Research Article

Molecular docking analysis of six major compounds from sweet basil (*Ocimum basilicum* L.) extract as potential anti-hypertension therapy

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Abstract

Hypertension is an abnormally high blood pressure condition that is the leading cause of preventable cardiovascular disease, chronic kidney disease, and cognitive impairment. In the case of hypertension, repressing the Angiotensin-Converting Enzyme (ACE) expression has been shown to be an effective method of controlling hypertension by inhibiting the conversion of angiotensin I to angiotensin II. Captopril is the most commonly used ACE inhibitor. It simultaneously inhibits the conversion of angiotensin I to the potent vasoconstrictor angiotensin II and the vasodilator peptide bradykinin. Sweet basil (*Ocimum basilicum* L.) on the other hand, is used in traditional Indian and Chinese medicine to treat a variety of diseases, including hypertension. The study aimed attempts to investigate the potency of 6 major compounds found in sweet basil (*Ocimum basilicum* L.) extract, as an anti-hypertension therapy. The analysis demonstrates that *Ocimum basilicum* L., extract is effective as an anti-hypertension therapy because it contains several compounds that may interact with ACE and inhibit its activity. The molecular docking analysis and drug-likeness prediction indicate that camphor could be a potential drug candidate because it does not violate the Lipinski rule, has a high Gastrointestinal (GI) absorption, a high affinity to interact with ACE, and a similar interaction site to the ACE-Captopril interaction.

Introduction

High blood pressure, or hypertension, is an abnormally arterial blood pressure with systolic blood pressure exceeding 140 mmHg and or diastolic blood pressure higher than 90 mmHg (Singh et al. 2017). Its prevalence increase with age, either in woman or man. Besides, low potassium intake, high sodium intake, obesity, lack of physical activity, and alcohol consumption also contribute to the risk of hypertension (Mills et al. 2020). On the other hand, hypertension is the most major contributing yet preventable risk factor for cardiovascular disease, chronic kidney disease, and cognitive impairment (Rahimi et al. 2015; Forouzanfar et al. 2016). Another study stated that high blood pressure has strong evidence showing its association with cardiovascular disease (Fuchs and Whelton, 2019). The effective treatment of hypertension is essential to reduce the disease burden and prevent it before causing other systemic effects (Oparil et al. 2018).

The renin–angiotensin–aldosterone system (RAAS) is one of the regulators of blood pressure with a wide range of regulation effects. Renin and prorenin are synthesized, stored, and released in response to stimuli by the juxtaglomerular cells of the kidney. Renin's primary function is to catalyze the angiotensinogen cleavage resulting in angiotensin I. Afterward, the Angiotensin-Converting Enzyme (ACE) cleaves angiotensin I to generate angiotensin II, which is central to the pathogenetic function of the RAAS in hypertension. The abnormality within the system may lead to dysregulation of blood pressure control which can directly or indirectly increase or decrease blood pressure (Singh and Williams, 2009). In the case of hypertension,

inhibiting the conversion of angiotensin I to angiotensin II by repressing ACE expression has been shown to be an effective method for controlling hypertension (Silva et al. 2015).

Captopril (D-3-mercapto-2-methyl-propionyl-L-proline) is the most widely used ACE inhibitor compound. Captopril inhibits the conversion of angiotensin I to the potent vasoconstrictor angiotensin II and the vasodilator peptide bradykinin simultaneously (Odaka and Mizuochi, 2000). On the other hand, sweet basil (*Ocimum basilicum* L.) is used in traditional Indian and Chinese medicine to treat various diseases, including hypertension (Ratta et al. 2021). Umar et al (2010) demonstrate the potential antihypertensive effects of *Ocimum basilicum* L., extract by decreasing angiotensin production in rats. Thus, the *Ocimum basilicum* L., extract might have the potency to inhibit ACE activity, and studying the potential compound within the extract is necessary to identify the potential drug candidates. This study evaluates the potency of 6 major compounds within *Ocimum basilicum* L., leaf extract to interact with ACE using the molecular docking approach. The six primary compounds include methyl cinnamate, linalool, beta-elemene, mono (2-ethylhexyl) phthalate, camphor, and anisole (Kathirvel and Ravi 2012). The binding affinity can be used to observe the potential interaction of small particles in the binding site of the proteins (Attique et al. 2019). Furthermore, the Lipinski rule of 5 will be utilized to observe the compound's suitability as a potential therapeutic agent.

