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In silico Investigations on Wound Healing Activity, Toxicity and Pharmacokinetic Profiles of *Cotinus Coggygria* Plant Biomolecules

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Abstract

Cotinus coggygria, commonly known as the "smoke tree," has been reported to exert positive effects on wound healing processes. This study aimed to evaluate the toxicity and pharmacokinetic properties of selected bioactive compounds, Gallic acid, Myricetin, Quercetin, Fisetin, Sulfuretin, Butin, and Taxifolin, present in the leaf extracts of *C. coggygria*, using comprehensive *in silico* approaches. Additionally, the study sought to identify the compounds with the most promising wound-healing potential. Toxicological assessments were conducted using computational modeling tools, while pharmacokinetic profiles were predicted based on structure–activity relationships (SAR). Molecular docking analyses were carried out to evaluate the interactions of the compounds with Transforming Growth Factor-beta (TGF-β), a key protein involved in the wound healing pathway. The results showed that Gallic acid, Butin, and Taxifolin exhibited the highest predicted LD_{50} values (~2000 mg/kg), suggesting a low toxicity profile. Among the investigated compounds, Butin, Sulfuretin, and Fisetin displayed the strongest binding affinities to the TGF-β protein, with docking scores of −6.12, −5.58, and −5.50 kcal/mol, respectively. These interactions indicate their potential contributions to the wound-healing effects attributed to *C. coggygria*. Overall, the findings provide valuable preliminary insights into the pharmacological potential of *C. coggygria* leaf-derived compounds and support their further investigation as candidates for wound-healing applications in biomedical research and drug development.

Keywords

Cotinus coggygria, Wound-healing, *In silico* predictions, ADMET, Molecular docking

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Cotinus Coggygria Bitki Biyomoleküllerinin Yara İyileştirme Aktivitesi, Toksisitesi ve Farmakokinetik Profilleri Üzerine Bilgisayar Destekli Araştırmalar

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Öz

Yaygın olarak "duman ağacı" olarak bilinen *Cotinus coggygria*'nın yara iyileşme süreçleri üzerinde olumlu etkileri olduğu bildirilmiştir. Bu çalışma, kapsamlı *in silico* yaklaşımlar kullanarak *C. coggygria* yaprak özütlerinde bulunan seçili biyoaktif bileşiklerin (Galik asit, Mirisetin, Kuersetin, Fisetin, Sülfüretin, Butin ve Taksifolin) toksisitesini ve farmakokinetik özelliklerini değerlendirmeyi amaçlamıştır. Ayrıca, çalışma en umut verici yara iyileştirme potansiyeline sahip bileşikleri belirlemeyi amaçlamıştır. Toksikolojik değerlendirmeler hesaplamalı modelleme araçları kullanılarak yürütülürken, farmakokinetik profiller yapı-aktivite ilişkilerine (SAR) dayanarak tahmin edilmiştir. Bileşiklerin yara iyileşme yolunda rol oynayan önemli bir protein olan Dönüştürücü Büyüme Faktörü-beta (TGF-β) ile etkileşimlerini değerlendirmek için moleküler yerleştirme analizleri gerçekleştirilmiştir. Sonuçlar, Gallik asit, Butin ve Taksifolinin en yüksek öngörülen LD_{50} değerlerini (~2000 mg/kg) gösterdiğini ve bunun düşük bir toksisite profiline işaret ettiğini göstermiştir. İncelenen bileşikler arasında Butin, Sülfüretin ve Fisetin, sırasıyla -6,12, -5,58 ve -5,50 kcal/mol yerleştirme puanlarıyla TGF-β proteinine en güçlü bağlanma afinitelerini göstermiştir. Bu etkileşimler, *C. coggygria*'ya atfedilen yara iyileştirici etkilere potansiyel katkılarını göstermektedir. Genel olarak, bulgular *C. coggygria* yaprağından elde edilen bileşiklerin farmakolojik potansiyeli hakkında değerli ön bilgiler sunmakta ve biyomedikal araştırma ve ilaç geliştirmede yara iyileştirici uygulamalar için aday olarak daha fazla araştırma yapılmasını desteklemektedir.

Anahtar kelimeler

Cotinus coggygria,
Yara iyileşmesi,
In silico tahminler,
ADMET,
Moleküler doking

1. INTRODUCTION

Cotinus coggygria Scop., also known as *Rhus cotinus L*., is a slow-growing deciduous shrub belonging to the Anacardiaceae family. This family is predominantly distributed throughout tropical regions of Asia, Africa, and the Americas, with several species extending into subtropical and temperate zones. In traditional Turkish medicine, infusions prepared from the leaves of *C. coggygria* have long been used for their therapeutic properties, including wound healing, hepatoprotective, antiviral, anti-inflammatory, antiseptic, cytotoxic, antioxidant, antihemorrhagic, and antibacterial activities, as supported by both *in vivo* and *in vitro* studies [1]. A number of pharmacological studies have reported the anticancer potential of *C. coggygria*. Extracts derived from various plant parts, especially from specimens found in Serbia, Italy, and Turkey, have shown notable cytotoxic and cytostatic effects against a variety of human cancer cell lines, including B lymphoblastoid, cervical, colon, breast, and lung cancers [2]. Phytochemical investigations have revealed that *C. coggygria* contains a rich diversity of bioactive constituents, particularly flavonoids (e.g., fisetin, sulfuretin, fustin, quercetin, myricetin), tannins, and phenolic compounds. Additional identified compounds include anthocyanins (e.g., leucodelphinidin, cyanidin 3-galactoside, delphinidin 7-glucoside), essential oils ((Z)-β-ocimene, limonene, (E)-β-ocimene), methyl gallate, gallic acid, biauron, and pentagalloylglucose [3]. The chemical structures of some of the major bioactive compounds present in *C. coggygria* leaves are illustrated in Figure 1.

