IN SILICO ANALYSIS OF HAWTHORN PHYTOCHEMICALS FOR CARDIOPROTECTIVE EFFECTS

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CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **"IN SILICO ANALYSIS OF HAWTHORN PHYTOCHEMICALS FOR CARDIOPROTECTIVE EFFECTS"** which is submitted by **Khushi Garg, 2k23/MSCBIO/28** in partial fulfilment of the requirement for the award of the Degree of Master in Biotechnology, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from January to May under the supervision of Professor Yasha Hasija. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

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Certified that Khushi Garg (2k23/MSCBIO/28) has carried out their search work presented in this thesis entitled **"In Silico Analysis of Hawthorn Phytochemicals for Cardioprotective Effects"** for the award of Master in Biotechnology from Department of Biotechnology, Delhi Technological University, Delhi, under my supervision.

The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/ Institution.

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ABSTRACT

A vital element of the Renin-Angiotensin Aldosterone System (RAAS) involves angiotensin. This cleavage process converts angiotensin I to the active octamer angiotensin II, a which act as a vasoconstrictor and elevates blood pressure and can cause hypertension, is catalysed by the angiotensin-converting enzyme (ACE). Bradykinin, an antihypertensive vasodilator that lowers blood pressure, is also rendered inactive by it. A key component of treating hypertension, cardiovascular conditions, and renal illnesses is ACE inhibition. Despite being approved for clinical use, a number of synthetic ACE inhibitors, including captopril, lisinopril, enalapril, and others, have been shown to have specific adverse effects and drug-drug interactions. Therefore, the quest for safer, nontoxic, and more affordable inhibitors is urgently needed. Numerous phytocompounds found in hawthorn, a plant used extensively in Chinese traditional medicine, including flavonoids, oligomeric procyanidins, triterpenoids, organic acids, and amines, are what give it its cardioprotective properties. Plant polyphenolic chemicals called flavonoids have been shown to have some ACE-inhibiting properties. Following the analysis of these phytocompounds' ADMET characteristics, PyRx software was used to do a molecular docking investigation on flavonoid derivatives with angiotensin converting enzymes in complex with synthetic medications, such as lisinopril. In contrast to the synthetic medication lisinopril, which had a binding affinity of 7.4 Kcal/mol, flavonoids demonstrated a binding affinity with ACE of up to -9.8 Kcal/mol. The protein-ligand interaction is then further visualized by BIOVIA Discovery Studio. With this knowledge, powerful medicinal medications made from natural plant flavonoids can be developed to control blood pressure.

Keywords - Hypertension, Angiotensin converting enzyme, Hawthorn flavonoids, Molecular Docking, Drug design.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

CVD	Cardiovascular Disease
RAAS	Renin-Angiotensin-Aldosterone System
HTN	Hypertension
BP	Blood Pressure
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
ACE	Angiotensin- Converting Enzyme
WHO	World Health Organization
ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
RCSB-PDB	Research Collaboratory for Structural Bioinformatics - Protein Data Bank
FDA	Food Drug and Administration
OPC	Oligomeric Procyanidins
JG cells	Juxtaglomerular cells
ACEI	Angiotensin- Converting Enzyme Inhibitors
Ang I	Angiotensin I
Ang II	Angiotensin II
AGTR1/ AT1R	Angiotensin II Type Receptor 1
AGTR2/ AT2R	Angiotensin II Type Receptor 2
APA	Aminopeptidase A
APN	Aminopeptidase N
FROG2 Server	FRee On line druG conformation generation 2 Server

CHAPTER 1

INTRODUCTION

Globally, cardiovascular diseases (CVDs) are the most prevalent noncommunicable diseases. According to the World Health Organization (WHO), 31% of fatalities worldwide each year are caused by CVDs [1]. The 2017 European Cardiovascular Disease Statistics indicate that CVDs are responsible for 45% of all deaths across Europe [2].

The phrase "cardiovascular disease," sometimes known as "heart disease," describes multiple conditions affecting cardiac and vascular arteries. These disorders cover a wide range of clinical presentations, such as congenital heart defects, peripheral arterial disease, rheumatic heart disease, coronary artery disease, and cerebrovascular illness [3]. The primary underlying cause of many CVDs is atherosclerosis, the gradual accumulation of lipid-rich plaques within artery walls, but there are many other contributing variables. This long-term inflammatory disease reduces blood flow and vascular integrity, which leads to serious side effects such heart failure, stroke, and myocardial infarction (heart attack).

The risk of developing CVD may be increased through various risk factors, both modifiable and non-modifiable. Smoking, poor eating habits, abdominal obesity, psychological issues, frequent alcohol use, sedentary lifestyles, and so on are all changeable risk factors. Conversely age, gender, and genetic predisposition are risk factors that cannot be changed. High blood pressure represents a primary risk factor for cardiovascular disorders that harm the heart, brain, kidneys, and other organs. According to the 2019 Global Burden of Disease Study, hypertensive cardiac disease ranks as the second leading cause of mortality for people between ages 50 to 74. In 2019, 18.6 million people worldwide suffered from hypertensive heart disease [4].

Hypertension, commonly referred to as elevated blood pressure, represents a persistent, age-associated disorder that impacts several organ systems simultaneously. The standard blood pressure measurement for healthy individuals is 120/80 mm Hg. Blood pressure readings exceeding 140/90 mm Hg are classified as hypertensive. Chronic hypertension stiffens the arteries, causes endothelial dysfunction, and imposes excessive strain on the arterial walls, all of which increase the risk of plaque formation. Since hypertension causes myocardial

infarction, left ventricular hypertrophy, and cerebrovascular accidents, it is one of the main causes of mortality from CVD [5].

A major hormonal signaling cascade that is crucial for controlling blood pressure, fluid homeostasis, and vascular tone, known as the Renin-Angiotensin-Aldosterone System (RAAS), is strongly linked to hypertension. An overactive RAAS is closely associated with hypertensive disorders, and RAAS inhibitors, such as angiotensin receptor blockers (ARBs) and angiotensin-converting enzyme (ACE) inhibitors, are commonly used to treat high blood pressure (BP) [6]. ACE inhibitors prevent the transformation of angiotensin I into angiotensin II by the ACE enzyme. Vasoconstrictor angiotensin II has a number of harmful effects, including endothelial and structural vascular damage. Angiotensin II represents a significant factor in cardiovascular damage affecting cardiac, cerebral, and renal organs. It also controls the hormone aldosterone, which elevates blood pressure through enhanced sodium absorption, fluid retention, and increased circulating volume.

Angiotensin II may result in pathological consequences including heart failure, cerebrovascular events, myocardial infarction (MI), and kidney failure. ACE inhibition decreases angiotensin II production, which has several beneficial impacts on the cardiovascular system.

Angiotensin II attenuation inhibits atherogenesis and lowers proinflammatory marker levels. It also lessens endothelial dysfunction and prevents fibrosis. Bradykinin, a vasodilatory peptide that ACE breaks down into inert peptides, is likewise more abundant when ACE inhibitors are present. This rise in bradykinin may be a factor in some of the side effects of ACE inhibitors, including dry cough and vasodilation [7]. There are several FDA-approved medications on the market that function as ACE inhibitors, but they may have adverse effects. Additionally, chronic synthetic medication usage may result in decreased patient compliance and further healthcare burdens.

The effects of plant-derived chemicals are frequently more multi-targeted and balanced, and they may alter several cardiovascular health pathways without substantially disrupting physiological homeostasis. Therefore, investigating herbal alternatives supports the growing need for natural, affordable, and comprehensive ways to disease treatment in addition to providing a safer therapeutic approach.

Herbal remedies and phytochemicals made from plants such as Hawthorn have demonstrated encouraging ACE-inhibitory action along with improved biocompatibility, fewer side effects, and extra cardioprotective, anti-inflammatory, and antioxidant qualities. Triterpenoids,

flavonoids, and oligomeric procyanidins (OPCs) are among the substances found in hawthorn. In contrast to synthetic medications, these natural compounds have ACE-inhibitory activity, which lowers blood pressure by gently and carefully modifying the renin-angiotensin system. Hawthorn phytochemicals lower the incidence of adverse effects including angioedema and dry cough since they do not effectively affect bradykinin breakdown, in contrast to synthetic ACE inhibitors [8].

Additionally, flavonoids and OPCs have anti-inflammatory, antioxidant, vasodilatory, and endothelial-protective properties which promotes cardiovascular wellness overall and hypertension control specifically [9]. Compared to synthetic medications, this multi-targeted method of action is thought to provide broader therapeutic effects by enhancing vascular function, lowering oxidative stress, and preventing platelet aggregation. More significantly, when eaten in little amounts, hawthorn phytocompounds do not cause any significant adverse effects. In contrast to the negative side effects observed with traditional ACE inhibitors, these effects are typically dose-dependent and noticeably milder.

Moreover, hawthorn has been utilized for generations in folk medicine to treating cardiac-related conditions, which supports its legitimacy as a safe herbal therapy when taken in prescribed dosages. Investigating Hawthorn's interaction with the target protein was crucial because of its numerous cardioprotective benefits and ACE-inhibitory activities. The molecular docking approach was used to achieve this. Molecular docking represents an excellent computational technique for predicting the binding interaction between a protein and a ligand. A lower docking score corresponds to a higher binding affinity, and vice versa. The best possible drug candidates can be predicted quickly, cheaply, and with less error-proneness by using in silico approaches. This saves time for additional analysis.

The binding interaction the Hawthorn phytocompounds and the target protein ACE is predicted through molecular docking technique. The RCSB-PDB is used to retrieve the crystal structure of ACE, which is in combination with the FDA-approved medication lisinopril (1O86), for this inquiry. A single chain with a sequence length of 589 makes up human ACE. Lisinopril is the complexed ligand that is present with ACE. The FDA-approved medication lisinopril binds to ACE at its active site. The Vina Wizard in PyRx program was used to carry out the docking approach. A phytocompound compound library is created to facilitate ligand accessibility during the docking process. After the docking process is finished, the binding affinities of the phytocompounds and the control medication can be compared [10].

