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RESEARCH ARTICLE

Flavonoid Profiling, Molecular Docking, and Modulatory Effects of *Artemisia herba- alba* Leaf Extract on ACE2 and TMPRSS2 Gene Expression

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ABSTRACT

Several studies have reported the potential antiviral activity of Artemisia species, which is related mainly to their flavonoid constituents. Transmembrane serine protease 2 (TMPRSS2) and angiotensin-converting enzyme 2 (ACE2) are critical SARS-CoV-2 entry factors. In this study, the phytochemical composition and modulatory effects of *Artemisia herba-alba* leaf extract on the gene expression of ACE2 and TMPRSS2 were investigated. To identify the flavonoid profile, HPLC was used. Cytotoxicity was estimated in A549 carcinoma and HDFn fibroblasts using an MTT assay ($IC_{50} = 114.8$ $\mu g/mL$). The real-time PCR results revealed significant upregulation of TMPRSS2 gene expression (2.4-fold, $p \leq 0.01$; p value = 0.0011), whereas ACE2 expression remained unchanged. Furthermore, a molecular docking study suggested the potential binding affinity of hesperidin and rutin for TMPRSS2 and ACE2. Therefore, both in vitro and in silico results indicated that *A. herba-alba* extract may interact with the viral entry pathway.

Keywords: ACE2, Artemisia herba-alba, COVID-19, Natural antiviral activity, TMPRSS2

Introduction

In 2019, the world faced a major health challenge caused by SARS-CoV-2, later known as coronavirus disease 2019.[1] Many studies have reported the effects of medicinal plants and their active constituents as important therapies against COVID-19.[2] TMPRSS2 was shown to be an important target for preventing and reducing the severity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[3] Additionally, angiotensin-converting enzyme 2 (ACE2) is a zinc- and chloride-dependent dipeptidyl carboxypeptidase I.[4] ACE2 is involved in heart function regulation and functions as a functional receptor on the cell membrane for coronavirus, which is related to SARS-CoV-2.[5] Both the ACE2 protein and the TMPRSS2 protein facilitate the attachment of the viral spike glycoprotein, thus allowing the virus to enter host cells.[6,7] ACE2 and TMPRSS2 genes are co-expressed on secretory cells in the nasal mucosa and absorptive enterocytes in the small intestine, as determined by single-stranded RNA sequencing analysis of goblet cells and lung type 2 pneumocytes.[8] The first contact point for the virus is the olfactory epithelium and respiratory tract in the nasal mucosa, where SARS-CoV-2 susceptibility and transmissibility are thought to be related to the expression of these genes.[9] The efficacy of many natural compounds against COVID-19 has been investigated. Most studies have used computational molecular docking techniques only.[10]

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The biological and mechanistic effects underlying the activities of these natural compounds and their ability to prevent the entry of SARS-CoV-2 into host cells are unclear.[11] Many researchers have referred to the ability of flavonoids to reduce the severity of viral infections that are mediated through a dual mechanism: direct interference with viral internalization and replication. The efficacy of many natural compounds against COVID-19 has been investigated. Flavonoids are used to treat this disease. Efforts to find antiviral drugs for COVID-19 have focused on various mechanisms, including preventing the virus from entering cells, inhibiting the interaction of viral proteins with host cells, and preventing viral replication. The aim is to stop the inflammatory responses caused by viral invasion. Therefore, treatments that can affect coronaviruses can be divided into three main categories: (i) preventing the virus from binding to human cell receptors or affecting specific receptors on host cells, thus blocking the virus from entering cells, i.e., preventing viral entry; (ii) limiting the synthesis and reproduction of viral RNA; and (iii) decreasing the expression of virulence factors to restore innate immunity. Therefore, computational studies have explored the use of flavonoid compounds as therapeutic candidates against COVID-19 by evaluating the cleavage of the S protein and the binding of the S protein to ACE2 receptors on the cell surface.[12] The *Artemisia* genus (Asteraceae) has numerous beneficial health effects; it may be a candidate against COVID-19.[11]

