Original Article

Ajwa date extract (*Phoenix dactylifera L.*)

Phytochemical analysis, antiviral activity against herpes simplex virus-I and coxsackie B4 virus, and in silico study

Ahmad K. Aljohani, *MSc, PhD,* ***Nader A. Maghrabi,*** *BSc,* ***Osama M. Alrehili,*** *BSc,* ***Abdulaziz S. Alharbi,*** *BSc,* ***Rawad S. Alsihli,*** *BSc,* ***Abdulrahman M. Alharthe,*** *BSc,* ***Rayan S. Albladi,*** *BSc,* ***Khalid A. Alosaimi,*** *BSc,* ***Bader M. Albadrani,*** *BSc,* ***Samar F. Miski,*** *MSc, PhD,* ***Hossein M. Elbadawy,*** *MSc, PhD,* ***Bandar D. Alrehaili,*** *MSc, PhD,* ***Fahd A. Abdelkarem,*** *MSc, PhD,* ***Modather F. Hussein,*** *MSc, PhD.*

ABSTRACT

الأهداف: التحقيق في التركيب الكيميائي النباتي لمستخلص تمر العجوة وتقييم نشاطه المضاد للفيروسات وآلية عمله.

المنهجية: تم استخدام تقنيات الكروماتوجرافيا السائلة عالية الأداء والكروماتوجرافيا الغازية–مطياف الكتلة والكروماتوجرافيا السائلة–مطياف الكتلة لتحليل التركيب الكيميائي النباتي لمستخلص تمر العجوة. تم تقييم النشاط المضاد للفيروسات باستخدام اختبار الامتصاص اللوني ضد فيروس الهربس البسيط (HSVI) وفيروس كوكساكي (CVB–4) حيث تم تقييم آلية العمل ضد فيروس الهربس البسيط باستخدام ثلاثة بروتوكولات مختلفة. كما تم إجراء دراسات الالتحام الجزيئي والحسابات الكيميائية الكمومية للتنبؤ بألفة الارتباط بين المركبات المحددة وجليكوبروتين دي الفيروسي.

النتائج: تم تحديد 17 مركبًا تنتمي إلى فئات مختلفة من المركبات، تتضمن بشكل رئيسي الفلافونويدات ومشتقات الأحماض الفينولية والأحماض الدهنية ومشتقات السكريات. أظهر مستخلص العجوة نشاطًا مضادًا للفيروسات ضد فيروس الهربس سيمبلكس 1 بتركيز IC50 يساوي 113.99±4.67ميكروغرام/مل، بينما أظهر نشاطًا محدودًا ضد فيروس كوكساكي يُعزى النشاط المضاد للفيروسات لمستخلص العجوة بشكل رئيسي إلى تأثيره الواقي على الخلايا من خلال منع التصاق الفيروس بالخلايا المضيفة، بتركيز IC50 يساوي 57.82±1.37 ميكروغرام/مل. وأشارت دراسات الالتحام الجزيئي إلى أن حمض الكلوروجينيك يمتلك أعلى ألفة ارتباط بجليكوبروتين دي الفيروسي، مما يشير إلى دوره المحتمل في تثبيط دخول الفيروس إلى الخلايا المضيفة.

الخلاصة: أظهر مستخلص تمر العجوة نشاطًا واعدًا كمضاد للفيروسات، خاصة ضد فيروس الهربس سيملكس الأول حيث قدمت التحليلات المخبرية والحاسوبية معًا رؤى قيمة حول آلية العمل. وقد مهدت هذه الدراسة الطريق لمزيد من البحث حول التطبيقات العلاجية لتمر العجوة.

Objectives: To investigate the phytochemical composition of Ajwa date extract and evaluate its antiviral activity and mechanism of action.

Methods: High perfomance liquid chromatography, gas chromatography-mass spectrometry, and liquid chromatography-mass spectrometry were used to analyze the phytochemical profile of Ajwa date extract. The antiviral activity was assessed using the MTT colorimetric assay against herpes simplex virus type I (HSV-I) and coxsackievirus B4 (CVB-4). Assessment of the mechanism of action against HSV-I was carried out using 3 protocols. Molecular docking and quantum chemical calculations were carried out to predict the binding affinities of the identified compounds to viral glycoprotein D.

Results: A total of 17 metabolites belonging to different classes of metabolites, mainly flavonoids, phenolic acid derivatives, fatty acids, and sugar derivatives. Ajwa extract exhibited antiviral activity against HSV-I with an IC: 50 of 113.99±4.67 µg/mL, whereas it showed limited activity against CVB-4. The antiviral activity of Ajwa extract was mainly attributed to its cell protectant activity by preventing adherence of viral to host cell with an IC: 50 equal to 57.82±1.37µg/mL. Molecular docking studies indicated that chlorogenic acid had the strongest binding affinity to viral glycoprotein D, which suggests its potential role in inhibiting viral entry into host cells.

Conclusion: The Ajwa date extract demonstrated promising antiviral activity, especially against HSV-I. Integrating in vitro and in silico analyses provided valuable insights into the mechanisms of action.

Keywords: Ajwa dates, antiviral activity, high-performance liquid chromatography, gas chromatography-mass spectrometry, molecular docking

Saudi Med J 2025; Vol. 46 (1): 26-35
doi: 10.15537/smj.2025.46.1.20240780

From the Department of Pharmacognosy and Pharmaceutical Chemistry (Aljohani), College of Pharmacy; from the College of Pharmacy (Maghrabi, Alrehili, Alharbi, Alsihli, Alharthe, Albladi, Alosaimi, Albadrani); from the Department of Pharmacology and Toxicology (Miski, Elbadawy, Alrehaili), College of Pharmacy, Taibah University, Al-Medinah Al-Munawarah, from the Departmet of Chemistry (Hussein), Collage of Science, Jouf University, Aljouf, Kingdom of Saudi Arabia; from the Graduate School of Bioresource and Bioenvironmental Science (Abdelkarem), Kyushu University, Kyushu, Japan; from the Department of Pharmacognosy (Abdelkarem), Faculty of Pharmacy; and from the Department of Chemistry (Hussein), Faculty of Science, Al-Azhar University, Assiut, Egypt.

Received 17th September 2024. Accepted 28th November 2024.