Method

Ligand and Protein Preparation
The 2D and 3D structure data of six major compounds in Ocimum basilicum L., and captopril as a control were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The six compounds include methyl cinnamate, linalool, beta-elemene, mono (2-ethylhexyl) phthalate, camphor, and anisole. Open babel was used for the energy minimization process during sample preparation. Afterward, the result was converted into pdbqt format for further analysis. The 3D structural data of ACE protein (1O8A) was obtained from the rscb protein data bank (https://www.rcsb.org/). Prior to the analysis, the 3D structure of the protein was visualized, and the water molecule and ligands were removed using PyMol. Following this, the protein clean structure data was exported in pdb file (Yuan et al. 2017).

Molecular Docking
PyRx (0.8 version), an Autodock Vina (1.1.2 version) based molecular docking program, was utilized to conduct the molecular docking analysis. The prepared ligand and protein data were imported to the PyRx. Following this, the grid box location was selected—the selected grid box location, including the whole room of the 3D protein structure. Afterward, the analysis was commenced and returned result was exported into a .pdb file to be analyzed using Discovery studio for binding interaction observation (Dallakyan and Olson, 2014).

Drug-Likeness Prediction
The drug-likeness properties of six major compounds in Ocimum basilicum L., were analyzed based on the Lipinski rule of 5. SwissADME (http://www.swissadme.ch/) was utilized to calculate the drug-likeness by using 2D data of the compounds as an input (Daina et al. 2017).

Results and Discussion

Ligand and Protein Preparation
The renin–angiotensin–aldosterone system regulates blood pressure and electrolyte homeostasis through the angiotensin-converting enzyme. ACE exists in somatic tissues as a glycoprotein composed of a single, long polypeptide chain of 1.277 amino acids. It comprises two homologous domains (N and C domains), each with an active site with a conserved HEXXH zinc-binding motif4, where two histidines are zinc ligands and a glutamate 24 residues downstream forms the third ligand. Both domains' active sites catalyze the hydrolysis of angiotensin I and the vasodilator bradykinin with comparable efficiency (Wei et al. 1992).

The 3D structure data of ACE was obtained from the Natesh et al (2003) study that is available on the protein data bank with id 1O8A (18). The data was utilized since it has good quality with a data resolution of about 2 Å (Gao et al. 2020). The protein visualization is illustrated in Fig.-1A. Since the original data has ligand

and water molecules within it, the data cleaning procedure was conducted, resulting in clean protein data visualized in Fig.-1B. The clean 3D structural data will then be used for the molecular docking analysis.
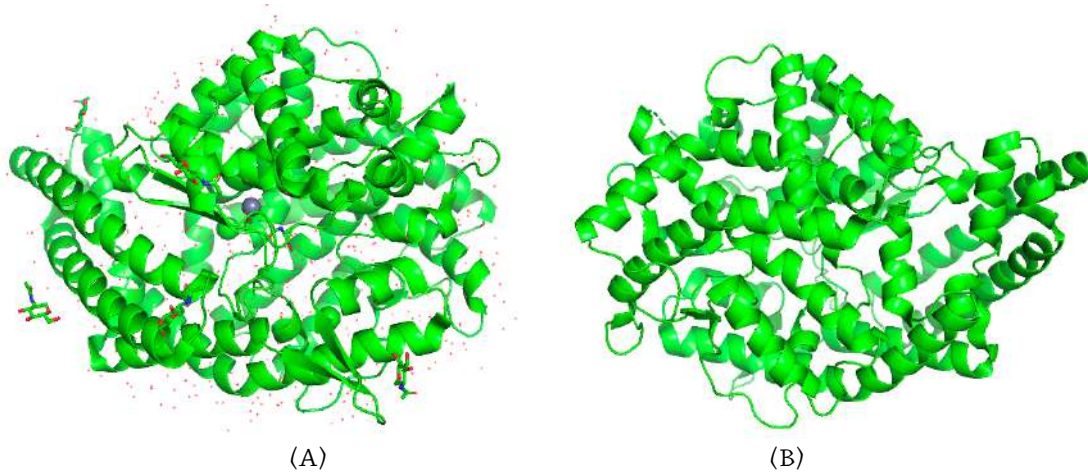

