**Original Investigation** | Özgün Araştırma

GMJ 2024;35:243-247

**DOI:** http://dx.doi.org/10.12996/gmj.2024.3846

# Antiviral Activity of *Pistacia atlantica* subsp. Kurdica Extract Against Herpes Simplex Virus Type 1

*Pistacia atlantica* subsp.'nin Antiviral Etkinliği Herpes Simplex Virüsü Tip 1'e Karşı Kurdica Ekstraktı

Shahin Gavanji[1], Hojjat Baghshahi[2], Azizollah Bakhtari[3], Ali Mohamadi[4], Zahra Hamami Chamgordani[5], Mojtaba Khandan[2], Javaher Sinaei[4]

[1]Department of Biotechnology, University of Isfahan Faculty of Advanced Sciences and Technologies, Isfahan, Iran
[2]Barij Medicinal Plants Research Center, Kashan, Iran
[3]Department of Reproductive Biology, Shiraz University of Medical Sciences School of Advanced Medical Sciences and Technologies, Shiraz, Iran
[4]Institute of Traditional Medicine and Herbal Plants of Iran, Isfahan, Iran
[5]Department of Adult Health Nursing, Isfahan University of Medical Sciences Faculty of Nursing and Midwifery, Isfahan, Iran

## ABSTRACT

**Objective:** Most research on the therapeutic effects of wild pistachio species has focused on bacteria and fungi. This study investigated the flavonoid and phenolic contents of different leaves and hulls of *Pistacia atlantica (P. atlantica)* subsp. Kurdica (*P. atlantica* subsp. Kurdica) extracts and their effect on herpes simplex virus 1 (HSV1).

**Methods:** In this study, aqueous, ethanolic, and methanolic extracts of the leaf and hull of *P. atlantica* subsp. Kurdica were prepared by the percolation method. The total phenolic and flavonoid contents in different extracts were measured by UV/Vis spectrophotometry using the Folin-Ciocâlteu reagent and the colorimetric method of aluminum chloride, respectively. On African green monkey kidney cells (VERO), the ethanolic extract of *P. atlantica* subsp. Kurdica was tested for toxicity. Furthermore, 50% cytotoxic and inhibitory concentrations ($CC_{50}$ and $IC_{50}$) were identified.

**Results:** Compared with aqueous and methanolic extracts, the ethanolic extract of the leaf and hull of *P. atlantica* subsp. Kurdica had the highest phenolic and flavonoid concentrations. In addition, our study showed that $CC_{50}$ values were 661.81 and 795.21 µg/mL. Also, $IC_{50}$ values were 97.51 and 110.82 µg/mL for leaf and hull extracts, respectively. The selectivity index for the ethanolic leaf and hull extracts were 6.79 and 7.18, respectively.

**Conclusion:** Our results showed that *P. atlantica* subsp. Kurdica had an anti-HSV1 effect in a dose-dependent manner but at a higher dose than acyclovir. Ethanolic extract of *P. atlantica* subsp. Kurdica is probably a suitable herbal medicine with anti-herpetic effects.

**Keywords:** Antiviral, herbal medicine, pistachio, tradition medicine, VERO

## ÖZ

**Amaç:** Yabani fıstık türlerinin tedavi edici etkileri üzerine yapılan araştırmaların çoğu bakteri ve mantarlara odaklanmıştır. Bu çalışmada, *Pistacia atlantica* (*P. atlantica*) subsp Kurdica (*P. atlantica* subsp. Kurdica) ekstraktlarının farklı yaprak ve kabuklarının flavonoid ve fenolik içerikleri ve bunların herpes simplex virüsü 1 (HSV1) üzerindeki etkileri araştırılmıştır.