RESEARCH OBJECTIVES AND SCOPE:

This study's primary goals are to:

- Examine the phytochemicals present in hawthorn plants.
- To investigate the pharmacokinetic properties of the Hawthorn phytocompounds.
- To forecast the tested ligands' toxicity.
- To forecast how the target protein and Hawthorn phytocompounds will interact.
- To compare the binding affinity of the phytocompounds and the synthetic drugs.

CHAPTER 2

LITERATURE REVIEW

2.1 CARDIOVASCULAR DISEASES (CVDS)

Multiple conditions impacting cardiac and vascular systems are collectively termed cardiovascular diseases (CVDs). These represents chronic illnesses that progress gradually throughout a person's lifetime and frequently remains asymptomatic. Furthermore, the primary cause of illness and mortality among patients globally is cardiovascular diseases (CVDs). Over 75% of CVD-related fatalities happen in 16 developing and economically disadvantaged countries. CVDs arise in a variety of forms, each with unique mechanisms, causes, and clinical consequences [11]. Hypertensive heart conditions, peripheral vascular disease, stroke, and cardiac artery disease are some of the most prevalent types. Atherosclerosis is a major pathological process that contributes to a number of CVDs. The persistent inflammation of big and medium-sized arteries at hemodynamically specified places is known as atherosclerosis.

It is distinguished by the buildup of cholesterol and its esters in the artery wall, either as extracellular lipids, including free cholesterol crystals, or in large lipid deposits of macrophage foam cells. It can be evaluated on two different levels. First, autopsy studies or advanced imaging techniques of the lesion dimension in living individual are the preferred methods for directly monitoring the lesion at the preclinical level. The second is when atherosclerosis and its complications have advanced to the point where they show up through clinical cardiovascular manifestations, such as chest pain, cardiac thrombosis, or sudden cardiac thrombosis, or sudden cardiac death, which are all commonly used as stand-ins for the underlying atherosclerosis [12], [13].

Various types of CVDs are:

- **Coronary heart disease (CHD) or cardiac artery disease (CAD) –** A disorder affecting the vascular system supplying the cardiac muscle.
- **Stroke –** A disorder impacting the vascular arteries that provide blood to the brain.
- **Hypertensive heart disease –** A group of cardiac disorders caused by chronically elevated blood pressure that impacts the cardiac and function.

- **Congenital heart disease –** Birth problems brought on by structural abnormalities of the heart from birth that impair the heart's normal development and function.
- **Peripheral vascular disease –** A disorder of blood vessels providing circulation to the extremities.
- **Rheumatic cardiac disease –** Streptococcal bacterial infections that trigger rheumatic fever may damage the cardiac muscle and valves.
- **Cardiomyopathies–** A condition of the heart muscle.
- **Arrythmias (Abnormal Heart Rhythm)–** A problem with the cardiac electrical system of the heart can result in irregular heart rates or rhythms.

Table 1 Different types of cardiovascular diseases (CVDs)

Cardiovascular Disease (CVDs)	Coronary artery Diseases	Angina Pectoris
		Myocardial Infarction (Heart Attack)
	Cerebrovascular Diseases	Stroke (Ischemic or Hemorrhagic)
		Transient Ischemic Attack (TIA)
	Hypertensive Heart Diseases	Left Ventricular Hypertrophy
		Heart Failure
	Congenital Cardiac Diseases	Septal Defects
		Valve Defects
		Coarctation of the Aorta
	Peripheral Artery Diseases	
	Rheumatic Heart Diseases	
	Cardiomyopathies	Dilated Cardiomyopathy
		Hypertrophic Cardiomyopathy
		Restrictive Cardiomyopathy
	Arrythmias (Irregular Cardiac Rhythms)	Atrial Fibrillation
		Ventricular Tachycardia
		Bradycardia / Heart Block

Multiple risk factors are linked with these conditions : elevated blood pressure (hypertension), elevated cholesterol (hyperlipidemia), tobacco consumption (including smoking), type 2 diabetes, family history of cardiac disease, lack of physical activity,

having excess weight or obesity, diet high in sodium, sugar and fat, overuse of alcohol, misuse of prescription or recreational drugs, preeclampsia or toxemia, gestational diabetes, chronic inflammatory or autoimmune conditions, chronic kidney disease etc.

One among the most important risk factors among these is hypertension, which puts the cardiovascular system at serious risk by continuously pressing against the cardiac and vascular system. According to the 2019 Global Burden of Disease Study, hypertensive cardiac disease represents the second leading cause of mortality for people between the ages of 50 to 74. In 2019, 18.6 million people worldwide suffered from hypertensive heart disease. A better knowledge of hypertension is crucial due to its high prevalence and detrimental effects on health [14].

2.2 HYPERTENSION (HTN)

A common, age-related, chronic condition, hypertension frequently results in crippling cardiovascular and kidney damage or even death. Hypertension is usually diagnosed by the World Health Organization when the systolic blood pressure (SBP) exceeds ≥140 mmHg and/or the diastolic blood pressure (DBP) reaches ≥90 mmHg. Often referred to as the "silent killer," hypertension, also known as high blood pressure, usually only manifests its grave repercussions later on, by which point major and frequently permanent harm to essential organs has already been done [4].

In addition to being a significant manageable risk factor for cardiovascular (CV), cerebrovascular, and chronic renal disease, hypertension represents a major renal disorder and primary contributor to mortality and disability. In addition to increasing the burden on the heart, which may result in heart failure, expansion of the heart chambers, or even sudden cardiac death, it can harm arterial walls, which contributes to atherosclerosis. Chronic hypertension stiffens the arteries, causes endothelial dysfunction, and places excessive strain on the arterial walls, all of which increase the risk of plaque formation. Since hypertension causes myocardial infarction, left ventricular hypertrophy, and cerebrovascular accidents, it is one of the main causes of mortality from CVD [15].

Essential (primary) hypertension and secondary hypertension are the two categories of hypertension. 90- 95% of cases are due to essential hypertension. Essential hypertension has been linked to numerous pathophysiologic variables, including: increased activity of

the sympathetic nervous system, possibly as a result of increased exposure to or reaction to psychosocial stress; overproduction of vasoconstrictors and sodium-retaining hormones; prolonged high sodium intake; insufficient potassium and calcium intake in the diet; increased or inappropriate renin secretion, which leads to increased production of angiotensin II and aldosterone; deficiencies of vasodilators, including prostacyclin, nitric oxide (NO), and the natriuretic peptides; abnormalities of resistance vessels, including specific lesions in the renal microvasculature; diabetes mellitus; insulin resistance; obesity; increased activity of vascular growth factors; changes in adrenergic receptors that affect heart rate, changed cellular ion transport, vascular tone, and the heart's inotropic characteristics [16]. Conversely, 5–10% of instances are secondary hypertension, which is caused by an underlying medical condition like renal disease, hormone imbalances, or pharmaceutical use.

It affects around one-third of Italian adults, almost 100 million Americans, and roughly 1.5 billion people worldwide, making it among the leading frequent causes of primary healthcare visit. Only 35–40% of treated hypertension patients achieve the indicated therapeutic objectives, indicating that BP control in the general population is still inadequate despite recent advancements in pharmacological and non-pharmacological therapy [17], [18].

According to estimates, 25% of the global population experiences hypertension independently, making it among the most serious conditions. Between thirty and forty-five percent of adults have hypertension. Nevertheless, it surpasses 60% in the population over 60 since blood pressure (BP) typically increases with aging through vascular resistance and blood vessel stiffening [19].

2.2.1 HYPERTENSION AND COMORBID METABOLIC DISORDERS

Between thirty and forty-five percent of adults have hypertension. Nevertheless, it surpasses 60% in the population over 60 since blood pressure (BP) typically increases with aging through vascular resistance and blood vessel stiffening. A clear association exists between high blood pressure and lipid problems, as over 40% of individual with essential hypertension also present hypercholesterolemia. Additional research has confirmed a significant correlation between dyslipidemia and hypertension. Furthermore, type 2

diabetes mellitus and hypertension often coexist. It is about twice as common in people with diabetes than in people without the disease, and it is even more common in Mexican American and African American communities.

Patients with type 2 diabetes continue to die from coronary heart disease, and having diabetes raises the risk of an acute myocardial infarction to a level similar to that of a previous heart attack in a person without the illness. Notably, hypertension is thought to be responsible for between 35% and 75% of cardiovascular problems in diabetes patients. Therefore, a comprehensive strategy that includes aggressive blood pressure control, treatment of dyslipidemia, and blood glucose regulation is necessary for the effective care of diabetic patients [20].

Cardiovascular (CV) morbidity and mortality are decreased when blood pressure is lowered with lifestyle changes and antihypertensive medications. Dual- and triple-combination treatments with calcium channel blockers, renin-angiotensin system blockers, and/or a diuretic are advised by guidelines. Management is frequently complicated by comorbidities. Cardiovascular and renal outcomes are improved by medications such as non-steroidal mineralocorticoid receptor antagonists, glucagon-like peptide-1 receptor agonists, sodium–glucose cotransporter 2 inhibitors, and angiotensin receptor-neprilysin inhibitors. In cases with concomitant hypertension linked to elevated sympathetic nerve activity, catheter-based renal denervation may provide an alternate therapeutic option [20].

2.3 RENIN- ANGIOTENSIN- ALDOSTERONE SYSTEM (RAAS)

Blood pressure, fluid homeostasis, and vascular tone are comprehensively controlled by the Renin-Angiotensin-Aldosterone System (RAAS), a crucial hormonal signaling cascade that is strongly linked to hypertension. One important endocrine/paracrine system that controls a wide range of cardiovascular functions is RAAS. It is essential for the homeostatic regulation of extracellular volume, tissue perfusion, and arterial pressure. It has a well-established involvement in the pathophysiology of atherosclerosis, cardiac hypertrophy, and hypertension. Through regulating arterial pressure and extracellular fluid volume, this mechanism maintains vascular homeostasis. This system closely regulates water, blood, plasma, lymphatic, and interstitial fluid to maintain the filtering organ kidneys and the pulsing organ heart without allowing either to overpower the other [21].