Address correspondence and reprint request to: Dr. Ahmad K. Aljohani, Pharmacognosy and Pharmaceutical Chemistry Department, College of Pharmacy, Taibah University, Al-Medinah Al-Munawarah, Kingdom of Saudi Arabia. E-mail: Akjohani@taibahu.edu.sa
ORCID ID: https://orcid.org/0000-0003-2332-1125

OPEN ACCESS

Palm dates (*Phoenix dactylifera L.*, family: Arecaceae) are widely cultivated and used as a food source and have been a staple diet in Saudi Arabia and the Middle East for thousands of years.[1] Ajwa dates, a unique type of date fruit grown in Al-Madinah Al-Munawarah, Saudi Arabia, are known for their diverse medicinal benefits. Ajwa dates in a different geographic locations contain various secondary metabolites, including glycosides, flavonoids, polyphenolics, and steroids, which identified using different chromatographic and analytical methods.[1,2] Recent investigations highlighted the antiviral potential of natural products rich in phenolic compounds, similar to those found in Ajwa dates, against various viral pathogens.[3] In addition, they enhance health and maintain well-being by offering a rich supply of proteins, vitamins, minerals, lipids, and dietary fibers.[4]

The nutraceutical value of Ajwa dates holds promising potential in managing several diseases, including inflammatory conditions, paralysis, nerve issues, and memory impairment.[5] Studies have reported that Ajwa dates possess anti-inflammatory, anticancer, kidney-protective, liver-protective, and antioxidant properties. These medicinal values are likely attributed to the presence of phenolics, flavonoids, and glycosides.[6]

Herpes simplex virus is classified under the *Hepesviridae* family and is an enveloped double strand DNA virus. It is classified into herpes simplex virus type I (HSV-I) and herpes simplex virus type II (HSV-II), which are primarily transmitted via oral and sexual contact with mucosal surfaces. The surface glycoproteins of this virus are highly conserved through evolution and possess a remarkable capacity to attach to multiple receptors on the host cell.[7] To enable viral entry and calcium signaling, HSV-II glycoprotein H interacts with integrin $\alpha v\beta 3$.[8] Moreover, 4 glycoproteins (namely, gB, gD, gH, and gL) are responsible for transporting the viral genomic material into the host cell.[6]

Infection with HSV-II increases the risk of acquiring and transmitting HIV.[9] If untreated, HSV infections can lead to serious complications, including meningoencephalitis, and widespread infection, especially in immunocompromised patients.[10] In contrast, coxsackie virus B4 (CVB-4) belongs to the genus *Enterovirus* and family *Picornaviridae* which is globally distributed as single-stranded RNA virus. Coxsackie virus B4 infection results in different proinflammatory cytokines production, such as the tumor necrosis factor α and interleukin-6.[11] Therefore, CVB-4 is associated with cardiomyopathy and type-1 diabetes. The CVB-4 can be localized in pancreatic cells, destroying their phenotype or gene expression.[12] Moreover, CVB-4 infection causes fatal thrombotic microangiopathy and acute pancreatitis in adults.[13] Nowadays, foods and their bioactive metabolites play a crucial role in preserving the body's normal physiological mechanism while boosting immunity to fight against viral illnesses. Consistent consumption of antiviral foods could help stop potential pandemics and epidemics, while combining pharmacological agents with natural products may lessen the intensity of viral illnesses.[14] Natural products, such as Ajwa, honey, avocado, and olives, which are used as food, are promising sources for prophylaxis and for the treatment of several diseases, with high degree of safety.[15-18]

Consequently, exploring natural products and diverse metabolites with novel mechanisms of action will open new therapeutic avenues.

There has been a growing interest in the benefits of Ajwa dates, particularly regarding their potential protective effects against viral infections.[19] Few reports are available on the antiviral activity of Ajwa dates against HSV-I, which discussed the virucidal activity of Ajwa date against HSV-1 via in-vivo and in-vitro studies. However, no previous reports regarding the antiviral activity of Ajwa dates cultivated from Saudi Arabia and no clarification for its possible mechanism of action.[20,21] Hence, the chemical characterization of Ajwa date extracts using high performance liquid chromatography (HPLC), gas chromatography mass spectrometry (GC-MS), and liquid chromatography-electrospray ionization-quadrupole time-of-flight-mass spectrometry (LC-ESI-Q-TOF-MS) has attracted immense attention. In this study, the antiviral activity of crude extracts was screened against HSV-I and CVB-4 viruses using MTT assay. Furthermore, the mechanism of action was elucidated employing in vitro and in silico methods, including molecular docking and quantum chemical calculations.

Disclosure. This study was funded by the Deanship of Scientific Research in Islamic University of Al-Madinah Al-Munawarah, Saudi Arabia. The Research Groups Grant (First Call): Number 867.

Methods. All experiments were carried out at Taibah University, Al-Madinah Al-Munawarah, Saudi Arabia, between March and September 2024. Ethical approval was obtained before commencing this work which was approved by the ethics committee at the

College of Pharmacy, Taibah University, Al-Madinah Al-Munawarah, Saudi Arabia

Ajwa dates (0.5 kg) were air-dried, sliced into small portions, and then macerated in 85% methanol (1 L × 3 T) at 25°C for 72 hours each time. Extract of Ajwa was filtered and then a rotary evaporator was used at 45°C to produce a dry and viscous extract (59.0 g).

The HPLC analysis of the Ajwa extract was carried out using the Agilent 1260 (USA) series. Separating samples was carried out using RP-8 (Zorbax eclipse plus, 4.6 mm × 250 mm, 5 µm). Water/0.05 trifluoroacetic acid and acetonitrile/0.05 trifluoroacetic acid (B) were selected for mobile phases in linear gradient manner under the following conditions: 18% B with a 0.09 mL/min of flow rate, 5 mL of injection volume, setting the column temperature at 40°C. The ultraviolet detector was accustomed to 280 nm, while 10 phenolics and 10 flavonoids were used as standards.

Ajwa samples were dissolved in methanol and analyzed using the GC-MS system (Agilent, USA) at Central Laboratories Network. The system comprised GC (7890B) and MS detector (5977A). The analysis was carried out under the following conditions: low bleed DB-5MS column (30 m × 0.25 mm id and 0.25 µm thickness) at a flow rate of 3.0 mL/min (constant), helium as a gas carrier, and an injection volume of 1 µL. The column temperature was elevated gradually by 10°C per minute to 200°C (held for one minute), increased at the rate of 20°C/min to 220°C (held for one minute); and further increased at the rate of 30°C/min to 320°C, which was the final temperature (held for 10 minutes).

The GC-MS spectrallines were detected using the ionization energy under the following conditions: an ionization energy of 70 electron volts, solvent delay time of 3 minutes, a ranging fragment of 50-600 m/z, an injection quantity of 1 µL (split ratio 10:1), and a constant temperature of 250°C. Temperature of injector was set at 250°C while temperature of detector was set at 320°C. The chemical compositions of Ajwa extracts were determined according to comparisons of their retention time, peak area, and peak height. The GC-MS spectral lines with the authenticated compounds database were stored in the National Institute of Standards and Technology (NIST) library.

Chemical profiling of Ajwa extract was carried out using the Agilent 6530 Q-TOF LC/MS (USA). A poroshell column 120 EC-C18 (2.1 × 100 mm, 2.7 µm) was emplyed to carry out the analysis, and mobile phase was employed as follows: A (0.5% acetic acid in H_2O), B (0.5% acetic acid in ACN). The gradient program was as follows: 10-100% B (0.00-50.00 min), with flow rate 200ul/min, and adjusted temperature at 40°C. A 1.00 µL of sample was injected and detected in negative ion mode setting the capillary voltage at 36 V.

