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In Silico Studies of Chemical Compounds from Punica Granatum's Peel as Angiotensin-I Converting Enzyme Inhibitor

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Abstract:

Salmonella, a prominent foodborne pathogen, poses significant health risks, causing both intestinal and extra-intestinal infections. Recognizing the potential of lactobacilli as probiotics due to their ability to produce substances inhibiting multidrug-resistant bacteria, this study aimed to assess antibiotic resistance, pathogenic gene frequency, antibacterial effects of lactobacillus supernatant from kefir, and its impact on resistance and pathogenicity gene expression.

In Tehran hospitals, 150 isolates from 240 clinical samples were collected and identified as Salmonella typhimurium using biochemical and serotype tests. Antibiotic sensitivity was assessed, and the frequencies of antibiotic resistance genes (tetA, tetB, and floR) and pathogenicity genes (sip, spvC, and invA) were investigated. Lactobacilli from kefir were isolated, and the minimum inhibitory concentration of lactobacillus supernatant was determined. The relationship between supernatant treatment and tetA and sip gene expression was examined using Real-time PCR.

Results revealed 38% of strains as Salmonella typhimurium serotype, displaying high resistance to ampicillin, tetracycline, and nitrofurantoin. Pathogenicity genes invA and sip exhibited high frequencies of 100% and 70.2%, respectively. Lactobacillus supernatant showed an MIC of 80 µg/ml, effectively reducing tetA and sip gene expression by 42.2% and 55.7%, respectively.

In conclusion, the study underscores the high antibiotic resistance in Salmonella typhimurium and suggests Meropenem, Trimethoprim Sulfamethoxazole, and Ampicillin-Sulbactam as effective treatments. Moreover, lactobacillus supernatant demonstrated significant potential against Salmonella typhimurium, highlighting lactobacilli as promising probiotics. This health-oriented strategy presents a viable solution for treating Salmonella infections and preventing their spread.

INTRODUCTION

High blood pressure (hypertension) is a common progressive disorder that results in diverse chronic diseases. The angiotensin-converting enzyme (ACE) produced from the lungs converts angiotensin I into angiotensin II which causes vasoconstriction followed by hypertension. At the moment, antioxidants are widely used for their preventive roles against cardiovascular diseases along with the potential for scavenging free radicals (1, 2). Since hypertension is an increasing health concern, and it poses a substantial risk to cardiovascular health and related complications, there is a tremendous need for developing new drugs. Several medications used to treat hypertension often target ACE, which is a crucial class of drugs for hypertension management (3). Inhibition of ACE has shown effectiveness in regulating and treating high blood pressure. While synthetic ACE inhibitors like lisinopril, captopril, and enalapril are commonly used for hypertension treatment, their regular use may be linked to undesirable drawbacks such as patient cough, postural hypotension, renal failure, and angioedema. Extensive studies have been conducted to search for ACE

inhibitors derived from natural products, as they may have better therapeutic profiles and fewer side effects (4). Anti-hypertensive synthetic drugs including ACE inhibitors, effectively control hypertension, however, unpleasant side effects emerge. In recent decades, studies on the role of food-derived compounds have provided a positive contribution to ACE regulation (5). The renin-angiotensin-aldosterone system (RAAS) plays a crucial role in maintaining arterial blood pressure. One of its primary components is ACE, a glycosylated zinc dipeptidyl carboxypeptidase that regulates arterial blood pressure and electrolyte balance through the renin-angiotensin-aldosterone system (6). Two isoforms of ACE are transcribed from the same gene in a specific manner. ACE is a glycoprotein composed of a single large polypeptide chain of 1,277 amino acids, whereas, in sperm cells, it occurs as a lower-molecular glycoform of 701 amino acids.

Punica granatum L., pomegranate, belongs to the Lythraceae family. Constituents of P. granatum fruits and peel are known to depict varied biological properties (7-9). P. granatum peel has several phytochemicals like polyphenolic compounds, phenolic acids, anthocyanins, and flavonoids as potent antioxidants (10-12). Studies in mortal and murine models have been shown significant anti-atherogenic, antioxidant, antihypertensive, and anti-inflammatory effects for pomegranate (13-15). Pomegranate is associated with several health advantages such as inhibition of ACE due to its high levels of antioxidant polyphenolic substances (16). Pomegranate peel extracts are particularly rich with phytobiotics such as hydrolysable tannins (ellagitannin, punicalagin, punicalin, gallic and ellagic acid), flavonoids, anthocyanins, and other phenols. These polyphenols possess a wide range of biologic properties including anti-inflammatory, antioxidant, hypoglycemic, lipid-lowering, antihypertensive, or antimicrobial effects (17-19). Pomegranate, mainly its peel, has been extensively researched and reviewed due to its numerous therapeutic effects. The tannin polyphenol compound in pomegranate has been demonstrated to induce nitric oxide synthase and act as an ACE inhibitor, exerting an antihypertensive effect (20). Particularly, P. granatum demonstrated antihypertensive effects (14).

