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Full Length Research Paper

Evaluation of anti-adenovirus activity of some plants from Lamiaceae family grown in Iran in cell culture

Horieh Saderi[1] and Maryam Abbasi[2]*

[1]Microbiology Department, School of Medicine, Shahed University, Tehran, Iran.
[2]Student Research Center, School of Medicine, Shahed University, Tehran, Iran.

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The family Lamiaceae included some plants such as thyme species which have a lot of medical properties even in the Iranian traditional medicine. Some of these properties have not been approved by this original article. The aim of this study was to evaluate the anti-adenovirus effect of the three plants from Lamiaceae family (*Thymus daenensis, Thymus vulgaris, Zataria multiflora*) that was grown in Iran. The aqueous extracts of the plants were prepared and the essential oil of *T. vulgaris* was purchased. Cytotoxicity effects of the tested compounds were determined. The anti-adenovirus effects of maximum non-cytotoxic concentration of tested compounds were evaluated in cell culture with 100 TCID50 of adenovirus type 5 by three methods: Simultaneous, pre-treatment and post-treatment. For antiviral effect, only those concentrations were accepted that completely inhibit cytopathic effect of virus in cell culture. In simultaneous and post-treatment methods, minimum concentration that showed anti-adenovirus activity was 12.5, 12.5, 25 and 50 µg/ml for *T. daenensis*, *T. vulgaris*, and *Z. multiflora* aqueous extracts and thyme essential oil, respectively. None of the tested compounds had shown anti-adenovirus effect in pre-treatment method. These results have suggested the potential use of these compounds for the treatment of the adenovirus infections.

Keywords: Adenovirus, antiviral effect, essential oil, extract, Lamiaceae family, *Thymus daenensis, Thymus vulgaris, Zataria multiflora*.

INTRODUCTION

Human adenoviruses have at least 51 serotypes; with prototype adenovirus type 5. These viruses are associated with a wide range of illnesses, including ocular, respiratory, gastrointestinal and urinary infections. Adenovirus infections are usually mild and always heal without the need of any special therapy. However, severe and life-treating adenovirus infections have been reported in some patients, including immunocompromised patients (Echavarría, 2008; Lenaerts et al., 2008). Although, some antiviral drugs are reported to inhibit adenovirus infection in vitro and used for empirical therapy in severe adenovirus infections, such as nucleoside analogues and even protease inhibitors, usefulness of them had not been approved (Lenaerts et al., 2008). In addition, the development of resistance to drugs has also been reported in adenoviruses (Gordon YJ, 1996). Therefore, new and more effective antiadenovirus agents are desired.

The family Lamiaceae (previously called Labiatae) has included some medicinal plants. Genus Thymus L., is represented in Iranian flora by 14 species, four of which (Thymus carmanicus, Thymus daenensis subsp. daenensis and T. daenensis subsp. lancifolius, Thymus persicus and Thymus trautvetteri) are endemic. The Persian name of this genus is “Avishan” and/or “Azorbeh” and the traditional name for plants in this genus is thyme. Infusion and decoction of aerial parts of thyme have been used medicinally to produce tonic, carminative, digestive, antispasmodic, anti-inflammatory, antitussive, and expectorant effects (Zargari, 1990; Nickavar et al., 2005). Recent studies have shown that, thyme have strong antibacterial, antifungal, antiviral, antiparasitic and antioxidant activities (Wild, 1994; Mojab et al., 2008;

*Corresponding author. E-mail: maramabbasi1986@gmail.com. Tel: 009821 88964792. Fax: 009821 88966310.

Rajbhandari et al., 2009, Stahl-Biskup and Saez., 2002). Thyme is also used as herbal tea and flavoring agent (condiment and spice) (Stahl-Biskup and Saez., 2002). The aromatic and medicinal properties of the genus thyme have made it one of the most popular medicinal plants (Nickavar et al., 2005). Another important genus of this family is Zataria; which its members are widely distributed in Iran, Afghanistan and Pakistan. The Persian name of this genus is "Shiraz Avishan". *Zataria multiflora* is used as a stimulant and is also prescribed for pre-mature labor pains (Ali et al., 2000). Antioxidant and anti-microbial activity of *Z. multiflora* was shown in previous studies (Saei-Dehkordi et al., 2010; Mahboubi and Bidgoli., 2010; Parsaeimehr et al., 2010; Gandomi et al., 2009).

Since there are some reports which indicate activities of the mentioned plants on some kind of viruses, such as herpes simplex virus (Koch et al., 2008; Nolkemper et al., 2006; Reichling et al., 2008), we had attempted to evaluate the anti-adenovirus activity of the aqueous extract of the three species grown in Iran (*Thymus vulgaris*, *Thymus daenensis* and *Z. multiflora*) and thyme essential oil (from *T. vulgaris*) in cell culture.