The production of renin (angiotensinogenase) through the juxtaglomerular cells (JG) surrounding the afferent (and occasionally efferent) arteriole of the renal glomerulus initiates the renin-angiotensin aldosterone hormonal cascade. After processing, the 406 amino acid protein known as prorenin—the precursor of renin—becomes the 340 amino acid active renin. Renin/prorenin receptors can non-proteolytically activate prorenin in various tissues, while neuroendocrine convertase 1 (proprotein convertase 1) or cathepsin B may proteolytically activate it in the kidney. The kidneys respond to low blood volume and low salt levels by releasing renin [22].

After binding to the (pro)renin receptor (ATP6AP2), renin acts on the chemical bond between leucine (Leu) and valine (Val) to cleave angiotensinogen into angiotensin- (1-10) (Ang I). This process is inhibited by the therapeutic medication Aliskiren, which is used to treat hypertension. While the liver serves as the primary source of systemic circulating angiotensinogen, the kidney, brain, heart, vascular system, adrenal gland, ovary, placenta, and adipose tissue have all been found to express angiotensinogen mRNA [23].

Plasma levels of angiotensinogen are usually stable and do not change abruptly because the liver secretes it constitutively. However, it has been demonstrated that glucocorticoids, estrogens and other sex steroids, thyroid hormone, inflammatory cytokines (such as TNF and IL-1), and Ang II can increase both hepatic and extrahepatic synthesis. The endothelium-bound angiotensin-converting enzyme (ACE) further cleaves the decapeptide angiotensin (1–10) (Ang I) in the kidney epithelial cells, lungs capillaries, and endothelial cells, converting angiotensin I into the peptide angiotensin-(1–8) (Ang II). Angiotensin II is created by removing two of the C terminal amino acids of Ang I [24]. Angiotensin converting enzyme inhibitors, or ACEIs, which are therapeutic agents used for treating hypertension, include captopril, lisinopril, enalaprilat, and ramiprilat (which are converted from the prodrugs enalapril and ramipril, respectively).

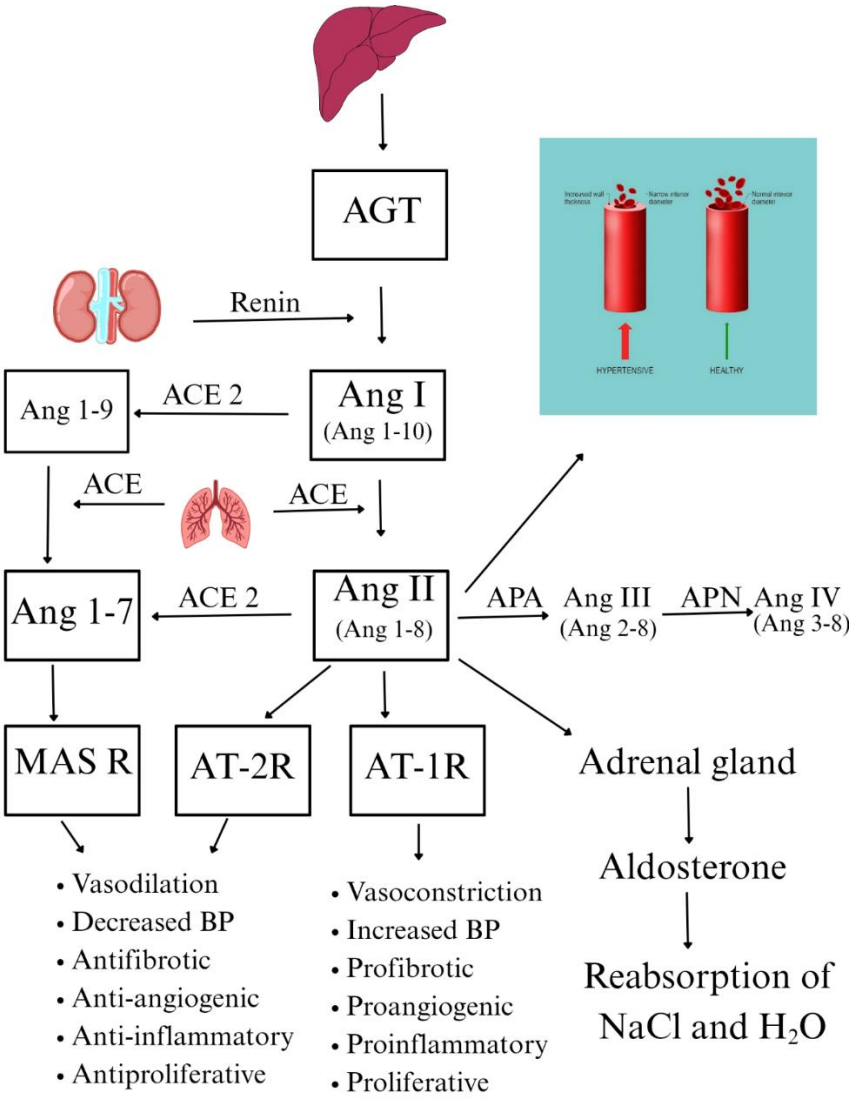


Fig. 1 Schematic Representation of RAAS Pathway

ACE is a shared part of the kinin-kallikrein system (KKS) and the RAAS. With its intracrine, autocrine, and paracrine functions, angiotensin II is a multipurpose effector molecule. In addition to controlling the generation of oxidative stress and the metabolism of multiple organs, such as the nervous system, digestive organs, skin, reproductive tract, sensory organs, lymphatic tissue, adipose tissue, adrenal glands, and kidneys, Ang II also functions as a vasoconstrictor on the cardiovascular system. Additionally, it stimulates the pituitary gland to secrete antidiuretic hormone (also known as vasopressin) and the adrenal gland cortex to secrete aldosterone. Together, aldosterone and ADH activate the kidney's proximal tubules to enhance sodium reabsorption, which causes potassium to be eliminated and sodium to be retained, so maintaining sodium-potassium homeostasis [25], [26].

Renal vasoconstriction may be influenced by prostaglandin release, which is another effect of angiotensin II. It has been determined that cyclooxygenase (COX) 1-derived prostaglandin and its receptor are essential for angiotensin II-dependent hypertension. Additionally, angiotensin II can increase the bulk of adipose tissue by promoting lipogenesis. As a result, this enzyme is connected to insulin resistance, glucose intolerance, and adipose inflammation.

The half-life of angiotensin II is thirty seconds. Red blood cells' aminopeptidase A (APA) enzymes can break it down into angiotensin III. With varying degrees of affinity for angiotensin receptors, there exist other degraded forms of angiotensin II. Aminopeptidase A (APA) further transforms angiotensin II into angiotensin III, which aminopeptidase N (APN) can then further transform into angiotensin IV. Angiotensin III can raise blood pressure via promoting sympathetic tone, vascular resistance, baroreflex function, and vasopressin release, which inhibits diuresis [27].

2.4 HUMAN ACE

A single-chain zinc metalloenzyme, ACE is synthesized as a 1,306-amino-acid polypeptide, matures to a 1,277-residue structure, is confined to the plasma membrane of endothelial, absorptive epithelium, and neuroepithelial cells, and is highly glycosylated (30% by weight). This carboxypeptidase enzyme is sometimes referred to as CD143, kininase II, or peptidyl-dipeptidase A.

A key player in the renin-angiotensin-aldosterone system (RAAS), ACE regulates the body's fluid balance to manage blood pressure. By releasing the terminal His-Leu, it transforms the precursor angiotensin I (ATI) into potent vasoconstrictor angiotensin II (ATII). Therefore, by causing vessels to contract, ACE indirectly raises blood pressure. By preventing ATI from becoming ATII, ACE inhibitors (ACEIs) reduce arteriolar resistance and boost venous capacity [25].

Additionally, it deactivates bradykinin, a powerful vasodilator. Bradykinin is a vasodilatory peptide that ACE breaks down into inert peptides, and ACE inhibitors raise its levels. Bradykinin triggers the release of endothelium-derived hyperpolarizing factor, prostacyclin, prostaglandins, and the vasodilator nitric oxide, among other calming substances. ACE inhibitors provide advantageous cardiovascular protection by blocking the breakdown of bradykinin and the enzyme ACE's ability to convert angiotensin I into angiotensin II.