**Yöntemler:** Bu çalışmada *P. atlantica* subsp. Kurdica'nın yaprak ve kabuğunun sulu, etanolik ve metanolik ekstraktları süzülme yöntemiyle hazırlandı. Farklı ekstraktlardaki toplam fenolik ve flavonoid içerikleri, sırasıyla; Folin-Ciocâlteu reaktifi ve alüminyum klorürün kolorimetrik yöntemi kullanılarak UV/Vis spektrofotometrisi ile ölçüldü. Afrika yeşil maymun böbrek hücrelerinde (VERO), *P. atlantica* subsp. Kurdica'nın etanolik ekstraktı toksisite açısından test edildi. Ayrıca %50 oranında sitotoksik ve inhibitör konsantrasyonlar ($CC_{50}$ ve $IC_{50}$) belirlendi.

**Bulgular:** Sulu ve metanolik ekstraktlarla karşılaştırıldığında, *P. atlantica* subsp. Kurdica'nın yaprak ve kabuğunun etanolik ekstraktı en yüksek fenolik ve flavonoid konsantrasyonlarına sahipti. Ayrıca çalışmamızda $CC_{50}$ değerlerinin 661,81 ve 795,21 µg/mL olduğu görüldü. Ayrıca yaprak ve kabuk ekstreleri için $IC_{50}$ değerleri sırasıyla 97,51 ve 110,82 µg/mL olarak bulunmuştur. Etanollü yaprak ve kabuk ekstraktlarının seçicilik indeksleri sırasıyla 6,79 ve 7,18 idi.

**Sonuç:** Sonuçlarımız *P. atlantica* subsp. Kurdica'nın doza bağlı olarak ancak asiklovire göre daha yüksek dozda anti-HSV1 etkisine sahip olduğunu gösterdi. *P. atlantica* subsp. Kurdica'nın etanolik ekstraktı muhtemelen anti-herpetik etkileri olan uygun bir bitkisel ilaçtır.

**Anahtar Sözcükler:** Antiviral, bitkisel ilaç, fıstık, geleneksel tıp, VERO

**Address for Correspondence/Yazışma Adresi:** Hojjat Baghshahi, MD, Barij Medicinal Plants Research Center, Kashan, Iran
**E-mail / E-posta:** Baghshahi_h1989@yahoo.com
**ORCID ID:** orcid.org/0000-0003-0540-471X

**Received/Geliş Tarihi:** 04.04.2023
**Accepted/Kabul Tarihi:** 13.05.2024

## INTRODUCTION

Herpes simplex virus 1 (HSV1) is a highly prevalent and contagious virus that can appear as permanent and latent infections with frequent outbreaks of oral lesions (1). Although there is currently no medication for the virus, antiviral drugs are frequently used to suppress and minimize outbreaks. Chemical antiviral drugs have caused the occurrence of allergies, side effects, and drug resistance, and finding new and effective treatments for the prevention and treatment of HSV outbreaks has been considered (2-5). It has been reported that approximately 65-80% of the world's population uses traditional medicine and medicinal plants as complementary medicine (6).

*Pistacia atlantica (P. atlantica)* subsp. Kurdica (*P. atlantica* subsp. Kurdica) is one of the subspecies of wild pistachio *P. atlantica* in the west (Zagros mountains) and less in the central and eastern regions of Iran (7,8). The leaves and fruits of this tree in folk medicine have many properties, including anti-atherogenic, blood sugar-lowering, liver protective, cell-protective, anti-genotoxic, anti-inflammatory, anti-ulcer, antipyretic, anti-fungal, anti-bacterial, anti-parasitic, Anti-mutagenic, antioxidant, and anti-cancer activity, as well as stimulant and diuretic (3,9,10). These pharmacological properties have been used in many diseases therapies (7,10).

Most studies have been conducted on the effects of this plant on bacteria and fungi (10-12), but there are very few reports on its antiviral effects (13). The current study determined the antiviral effects of *P. atlantica* subsp. Kurdica hull and leaf extracts on HSV1.

## MATERIALS AND METHODS

### *Plant Collection and Extraction*

Hulls and leaves of *P. atlantica* subsp. Kurdica were obtained from Ilam Province, Iran. Under sterile conditions, the samples were dried for 10 days in the shade. After that, a grinding machine ground them. Extraction was performed by the percolation method using different solvents such as ethanol, methanol 70%, and water. In brief, 50 g of plant powder was poured into the percolator and soaked in various solvents for 72 h. Then, the solid part was separated from the solvent part using Whatman paper with 11 µm pore sizes. The obtained extracts were then concentrated using a rotary evaporator under vacuum at a temperature of 40 °C. The extracts were kept in small sterile containers at a temperature of 4 °C in a dark place for further tests.