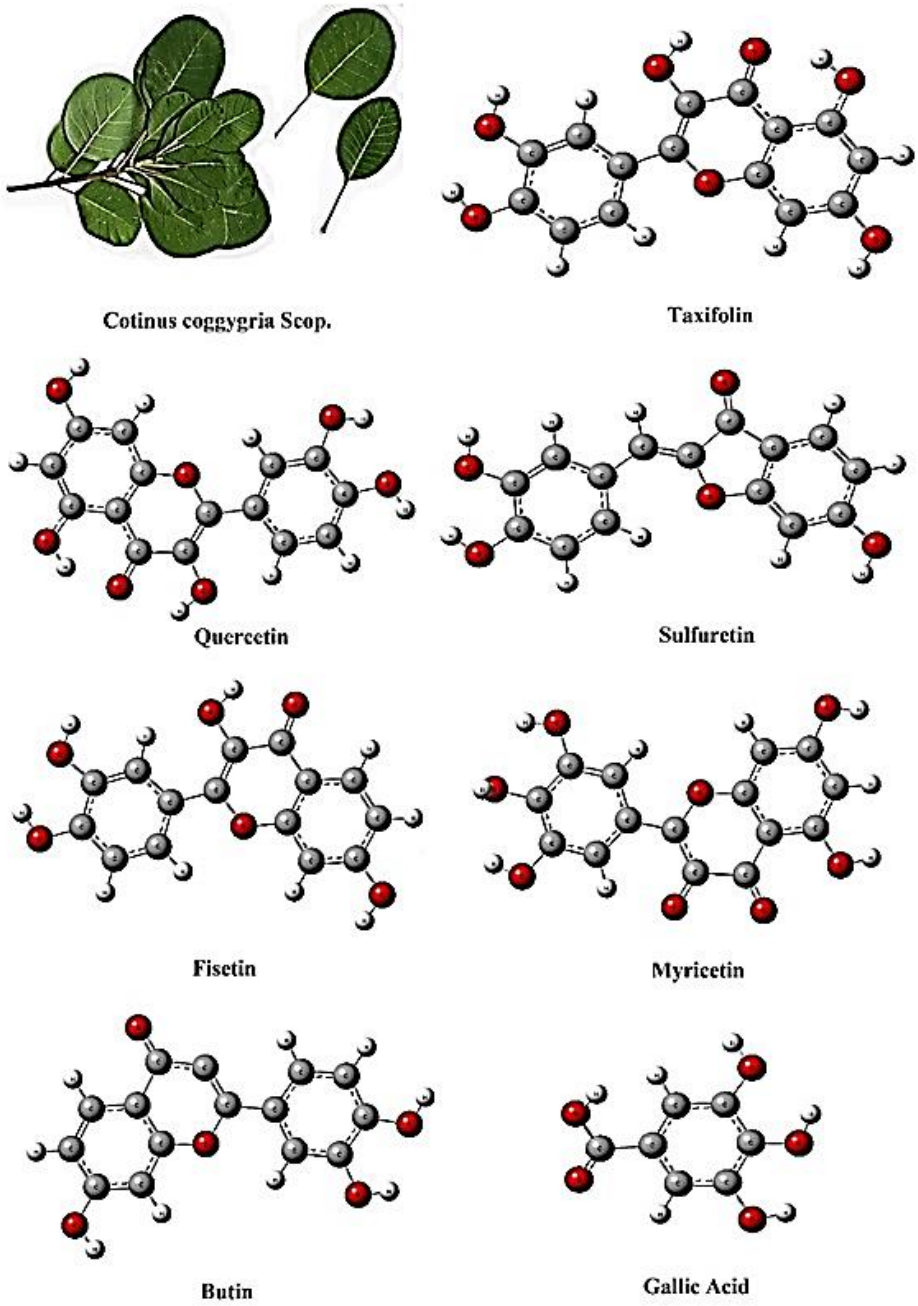

Figure 1. Chemical structures of bioactive molecules isolated from the leaves of the *Cotinus coggygria Scop*. plant. The figure illustrates key phytochemical constituents, including flavonoids, tannins, and other secondary metabolites, which are known to contribute to the plant's reported pharmacological properties such as antioxidant, anti-inflammatory, and antimicrobial activities

The skin, as the body's largest and most externally exposed organ, performs essential physiological functions such as thermoregulation, immune surveillance, sensory perception, and protection against environmental insults including ultraviolet radiation and pathogens [4]. Due to its constant exposure, the skin is especially susceptible to physical injury [5]. When the integrity of the skin barrier is disrupted, wounds can develop, often leading to microbial invasion that compromises both the surface and deeper tissues, increasing the risk of infection [6]. Wound healing is a complex and dynamic biological process aimed at restoring the structural and functional integrity of damaged tissue. This process involves multiple overlapping and tightly regulated stages, including hemostasis, inflammation, proliferation, and remodeling. Various cell types contribute to these stages, including inflammatory cells (e.g., neutrophils, macrophages), fibroblasts, keratinocytes, and epithelial cells, all of which play a central role in re-establishing tissue homeostasis [7,8]. Despite significant progress in pharmaceutical development, impaired or delayed wound healing remains a major clinical challenge. According to the World Health Organization, ineffective wound management contributes to nearly five million deaths annually worldwide [9]. Although numerous synthetic agents have been developed to facilitate wound healing, many are associated with undesirable side effects or limited long-term efficacy. As a result, interest in plant-based therapeutic alternatives has increased substantially in recent years. Experimental evidence suggests that natural products derived from medicinal plants can enhance various phases of wound repair. Specifically, plant-derived antioxidants have been shown to modulate keratinocyte activity, promote collagen synthesis, and support extracellular matrix (ECM) remodeling, key events in the wound healing cascade [10]. These findings support the potential of phytocompounds as effective agents in managing skin injuries. Given the traditional usage and rich phytochemical profile of *C. coggygria*, the present study aims to evaluate the toxicity parameters, ADME (Absorption, Distribution, Metabolism, and Excretion) profiles, and wound-healing potential of selected bioactive compounds found in its leaves using *in silico* approaches. To this end, the molecular structures of quercetin, fisetin, myricetin, gallic acid, sulfuretin, butin, and taxifolin, among the most abundant constituents, were first optimized using Density Functional Theory (DFT). Based on these optimized structures, ADME and toxicity profiles were predicted computationally. Furthermore, molecular docking analyses were conducted to assess the interaction of these compounds with transforming growth factor beta (TGF-β), a multifunctional cytokine from the TGF superfamily that plays a key role in re-epithelialization, inflammation, angiogenesis, and granulation tissue formation during wound healing [11].