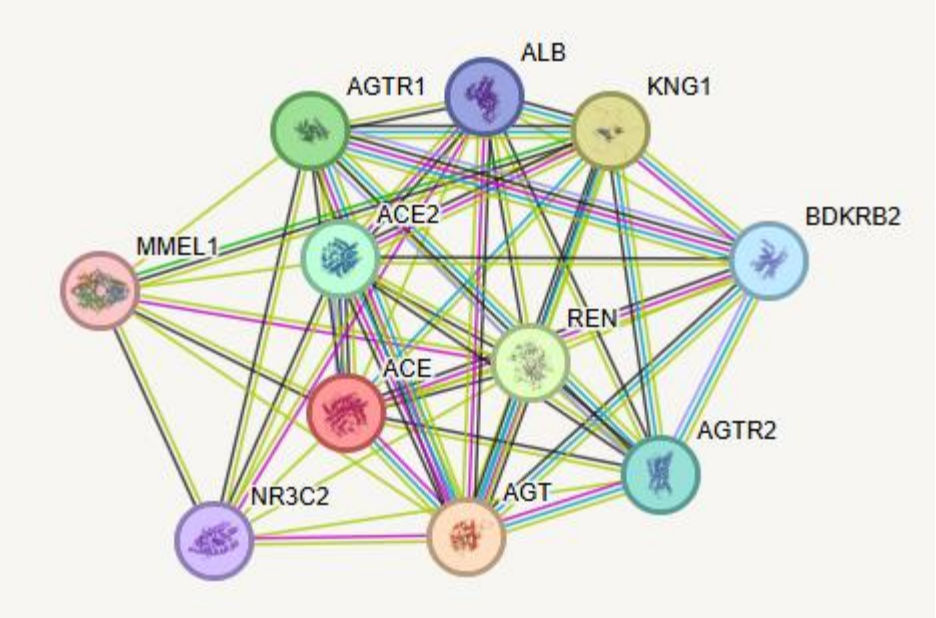


Fig. 2 String Analysis of ACE enzyme [28]

Predicted Functional Partners of ACE:

AGT= Angiotensinogen

REN= Renin

AGTR1= Type-1 angiotensin II receptor

ACE2= Angiotensin Converting Enzyme 2

AGTR2= Type-2 angiotensin II receptor

BDKRB2= B2 bradykinin receptor

KNG1= Low molecular weight growth-promoting factor

ALB= Serum albumin

NR3C2= Mineralocorticoid receptor

MMEL1= Membrane Metallo-endopeptidase-like 1

The network of protein-protein interactions (PPIs) is revealed by the STRING analysis of ACE. These correlations make it evident how ACE affects the kinin-kallikrein and renin-angiotensin-aldosterone (RAAS) systems, which are crucial for maintaining electrolyte balance, controlling blood pressure, and maintaining vascular tone.

For example, ACE transforms the angiotensin I produced by AGT and REN into angiotensin II, a strong vasoconstrictor that operates through AGTR1/2. A homolog of ACE, ACE2, is present because it counteracts ACE function by changing angiotensin II into vasodilatory peptides. Additionally, ACE's function in bradykinin degradation is highlighted by its interaction with BDKRB2 and KNG1, which connects it to vasodilatory and inflammatory pathways. Proteins including albumin (ALB), mineralocorticoid receptor (NR3C2), and membrane metallo-endopeptidase (MMEL1) imply that ACE might be a component of larger regulatory networks involving protein transport and hormonal signaling. All things considered, this PPI network highlights the versatility of ACE and offers a basis for comprehending the potential effects of phytochemical ACE modulation on various cardiovascular and renal pathways.

2.5 ACE INHIBITORS (ACEIs)

Captopril, initial ACEI inhibitor, was developed from a popular precursor in pit viper venom, which is employed as an arrowhead poison by native Brazilian tribes. Because ACEIs reduce ATII synthesis, the adrenal cortex now cannot release aldosterone. This enables the kidney to retain potassium ions while excreting sodium ions and obligatory water. Because of this, blood volume decreases, which lowers blood pressure. ACEIs are common pharmacological treatment options primarily used primarily heart protective benefits and HTN. The angiotensin II type 1 (AT1) receptor (vasoconstriction, cell proliferation, salt and water retention, sympathetic activity) and the angiotensin II type 2 (AT2) receptor are the downstream effects that ACE inhibitors prevent by blocking the production of angiotensin II. One drawback of ACE inhibitors is that, even when ACE is inhibited, low-level angiotensin II synthesis persists when non-ACE routes are present [28].

Three categories of ACEIs can be distinguished by their molecular makeup [29]:
1. Agents that contain sulfhydryl (captopril, zofenopril)

2. Dicarboxylate-containing agents (includes enalapril, ramipril, quinapril, perindopril, lisinopril, benazepril, imidapril, trandolapril, cilizapril and spirapril)

3. Phosphonate-containing agent fosinopril.

2.5.1 FDA Approved Medications for ACE Inhibition

Captopril /Capoten: The first ACE inhibitor, captopril, was created in 1975 and approved by the FDA in 1981. Captopril is not given as a prodrug like most ACE inhibitors (lisinopril is the only one that is). However, it is the only one that is applied to manage certain types of congestive Additionally, it is utilized to maintain kidney function in diabetic nephropathy and to increase survival following myocardial infarction.

Zofenopril: When taken as a prodrug, zofenopril undergoes liver metabolism to become zofenoprilat, the active form. It is prescribed for treating hypertension and ischemic heart conditions.

Enalapril: The liver converts the prodrug enalapril into the active form, enalaprilat. It is prescribed for treat hypertension, diabetic nephropathy, and heart failure.

Ramipril is an ACE inhibitor that is given as a prodrug and converted to its active form by the liver. It is used to treat congestive heart failure and mild to moderate hypertension.

Quinapril is a second-generation ACE inhibitor that is taken as a prodrug and is transformed in the liver into quinaprilat, its active metabolite. In research involving healthy participants, it suppresses plasma ACE activity. Patients with congestive heart failure and hypertension are treated with quinapril. Similar in effectiveness to other ACE inhibitors, but less likely to cause side effects or withdrawals than captopril or enalapril.

Perindopril: The liver converts the ester prodrug perindopril into its active form, perindoprilat. It is used to treat stable coronary artery disease, heart failure, and hypertension.

Lisinopril: After enalapril and captopril, lisinopril became the third ACE inhibitor to be authorized for clinical use in the management of congestive heart failure and hypertension. In terms of chemistry, it is enalapril's lysine analog. It is not a prodrug and is eliminated unaltered in the urine, in contrast to other ACE inhibitors.

Benazepril: When benazepril is taken as an ester prodrug, the liver converts it to benazeprilat, the active form. It is mostly used to treat heart attacks, congestive heart failure, and hypertension.

Imidapril is an ACE inhibitor that is taken as a prodrug and converted to imidaprilat, the active form, in the liver. It is used to treat mild to severe essential hypertension and to keep patients from developing heart failure following myocardial infarction.

Trandolapril: The liver converts the prodrug trendolapril into its active form. An ACE inhibitor called trandolaprilat is used to treat congestive heart failure and hypertension.

Cilazapril is a prodrug converted to the active drug cilazaprilat in the liver. It is used for the treatment of hypertension and congestive heart failure. In several countries, cilazapril is marketed under the names Dynorm, Inhibace, and Vascace; however, it is not accessible in the United States.

Spirapril: The active metabolite of the prodrug spirapril is spiraprilat. Because of its extended duration of action and limited dose range, it is used once day to treat mild to moderate hypertension.

Fosinopril: The only member of a phosphinic acid derivative that rapidly hydrolyzes to the active form fosinoprilat is fosinopril, primarily in the liver and gastrointestinal mucosa. It is used to treat certain forms of chronic heart failure and hypertension [31].

Side effects

ACE inhibitors may cause the following negative effects:

- Dry cough
- Angioedema (face, lip, tongue, or throat swelling)
- Hyperkalemia, or increased blood potassium levels
- Taste loss
- Rarely, renal function deteriorates temporarily.

2.6 HAWTHORN (https://www.aafp.org/pubs/afp/issues/2010/0215/p465.html)

Traditional medications, which are frequently used to treat heart conditions, are expensive and have drawbacks. Thus, a more dependable, reasonably priced, and potent substitute is required. Therefore, the most crucial type of treatment for cardiovascular illness is the use of medicinal plants. Medicinal plants are more likely to be used for managing and treating CVDs [32]. Herbal medicine has achieved significant recognition in the medical

community as a result of growing knowledge about the mechanism by which herbs enhance health and improve quality of life. Growing research suggests that utilizing phytochemicals and plant-derived whole foods represents a promising alternative approach for preventing cardiovascular disease.

Hawthorn, sometimes referred to as haw, may bush, or whitehorn, is part of a family of prickly trees and bushes native to temperate climates in North America, Asia, and Europe. It is part of the Rosaceae plant family and produces vibrant red berries, white blooms, and bright green foliage [33].

In folk medicine, hawthorn has traditionally been employed for treating sleeplessness, gall bladder illness, diarrhea, and asthma by acting as an antispasmodic. Hawthorn was also utilized in Chinese medicine to treat a number of ailments, such as dyspnea, poor circulation, hyperlipidemia, and digestive issues. Conditions including anxiety, asthma, hypertension, dyslipidemia, hypotension, angina, arrhythmias, heart failure, and indigestion have all been treated with hawthorn in the past [34]. Research on hawthorn for managing congestive heart failure (CHF) provides the strongest evidence for its therapeutic advantages.

In Europe, hawthorn medical extract has long been a popular herbal medicine. This gentle cardiotonic compounds' active ingredients are thought to include flavonoids and oligomeric procyanidins. Additional research on hawthorn in heart failure patients has shown improvements in the patients' subjective sense of well-being, cardiac output efficiency and heart rate-pressure index. Nevertheless, there is no proof that mortality or unexpected deaths have significantly decreased. Vertigo and light-headedness represent the primary frequent adverse reactions, but hawthorn is often well tolerated.

The oldest known medicinal plant used in European medicine is hawthorn. Another well-liked herbal supplement in the US market is hawthorn. Hawthorn's pharmacologic impact is believed to be caused by the quantity of oligomeric procyanidins and flavonoids found in its leaves, flowers, and berries. Although numerous in vivo research has also shown the physiologic effects of hawthorn extract, most pharmacological studies conducted on the extract utilize laboratory and on animals.

The impact of oxidative stress and neutrophils elastase activation in neutrophils could contribute to cardiac tissue damage during ischemic events. Hawthorn's leaves and blooms possess oligomeric procyanidins that serves as antioxidants and suppress neutrophil

elastase activity, thereby potentially minimizing ischemia-related cardiac injury. Studies on animals have also demonstrated that oligomeric procyanidins improve coronary blood flow [35].

Flavonoids increase vasodilation via inhibiting phosphodiesterase and activating endothelium-derived relaxing factor. The antiatherogenic qualities of hawthorn extract may be explained by flavonoids, which have been investigated for preventing lipid peroxidation in laboratory settings. It has also been demonstrated that flavonoids prevent platelet adhesion and aggregation [36], [37].

