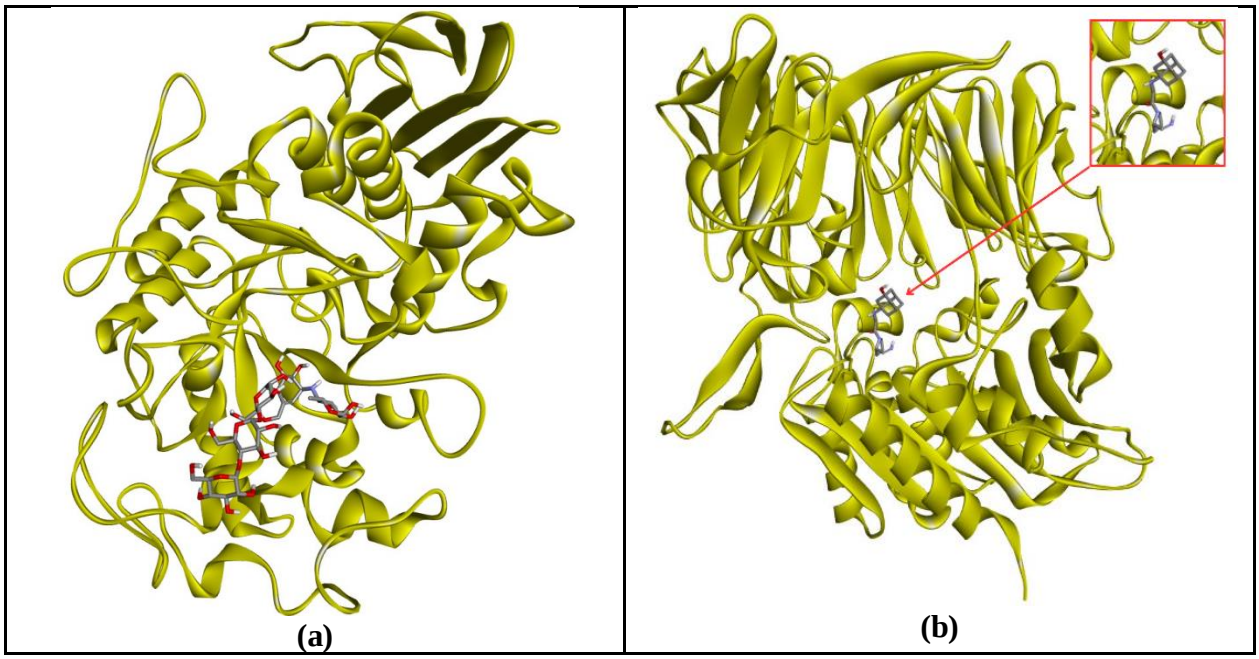

Figure 2. 3D Structure of pancreatic α-amylase (PDB ID: 2QV4) (a) and DPP-4 (PDB ID: 3W2T) (b)

The 3D structures of trans-anethole, fenchone, and estragole were optimized using Hyperchem 8 with the semiempirical AM1 method. Preparation of the target proteins using the BIOVIA Discovery Studio began with the removal of water molecules (H_2O) from the target proteins. Subsequently, the native ligands on the target proteins were removed to create a binding cavity. The separated native ligands were saved with .pdb format. Before molecular docking using Autodock 4.2, the ligand and protein were prepared with the 40 × 40 × 40 Å grid box dimensions and a grid spacing of 0.375 Å using AutiGrid in AutodockTools 1.5.6. The docking protocol was set with flexible ligand and fixed protein. The accuracy of the docking protocol was validated by redocking the ligand to the binding cavity of the protein, and the root-mean-square deviation (RMSD) value was calculated. The best conformation with an rmsd value <2.0 Å indicates that the docking prediction was successful. Binding energy and interaction data were then analyzed to evaluate the affinity and interaction models of the test compounds against the target proteins. Toxicity prediction was carried out using the 2D structure of the test compound with SDF file format (*.sdf) in Toxtree v3.1.0 software. Testing was carried out using Cramer Rules, Kroes TTC Decision Tree, Benigni/Bossa Rulebase, and Verhaar Scheme. Data analysis was carried out descriptively.