### *Determination of the Total Phenolic Content*

The total phenolic content in samples of different hull and leaf extracts was measured by UV-Vis spectrophotometer using Folin-Ciocâlteu reagent (FCR). Briefly, 1 mg/mL of the various hull and leaf extracts were added to 0.5 mL of FCR and kept for 7 min at room temperature. Then, 0.4 mL of 7.5% sodium carbonate solution was added. After 1 h, the absorbance of different samples was read using a spectrophotometer at 765 nm. Gallic acid was used as a standard to draw the calibration curve. The total phenolic content was reported on the basis of mg of gallic acid equivalent/g of extract powder.

### *Determination of the Total Flavonoid Content*

The aluminum chloride colorimetric assay was used to determine the total flavonoid content in the samples. Thus, 1 mg/mL of each extract was added to 0.1 mL of 10% aluminum chloride and 0.1 mL of 1 M potassium acetate. The solutions were placed at room temperature for 30 min. The total flavonoid content was measured on the basis of mg RE/g of extract powder using the absorbance of a spectrophotometer at 415 nm.

### *Cells and Viruses*

African green monkey kidney (VERO) cells and HSV1 were obtained from the Traditional Medicine Research Institute, Isfahan, Iran. VERO cells were cultured in Eagle's minimum essential medium enriched with 10% newborn calf serum, 100 U/mL penicillin (Gibco), and 100 µg/mL streptomycin sulfate (Gibco).

### *Cytotoxicity Assay*

The cytotoxic effects of different extracts were determined by a photometric technique using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (14). In each well of the 96-well plate, 180 microliters of cell suspension (VERO cells) equal to $3x10^4$ µL cells were added. The extracts were added to the wells at different concentrations from 50 to 1000 µg/mL. The culture medium containing 0.5% dimethyl sulfoxide (DMSO) without plant extract was considered a negative control, whereas the antiviral drug acyclovir was considered a positive control. The plate was incubated for 48 h in an incubator with 5% $CO_2$ at 37 °C. Then 20 µL of MTT solution was added to the wells and incubated for 2 h. The 100 µL of DMSO was poured into the wells and mixed well to dissolve the formazan crystals. The absorbance was determined at 560 nm using an ELISA reader. The VERO cell survival percentage was estimated using the following equation, and for the positive and negative control groups, 100 was considered. A concentration of the tested compounds that reduces the cell survival rate by half was considered $IC_{50}$.

$$\text{VERO cell survival percentage} = \left[\frac{\text{Test compound OD} - \text{Blank OD}}{\text{Negative control OD} - \text{Blank OD}}\right] \times 100$$

### *Antiviral Activity*

A plaque reduction test on VERO cells was used to determine the anti-HSV1 activity of the hull and leaf extracts. In each well of a 24-well plate, $400x10^3$ µL of VERO cells were incubated in 1 mL of Dulbecco's Modified Eagle Medium (DMEM) containing 3% inactivated FBS for 24 h. Then one µL of thawed viral suspension was added to each well. After 10 min, 20 µL of different concentrations of extracts were added to each well along with 2 mL of preheated DMEM medium with methylcellulose. In the negative and positive control groups, 20 µL DMSO diluted with deionized water and 20 µL acyclovir were added to the wells. The plates were incubated for 48 h and stained. Plaques were counted in each well, and the inhibition percentage of each extract concentration was determined using the following equation:

$$\text{Percentage of plaque inhibition} = \left[1 - \frac{(\text{number of plaque}) \text{ tested}}{\text{Number of control plaque}}\right] \times 100$$

### *Statistical Analysis*

ANOVA was used to analyze the data using GraphPad Prism 6 software (GraphPad Software, La Jolla, CA, USA), and Tukey's multiple comparison tests were used to compare means. Differences with a $p<0.05$ were considered significant.