Hypersensitivity to Crataegus products is the only known absolute contraindication to using hawthorn. Due to the potential for uterine contractions, it should not be used during pregnancy. There is no scientific proof that Crataegus may be used safely in young people or during lactation. As a result, it is not currently advised for infants or nursing mothers. According to most research, oral hawthorn is generally safe; common adverse reactions include vertigo and light-headedness. Nausea, fatigue, excessive sweating, rapid heartbeat, headache, light-headedness, dyspnea, sleeplessness, restlessness and stomach upset occur less commonly. There were reportedly few negative effects. Hawthorn phytochemicals have minimal risk of drug interactions including angioedema and dry cough since they do not substantially disrupt bradykinin breakdown, in contrast to synthetic ACE inhibitors.

Notwithstanding the enormous potential of phytochemicals, several challenges still need to be addressed, including determining standardization, bioavailability, efficacy, and safety when applying phytochemicals in therapeutic settings. However, developments in computational methods, like molecular docking, have made it easier to identify and screen phytochemicals, speeding up efforts to find new drugs in this area.

2.7 MOLECULAR DOCKING

Nowadays, it is a common computational method in virtual screening studies to identify new physiologically active compounds and in drug discovery for lead compound optimization. The docking approach aims to forecast how small molecules will interact with specific receptor binding sites, including their binding strengths and orientational preferences. The core elements of any docking procedure consist of a computational search

method and an energetic evaluation system that generates and analyze different ligand conformations.

This computational procedure determines how a small molecule acting as a ligand will interact and bind to the specified target protein's active site. Molecular docking models the three-dimensional structures of proteins and ligands to determine how they will interact to form stable complexes using prediction methods. As a result, their binding affinities and interaction mechanism are evaluated.

A lower docking score corresponds to a higher binding affinity, and vice versa. Molecular docking is essential for identifying lead compounds, refining their chemical structures, and supporting additional experimental research in the early stages of drug discovery. Time can be saved for more research by using in silico algorithms to forecast the best possible drug candidates because they are effective, economical, and less prone to errors.

The molecular docking approach is used to predict how the Hawthorn phytocompounds would interact with the target protein ACE [38]. The RCSB-PDB is used to retrieve the crystal structure of ACE, which is in combination with the FDA-approved medication lisinopril (1O86), for this inquiry. A single chain with a sequence length of 589 makes up human ACE. Lisinopril is the complexed ligand that is present with ACE. The FDA-approved medication lisinopril binds to ACE at its active site. The Vina Wizard in PyRx program was used to carry out the docking approach. A phytocompound compound library is created to facilitate ligand accessibility during the docking process. After the docking process is finished, the binding affinities of the phytocompounds and the control medication can be compared [39].

CHAPTER 3

METHODOLOGY

This study's methodology combines molecular docking simulations and computational tools to find possible phytochemicals of Crataegus sp. that target the 1O86 protein for the treatment of cardiovascular disorders.
Here, we virtually screened a number of ligands (phytochemicals) that target the protein 1O86 using PyRx. The methods used for ligand selection, protein preparation, molecular docking, and visualization processes are described in detail in the paragraphs that follow.

3.1 SOFTWARE AND DATABASES

To conduct the study, the following database were used:

- PubChem (https://pubchem.ncbi.nlm.nih.gov/)
- RCSB PDB (https://www.rcsb.org/)
- DrugBank (https://go.drugbank.com/)
- SwissADME (http://www.swissadme.ch/)
- pkCSM Server (https://biosig.lab.uq.edu.au/pkcsm/prediction)
- FRoG2 Server (https://bioserv.rpbs.univ-paris-diderot.fr/services/Frog2/)

The software's that were used to predict the results were:

- Notepad ++
- Swiss PDB Viewer
- Open Babel
- Vina Wizard in Pyrx
- BIOVIA Discovery Studio

3.2 LIGAND PREPARATION

3.2.1 ADMET ANALYSIS

Following the literature analysis, a list of the phytochemicals found in hawthorn plants was created. The 3D structures of the phytochemicals were obtained in sdf format from the

DrugBank and PubChem databases [40]. These structures' SMILES notation was also created in a different notepad document. The pharmacokinetic characteristics of the phytochemicals are subsequently examined. SwissADME server [41] was used for ADME study of these structures, while pkCSM server [42] was used for toxicity analysis. Following a thorough examination of their pharmacokinetic characteristics, phytochemicals that met the necessary requirements were chosen, and the information was stored in an Excel document.

3.2.2 COMPOUND LIBRARY PREPARATION

FRoG2 Server was used to construct a compound library of the chosen phytocompounds using the corresponding SMILES notation [43]. After downloading the file, Notepad + + was used to change the ligand or compound names for easier comprehension. After that, the file was then opened in Open Babel, which reduced the ligands' energy and produced a new file in PDBQT format.

3.3 PROTEIN PREPARATION

The target protein (1O86), which is in complex with the control medication (lisinopril), was found to have the correct structure using the RCSB PDB database. The protein's 3D structural data was acquired from the PDB database. The Swiss PDB Viewer was then used to open the protein file [44]. Water molecules, heteroatoms, and the control medication (LPR), which was in association with the ACE protein, were removed in order to prepare the protein. After adding polar hydrogen atoms, the protein was given Kollman charges. An additional file containing the produced protein was saved. The target protein and lisinopril were stored in a different file that served as a control medication.

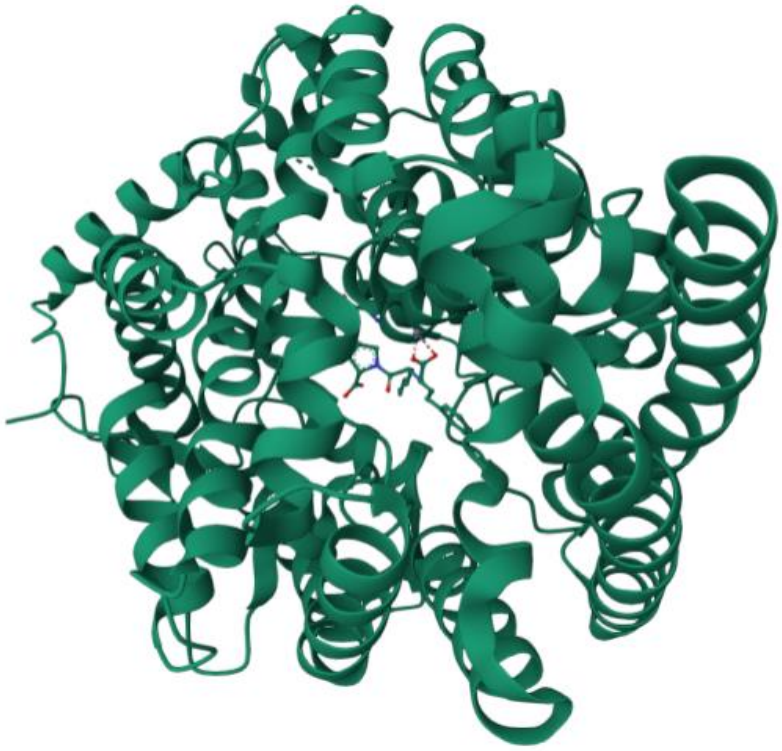


Fig. 3. The crystallographic structure of human ACE bound to lisinopril (PDB code: 1O86).

3.4 PROTEIN-LIGAND MOLECULAR DOCKING

Molecular docking was carried out using PyRx Software's Vina Wizard [45]. After loading, the control medication was designated as a ligand and the protein as a macromolecule. Clicking "ligand selection" further loaded the chemical library. Now, a significant number of ligands were supplied through a single file, making it impossible to pick each one separately. The grid box was positioned at the target protein's active site. To verify or authenticate the protein's real active sites, two methods were used. In the first place, the grid was arranged so that the control medication was in the middle. Second, in order to confirm the decision, each active site—which now showed up in the protein as pink—was chosen separately and the grid was resized to encompass each active site. The docking was now completed. The docking postures, binding affinities, and RMSD values were shown in the bottom panel once the docking operation was finished. The results were subsequently exported as a CSV file. BIOVIA Discovery Studio software was employed to retrieve and visualize the docking conformation exhibiting the strongest binding interaction.[46].

3.5 VISUALIZATION

The docking poses were downloaded and opened in BIOVIA Discovery Studio and the protein interaction was then examined in more detail. For a deeper comprehension of the interaction and the bonds involved, a 2D schematic of the protein-ligand interaction was then further displayed. To compare the outcomes, the file was then stored in a different file.

