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In vitro antiviral activity of thymoquinone and its synergistic effect with acyclovir against herpes simplex virus type 1 (HSV-1)

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ABSTRACT

Introduction: Drug resistance has heightened the demand for natural antiviral agents. Thymoquinone (TQ), a major bioactive component of black seed oil, has demonstrated antiviral activity in numerous studies. However, its potential efficacy against herpes simplex virus type 1 (HSV-1) has not been extensively explored. This research aimed to assess the in vitro antiviral effect of TQ and its synergistic relationship with acyclovir against HSV-1.

Methods: In this experimental study, the cytotoxic effects of TQ and acyclovir on Vero cells were evaluated using the MTT test. The antiviral activity of TQ and acyclovir with sub-cytotoxic doses and combinations with TQ (CC10) on HSV-1 virus replication was investigated using both MTT and 50% tissue culture infectious dose ($TCID_{50}$) assays. Compusyn software was used to analyze the combination index (CI) of TQ and acyclovir.

Results: The 50% cytotoxic concentration (CC_{50}) values of acyclovir and TQ were calculated to be 537.7 and 57.5 µM, respectively. The 50% inhibitory concentration (IC_{50}) values of acyclovir, TQ, and acyclovir together with 10 µM TQ (CC_{10}) were obtained to be 0.25, 13.38, and 0.04 µM, respectively. Based on the $TCID_{50}$ findings, both drugs, whether used separately or in combination, significantly diminished viral titers. Synergistic effects were identified at most concentrations, with the strongest synergy observed at 0.3 µM TQ in combination with 0.03 µM acyclovir.

Conclusion: TQ possesses potent antiviral activity against HSV-1 virus and has synergistic effects with acyclovir to inhibit virus replication. Further studies need to be carried out to assess its utility and safety profile.

Implication for health policy/practice/research/medical education:
The demonstrated antiviral activity of thymoquinone and its synergistic interaction with acyclovir highlight its potential as a promising complementary therapeutic candidate against HSV-1 infection. These findings support further investigation into thymoquinone-based combination therapies to enhance antiviral efficacy, reduce the required drug doses, and minimize the development of drug resistance. Future studies are warranted to evaluate its safety, clarify its mechanisms of action, and assess its translational applicability.

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Introduction

The herpes simplex viruses (HSVs) are among the widespread human pathogens, known to establish lifelong latent infections within hosts that can periodically recur (1,2). They cause a range of infections, varying from mild to severe, including mucocutaneous herpes infection, genital herpes infections, herpes simplex encephalitis, recurrent miscarriage, and meningitis. The manifestation of these infections shows variability according to several factors, such as how the virus enters the human host, how

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strong the host's immunity is, and if this is a primary or recurrent episode. Amongst hosts with reduced immunity and babies, HSV infections often lead to excruciating and debilitating rashes on the skin that can prove to be life-threatening themselves (2,3). Acyclovir and its derivatives are established as first-line therapeutic agents for HSV infections. However, sustained or frequent administration of acyclovir has been associated with an increased incidence of acyclovir-resistant HSV strains (4). The emergence of drug resistance in HSV presents a significant clinical challenge, particularly in immunocompromised individuals. These patients often necessitate prolonged antiviral regimens, and this, coupled with persistent viral replication, escalates the risk of resistance development. The restricted repertoire of available antiviral drugs for HSV infections further complicates the management of drug-resistant cases. Moreover, current antiviral agents are known to induce various adverse effects. Consequently, the identification of natural antiviral compounds exhibiting fewer side effects or the implementation of combination therapies utilizing diverse antiviral drugs holds considerable importance (1).

Numerous studies have demonstrated the significant impact of herbal medicines in the safe treatment of HSV infections (5-7). Among herbal antivirals against HSV are *Aloe vera* extracts (8), *Equisetum arvense* L. and clove (*Syzygium aromaticum*) (5). Medicinal plants can serve as potent alternatives in acyclovir-resistant HSV infections. Further studies are required to discover new uses of antiviral medications, such as structure refinement, synergy between herbal medicine and acyclovir, and confirmation of evidence regarding in vitro and animal studies (7,9).

