Journal of Shahrekord University of Medical Sciences
doi:10.34172/jsums.2022.17 2022;24(3):104-110 http://j.skums.ac.ir

Original Article

Phytochemical properties and antiviral effect of green tea *(Camellia sinensis)* extract on adenovirus *in vitro*

Shahrzad Mirmojarabian[1], Ali Karimi[2], Zahra Lorigooini[3], Fatemeh Javadi-Farsani[4], Amin Soltani[5], Mohammad Taghi Moradi[3*]

[1]Student Research Committee, Shahrekord University of Medical Sciences, Shahrekord, Iran
[2]Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran
[3]Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran
[4]School of Medicine, Golestan University of Medical Sciences, Gorgan, Iran
[5]Clinical Biochemistry Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran

*Corresponding Author: Mohammad-Taghi Moradi, Email: mtmoradi65@gmail.com

Abstract

Background and aims: The lack of effective antiviral drugs for adenoviruses is one of the most important problems in this area. The aim of this study was to investigate the phytochemical properties and antiviral effect of the green tea extract (GTE) on adenovirus in HEp2 cells *in vitro*.

Methods: In this experimental study, dried leaves of green tea were extracted by maceration. Total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity of the extract were measured by Folin-Ciocalteu, aluminum chloride, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) colorimetric methods, respectively. The amounts of some phenolic compounds in the extract were also determined using high-performance liquid chromatography. The toxicity of the extract on Hep2 cells and antiviral activity of the extract on adenovirus were assessed by the MTT colorimetric method. The half-maximum cytotoxicity concentration (CC_{50}) and the 50% inhibitory concentration (IC_{50}) of the extract were calculated as well.

Results: Phytochemical investigations showed that the IC_{50} of DPPH radical was 42.1 ± 3.2 µg/mL compared with butylated hydroxytoluene (IC_{50} of 33.5 ± 3.67 µg/mL). The TPC and TFC of the extract were 74.2 mg GAE/g and 16.3 mg RE/g of the dry extract, respectively. The extract demonstrated the highest amounts of syringic acid, gallic acid, 3,4-dihydroxybenzoic acid, and rutin levels (67.27, 20.12, 7.39, and 2.97 mg/g DW, respectively). Based on the results of cell culture, the CC_{50} and IC_{50} of GTE were 103.3 µg/mL and 25.16 µg/mL, respectively.

Conclusion: GTE with phenolic and flavonoid compounds can exert dose-dependent inhibitory effects on adenoviruses.

Keywords: Adenovirus, *Camellia sinensis*, Antiviral effect, Green tea

Received: 13 October 2021, Accepted: 27 November 2021, ePublished: 18 August 2022

Introduction

Viral diseases are among the major health issues in many countries. One of the most important problems in this area is the lack of appropriate antiviral drugs for some viruses such as adenoviruses and the acquisition of resistance to available drugs (1,2). Human adenoviruses are non-enveloped viruses that have double-strand DNA and belong to the genus *Mastadenovirus* from the Adenoviridae family. Overall, 52 serotypes of these viruses have so far been identified and divided into seven subgroups (A-G). Adenoviruses can multiply and cause disease in the eyes and respiratory, gastrointestinal, and urinary tracts. These viruses cause a wide range of diseases from self-limiting infections and mild diseases, including conjunctivitis and gastrointestinal diseases to some life-threatening infections (3,4). Adenoviruses are spread all over the world and are present all year round and usually do not cause epidemics in the communities. The most common serotypes in clinical specimens include low-numbered respiratory and gastrointestinal types. These viruses are mainly transmitted through the fecal-oral route but are also sometimes transmitted through small respiratory droplets or contaminated objects. Most adenoviral diseases are left undiagnosed because they have no clinical symptoms and many infection cases are asymptomatic (5). Despite many advances in cysteine protease inhibitors and nucleoside synthesis analogues to inhibit adenovirus replication, no chemical treatment has yet been identified to effectively assist in preventing and treating adenovirus infection. Alongside chemical drugs, the advent of antiviral, especially anti-adenoviral, drugs could promise more effective treatments with fewer side effects. Medicinal plants have long been used to treat human diseases, and several herbal medicines have been converted into therapeutic compounds with reportedly remarkable outcomes.