RESULTS

The docking protocols have good accuracy for redocking the native ligands into α-amylase and DPP-4 and are declared valid based on the RMSD value. The redocking of native ligand into α-amylase produced RMSD values of 1.35 and 1.74 Å for DPP-4. Molecular docking results, as shown in Table 1, reveal the interactions between target proteins and ligands. It provides a quantitative prediction of binding energy and ranks compounds based on ligand-receptor binding affinities[15].

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Table 1. Docking Results of Native Ligand and Test Compounds against Protein

Protein	Ligand	Binding Energy (Kkal/mol)	Hydrogen Bond	
			Amino Acid Residue	Donor-Acceptor
α-amylase (PDB ID: 2QV4)	*Native ligand*	-6.88	GLN63	HE21-O6B
			ARG195	HH22-O2T
			HIS305	HD1-O3B
			TRP59	H10-O
			HIS299	H15-NE2
			ASP300	H15-OD2
			GLU233	H13-OE2
			HIS201	H11-NE2
			TYR62	H16-O
			HIS101	H16-NE2
	trans-anethole	-4.67	GLN63	HE22-O1
	fenchone	-4.66	GLN63	HE21-O1
	estragole	-4.34	LYS200	HZ3-O1
DPP-4 (PDB ID: 3W2T)	*Native ligand*	-9.51	ARG125	HH11-O20
			ASN710	HD22-O20
			SER209	HG-O4
			GLU205	H3-O
			GLU206	H2-OE2
	trans-anethole,	-4.70	ARG125	HH12-O1
	fenchone	-5.10	ASN710	HD22-O1
	estragole	-4.45	ARG125	HH12-O1

Information: ASN = Asparagin, ASP = Aspartat, ARG = Arginin, ALA = Alanin, GLN = Glutamine, GLU = Glutamate acid, TRP = Triptofan, SER = Serin, LYS = Lisin, HIS = Histidine, ILE = Isoleusin, LEU = Leusin.

Visualization of the interaction of the native ligand and the test compounds at the target protein binding site is shown in Figure 3. The results show that the test compounds bind at the same site as the native ligand of each protein. Estragole did not form hydrogen bonds with the same amino acid residue as native ligands but binds to the same binding site as native ligands. The similarity of the binding site indicates that the test compounds may have the same mechanism of action as the native ligand.

In silico toxicity, prediction results of test compounds for the four test parameters are shown in Table 2. The trans-anethole did not show any structural features indicating potential toxicity. Fenchone has the class III category for the Cramer Rules parameters, and estragole has a structural alert for genotoxic carcinogenicity based on the Benigni/Bossa Rulebase parameter.

Table 2. Toxicity Test Result

Parameters		Trans-anethole	Fenchone	Estragole
Cramer Rules		Low (Class I)	High (Class III)	Low (Class I)
Kroes TTC Decision Tree		Save	Save	Negligible risk
Verhaar Scheme		Class 1	Class 1	Class 1
Benigni/Bossa Rulebase	genotoxic carcinogenicity	Negative	Negative	Structural alert
	nongenotoxic carcinogenicity	Negative	Negative	Negative