Thymoquinone (TQ) is a monoterpene and is identified as the main active compound in *Nigella sativa* oil, which is synthesized via the oxidation of thymol under an acidic medium with the help of manganese oxide. TQ possesses promising therapeutic uses against a wide range of diseases (Figure 1).

It has been proven that TQ possesses antiviral properties, especially against the Ebola virus, influenza virus, human immunodeficiency virus, and Zika virus (10).

Combination therapy involving the use of many pharmacological agents offers substantial advantages compared to the use of one drug alone. Using a combination of drugs decreases the likelihood of drug-resistant mutations and reduces the potential for toxicity, since smaller doses of each drug can be used. Drugs used in combination treatments should also have different mechanisms of action, taking into consideration the seriousness of the disease in question (9). Given the documented antiviral properties of TQ against a wide variety of viruses and the lack of studies focusing on its anti-herpesvirus properties, this laboratory-based experiment examined the antiviral activity of TQ and its synergistic interaction with acyclovir against HSV-1.

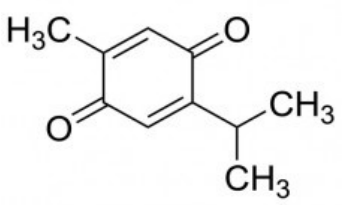

Figure 1. Chemical structure of thymoquinone.

Materials and Methods

Cells and viruses

Vero cells provided by the Cell Bank of the Pasteur Institute of Iran were used for HSV-1 propagation. HSV-1 (KOS strain) was provided by the Virology Laboratory at SKUMS (Iran). A virus stock was prepared on Vero cells, and the virus titer was determined as 50% tissue culture infectious dose ($TCID_{50}$)/mL based on the cytopathic effects (CPEs) in Vero cells. The infective titer of the stock solution was $10^{6/7}$ $TCID_{50}$/mL. Acyclovir (Sigma, USA) and TQ (Sigma-Aldrich, USA) were solubilized initially in dimethyl sulfoxide (DMSO; Samchun Chemical, Korea), followed by dilution in phosphate-buffered saline (PBS) to achieve a uniform, stable stock solution. The final concentration of DMSO in cell culture media was maintained below 0.02%.

Evaluation of cytotoxicity

The cytotoxic effects of the compounds on Vero cells were tested using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma, USA] assay prior to antiviral testing in order to determine their non-toxic concentrations. In summary, confluent Vero cell monolayers grown in 96-well plates were treated with 100 µL of the compounds at various concentrations and incubated for 48 hours. After this time, 50 µL of MTT solution (1 mg/mL in PBS) was added to each well, and incubation continued for a further 4 hours at 37 °C. The medium was then removed and replaced with 100 µL of DMSO to solubilize the formazan crystals. The plates were then shaken for 15 min, after which the absorbance was measured at 640 nm using a microplate reader (StataFax 2100, USA). Cell viability was determined by the equation: Toxicity (%) = [100 – (OD treated /OD control) × 100]. The 50% cytotoxic concentration (CC_{50}) and the 10% cytotoxic concentration (CC_{10}) were calculated from the dose–response curve by regression analysis using GraphPad software (5,11). CC_{10} was considered the maximum non–toxic concentration for antiviral assays in the combination therapy of TQ and acyclovir (12).

Evaluation of antiviral activity by MTT assay

The antiviral activity of the tested compounds was determined by the MTT colorimetric assay, which is the basis for reduction of CPE induced by HSV-1.

Briefly, confluent Vero cell monolayers grown in 96-well microplates were infected with 100 $TCID_{50}$ of HSV-1 for one hour. Following the adsorption period, the viral solution was removed and the cells were treated with 100 µL of culture medium containing different non-cytotoxic concentrations (below the CC_{50}) of acyclovir, TQ, or their combinations. Combination treatments included different concentrations of acyclovir with a concentration of CC_{10} of TQ. The CC_{10} value, representing the maximum non-toxic concentration, was designated as the reference point for evaluating antiviral activity (12,13). Untreated cell controls (no virus or drugs) and virus controls (virus only without treatment) were performed at each step to confirm the validity of the assay. After a 48-hour incubation period at 37 °C in a humid atmosphere containing 5% CO2, cell viability was measured using the MTT assay as described earlier. The viral inhibition percentage was calculated as follows: Antiviral activity (%) = [(OD treated—OD positive control) / (OD cell control—OD virus control)] × 100. All experiments were done in triplicate. The 50% inhibitory concentration (IC_{50}), which is defined as the concentration of compound that blocks viral replication by 50%, was calculated from the dose-response curve by utilizing regression analysis in GraphPad software. SI, calculated as the ratio of CC50 to IC50, was used to assess the therapeutic potential of each compound as an antiviral candidate (5).