Metabolomic non-target screening framework applied through the molecular feature extraction of the ions with similar elution profiles and m/z values using MassHunter Profinder software B.10.00, The data between retention time 0.5 and 50.0 minutes were collected, with mass range of 100-1700 m/z. Resulting data were generated as compound exchange format to Agilent Mass Profiler Professional software package, version 15.1 (Agilent Technologies, CA, USA) for more processing of the differential analysis, also exported to MetaboAnalyst 5.0 for visualization and statistical analysis.

Viruses (HSV-I and CVB-4) and Vero cell lines (ATCC, USA) were maintained in Dulbecco's Modified Eagle Medium (DMEM) with 10% serum, 100 mg/mL streptomycin, and 0.25 mg/mL amphotericin B, incubated at 5% CO_2 and 37°C, all purchased from Thermo Fisher (Waltham, USA). For cytotoxicity and antiviral assays, DMEM with 1.5% fetal bovine serum was used. Cytopathic effect in Vero cells was used to determine the viral titer in triplicates.

The Ajwa's cytotoxic action was determined by MTT colorimetric assay on Vero cell lines.[21-24] Briefly, Vero cells were subjected to 2-times serial dilutions using the crude extract after 24 hours of incubation, and then dissolved in dimethylsulfoxide. A positive control was acyclovir (Merck, USA). Cell viability was checked using the MTT assay in triplicates. Absorbance was recorded at 560 nm. Percentage cell viability (v%) = (V treated) / (V cont.) × 100

The antiviral activity of the Ajwa extract on HSV-I and CVB-4 was assessed using the MTT assay, and the IC50 value was calculated.[22-25]

Briefly, samples of different concentrations and the virus suspension were divided into equal volume (1:1 v/v) and incubated for one hour. Afterwards, 100 µL of the sample/viral suspension was added and placed on a shaking surface which was set at 150 rpm for 5 minutes. The MTT assay was run in triplicates. Antiviral activity was calculated using the following equation:

Percentage of antiviral activity = (Atv-Acv)/(Acd-Acv)×100

The Atv denotes the absorbance of the tested mixtures on the infected cells, Acv denotes the absorbance of the virus control, and Acd refers to the absorbance of the cell control. The minimum inhibitory concentration IC50 was determined from the dose-response curve using a

program based on Microsoft Excel. The procedure was carried out in triplicates.

The challenge dose of the virus (CDV) was applied to Vero cell cultures pretreated with the Ajwa extract in serial dilutions, followed by 3 different protocols to determine its antiviral mechanism. Each protocol was carried out independently to verify consistency, and the average results from the 3 trials for each protocol were reported. Acyclovir was used as a positive control.[25-27]

In protocol A (virucidal), to evaluate the virucidal activity of Ajwa date extract, the CDV was incubated with an equal volume of different concentrations of the extract for one hour.

Protocol B (anti-replicative) was carried out to study the anti-replicative activity of the compound, where the CDV was incubated with Vero cell lines for one hour, followed by the addition of an equal volume of different concentrations of Ajwa date extract.

Protocol C (protective) was intended to evaluate the protective activity and the prevention of adhesion to the cell surface, where different concentrations of Ajwa date extract were initially incubated for one hour with the Vero cell lines, followed by the addition of the CDV. All procedures were then completed as previously mentioned.

To continue exploring the mechanism of antiviral activity of Ajwa, in silico simulations of phenolic acids and flavonoids were achieved using the Molecular Operating Environment (MOE® 2015) within 3 different crystalline forms of proteins in HSV-I (PDB ID: 1JMA, 2C36, and 1LG2) from the Protein Data Bank. The results revealed a mechanism for receptor-mediated activation of viral entry and attachment to host cells. The 3 structures of proteins were assembled based on the MOE LigX protocol and were protonated at a cutoff value of 15 Å. The protein's crystal structure was validated by re-docking the co-crystallized ligand with the docking score and root mean square deviation (Å). The compound's chemical structure of each compound was built, and the energy was minimized using the Chem®Draw and MOE software.

Estimating quantum-chemical of the identified composites were carried out to measure their molecular possessions by the Gauss View 06 and Gaussian 09 program packages.[30,31] Molecular structure, frontier molecular orbital analysis, and surface electrostatic potential of the obtained compounds were determined using Becke-3-Lee-Yang Parr (B3LYP) using the DFT/B3LYP method with the density functional theory (DFT), Becke, 3-parameter, B3LYP level with a basis set of 6-31G in the ground state.[28-30,32]

Statistical analysis. All experiments were carried out in triplicate. Data were shown as the mean and standard deviation. The IC50 was calculated using dose-response curve. Differences between groups were evaluated with simple t-test.

Results.

Phytochemical characterization of the Ajwa crude extract showed the presence of 5 main phenolic acids: coumaric acid, ferulic acid, gallic acid, caffeic acid, and chlorogenic acid, in addition to one flavonoid, quercetin (Figure 1). The phenolic acids present in the crude extract at the highest concentration was gallic acid.

Relating obtained spectral fragmentation patterns with those stored in Wiley and NIST Mass Spectral Library aided in identifying 8 compounds from the crude extract of Ajwa dates. These compounds were mainly sugar derivatives and fatty acids. Two fatty acid derivatives, namely, decanoic acid-9-oxo-methyl ester (14.16 Rt. min.) and hexadecanoic acid (17.68 Rt. min.) were identified, along with 7 metabolites related to sugar, namely, 2-furanomethanol (4.02 Rt. min.), levoglucosenone (4.13 Rt. min.), 2-furancarboxaldehyde-5-methyl (5.18 Rt. min.), 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (5.41 Rt. min.), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (8.41 Rt. min.), and 5-hydroxymethylfurfural (9.71 Rt. min.).

As listed in Table 1, 20 compounds were tentatively identified from the crude extracts of Ajwa dates using LC-ESI-Q-TOF-MS. The identified compounds mainly glycosides and includes 7 flavonoid derivatives, 3 phenolic acid, 5 fatty acid derivatives, and 2 sugars.

The crude extract of Ajwa dates did not exhibit cytotoxic activity against Vero cell lines. The IC50 value was 336.31±4.45 µg/mL (Figure 2), which confirmed its safety in cell lines compared with acyclovir (IC50=362.9±1.92).

The virucidal activity of the crude extract at different concentrations of Ajwa dates against HSV-I and CVB-4 was determined using the MTT assay (Figure 3). The extract showed an antiviral activity against HSV-I, with an IC50 of 113.99±4.67 µg/mL in comparison with acyclovir (IC50 of 29.3±3.16 µg/mL). Conversely, Ajwa extract did not show any activity versus CVB-4, with an inhibition percentage of 6.2±0.23% at the concentration of 125 µg/mL.