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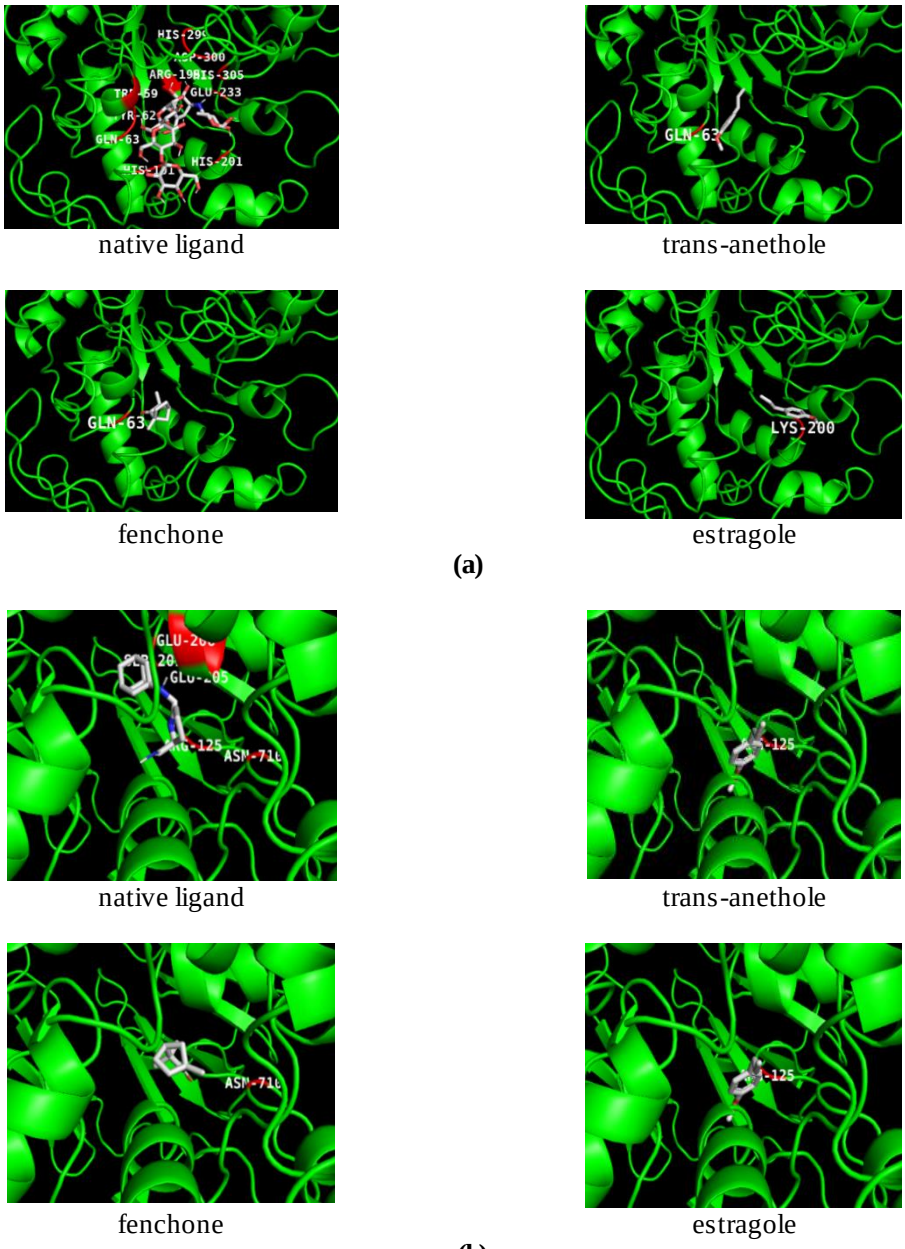

Figure 3. Visualization of the interactions of native ligand, trans-anethole, fenchone, and estragole in the binding pockets of α-amylase (a) and DPP-4 (b).

DISCUSSION

In-silico study can be done as a preliminary test to determine compounds' potential activity and toxicity before further development stages are carried out. In-silico study can also be used to determine molecular activity mechanisms directly related to the molecular target of a compound. Based on the docking data results, as shown in Table 1, the binding energies between the test compounds trans-anethole, fenchone, and estragole and the target proteins α-amylase and DPP-4 are all negative. Negative binding energies indicate that the test compounds have an affinity to the target proteins[17]. The binding energies of the

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test compounds are greater than those of the native ligands, indicating that the test compounds have a lower binding affinity to the target proteins than the native ligands. The more negative the binding energy, the more stable the formed bond[18]. A positive binding energy indicates that a formed bond is very weak or nonexistent[19].