2. MATERIAL AND METHOD

In silico studies are a valuable source of preliminary data before *in vitro* and *in vivo* studies. In this study, the wound-healing potential, toxicity profiles, and pharmacokinetic properties of molecules found in *C. coggygria* were conducted using *in silico* techniques. The DFT method at the 6-311++G(d,p) level was used to obtain optimized structures. The calculations were made using the Gaussian09 software package [12] on high-performance computing systems. The 3D structures used in optimizations were taken from the PubChem chemical database (https://pubchem.ncbi.nlm.nih.gov/). The optimized structures were subsequently used in the ProTox-III (version 3.0) algorithm to predict the toxicities of the compounds [13]. The PreADMET online tool was utilized to determine the ADME parameters of the compounds [14]. The 3D structure of the TGF-β Receptor Type I Kinase Domain (PDB ID: 6B8Y) was retrieved from the RCSB Protein Data Bank, and the protein was prepared using the BIOVIA Discovery Studio Visualizer software (version 24). Molecular docking simulations were performed with AutoDock Tools (version 1.5.7) to determine the binding affinities between the protein and the ligands. Furthermore, the tool BIOVIA Discovery Studio Visualizer was used to analyze the 3D interactions between ligands and amino acid residues within the binding site.

3. RESULTS AND DISCUSSION

3.1. Pharmacokinetic Profiles and Drug-Likeness of Compounds

The drug-likeness parameter assesses the structural and physicochemical similarities between newly investigated compounds and approved pharmaceutical agents. It provides preliminary insights into whether a compound has the potential to become a viable drug candidate. This evaluation is primarily based on a comparison of the functional groups and molecular frameworks of known drugs with those of the test compounds. The information derived from such analyses enables highly accurate predictions regarding the likelihood that a compound may exhibit drug-like behavior. Accordingly, the probability of a compound being further developed into a therapeutic agent can be estimated. Drug-likeness is typically evaluated using a set of well-established rules, including those proposed by Lipinski's Rule of Five [15] and Ghose's criteria [16], which define the molecular features essential for favorable pharmacokinetic behavior in the human body.

Table 1 summarizes the drug-likeness evaluations and key physicochemical properties of the compounds identified in *C. coggygria* leaf extracts.

Table 1. Druglikeness profiles and key physicochemical parameters of the selected compounds. The table summarizes essential molecular descriptors including molecular weight, lipophilicity (LogP), hydrogen bond donors and acceptors, topological polar surface area (TPSA), and compliance with drug-likeness rules such as Lipinski's Rule of Five. These parameters provide insight into the compounds' potential oral bioavailability and pharmacokinetic behavior

Parameter	**Taxifolin**	**Quercetin**	**Sulfuretin**	**Fisetin**
Formula	$C_{15}H_{12}O_7$	$C_{15}H_{10}O_7$	$C_{15}H_{10}O_5$	$C_{15}H_{10}O_6$
Molecular weight (g/mol)	304.25	302.24	270.24	286.24
Num. heavy atoms	22	22	20	21
Num. aromatic heavy atoms	12	16	12	16
Num. H-bond acceptors	7	7	5	6
Num. H-bond donors	5	5	3	4
Molar Refractivity	127.45	78.04	71.89	76.01
Druglikeness				
Lipinski Rules	OK	OK	OK	OK
Ghose Rules	OK	OK	OK	OK
Bio. Score	0.55	0.55	0.55	0.55
Parameter	**Myricetin**	**Butin**	**Gallic Acid**	
Formula	$C_{15}H_{10}O_8$	$C_{15}H_{12}O_5$	$C_7H_6O_5$	-
Molecular weight (g/mol)	318.24	272.25	170.12	-
Num. heavy atoms	23	20	12	-
Num. aromatic heavy atoms	16	12	6	-
Num. H-bond acceptors	8	5	5	---
Num. H-bond donors	6	3	4	-
Molar Refractivity	80.06	71.57	39.47	-
Druglikeness				
Lipinski Rules	Yes; 1 violation OH>5	OK	OK	-
Ghose Rules	OK	OK	No; 2 violations MR<40 atoms<20	-
Bio. Score	0.55	0.55	0.56	-

Studies on currently used antibiotics, vitamins, antipyretics, and other drugs have demonstrated that these compounds generally share certain common physicochemical properties. These characteristics are encapsulated in Lipinski's Rule of Five, which states that drug-like molecules typically have: hydrogen bond donors ≤ 5, hydrogen bond acceptors ≤ 10, molecular weight ≤ 500 g/mol, and log P ≤ 5 [17].

As shown in Table 5, all compounds except myricetin comply with Lipinski's criteria. Myricetin exhibits only a single violation, exceeding the limit for hydrogen bond donors by one. Given that it satisfies all other conditions, myricetin can still be considered to possess favorable drug-like properties. Furthermore, Ghose et al. analyzed 6,304 compounds from the Comprehensive Medicinal Chemistry Database and proposed a set of physicochemical property ranges that characterize drug-likeness in over 80% of the compounds studied. These ranges include: log P between -0.4 and 5.6, molar refractivity between 40 and 130, molecular weight between 160 and 480 g/mol, and atomic count between 20 and 70. Based on these criteria, gallic acid is the only compound in the present study that violates two parameters molar refractivity and atomic count suggesting a comparatively lower drug potential relative to the other compounds analyzed.

Another critical parameter in drug analysis is the evaluation of ADME profiles. An effective drug candidate must possess several desirable features. Pharmacokinetic (PK) properties are essential not only for the efficacy of a compound but also for its safety. The term "pharmacokinetics" refers to the characteristics of ADME that determine how a drug is absorbed, distributed, metabolized, and ultimately eliminated from the body [18]. Figure 2 schematically illustrates the ADME processes of a drug within the human body.

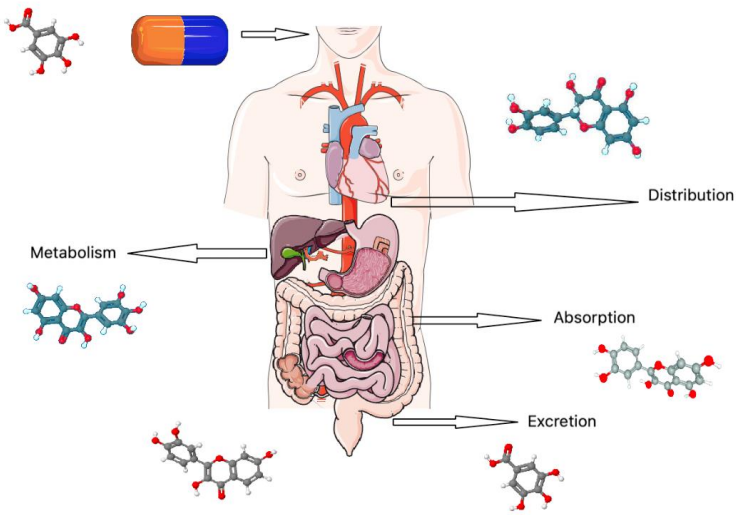

Figure 2. Schematic representation of the ADME processes (Absorption, Distribution, Metabolism, and Excretion) of a drug within the human body. This figure illustrates the pharmacokinetic journey of a compound after administration, including its absorption into systemic circulation, distribution across tissues and organs, metabolic transformation (primarily in the liver), and eventual excretion through renal or biliary pathways. Understanding these processes is critical for evaluating the drug's bioavailability, efficacy, and potential toxicity.