Evaluation of antiviral activity by the $TCID_{50}$ assay

The antiviral activity of acyclovir, TQ, or their combinations was further investigated by determining their effect on viral infectivity by the $TCID_{50}$ method. Briefly, confluent Vero cell monolayers grown in 48-well microplates were incubated with 200 µL of culture medium containing different noncytotoxic concentrations of the compounds (below the CC_{50}) in the presence of 100 $TCID_{50}$ of HSV-1. Following incubation at 37°C in a humidified atmosphere with 5% CO2 for 48 hours, supernatants from each well were separately harvested for viral titration. For the determination of viral titer, Vero cells were seeded in 96-well microplates and allowed to form confluent monolayers. Serial tenfold dilutions of the collected viral suspensions were prepared in culture medium containing 2% fetal bovine serum (FBS, Gibco), and 100 µL from each dilution was added to the wells. The plates were further incubated at 37 °C with 5% CO2 until characteristic CPE appeared. The viral titer, expressed as $TCID_{50}$/mL, was estimated using the Reed–Muench formula (14). This assay made it possible to measure the reduction of infectious viral particles in the presence of each compound, providing another determination of their antiviral activity (15).

Evaluation of synergistic effects using CompuSyn software

HSV-1-infected Vero cells were treated with different concentrations of acyclovir and TQ, alone and in combination, for a period of 48 hours. Afterwards, the viral growth inhibition percentage was determined by using the MTT assay as mentioned previously. The interaction between acyclovir and TQ was calculated by CompuSyn software, version 1.0. The combination index values were calculated with this software, and the experiments were done in triplicate. The combination index was interpreted as follows: A CI value less than 1 is considered synergistic; a value of exactly 1 is additive, whereas a combination index value greater than 1 suggests an antagonistic effect between compounds tested (16).

Data analysis

All the experiments were done in triplicate, and the data were expressed as mean and 95% confidence interval (CI). IC_{50} and CC_{50} values were obtained by nonlinear regression analysis using GraphPad Prism version 6.0 (USA). The combination index for drug interaction was analyzed with CompuSyn software version 1.0 (ComboSyn Inc., Paramus, NJ, USA).

Results

Cytotoxicity and antiviral activity using MTT assay

As estimated by nonlinear regression analysis of the MTT assay, there was a significant difference ($P < 0.05$) between the tested concentrations of the compounds regarding Vero cell viability. The CC50 values were 537.7 µM (95% CI: 448.7–705.7) for acyclovir and 57.5 µM (95% CI: 38.6–84.6) for TQ (Figure 2).

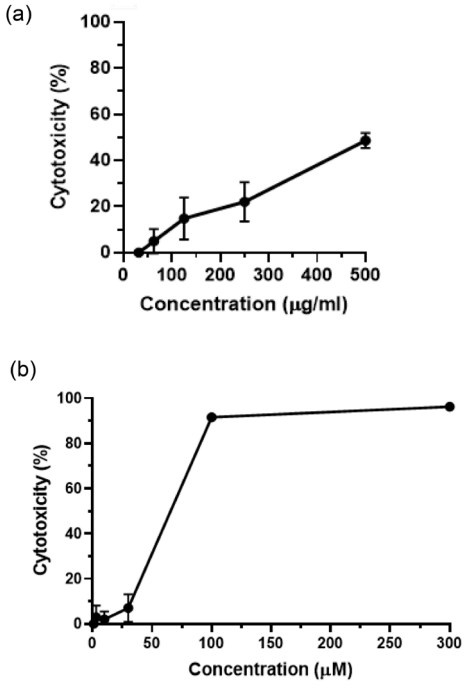

Figure 2. The cytotoxicity effects of acyclovir and thymoquinone. Cytotoxicity was evaluated using the MTT assay. All experiments were performed in triplicate, and the results are presented as the mean± SD; a: Acyclovir, b: Thymoquinon.