MATERIALS AND METHODS

Cell culture

Hela cells obtained from National Cell Bank of Iran (NCBI, Pasteur Institute of Iran) were grown as monolayer in 50 ml cell culture flasks or 24-well cell culture microplates. Dulbecco's Minimum Essential Medium (DMEM) containing 10% and 2% fetal bovine serum were used for growth and maintenance of cells, respectively. Penicillin G (100 U/ml), streptomycin (100 µg/ml) and amphotericin B (0.025 µg/ml) were added to the culture medium to avoid contamination. The cell cultures were maintained in a humidified atmosphere under 5% CO_2 at 37˚C.

Virus

Stock human adenovirus serotype 5 provided by Dr. J. C. de Jong (Erasmus University, Netherlands) was propagated in Hela cells and the infected cells supernatant fluids were harvested, titrated and stored at -80˚C until use.

For titration of viruses, Hela cells were seeded in 24-well culture plates and then incubated. After 24 h, serial dilutions of virus stock were prepared in culture medium, and each dilution was added to four wells. After an additional 72 h of incubation, the cytopathic effect in each well was recorded. Titer of adenovirus was calculated as described previously by Reed and Muench method (Reed and Muench., 1938) and expressed as tissue culture infective dose 50% per ml (TCID50/ml).

Extract and essential oil

Essential oil of *T. vulgaris* was purchased from Barij Essence Co. Ltd, Iran. *T. daenensis* subsp. *Daenensis* was provided and identified by Dr. F. Sefidkon (Research Institute of Forest and Rangelands, Iran). *T. vulgaris* and *Z. multiflora* were a gift from Dr. F. Jookar Kashi (Essential Oils Research Institute, University of Kashan, Iran). For preparing aqueous extracts of the plants, 50 g of air dried and powdered aerial parts of plants were boiled with 200 ml of distilled water for 20 min. The aqueous was collected and filtered by Whatman No.1 filter paper, and dried by incubation in 37˚C for 12 h. Stock solution (10 mg/ml) of aqueous extracts and essential oil were prepared in dimethylsulfoxide (DMSO) and tested compounds were prepared by appropriate dilution in maintenance medium.

Cytotoxicity Assays

Serial dilutions of each tested compounds (from 50 to 4000 µg/ml) were prepared in the maintenance medium and 200 µl of each dilution added was to 24 h growth Hela cells in a 96-well plate. Each dilution was tested in duplicate. The wells were observed by light microscope after 48 h for any signs of cytopathic effect compared with the untreated cells as controls to determine minimum dilution of each tested compounds with no apparent cytotoxicity.

Antiviral Assay

For cytopathic effect inhibition assay, Hela cells were grown in 24-well microplates and incubate at 37˚C in the presence of 5% CO_2 until the cells became confluent. After removing the culture medium, 200 µl of 1:1 mixture of virus suspension containing 100 TCID50 and serial dilution of each tested compounds (prepared from the maximum non-cytotoxic concentration) and incubated for 24 h in 4˚C were added to each well. For the virus control, 100 µl of virus suspension and 100 µl of culture medium without tested compound were added. For the cell controls, 100 µl of maximum non-cytotoxic concentration of each extracts and/or essential oil and 100 µl of culture medium were added. The plates were incubated again for 48 h and then were observed for cell cytopathic effect. In addition to this simultaneous treatment method, pre-treatment (adding 24 h before virus infection) and post-treatment (adding 24 h after virus infection) of extracts or essential oil were also attempted in the same protocol as aforementioned except the variation in the time of addition of test substances.

The concentrations of tested compound which completely inhibit adenovirus cytopathic effect with respect to virus control well were recorded as effective concentration.

RESULTS

The results of cytotoxicity assay for tested compounds were shown in Table 1. The cytopathic effects were observed in 500 µg/ml concentrations for all tested aqueous extracts (*T. vulgaris*, *T. daenensis*, and *Z. multiflora*) and in 2000 µg/ml concentration for thyme essential oil at first time; therefore, maximum non-cytotoxic concentration for all three extracts were 200 µg/ml and for essential oil was 1000 µg/ml.

The results of anti-adenovirus assays for different concentrations of the *T. vulgaris*, *T. daenensis*, and *Z. multiflora* aqueous extracts were shown in Table 2 and for *T. Vulgaris*, the essential oil was shown in Table 3. In the pre-treatment method, neither of the concentrations of tested compounds has shown cytopathic effect inhibition. Minimum concentration of *Z. multiflora* aqueous extract with cytopatthic effect inhibition activity was 25 µg/ml in simultaneous method and 12.5 µg/ml in post-treatment method. For *T. vulgaris* and *T. daenensis*

Table 1. The results of cytotoxicity assay for each tested compounds.