The structure-activity relationship (SAR) has been used to identify the chemical structures and natural elements of bioactive compounds and their derivatives. Additionally, since peels of fruit and vegetables are often considered waste, they are also cost-effective. Accordingly, several experiments have concentrated on the natural waste parts of pomegranate, to discover numerous miraculous properties for human health. The implicit remedial parcels of pomegranate peel are wide-ranging and include both prevention and treatment of cancer, cardiovascular complaints, diabetes, dental conditions, erectile dysfunction, protection from ultraviolet (UV) radiation, and antimicrobial (15, 21-23). Other implicit applications include child brain ischemia, Alzheimer's disease (AD), male infertility, arthritis, dermal injuries, and obesity (24-28).

With the fleetly aging and growing world population comes a critical demand for new and better medicines. In silico studies can now dominate virtually every aspect of drug discovery and development (29, 30). To discover new lead compounds, conventional in vitro screening assays are required to evaluate compounds against the target of interest. Researchers began to use computational models to find the relations between medicines and natural systems, the so-called pharmacokinetic and pharmacodynamic processes. Computational tools applicable to medicine development are now extensively honored (29). Reducing the cost of the study and adopting a better ethical approach compared to animal models of exploration are among the numerous benefits of in silico methods, such as molecular docking, for predicting ligand-receptor relationships. Molecular docking has been successful in identify binding mechanisms, has explained experimental results, and has been suitable to identify binding sites of new molecular targets (30, 31).

The success of docking is determined by the ability to distinguish between correct and incorrect conformations and the ability to rank the produced conformations. Selected compounds of pomegranate peel (rich in polyphenols similar to tannins and flavonoids) were shown to have antioxidant and antihypertensive effects (32).

The idea of this study is to identify the active compounds present in P. pomegranate peel as potential inhibitors of angiotensin- I converting enzyme (ACE) using an in-silico screening method. The chemical compounds to be evaluated using docking programs are kaempferol-3-O-rutinoside, luteolin, and rutin (the structures are illustrated in Figure 1), and they will be compared to the well-known ACE inhibitor medicines such as captopril and lisinopril. Each of these mentioned ligands has demonstrated some ACE inhibitory properties in in vitro studies.

MATERIALS AND METHODS

The chemical structures of ACE inhibitors were designed using HyperChem software (version 7, Hypercube Inc.) and are shown in Table 1. Conformational analysis of the desired compounds was performed through the semi-empirical molecular orbital calculations (PM3) method using HyperChem software. The molecular structures were optimized

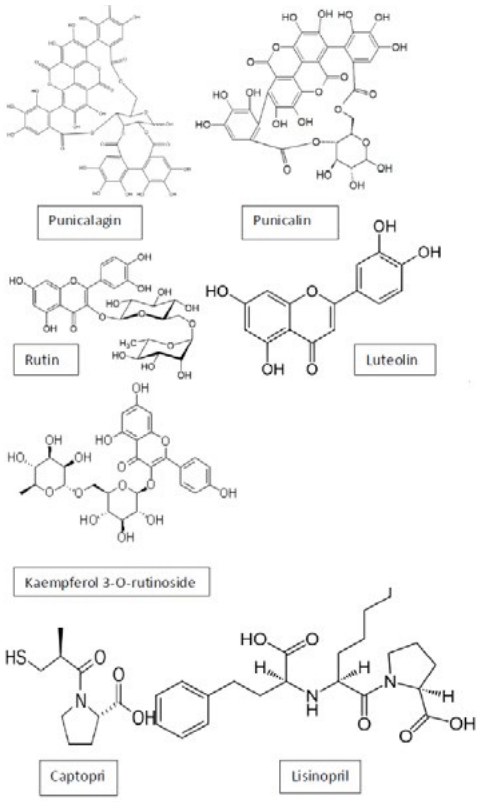

Fig 1. Ligands structures

Table 1. The docking between a ligand and the angiotensin-I converting enzyme (ACE) using AutoDock 4.2 software

