Archive of SID

Research Journal of Pharmacognosy (RJP) 7(2), 2020: 53-60
Received: 24 Nov 2019
Accepted: 1 Mar 2020
Published online: 7 Mar 2020
DOI: 10.22127/rjp.2020.208324.1534

Original article

In Vitro Anti-adenovirus Activity and Antioxidant Potential of *Pistacia atlantica* Desf. Leaves

Ali Karimi[1], Mohmmad Taghi Moradi[2*], Asghar Gafourian[3]

[1]Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran.
[2]Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran.
[3]Student Research Committee, Shahrekord University of Medical Science, Shahrekord, Iran.

Abstract

Background and objectives: Human adenoviruses cause a wide range of diseases, from self-limiting and mild infections to some life-threatening infections. Many studies have shown that components derived from medicinal plants have antiviral activity. *Pistacia* genus is rich in phenolic compounds and has antioxidant and antimicrobial effects. The aim of this study was to investigate antioxidant potential and antiviral effects of ethanol and crude extracts and different fractions of *Pistacia atlantica* Desf. leaves on adenovirus. **Methods:** Crude *P. atlantica* leaf extract was prepared by maceration with 80% ethanol. Hexane, chloroform, ethyl acetate and n-butanol fractions were prepared using liquid-liquid extraction method. Toxicity on HEp-2 cells and anti-adenoviral activity of the extract/fractions were evaluated by MTT colorimetric methods. The concentration that caused 50% viral inhibition (IC_{50}) and 50 % cytotoxicity concentration (CC_{50}) were evaluated using regression analysis. Selectivity index (SI), as a marker of antiviral activity, was calculated. To determine antioxidant activity the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay was used. **Results:** Ethyl acetate fraction showed the highest antioxidant activity with IC_{50} of 1.54±0.12 µg/mL in DPPH scavenging assay. Based on our results, IC_{50} of *P. atlantica* crude extract on adenovirus was 15.72 µg/mL, with SI of 8.09; n-butanol fraction showed the highest anti-adenoviral activity among the fractions with IC_{50} of 16.38 µg/mL and SI of 26.5. **Conclusion:** The ethanol extract and n-butanol fraction of *P. atlantica* leaves showed inhibitory effects on adenovirus and could be a new promising anti-adenovirus agent.

Keywords: adenovirus; antioxidant effect; antiviral; *Pistacia atlantica* Desf.

Citation: Karimi A, Moradi MT, Gafourian A. In vitro anti-adenovirus activity and antioxidant potential of *Pistacia atlantica* Desf. leaves. Res J Pharmacogn. 2020; 7(2): 53-60.

Introduction

Adenovirus (ADV) is a non-enveloped virus with a linear double-stranded DNA (dsDNA) that encodes 30-40 proteins. Similar to with other mammalian adenoviruses, human ADVs are classified into the genus *Mastadenovirus* (mastós, breast) [1]. Human ADV is associated with various human diseases such as myocarditis, conjunctivitis, gastroenteritis, hepatitis, and pneumonia. Generally, human ADVs are not highly pathogenic viruses because they are mostly associated with self-limiting respiratory infections, hemorrhagic cystitis and gastroenteritis, particularly in infants and young children [2]. In addition, ocular ADV infection is one of the leading reasons for viral conjunctivitis.

During the last two decades, the incidence of severe and life-threatening ADV infections has risen due to the growing transplantation, strong immunosuppressive therapies and the emergence of the HIV epidemic.

*Corresponding author: mtmoradi@skums.ac.ir

© 2020. Open access. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by-nc/4.0/)