Flavonoids are polyphenolic natural compounds that represent major constituents of *Artemisia* and are widely recognized for a variety of activities, including anti-inflammatory and immunomodulatory effects mediated through the inhibition of different inflammatory cytokines.[13] Many researchers have referred to the ability of flavonoids to reduce the severity of viral infections that are mediated through a dual mechanism: direct interference with viral internalization and replication.[14] Recent studies of hesperidin (HD) have confirmed its activity against SARS-CoV-2 infection in a laboratory cell line model. According to molecular simulation analysis, this may be due to its binding to the virus's 3CLpro and S proteins, as well as to the cellular ACE2 receptor in host cells, suggesting the potential of HD in combating SARS-CoV-2. However, there is insufficient evidence to determine the efficacy of the hesperidin or hesperetin compound against SARS-CoV-2 infection.[15] Acute treatment of Calu-3 cells with quercetin reduced the expression of ACE2 and TMPRSS2 mRNA and protein; however, this inhibition was not sufficient to prevent viral entry into the cells.[16] In addition, a virtual check of 2030 natural compounds was proposed, with rutin being selected as a potential antibody to SARS-CoV-2 Mpro.[17] In this study, we examined the effect of the leaf extract of *Artemisia herba-alba* on the entry receptor for the virus by affecting the expression of the "ACE2 and TMPRSS2" genes *in vitro*.

Materials and methods

Plant sampling

The plant material, Artemisia leaf, was collected from Altib, East of Basrah, Iraq, in Jan 2024, with a team under the supervision of Dr. Ola Al-Moussawi, College of Pharmacy, University of Basrah. The plants were cleaned, dried at room temperature, and powdered.

Extraction methods

Powdered plant material (15 g) was soaked in 120 mL of hexane at room temperature for defatting. The menstruum was then filtered, and the residue was extracted using the method described previously.[18]

High-performance liquid chromatography for flavonoids analysis

HPLC analysis is among the best methods for identifying, separating, and quantifying target compounds present in plant samples.[19] The leaf extract of *A. herba-alba* was subjected to high-performance liquid chromatography (HPLC);)SYKAM- Germany), provided with a UV spectrometer, to match and quantify the flavonoids in the crude extract. A C18 Octadecylsilane (ODS) column was used to separate the flavonoids. The gradient elution method, with a mobile phase as follows: initial 40% of 1% formic acid in water (v/v)) at 0–5 min, 50% methanol at 6–15 min, and a flow rate of 1.0 mL/min was used. The injected volume of the sample and standards was 100 μL.[20] The flavonoid type was determined by comparing the peak retention times of the standards with those of the samples at 280 nm. Standard compounds from Medochiem (China) were used. Flavonoid compounds, including kaempferol, quercetin, luteolin, apigenin, rutin, hesperidin, and vitexin, were identified. Flavonoid standard working solutions of 1–10 μg/mL were prepared in HPLC-grade methanol.

Cytotoxicity evaluation

Reagents

The cell culture reagents and chemicals used were from Ajenta Pharm (India) and Sigma–Aldrich (Poole, Dorset, UK). An MTT kit from Intron Biotech (Korea) was used. Human carcinoma (A549) cells and normal human dermal fibroblasts (HdFn) were obtained

from the Pharmacology Department/Medicine College/Malaya University/Kualalampur, Malaysia. The media and solutions were made according to.[21]

Cytotoxicity assay of A. herba-alba leaf extract using the MTT method

Tumor cells (1×10^4 cells/ml) were cultured in 96-well plates with complete medium (200 μL per well). After 24 hours of incubation at 37°C and 5% CO_2, serial dilutions of the extract (100–500 μg/mL) with medium were added. Each concentration and control (serum-free medium) was tested in triplicate. After 24 hours of treatment, the cells were washed once with PBS and dissociated with trypsin-EDTA, after which fresh medium was added to resuspend the cells. MTT solution (10 μL) was added to the wells of the plate, which was subsequently incubated for 4 hours. After the medium was removed, the solubilization solution (100 μL) was added, and the plates were read at 575 nm using an ELISA reader. The IC_{50} concentration of the methanol extract against A549 cells was compared with that against normal HDFn cells to determine its cytotoxicity.

Real-time PCR experiment

Total RNA from A549 cells treated with the IC_{50} extract was extracted using the "QIAGEN RNeasy Mini Kit" (cat. 74106). A Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific, cat. K1621) was used for cDNA synthesis. Real-time PCR was conducted using "SYBR Green PCR Supermix (ELK Biotechnology, EQ001)". The cycling conditions were.[22] Amplified ACE2 NCBI Reference Sequence: NM_021804.1 and TMPRSS2 NCBI Reference Sequence: NM_005656.4 using primers.[23,24]