The crude extract predominantly exhibited prophylactic activity by preventing viral adherence to the cell, with an IC50 of 57.82±1.37 µg/mL in protocol C, and to a lesser extent, with IC50 values of 118.31±2.28 in protocol B and 123.87±4.44 in protocol A. In comparison, acyclovir showed IC50 values of 27.8±1.1

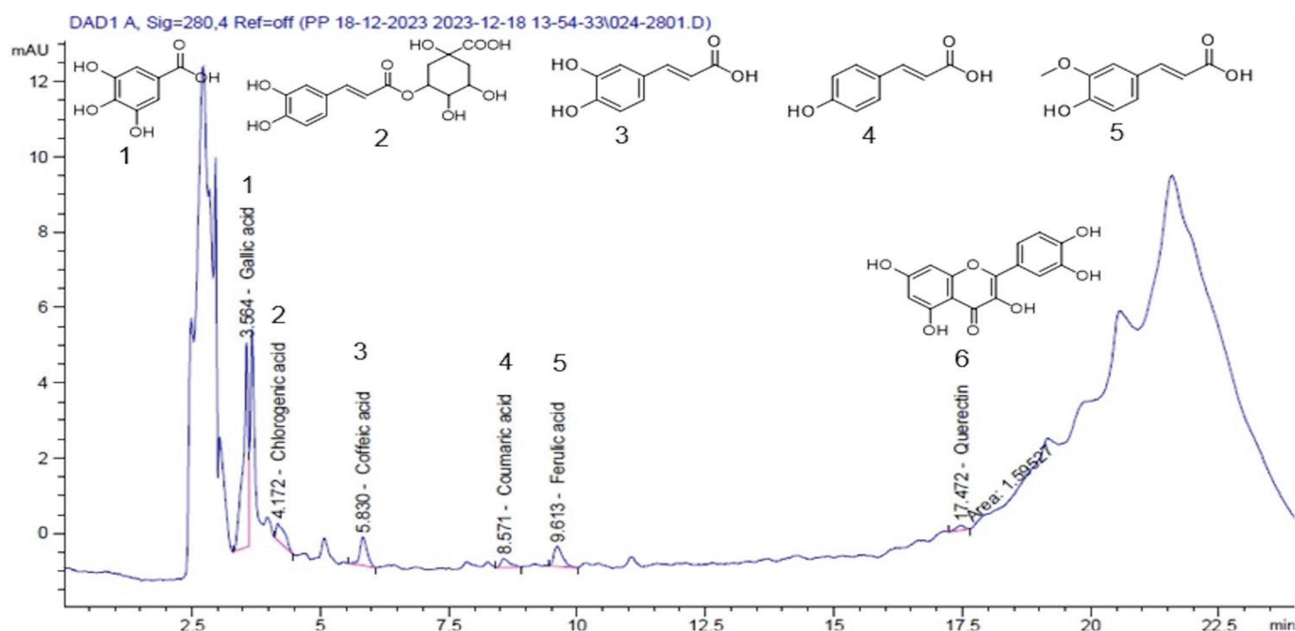

Figure 1 - Chemical structures of the identified secondary metabolites of the crude extract of Ajwa and their quantities (gallic acid 3.56 Rt.min. 60.05% area under the peak, chlorogenic acid 4.17 Rt.min. 9.55% area under the peak, caffeic acid 5.83 Rt.min. 12.49% area under the peak, coumaric acid 8.57 Rt.min. 4.86% area under the peak, ferulic acid 9.61 Rt.min. 10.36% area under the peak, and quercetin 17.47 Rt.min. 2.67% area under the peak) using high performance liquid chromatography.

Table 1 - Liquid chromatography-electrospray ionization-quadrupole time-of-flight-mass spectrometry data of Ajwa extract in negative ionization modes.

Compounds	Molecular formula	Rt. (min)	m/z	Ion species	Error ppm
Flavonoid and their derivatives					
Chrysoeriol hexosyl sulfate	$C_{22}H_{22}O_{14}S$	13.896	541.065	$[M-H]^-$	-0.72
Chrysoeriol rhamnosyl hexoside	$C_{28}H_{32}O_{15}$	14.25	607.1658	$[M-H]^-$	-1.1
Dihydrokaempferol hexoside	$C_{21}H_{22}O_{11}$	4.243	449.1083	$[M-H]^-$	-0.62
Isorhamnetin hexoside	$C_{22}H_{22}O_{12}$	13.781	477.1028	$[M-H]^-$	-1.17
Luteolin rhamnosyl hexoside	$C_{27}H_{30}O_{15}$	12.656	593.15032	$[M-H]^-$	0.50
Orientin	$C_{21}H_{20}O_{11}$	12.14	447.0924	$[M-H]^-$	-0.91
Tectoridin	$C_{22}H_{22}O_{11}$	14.205	461.1084	$[M-H]^-$	-0.52
Phenolic acid and their derivatives					
Caffeoyl shikimic acid	$C_{16}H_{16}O_8$	3.488	335.0771	$[M-H]^-$	-0.11
Caffeoyl shikimic acid hexoside	$C_{22}H_{26}O_{13}$	2.564	497.1304	$[M-H]^-$	0.35
Ferulic acid	$C_{10}H_{10}O_4$	7.61	193.0501	$[M-H]^-$	-0.52
Fatty acid and their derivatives					
Hydroxy octadecenoic acid	$C_{18}H_{34}O_3$	34.024	297.2425	$[M-H]^-$	-1.12
Hydroxy octadecatrienoic acid	$C_{18}H_{30}O_3$	34.058	293.2108	$[M-H]^-$	-1.36
Dihydroxy octadecanoic acid	$C_{18}H_{36}O_4$	41.476	375.2738	$[M-H]^-$	-1.5
Dihydroxy octadecenoic acid	$C_{18}H_{34}O_4$	26.968	313.2369	$[M-H]^-$	-1.6
Trihydroxy octadecenoic acid	$C_{18}H_{34}O_5$	20.303	329.2329	$[M-H]^-$	-0.51
Sugar derivatives					
Glucose	$C_6H_{12}O_6$	0.697	179.0558	$[M-H]^-$	-0.32
Gluconic acid	$C_6H_{12}O_7$	0.821	195.0507	$[M-H]^-$	-0.17

μg/mL in protocol C, 23.4.3±0.63 μg/mL in protocol B, and 17.1±0.37 μg/mL in protocol A.