CHAPTER 4

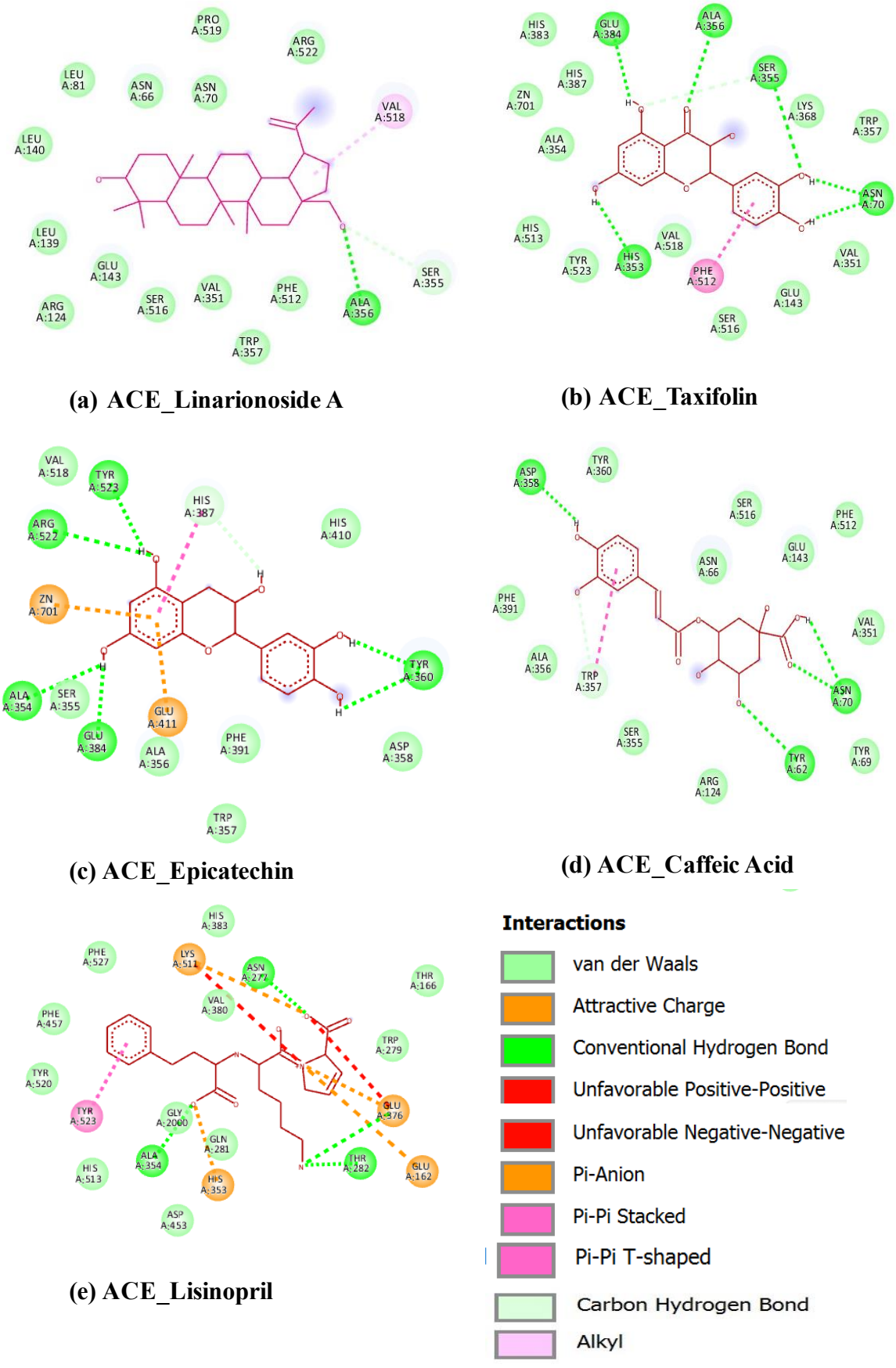
RESULTS

4.1 MOLECULAR DOCKING STUDIES

Based on molecular docking investigations, four phytocompounds were found to have a better binding affinity with the ACE protein than lisinopril and to satisfy the ADMET criterion. Lisinopril's binding affinity for the ACE protein was -7.4 Kcal/mol (see fig. 4e). while Linarionoside A had the highest affinity at -9.8 Kcal/mol (Refer fig. 4a), followed by Taxifolin (-8.7 Kcal/mol, refer fig. 4b), Epicatechin (-7.8 Kcal/mol, refer fig. 4c), and Caffeic Acid (-7.7 Kcal/mol, refer fig. 4d). These findings imply that natural substances have higher binding affinities to the ACE active site than lisinopril, suggesting that they may be useful inhibitors.

Table 2 Docking Scores of Phytochemicals with ACE

S. No.	**Phytochemicals/ Drug**	**Molecular Binding Energy (Kcal/mol)**
1	ACE_Linarionoside_A	-9.8
2	ACE_Taxifolin	-8.7
3	ACE Epicatechin	-7.8
4	ACE_Caffeic_Acid	-7.7
5	ACE_Lisinopril	-7.4



(a) ACE_Linarionoside A

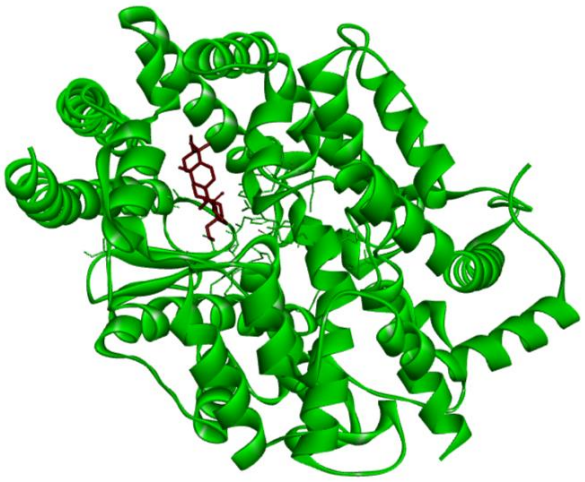
(b) ACE_Taxifolin

(c) ACE_Epicatechin

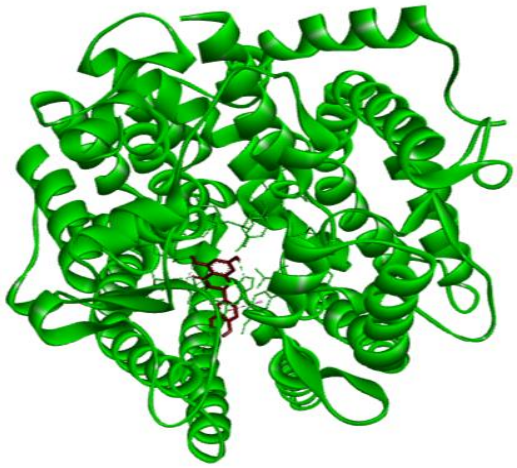
(d) ACE_Caffeic Acid

(e) ACE_Lisinopril

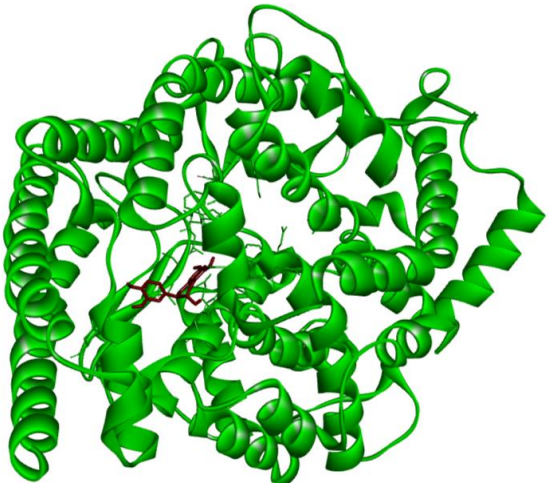
Fig. 4 2D interactions of ligands/ drug with ACE protein



(a) ACE_Linarionoside A

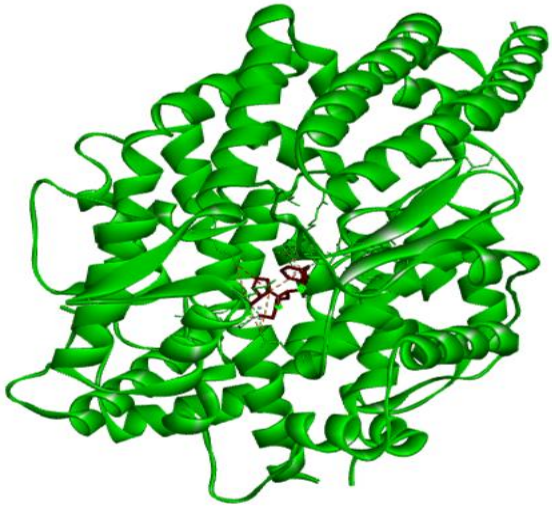


(b) ACE_Taxifolin



(c) ACE_Epicatechin

(d) ACE_Caffeic Acid



(e) ACE_Lisinopril

Fig. 5 3D interactions of ligands with ACE protein (green: ACE Protein, red: Ligand/ Drug)

4.2 PHARMACOKINETICS SCREENING OF PHYTOCHEMICALS

A. Physicochemical Properties

The molecular weight of each phytocompound was less than 500 Da, which is in line with Lipinski's rule of five for drug-likeness. The phytocompounds might be better options in terms of oral bioavailability and stable target binding than lisinopril because they have lower molecular flexibility (fewer rotatable bonds) and more favorable lipophilicity (LogP within the optimal range).

Table 3 Physicochemical properties of Ligands

Ligands/ Reference Drug	**Molecular Weight (Da)**	**LogP**	**Rotatable Bonds Count**	**H-bond Count**	**H-bond Donors Count**	**Surface Area ($A^{\circ 2}$)**
Lisinopril	441.525	-0.4142	12	5	4	181.584
Epicatechin	290.271	1.5461	1	6	5	119.662
Taxifolin	304.254	1.1863	1	7	5	123.824
Caffeic Acid	180.159	1.1956	2	3	3	74.381
Linarionoside A	374.474	0.469	6	7	5	154.805

B. Pharmacokinetic Parameters

Absorption parameters

All of the selected phytocompounds demonstrated compliance with the optimal gastrointestinal absorption requirements in terms of absorption, despite the fact that lisinopril's weak lipophilicity may hinder its passive absorption. The intestinal absorption of taxifolin and caffeic acid was the highest, suggesting good oral bioavailability (Refer to Table 4). Additionally, caffeine does not act as a substrate for P-glycoprotein, increasing its bioavailability and absorption. They are less likely to obstruct drug-drug interactions associated with the P-gp efflux transporter, though, as none of the ligands block P-gp I or II.