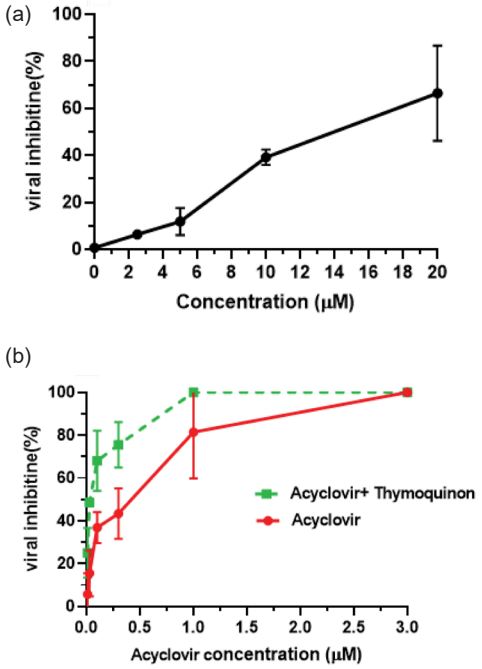

Figure 3. Antiviral activity of acyclovir, thymoquinone, and their combination against HSV-1 based on MTT assay results. The experimental results are presented as the mean± SD, derived from two separate experiments with triplicate measurements in each; a: Thymoquinon, b: Acyclovir and acyclovir with CC_{10} of thymoquinone.

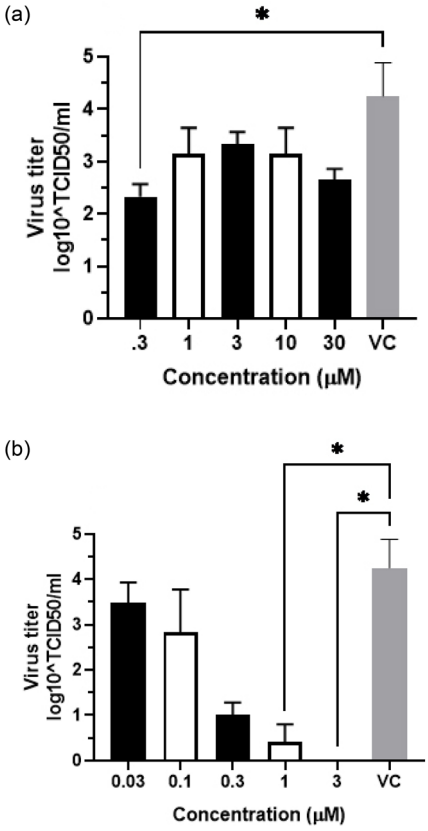

Figure 4. Comparison of viral titers at different concentrations of thymoquinone and acyclovir using the $TCID_{50}$ assay. Statistical analysis was conducted using the Kruskal-Wallis test (*$P < 0.05$); a: Thymoquinon, b: Acyclovir, VC: Virus Control.

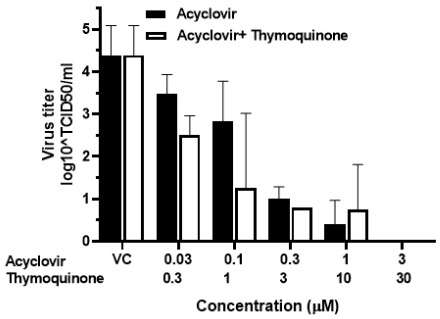

Figure 5. Comparison of viral titers at different concentrations of the acyclovir alone or combined with thymoquinone using the $TCID_{50}$ assay. Results are expressed as the mean ± SD from three separate experimental replicates; VC: Virus Control.

Antiviral activity was tested by an MTT-based CPE reduction assay. The IC_{50} values determined for acyclovir, TQ, and acyclovir combined with the CC10 concentration of TQ were 0.25 µM (95% CI: 0.156–0.39), 13.38 µM (95% CI: 10.95–16.67), and 0.04 µM (95% CI: 0.026–0.05), respectively (Figure 3).

These findings indicate that TQ exhibits significant antiviral activity against HSV-1 in vitro at an SI of 4.3. In addition, the co-administration of 10 μM TQ (cc_{10}) with acyclovir resulted in a sixfold decrease in the effective dose of acyclovir.