www.SID.ir

Archive of SID

Much evidence is available regarding the in vivo efficacy of antiviral therapy against human ADV in the preemptive setting for cidofovir [3], but the clinical effect of the drug for treatment of overt viral disease has not yet been widely demonstrated [4]. Although resistant mutants have been described in vitro, cidofovir is obviously effective on all human ADV species [5] and is currently the primary anti-HAdV agent for preemptive therapy [3,6].
The challenges facing cidofovir treatment include its low bioavailability and poor correlation of pharmacological effects with the prescribed dose [7]. Besides, cidofovir can show a dose-limiting nephrotoxicity, and repeated monitoring of renal and tubular function along with hydration using probenecid is also sensible [4].
In regards to the growth of numbers of immunosuppressed patients, it is necessary to develop alternative anti-ADV treatment drugs. Medicinal plants have long been used to treat human diseases and a number of herbal medicines have been developed into therapeutic agents with satisfactory results. Plants are able to synthesize a wide spectrum of compounds and have long been used as remedies. Numerous plants are also being studied to detect drug sources.
Pistacia species appear as tree or shrub and creeping plants that include over 17 species across the world. *Pistacia atlantica* Desf. (wild pistachio), *Pistacia vera* and *Pistacia khinjuk* are three species that grow in Iran [8]. Different parts of *Pistacia* are widely used in traditional medicine of Iran and other countries. In Turkish native medicine, *P. atlantica* gum is used for respiratory and urinary tract infections and in Iran, it is used as a potent laxative in the treatment of constipation and gastrointestinal disorders. Aerial parts of the plant are traditionally used to treat eczema, tremor, throat infection, diarrhea, kidney stones, asthma, and gastric disease. The leaves of the plant have traditionally been used in Jordan for diabetes and eye infection. The fruit are used in Asian countries like Jordan and Turkey for gastric pain, oral diseases and to treat cough, diarrhea and stress [8].
In different studies, antimicrobial effects of *Pistacia* on several bacterial species, fungi and yeast have been evaluated and the results have shown that the leaf extract of different species of *Pistacia* shows inhibitory effects on the Gram-positive and Gram-negative bacteria, *Candida albicans*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Trichoderma*, and *Fusarium* [8,9].
So far, no study has evaluated the antiviral effects of *P. atlantica*, but the antiviral effects of other species of *Pistacia* have been studied. Özçelik et al. investigated the antibacterial, antifungal and antiviral effects of the extracts of different parts of *P. vera* and found that these extracts showed low antibacterial and antifungal effects. These extracts also showed high antiviral effects against herpes simplex and para-influenza viruses; the seed and kernel extract showed comparably more potent effects than other plant organs [10]. Besides, another study on the antiviral effects of 75 plant species against herpes virus, Sindbis virus and Poliovirus showed that *P. lentiscus* extract exhibited the highest antiviral activity against herpes virus [11].
The study by Rashed et al. also showed the antiviral effects of flavonoids isolated from *Pistacia chinensis* extract against hepatitis C virus [12]. Due to the lack of effective treatment for the above-mentioned viral infections and the numerous side effects of drugs currently used, there is an increasing demand for novel antiviral compounds.
Regarding the antimicrobial and antiviral effects of other species of this plant, in the present study, the phytochemical properties and antiviral effects of ethanol extract and various components of *P. atlantica* leaf against adenovirus were investigated in vitro.

Materials and Methods

Ethical considerations

The protocol of study was reviewed and confirmed by the Ethics Committee of Shahrekord University of Medical Sciences (registration code: IR.SKUMS.REC.1396.197).

Plant collection, extraction and fractionation

Pistacia atlantica leaves were collected in summer 2017 from the mountains around Lordegan, southwest of Iran. Then plant was identified and confirmed (herbarium number MPSKUMS-93) at the Herbarium of Medical Plants Research Center, Shahrekord University of Medical Sciences (Iran). The leaves were pulverized (100 g) and dissolved in 80% ethanol (400 mL) and left at room temperature for 96 h. Then, the mixture was filtered and concentrated under vacuum pressure at 40 °C in a rotary evaporator. Four fractions of the crude extract

www.SID.ir

Archive of SID

with different polarities were prepared by in-solution isolation using the difference in the polarities of various secondary metabolites (figure 1). The crude extract was dissolved in ethyl alcohol/H_2O and fractionated by consecutive liquid/liquid partitioning with n-hexane (Merck, Germany), and then with chloroform, ethyl acetate, and n-buthanol (Merck, Germany) by increasing polarity order [13].