Green tea (*Camellia sinensis*) belongs to the Theaceae family and has green and leathery leaves, as well as

white and fragrant flowers. Different types of teas are obtained from this plant depending on the process of auto-oxidation catalysis with polyphenol oxidase and peroxidase. Therefore, teas are classified as non-oxidized (green and white), semi-oxidized (oolong), oxidized (black), and post-fermented (pu-erhtea) types. Green tea powder, which is the result of the process of drying the fresh leaves of the plant, is obtained without going through oxidation and fermentation. Green tea has a variety of compounds, especially from the family of catechins (6,7), which have antioxidant, anti-inflammatory, antiseptic, and hypolipidemic properties, prevent postoperative abdominal adhesions, produce antibacterial effects and protect against contrast-induced nephropathy (6,8-10). The antiviral activity of *C. sinensis* extracts against the herpes simplex virus and influenza virus has been reported in previous research (11). Given the antiviral and antimicrobial effects of green tea, this study aimed to evaluate the phytochemical properties and anti-adenoviral effects of the hydroalcoholic green tea extract (GTE) *in vitro*.

Materials and Methods

Cells and viruses

Human adenovirus serotype 5 was procured from a virology laboratory, Shahrekord University of Medical Sciences, Iran. Hep2 cells were purchased from the Pasteur Institute of Iran and cultured in DMEM containing 10% fetal bovine serum.

Extraction

To prepare the extract, green tea leaves were purchased from reputable groceries, and after being authenticated by a botanist, were registered in the herbarium of the Medical Plants Research Center, Shahrekord University of Medical Sciences, Iran (Voucher number: MPSKUMS-206). The plant was pulverized by an electric mill, and then the resulting powder was mixed with ethyl alcohol (70%) ratios of 1:5 after 48 hours. The contents of the container were filtered using filter paper and a glass funnel. The solvent was concentrated by a rotary evaporator and vacuum pump (set at < 40°C). The concentrated liquid was dried in an oven at 37°C. Finally, the extract was dissolved in DMSO, and different concentrations of the extract were prepared using a culture medium.

Measuring antioxidant activity by DPPH method

Antioxidant activity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) based on the percentage of free radical scavenging. First, different concentrations of the extract were prepared. Two hundred microliters of samples at different concentrations was mixed with 1 mL of a 90 µM DPPH solution to a final volume of 95 mL with the addition of 95% methanol and shaken in the dark for 60 minutes. Then, the absorbance of the samples containing the extract and the control was read at 517-nm wavelength using a UV-Vis spectrophotometer (UNICO 2100: USA). The absorbance was read at 517 nm using the high absorbance of the reaction mixture indicating low free radical scavenging activity. A sample containing methanol and DPPH solution was used as a control. Butylated hydroxytoluene (BHT) was applied as a positive control. The inhibition of free radicals by DPPH was calculated according to the following formula:

Antiradical activity (%) = (A control−A sample)/A control × 100.

The 50% inhibitory concentration (IC_{50}) value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, was computed based on the linear regression of the plots of the percentage antiradical activity against the concentration of the tested compounds (12).

Measuring total phenolic content (TPC)

TPC was measured by the Folin-Ciocalteu colorimetric method, and gallic acid was used as the reference standard. For this purpose, standard solutions of gallic acid were prepared in a solution of 60% methanol, and then 0.5 mL of each solution was transferred to a test tube, to which 0.5 mL of Folin-Ciocalteu 10% solution was added, and 0.4 mL of sodium carbonate 7.5% solution was added after 3-8 minutes. Next, the tubes were left at laboratory temperature for 30 minutes, and the optical absorbance was read by a spectrophotometer at 765-nm wavelength, and finally, a standard curve was plotted accordingly.
Then, 0.01-0.02 g of the dried sample was dissolved in 60% methanol to a final volume of 10 mL, and TPC was measured per the above-described method except that the extract solution was added instead of the standard solution. Subsequently, the optical absorbance was added to the standard curve, and the TPC of the extract was obtained in mg gallic acid (GAE) equivalent to g of the dry extract (13).

Measuring total flavonoid content (TFC)

TFC was measured by the aluminum chloride colorimetric method. Briefly, 0.5 mL of each extract solution (0.01 g in 10 mL of 60% methanol) was added to 0.5 mL of 2% aluminum chloride and 3 mL of 5% potassium acetate. After 40 minutes, the optical absorbance of samples was read against distilled water at a 415-nm wavelength. Simultaneously, different dilutions of rutin were made, and the standard curve and experiment were prepared per the above-described method. Moreover, the optical absorbance of the samples was compared with the standard curve, and the TFC of the extract was calculated in mg rutin (RE) equivalent to g of the dry extract (14).