Antiviral activity by the $TCID_{50}$ method

The $TCID_{50}$ method was used to determine the viral titer in this study. The outcome showed that the administration of acyclovir and TQ significantly reduced viral titers. Concurrent administration of acyclovir and TQ also enhanced the reduction of viral titer compared to acyclovir administered alone at most tested concentrations ($P < 0.05$; Figures 4 and 5).

Synergistic effect analysis

In an attempt to understand the potential synergistic effect of acyclovir and TQ against the HSV-1 virus, the present study was conducted using CompuSyn software under in vitro conditions. Acyclovir was tested within a concentration range of 0.03-3 µM, while for TQ it was within the range of 0.3-30 µM. The results indicated that the combination of acyclovir and TQ demonstrates a synergistic antiviral effect at most tested concentrations. Maximum synergy was observed with 0.3 µM TQ combined with 0.03 µM acyclovir; the value of combination index obtained was 0.34 (Table 1).

Discussion

The present study aimed to evaluate the in vitro antiviral effect of TQ and its synergistic relationship with acyclovir

Table 1. Synergistic effect of thymoquinone and acyclovir against HSV-1 as determined by CompuSyn software

Acyclovir (μM)	Thymoquinone (μM)	Combination index	Result
3	30	0.47	Synergism
1	10	3.15	Antagonism
0.3	3	0.8	Synergism
0.1	1	0.6	Synergism
0.03	0.3	0.34	Synergism

The combination index interprets the drug interaction as: synergy (combination index < 1), additivity (combination index = 1), or antagonism (combination index > 1). The antiviral effect was measured using the MTT assay.

against HSV-1. The experimental data indicated that exposure to 57.5 μM TQ for 48 hours resulted in a 50% reduction in Vero cell viability. Furthermore, the compound suppressed viral replication within infected cells in a concentration-dependent fashion, with a half-maximal inhibitory concentration (IC_{50}) determined to be 13.38 μM. In addition, TQ exhibited antiviral activity against HSV-1 in vitro, with a selectivity index of 4.3. According to the $TCID_{50}$ results, treatment with TQ resulted in a significant reduction of viral titer, indicating an inhibiting effect on the replication of HSV-1. The herbal compound TQ has been shown to exhibit a wide range of therapeutic properties, particularly antibacterial, antiviral, and antifungal activities (10, 17-19). While the specific anti-HSV efficacy of TQ or black seed oil has not been previously documented in the literature, contemporary research has elucidated its mechanism of action against other viral pathogens. He et al. reported that TQ could inhibit the entry of SARS-CoV-2 by targeting the host protein ADAM17 (20), while another comprehensive review highlighted its ability to modulate oxidative stress and inflammatory signaling pathways, such as NF-κB and MAPK, crucial for viral growth (21). Kumar et al. identified that TQ inhibits Chikungunya virus by affecting the envelope's hydrophobic pocket (22), and Zihlif et al demonstrated that TQ suppressed the proliferation of Epstein–Barr virus in B cells (23). Moreover, in vivo studies conducted by Salem et al. have shown that Nigella sativa oil significantly reduces murine cytomegalovirus (MCMV) titers in infected mice, supporting further the potential antiviral activity of TQ (24).

Our results further showed that the combination of acyclovir with 10 μM TQ, which corresponds to its maximal non-cytotoxic concentration, significantly enhanced the antiviral activity of acyclovir, reducing its IC_{50} value by approximately 6-fold. This combination significantly decreased the HSV-1 viral titer compared with acyclovir alone. Synergy analysis using CompuSyn software revealed a synergistic relationship between TQ and acyclovir at several concentrations tested, with the most notable synergism observed at 0.3 μM TQ combined with 0.03 μM acyclovir. Collectively, these data suggest that TQ acts in synergy with acyclovir to inhibit HSV-1 replication and may be useful as part of a combination therapy.

Preclinical studies have also documented the synergistic interactions between TQ and conventional drugs in various disease models. TQ enhanced the anticancer effect of cisplatin (25), doxorubicin (26), and paclitaxel (27) through increased sensitivity and reduced drug resistance of cancer cells. In addition, Özdal et al reported that TQ showed a synergistic antifungal effect with nystatin against several Candida species (28). In a similar vein, TQ was found to enhance the antibacterial action of antibiotics such as oxacillin, penicillin, and tetracycline, thereby reducing bacterial resistance (29).

