



Characterization of Waste *Morus alba* Leaf Extracts and Their Binding Affinities Toward the VEGF Receptor

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ARTICLE INFO		Abstract
Article Type	: Research Article	Wound healing is a complex biological process regulated by coordinated cellular and molecular mechanisms, in which angiogenesis plays a pivotal role. Plant-derived bioactive compounds have gained increasing attention as potential therapeutic agents due to their biocompatibility and diverse pharmacological properties. In the present study, aqueous, methanolic, and ethanolic extracts of <i>Morus alba</i> leaves were chemically characterized, and the binding potential of major phytochemicals toward the Vascular Endothelial Growth Factor (VEGF) receptor was evaluated using in silico molecular docking. Leaf extracts were prepared using soxhlet extraction and analyzed by high-performance liquid chromatography (HPLC). Among 17 reference standards, ethanol extracts exhibited the highest phytochemical diversity, while aqueous extracts contained the fewest components. Apigenin, curcumin, and 4-hydroxybenzoic acid were identified as the predominant constituents in ethanol, methanol, and aqueous extracts, respectively, with trans-p-coumaric acid present as a common major compound across all extracts. Molecular docking analyses were conducted using AutoDock Vina to assess ligand–receptor interactions with the VEGF receptor. Docking results revealed that trans-p-coumaric acid exhibited the strongest binding affinity toward the VEGF receptor, with a minimum binding energy of –5.9 kcal/mol, followed by curcumin (–5.7 kcal/mol) and hydroxybenzoic acid (–4.7 kcal/mol). Interaction analyses indicated that complex stability was primarily governed by hydrogen bonding and hydrophobic π-interactions within the receptor binding pocket. Comparative evaluation suggested that the planar geometry and favorable interaction profile of trans-p-coumaric acid enabled stable and energetically favorable receptor binding. Overall, these findings highlight trans-p-coumaric acid as a promising lead compound for VEGF modulation and support the potential of <i>Morus alba</i> leaf-derived phytochemicals as candidates for further experimental validation and wound-healing-oriented drug development.
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1. Introduction

A wound is defined as visible or invisible damage occurring in a living organism as a result of forces or agents of varying nature (Ekizoğlu & Arıcan, 2009; Parsak, Sakman, & Çelik, 2007). Structural disruption of the skin, muscle, and mucosal tissues caused by trauma originating from such forces, which subsequently lead to infection, is defined as an injury (URL-1). It is well established that a variety of complex mechanisms are activated within the organism during the wound healing process. This process is regulated through the coordinated involvement of immune system components and biological events such as cell adhesion, cell migration, cell proliferation, cell differentiation, and apoptosis (Bahar, 2021). Due to their intrinsic complexity, the classification of wounds is crucial for accurate diagnosis and effective treatment strategies (Abazari, Ghaffari, Rashidzadeh, Badeleh, & Maleki, 2022).

In addition to endogenous mechanisms, exogenous interventions may be required to promote rapid healing of injured tissues. Throughout history, numerous approaches have been employed within the scope of wound care. These have included the use of natural and artificial dressings, hydrophilic cotton, sterile gauze, hot water-soaked compresses, suturing with bone or metal needles, and cauterization using heated metals applied directly to the wound (Kurtoğlu & Karataş, 2009). Contemporary wound management strategies emphasize the critical roles of moisture and temperature in the healing process. Accordingly, various predominantly synthetic materials—such as alginate, hydrogel, foams, hydrocolloid dressings, and polyurethane films—are widely used. In contrast, traditional approaches have primarily relied on plant-based materials (Vural & Savcı, 2017). In underdeveloped societies with limited access to medical care, wound healing is often supported by locally prepared herbal remedies. Importantly, exogenous components used for this purpose should be biocompatible and not perceived as foreign substances by the body (Bahar, 2021). In this context, plants represent one of the most frequently utilized natural sources.

Phytotherapy is among the oldest therapeutic practices known to humanity. The foundations of modern herbal medicine trace back to China and India, while in Western societies, plants traditionally used in folk medicine were later adopted by physicians and continue to be incorporated into alternative and complementary medical practices (Durusoy & Ulusal, 2007). Although modern medical approaches have historically approached alternative and complementary therapies with skepticism, it is now widely acknowledged that a substantial proportion of

pharmaceutical drugs are derived from natural compounds, many of which originate from plant sources (Shedoeva, Leavesley, Upton, & Fan, 2019). The ease of accessibility of plants, their diverse biological activities, and their ability to act synergistically with other compounds further underscore their therapeutic significance (Budovsky, Yarmolinsky, & Ben-Shabat, 2015). Antioxidants present in plants are molecules that prevent harmful free radicals from causing metabolic damage (İşbilir & Çelik, 2021). Numerous secondary metabolites produced by plants have been reported to exert beneficial effects on wound healing. For instance, constituents of *Helianthemum canum* have been shown to enhance wound healing in both *in vivo* and *in vitro* studies (Küpeli Akkol et al., 2023). Similarly, several studies have highlighted the wound-healing potential of plants such as *Phlomis rigida* (Okur et al., 2021), Echinacea (Maver et al., 2015), and *Hydnocarpus wightiana* (Sharma & Hall, 1991).