Separation of flavonoids and phenolic acids using high-performance liquid chromatography (HPLC)

Two flavonoids (quercetin and rutin) and fifteen phenolic acids (gallic acid, 3,4-dihydroxybenzoic acid, syringic

acid, chlorogenic acid, vanillic acid, caffeic acid, 2,5-dihydroxybenzoic acid, catechin, p-coumaric acid, ferulic acid, chicoric acid, rosmarinic acid, salicylic acid, and kaempferol, apigenin) in the extract were analyzed using the HPLC method. The qualitative analysis was performed using reverse-phase HPLC coupled with a UV-visible detector (UV PDA 2800) and column C18 (5 µm particle size, i.d. 250 mm × 4.6 mm). First, 2.5 mg of dry extract was dissolved in 1 mL methanol and water (a ratio of 1:4) to prepare a concentration of 2500 ppm, and then 20 µL of the samples were injected into HPLC and isolated by the fixed phase C18 and the binary mobile phase of deionized water and methanol containing trifluoroacetic acid 0.05%. Detection was performed by scanning from 190 to 800 nm and reading in the range of 280-372 nm. The results were calculated using the calibration curve equation of the analytical standards of each compound to identify and determine the amount. The results were expressed as µg/mg of the extract (15).

Investigating toxicity by MTT assay

MTT assay was performed to investigate the extract's cytotoxic effect on Hep2 cells (percentage of cell death) and to calculate its half-maximum cytotoxicity concentration (CC_{50}) on this cell line**.** For this purpose, cell suspension containing 10^4 cells in a volume of 100 µL was added to all wells of a 96-well plate. After 24 hours and cell monolayer formation, cells were incubated with the extract at concentrations of 3.125, 6.25, 12.5, 25, 50, 100, and 200 µg/mL for four days. Then, the cell supernatant was removed and each well was washed with 100 µL of PBS. Subsequently, 60 µL of 1 mg/mL of the MTT solution was added to the wells and incubated for four hours at 37°C. Next, the contents of the wells were gently removed and 100 µL of DMSO was added to them, and the microplate was incubated at room temperature for 10 minutes. The optical absorbance of the plates was read at a 570-nm wavelength. The optical absorbance recorded by the ELISA reader was converted to cell death percentage using the following formula:

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

where *At* and *As* denote the absorbance of the test substance and the solvent control, respectively (16). CC_{50}, or the concentration of the extract that killed 50% of the cultured cells, was obtained by the Probit test.

Investigating antiviral effects

Hep2 cells in appropriate numbers were transferred to 96-well plates. After the incubation and formation of a cell monolayer, 100 $TCID_{50}$ viruses were added to each well and incubated for 2 hours. In the next step, the media on the wells were aspirated, and non-cytotoxic concentrations of the extract (0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL) were added to the wells. Six wells were considered cell control (free of any virus and extract) and six wells were considered virus control (infected with a virus and free of the extract). After 96 hours of incubation under standard cell culture conditions, the MTT assay was performed according to the above-mentioned procedure, and the percentage of viral inhibition at different doses of extracts was calculated concerning virus control using the following formula:

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

where *Atv*, *Acv*, and *Acd* are the absorbance of test compounds on virus-infected cells, the absorbance of the virus control, and the absorbance of the cell control, respectively. The procedure was performed three times (17). IC_{50} was calculated as the minimum concentration of the extract with 50% viral inhibition using the Probit test.

Data analysis

The CC_{50} or IC_{50} and their confidence intervals were obtained by Probit test in SPSS software, version 23. Each experiment was performed in triplicate, and the graphs were plotted using GraphPad Prism 6 software**.**

Results

Antioxidant capacity, TPC, and TFC

Phytochemical investigations showed that the IC_{50} of DPPH radical was 42.1 ± 3.2 µg/mL compared with butylated hydroxytoluene (33.5 ± 3.67 µg/mL) . The TPC and TFC of the extract were 74.2 mg GAE/g and 16.3 mg RE/g of the dry extract, respectively (Table 1).