Name	Binding energy	Ligand efficiency	Inhibit constant	Internal energy	Vdw-hb-dissolve-energy	Electrostatic energy	Total-internal	Torsional energy	Unbound energy
Kaempferol	-6.72	-0.16	-11.9 µM	-11.19	-10.5	-0.69	-7.73	4.47	-7.73
Lisinopril	-8.81	-0.3	-345.89 nM	-12.39	-7.99	-4.41	-2.06	3.58	-2.06
Punicalagin	-5.7	-0.07	-0.03 µM	-0.03	-0.69	-0.66	-12.17	5.67	-12.17
Punicalin	-8.03	-0.14	-1.3 µM	-12.8	-12.36	-0.44	-8.72	4.77	-8.72
Captopril	-6.99	-0.5	-7.56 µM	-8.48	-6.84	-1.64	-0.94	1.49	-0.94
Luteolin	-8.32	-0.4	-802.86 nM	-9.51	-8.59	-0.92	-0.9	1.19	-0.9
Rutin	-6.74	-0.16	-11.41 µM	-11.52	-11.26	-0.25	-11.74	4.77	-11.74

using the Polak-Ribiere (conjugate gradient) algorithm until the root mean square (RMS) gradient was 0.01 kcal mol^{-1}. Among all energy minima conformers, the global minimum of compounds was used in docking calculations and the resulting geometry was transferred into the Autodock (version 4.2) program package, which was developed by Arthur J. Olson Chemometrics Group [33]. It is a widely used software for docking between chemical compounds, such as ligands and receptors, which are macromolecules. The 3D structure of ACE can be obtained from the website Protein Data Bank (PDB) with the accession number 1O86. ACE is an enzyme that forms a complex with Zn and Cl as essential elements of the enzyme.

RESULTS

In the docking process, six chemical compounds from Punica peel, and a reference compound (lisinopril) were subjected to docking. Further, the binding site on the enzyme ACE (Zn701) and interactions were accessed. The docking results are presented in Table 1.

The table compares ligands based on their binding energy and efficacy, as well as their affinity toward receptors. A higher value for the binding energy difference indicates a more favorable outcome.

Moreover, interactions of the chemical compounds kaempferol, luteolin and punicalin with the receptor were illustrated in Figures 2, 3 and 4.

DISCUSSION

This study assessed chemical compounds from pomegranate peel due to their reported anti-hypertensive and ACE inhibitory activities. In total, six chemical compounds from the peel were subjected to molecular docking. Further, the binding sites on the enzyme (Zn701) and interactions were accessed. Our research indicates that all the selected ligands, primarily flavonoids, in the computational model especially leuteolin could interact with the ACE receptors, thereby inhibiting the ACE.

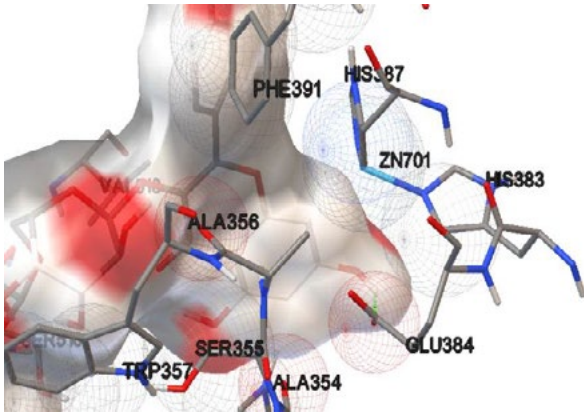

Fig 2. Kaempferol interaction with the receptor (spheres represent hydrogen bindings)

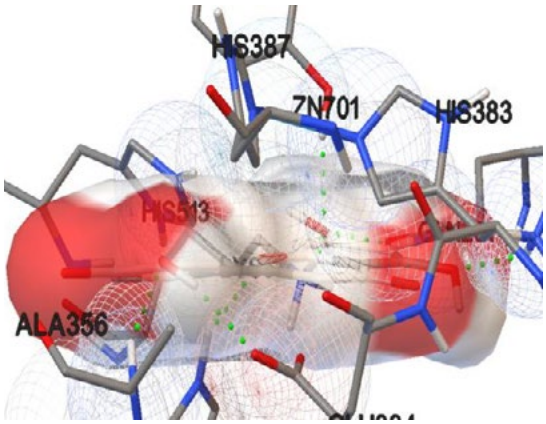

Fig 3. Luteolin interaction with the receptor (direct hydrogen bonding)