## RESULTS

The phenolic and flavonoid contents of the ethanolic, methanolic, and aqueous extracts of the leaf and hull are shown in Figure 1. The present results showed that the phenolic and flavonoid contents were the highest in the ethanolic extract and the lowest in the aqueous extract.

Antiviral activity indices of ethanolic extracts of the leaves and hulls of *P. atlantica* subsp. Kurdica are shown in Table 1. The ethanolic extract of the hull had higher indices than the leaf. The antiviral activity of each extract was expressed as a selectivity index (SI), which was determined to be 7.18 and 6.79 for hull and leaf extracts, respectively (Table 1).

$CC_{50}$ leaf: y=-0.2027x + 184.15,

$CC_{50}$ hull: y=-0.1627x + 179.38,

$IC_{50}$ leaf: y=1.5445x - 100.61,

$IC_{50}$ hull: y=1.8442x - 154.37.

The survival rate of VERO cells at concentrations of 400 to 900 µg/mL for leaf and 400 to 1100 µg/mL for hull showed that the cell survival rate decreased with increasing dose (Figure 2). Ethanolic extracts of the leaves and hulls of *P. atlantica* subsp. Kurdica at the concentration of 400-500 and 400 µg/mL, respectively, caused the maximum survival rate, and at concentrations of 900 and 1100 µg/mL, respectively, the survival rate was equal to zero.

The anti-herpes activity of the ethanolic extracts of the leaf and hull has been shown in comparison with that of acyclovir (Figure 3). The results of this study stated that the concentration range of anti-herpes activity of leaf and hull extracts was from 60 to 130 and 80 to 140 µg/mL, respectively, which increased with the dose of this antiviral activity. In the positive control group (acyclovir), this range was 0.04 to 0.4 µg/mL.

**Table 1.** Assessment of $CC_{50}$, $IC_{50}$, and selectivity index in ethanolic extracts of the leaves and hulls of *P. atlantica* subsp. Kurdica

| Extracts | $CC_{50}$ (µg/mL) | $R^2$ (%) | $IC_{50}$ (µg/mL) | $R^2$ (%) | $CC_{50}/IC_{50}$ (SI) |
|---|---|---|---|---|---|
| Leaf | 661.81 | 99.51 | 97.51 | 98.12 | 6.79 |
| Hull | 795.21 | 94.92 | 110.82 | 97.53 | 7.18 |

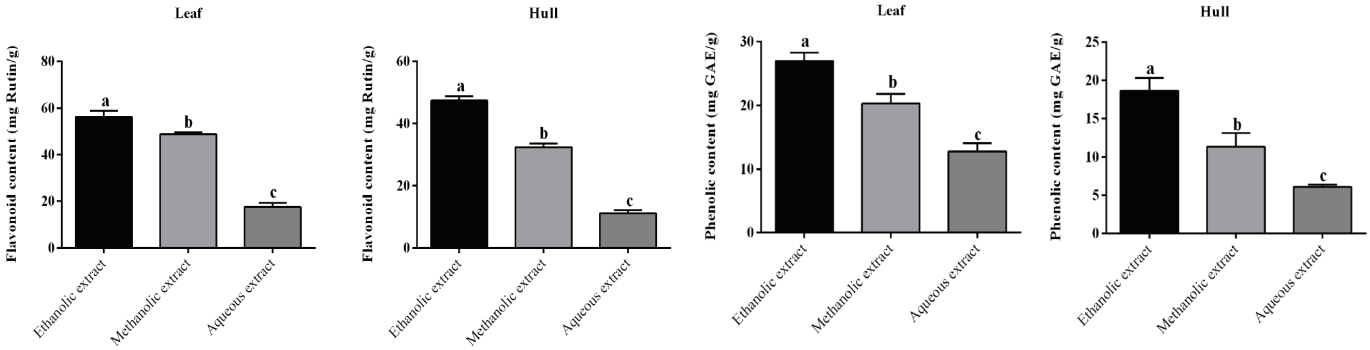


**Figure 1.** Phenolic and flavonoid contents of different leaf and hull extracts of *P. atlantica* subsp. Kurdica. Each value refers to the mean ± standard variation. [a-c]Different letters in each bar indicate significant differences at the 5% level.