Other studies have also been conducted using acyclovir as a combination therapy to improve efficacy and mitigate the emergence of viral resistance. Combination therapies, such as acyclovir with IFN-α, have proven to have more significant antiviral activity against HCMV than as a monotherapy (30). In a similar vein, the combination of acyclovir and CMX001 (a lipid-conjugated, orally bioavailable derivative of cidofovir) showed synergistic inhibition of HSV replication in cell cultures (31). Gong et al. also found that acyclovir combined with the pentacyclic triterpenoid betulin exhibited enhanced antiviral activity against HSV-1 and HSV-2 (32). Such combination therapies are advantageous as they can decrease the likelihood of drug-resistant viral strains emerging and lessen cytotoxicity by allowing for lower dosages of each drug.

Despite these promising findings, several considerations must be addressed. First, since the present study was conducted in vitro, in vivo validation using animal models is essential to evaluate pharmacokinetics, bioavailability, and tissue distribution, particularly in neural and epithelial tissues typically affected by HSV-1. Second, mechanistic studies are warranted to elucidate the molecular pathways involved in viral replication. Given prior evidence that TQ modulates NF-κB, MAPK, and oxidative stress pathways, it is possible that its antiviral activity involves host-directed mechanisms that complement acyclovir's direct inhibition of viral DNA polymerase. Future studies should also assess TQ's potential against acyclovir-resistant HSV strains. Furthermore, formulation development (e.g., TQ-loaded nanoparticles or topical gels) could enhance bioavailability and enable localized delivery, minimizing systemic toxicity.

This study has several limitations. As the experiments were conducted in vitro, the findings may not fully reflect in vivo conditions, including pharmacokinetics and host immune responses. In addition, the precise molecular mechanisms underlying the antiviral and synergistic effects of TQ were not investigated. Further studies are warranted to evaluate its efficacy in vivo and against drug-resistant HSV strains.

Conclusion

In conclusion, this study revealed that TQ possesses significant antiviral activity against HSV-1 in vitro and acts synergistically with acyclovir to inhibit viral replication. These findings support the potential use of TQ as a promising adjuvant in combination therapy for the treatment of infections caused by HSV. However, further in vivo and clinical studies are needed to determine the therapeutic safety, efficacy, and molecular mechanisms of the TQ–acyclovir combination treatment.

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Declaration of AI-assisted tools in the writing procedure

The authors used ChatGPT (OpenAI) solely for minor language editing and stylistic refinement. The tool was employed to enhance the clarity and readability of the manuscript. All scientific aspects of the study, including its design, data acquisition, analysis, and interpretation, were carried out independently by the authors, who assume full responsibility for the accuracy and integrity of the content.

Authors' contribution

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Writing–review & editing: All authors.

Conflict of interests

The authors declare that there is no conflict of interest.

Ethical considerations

Ethical considerations in this study included obtaining permission from the Ethics Committee of Shahrekord University of Medical Sciences (Ethical Code IR.SKUMS.MED.REC.1402.029).