The ADME properties of a drug are significantly influenced by various physicochemical and biological factors, including apparent permeability, cell type, transporter expression levels, compound concentration, penetration direction, pH, and the presence of inhibitors. Table 2 presents the predicted ADME profiles for taxifolin, quercetin, sulfuretin, fisetin, myricetin, butin, and gallic acid.

Table 2. In silico predicted ADME (Absorption, Distribution, Metabolism, and Excretion) profiles of selected phytochemicals: taxifolin, quercetin, sulfuretin, fisetin, myricetin, butin, and gallic acid. The table presents key pharmacokinetic parameters such as gastrointestinal absorption, blood–brain barrier (BBB) permeability, and bioavailability scores. These predictions provide insight into the compounds' drug-likeness and their potential suitability for further development as orally active therapeutic agents

ID	Taxifolin	Quercetin	Sulfuretin	Fisetin
BBB (C_{brain}/C_{blood})	0.17	0.17	0.16	0.32
Caco-2 (nm/sec)	3.42	3.41	13.37	9.58
MDCK (nm/sec)	9.57	13.35	84.69	68.19
HIA (%)	60.16	63.49	89.55	79.43
Plasma Protein Bind. (%)	95.16	93.24	100.00	88.73
Pure water sol. (g/L)	0.24	0.10	0.01	0.06
Skin perm. (cm/hour)	-4.43	-4.43	-4.19	-4.33
ID	Myricetin	Butin	Gallic acid	
BBB (C_{brain}/C_{blood})	0.11	0.66	0.35	-
Caco-2 (nm/sec)	0.99	10.52	13.85	-
MDCK (nm/sec)	4.88	55.40	9.54	-
HIA (%)	40.96	87.31	53.70	
Plasma Protein Bind. (%)	96.79	100.00	65.39	-
Pure water sol. (g/L)	0.43	0.13	72.33	-
Skin perm. (cm/hour)	-4.53	-4.19	-3.63	-

The blood-brain barrier's permeability (BBB) is a critical determinant in assessing a drug's potential ability to penetrate the central nervous system (CNS). This parameter is crucial for minimizing undesired CNS-related side effects [19]. Molecules with a C_{brain}/C_{blood} value of 2 or above can penetrate the CNS effectively. BBB values below 0.1 indicate low absorption, and those between 0.1 and 2.0 indicate moderate absorption [20]. As seen in the table, the BBB parameters of the molecules have middle permeability on CNS with 0.1-2.0 C_{brain}/C_{blood} values. Caco-2 and MDCK cell models are widely used in vitro systems developed to predict oral drug absorption [21]. The most popular cell lines for measuring apparent permeability are Caco-2 cell lines, derived from human colon cells and mainly used to evaluate the intestinal absorption of given drugs. *In silico* models have been created to predict the apparent permeability observed in Caco-2 cells [22]. The predicted intestine absorption rate of gallic acid was determined to be 13.85 nm/sec, the molecule with the highest absorption between investigated compounds. With values of 13.37 and 10.52 nm/sec, respectively, sulfuretin and butin were the second and third molecules with the highest intestine absorption. The MDCK refers to the Madin-Darby canine kidney cell line. MDCK cells are commonly used in early drug discovery despite their nonhuman and nonintestinal origin because of a much quicker differentiation period (3-4 days), durability, and accurate prediction of human intestinal absorption, as demonstrated in multiple studies [23]. Both parameters estimate the substance transfer in nanomoles/second. The highest absorption from kidney cells occurs for sulfuretin and fisetin molecules, while Caco2 absorption is highest for sulfuretin and gallic acid.

Another critical parameter is the estimation of human intestinal absorption (HIA), which is a critical step in developing new pharmacological compounds [24]. The highest HIA value belongs to sulfuretin. It was predicted to be 89.55 percent. After sulfuretin, Butin (87.31 percent) and Fisetin (79.43 percent) were found to have the highest absorption percentage. One of the most important characteristics of pharmacokinetics is the binding of pharmaceuticals to plasma proteins, which can impact various critical pharmacological features, including clearance rate, therapeutic index, drug-drug interaction, and distribution volume. A drug's binding to plasma protein, attribute directly influences its toxicity, distribution, metabolism, excretion, and absorption. For this reason, plasma protein binding ratio (PPB) evaluation is crucial to both the safe administration of existing medications and the creation of novel ones [25]. In addition, for a drug to show its full effectiveness, it should be found free in plasma. It is seen from Table 2 that sulfuretin and butin have the highest plasma protein binding percentage, at 100 %. The most abundant molecule in plasma nonbonding is gallic acid. Poor solubility is a major challenge in drug development and is closely related to target selection. Since solubility is key to absorption, achieving therapeutic blood concentrations for systemic effects requires adequate solubility in intestinal fluids. A compound's solubility significantly affects its ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profile [26]. The results show that gallic acid has high water solubility, while the dissolution rates of other molecules in pure water are quite low. Considering that almost 70% of the human body consists of water, this result also shows that gallic acid would be more suitable for designing as an oral drug than other studied molecules. The last important parameter in the table is skin permeability. Skin permeability is a parameter that should be considered, especially for drugs that need to be applied to the skin, such as creams and ointments. It refers to the progress of the drug through the epidermis and dermis layers in cm/hour. The *in silico* predicted results show that molecules other than gallic acid have excellent skin permeability values and can be easily used in a cream-like drug form.

3.2. Toxicity Profiles of the Compounds

Unlike in vitro/in vivo approaches, which are often limited by ethical concerns, time, budget, and other resource constraints, *in silico* toxicity prediction plays a significant role in regulatory decision-making and the efficient selection of lead compounds in drug design [27]. *in silico* predictions are widely used in toxicity studies and rely on data from chemicals whose toxicological profiles have already been determined [28]. It generally utilizes a range of computational techniques that establish a relationship between a chemical's structure and its efficacy or toxicity. The toxicity of various substances, including mixtures, nanomaterials, and chemicals, can be predicted both quantitatively and qualitatively thanks to computational approaches [29].