Initially, analysis of the top-ranked pose of the tested compounds with viral glycoprotein D (1JMA) showed possible molecular interactions and different binding patterns. Chlorogenic acid displayed the strongest connection by forming many important bonds with amino acids such as Asp 147 and Glu 226, with bond

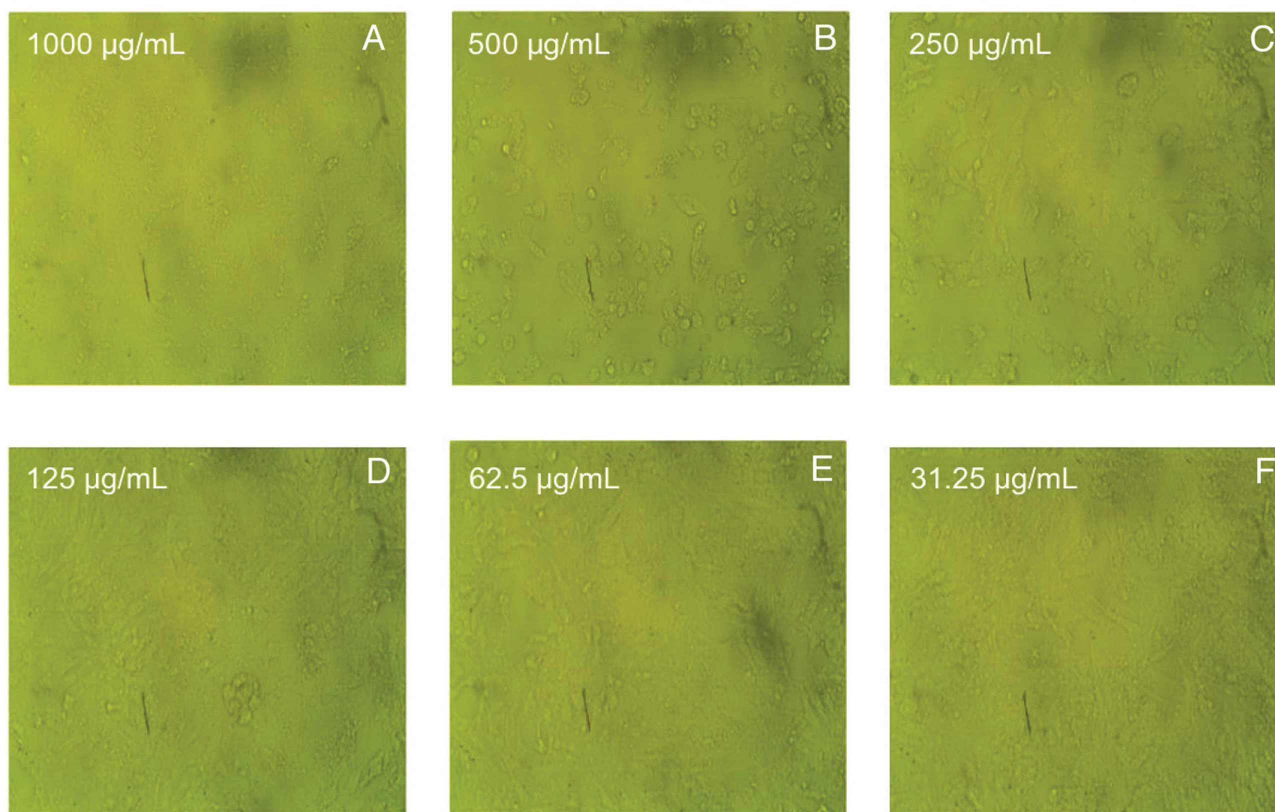

Figure 2 - The cytotoxic activity of crude extract of Ajwa dates at different concentrations; **A**) 1000 µg/mL, **B**) 500 µg/mL, **C**) 250g/mL, **D**) 125 µg/mL, E) 62.5 µg/mL, **F**) 31.25 µµg/mL on VERO cell lines.

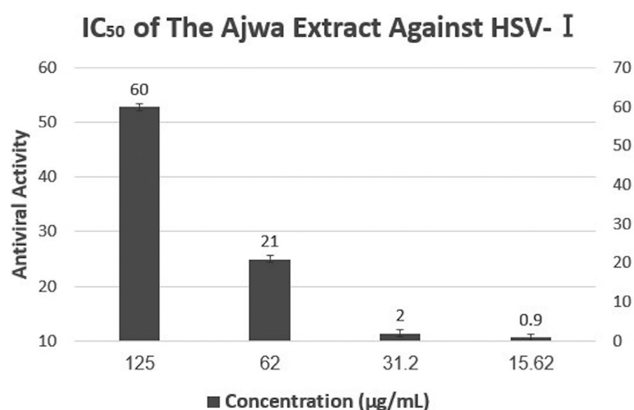

Figure 3 - Virucidal activity of Ajwa date crude extract at different concentrations using the MTT assay.

lengths ranging between 2.99-3.25 Å (Figure 4). In the second order, quercetin interacted with Arg 130 and Thr 29. Coumaric acid, ferulic acid, gallic acid, and caffeic acid had slightly weaker binding energies.

Furthermore, the analysis of the interaction of viral glycoprotein D (2C36) with the identified secondary metabolites revealed that all 6 compounds interacted with multiple residues from sub-pockets. Chlorogenic acid exhibited the strongest connection by forming several important bonds with amino acids such as Asp 30 and Ile 224, with bond lengths ranging between 2.91-3.32 Å (Figure 4). In the second order, quercetin interacted with Arg 130 and Thr 29. Coumaric acid, ferulic acid, gallic acid, and caffeic acid showed slightly weaker binding energies.

For further analysis, 6 selected compounds with glycoprotein D (1L2G) were examined, which indicated the number of molecular interactions and various binding patterns. Chlorogenic acid had superior docking scores by forming several important bonds with amino acids such as Glu 226 and Asn 148, with bond lengths ranging from 2.86-3.34 Å (Figure 4). In the second order, quercetin interacted with Arg 130 and Thr 29. Coumaric acid, ferulic acid, gallic acid, and caffeic acid displayed slightly weaker binding energies.

The molecular properties and reactivity of the identified phenolic acids and flavonoids were measured based on DFT parameters.[28-30] The important DFT