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References

1. Tognarelli EI, Palomino TF, Corrales N, Bueno SM, Kalergis AM, González PA. Herpes simplex virus evasion of early host antiviral responses. Front Cell Infect Microbiol. 2019;9:127. doi: 10.3389/fcimb.2019.00127
2. Piperi E, Papadopoulou E, Georgaki M, Dovrat S, Bar Illan M, Nikitakis NG, et al. Management of oral herpes simplex virus infections: The problem of resistance. A narrative review. Oral Dis. 2024;30(3):877-94. doi: 10.1111/odi.14635
3. Duke ER, Boshier FAT, Boeckh M, Schiffer JT, Cardozo-Ojeda EF. Mathematical modeling of within-host, untreated, cytomegalovirus infection dynamics after allogeneic transplantation. Randomized Controlled Trial. 2021;13(11):2292. doi: 10.3390/v13112292
4. Schalkwijk HH, Snoeck R, Andrei G. Acyclovir resistance in herpes simplex viruses: Prevalence and therapeutic alternatives. Biochem Pharmacol. 2022;206:115322. doi: 10.1016/j.bcp.2022.115322
5. Moradi MT, Karimi A, Alidadi S, Hashemi L. In vitro anti-herpes simplex virus activity, antioxidant potential and total phenolic compounds of selected iranian medicinal plant extracts. Indian J Tradit Know. 2018;17:255-62.
6. Moradi MT, Rafieian-Kopaei M, Karimi A. A review study on the effect of Iranian herbal medicines against in vitro replication of herpes simplex virus. Avicenna J Phytomed. 2016;6(5):506-15. doi: 10.22038/ajp.2016.6567
7. Xu L, Zhong XL, Xi ZC, Li Y, Xu HX. Medicinal plants and natural compounds against acyclovir-resistant HSV infections. Frontiers in microbiology. 2022;13:1025605. doi: 10.3389/fmicb.2022.1025605
8. Ebrahimi E, Mousavi-Jazayeri SM, Rezaee M-B, Parsania M. Antiviral effects of *Aloe vera* (L.) Burm.f. and *Ruta graveolens* L. extract on acyclovir-resistant herpes simplex virus type 1. J Med Plants By-Prod. 2021;10(1):103-8. doi: 10.22092/jmpb.2021.352463.1286
9. Kurokawa M, Nagasaka K, Hirabayashi T, Uyama S, Sato H, Kageyama T, et al. Efficacy of traditional herbal medicines in combination with acyclovir against herpes simplex virus type 1 infection in vitro and in vivo. Antiviral Res. 1995;27(1-2):19-37. doi: 10.1016/0166-3542(94)00076-k.
10. Tania M, Asad A, Li T, Islam MS, Islam SB, Hossen MM, et al. Thymoquinone against infectious diseases: Perspectives in recent pandemics and future therapeutics. Iran J Basic Med Sci. 2021;24(8):1014-22. doi: 10.22038/ijbms.2021.56250.12548
11. Jadhav P, Kapoor N, Thomas B, Lal H, Kshirsagar N. Antiviral potential of selected Indian medicinal (ayurvedic) plants against herpes simplex virus 1 and 2. N Am J Med Sci. 2012;4(12):641-7. doi: 10.4103/1947-2714.104316.
12. Cheng J, Sun N, Zhao X, Niu L, Song M, Sun Y, et al. In vitro screening for compounds derived from traditional Chinese medicines with antiviral activities against porcine reproductive and respiratory syndrome virus. J Microbiol Biotechnol. 2013;23(8):1076-83. doi: 10.4014/