Flavonoids and phenolic acids in GTE

Two flavonoids and five phenolic acids were analyzed in the GTE. The results revealed that the major phenolic

Table 1. DPPH radical-scavenging activity of the green tea extract and BHT

Sample	Concentration (µg/mL)	Scavenging of DPPH radical activity inhibition (%) (Mean ± SEM)	DPPH-radical scavenging activity IC_{50}/(µg/mL)
Green tea extract	70	72.5±2.5	42.1±3.2
	60	67.1±2.7	
	50	53.9±1.8	
	40	49.1±2.2	
	30	43.8±2.6	
	20	35.6±2.3	
	10	26.6±1.9	
BHT	200	90.2±1.2	33.5±3.67
	100	78.2±3.5	
	50	55.6±0.99	
	25	37±2.35	
	12.5	22.9±1.63	
	6.25	15.2±2	
	3.125	12.3±1.76	

Note. All results are presented as mean ± SEM of three assays. SEM: Standard mean error; DPPH: 1,1-Diphenyl-2-picrylhydrazyl; BHT: Butylated hydroxytoluene.

and flavonoid compounds in the extract were syringic acid, gallic acid, 3,4-dihydroxybenzoic acid, and rutin levels with 67.27, 20.12, 7.39, and 2.97 mg/g, respectively (Figure 1). On the other hand, chlorogenic acid, vanillic acid, caffeic acid, 2,5-dihydroxybenzoic acid, catechin, P-coumaric acid, ferulic acid, chicoric acid, rosmarinic acid, salicylic acid, quercetin, kaempferol, and apigenin were not detected in GTE.

Cytotoxicity of GTE

The cytotoxicity of GTE at different concentrations (3.125, 6.25, 12.5, 25, 50, 100, and 200 µg/mL) on Hep2 cells was evaluated at 96 hours using an MTT assay. Based on Probit analysis, the concentration of the extract that caused the death of 50% of cultured cells (CC_{50}) was calculated at 103.3 µg/mL (95% CI: 94.98-112.3). The Probit model demonstrated a significant relationship between the concentration of the extract, and more cell death was found with more increase in the extract concentration ($P<0.001$, Figure 2).

Anti-viral effect of GTE

The anti-viral effect of GTE at different non-cytotoxic concentrations of the extract (0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL) on adenovirus was evaluated 96 hours post-treatment using an MTT assay. According to the Probit model, the extract reduced the replication of virus-infected cells dose-dependently ($P<0.001$). Based on Probit analysis, the IC_{50} of the extract was calculated at 25.16 µg/mL (95% CI: 23.57-26.86, Figure 3).

Discussion

The present study aimed to evaluate the anti-adenoviral activity of hydroalcoholic GTEs. The results showed that the extract at 103.3 µg/mL had a toxic effect of 50% on Hep2 cells after 96 hours of exposure.

Additionally, it inhibited the proliferation of virus-infected cells in a dose-dependent manner so that the IC50 of the extract was calculated at 25.16 µg/mL.

Several types of compounds have been identified in different types of green tea, the most important of which include secondary metabolism products such as ascorbic acid, terpenes, alkaloids, and especially phenolic compounds, as well as volatile compounds, carotenoids, tocopherols, vitamins A, K, and B, and minerals such as fluorine, potassium, magnesium, iron, manganese, and phosphorus. Primary metabolism products such as polysaccharides, proteins, and lipids have been identified as well (18-20).

In addition, compounds in teas have various biological properties that have been confirmed in several *in vivo* and *in vitro* studies (21), the most important of which are antioxidant (8), anti-inflammatory, antiseptic, hypolipidemic, preventive of postoperative intra-