Morus alba, one of approximately 150 species belonging to the *Morus* genus, is a plant that has been utilized in various forms beyond its fruit. Mulberry leaves, commonly used as feed for silkworms, also serve as a food source for certain herbivores (Butt, Nazir, Sultan, & Schroën, 2008). Although the leaves are valuable, their collection is often labor-intensive due to their prevalence in agricultural areas, and they are therefore frequently left to decompose as organic waste. Nevertheless, species within the *Morus* genus are known to contain ascorbic acid, B-complex vitamins, vitamin D, folic acid, tannins, and alkaloid derivatives. Owing to these properties, *Morus* species have been traditionally used for the treatment of respiratory disorders, dizziness, ear ailments, indigestion, and various other conditions, and their pharmacological potential has been well documented. In particular, their antioxidant content has been reported to confer protective effects against several skin-related disorders (Seep et al., 2013). It has been demonstrated that extracts from black mulberry leaves accelerate the healing of skin wounds (Zhou et al., 2019), while white mulberry extracts have been shown to promote superior wound healing in rats compared to silver sulfadiazine creams (Bhatia et al., 2014).

In the present study, we aimed to characterize aqueous, methanolic, and ethanolic extracts obtained from *Morus alba* leaves using high-performance liquid chromatography (HPLC) and to evaluate the binding affinity of the major identified compounds (curcumin, trans-p-coumaric acid, and hydroxybenzoic acid) toward the Vascular Endothelial Growth Factor (VEGF) receptor through *in silico* molecular docking analyses.

2. Materials and methods

2.1. Materials

Morus alba leaves used in this study were collected from the Muş Alparslan University campus. The collected samples were air-dried and subsequently ground into a fine powder using a blender. The receptor employed in the *in-silico* studies was obtained from the RCSB Protein Data Bank (RCSB, 2025), while the ligands were retrieved from the National Library of Medicine database (PubChem, 2025). Detailed information regarding the receptor and ligands are provided in Table 1.

Table 1. Information on the receptor and ligands used for *in silico* docking study

No	Molecule	Type	Code
1	Vascular endothelial growth factor VEGF	Receptor	3KVQ
2	Curcumin	Ligand	969516
3	Trans-p-coumaric acid	Ligand	13783633
4	Hydroxybenzoic acid	Ligand	135

2.2. Methods

2.2.1. Extraction and Characterization

The dried herbal material was weighed in 20 g portions and subjected to soxhlet extraction for 4 h. Following extraction, the solvents were removed by evaporation, as described previously (Santos et al., 2020). The chemical composition of the obtained extracts was analyzed using high-performance liquid chromatography (HPLC) through outsourced analytical services.

Preparation of Receptors Ligands and Molecular Docking

Receptor and ligand structures were converted into PDB format using PyMOL software (Schrödinger & DeLano, 2020). The receptor was prepared in AutoDock Vina (Trott &

Olson, 2010) by removing water molecules and adding polar hydrogens and Kollman charges. The prepared receptor was saved in .pdbqt format and rendered ready for docking. The ligand-binding site of the receptor was determined using Discovery Studio Visualizer (Biovia, 2023). The receptor contained a single binding pocket, with grid box center coordinates defined as X = 15.194284, Y = 21.851905, and Z = 29.000164, and grid dimensions set to 50 × 50 × 50 (Figure 1). Interactions between the receptor and ligands were visualized in both two- and three-dimensional formats using Discovery Studio Visualizer.

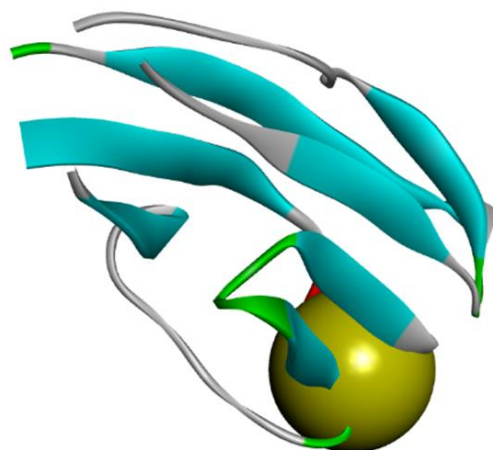


Figure 1. Binding region of ligands within the VEGF receptor

3. Results

According to HPLC analyses of the 17 reference standards, the ethanol extract contained the highest number of identified components, whereas the aqueous extract exhibited the lowest. The most abundant compounds were identified as apigenin in the ethanol extract, curcumin in the methanolic extract, and 4-hydroxybenzoic acid in the aqueous extract. Trans-p-coumaric acid was detected as a common major component across all three extracts (Table 2).