Molecular docking study

This study investigated the effects of several flavonoid products against two important viral target proteins. MOE 22 software was used to study the binding mode of the selected compounds against target proteins (ACE2 and TMPRSS2). The cocrystallized structures of the proteins were downloaded from the RCSB PDB, ACE2 (PDB ID: 6M0J; resolution: 2.45 Å) and TMPRSS2 (PDB ID: 7MEQ; resolution: 1.95 Å). The proteins were prepared, and the energy was minimized using MMFF94 force fields. The binding site was defined on the basis of the cocrystallized ligand present in the selected PDB structure. The Site Finder tool (MOE 22 software) was used to determine the active pocket corresponding to the reference ligand binding region. The amino acid residues that constitute the binding site are listed in Table S1. The validation of the docking process was performed by redocking the original cocrystallized ligand into the corresponding protein binding pocket using identical docking parameters. The RMSD values between the experimental and redocked conformations were calculated and found to be less than 2.0 A°, confirming the reliability of the docking protocol. Each ligand was docked into both proteins to produce ten binding poses. The binding score (ΔG) kcal/mol and RMSD values were subsequently calculated.

Statistical analysis

The data were analyzed using GraphPad Prism (v9.4) and are presented as the mean $\pm$ SD. One-way ANOVA with Duncan's test was applied, and $p \leq 0.01$ was considered to indicate statistical significance. All the experiments were performed in three biological replicates.

Results and discussion

HPLC

To improve the separation of the studied extract, high-performance chromatography was used. HPLC chromatograms of the extract and standard compounds are shown in Figs. 1 and 2. Quantification was carried out by the process of standard calibration. The amount of each compound in the analyzed sample is shown in Table 1. The *A. herba-alba* extract identified seven compounds, accounting for 58.5% of the overall content of the extract. Seven standards of phenolic compounds, namely, kaempferol (2.30 min), quercetin (3.52 min), luteolin (4.02 min), apigenin (5.17 min), rutin (7.89 min), hesperidin (10.18 min), and vitexin (12.08 min), were detected and eluted successfully at different retention times, and the results were recorded at 280 nm. To determine the content of the flavonoids, calibration curves were prepared in the range of 2–10 ppm for each standard solution. The greatest amounts of flavonoids detected were hesperidin and kaempferol.

The high concentrations of some identified flavonoids in the methanol extract demonstrated the efficiency of using methanol at 80% in extracting flavonoids. Previous studies have used methanol as a good solvent for efficiently extracting phenolic compounds.[19] Polyphenols are among the most important phytochemicals, with broad-spectrum properties and involvement in various chemical, physiological, and biological processes. They are categorized as flavonoids, hydroxycinnamic acids, hydroxybenzoic acids, lignin, and stilbenes. Many of them are therapeutically effective.[25,26] Their antiviral efficacy has been studied, as well as their

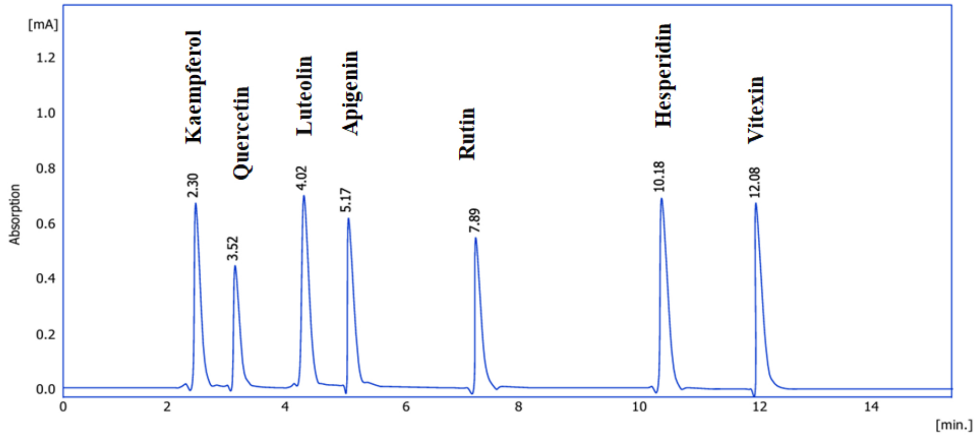

Fig. 1. HPLC chromatograms of *A. herba-alba* leaf extracts with different retention times.