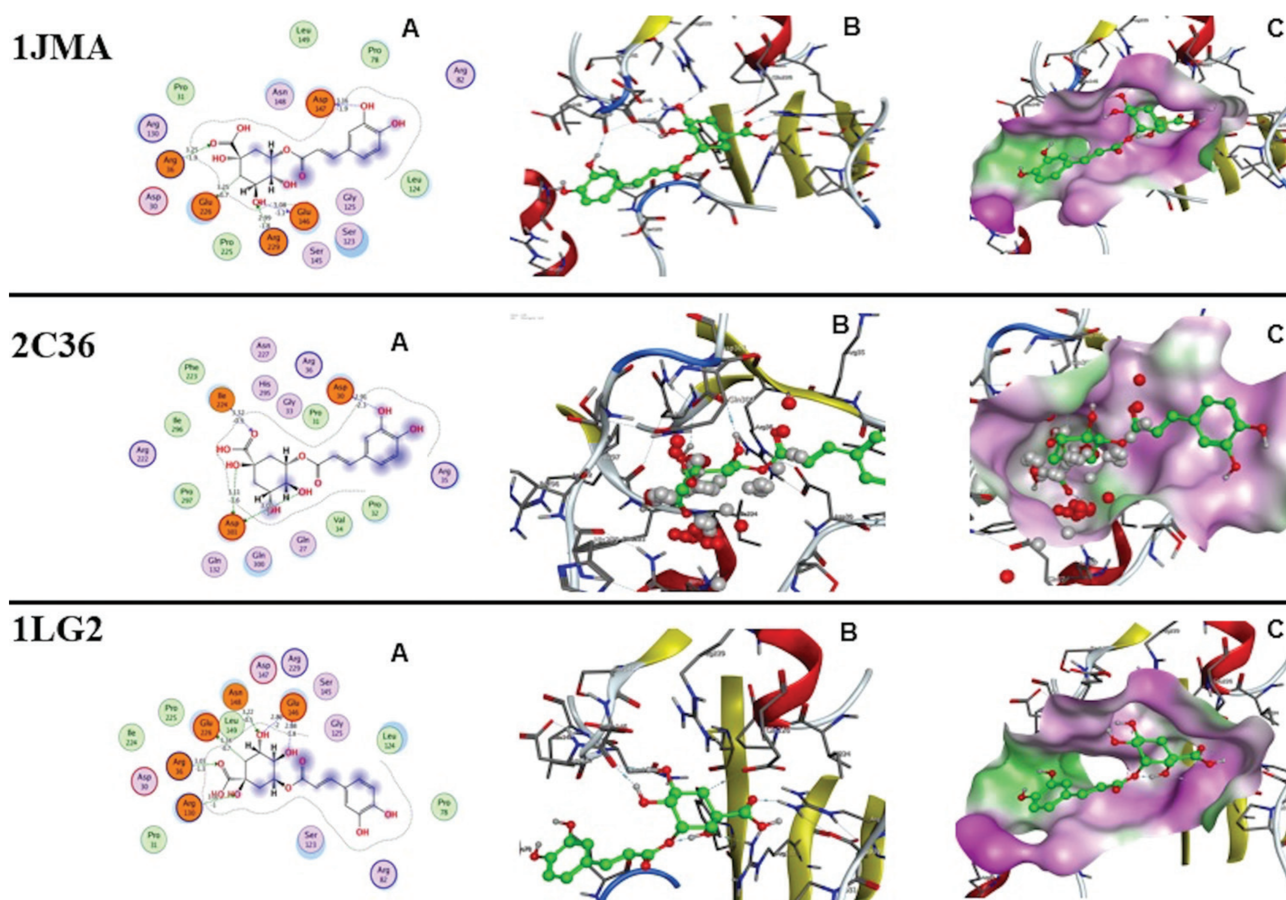

Figure 4 - The chlorogenic acid moiety substrate binding channel formed by several important bonds against 3 different glycoproteins D. **A**) The 2D structure of the formed interaction, **B**) the 3D structure, and **C**) the surface structure.

parameters for measuring the molecular interactions and reactivity of the molecules were chemical hardness (η) and softness (σ). A higher value for softness and a lower value for hardness indicate that the molecule is more reactive, which suggests that it can easily adjust its electron density in response to chemical environments. The electrophilicity parameter is important for understanding the molecule's behavior in charge-transfer reactions.

As shown in Figure 5, all phenolic acids showed higher reactivity than the flavonoid quercetin, with smaller values of (ΔE) and I.

The electrostatic potential profiles of phenolic acids and quercetin differed owing to variations in functional groups, such as the hydroxyl group, as follows: caffeic acid, ferulic acid, chlorogenic acid, and quercetin had multiple hydroxyl groups, which led to a substantial negative charge density around the oxygen atoms. Gallic acid had 3 hydroxyl groups, which resulted in an even more pronounced negative charge distribution than others. Finally, coumaric acid had only one hydroxyl group; hence, the negative charge distribution was less intense. Caffeic acid, ferulic acid, and chlorogenic acid acquired a carboxyl group, contributing to a more negative charge density around the oxygen atom, whereas gallic acid, coumaric acid, and quercetin lacked a carboxyl group, resulting in a less negative charge in the corresponding region. The methoxy group of ferulic acid can contribute to a slight increase in electron density and a change in charge distribution compared with caffeic acid. Consequently, the quinone structure represented by quercetin can lead to a charge distribution different from that of other molecules such as proteins or receptors that may contribute to their activity and interaction with biological macromolecules.

Discussion. The findings from this study highlight the significant antiviral potential of methanolic Ajwa date extract against HSV-I, supporting its usage as antiviral drug. Ajwa extract was analysed by HPLC