Determination of free-radical scavenging activity

The free-radical scavenging activity of *P. atlantica* extract and fractions were measured by 2, 2 diphenyl-1-picrylhydrazyl (DPPH) method described by Moon and Terao [14] with some modifications. Briefly, different amounts of the extract and methanol were added to 0.3 mg/mL methanolic solution of DPPH to a total volume of 3.0 mL. After the solution was left for 15 min at room temperature, its absorbance was read at 517 nm by UV-Vis spectrophotometer (UNICO 2100, USA). Butylated hydroxytoluene (BHT) was used as the positive control. Inhibition of free radical by DPPH was calculated as follows:

$$\text{Antiradical activity (\%)} = [(\text{A control} - \text{A sample})/\text{A control}]\times 100$$

The IC_{50} value was defined as the amount of antioxidant needed to reduce the initial DPPH concentration by 50%. The value was determined based on linear regression of plots of the percentage of antiradical activity against the concentration of the compounds [15]. The experiment was carried out in triplicate.

Cell culture and virus propagation

HEp-2 (cervix adenocarcinoma) cells was purchased from Pasteur Institute of Iran. The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, USA), 100 µg/mL of streptomycin, 100 UI/mL of penicillin, and 0.25 µg/mL amphotericin B (Gibco, USA) at 37 °C and 5% CO2. The same medium containing 1.5% FBS was used for cytotoxicity and antiviral assays. ADV (type 5) was kindly provided by the Health Faculty of Tehran University of Medical Sciences, Tehran, Iran. Virus stock was prepared by infection of confluent monolayer HEp-2 cells in 75 cm^2 culture flasks using DMEM medium with 1.5% FBS, at 37 °C in 5% CO2. Virus titer was measured by the cytopathic effect (CPE) of ADV in HEp-2 cells and was expressed as the 50% tissue culture infective dose ($TCID_{50}$) per mL.

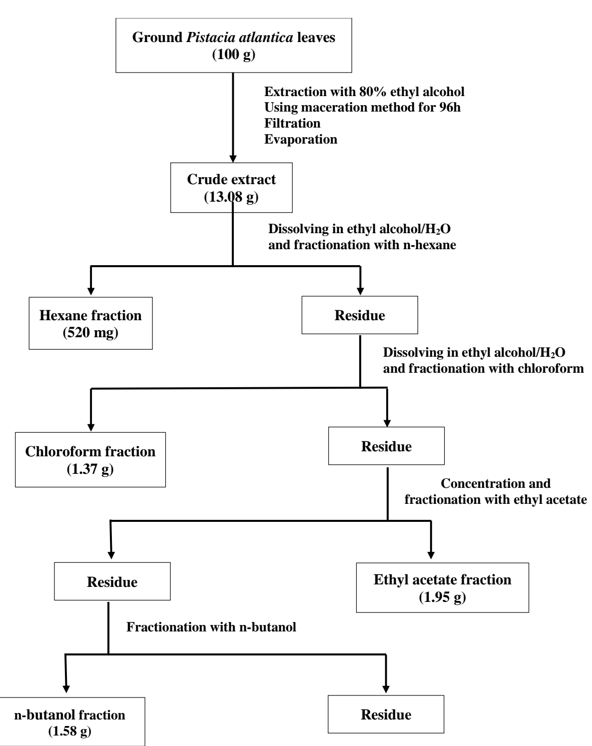

Figure 1. Flow chart for the extraction and fractionation of *Pistacia atlantica* leaves

www.SID.ir

Archive of SID

Cytotoxicity assay

The effects of the extract and fractions on the viability of HEp-2 cells were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbrome (MTT; Sigma, USA) assay according to a previously described method [16,17] with certain modifications. Briefly, when the cell monolayer became confluent onto 96-well plates, the cells were incubated with 150 µL/well of various concentrations of the extract/fractions at 37 °C with 5% CO_2 for 96 h (in triplicate). Afterwards, cell monolayers were incubated with 50 µL of 1 mg/ml MTT in PBS at 37 °C for 4 h, and then treated with 100 µL of acidic isopropanol (0.05 N HCl in absolute isopropanol). After the plates were shaken for 15 min, the absorbance was read using a reference filter at 570 nm with a microplate reader (StataFax2100, USA). Data were expressed as the percentage of toxicity using the following formula:

$$\text{Toxicity (\%)} = [100 - (At/As) \times 100]\%$$

Where At and As represent the absorbance of the test substance and the solvent control, respectively. The 50% cytotoxicity concentration (CC_{50}) was defined as the cytotoxic concentration of the crude extract/fractions according to regression analysis.