Table 2. HPLC-determined composition of *Morus alba* extracts (ng/μL)

Extract	Ascorbic acid	Galllic acid	3,4-Dihydroxybenzoic acid	4-Hydroxybenzoic acid	trans-p-coumaric acid	Myricetin	Absciscic acid	Quercetin	Apigenin	Kaempferol	Curcumin	Catechol	Vanillin	Caffeic acid	Cinnamic acid	Rosmarinic acid	Salicylic acid
Ethanol	0.970	-	-	5.31	17.68	-	19.18	-	51.60	2.34	9.01	-	2.36	-	2.38	-	1.33
Methanol	-	-	-	4.88	20.65	-	-	-	-	-	56.71	0.66	0.77	-	3.10	1.70	1.91
Water	-	-	-	3.14	1.44	-	-	0.46	-	-	-	-	-	-	0.90	1.67	-

Docking calculations were performed for the VEGF receptor using nine distinct binding poses for each ligand within the defined grid coordinates. Trans-p-coumaric acid exhibited binding energies ranging from -4.9 to -5.9 kcal/mol (Table 3). The amino acid residues involved in binding and the interaction modes of trans-p-coumaric acid within the

receptor pocket are illustrated in Figure 2. Hydroxybenzoic acid demonstrated binding energies between -3.5 and -4.7 kcal/mol (Table 3), with its interaction profile presented in Figure 3. Finally, curcumin showed binding energies ranging from -4.7 to -5.7 kcal/mol (Table 3), and the corresponding binding modes are depicted in Figure 4.

Table 3. Binding affinities (kcal/mol) of trans-*p*-coumaric acid, hydroxybenzoic acid, and curcumin to the VEGF receptor across nine docking poses

	Trans- <i>p</i> -coumaric acid	Hydroxybenzoic acid	Curcumin
1	-5.9	-4.7	-5.7
2	-5.7	-4.5	-5.2
3	-5.2	-4.5	-5.0
4	-5.2	-4.2	-4.9
5	-4.9	-4.0	-4.8
6	-4.9	-4.0	-4.7
7	-4.9	-4.0	-4.7
8	-4.9	-3.9	-4.7
9	-4.9	-3.5	-4.7

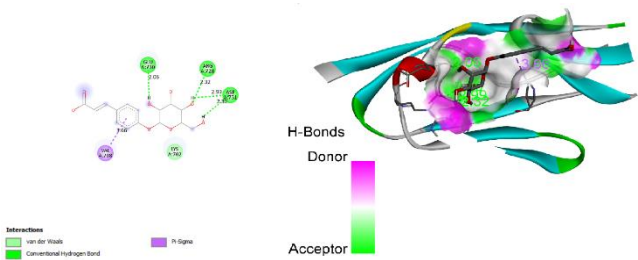


Figure 2. Optimal binding region and pose of trans-*p*-coumaric acid within the VEGF receptor (2D/3D)

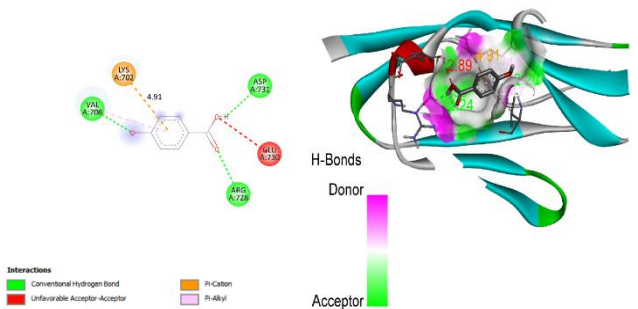


Figure 3. Optimal binding region and pose of hydroxybenzoic acid within the VEGF receptor (2D/3D).

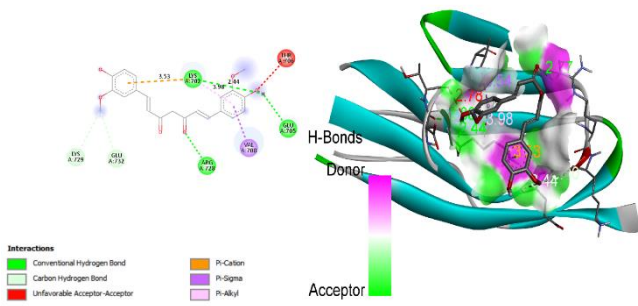


Figure 4. Optimal binding region and pose of curcumin within the VEGF receptor (2D/3D).