Table 4 Absorption Parameters of Ligands

Ligands/ Reference drugs	**Aqueous Solubility (log mol/L)**	**Intestinal Permeability (log Papp in 10-6 cm/s)**	**Gastrointestinal Absorption (% Absorbed)**	**Dermal Permeability (log Kp)**	**P-glycoprotein substrate**	**P-glycoprotein I & II inhibitor**
Lisinopril	-2.759	-0.525	3.751	-2.735	Yes	No
Epicatechin	-3.178	-0.411	66.773	-2.735	Yes	No
Taxifolin	-3.1	-0.411	58.999	-2.735	Yes	No
Caffeic Acid	-1.671	0.264	68.465	-2.616	No	No
Linarionoside A	-2.31	-0.157	36.585	-3.19	Yes	No

Metabolic Parameters

None of the ligands inhibit CYP1A2, CYP2C19, CYP2C9, CYP2D6, or CYP3A4 (Refer to Table 5). This implies that there is less likelihood of harmful drug-drug interactions because these ligands are unlikely to obstruct the liver's ability to metabolize drugs.

Table 5 Metabolic Parameters of Ligands

Ligands/ Reference drugs	**CYP1A2 inhibitor**	**CYP2C19 inhibitor**	**CYP2C9 inhibitor**	**CYP2D6 inhibitor**	**CYP3A4 inhibitor**
Lisinopril	No	No	No	No	No
Epicatechin	No	No	No	No	No
Taxifolin	No	No	No	No	No

Caffeic Acid	No	No	No	No	No
Linarionoside A	No	No	No	No	No

Distribution and Excretion Parameters

Based on the volume of distribution (VDss) values, the phytocompounds Epicatechin (0.668 log L/Kg) as well as and Taxifolin (0.929 log L/Kg) demonstrated showed moderate distribution, while Caffeic Acid (-0.446 log L/Kg) and Linarionoside A (-0.342 log L/Kg) exhibited limited distribution (Refer to Table 6). Lisinopril (1.832 log L/Kg) showed the highest tissue penetration. According to fraction unbound values, linarionoside A and lisinopril were more readily available in the bloodstream than the other phytocompounds. The likelihood of neurotoxic consequences was reduced because none of the chemicals were able to effectively cross the blood-brain barrier (BBB), based on blood-brain barrier permeability and CNS permeability values. According to the total clearance values, epicatechin (0.179 log ml/min/Kg) and taxifolin (-0.034 log ml/min/Kg) had slower removal rates, whereas linarionoside A (1.329 log ml/min/Kg) and caffeine (0.52 log ml/min/Kg) showed higher clearance rates. This could be one reason for the systemic effects' prolonged duration.

Table 6 Distribution and Excretion Parameters of Ligands

	Distribution				**Excretion**	
Ligands/ Reference drugs	**Volume of Distribution (human) (log L/Kg)**	**Unbound Fraction (human) (Fu)**	**Blood Brain Barrier permeability (log BB)**	**CNS permeability (log PS)**	**Systemic Clearance (log ml/min/kg)**	**Renal OCT2 substrate**
Lisinopril	1.832	0.634	-1.004	-4.167	0.83	No
Epicatechin	0.668	0.295	-1.005	-3.359	0.179	No
Taxifolin	0.929	0.258	-1.046	-3.487	-0.034	No
Caffeic Acid	-0.446	0.522	-0.804	-2.647	0.52	No

Linarionoside A	-0.342	0.614	-1.014	-3.919	1.329	No

Toxicity Parameters

None of the chosen phytocompounds showed signs of skin sensitization, hERG inhibition, or AMES toxicity, according to the toxicity profile. Interestingly, all phytocompounds were non-hepatotoxic, suggesting a possibly safer toxicity profile, although lisinopril was warned for hepatotoxicity. The substances' T. Pyriformis and Minnow toxicity values differed, with epicatechin and taxifolin showing somewhat higher toxicity levels than caffeine and linarionoside A (Refer to Table 7).

Table 7 Toxicity Parameters of Ligands

Ligands/ Reference drugs	**AMES toxicity**	**Max. tolerated dose (human) (log mg/kg/day)**	**hERG I & II inhibitor**	**Hepatotoxicity**	**Skin Sensitisation**	**T. Pyriformis toxicity (log ug/L)**	**Minnow toxicity (log mM)**
Lisinopril	No	0.441	No	Yes	No	0.285	3.015
Epicatechin	No	0.244	No	No	No	0.605	2.869
Taxifolin	No	0.247	No	No	No	0.389	3.368
Caffeic Acid	No	-0.106	No	No	No	0.023	2.22
Linarionoside A	No	0.874	No	No	No	0.285	2.792

CHAPTER 5

DISCUSSION

For predicting possible ligands for a target protein, molecular docking is a great method. In this work, we investigated ligands from the Hawthorn plant that may function as an ACE (angiotensin-converting enzyme) inhibitor. The literature review provided a list of Hawthorn ligands, from which those that met the ADMET criteria were chosen and adopted to investigate the interaction with the target protein ACE using PyRx. To maximize the outcomes, lisinopril was utilized as a reference medication and control. Since each ligand was made with a potential three-dimensional orientation, they were each docked with the enzyme in its active site independently. In order to show how well ligands bound to ACE, the docking score was taken into consideration. Better ligand-protein binding site fit was indicated by a negative docking score. Only four Hawthorn ligands met the ADMET requirements and had superior binding affinity when compared to lisinopril, according to the docking study. Lisinopril exhibited a binding energy of -7.4 Kcal/mol against the ACE protein, whereas Linarionoside A showed the strongest binding interaction at -9.8 Kcal/mol, succeeded by Taxifolin (-8.7 Kcal/mol), Epicatechin (-7.8 Kcal/mol), and Caffeic Acid (-7.7 Kcal/mol). The Lipinski, Ghose, Veber, and Egan rules were all upheld by these ligands. They are characterized by a strong bioavailability score of 0.55 and high GI absorption. Furthermore, as shown by its toxicity qualities, synthetic ACE inhibitors like lisinopril may be hepatotoxic; however, none of the natural ligands mentioned here showed any hepatotoxicity in their ADMET properties. Based on the above results, Caffeic acid outperformed these ligands in terms of absorption and bioavailability, and it may be a better ACE inhibitor that lowers blood pressure and associated cardiovascular diseases. However, other chosen ligands also demonstrated a promising future as ACE inhibitors but certain pharmacokinetic properties limit their usage. These findings emphasize the necessity of additional optimization to improve the pharmacokinetic profiles of promising Hawthorn flavonoids for cardiovascular therapies, whether through prodrug methods or formulation changes.

CHAPTER 6

CONCLUSION AND FUTURE PROSPECTS

Several conclusions are drawn from the ADMET analysis and the total molecular docking analysis. It is evident from the binding affinity between ACE and ligands that natural substances have a greater potential to serve as therapeutic targets for cardio-protection. Despite having a high binding affinity for ACE, lisinopril's pharmacokinetic characteristics (ex-hepatotoxicity) point to potential limitations in its chemical makeup. However, caffeic acid showed encouraging pharmacokinetics, including favorable skin penetration, high gastrointestinal absorption, and a reasonably safe toxicity profile. As a result, it could be a lead compound for additional research into the treatment of hypertension with some modifications. Though their ADMET criterion of acting as P glycoprotein substrate may reduce the drug's bioavailability, linarionoside A, epicatechin, and taxifolin found in hawthorn demonstrated a higher binding affinity than lisinopril. This can be addressed by formulating the medication with a P-glycoprotein inhibitors. Therefore, compared to synthetic medications, natural ligands may be a superior alternative for cardiovascular problems with less adverse effects. However, further research is needed to help with their clinical translation. To confirm these medications' pharmacokinetic properties and enzymatic inhibitory activity, future research should focus on verifying them both in vitro and in vivo. To improve their metabolic stability and bioavailability, prodrug strategies and structural alterations can also be investigated.

REFERENCES

[1] "World Health Organization (WHO). Non-communicable Diseases. Fact sheets. Retrieved September 2, 2021, from WHO website.pdf."

[2] W. Martinet, I. Coornaert, P. Puylaert, and G. R. Y. De Meyer, "Macrophage Death as a Pharmacological Target in Atherosclerosis," *Front. Pharmacol.*, vol. 10, p. 306, Apr. 2019, doi: 10.3389/fphar.2019.00306.

[3] A. Shaito *et al.*, "Herbal Medicine for Cardiovascular Diseases: Efficacy, Mechanisms, and Safety," *Front. Pharmacol.*, vol. 11, p. 422, Apr. 2020, doi: 10.3389/fphar.2020.00422.

[4] K. Sawicka, M. Szczyrek, I. Jastrzębska, M. Prasał, A. Zwolak, and J. Daniluk, "Hypertension – The Silent Killer," vol. 5, no. 2, 2011.

[5] K. Kario, A. Okura, S. Hoshide, and M. Mogi, "The WHO Global report 2023 on hypertension warning the emerging hypertension burden in globe and its treatment strategy," *Hypertens Res*, vol. 47, no. 5, pp. 1099–1102, May 2024, doi: 10.1038/s41440-024-01622-w.

[6] Jose Martinez-Gonzalez and Lina Badimon, "Mechanisms Underlying the Cardiovascular Effects of COX-Inhibition: Benefits and Risks," *CPD*, vol. 13, no. 22, pp. 2215–2227, Aug. 2007, doi: 10.2174/138161207781368774.

[7] K. R. Acharya, E. D. Sturrock, J. F. Riordan, and M. R. W. Ehlers, "Ace revisited: A new target for structure-based drug design," *Nat Rev Drug Discov*, vol. 2, no. 11, pp. 891–902, Nov. 2003, doi: 10.1038/nrd1227.

[8] W. Guo, T. Shao, Y. Peng, H. Wang, Z.-S. Chen, and H. Su, "Chemical composition, biological activities, and quality standards of hawthorn leaves used in traditional Chinese medicine: a comprehensive review," *Front. Pharmacol.*, vol. 14, p. 1275244, Oct. 2023, doi: 10.3389/fphar.2023.1275244.

[9] P. R. Venskutonis, "Phytochemical composition and bioactivities of hawthorn (*Crataegus* spp.): review of recent research advances," *Journal of Food Bioactives*, pp. 69–87, Dec. 2018, doi: 10.31665/JFB.2018.4163.