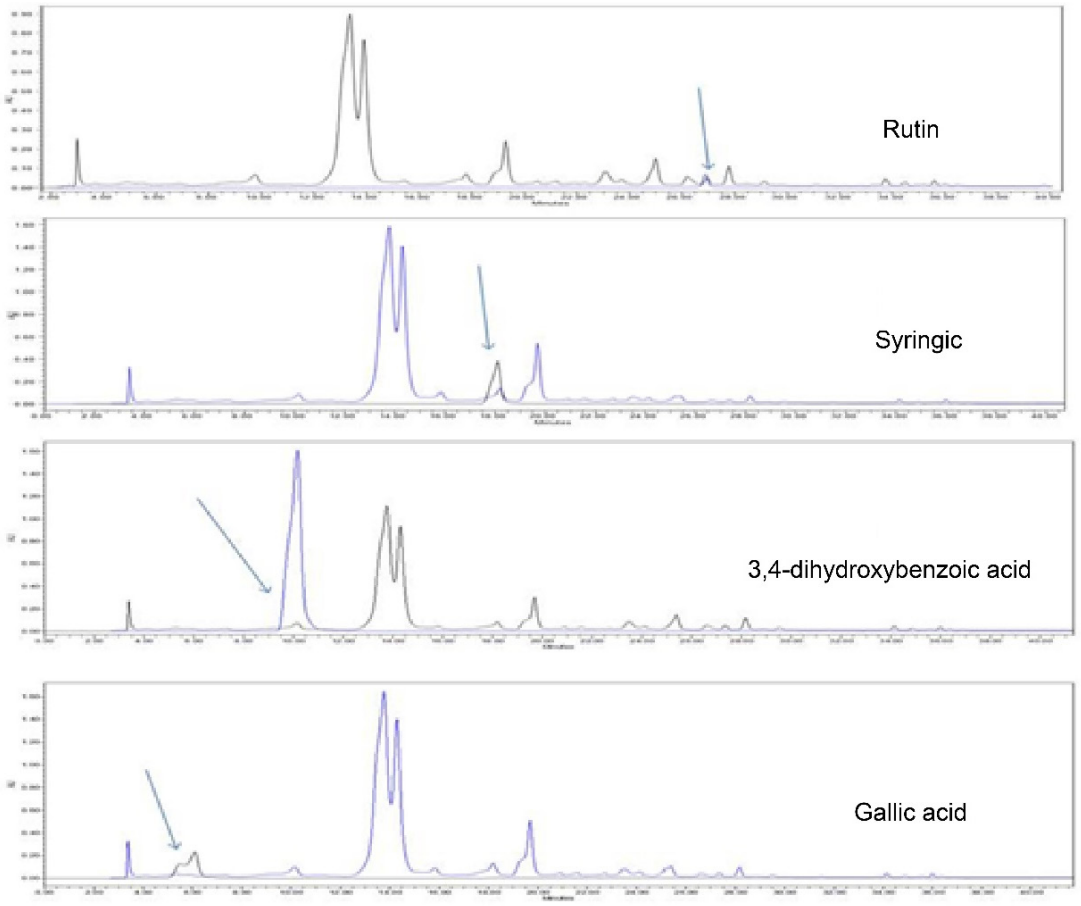

Figure 1. High-performance liquid chromatography curve for the major phenolic and flavonoid compounds (syringic acid, gallic acid, 3,4-dihydroxybenzoic acid, and rutin) of the green tea extract.

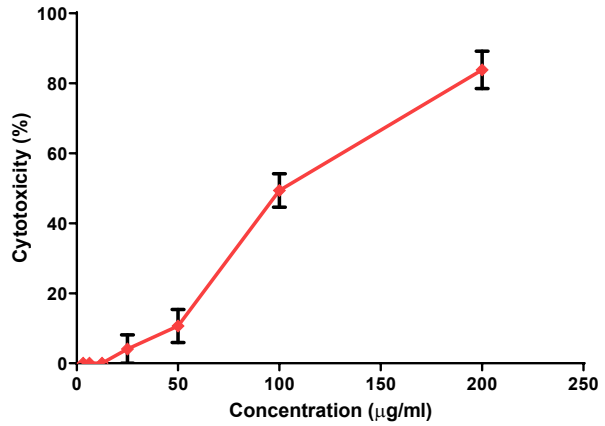

Figure 2. Cytotoxicity of GTE on HEp2 cells. *Note*. GTE: Green tea extract. Confluent HEp2 cells were exposed to different concentrations of the extract for 96 hours. Cytotoxicity was measured in an MTT assay, and experiments were performed in triplicate.

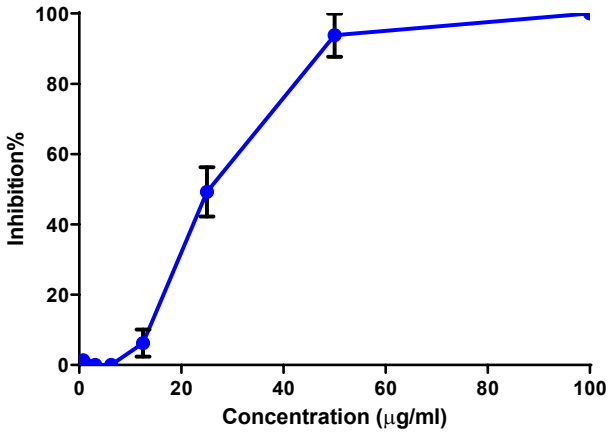

Figure 3. Anti-adenovirus activity of the green tea extract on HEp2 cell lines. Confluent HEp2 cells after virus infection were exposed to different concentrations of the extract for 48 hours. Cell viability was measured with MTT assay, and the results were presented as means ± standard deviations from three experiments.

abdominal adhesion, antibacterial, and protective properties against contrast-induced nephropathy (22-24). The anti-herpes effects of *Camellia sinensis* and the adenoviral effects of black tea have also been reported in various studies (11,17,25,26).