Antiviral assay

Antiviral activity of *P. atlantica* extract and fractions were evaluated using inhibitory activity assay by means of MTT method, as described previously [16]. In summary, when the cell monolayer became confluent in 96-well plates, the cell culture medium was aspirated, rinsed with PBS, and then infected with 100 $TCID_{50}$ (100 µL) of virus suspension for 2 h to allow virus adsorption, and then the virus was removed and the cells were treated with serial two fold dilutions of nontoxic concentration of the extract/fractions in triplicate. To obtain positive control, cells were infected with the same concentration of virus but without the addition of extract. To obtain negative or cell control, only DMEM and 1.5% FBS were added to the cells. The plates were incubated at 37 °C in a humidified CO_2 atmosphere for 4 days [13]. Cell viability was also determined using previously described MTT assay [16]. At each stage, negative control (without viruses and plant compounds) and virus control (viruses without plant components) were also designed. The percentage of viral inhibition was calculated as the percentage of inhibition using the following formula [17,18]:

$$\text{Antiviral activity (\%)} = (Atv - Acv)/(Acd - Acv) \times 100\ \%$$

Where Atv, Acv, and Acd are the absorbance of the test compounds on virus-infected cells, the absorbance of the virus control, and the absorbance of the cell control, respectively. Finally, after three replications, IC_{50} was calculated as the minimum concentration of plant compounds capable of inhibiting 50% of viruses using a regression plot. Selectivity index (SI), as a marker of antiviral activity, was determined as the ratio of CC_{50} to IC_{50}.

Statistical analysis

The IC_{50} and CC_{50} values were calculated by regression analysis using GraphPad Prism 6 (GraphPad Software, La Jolla, CA). All measurements were done in triplicate and statistical analysis was done by statistical software using one-way analysis of variance (ANOVA) and the post-hoc Tukey'stest.

Results and Discussion

Our results showed that the scavenging effect of the extract/fractions increased as the concentration increased. The extract/ fractions of *P. atlantica* significantly reduced the DPPH. The Ethyl acetate fraction showed the highest antioxidant activity with IC_{50} of 1.54±0.12 µg/mL for DPPH radicals, the chloroform fraction, n-butanol fraction, and the total extract had IC_{50} values of 3.4±0.11, 3.66± 1.52, and 4.6±0.66µg/mL, respectively, and the n-hexane fraction showed the highest value (14.84±2.07). The results were expressed relative to butylated hydroxytoluene (BHT), a reference standard having IC_{50} of 33.5±3.67 µg/mL. The IC_{50} values of the chloroform fraction, the n-butanol fraction, and the total extract, were found to be significant ($p<0.05$) as compared with BHT. Cytotoxic activities of the extract and fractions were investigated using HEp-2 cell line. The CC_{50} of *P. atlantica* crude extract on HEp-2 cells was 127.2 µg/mL. The n-butanol fraction showed the lowest cytotoxic activity with the highest CC_{50} of 434.7 µg/mL. Other fractions, including ethyl acetate, chloroform and n-hexane, showed CC_{50} of 32.38, 64.5 and 302.2 µg/mL, respectively (table 1).

www.SID.ir

Archive of SID

The analysis demonstrated that the concentrations of extract and fractions were significantly associated with the cell death ($p<0.05$, figure 2). To evaluate the effect of *P. atlantica* extract and fractions on adenovirus-infected HEp-2 cells, the cells were treated with non-cytotoxic concentrations of extract/fractions for 4 days to investigate the cytopathic effect and viability of uninfected and infected cells using MTT. Based on nonlinear regression analysis, IC_{50} of *P. atlantica* crude on ADV was 15.72 (CI 95%:13.02-18.99) µg/mL, with SI value of 8.09 (figure 1, table 1). Among fractions, the n-butanol fraction demonstrated the highest anti-adenoviral activity, with IC_{50} of 16.38 (CI 95%: 12.73-21.07) µg/mL and SI of 26.5 (table 1).