*GAE: Gallic acid equivalent.*

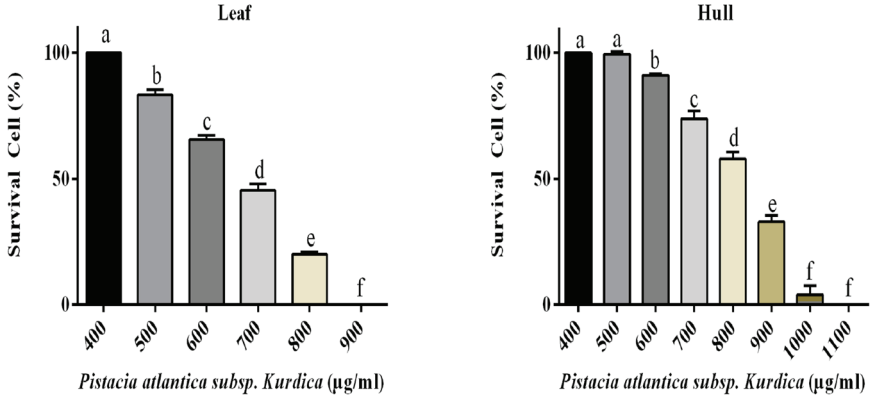


**Figure 2.** VERO cell survival at different concentrations of ethanolic extracts of leaf and hull of *P. atlantica* subsp. Kurdica. Each value refers to the mean ± standard variation. [a-f]Different letters in each bar indicate significant differences at the 5% level.

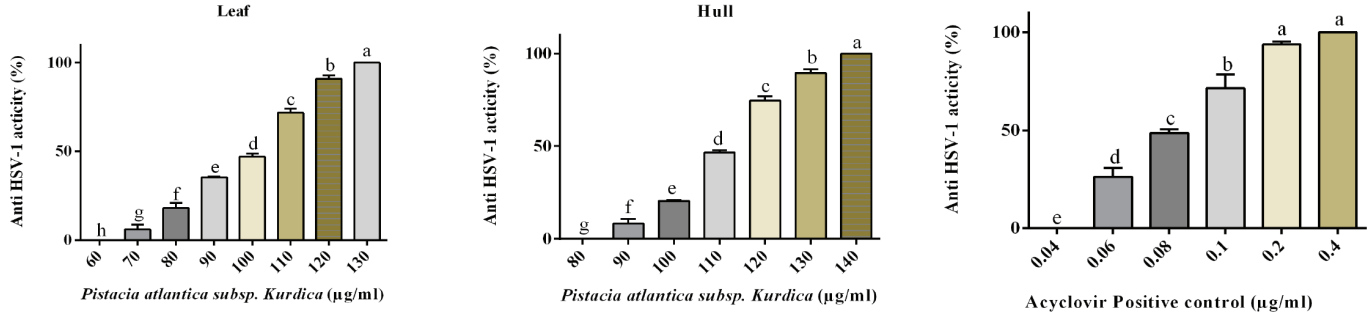


**Figure 3.** Antiherpes simplex virus-1 activity of ethanolic extracts of the leaves and hulls of *P. atlantica* subsp. Kurdica compared with acyclovir. Each value refers to the mean ± standard variation. [a-e]Different letters in each bar indicate significant differences at the 5% level.

*HSV1: Herpes simplex virus 1.*

## DISCUSSION

According to reports, many traditional medicinal plants and herbs have potent antiviral activity (15). Some antiviral constituents include various structural classes such as flavonoids, tannins, alkaloids, terpenes, coumarins, proteins, peptides, polysaccharides, lignans, naphtho- and anthraquinones (16,17). The antiviral activity, and the antimicrobial and antifungal activities of pistachio, is related to phenolic and flavonoid compounds (18-20). The main phenolic compounds of the *Pistacia* genus include caffeic acid, catechin, eryodictiol-7-O-glucoside, gallic acid, protocatechuic acid, ellagic acid, ursolic acid, chlorogenic acid, ferulic acid, juglone, synaptic acid, and vanillic acid (13,18). However, there have been few studies on the antiviral effect of wild pistachio species (13). Our findings confirm the anti-HSV1 effect of *P. atlantica* subsp. Kurdica. Chlorogenic acid and caffeic acid have antiviral effects (21).