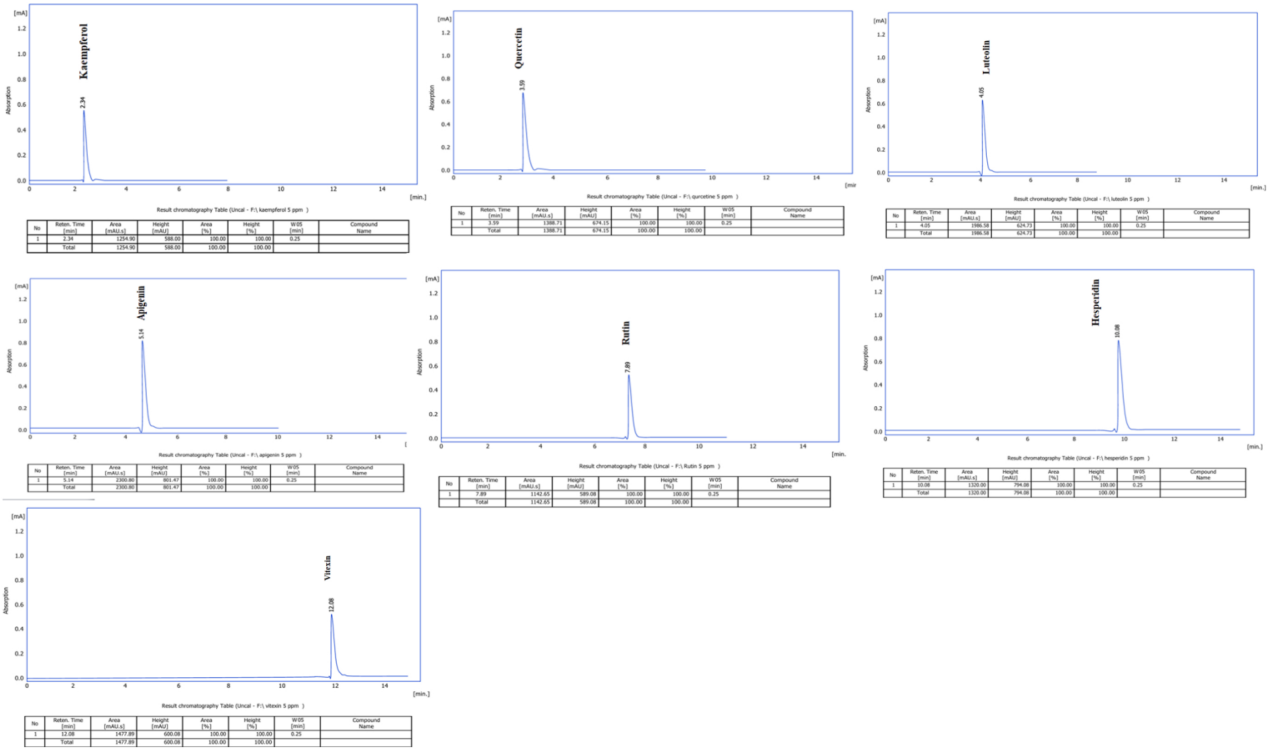

Fig. 2. HPLC chromatogram of flavonoid standards with their different retention time.

Table 1. Retention times of flavonoid standards and identical flavonoids at different concentrations (ppm) in *A. herba-alba* leaf extract.

No.	Retention Time[min]for standards	Retention Time[min]for identified flavonoids	Identical Flavonoids	Concentration [ppm]
1	2.34	2.30	kaempferol	62.9
2	3.59	3.52	Quercetine	40.1
3	4.05	4.02	Luteolin	60.7
4	5.14	5.17	Apigenin	51.9
5	7.89	7.89	Rutin	44.7
6	10.08	10.18	Hesperidin	68.9
7	12.08	12.08	Vitexin	60

Table 2. The binding affinity (Kcal/mol) of flavonoid products for target proteins.

	ACE2: ID 6M0J		Protease2: ID 7MEQ	
Compound	S score kcal/mol	RMSD	S Kcal/mol	RMSD
kaempherol	–6.54	1.177	–4.15	1.539
Quercetin	–5.33	1.613	–5.37	1.418
Luteolin	–5.07	1.244	–5.44	1.288
Apigenin	–4.96	0.849	–5.21	1.590
Rutin	–7.49	1.853	–6.96	1.647
Hesperidin	–7.89	1.736	–7.30	2.150
Vitexin	–5.56	0.316	–6.22	1.080
ligand	–4.636	1.223	–5.12	1.674