Table 1. Cell cytotoxicity, anti-adenoviral activity, and selectivity index of the crude extract and various fractions of *Pistacia atlantica*

Extract/fractions (µg/mL)	CC_{50} (CI95%) (µg/mL)	IC_{50} (CI95%) (µg/mL)	SI
Crude extract	127.2(110.2-146.8)	15.7(13.02-18.99)	8.09
n-Hexane fraction	302.2(258-353)	>302.2	<1
Chloroform fraction	64.5(62.03-67.2)	8.39(7.55-9.31)	7.68
Ethyl acetate fraction	32.38(28.7-36.5)	5.85(5.2-6.58)	5.5
n-Butanol fraction	434.7(424-445.7)	16.38(12.7-21.1)	26.5

CC_{50}: 50% cytotoxicity concentration; IC_{50}: 50% inhibitory concentration; CI 95 %: confidence interval 95 %; SI: selectivity index

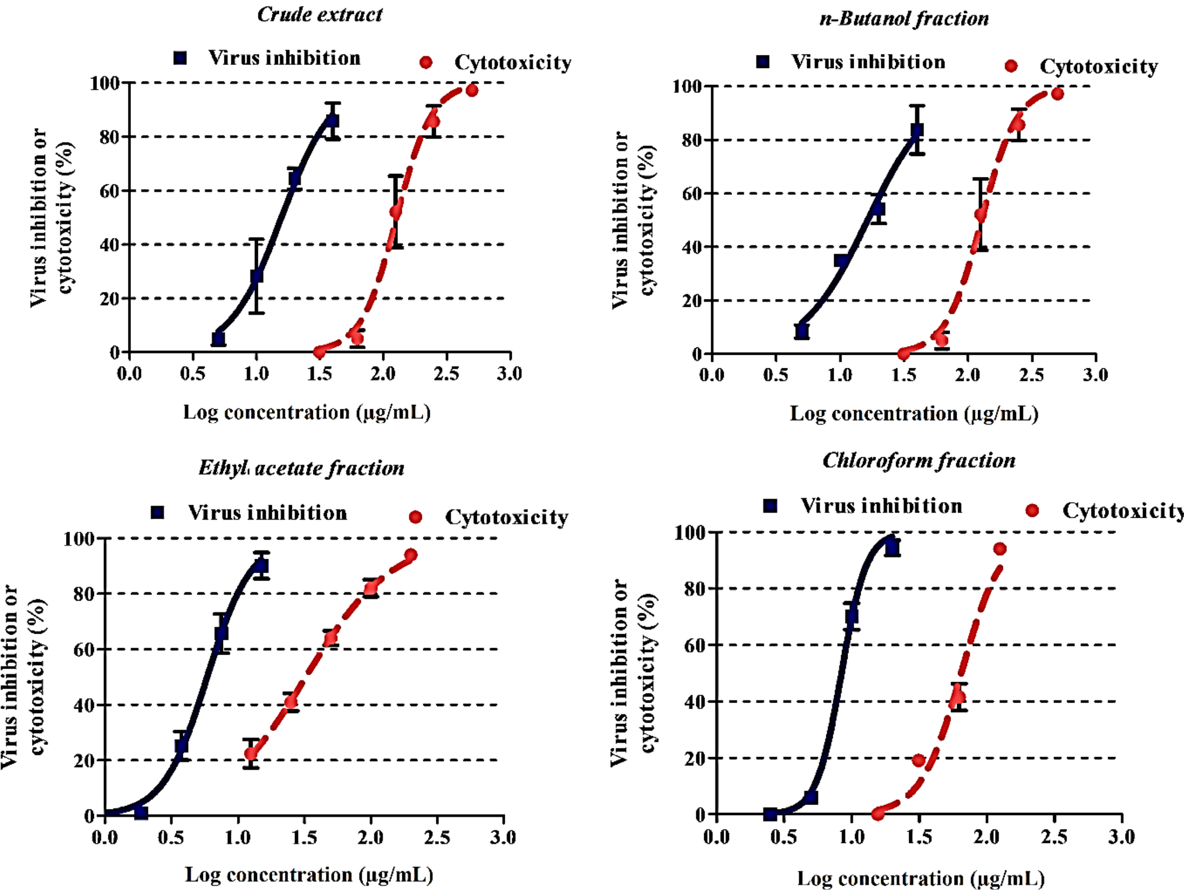

Figure 2. Cytotoxicity and anti-adenoviral activity of crude extract and various fractions of *Pistacia atlantica*. Confluent HEp-2 cells without virus infection or infected with virus were exposed to different concentrations of the extract/ fractions for 96 h. Cell viability was measured by the MTT test. Values are expressed as mean (± standard error of measurement) of three experiments.

www.SID.ir

Archive of SID

Our results showed that with increasing the concentration of extract/fractions, CPE inhibition became more pronounced (figure 1, $p < 0.05$).

In the present study, ethyl acetate fraction showed the lowest IC_{50} for inhibition of DPPH free radicals (1.54±0.12) followed by chloroform fraction, butanol fraction, total extract and hexane. These results are comparable to the concentration of BHT that inhibited 50% of DPPH radicals (IC_{50}) in the present study (33.5±3.67 µg/mL), indicating high antioxidant effects of ethyl acetate extract. Other studies have also confirmed the antioxidant effects of pistachio extract. The antioxidant effects and the amounts of phenolic compounds of *P. atlantica* leaf extract have been reported in various studies [19,20].

The antiviral effects of ethanol extract and different fractions of *P. atlantica* leaves on adenovirus were investigated *in vitro*, and total extract and all fractions, except for hexane, showed antiviral activity. The CC_{50} and IC_{50} values (on adenovirus) of the crude extract were 127.2 µg/mL (CI95%:110.2-146.8) and 15.7 µg/mL (CI95%:13.02-18.99), respectively. The selectivity index (SI) of the extract on adenovirus was 8.09. The recommended IC_{50} value, characteristic of herbal extract against infectious diseases, is less than 100 µg/mL [21]. The crude extract revealed an IC_{50} value of 15.7µg/mL that is far below the recommended cut-off. Among fractions, the n-butanol fraction showed the highest anti-adenoviral activity, with an IC_{50} of 16.38 (CI 95%: 12.73-21.07) µg/mL and SI of 26.5. The SI value is a ratio that measures the window between cytotoxicity and antiviral activity by