Camellia sinensis can be considered an important source of polyphenols, especially flavonoids. The most important flavonoids in *C. sinensis* include catechins (7, 27). According to the results of some studies, catechins have strong antiviral effects so that they can inhibit herpes virus replication at low concentrations (28, 29). Further studies have shown that catechins in green tea, especially epigallocatechin gallate (EGCG), inhibit virus growth at various stages, including direct inactivation of virus particles, inhibition of intracellular virus growth, and inhibition of the viral protease, adenain, in cell culture (30). Regarding the antiviral effects of EGCG, Yamaguchi et al concluded that this compound could prevent HIV-1 replication by acting on different stages of its life cycle (31).

In the current study, the TPC of GTE and its TFC were calculated at 74.2 mg GAE/g and 16.3 mg RE/g of the dry extract, respectively; therefore, the presence of phenolic and flavonoid compounds in this extract can be the reason for its antiviral effects, which is consistent with the results of other studies. Some studies demonstrated that phenolic compounds have antibacterial and antiviral properties (32,33). Other studies also indicated that flavonoids derived from different plants can exhibit antiviral properties (34-36).

In the present study, the identification of flavonoids and phenolic acids in GTE using HPLC revealed that the extract contains large amounts of flavonoid compounds and phenolic acids, including syringic acid, gallic acid, 3,4-dihydroxybenzoic acid, and rutin. Other compounds such as quercetin, ferulic acid, rosmarinic acid, apigenin, and kaempferol have also been identified in the extract of this plant (6,37,38).

Conclusion

According to the results of the current study, the GTE can reduce the proliferation of adenovirus in Hep2 cells in a dose-dependent manner, and the presence of phenolic and flavonoid compounds can be the reason for the antiviral effect of the extract. It is hoped that by conducting further studies and isolating the active ingredients of green tea, this plant will be used as a complementary drug alongside anti-adenoviral drugs in controlling and preventing adenovirus infection.

Acknowledgments

This work was financially supported by Shahrekord University of Medical Sciences, Shahrekord, Iran (Grant No.: 3046). The authors are thankful to all who provided support in conducting this research.

Authors' contribution

SM, FJF, and AS: Data acquisition, analysis, and interpretation, and draft writing. AK and ZL: Study designing, and critical revision of the manuscript for important intellectual content. MTM: Study designing, data analysis and interpretation, and supervision. All authors wrote the draft of the manuscript and approved the final manuscript.

Conflict of interests

The authors of the present work declare no conflict of interests.

Ethical approval

This study was approved by the Ethics Committee of Shahrekord University of Medical Sciences (the ethical code number of IR.SKUMS.REC.1398.005).

Funding/Support

This work was funded by Shahrekord University of Medical Sciences, Shahrekord, Iran (grant no. 3046).

References

1. Russell WC. Adenoviruses: update on structure and function. J Gen Virol. 2009;90(Pt 1):1-20. doi: 10.1099/vir.0.003087-0.
2. van der Eb AJ, van Kesteren LW, van Bruggen EF. Structural properties of adenovirus DNA's. Biochim Biophys Acta. 1969;182(2):530-41. doi: 10.1016/0005-2787(69)90205-6.
3. Arabzadeh SAM, Daei Parizi MH, Molaei HR, Aghaei Afshar A,