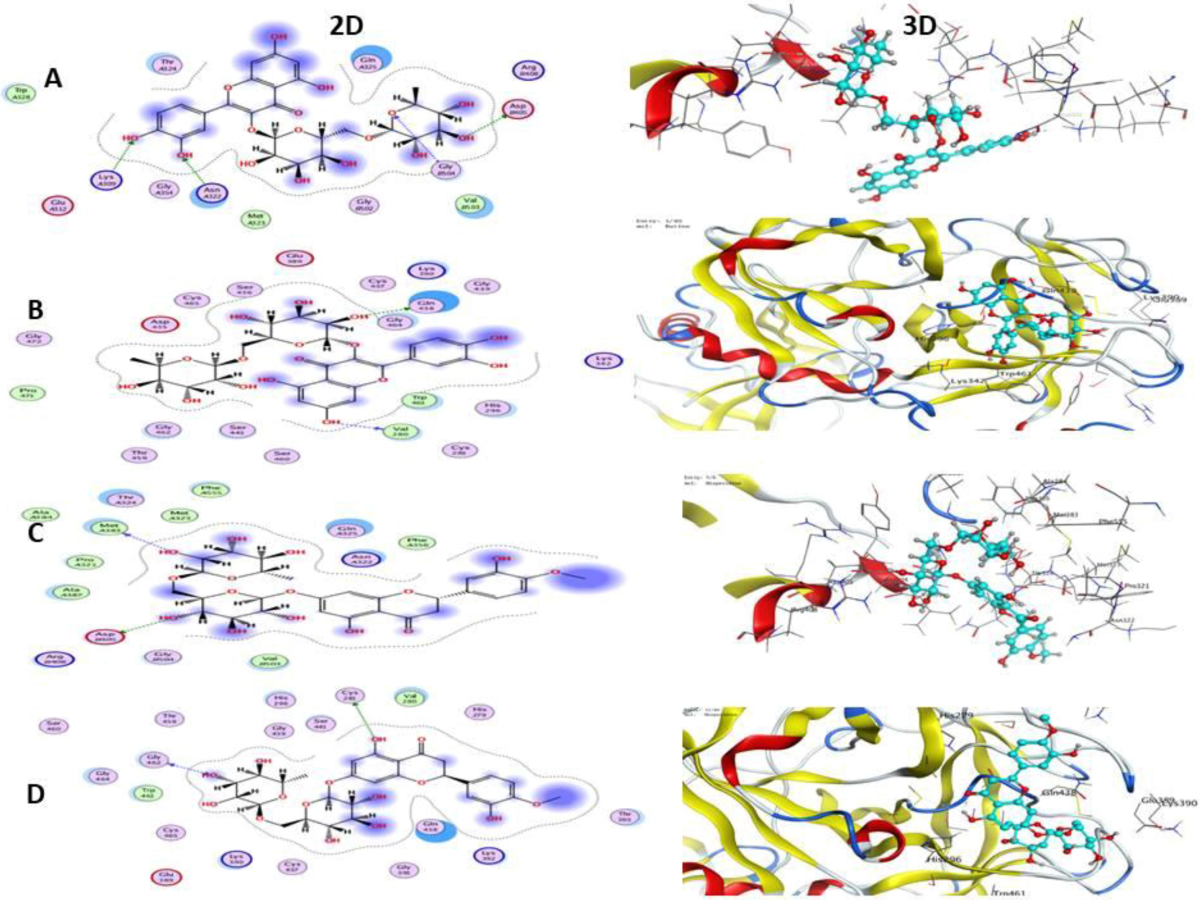

Fig. 3. 2D and 3D binding modes of Rutin (A, B) and Hesperidin (C, D) inside the validated ligand-binding site of ACE2 and TMPRSS2, respectively.

safety and efficacy in integrative and complementary therapeutic approaches.[27]

The molecular docking

Molecular docking simulations were performed to evaluate the potential interactions of the selected flavonoid compounds with two key viral molecules, ACE2 and TMPRSS2. The binding mode of the tested flavonoid products was good for the ACE2 receptor, with binding energies ranging from –4.96 to –7.89 kcal/mol, as shown in Table 2 and Fig. S1. Rutin exhibited good binding energy (–7.49 kcal/mol) and formed four hydrogen bonds, two between phenol and (OD1 ASP 405 and N GLY 504) and two between the hydroxyl groups in the disaccharide rutinose moiety and (NZ LYS 309 and ND2 ASN 322). Hesperidin has a relatively high binding energy to the ACE2 receptor (–7.89) kcal/mol. It formed two hydrogen bonds between the hydroxyl groups in the disaccharide rutinose moiety and (OD1 ASP 405 and O MET 383), as shown in Table 3 and Fig. 3. This multipoint

Table 3. Types of interactions of selected flavonoids with amino acid residues in the validated ligand-binding site in the target proteins.