4. Discussion

Morus alba, a member of the Moraceae family, is a widely cultivated medicinal plant valued both for its fruit and in sericulture. The species has been traditionally employed in

the treatment of asthma, cough, bronchitis, edema, insomnia, wound healing, diabetes, influenza, ocular infections, and epistaxis, and is cultivated across many regions worldwide (Devi et al., 2013). Owing to its richness in secondary metabolites, *M. alba* is characterized by high levels of phenolic and flavonoid compounds. In the present study, apigenin was detected at a concentration of 51.60 ng/ μ L in the ethanol extract. This compound has been reported to inhibit lipopolysaccharide-induced nitric oxide synthase activity (Tran et al., 2017). Furthermore, compounds derived from this genus have been shown to promote healing in both infected and non-infected wounds (Sharif et al., 2024).

Curcumin, identified as another major constituent, has also been reported to play a significant role in wound healing (Bhatia et al., 2015), with curcumin-containing hydrogel dressings demonstrating notable therapeutic efficacy (Chen et al., 2025). Similarly, phytochemicals including coumaric acid derivatives have been highlighted as potential active pharmaceutical ingredients for dermal and mucosal applications due to their bioadhesive and thermoresponsive properties (de Souza Ferreira et al., 2024). Hydroxybenzoic acid, another major compound identified in this study, has been reported to reduce inflammatory responses induced by organisms such as jellyfish (Geng et al., 2024).

In the present work, these major compounds were subjected to *in-silico* docking analyses to evaluate their binding affinities toward VEGF. The calculated binding free energy of -5.9 kcal/mol for trans-*p*-coumaric acid indicates a strong penetration capability within the receptor pocket. The clustering of energy scores within a narrow upper range suggests a stable binding mode, indicating that this molecule may represent a promising candidate for molecular optimization studies.

Hydroxybenzoic acid exhibited a stable yet low-energy barrier binding profile within the receptor pocket, suggesting that it may be more suitable for evaluation within fragment-based drug design approaches rather than as a standalone potent inhibitor. Molecular docking simulations targeting the VEGF receptor revealed that curcumin achieved a binding free energy of -5.7 kcal/mol in its most stable conformation. The clustering of subsequent conformations within a narrow energy range indicates thermodynamically favorable and sterically compatible binding. Two-dimensional interaction maps revealed that complex stability was primarily governed by critical hydrogen bonds mediated by phenolic hydroxyl and ketone

groups, as well as hydrophobic π -interaction networks involving aromatic rings.

Comparative docking analysis of the three ligands demonstrated that trans-p-coumaric acid formed the most thermodynamically stable and specific complex with the VEGF receptor (−5.9 kcal/mol), followed by curcumin (−5.7 kcal/mol) and hydroxybenzoic acid (−4.7 kcal/mol). The broader hydrophobic interaction network of trans-p-coumaric acid relative to the limited van der Waals surface of hydroxybenzoic acid, combined with its more planar geometry compared to the flexible and bulky scaffold of curcumin, facilitated sterically unhindered penetration into the receptor pocket and minimized entropic penalties during binding. From a structure–activity relationship perspective, the trans-p-coumaric acid scaffold most effectively embodies the pharmacophoric features required for receptor modulation. These findings suggest that this molecule may be prioritized in drug development pipelines, while the remaining compounds may serve as secondary building blocks in derivatization and lead optimization studies.

Conclusion

Comparative docking analysis of the three ligands demonstrated that trans-p-coumaric acid formed the most thermodynamically stable and specific complex with the VEGF receptor (−5.9 kcal/mol), followed by curcumin (−5.7 kcal/mol) and hydroxybenzoic acid (−4.7 kcal/mol). The broader hydrophobic interaction network of trans-p-coumaric acid relative to the limited van der Waals surface of hydroxybenzoic acid, combined with its more planar geometry compared to the flexible and bulky scaffold of curcumin, facilitated sterically unhindered penetration into the receptor pocket and minimized entropic penalties during binding. From a structure–activity relationship perspective, the trans-p-coumaric acid scaffold most effectively embodies the pharmacophoric features required for receptor modulation. These findings suggest that this molecule may be prioritized in drug development pipelines, while the remaining compounds may serve as secondary building blocks in derivatization and lead optimization studies

Authorship Contribution Statement

SÇ: Investigation, data curation, formal analysis, writing – original draft preparation.

Declaration of Competing Interest

The author declares no conflict of interest.

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