The antibacterial, antifungal, and antiviral effects of various sections of cultivated Vera pistachios were investigated by Ozçelik et al. (22). Their results showed that these extracts have significant antibacterial and antifungal effects. They also showed high antiviral effects against HSV and parainfluenza virus; therefore, its seed and kernel extracts showed higher antiviral effects than other plant organs (22). In addition, the antiviral effects of 75 plant species have been investigated against herpes virus, Sindbis virus, and poliovirus. Owing to its phenolic compounds, *Pistacia lentiscus* extract was found to have the greatest antiviral action against the herpes virus (23).

Our study showed that $CC_{50}$ values were 661.81 and 795.21 µg/mL. Also, $IC_{50}$ values were 97.51 and 110.82 µg/mL for leaf and hull extracts, respectively. The SI for the ethanolic leaf and hull extracts were 6.79 and 7.18, respectively. In line with our study, Karimi et al. (13) showed anti-adenovirus effects of ethanolic extract and n-butanol fraction of *P. atlantica* leaf. They stated that $CC_{50}$, $IC_{50}$, and SI on Hep-2 cells were 434.7, 16.37 µg/mL, and 26.5.

Musarra-Pizzo et al. (18) demonstrated that polyphenol-rich extracts of natural *Pistacia vera L.* shelled at 0.4, 0.6, and 0.8 mg/mL concentration reduced the expression of the viral proteins ICP8, UL42, and US11, and finally lead to minimize of viral DNA synthesis in VERO cells. They reported values of $CC_{50}$, $IC_{50}$, and SI equal to 1.2, 0.4, mg/mL, and 3. These differences may be caused by the pistachio species or the tested cells.

## CONCLUSION

The results of this study showed that the ethanol extract of wild pistachio leaves and hull both have similar anti-HSV1 effects. These extracts are comparable to acyclovir at a higher dose. Further clinical research is required to confirm the therapeutic effects of anti-HSV1.

### *Ethics*

**Ethics Committee Approval:** Ethic committee approval is not required.

**Informed Consent:** Informed consent approval is not required.

### *Author Contributions*

Concept: S.G., H.B., Design: S.G., H.B., A.B., Analysis or Interpretation: A.B., Literature Search: A.M., Z.H.C., M.K., J.S., Writing: S.G., H.B.

**Conflict of Interest:** No conflict of interest is declared by the authors.

**Financial Disclosure:** The authors declared that this study received no financial support.

## REFERENCES

1. Bandara HMHN, Samaranayake LP. Viral, bacterial, and fungal infections of the oral mucosa: Types, incidence, predisposing factors, diagnostic algorithms, and management. Periodontol 2000. 2019; 80: 148-76.
2. Crimi S, Fiorillo L, Bianchi A, D'Amico C, Amoroso G, Gorassini F, et al. Herpes Virus, Oral Clinical Signs and QoL: Systematic Review of Recent Data. Viruses. 2019; 11: 463.
3. Rahman HS. Phytochemical analysis and antioxidant and anticancer activities of mastic gum resin from Pistacia atlantica subspecies kurdica. Onco Targets Ther. 2018; 11: 4559-72.
4. Ambika AP, Nair SN. Wound Healing Activity of Plants from the Convolvulaceae Family. Adv Wound Care (New Rochelle). 2019; 8: 28-37.
5. Mulat M, Pandita A, Khan F. Medicinal Plant Compounds for Combating the Multi-drug Resistant Pathogenic Bacteria: A Review. Curr Pharm Biotechnol. 2019; 20: 183-96.
6. Pan SY, Litscher G, Chan K, Yu ZL, Chen HQ, Ko KM. Traditional medicines in the world: where to go next? Evid Based Complement Alternat Med. 2014; 2014: 739895.
7. Hatamnia AA, Rostamzad A, Hosseini M, Abbaspour N, Darvishzadeh R, Malekzadeh P, et al. Antioxidant capacity and phenolic composition of leaves from 10 Bene (Pistacia atlantica subsp. kurdica) genotypes. Nat Prod Res. 2016; 30: 600-4.