Protein ID	Compounds	Interaction Atom	Amino acid residues	Type of Interaction	Distance Å	E kcal/mol
6M0J	kaempherol	O 25	O CYS 336	H-donor	2.93	–2.9
		O 6	ND2 ASN 343	H-acceptor	3.67	–0.6
		O 9	CB ASN 343	H-acceptor	3.47	–0.6
		O 9	ND2 ASN 343	H-acceptor	2.77	–4.5
		O 9	ND2 ASN 343	ionic	2.77	–6.2
	Quercetin	O 9	ND2 ASN 343	H-acceptor	3.26	–0.7
		O 9	ND2 ASN 343	ionic	3.26	–2.9
	Luteolin	O 13	O MET 323	H-donor	2.97	–3.2
		6-ring	N GLN 325	pi-H	4.62	–1.5
	Apigenin	O 8	O CYS 336	H-donor	3.11	–0.6
		O 13	O ASP 364	H-donor	2.94	–2.4
	Rutin	O 70	OD1 ASP 405	H-donor	3.17	–2.0
		O 15	N GLY 504	H-acceptor	3.01	–2.1
		O 58	NZ LYS 309	H-acceptor	3.26	–0.9
		O 60	ND2 ASN 322	H-acceptor	3.00	–2.6
	Hesperidin	O 46	OD1 ASP 405	H-donor	2.85	–3.4
		O 67	O MET 383	H-donor	3.23	–1.1
	Vitexin	O 22	O MET 383	H-donor	3.04	–1.2
	Ligand	N2 19	OG SER 420	H-donor	2.89	–2.9
		O3 21	OG SER 420	H-donor	2.79	–1.8
		O7 28	NZ LYS 416	H-acceptor	2.79	–10.5
		O7 28	ND2 ASN 546	H-acceptor	2.69	–5.5
7MEQ	kaempherol	O 25	OG SER 436	H-donor	2.87	–1.8
		6-ring	N GLY 462	pi-H	4.23	–1.0
	Quercetin	O 23	OG SER 436	H-donor	3.03	–1
	Luteolin	C 15	SG CYS 465	H-donor	3.75	–0.8
		6-ring	CB GLN 438	pi-H	4.25	–0.7
	Apigenin	O 8	SG CYS 465	H-donor	3.21	–2.9
	Rutin	O 47	O VAL 280	H-donor	2.81	–1.8
		O 62	OE1 GLN 438	H-donor	2.81	–2.6
	Hesperidin	O 69	O GLY 462	H-donor	2.92	–1.8
		O 76	SG CYS 281	H-donor	4.46	–0.7
		O 69	N GLY 462	H-acceptor	2.86	0.2
	Vitexin	O 38	O VAL 280	H-donor	2.74	–1.5
		O 50	5-ring HIS 296	H-pi	4.13	–1.3
		6-ring	CB GLN 438	pi-H	3.98	–1.1
	ligand	N 17	O GLY 464	H-donor	2.73	–1.4
		N 20	OD2 ASP 435	H-donor	3.43	–2.0
		N 20	OG SER 436	H-donor	2.95	–3.9
		N 20	OD1 ASP 435	Ionic	2.86	–5.5
		N 20	OD2 ASP 435	ionic	3.43	–2.2

interaction may improve binding stability and prolong receptor occupancy. ACE2 is known to undergo significant conformational changes characterized by hinge-like motions between its subdomains;[28,29] therefore, conformational flexibility can be influenced by ligand interactions. Studies have shown that binding at various sites on ACE2 influences both the receptor's interaction with viral spike proteins and its intrinsic enzymatic activity via allosteric mechanisms,[30] suggesting that both rutin and hesperidin may contribute to antiviral effects without completely abolishing the physiological function of ACE2.

Additionally, the tested product showed a high affinity for TMPRSS2, with binding energy values ranging from –4.15 to –7.30 kcal/mol, as shown in Table 2 and Fig. S2. Hesperidin displays good affinity toward TMPRSS2, with a binding energy of –7.30 kcal/mol; it formed two hydrogen bonds between the hydroxyl group in the disaccharide rutinose moiety and (O GLY 462 and N GLY 462) and one additional

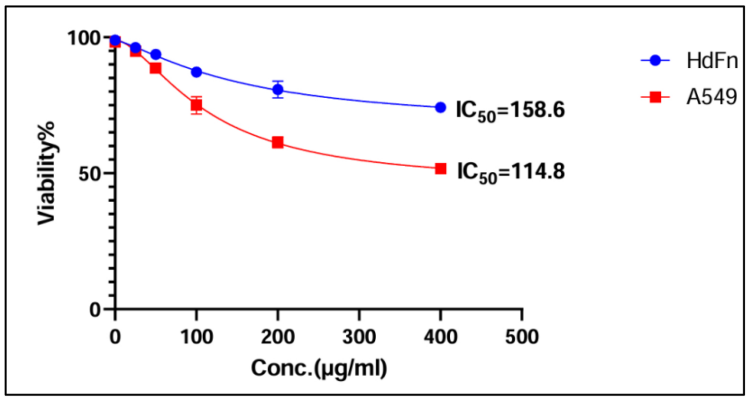

Fig. 4. IC_{50} values of *A. herba-alba* leaf extract against A549 and HdFn cell lines.