jmb.1303.03074
13. Cavicchioli VQ, Carvalho OV, Paiva JC, Todorov SD, Silva Júnior A, Nero LA. Inhibition of herpes simplex virus 1 (HSV-1) and poliovirus (PV-1) by bacteriocins from *Lactococcus lactis* subsp. *lactis* and *Enterococcus durans* strains isolated from goat milk. Int J Antimicrob Agents. 2018;51(1):33-7. doi: 10.1016/j.ijantimicag.2017.04.020.
14. Baid K, Chiok KR, Banerjee A. Median Tissue Culture Infectious Dose 50 ($TCID_{50}$) Assay to Determine Infectivity of Cytopathic Viruses. In: Thakur A, editor. Intracellular Pathogens: Methods and Protocols. New York, NY: Springer US; 2024. p. 117-23.
15. Ramakrishnan MA. Determination of 50% endpoint titer using a simple formula. World J Virol. 2016;5(2):85-6. doi: 10.5501/wjv.v5.i2.85
16. Chou TC. Drug Combination Studies and Their Synergy Quantification Using the Chou-Talalay Method. Cancer Research. 2010;70(2):440-6. doi: 10.1158/0008-5472.Can-09-1947
17. Houssein M, Fatfat M, Habli Z, Ghazal N, Moodad S, Khalife H, et al. Thymoquinone synergizes with arsenic and interferon alpha to target human T-cell leukemia/lymphoma. Life Sci. 2020;251:117639. doi: 10.1016/j.lfs.2020.117639
18. Khazdair MR, Ghafari S, Sadeghi M. Possible therapeutic effects of Nigella sativa and its thymoquinone on COVID-19. Pharm Biol. 2021;59(1):696-703. doi: 10.1080/13880209.2021.1931353
19. Chatterjee G, Saha AK, Khurshid S, Saha A. A Comprehensive Review of the Antioxidant, Antimicrobial, and Therapeutic Efficacies of Black Cumin (*Nigella sativa* L.) Seed Oil and Its Thymoquinone. J Med Food. 2025;28(4):325-39. doi: 10.1089/jmf.2024.k.0149
20. He J, Liu S, Tan Q, Liu Z, Fu J, Li T, et al. Antiviral Potential of Small Molecules Cordycepin, Thymoquinone, and N6, N6-Dimethyladenosine Targeting SARS-CoV-2 Entry Protein ADAM17. Molecules. 2022;27(24):9044.
21. Shoaib A, Javed S, Wahab S, Azmi L, Tabish M, Sultan MH, et al. Cellular, Molecular, Pharmacological, and Nano-Formulation Aspects of Thymoquinone—A Potent Natural Antiviral Agent. Molecules. 2023;28(14):5435.
22. Kumar R, Nehul S, Singh A, Tomar S. Identification and evaluation of antiviral potential of thymoquinone, a natural compound targeting Chikungunya virus capsid protein. Virology. 2021;561:36-46. doi: 10.1016/j.virol.2021.05.013
23. Zihlif MA, Mahmoud IS, Ghanim MT, Zreikat MS, Alrabadi N, Imraish A, et al. Thymoquinone efficiently inhibits the survival of EBV-infected B cells and alters EBV gene expression. Integr Cancer Ther. 2013;12(3):257-63. doi: 10.1177/1534735412458827
24. Salem ML, Hossain MS. Protective effect of black seed oil from *Nigella sativa* against murine cytomegalovirus infection. Int J Immunopharmacol. 2000;22(9):729-40. doi: 10.1016/s0192-0561(00)00036-9
25. Kotowski U, Heiduschka G, Kadletz L, Fahim T, Seemann R, Schmid R, et al. Effect of thymoquinone on head and neck squamous cell carcinoma cells in vitro: Synergism with radiation. Oncol Lett. 2017;14(1):1147-51. doi: 10.3892/ol.2017.6189
26. Brown RK, Wilson G, Tucci MA, Benghuzzi HA. The effects of thymoquinone and Doxorubicin on leukemia and cardiomyocyte cell lines. Biomed Sci Instrum. 2014;50:391-6
27. Khazaei MR, Bozorgi M, Khazaei M, Moradi A, Bozorgi A. Computational and in vitro analyses on synergistic effects of paclitaxel and thymoquinone in suppressing invasive breast cancer cells. Mol Biol Rep. 2024;51(1):388. doi: 10.1007/s11033-024-09328-5.
28. Özdal Zincir Ö, Özdal U, Ünlü Ö, Demirci M, Katiboğlu AB, Egil E, et al. Synergistic effect of thymoquinone and nystatin in the treatment of oral candidiasis; an in vitro study. Odontology. 2022;110(2):330-7. doi: 10.1007/s10266-021-00667-4
29. Rondevaldova J, Novy P, Urban J, Kokoska L. Determination of anti-staphylococcal activity of thymoquinone in combinations with antibiotics by checkerboard method using EVA capmat™ as a vapor barrier. Arabian J Chem. 2017;10(4):566-72. doi: 10.1016/j.arabjc.2015.04.021
30. Smith CA, Wigdahl B, Rapp F. Synergistic antiviral activity of acyclovir and interferon on human cytomegalovirus. Antimicrob Agents Chemother. 1983;24(3):325-32. doi: 10.1128/aac.24.3.325
31. Prichard MN, Kern ER, Hartline CB, Lanier ER, Quenelle DC. CMX001 potentiates the efficacy of acyclovir in herpes simplex virus infections. Antimicrob Agents Chemother. 2011;55(10):4728-34. doi: 10.1128/aac.00545-11
32. Gong Y, Raj KM, Luscombe CA, Gadawski I, Tam T, Chu J, et al. The synergistic effects of betulin with acyclovir against herpes simplex viruses. Antiviral Res. 2004;64(2):127-30. doi: 10.1016/j.antiviral.2004.05.006