Salari AA. The frequency distribution of parainfluenza, adeno and respiratory syncytial virus infections in children below 2 years old with bronchiolititis, by multiplex polymerase chain reaction method, Afzalipoor hospital, Kerman, 2006. J Kerman Univ Med Sci. 2008;15(4):305-11. [Persian].
4. Henquell C, Boeuf B, Mirand A, Bacher C, Traore O, Déchelotte P, et al. Fatal adenovirus infection in a neonate and transmission to health-care workers. J Clin Virol. 2009;45(4):345-8. doi: 10.1016/j.jcv.2009.04.019.
5. Schmitz H, Wigand R, Heinrich W. Worldwide epidemiology of human adenovirus infections. Am J Epidemiol. 1983;117(4):455-66. doi: 10.1093/oxfordjournals.aje.a113563.
6. Zhang L, Ho CT, Zhou J, Santos JS, Armstrong L, Granato D. Chemistry and biological activities of processed *Camellia sinensis* teas: a comprehensive review. Compr Rev Food Sci Food Saf. 2019;18(5):1474-95. doi: 10.1111/1541-4337.12479.
7. Tao W, Zhou Z, Zhao B, Wei T. Simultaneous determination of eight catechins and four theaflavins in green, black and oolong tea using new HPLC-MS-MS method. J Pharm Biomed Anal. 2016;131:140-5. doi: 10.1016/j.jpba.2016.08.020.
8. Nasri H, Hajian S, Ahmadi A, Baradaran A, Kohi G, Nasri P, et al. Ameliorative effect of green tea against contrast-induced renal tubular cell injury. Iran J Kidney Dis. 2015;9(6):421-6.
9. Yam TS, Shah S, Hamilton-Miller JM. Microbiological activity of whole and fractionated crude extracts of tea (*Camellia sinensis*), and of tea components. FEMS Microbiol Lett. 1997;152(1):169-74. doi: 10.1111/j.1574-6968.1997.tb10424.x.
10. Sánchez M, González-Burgos E, Iglesias I, Lozano R, Gómez-Serranillos MP. The pharmacological activity of *Camellia sinensis* (L.) Kuntze on metabolic and endocrine disorders: a systematic review. Biomolecules. 2020;10(4):603. doi: 10.3390/biom10040603.
11. Farahani M. Anti-herpes simplex virus effect of *Camellia sinesis*, *Echium amoenum* and *Nerium oleander*. J Appl Environ Microbiol. 2014;2(4):102-5. doi: 10.12691/jaem-2-4-3 .
12. Nahak G, Sahu RK. In vitro antioxidative acitivity of *Azadirachta indica* and *Melia azedarach* leaves by DPPH scavenging assay. Nat Sci. 2010;8(4):22-8.
13. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat Protoc. 2007;2(4):875-7. doi: 10.1038/nprot.2007.102.
14. Asgari S, Setorki M, Rafieian-Kopaei M, Heidarian E, Fard S, Ansari Samani R, et al. Postprandial hypolipidemic and hypoglycemic effects of *Allium hertifolium* and *Sesamum indicum* on hypercholesterolemic rabbits. Afr J Pharm Pharmacol. 2012;6(15):1131-5. doi: 10.5897/ajpp12.092.
15. Jamshidi-Kia F, Saeidi K, Lorigooini Z, Maggi F. Phenolic profile, chemical relationship and antioxidant activity of Iranian *Verbascum* species. Acta Biol Crac Ser Bot. 2020;62(2):31-42. doi: 10.24425/abcsb.2020.131669.
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. doi: 10.1016/0022-1759(83)90303-4.
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 Knowl. 2018;17(2):255-62.
18. Nie SP, Xie MY. A review on the isolation and structure of tea polysaccharides and their bioactivities. Food Hydrocoll. 2011;25(2):144-9. doi: 10.1016/j.foodhyd.2010.04.010.
19. Bahmani M, Zargaran A, Rafieian-Kopaei M, Saki K. Ethnobotanical study of medicinal plants used in the management of diabetes mellitus in the Urmia, Northwest Iran. Asian Pac J Trop Med. 2014;7 Suppl 1:S348-54. doi: 10.1016/s1995-7645(14)60257-1.
20. Asadi SY, Parsaei P, Karimi M, Ezzati S, Zamiri A, Mohammadizadeh F, et al. Effect of green tea (*Camellia sinensis*) extract on healing process of surgical wounds in rat. Int J Surg. 2013;11(4):332-7. doi: 10.1016/j.ijsu.2013.02.014.
21. Sharangi AB. Medicinal and therapeutic potentialities of tea (*Camellia sinensis* L.)–a review. Food Res Int. 2009;42(5-6):529-35. doi: 10.1016/j.foodres.2009.01.007.
22. Parsaei P, Karimi M, Asadi SY, Rafieian-Kopaei M. Bioactive components and preventive effect of green tea (*Camellia sinensis*) extract on post-laparotomy intra-abdominal adhesion in rats. Int J Surg. 2013;11(9):811-5. doi: 10.1016/j.ijsu.2013.08.014.
23. Memarzadeh S, Validi M, Mobini G, Rafeian-Kopaei M, Mansouri S. The antibacterial effect of *Camellia sinensis* extract on bacterias, conjunctivitis in vitro. J Shahrekord Univ Med Sci. 2012;14(4):61-9. [Persian].
24. Nasri H, Ahmadi A, Baradaran A, Nasri P, Hajian S, Pour-Arian A, et al. A biochemical study on ameliorative effect of green tea (*Camellia sinensis*) extract against contrast media induced acute kidney injury. J Renal Inj Prev. 2014;3(2):47-9. doi: 10.12861/jrip.2014.16.
25. Deepika G, Durgadevi H, Narayan R, Sudanthira M, Manickan E. Anti-herpes simplex viruses activity of *Camellia sinensis*, member of the family Theaceae (green tea). BMC Infect Dis. 2014;14(Suppl 3):47. doi: 10.1186/1471-2334-14-s3-p47.
26. Karimi A, Moradi MT, Alidadi S, Hashemi L. Anti-adenovirus activity, antioxidant potential, and phenolic content of black tea (*Camellia sinensis* Kuntze) extract. J Complement Integr Med. 2016;13(4):357-63. doi: 10.1515/jcim-2016-0050.
27. Koch W, Kukula-Koch W, Komsta Ł, Marzec Z, Szwerc W, Głowniak K. Green tea quality evaluation based on its catechins and metals composition in combination with chemometric analysis. Molecules. 2018;23(7):1689. doi: 10.3390/molecules23071689.
28. Isaacs CE, Wen GY, Xu W, Jia JH, Rohan L, Corbo C, et al. Epigallocatechin gallate inactivates clinical isolates of herpes simplex virus. Antimicrob Agents Chemother. 2008;52(3):962-70. doi: 10.1128/aac.00825-07.
29. Cheng HY, Lin CC, Lin TC. Antiviral properties of prodelphinidin B-2 3′-O-gallate from green tea leaf. Antivir Chem Chemother. 2002;13(4):223-9. doi: 10.1177/095632020201300403.
30. Weber JM, Ruzindana-Umunyana A, Imbeault L, Sircar S. Inhibition of adenovirus infection and adenain by green tea catechins. Antiviral Res. 2003;58(2):167-73. doi: 10.1016/s0166-3542(02)00212-7.
31. Yamaguchi K, Honda M, Ikigai H, Hara Y, Shimamura T. Inhibitory effects of (-)-epigallocatechin gallate on the life cycle of human immunodeficiency virus type 1 (HIV-1). Antiviral Res. 2002;53(1):19-34. doi: 10.1016/s0166-3542(01)00189-9.
32. Abdelkebir R, Alcántara C, Falcó I, Sánchez G, Garcia-Perez JV, Neffati M, et al. Effect of ultrasound technology combined with binary mixtures of ethanol and water on antibacterial and antiviral activities of *Erodium glaucophyllum* extracts. Innov Food Sci Emerg Technol. 2019;52:189-96. doi: 10.1016/j.ifset.2018.12.009.
33. Fabra MJ, Falcó I, Randazzo W, Sánchez G, López-Rubio A. Antiviral and antioxidant properties of active alginate edible films containing phenolic extracts. Food Hydrocoll.