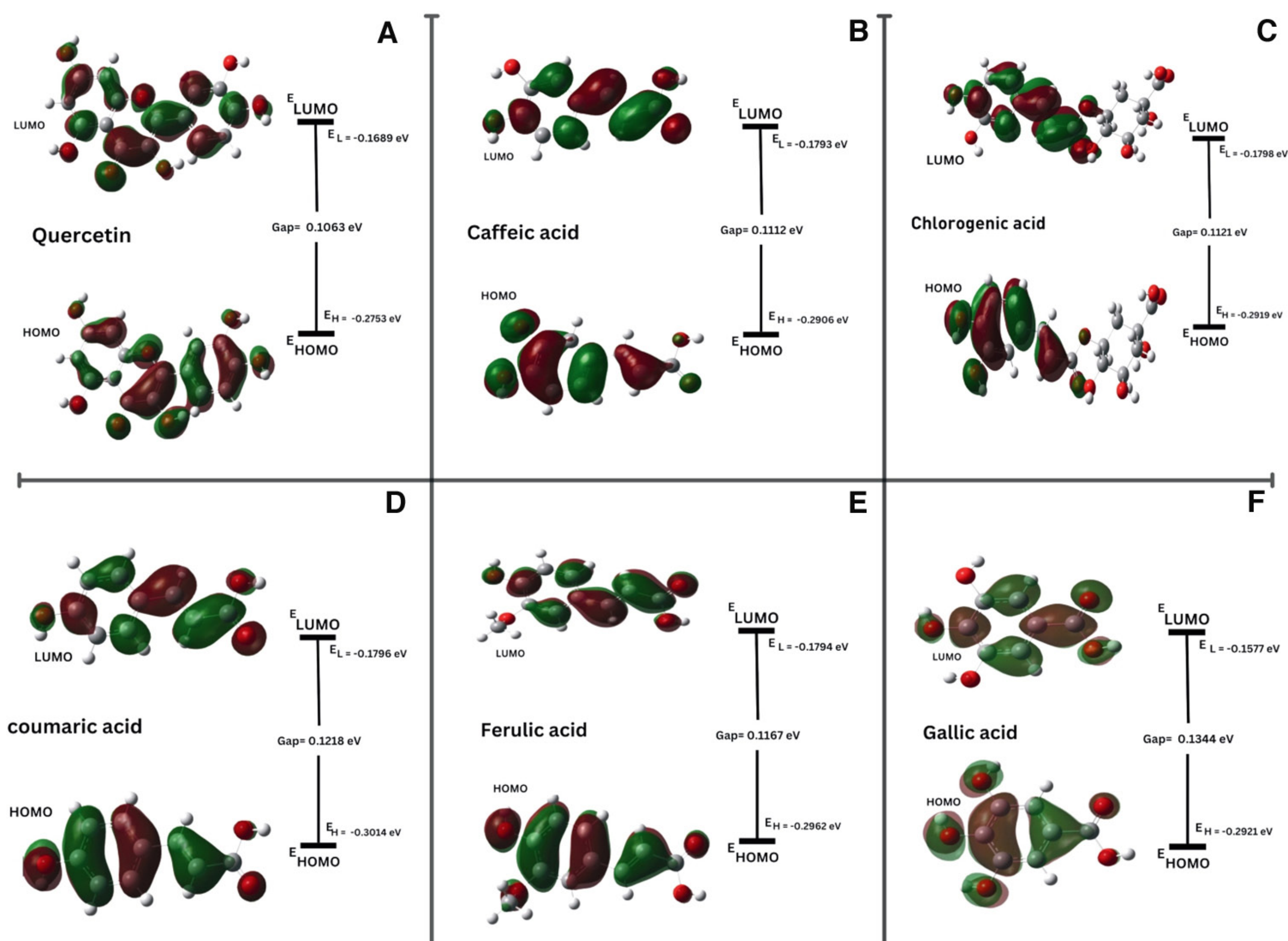

Figure 5 - The energy gaps of the complexes followed an ascending order: **A**) quercetin, **B**) caffeic acid, **C**) chlorogenic acid, **D**) coumaric acid, **E**) ferulic acid, and **F**) gallic acid.

and compared with 20 standards reflected the presence of both 5 phenolic acids and one flavonoid which they can contribute to the observed antiviral activity. Gallic acid was the phenolic acid present in the highest concentration in the crude extract of Ajwa date. Furthermore, the GC-MS analysis clarified that sugar derivatives and fatty acids were the major biomolecules. The sugar derivatives were mainly monosaccharides, with only one trisaccharide, melezitose. Of the 2 identified fatty acids, one was palmitic acid, a saturated fatty acid, and the second was a fatty acid ester. Additionally, 17 metabolites were tentatively identified using LC-Q-TOF-MS from the crude extract of Ajwa. As listed in Table 1, The identified flavonoid glycoside belong to flavone nucleus, one flavonol, in addition to one sulphated flavone.[1-3,5-7] The phenolic acids identified include one aglycone, ferulic acid, and 2 caffeic acid derivatives.[9,10] A total of 5 polyhydroxylated fatty acid and 2 sugar derivatives were also characterized.[11-15]

A total of 3 protocols were used, which differed in the time and priority of adding the virus and the crude extract to Vero cell lines, to study possible mechanisms of action of the extract against HSV-I. The obtained results implied that Ajwa dates exerted antiviral activity, particularly against HSV-I, via various mechanisms but mainly by protecting the cells and preventing viral adherence. This activity can partially be attributed to secondary metabolites such as phenolic acids and flavonoids. These findings came in agreement with previous findings, hinting at the possibility of the secondary metabolites binding to HSV-I adherence proteins.[31-33] Phenolic acid derivatives and flavonoidal glycosides detected by HPLC, LC-MS in Ajwa extract might be synergistically exert its antiviral activity against HSV-I and this conclusion coherent with the previous report regarding virucidal activity of phenolic acid and flavonoids against herpes viruses.[34,35] As mentioned

earlier, one or more identified metabolites could bind to glycoprotein D on HSV-I and prevent its adherence to the host cell membrane. The safety of the extract was confirmed by the results from the cytotoxicity assay in which the maximum non-toxic concentration and IC50 were recorded.

A previous investigation of the most potent compound into the active site of viral glycoprotein D (PDB ID: 1JMA, 2C36, and 1LG2) was carried out to clarify any inhibitory mechanisms, predict binding methods, and interactions. Of the various metabolites, chlorogenic acid demonstrated the strongest binding affinity to viral glycoprotein D in molecular docking studies, suggesting its role in inhibiting viral entry into host cells. The integration of in vitro antiviral assays with in silico molecular docking and quantum-chemical calculations offers a comprehensive understanding of the mechanisms by which Ajwa date extract exerts its effects.

Quantum-chemical calculations were carried out, as shown in **Figure 5** to clarify the degree of reactivity of the phenolics and flavonoid detected by HPLC (**Figure 1**). All of the identified phenolic acids showed variable degree of reactivity from moderate to low with energy difference ranging from (ΔE: 0.11127-0.134404). Furthermore, quercetin showed high degree of reactivity with ΔE equal to 0.10637.

Study limitations. The lack of animal or human testing to elucidate pharmacokinetics, bioavailability, and potential toxicity. Additionally, the study focused solely on 2 viruses, leaving its broader antiviral potential uncertain. While molecular docking provided predictive insights into compound-virus interactions, confirmatory methods, such as binding assays, would strengthen these findings.

In conclusion, the findings from this study are significant as they not only establish the antiviral efficacy of Ajwa dates but also provide a scientific basis for their traditional use in enhancing health and preventing viral infections.

Acknowledgment. *The authors gratefully acknowldege the College of Pharmacy at Taibah University, Al-Madinah Al-Munawarah, Saudi Arabia, for providing a helpful environment to carry out this work. The authors also would like to thank ContentConcepts (www.ContentConcepts.com) for the English language editing.*