dividing the given antiviral activity value by the cytotoxicity value [22]. The higher the SI ratio, the theoretically more effective and safer a drug would be during in vivo treatment for a given viral infection. The ideal drug would be cytotoxic only at very high concentrations and shows antiviral activity at very low concentrations, thus yielding a high SI value and being able to eliminate the target virus at concentrations far below its cytotoxic concentration. The SI of a compound is a widely accepted measure of its in vitro efficacy for the inhibition of virus replication [22].

Although no study has yet evaluated the antiviral effects of *P. atlantica*, the antiviral effects of other *Pistacia* species have been studied. Özçelik et al. investigated the antibacterial, antifungal and antiviral effects of the extracts from different parts of *P. vera* and found that these extracts had low antibacterial and substantial antifungal effects. These extracts also showed high antiviral effects against herpes simplex virus and parainfluenza virus, with seed and kernel extracts showing comparably more potent effects than other plant organs [10]. They suggested that these fatty acids may be associated with the antiviral, antibacterial and antifungal activities observed due to the presence of palmitic acid and linoleic acid in the extracts of different parts of *P. vera*. They also found that palmitic acid exhibited potent antiviral effects against HIV-1 and HIV-2 [10]. Besides, in a study on the antiviral effects of 75 plant species against herpes virus, Sindbis virus and Poliovirus, *P. lentiscus* extract showed the highest antiviral activity against herpes virus because of phenolic compounds in the plant [22]. The study of Rashed et al. also showed the antiviral effects of *P. chinensis* against herpes virus [12]. The antiviral activity of the pistachio extract and its various fractions, as well as the antimicrobial and antifungal activity of the pistachio in previous studies, have been shown to be related to the presence of phenolic and flavonoid compounds. The main phenolic compounds of leaf extracts of *Pistacia* genus include ferulic acid, vanillic acid, ursolic acid, caffeic acid [21], gallic acid, chlorogenic acid, ellagic acid, synaptic acid, juglone and catechin [23,24]. Various studies have demonstrated the antiviral effects of the above compounds. The inhibitory effects of caffeic acid and chlorogenic acid against ADV and the substantial antiviral effects of ursolic acid against adenovirus, herpes virus, coxsackievirus, enterovirus and hepatitis virus have been reported [24]. In the study of Wang et al., the antiviral effects of chlorogenic acid, caffeic acid, and quinic acid were demonstrated both in vitro and in vivo [25].