8. Shahghobadi H, Shabanian N, Rahmani M-S, Khadivi A. Genetic characterization of Pistacia atlantica subsp. kurdica from northern Zagros forests in Iran. Trees. 2019; 33: 481-90.
9. Peksel A, Arisan-Atac I, Yanardag R. Evaluation of antioxidant and antiacetylcholinesterase activities of the extracts of Pistacia atlantica Desf. Leaves. J Food Biochem. 2010; 34: 451-76.
10. Ben Ahmed Z, Yousfi M, Viaene J, Dejaegher B, Demeyer K, Heyden YV. Four Pistacia atlantica subspecies (atlantica, cabulica, kurdica and mutica): A review of their botany, ethnobotany, phytochemistry and pharmacology. J Ethnopharmacol. 2021; 265: 113329.
11. Gerchman Y, Inbar M. Distinct antimicrobial activities in aphid galls on Pistacia atlantica. Plant Signal Behav. 2011; 6: 2008-12.
12. Azeez SH, Gaphor SM. Evaluation of Antibacterial Effect against Porphyromonas gingivalis and Biocompatibility of Essential Oil Extracted from the Gum of Pistacia atlantica Kurdica. Biomed Res Int. 2019; 2019: 9195361.
13. Karimi A, Moradi MT, Gafourian A. In vitro anti-adenovirus activity and antioxidant potential of Pistacia atlantica Desf. leaves. Res J Pharmacogn. 2020; 7: 53-60.
14. Batool S, Javaid S, Javed H, Laiba Asim, Shahid I, Khan M, et al. Addressing artifacts of colorimetric anticancer assays for plant-based drug development. Med Oncol. 2022; 39: 198.
15. Garber A, Barnard L, Pickrell C. Review of Whole Plant Extracts With Activity Against Herpes Simplex Viruses In Vitro and In Vivo. J Evid Based Integr Med. 2021; 26: 2515690X20978394.
16. Lozada-Ramírez JD, Ortega-Regules AE, Hernández LR, Anaya de Parrodi C. Spectroscopic and spectrometric applications for the identification of bioactive compounds from vegetal extracts. Appl Sci. 2021; 11: 3039
17. Liu AL, Du GH. Antiviral Properties of Phytochemicals. Dietary Phytochemicals and Microbes. 2012: 93–126.
18. Musarra-Pizzo M, Pennisi R, Ben-Amor I, Smeriglio A, Mandalari G, Sciortino MT. In Vitro Anti-HSV-1 Activity of Polyphenol-Rich Extracts and Pure Polyphenol Compounds Derived from Pistachios Kernels (Pistacia vera L.). Plants (Basel). 2020; 9: 267.
19. Mandalari G, Barreca D, Gervasi T, Roussell MA, Klein B, Feeney MJ, et al. Pistachio Nuts (Pistacia vera L.): Production, Nutrients, Bioactives and Novel Health Effects. Plants (Basel). 2021; 11: 18.
20. Rezaie M, Farhoosh R, Pham N, Quinn RJ, Iranshahi M. Dereplication of antioxidant compounds in Bene (Pistacia atlantica subsp. mutica) hull using a multiplex approach of HPLC-DAD, LC-MS and (1)H NMR techniques. J Pharm Biomed Anal. 2016; 117: 352-62.
21. Jassim SA, Naji MA. Novel antiviral agents: a medicinal plant perspective. J Appl Microbiol. 2003; 95: 412-27.
22. Ozçelik B, Aslan M, Orhan I, Karaoglu T. Antibacterial, antifungal, and antiviral activities of the lipophylic extracts of Pistacia vera. Microbiol Res. 2005; 160: 159-64.
23. Agut H, Bonnafous P, Gautheret-Dejean A. Human Herpesviruses 6A, 6B, and 7. Microbiol Spectr. 2016;4.