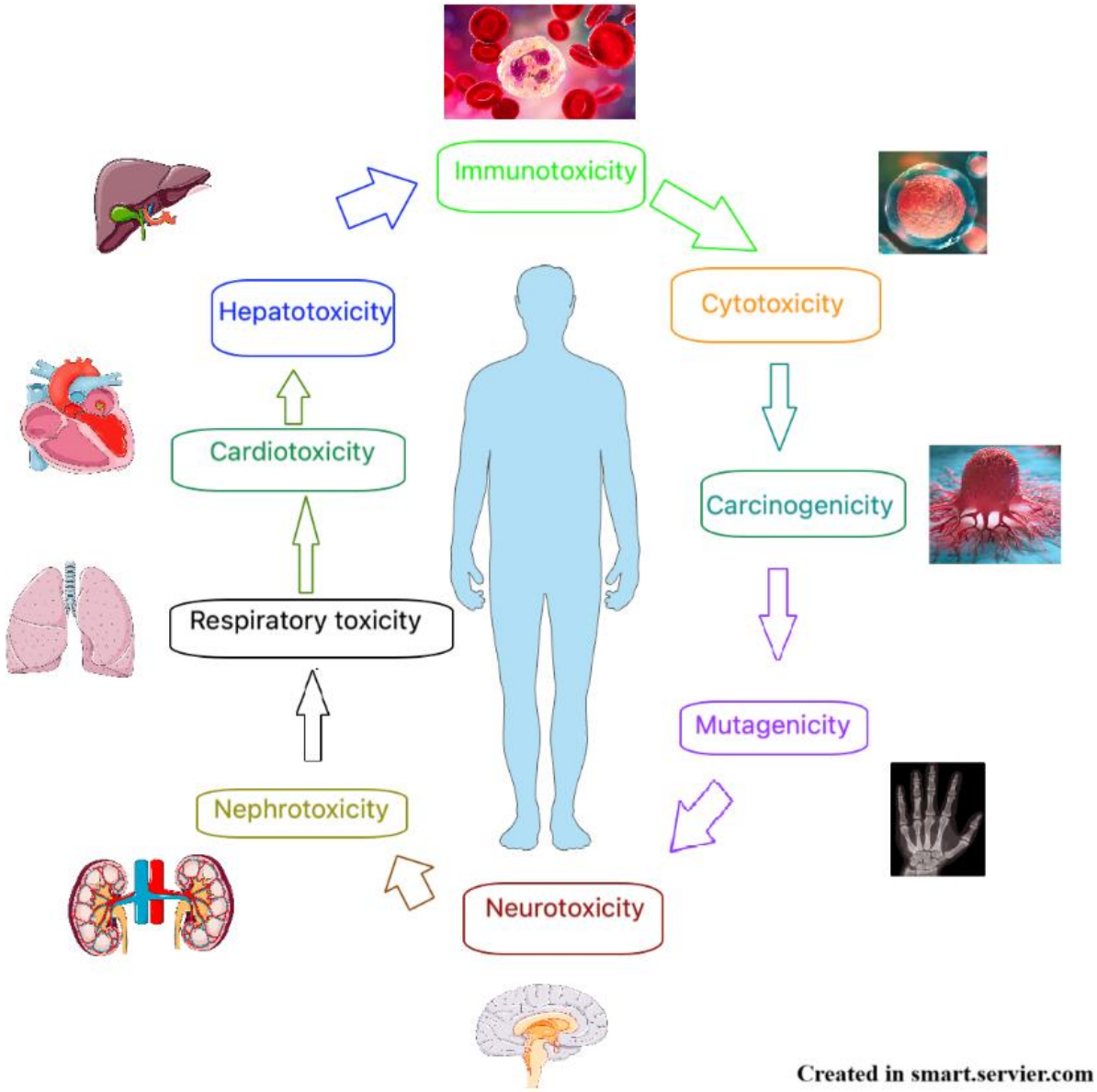

Figure 3. Summary of predicted organ-specific toxicities and key toxicity endpoints associated with the selected compounds. The figure highlights potential adverse effects on major organs such as the liver, kidneys, heart, and central nervous system, along with toxicity endpoints including hepatotoxicity, cardiotoxicity, carcinogenicity, mutagenicity, and cytotoxicity. These predictions, derived from *in silico* models, provide early insight into the safety profiles of the compounds prior to experimental validation

In silico-determinable organ toxicity and toxicity endpoints are shown in Figure 3. As can be seen from the figure, living beings can be exposed to different types of toxicity under the threat of many toxic factors originating from external physical effects. These effects vary from species to species and are dose-dependent. The dose is a very decisive and essential concept in toxicity studies, and the median lethal dose (lethal dose 50; LD50) is used in reporting the results of the study, which is equivalent to the dose that causes death in half of the individuals in the studied population [30]. The toxicity decreases as the LD50 value increases. Toxicity predictions for molecules obtained from *C. coggygria* are given in Table 3.

Table 3. *In silico* predicted toxicity parameters of the selected compounds based on computational toxicity models. The table includes key endpoints such as LD_{50} values, hepatotoxicity, carcinogenicity, mutagenicity, immunotoxicity, and potential for skin or reproductive toxicity. These predictions help assess the preliminary safety profiles of the compounds and guide their suitability for further preclinical development