In the present study, the greatest inhibitory effect against adenovirus was obtained for n-butanol fraction. which showed the least toxic effect on HEp-2 cells; its concentration for inhibition of 50% of HEp-2 cells (CC_{50}) was 434.7 µg/mL and thus had the lowest cytotoxic effect. The IC_{50} of this fraction against adenovirus was 16.37 µg/mL with the selectivity index of 26.5, indicating that it showed the highest antiviral effect.

According to the results of this study, erhanol extract and n-butanol fraction of *P. atlantica*

www.SID.ir

Archive of SID

leaves showed inhibitory effects on adenovirus, and therefore could serve as new promising anti-adenovirus agents; however, further studies on the inhibitory effects of purified compounds of this plant are needed to confirm this argument.

Acknowledgements

This work was financially supported by Shahrekord University of Medical Sciences, Shahrekord, Iran (Grant No.: 2594). Authors are thankful to the Director of Medical Plants Research Center and to the Deputy of Research and Technology of Shahrekord University of Medical Sciences.

Author contributions

Ali Karimi contributed to the design of the study, supervised the work scientifically and edited the manuscript. Mohammad Taghi Moradi contributed to the design and protocol, developed the original idea, analyzed and abstracted the data and prepared the manuscript. Asghar Gafourian contributed to the data collection and laboratory testing. All authors read and approved the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

References

[1] Liu EB, Ferreyra L, Fischer SL, Pavan JV, Nates SV, Hudson NR, Tirado D, Dyer DW, Chodosh J, Seto D, Jones MS. Genetic analysis of a novel human adenovirus with a serologically unique hexon and a recombinant fiber gene. *PLoS One*. 2011; Article ID 24491.

[2] Lenaerts L, De Clercq E, Naesens L. Clinical features and treatment of adenovirus infections. *Rev Med Virol*. 2008; 18(6): 357-374.

[3] Lindemans CA, Leen AM, Boelens JJ. How I treat adenovirus in hematopoietic stem cell transplant recipients. *Blood*. 2010; 116(25): 5476-5485.

[4] Lion T. Adenovirus infections in immunocompetent and immunocompromised patients. *Clin Microbiol Rev*. 2014; 27(3): 441-462.

[5] Morfin F, Dupuis-Girod S, Mundweiler S, Falcon D, Carrington D, Sedlacek P, Bierings M, Cetkovsky P, Kroes AC, Van Tol MJ, Thouvenot D. In vitro susceptibility of adenovirus to antiviral drugs is species-dependent. *Antivir Ther*. 2005; 10(2): 225-229.

[6] Matthes-Martin S, Feuchtinger T, Shaw PJ, Engelhard D, Hirsch HH, Cordonnier C, Ljungman P. European guidelines for diagnosis and treatment of adenovirus infection in leukemia and stem cell transplantation: summary of ECIL-4 (2011). *Transpl Infect Dis*. 2012; 14(6): 555-563.

[7] Cundy KC. Clinical pharmacokinetics of the antiviral nucleotide analogues cidofovir and adefovir. *Clin Pharmacokinet*. 1999; 36(2): 127-143.

[8] Bozorgi M, Memariani Z, Mobli M, Salehi Surmaghi MH, Shams-Ardekani MR, Rahimi R. Five *Pistacia* species (*P. vera, P. atlantica, P. terebinthus, P. khinjuk,* and *P. lentiscus*): a review of their traditional uses, phytochemistry, and pharmacology. *Sci World J*. 2013; Article ID 219815.