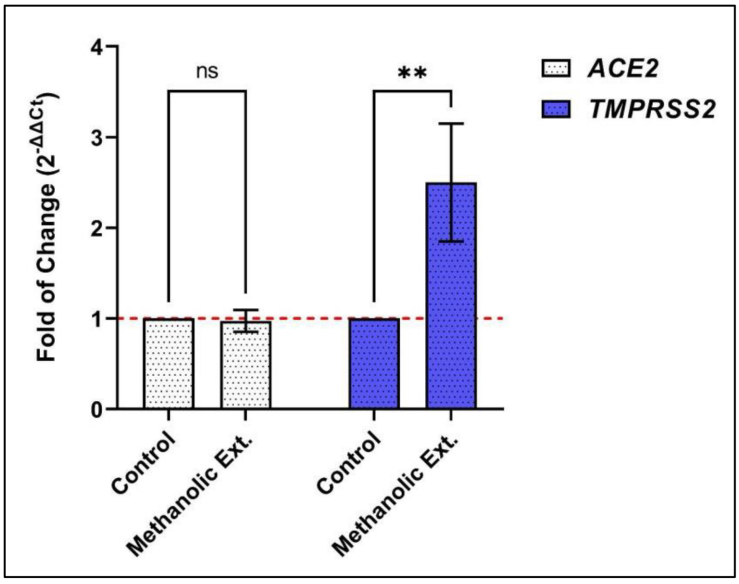

Fig. 5. Expression rate of the ACE2 and TMPRSS2 genes in the 24-treatment NS: Nonsignificant, p = 0.9940, ** p = 0.0011.

hydrogen bond between the phenol group and SG CYS 281 Fig. 3, whereas rutin has the next lowest binding energy (–6.96) kcal/mol. It formed two hydrogen bonds, one between the hydroxyl of the disaccharide rutinose moiety and OE1 GLN 438 and one between phenol and O VAL 280, as shown in Table 3 and Fig. 3. The interaction with residues in the active pocket suggests that these flavonoids may occupy the substrate-binding site or sterically interfere with the catalytic function of the protease, thereby preventing the proteolytic conversion required for SARS-CoV-2 spike protein activation and viral entry.[31] The contribution of the disaccharide rutinose moiety to these binding profiles confirms the importance of glycosylation in improving the binding affinity and position within the active site of the protease.[3]

These results support the potential effects of the flavonoids rutin and hesperidin on viral entry. The presence of disaccharide rutinose moieties may promote hydrogen bonding and binding specificity.

Evaluation of artemisia plant extract on the ACE2 and TMPRSS2 gene expression

This study investigated the effect *of A. herba-alba* leaf extract on virus binding to host cells by evaluating its effect on the gene expression patterns of the ACE2 and TMPRSS2 genes in human lung cell lines that were treated with an IC_{50} (114.8 μg/mL). Fig. 4. The expression of the TMPRSS2 gene was significantly increased by 2.4 ($p \leq 0.01$, p value = 0.0011) at 24 h after treatment with methanol extract, whereas the expression of the ACE2 gene did not significantly differ from that of the B-actin gene ($p \leq 0.01$, p value = 0.9940) Fig. 5.

Nikiforuk et al.[32] and Chen et al.[33] studied the relationship between the ACE2 and TMPRSS2 genes and viral entry into host cells and reported a significant decrease in the expression of these genes in SARS-CoV-2 patients compared with that in uninfected individuals during the acute phase. This decrease may be a response by the host body to block the spread of the virus by reducing ACE2 and TMPRSS2 expression, or the decreased expression of these genes may be a method used by the virus to facilitate its entry and reproduction.[34] In any case, the ability of the SARS-CoV-2 S protein in all variants to decrease ACE2 expression plays an important role in mediating clinically harmful physiological effects.[35]