ID	**Taxifolin**		**Quercetin**		**Sulfuretin**	
Predict LD_{50} (mg/kg)	2000		159		500	
Predict Tox. Class	4	harmful if swall.	3	toxic if swall.	4	harmful i if swall.
	Probab.	Predict.	Probab.	Predict.	Probab.	Predict.
Hepatotox.	0.69	Inactive	Inactive	0.69	Inactive	0.58
Immunotox.	0.76	Inactive	Inactive	0.89	**Active**	0.85
Cytotox.	0.99	Inactive	Inactive	0.99	Inactive	0.88
Carcinogen.	0.68	**Active**	**Active**	0.68	**Active**	0.71
Mutagen.	0.51	**Active**	**Active**	0.51	**Active**	0.61
Neurotox.	0.89	Inactive	Inactive	0.89	Inactive	0.87
Nephrotox.	0.62	**Active**	**Active**	0.62	**Active**	0.50
Respiratory tox.	0.83	**Active**	**Active**	0.83	**Active**	0.72
Cardiotox.	0.99	Inactive	Inactive	0.99	Inactive	0.91
ID	**Fisetin**		**Myricetin**			
Predict LD_{50} (mg/kg)	159		159			
Predict Tox. Class	3	toxic if swall.	3	toxic if swall.		
	Probab.	Predict.	Probab.	Predict.		
Hepatotox.	0.7	Inactive	0.69	Inactive		
Immunotox.	0.51	Inactive	0.86	Inactive		
Cytotox.	0.98	Inactive	0.99	Inactive		
Carcinogen.	0.71	**Active**	0.68	**Active**		
Mutagen.	0.53	Inactive	0.51	**Active**		
Neurotox.	0.88	Inactive	0.89	Inactive		
Nephrotox.	0.57	**Active**	0.62	**Active**		
Respiratory tox.	0.82	**Active**	0.83	**Active**		
Cardiotox.	0.93	Inactive	0.99	Inactive		
ID	**Butin**		**Gallic acid**			
Predict LD_{50} (mg/kg)	2000		2000			
Predict Tox. Class	4	harmful if swall.	4	harmful if swall.		
	Probab.	Predict.	Probab.	Predict.		
Hepatotox.	Inactive	0.65	0.61	Inactive		
Immunotox.	Inactive	0.69	0.99	Inactive		
Cytotox.	Inactive	0.89	0.91	Inactive		
Carcinogen.	**Active**	0.66	0.56	**Active**		
Mutagen.	Inactive	0.56	0.94	Inactive		
Neurotox.	Inactive	0.88	0.88	Inactive		
Nephrotox.	**Active**	0.58	0.69	**Active**		
Respiratory tox.	**Active**	0.79	0.52	**Active**		
Cardiotox.	Inactive	0.86	0.89	Inactive		

The table shows that the predicted LD50 for Taxifolin, Gallic acid, and Butin is 2000 mg/kg. Compared to other molecules, these three chemicals have less toxicity. Quercetin, myricetin and fisetin have very similar structures, so the LD50 values for all three were obtained as 159 mg/kg. These LD50 values are relatively high, and it is seen that the drugs to be used orally should be designed to be suitable for use in minimum doses. In addition, as expected, all molecules show active toxic properties when evaluated in terms of respiratory toxicity and nephrotoxicity. It should be noted that Protox 3.0 predictions are based on a trained dataset and, while providing valuable insights into toxicity profiles, may have limitations in accurately predicting certain specific toxicological endpoints such as chronic toxicity or liver-specific toxicity. Therefore, Protox 3.0 results were interpreted cautiously and complemented with other *in silico* tools and available literature data to ensure a comprehensive toxicity assessment.

3.3. Determination of Wound Healing Activity by Molecular Docking Results

Molecular docking is a widely used *in silico* computational technique designed to predict the binding affinities between ligands and receptor proteins. The technique can generate predictions that closely reflect experimental outcomes, especially for complexes formed between proteins and drugs or drug-like chemical compounds. In addition, it is commonly employed in drug research and development, and its use has become nearly indispensable.

In this study, TGF-β were selected as target receptor. TGF-β plays a pivotal regulatory role in the physiological processes involved in wound healing. It orchestrates critical cellular activities such as proliferation, migration, differentiation, and extracellular matrix (ECM) deposition, which are essential for tissue regeneration [31, 32]. Given its active involvement across all three phases of wound repair, namely inflammation, proliferation, and remodeling, TGF-β represents a rational and biologically relevant docking target in the context of wound-related drug discovery. One of the primary reasons for selecting TGF-β as a docking target is its central involvement in fibroblast activation and collagen biosynthesis, which are key processes in tissue reconstruction. TGF-β1, in particular, is known to induce fibroblast-to-myofibroblast differentiation and stimulate ECM production via the Smad signaling cascade [33, 34]. Importantly, dysregulation of TGF-β signaling has been implicated in pathological fibrosis and excessive scar formation, suggesting that small molecules capable of modulating this pathway may exert therapeutic benefits in wound management [35, 36]. Moreover, the well-defined structure of the TGF-β receptor-ligand complex and the downstream Smad transcription factors offers an accessible and structurally characterized platform for computational modeling and molecular docking studies [37]. The availability of crystallographic data and ligand-

binding information enhances the reliability of *in silico* screening efforts aimed at identifying potential TGF-β modulators. In summary, the multifaceted role of TGF-β in wound healing, its druggable molecular features, and its broader relevance in disease contexts collectively support its selection as the primary docking target in this study. The binding poses of the compounds and target receptor were given in Figure 4 and Figure 5.