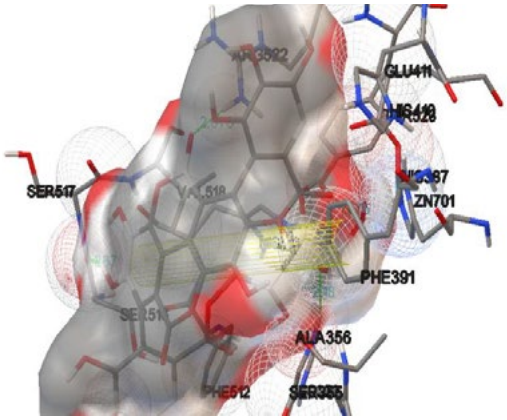

Fig 4. Punicalin interactions with the receptor

The binding energy indicates the stability of the interaction (bonding) between the angiotensin receptor and ligands such as ACE in their binding site. The most stable and optimal conformation for drug design is the one with the lowest energy, as it possesses the highest affinity. Molecular docking's primary objective is to identify the most stable conformation between the two molecules (34).

Molecular docking, a computational procedure, aims to predict the favored orientation of a ligand to its macromolecular target (receptor) when they are bound to each other to form a stable complex (35). Our docking results demonstrated the effectiveness of the chemical compound luteolin, compared to the standard drug, likely due to relatively smaller structure of the compound and its ability to directly interact with Zn in the binding site. Luteolin exhibited substantial activity, due to its ability to directly interact with Zn in the binding site, despite not having pi-pi interactions. Besides, it demonstrated relatively strong hydrogen bonding with the receptor, similar to lisinopril Luteolin exhibits interactions with the active site Zn that are very similar to those of lisinopril. Punicalin another compound exhibited appropriate hydrogen bonds and (\pi -\pi) interactions with the macromolecule. The outcomes showed that all compounds could interact and have some enzyme inhibition, however, leuteolin has excellent inhibition and stronger hydrogen bind with Zn 701 of the receptor, and it is comparable with lisinopril which was the standard ligand for the study. Our findings reveal a robust interaction between the selected compounds as ligands and their targeted receptor.

Consistent with our findings, the in vitro ACE inhibitory impact as well as antioxidant, anti-diabetic, and anti-obesity traits of P. granatum fruit peel extract were assessed. Ethanolic extract (100–1000 mg/mL) showed increased inhibition of ACE in a concentration-dependent manner with an IC50 value of 519.45 mg/mL. In this regard, P. granatum fruit peel extract revealed promising antioxidant, anti-diabetic, anti-obesity, and anti-hypertensive properties (36). Similarly, a potent ACE inhibitor in pomegranate as a treatment for hypertension has been stated. The activities of 24 major compounds, the majority of which inhibited ACE. Of note, pedunculagin, punicalin, and gallagic acid were the most effective ACE inhibitors with an IC_{50} values of 0.91, 1.12, and 1.77 µM, respectively. As demonstrated in molecular docking studies, compounds block ACE by forming multiple hydrogen bonds and hydrophobic interactions

with catalytic residues and zinc ions in ACE's C- and N-domains, consequently, inhibiting ACE's catalytic activity (16).

Previously it has been stated that consuming pomegranate juice could remarkably lower hypertension and inhibit the serum ACE activity (37, 38). The soluble polyphenols in the juice generally consists of tannins, ellagic tannins, anthocyanins, catechins, gallic, and ellagic acids. All these compounds have been confirmed contributing to in vivo ACE inhibition and subsequently anti-hypertension (13, 39-41). Molecular docking analysis has been employed to clarify the combination mode of ACE and phenolic compounds (13).

The anti-hypertensive activities of medicinal plants act by inhibition of ACE. A wide range of medicinal plants with ACE inhibitory activities have been demonstrated (7), and this activity was related to the synergistic action of secondary metabolites viz. alkaloids, flavonoids, tannins, proanthocyanidins, fatty acids, and terpenoids (42, 43). The ACE inhibitory activities of extracts might be related to flavonoid, alkaloid, and tannin contents, probably by sequestration of enzyme metal co-factor, protein precipitation, or through other mechanisms.

The significance of in-silico studies is that they are economical allowing for precise predictions of the effects of P. pomegranate peel components as ACE inhibitors and the development of new lead compounds. The study demonstrated a strong correlation between computational models and clinical findings.

CONCLUSION

The results of molecular dynamic simulations confirmed the accuracy of docking, the binding mode of ligands, and the reliability of active conformations obtained by AutoDock. Our findings indicate that pomegranate peel contains compounds that are well-known for their anti-hypertensive properties, and several compounds in Punica peel, primarily flavonoids, can have ACE inhibitory effects in a computational model. This study highlights the effectiveness of in-silico models in conjunction with in vivo studies. Further studies will show Punica peel usefulness as a nutritional supplement or pharmaceutical formulation for inhibiting ACE inhibitory activity, making it a promising candidate for future drug development.

Statements and Declarations

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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