References

1. Echegaray N, Gullón B, Pateiro M, Amarowicz R, Misihairabgwi JM, Lorenzo JM. Date fruit and its by-products as promising source of bioactive components: a review. *Food Reviews International* 2023; 39: 1411-1432.
2. Alsuhaymi S, Singh U, Al-Younis I, Kharbatia NM, Haneef A, Chandra K, et al. Untargeted metabolomics analysis of four date palm (*Phoenix dactylifera L.*) cultivars using MS and NMR. *Nat Prod Bioprospect* 2023; 13: 44.
3. Montenegro-Landívar MF, Tapia-Quirós P, Vecino X, Reig M, Valderrama C, Granados M, et al. Polyphenols and their potential role to fight viral diseases: an overview. *Sci Total Environ* 2021; 801: 149719.
4. Mirza MB, Syed FQ, Khan F, Elkady AI, Al-Attar AM, Hakeem KR. Ajwa dates: a highly nutritive fruit with the impending therapeutic application. *Plant and Human Health* 2019; 3: 209-230.
5. Anwar S, Raut R, Alsahli MA, Almatroudi A, Alfheeaid H, Alzahrani FM, et al. Role of Ajwa date fruit pulp and seed in the management of diseases through in vitro and in silico analysis. *Biology (Basel)* 2022; 11: 78.
6. Hamad I, AbdElgawad H, Al Jaouni S, Zinta G, Asard H, Hassan S, et al. Metabolic analysis of various date palm fruit (*Phoenix dactylifera L.*) cultivars from Saudi Arabia to assess their nutritional quality. *Molecules* 2015; 20: 13620-13641.
7. Madavaraju K, Koganti R, Volety I, Yadavalli T, Shukla D. Herpes simplex virus cell entry mechanisms: an update. *Front Cell Infect Microbiol* 2021; 10: 617578.
8. Cheshenko N, Trepanier JB, González PA, Eugenin EA, Jacobs WR Jr, Herold BC. Herpes simplex virus type 2 glycoprotein H interacts with integrin $\alpha v\beta 3$ to facilitate viral entry and calcium signaling in human genital tract epithelial cells. *J Virol* 2014; 88: 10026-10038.
9. Sacks SL, Griffiths PD, Corey L, Cohen C, Cunningham A, Dusheiko GM, et al. HSV-2 transmission. *Antiviral Res* 2004; 63: S27-S35.
10. Altammami GS, Alswayed SK, AlJasser MI, Alkhodair RA. Dermatological conditions in the intensive care unit at a tertiary care hospital in Riyadh, Saudi Arabia. *Saudi Med J* 2024; 45: 834-839.
11. Alidjinou EK, Sané F, Trauet J, Copin MC, Hober D. Coxsackievirus B4 can infect human peripheral blood-derived macrophages. *Viruses* 2015; 7: 6067-6079.
12. Nekoua MP, Bertin A, Sane F, Gimeno JP, Fournier I, Salzet M, et al. Persistence of coxsackievirus B4 in pancreatic β cells disturbs insulin maturation, pattern of cellular proteins, and DNA methylation. *Microorganisms* 2021; 9: 1125.
13. Landers CT, Kuppuswamy V, Evers B, Cardenas CP. A case of fatal thrombotic microangiopathy and acute pancreatitis due to coxsackie B4 infection. *Chest* 2023; 164: A1867.
14. Azam MS, Islam MN, Wahiduzzaman M, Alam M, Dhrubo AAK. Antiviral foods in the battle against viral infections: understanding the molecular mechanism. *Food Sci Nutr* 2023; 11: 4444-4459.
15. Bukhari MA, Qamash RA, Bulkhi RA, Bifari JA, Bakhsh OS, Hawsawi KO, et al. Biological studies of the activity of Manuka honey against *Carbapenem*-resistant *Enterobacterales* (CRE) bacteria. *Saudi Med J* 2024; 45: 876-887.
16. Alshehri FF. Understanding the prevalence, progression, and management of metabolic syndrome in Saudi Arabia. *Saudi Med J* 2023; 44: 973-986.
17. Darshana D, Chutiwitoonchai N, Paemanee A, Sureram S, Mahidol C, Ruchirawat S, et al. Virucidal and antiviral polyhydroxylated fatty alcohols in avocado fruit pulp (*Persea americana*) of booth 7 Cultivar¶. *ACS Food Science & Technology* 2023; 3: 1759-1767.

18. Emwas AM, Al-Rifai N, Szczepski K, Alsuhaymi S, Rayyan S, Almahasheer H, et al. You are what you eat: application of metabolomics approaches to advance nutrition research. ***Foods*** 2021; 10: 1249.
19. Soleman DM, Eldahshan OA, Ibrahim MH, Ogaly HA, Galal HM, Batiha GE, et al. GC/MS analysis, cytotoxicity, and antiviral activities of Annona glabra hexane extract supported by in silico study. ***Molecules*** 2023; 28: 1628.
20. Gomaa HH, Amin DY, Ahmed AR, Ismail NA, El Dougdoug KA, Abd-Elhalim BT. Antimicrobial, antibiofilm, and antiviral investigations using egyptian *Phoenix dactylifera L.* pits extract. ***AMB Express*** 2024; 14: 44.
21. Allahyari S, Pakbin B, Amani Z, Mahmoudi R, Hamidiyan G, Peymani A, et al. Antiviral activity of *Phoenix dactylifera* extracts against herpes simplex virus type 1: an animal study. ***Comparative Clinical Pathology*** 2021; 30: 945-951.
22. Flechas MC, Ocazionez RE, Stashenko EE. Evaluation of in vitro antiviral activity of essential oil compounds against dengue virus. ***Pharmacogn J*** 2018; 10: 55-59.
23. Gescher K, Kühn J, Hafezi W, Louis A, Derksen A, Deters A, et al. Inhibition of viral adsorption and penetration by an aqueous extract from *Rhododendron ferrugineum L.* as antiviral principle against herpes simplex virus type-1. ***Fitoterapia*** 2011; 82: 408-413.
24. Jassim SA, Naji MA. In vitro evaluation of the antiviral activity of an extract of date palm (*Phoenix dactylifera L.*) pits on a *Pseudomonas* phage. ***Evid Based Complement Alternat Med*** 2010; 7: 57-62.
25. Chu Y, Lv X, Zhang L, Fu X, Song S, Su A, et al. Wogonin inhibits in vitro herpes simplex virus type 1 and 2 infection by modulating cellular NF-κB and MAPK pathways. ***BMC Microbiol*** 2020; 20: 227.
26. Luo Z, Kuang XP, Zhou QQ, Yan CY, Li W, Gong HB, et al. Inhibitory effects of baicalein against herpes simplex virus type 1. ***Acta Pharm Sin*** B 2020; 10: 2323-2338.
27. Fahmy NM, Al-Sayed E, Moghannem S, Azam F, El-Shazly M, Singab AN. Breaking down the barriers to a natural antiviral agent: antiviral activity and molecular docking of *Erythrina speciosa* extract, fractions, and the major compound. ***Chem Biodivers*** 2020; 17: e1900511.
28. Parr RG, Yang W. Density-functional theory of atoms and molecules. [Updated 1989; Accessed 2024 July 3). Available from: https://global.oup.com/academic/product/density-functional-theory-of-atoms-and-molecules-9780195092769
29. Parr RG, Szentpály LV, Liu S. Electrophilicity index. ***J Am Chem Soc*** 1999; 121: 1922-1924.
30. Pearson RG. Absolute electronegativity and hardness: application to inorganic chemistry. ***Inorg Chem*** 1986; 27: 734-740.
31. Ortega JT, Serrano ML, Suárez AI, Baptista J, Pujol FH, Cavallaro LV, et al. Antiviral activity of flavonoids present in aerial parts of *Marcetia taxifolia* against hepatitis B virus, poliovirus, and herpes simplex virus in vitro. ***EXCLI J*** 2019; 18: 1037-1048.
32. Kim TI, Kwon EB, Oh YC, Go Y, Choi JG. *Mori ramulus* and its major component Morusin inhibit herpes simplex virus type 1 replication and the virus-induced reactive oxygen species. ***Am J Chin Med*** 2021; 49: 163-179.
33. Čulenová M, Sychrová A, Hassan STS, Berchová-Bímová K, Svobodová P, Helclová A, et al. Multiple in vitro biological effects of phenolic compounds from *Morus alba* root bark. ***J Ethnopharmacol*** 2020; 248: 112296.
34. Šudomová M, Hassan STS. Flavonoids with anti-herpes simplex virus properties: deciphering their mechanisms in disrupting the viral life cycle. ***Viruses*** 2023; 15: 2340.
35. Zangooie S, Ghanbari R, Jalilian FA, Mohammadvand S, Teimoori A. Antiviral potential of phenolic compounds against HSV-1: in-vitro study. ***Antivir Ther*** 2024; 29: 13596535241271589.