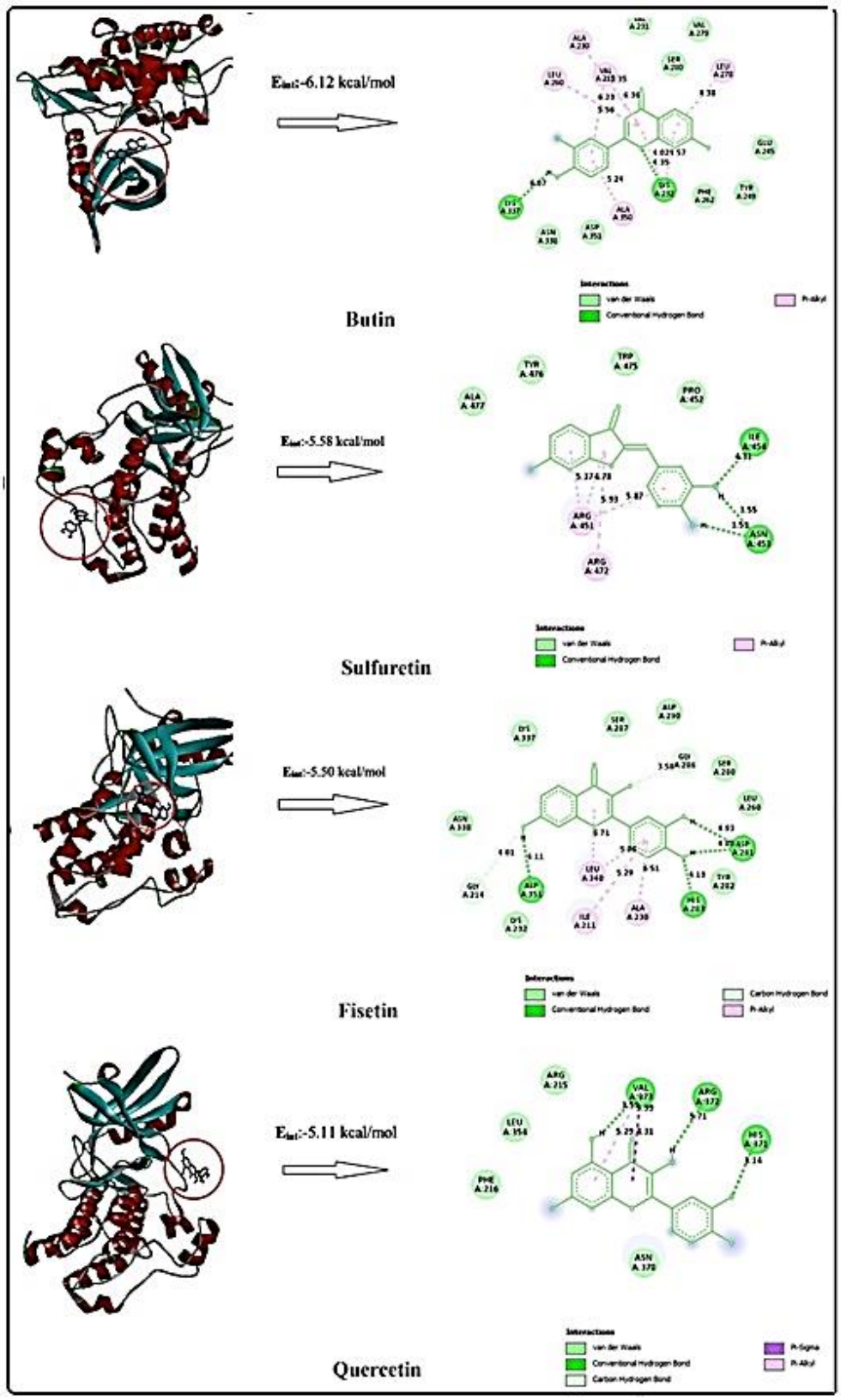

Figure 4. Visualization of intermolecular binding modes and key interaction parameters resulting from ligand–protein docking studies for butin, sulfuretin, fisetin and quercetin. This figure illustrates the predicted binding poses of the ligands within the active site of the target protein, highlighting critical interactions such as hydrogen bonds, hydrophobic contacts, π–π stacking, and electrostatic interactions. Binding affinity scores and interaction distances are also indicated, providing insights into the strength and specificity of ligand–receptor associations that may underlie potential biological activity

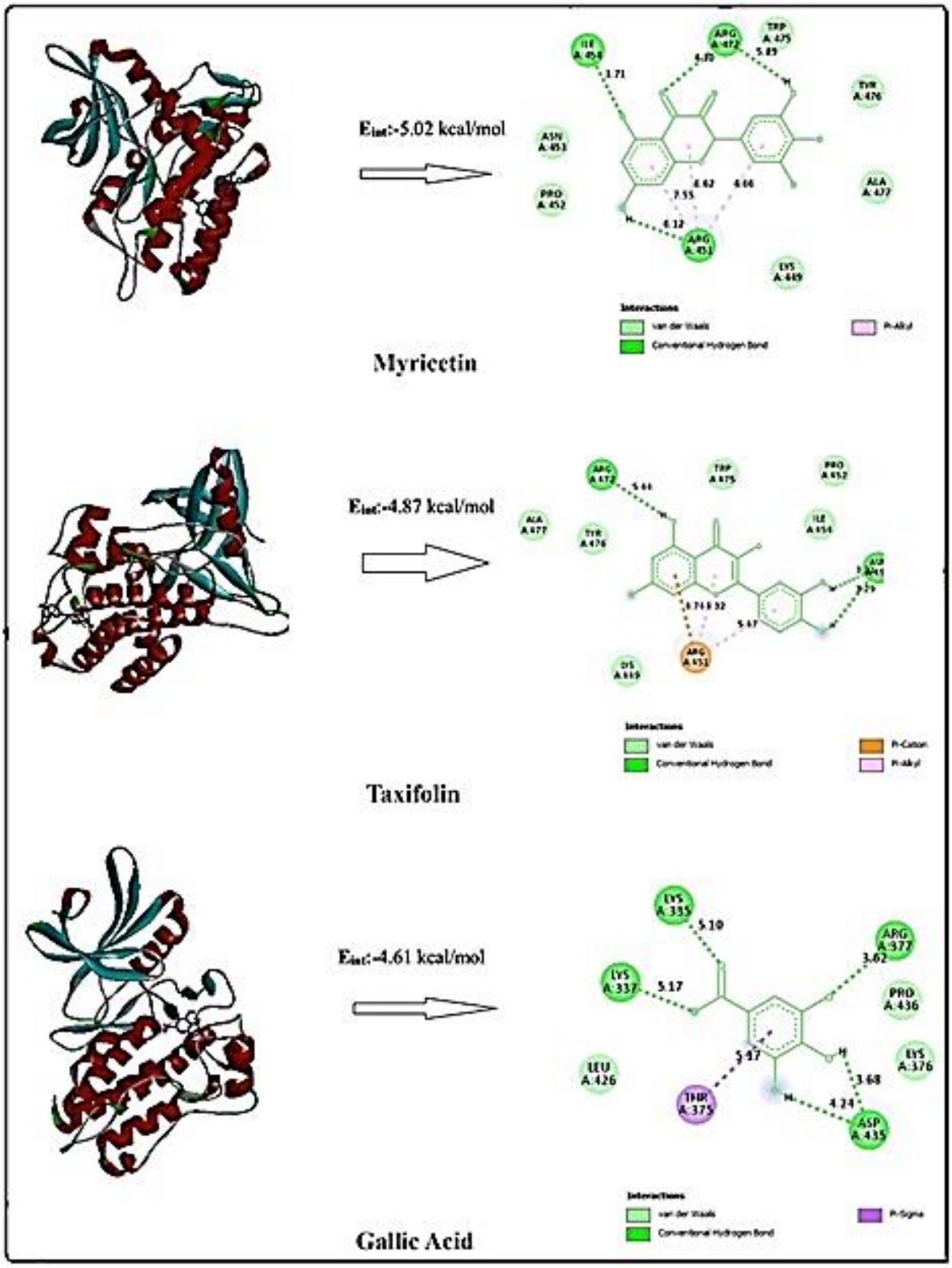

Figure 5. Visualization of intermolecular binding modes and key interaction parameters resulting from ligand–protein docking studies for myricetin, taxifolin and gallic acid molecules

BIOVIA Discovery Studio Visualizer tool (v24) was utilized to monitor the interactions among the chosen seven ligands (molecules under study) and the protein of preference. Hydrogen bonds, Pi-sigma bonds, Pi-alkyl bonds, and van der Walls bonds established between ligands and proteins are also seen in Figure 4 and Figure 5. Ligand-protein interaction bond types between compounds and amino acids are given in Table 4.