Fig.-1. Angiotensin-Converting Enzyme (ACE) Protein Structure: (A) Before Ligand and Water Removal, (B) After Ligand and Water Removal.

Molecular Docking

The molecular docking of ACE against ligands contained by *Ocimum basilicum* L., was conducted to predict the optimal conformation of ligand and its potency to have an interaction with the protein, which is measured by Gibbs free energy at di particular interaction site (Godoy et al. 2015). Gibbs free energy demonstrates binding affinity between ligand and protein. The lower energy has a higher possibility of spontaneous reaction between protein and ligand (Du et al. 2016; Wang and Zhu 2016). The six major compounds demonstrate a potency to interact with ACE with a binding affinity comparable to Captopril as the control. Beta elemene has the lowest gibs free energy value among the others, even lower than the control, as shown in Table 1. Besides Beta elemene, Mono (2-ethylhexyl) phthalate, Methyl cinnamate, and Camphor have lower Gibbs free energy compared to the control with values -6.7 Kcal/Mol, -6 Kcal/Mol, and -5.9 Kcal/Mol, respectively. Furthermore, linalool and anisole have relatively lower potency to interact with ACE. However, since their Gibbs free energy of them is still negative, there is still a possibility for them to interact with ACE.

Table 1. Gibbs Free Energy Ligand and Protein Interaction

No	Protein	Ligand	Binding Affinity (Kcal/Mol)
1		captopril	-5.7
2		Beta elemene	-6.8
3		Mono (2-ethylhexyl) phthalate	-6.7
4	Angiotensin-converting	Methyl cinnamate	-6
5	enzyme (ACE)	Camphor	-5.9
6		Linalool	-5.3
7		Anisole	-4.8