Hydrogen bonds are formed between the target protein and native ligands, as well as the test compounds. As shown in Table 1, the hydrogen bonds are formed between the native ligand and GLN63, ARG195, HIS305, TRP59, HIS299, ASP300, GLU233, HIS201, TYR62, HIS101 residues of target protein α-amylase. A hydrogen bond was formed between trans-anethole and fenchone with GLN63 residue of α-amylase, meanwhile estragole with LYS200 residue. All three test compounds, as well as the native ligands, form hydrogen bonds in the α-amylase binding pocket (Fig. 3a). GLN63 is an amino acid residue that also formed hydrogen bonds with the native ligand, while LYS200 is in the α-amylase binding pocket even though it did not form hydrogen bonds with the native ligand.

The native ligand forms hydrogen bonds with ARG125, ASN710, SER209, GLU205, and GLU206 residues of DPP-4. Trans-anethole and estragole formed hydrogen bonds with ARG125 residue, while fenchone formed hydrogen bonds with ASN710 residue of DPP-4, which are the same residue that binds to the native ligand. All three test compounds, as well as the native ligands, form hydrogen bonds in the binding pocket of DPP-4 (Fig. 3b). The similarity of amino acid residues that formed hydrogen bonds between the native ligand and the test compound indicates that these compounds have the same interaction with the native ligand in the binding pocket of the target proteins. The hydrogen bond is a type of noncovalent interaction that plays a crucial role in forming stronger binding energies. In general, molecular interactions that occur in the body are noncovalent interactions, which are interactions between atoms that are not covalently bonded to each other[20]. The results of another study showed that administration of trans-anethole with a dose of 80 mg/kg BW showed a significant reduction in the level of glycosylated hemoglobin (HbA1c) and plasma glucose and increased insulin and hemoglobin in diabetic male albino Wistar rats[21]. These suggest that trans-anethole, as well as fenchone and estragole, can be developed further as a therapeutic agent for type 2 diabetes mellitus.

Toxicity test results (Table 2), according to the Cramer Rules, fenchone was classified into the class III category. According to Cramer Rules, fenchone is classified as class III. This was based on the fact that fenchone did not meet any of the criteria in the Cramer Rules, such as containing atoms other than C, H, O, N, and S, aliphatic hydrocarbon, benzene derivative, heterocyclic, terpene, and sulfonate groups[22]. The Kroes TTC Decision Tree Parameter is a method for setting threshold limits for human exposure to chemicals[23]. Based on the results obtained, trans-anethole and fenchone were classified as safe, while estragole was classified as a negligible risk with a low probability of cancer risk (1:1,000,000). Estragole has an alkenyl benzene structure, which is a structure that is genotoxic carcinogenic[24]. The Verhaar Scheme approach is a method for classifying compounds based on their environmental toxicity[25]. The three test compounds are classified as Class 1, indicating that they are non-reactive and do not interact with specific receptors to cause toxicity. Prediction of the mutagenic and carcinogenic potential of compounds was carried out using the Benigni/Bossa Rulebase parameters[26]. Trans-anethole and fenchone showed negative results for genotoxic

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carcinogenicity and non-genotoxic carcinogenicity. Estragole, on the other hand, showed structural alert for genotoxic carcinogenicity and negative results for non-genotoxic carcinogenicity. Estragole has an alkenylbenzene structure, which is a genotoxic carcinogenic structure and has been shown to cause liver tumors in test animals when administered in high doses[26]. Toxicity prediction in this study uses the two-dimensional structure of the compound, so it is necessary to know more about the possibility of toxicity, one of which is by using the three-dimensional structure of the compound to determine the interactions that allow toxicity to occur.

CONCLUSION

Trans-anethole, fenchone, and estragole have shown potential as antidiabetic agents based on their interaction and affinity to target proteins α-amylase and dipeptidyl peptidase IV (DPP-4), which are indicated by negative binding energies. The trans-anethole did not show any structural features indicating potential toxicity. At the same time, fenchone has the class III category for the Cramer Rules parameters, and estragole has a structural alert for genotoxic carcinogenicity based on the Benigni/Bossa Rulebase parameter.

CONFLICT OF INTEREST

There is no conflict of interest in the preparation of this article.

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