[10] S. M. A. Shahid *et al.*, "ANALYSIS OF BINDING PROPERTIES OF ANGIOTENSIN-CONVERTING ENZYME 2 THROUGH IN SILICO MOLECULAR DOCKING".

[11] D. Mozaffarian *et al.*, "Heart Disease and Stroke Statistics—2015 Update".

[12] W. Frąk, A. Wojtasińska, W. Lisińska, E. Młynarska, B. Franczyk, and J. Rysz, "Pathophysiology of Cardiovascular Diseases: New Insights into Molecular Mechanisms of Atherosclerosis, Arterial Hypertension, and Coronary Artery Disease," *Biomedicines*, vol. 10, no. 8, p. 1938, Aug. 2022, doi: 10.3390/biomedicines10081938.

[13] J. Scott, "Pathophysiology and biochemistry of cardiovascular disease," *Current Opinion in Genetics & Development*, vol. 14, no. 3, pp. 271–279, Jun. 2004, doi: 10.1016/j.gde.2004.04.012.

[14] T. Vos *et al.*, "Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019," *The Lancet*, vol. 396, no. 10258, pp. 1204–1222, Oct. 2020, doi: 10.1016/S0140-6736(20)30925-9.

[15] "The heart in HTN.pdf."

[16] S. Oparil, M. A. Zaman, and D. A. Calhoun, "Pathogenesis of Hypertension".

[17] P. Minuz *et al.*, "Telemedicine and Digital Medicine in the Clinical Management of Hypertension and Hypertension-Related Cardiovascular Diseases: A Position Paper of the Italian Society of Arterial Hypertension (SIIA)," *High Blood Press Cardiovasc Prev*, vol. 30, no. 5, pp. 387–399, Aug. 2023, doi: 10.1007/s40292-023-00595-0.

[18] G. Tocci *et al.*, "Blood pressure levels and control in Italy: comprehensive analysis of clinical data from 2000–2005 and 2005–2011 hypertension surveys," *J Hum Hypertens*, vol. 29, no. 11, pp. 696–701, Nov. 2015, doi: 10.1038/jhh.2015.4.

[19] C. Fiuza-Luces *et al.*, "Exercise benefits in cardiovascular disease: beyond attenuation of traditional risk factors," *Nat Rev Cardiol*, vol. 15, no. 12, pp. 731–743, Dec. 2018, doi: 10.1038/s41569-018-0065-1.

[20] L. Lauder *et al.*, "Hypertension management in patients with cardiovascular comorbidities," *European Heart Journal*, vol. 44, no. 23, pp. 2066–2077, Jun. 2023, doi: 10.1093/eurheartj/ehac395.

[21] L. Te Riet, J. H. M. Van Esch, A. J. M. Roks, A. H. Van Den Meiracker, and A. H. J. Danser, "Hypertension: Renin–Angiotensin–Aldosterone System Alterations," *Circulation Research*, vol. 116, no. 6, pp. 960–975, Mar. 2015, doi: 10.1161/CIRCRESAHA.116.303587.

[22] W. W. Batenburg and A. H. Jan Danser, "The (pro)renin receptor: A new addition to the renin–angiotensin system?," *European Journal of Pharmacology*, vol. 585, no. 2–3, pp. 320–324, May 2008, doi: 10.1016/j.ejphar.2008.02.092.

[23] G. L. Schwartz and S. T. Turner, "Pharmacogenetics of Antihypertensive Drug Responses:," *American Journal of PharmacoGenomics*, vol. 4, no. 3, pp. 151–160, 2004, doi: 10.2165/00129785-200404030-00002.

[24] S. A. Atlas, "The Renin-Angiotensin Aldosterone System: Pathophysiological Role and Pharmacologic Inhibition," *JMCP*, vol. 13, no. 8 Supp B, pp. 9–20, Oct. 2007, doi: 10.18553/jmcp.2007.13.s8-b.9.

[25] S. Patel, A. Rauf, H. Khan, and T. Abu-Izneid, "Renin-angiotensin-aldosterone (RAAS): The ubiquitous system for homeostasis and pathologies," *Biomedicine & Pharmacotherapy*, vol. 94, pp. 317–325, Oct. 2017, doi: 10.1016/j.biopha.2017.07.091.

[26] N. R. Pugliese, S. Masi, and S. Taddei, "The renin-angiotensin-aldosterone system: a crossroad from arterial hypertension to heart failure," *Heart Fail Rev*, vol. 25, no. 1, pp. 31–42, Jan. 2020, doi: 10.1007/s10741-019-09855-5.

[27] I. Hamming *et al.*, "The emerging role of ACE2 in physiology and disease," *The Journal of Pathology*, vol. 212, no. 1, pp. 1–11, May 2007, doi: 10.1002/path.2162.

[28] D. Szklarczyk *et al.*, "The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest," *Nucleic Acids Research*, vol. 51, no. D1, pp. D638–D646, Jan. 2023, doi: 10.1093/nar/gkac1000.

[29] F. H. Messerli, S. Bangalore, C. Bavishi, and S. F. Rimoldi, "Angiotensin-Converting Enzyme Inhibitors in Hypertension," *Journal of the American College of Cardiology*, vol. 71, no. 13, pp. 1474–1482, Apr. 2018, doi: 10.1016/j.jacc.2018.01.058.

[30] Mil. osz Regulski *et al.*, "Chemistry and Pharmacology of Angiotensin-Converting Enzyme Inhibitors," *CPD*, vol. 21, no. 13, pp. 1764–1775, Mar. 2015, doi: 10.2174/1381612820666141112160013.

[31] M. Gillespie *et al.*, "The reactome pathway knowledgebase 2022," *Nucleic Acids Research*, vol. 50, no. D1, pp. D687–D692, Jan. 2022, doi: 10.1093/nar/gkab1028.

[32] J. Ding *et al.*, "Exploring the Mechanism of Hawthorn Leaves Against Coronary Heart Disease Using Network Pharmacology and Molecular Docking," *Front. Cardiovasc. Med.*, vol. 9, p. 804801, Jun. 2022, doi: 10.3389/fcvm.2022.804801.

[33] Q. Chang, Z. Zuo, F. Harrison, and M. S. S. Chow, "Hawthorn," *The Journal of Clinical Pharma*, vol. 42, no. 6, pp. 605–612, Jun. 2002, doi: 10.1177/00970002042006003.

[34] J. M. Rigelsky and B. V. Sweet, "Hawthorn: Pharmacology and therapeutic uses," *American Journal of Health-System Pharmacy*, vol. 59, no. 5, pp. 417–422, Mar. 2002, doi: 10.1093/ajhp/59.5.417.

[35] M. Yavuz, F. Ç. Çelikezen, M. Firat, Z. Baş, and V. Türkoğlu, "The investigation of hawthorn (*Crataegus orientalis*) plant's inhibition effect on angiotensin converting enzyme and *in silico* studies," *Natural Product Research*, vol. 39, no. 11, pp. 3079–3085, Jun. 2025, doi: 10.1080/14786419.2024.2324467.

[36] P.-G. Pietta, "Flavonoids as Antioxidants," *J. Nat. Prod.*, vol. 63, no. 7, pp. 1035–1042, Jul. 2000, doi: 10.1021/np9904509.

[37] P.-G. Pietta, "Flavonoids as Antioxidants," *J. Nat. Prod.*, vol. 63, no. 7, pp. 1035–1042, Jul. 2000, doi: 10.1021/np9904509.

[38] R. Hasan and R. Herowati, "Molecular Docking and Pharmacokinetic Studies of Moringa oleifera As Angiotensin-Converting Enzyme Inhibitors," *JFIKI*, vol. 11, no. 1, pp. 80–88, Apr. 2024, doi: 10.20473/jfiki.v11i12024.80-88.

[39] S. M. A. Shahid *et al.*, "ANALYSIS OF BINDING PROPERTIES OF ANGIOTENSIN-CONVERTING ENZYME 2 THROUGH IN SILICO MOLECULAR DOCKING".

[40] S. Kim *et al.*, "PubChem 2025 update," *Nucleic Acids Research*, vol. 53, no. D1, pp. D1516–D1525, Jan. 2025, doi: 10.1093/nar/gkae1059.

[41] A. Daina, O. Michielin, and V. Zoete, "SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules," *Sci Rep*, vol. 7, no. 1, p. 42717, Mar. 2017, doi: 10.1038/srep42717.

[42] D. E. V. Pires, T. L. Blundell, and D. B. Ascher, "pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures," *J. Med. Chem.*, vol. 58, no. 9, pp. 4066–4072, May 2015, doi: 10.1021/acs.jmedchem.5b00104.

[43] M. A. Miteva, F. Guyon, and P. Tuffery, "Frog2: Efficient 3D conformation ensemble generator for small compounds," *Nucleic Acids Research*, vol. 38, no. Web Server, pp. W622–W627, Jul. 2010, doi: 10.1093/nar/gkq325.

[44] N. Guex and M. C. Peitsch, "SWISS-MODEL and the Swiss-Pdb Viewer: An environment for comparative protein modeling," *Electrophoresis*, vol. 18, no. 15, pp. 2714–2723, Jan. 1997, doi: 10.1002/elps.1150181505.

[45] S. Dallakyan and A. J. Olson, "Small-Molecule Library Screening by Docking with PyRx," in *Chemical Biology*, vol. 1263, J. E. Hempel, C. H. Williams, and C. C. Hong, Eds., in Methods in Molecular Biology, vol. 1263. , New York, NY: Springer New York, 2015, pp. 243–250. doi: 10.1007/978-1-4939-2269-7_19.

[46] "D. Biovia, H. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. Bhat, T.J.T.J.o.C.P. Richmond, Dassault Systèmes BIOVIA, Discovery Studio Visualizer, v. 17.2, San Diego: Dassault Systèmes, 2016, 10 (2000) 0021-9991.".