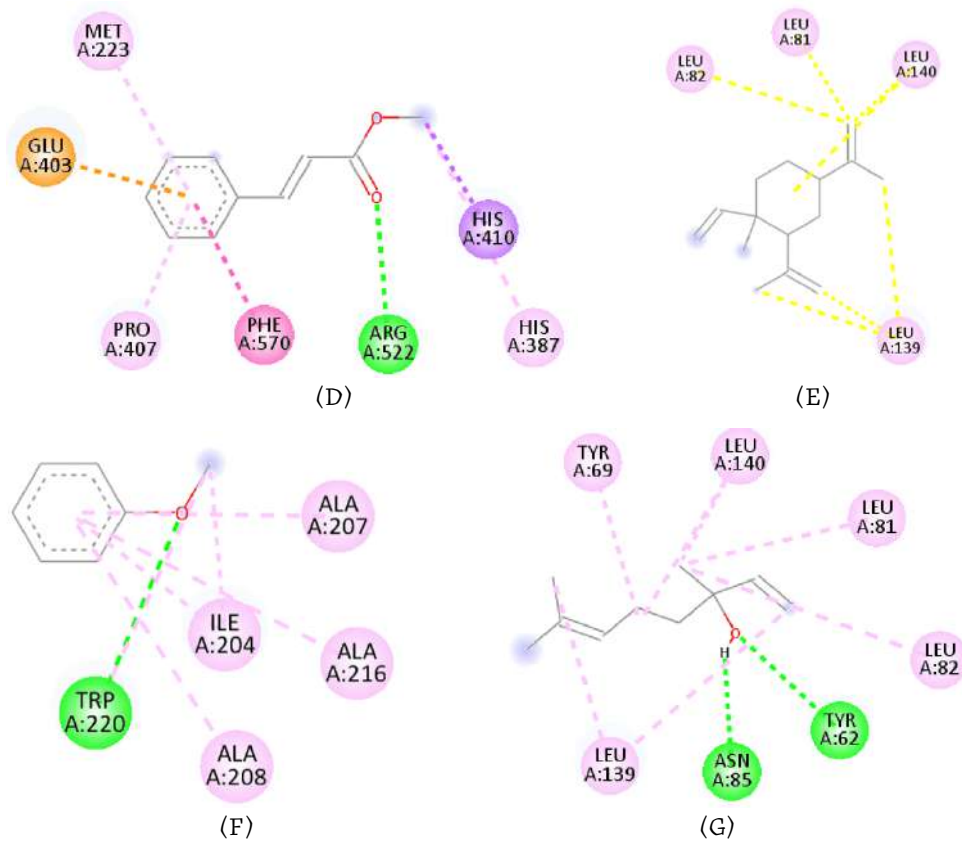(A)   (B)   (C)
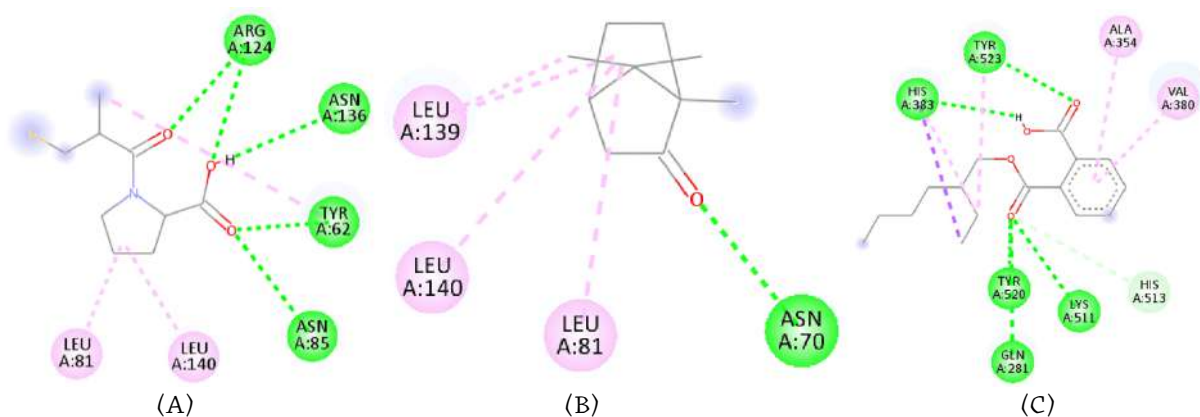
Fig.-2. Visualization of ACE and Ligand Interaction: (A) Captopril, (B) Beta elemene, (C) Mono (2-ethylhexyl) phthalate, (D) Methyl cinnamate, (E) Camphor, (F) Linalool, and (G) Anisole.

The interaction of enzyme and substrate at a particular location will initiate an enzyme-substrate reaction (Chen et al. 2016). Hence, interaction site information is essential for predicting ligand potency from *Ocimum basilicum* L., compounds to conduct a similar activity with the control. Table 2 describes the location and interaction type between protein and ligand within the interaction sites. The ligand-protein interaction is visualized in Fig.-2.

Table 2. Interaction Type and Location

Ligand	Interaction type	Location
	Hydrogen Bond	TRP220
	Hydrophobic	ILE204
	Hydrophobic	TRP220
Anisole	Hydrophobic	ILE204
	Hydrophobic	ALA207
	Hydrophobic	ALA208
	Hydrophobic	ALA216
	Hydrophobic	LEU140
	Hydrophobic	LEU139
	Hydrophobic	LEU139
Beta elemane	Hydrophobic	LEU81
	Hydrophobic	LEU82
	Hydrophobic	LEU140
	Hydrophobic	LEU139
Linalool	Hydrogen Bond	TYR62