[9] Rigane G, Ghazghazi H, Aouadhi C, Ben Salem R, Nasr Z. Phenolic content, antioxidant capacity and antimicrobial activity of leaf extracts from *Pistacia atlantica*. *Nat Prod Res*. 2017; 31(6): 696-699.

[10] Ozcelik B, Aslan M, Orhan I, Karaoglu T. Antibacterial, antifungal, and antiviral activities of the lipophylic extracts of *Pistacia vera*. *Microbiol Res*. 2005; 160(2): 159-164.

[11] Mouhajir F, Hudson JB, Rejdali M, Towers GHN. Multiple antiviral activities of endemic medicinal plants used by berber peoples of Morocco. *Pharm Biol*. 2008; 39(5): 364-374.

[12] Rashed K, Calland N, Deloison G, Rouille Y, Séron K. In-vitro antiviral activity of *Pistacia chinensis* flavonoids against hepatitis c virus (HCV). *J Appl Pharm*. 2014; 6(1): 8-18.

[13] Mohammadi-Kamalabadi M, Karimi A, Rafieian M, Amjad L. Phytochemical study and anti viral effect evaluation of methanolic extract with fractions of aerial parts of *Euphorbia spinidens*. *J Babol Uni Med Sci*. 2014; 16(5): 25-34.

[14] Moon JH, Terao J. Antioxidant activity of caffeic acid and dihydrocaffeic acid in lard and human low-density lipoprotein. *J Agri Food Chem*. 1998; 46(12): 5062-5065.

[15] Nahak DG, Sahu R. In vitro antioxidative acitivity of *Azadirachta indica* and *Melia azedarach* leaves by DPPH scavenging assay. *Nature Sc*i. 2010; 8(4): 22-28.

www.SID.ir

Archive of SID

[16] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods*. 1983; 65(1-2): 55-63.
[17] 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(2): 255-262.
[18] 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): 641-647.
[19] 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(3): 451-476.
[20] Hatamnia AA, Rostamzad A, Hosseini M, Abbaspour N, Darvishzadeh R, Malekzadeh P, Aminzadeh BM. Antioxidant capacity and phenolic composition of leaves from 10 Bene (*Pistacia atlantica* subsp. *kurdica*) genotypes. *Nat Prod Res*. 2016; 30(5): 600-604.
[21] Cos P, Vlietinck AJ, Berghe DV, Maes L. Antiinfective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. *J Ethnopharmacol*. 2006; 106(3): 290-302.
[22] Pritchett JC, Naesens L, Montoya J. Treating HHV-6 infections: the laboratory efficacy and clinical use of anti-HHV-6 agents. In: Flamand L, Lautenschlager I, Krueger GRF, Ablashi DV, Eds. Human herpesviruses HHV-6A, HHV-6B & HHV-7. 3rd ed. Boston: Elsevier, 2014.
[23] 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: 352-362.
[24] Jassim SAA, Naji MA. Novel antiviral agents: a medicinal plant perspective. *J Appl Microbiol.* 2003; 95(3): 412-427.
[25] Wang GF, Shi LP, Ren YD, Liu QF, Liu HF, Zhang RJ, Li Z, Zhu FH, He PL, Tang W, Tao PZ. Anti-hepatitis B virus activity of chlorogenic acid, quinic acid and caffeic acid in vivo and in vitro. *Antiviral Res*. 2009; 83(2): 186-190.

Abbreviations

ADV: Adenovirus; DPPH: 2, 2 diphenyl-1-picrylhydrazyl; BHT: butylated hydroxytoluene; IC_{50}: 50% viral inhibition; CC_{50}: 50% cytotoxicity concentration; SI: selectivity index; HEp-2: human epithelial type 2; DMEM: Dulbecco's Modified Eagle's Medium; FBS: fetal bovine serum; $TCID_{50}$: 50% tissue culture infective dose; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbrome; CI 95 %: confidence interval 95 %

www.SID.ir