Table 4. Types of ligand–protein interaction bonds identified between the investigated compounds and target amino acid residues. This table presents detailed information on the nature of molecular interactions such as hydrogen bonds, hydrophobic interactions, van der Waals forces, π–π stacking, and electrostatic contacts formed between the ligands and specific amino acids within the active site of the target protein. These interactions contribute to the binding stability and specificity, offering insights into the molecular basis of potential bioactivity

Compound	Hydrogen	Pi Sigma Pi Cation
Butin	Lys 337, Lys 232	-
Sulfuretin	Ile 454, Asn 453	-
Fisetin	Hıs 283, Asp 281, Asp 351, Gly 214, Gly 286	-
Quercetin	Val 373, Arg 372, Hıs 371	
Myricetin	Arg 472, Arg 451, Ile 454	
Taxifolin	Arg 472, Asn 453	Arg 451
Gallic acid	Lys 335, Lys 337, Asp 435, Arg 377	Thr 375
Compound	**Pi Alkly**	**Van der Waals**
Butin	Ala 350, Leu 278, Val 219, Ala 230, Leu 260	Val 231, Val 279, Ser 280, Glu 245, Tyr 249, Phe 262, Asp 351, Asn 338
Sulfuretin	Arg 451, Arg472	Ala 477, Tyr 476, Trp 475, Pro 452
Fisetin	Leu 340, Ile 211, Ala 230	Asn 338, Lys 337, Ser 287, Asp 290, Ser 280, Tyr 282, Lys 232,Leu 260
Quercetin	-	Arg 215, Leu 354, Phe 216, Asn 370
Myricetin	-	Asn 453, Pro 452, Lys 449, Ala 477, Tyr 476, Trp 475
Taxifolin	-	Ala 477, Tyr 476, Lys 449, Trp 475, Ile 454, Pro 452
Gallic acid	-	Leu 426, Pro 436, Lys 376

Molecular docking studies were performed using the following parameters: the grid box dimensions were set to 36 Å, 68 Å, and 58 Å along the X, Y, and Z axes, respectively. The grid spacing was fixed at 0.417 Å. The grid box center coordinates were positioned at X = -0.138, Y = -0.028, and Z = 0.0. Ligand flexibility was accounted for during the docking process. BIOVIA Discovery Studio Visualizer tool (v24) was utilized to monitor the interactions among the chosen seven ligands and the proteins of preference.

The ligand-receptor hydrogen bond is an essential connection that influences the molecule's affinity for the target protein. The ligand's stability with the target protein is determined by hydrophobic and non-covalent bonds, which form groups on the polar side of the protein structure as a result of non-polar chains linking combined [19]. With the TGFβ protein, butin was found to form several conventional and carbon-hydrogen bonds. Two conventional hydrogen bonds were found to form at positions of Lys 337 (6.07 Å) and Lys 232 (4.35 Å). Five pi-alkyl bonds were also found at the positions of Ala 350 (5.24 Å), Leu 260 (5.56 Å), Val 219 (5.35 Å), Ala 230 (6.36 Å), and Leu 260 (5.56 Å). In addition, the docking score of the TGFβ with butin was -6.12 kcal/mol. For the sulfuretin-receptor interaction, three conventional hydrogen bonds were found at the positions of Ile 454 (4.31 Å), Asn 453 (3.53 Å), and Asn 453 (3.55 Å). Four pi-alkyl bonds were also seen as Arg 451 (5.37 Å), Arg 451 (4.78 Å), Arg 472 (5.93 Å) and Arg 451 (5.87 Å). The interaction energy of TGFβ protein docked with sulfuretin obtained -5.58 kcal/mol. For the Fisetin, four conventional hydrogen bonds were found at the positions of Asp 351 (4.11 Å), Hıs 283 (4.13 Å), Asp 281 (4.88 Å), and Asp 281 (4.93 Å). Two carbon-hydrogen bonds were also found at the positions of Gly 214 (4.01 Å) and Gly 286 (3.58 Å). Additionally, four Pi-Alkyl bonds were also obtained at the positions of Leu 340 (6.71 Å), Ile 211 (5.29 Å), Leu 340 (5.06 Å) and Ala 230 (6.51 Å). The docking result between TGFβ protein and fisetin was -5.50 kcal/mol. According to these results, the molecules with the highest binding energy values were found to be butin (-6.12 kcal/mol), sulfuretin (-5.58 kcal/mol) and fisetin (-5.50 kcal/mol). Conventional hydrogen bonds, Pi Alkali bonds, and Van der Waals interactions formed by butin, sulfuretin, and fisetin molecules with TGF-β protein have been shown to play a role in the wound healing process by promoting angiogenesis at the beginning of the epithelialization phase, activating the TGF-β pathway and inhibiting cytokine release and inflammatory response.

4. CONCLUSION

In this study, the aim was to identify which molecules are most active in the wound-healing activity of the *Cotinus coggygria* plant, also known as the "smoke tree" in popular usage, and whose wound-healing properties have been confirmed in numerous studies. The investigations were conducted *in silico* on Gallic acid, Myricetin, Quercetin, Fisetin, Sulfuretin, Butin, and Taxifolin, which are the most abundant molecules in the leaves of the plant. The results obtained from the study can be summarized as follows. Among the compounds examined, Gallic acid, Butin, and Taxifolin were found to have the highest LD_{50} values (~2000 mg/kg), indicating the lowest toxicity. It was found that the wound-healing effects of the plant are primarily attributed to Butin, Sulfuretin, and Fisetin, with

binding energies of −6.12 kcal/mol, −5.58 kcal/mol, and −5.50 kcal/mol, respectively, in their interactions with the TGF-β protein, which is a key component of the relevant pathway. Since these molecules have been shown to possess wound-healing effects in multiple previous studies, molecular docking successfully reflected these properties. Gallic acid, which is abundant in the plant extract, was shown to have a very low plasma protein binding rate, lower toxicity, and higher water solubility compared to the others. Thus, according to the results of the *in silico* study, Gallic acid appears to be a promising candidate for the development of an orally administered drug. The ADME profiles of the molecules were evaluated, and it was found that all compounds except Gallic acid exhibited high skin permeability. This suggests that topical wound-healing formulations (e.g., creams) based on these molecules could be pharmacologically effective. The toxicity and wound-healing effects of the compounds present in the extract obtained from *Cotinus coggygria* leaves were identified, providing valuable preliminary data for future pharmacological research and drug development involving these molecules.

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