	Interaction	Residue
	Hydrogen Bond	ASN85
	Hydrophobic	LEU81
	Hydrophobic	LEU82
	Hydrophobic	LEU140
	Hydrophobic	LEU140
	Hydrophobic	LEU139
	Hydrophobic	LEU139
	Hydrophobic	TYR69
Methyl cinnamate	Hydrogen Bond	ARG522
	Electrostatic	GLU403
	Hydrophobic	HIS410
	Hydrophobic	PHE570
	Hydrophobic	HIS387
	Hydrophobic	MET223
	Hydrophobic	PRO407
Mono (2-ethylhexyl) phthalate	Hydrogen Bond	GLN281
	Hydrogen Bond	LYS511
	Hydrogen Bond	TYR520
	Hydrogen Bond	TYR523
	Hydrogen Bond	HIS383
	Hydrogen Bond	HIS513
	Hydrophobic	HIS383
	Hydrophobic	HIS383
	Hydrophobic	TYR523
	Hydrophobic	ALA354
	Hydrophobic	VAL380
Camphor	Hydrogen Bond	ASN70
	Hydrophobic	LEU81
	Hydrophobic	LEU139
	Hydrophobic	LEU140
	Hydrophobic	LEU139
Captopril	Hydrogen Bond	TYR62
	Hydrogen Bond	ASN85
	Hydrogen Bond	ARG124
	Hydrogen Bond	ARG124
	Hydrogen Bond	ARG124
	Hydrogen Bond	ASN136
	Hydrophobic	LEU81
	Hydrophobic	LEU140
	Hydrophobic	TYR62

Interaction site observation between ligand and protein shows that beta elemene, camphor, and linalool interact similarly with control to ACE protein. Three of them have hydrophobic interaction with ACE protein at amino acid leucine 140, and leucine 81, and linalool has hydrogen bonds at tyrosine 62 and asparagine 85. According to this, the three compounds might potentially have inhibition activity to ACE protein. In general,

the molecular docking results demonstrate the potency of the major compound from *Ocimum basilicum* L., as an antihypertension agent by inhibiting the ACE protein activity. Afterward, the drug-likeness prediction is essential to identify the potential compound to be developed as a drug.

Drug-Likeness Prediction

SwissADME predicted drug-likeness using Lipinski's rule of five to determine how a compound meets the pharmacological requirements for an oral drug that enters circulation and can have an active effect. As shown in Table 3, all of the compounds meet the requirements of Lipinski's rule of five, except for Beta elemene, which has one violation, MLOGP > 4.15. Except for Beta elemene, the pharmacokinetic properties prediction revealed that all compounds had high GI absorption. Except for Beta elemene, the majority of the compounds found in *Ocimum basilicum* L., have the potential to be drug candidates (Lipinski et al. 2001).

Table 3. Drug-Likeness Prediction

Compound	Molecular weight (g/mol)	Lipinski violation	GI absorption	Hydrogen Acceptor	Hydrogen Donor	Rotatable bonds	Remark
Beta elemene	204.35	1	Low	0	0	3	MLOGP > 4.15
Mono (2-ethylhexyl) phthalate	277.34	0	High	4	0	9	
Methyl cinnamate	162.19	0	High	2	0	3	
Campor	152.23	0	High	1	0	0	
Linalool	154.25	0	High	1	1	4	
Anisole	108.14	0	High	1	0	1	

Conclusion

The study evaluated the potential of six major compounds within *Ocimum basilicum* L., leaf extract to interact with ACE using molecular docking analysis. The results demonstrate that camphor might be a potential drug candidate since it has a high affinity to interact with ACE and a similar interaction site to the ACE-Captopril interaction. Besides, it also has no violation of the Lipinski rule and has a high Gastrointestinal (GI) absorption. However, further in vitro, and in vivo studies are necessary to validate its efficacy and safety. Overall, this study highlights the potency of 6 major compounds contained in *Ocimum basilicum* L., leaf extract as sources of potential therapeutics for the treatment of hypertension.

Conflict of Interests

The author (s) declares that there is no conflict of interest in this research and manuscript.

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