On the other hand, in both SARS-CoV and SARS-CoV-2, a decrease in TMPRSS2 expression was detected, which contributed to SARS-CoV and SARS-CoV-2 pathology, as it led to the infiltration of leukocytes. These findings support the hypothesis that decreases in ACE2 and TMPRSS2 expression are caused by viruses facilitating infection, replication, and disease progression. In other words, decreased expression of these genes contributes to the severity and spread of the virus.[33] Ali and Khaleel et al.[36] reported that COVID-19 reduces the secretion of ACE2 in the body, leading to damage to the respiratory system. Therefore, the results of this study suggest that *A. herba-alba* leaf extract can produce a compensatory cellular response, which helps restore normal host gene expression. The results in Table 2 refer to hesperidin and rutin, which were the most effective constituents, and in light of the evidence we obtained in this study, the flavonoids studied from *A. herba-alba* leaf extract did not affect the gene expression of ACE2; however, these results contradict the results of Houghton et al. (2024),[16] who reported that quercetin reduced ACE2 gene expression and TMPRSS2 expression in both Calu-3-ALIs and PBECs after 4 hours, but these findings were in good agreement with those of other studies that have proven the effectiveness of some flavonoid extracts against coronavirus. Agrawal et al. (2021)[37] reported the effect of rutin on the virus. In addition, many studies have addressed the role of polyphenols in SARS-CoV-2 infection using kaempferol, quercetin, rutin and hesperidin.[38,39]

Conclusion

Artemisia herba-alba extract was analyzed for its flavonoid profile, and its modulatory effect on two key viral entry genes was studied. Both in vitro and in silico methods were applied to explore the effect of this gene on the expression of the ACE2 and TMPRSS2 genes. Several flavonoid compounds were identified in the extract, with the major ones being kaempferol and hesperidin. *The* in vitro results revealed that "TMPRSS2 gene expression" significantly increased ($p \leq 0.01$) after 24 hours of treatment with the plant extract, with a 2.4-fold increase (p value = 0.0011), whereas ACE2 gene expression did not significantly change compared with that of the reference gene β-actin, and future studies should investigate the protein expression and activity of TMPRSS2 to further validate these findings.

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Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for republication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Basrah.

Authors' contributions statements

K.K.T and Z.T.A designed the study, conducted the methodology and wrote the manuscript. U. M. N. collected and classified the sample, and H. N. A. conducted the molecular docking and interpreted the data. E. Y. A. Prepare the research in accordance with the journal's template and he is corresponding author. All authors have carefully reviewed and approved the final version of the manuscript.

Supplementary materials

Supplementary materials available online at https://bsj.uobaghdad.edu.iq/cgi/viewcontent.cgi?filename=0&article=5315&context=home&type=additional&preview_mode=1.

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تحليل الفلافونويدات، والالتحام الجزيئي، والتأثيرات المعدلة لمستخلص أوراق نبات الشيح الأبيض على التعبير الجيني لـ ACE2 و TMPRSS2

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الخلاصة

أشارت العديد من الدراسات إلى النشاط المضاد للفيروسات المحتمل لأنواع نبات الشيح، والذي يرتبط بشكل أساسي بمكوناتها من الفلافونويدات. يُعد كل من بروتين سيرين بروتياز 2 عبر الغشاء (TMPRSS2) وإنزيم تحويل الأنجيوتنسين 2 (ACE2) من عوامل دخول فيروس SARS-CoV-2 الحاسمة. في هذه الدراسة، تم فحص التركيب الكيميائي النباتي والتأثيرات المعدلة لمستخلص أوراق نبات الشيح الأبيض (Artemisia herba-alba) على التعبير الجيني لكل من ACE2 وTMPRSS2. ولتحديد خصائص الفلافونويدات، استُخدمت تقنية كروماتوغرافيا السائل عالي الأداء (HPLC). قُدِّرت السمية الخلوية في خلايا سرطان A549 وخلايا الأرومة الليفية HdFn باستخدام اختبار MTT (IC50 = 114.8 ميكروغرام/مل). كشفت نتائج تفاعل البوليميراز المتسلسل الكمي (PCR) عن زيادة ملحوظة في التعبير الجيني لـ TMPRSS2 (بمقدار 2.4 ضعف، $p \leq 0.01$؛ قيمة p.v. = 0.0011)، بينما بقي التعبير الجيني لـ ACE2 دون تغيير. علاوة على ذلك، أشارت دراسة المحاكاة الجزيئية إلى احتمالية ارتباط الهيسبيريدين والروتين بـ TMPRSS2 وACE2. لذا، أشارت نتائج كل من التجارب المخبرية والمحاكاة الحاسوبية إلى أن مستخلص نبات A. herba-alba قد يتفاعل مع مسار دخول الفيروس.

الكلمات المفتاحية: إنزيم ACE2، نبات الشيح الأبيض، كوفيد-19، نشاط مضاد للفيروسات طبيعي، TMPRSS2.