2018;81:96-103. doi: 10.1016/j.foodhyd.2018.02.026.
34. Khazeei Tabari MA, Iranpanah A, Bahramsoltani R, Rahimi R. Flavonoids as promising antiviral agents against SARS-CoV-2 infection: a mechanistic review. Molecules. 2021;26(13):3900. doi: 10.3390/molecules26133900.
35. Ninfali P, Antonelli A, Magnani M, Scarpa ES. Antiviral properties of flavonoids and delivery strategies. Nutrients. 2020;12(9):2534. doi: 10.3390/nu12092534.
36. Lalani S, Poh CL. Flavonoids as antiviral agents for enterovirus A71 (EV-A71). Viruses. 2020;12(2):184. doi: 10.3390/v12020184.
37. Zareef M, Chen Q, Ouyang Q, Arslan M, Hassan MM, Ahmad W, et al. Rapid screening of phenolic compounds in congou black tea (*Camellia sinensis*) during in vitro fermentation process using portable spectral analytical system coupled chemometrics. J Food Process Preserv. 2019;43(7):e13996. doi: 10.1111/jfpp.13996.
38. Amoros M, Simões CM, Girre L, Sauvager F, Cormier M. Synergistic effect of flavones and flavonols against herpes simplex virus type 1 in cell culture. Comparison with the antiviral activity of propolis. J Nat Prod. 1992;55(12):1732-40. doi: 10.1021/np50090a003.