Tested compound Concentration (µg/ml)	Percent of cytopathic effect in well containing			
	***T. vulgaris* aqueous extract**	***T. vulgaris* aqueous extract**	***T. vulgaris* aqueous extract**	***T. vulgaris* aqueous extract**
0	0	0	0	0
50	0	0	0	0
100	0	0	0	0
200	0	0	0	0
500	25%	25%	25%	0
1000	25%	50%	25%	0
2000	50%	50%	50%	75%
4000	100%	100%	100%	100%

aqueous extracts, minimum concentration for cytopathic effect inhibition was 25 µg/ml; while the thyme essential oil was 50 µg/ml in both simultaneous and post-treatment methods.

DISCUSSION

Interest in employing antiviral compounds from natural sources (like plants) has been enhanced by researchers and the consumers' preference for natural medicines and concerns about the toxic effects of synthetic antiviral drugs (Zandi et al., 2007). There are reports about antiviral properties of plants belonging to Lamiaceae family, including *Thymus* and *Zataria* genera (Stahl-Biskup and Seaz., 2002; Koch et al., 2008; Nolkemper et al., 2006; Reichling et al., 2008), but until now, there has been no other study on the anti-adenovirus effect of these plants. This was valuable especially for us because we had easy access to these plants in Iran. This research has shown for the first time the inhibitory effects of *Z. multiflora, T. daenensis, T. vulgaris* aqueous extracts and *T. vulgaris* essential oil on adenovirus infection of cell culture. This finding and previous reports about the expectorant, antioxidant and anti-inflammatory activities of the plants belong to Lamiaceae family **(**Miura and Nakatami., 1989; Ismaili et al., 2002; Ismaili et al., 2004); and they are useful for the treatment of viral infections.

Anti-adenovirus effects of *Z. multiflora*, *T. daenensis, T. vulgaris* aqueous extracts and *T. vulgaris* essential oil is shown in simultaneous and post-treatment methods but not in pre-treatment method; therefore, these tested compounds could inhibit replication cycle of adenovirus in cell culture but not affect the resistance of cells to these viruses. These results suggested that *Z. multiflora, T. daenensis, T. vulgaris* aqueous extracts and thyme essential oil might be used for the production of natural anti-adenovirus drug after *in vivo* studies.

The phytochemical characterization of the used substances and the identification of the responsible compounds in anti-adenovirus activity are necessary. In a study, it has been shown that the main constituents of the dry *Z. multiflora* were thymol, carvacrol, while the main constituents of the fresh plant were thymol, carvacrol, p-cymene, linalool and γ-terpinene (Shafiee and Javidnia., 1997). In another study, the chemical constituents of five ecotypes of *Z. multiflora* essential oil with respect to main phytogeographic grown towns in Iran were studied. This investigation showed that, there were qualitative similarities among the oils from different origins, whereas the amounts of some components were varied. In this study, 34, 34, 32, 29 and 53 various compounds were identified from different samples, with the highest oxygenated monoterpens value at 72.99% and thymol a phenolic compound of oxygenated monoterpens, as the most abundant component ranging from 27.05% to 64.87% (Saei-Dehkordi et al. 2010). Also, it had been shown that the major volatile constituents obtained from the aerial parts of the *T. vulgaris* are thymol (44.7%), p-cymene (18.6%) and γ-terpinene (16.5%) and for *T. daenensis* subsp. *Daenensis* are thymol (74.7%), *p*-cymene (6.5%), β-caryophyllene (3.8%) and methyl carvacrol (3.6%) and this oil is rich in monoterpene phenols, especially thymol and carvacrol (Porte and Godoy., 2008, Nickavar et al., 2005). Since the amount of active constituents present in the plants depend on the geographical distribution, season of collection, climate and other environmental factors at the collection site (Rajbhandari et al., 2009, Nickavar et al., 2005; Saei-Dehkordi et al., 2010), it has been recommended to confirm the shown activity of studied substances on adenovirus and future studies for determination and purification of the effective components.

This study had studied for the first time, anti-adenovirus activity of aqueous extract of *Z.multiflora, T. daenensis, T. vulgaris* and *T. vulgaris* essential oil. Use of these plants belong to the Lamiaceae family, including *Z. multiflora*, *T. daenensis* and *T. vulgaris* for the treatment of various diseases, especially in common cold in Iranian traditional medicine (Nickavar et al., 2005), is confirmed

Table 2. The results of anti-adenovirus assays for *Zataria multiflora*, *Thymus daenensis* and *Thymus vulgaris* aqueous extracts.

Tested compound concentration (µg/ml)	*Z. multiflora* aqueous extract cytopathic effect inhibition:			*T. daenensis* aqueous extract cytopathic effect inhibition:			*T. vulgaris* aqueous extract cytopathic effect inhibition:		
	Simultaneous treatment	Pre-treatment	Post-treatment	Simultaneous treatment	Pre-treatment	Post-treatment	Simultaneous treatment	Pre-treatment	Post-treatment
200	+	_	+	+	_	+	+	_	+
100	+	_	+	+	_	+	+	_	+
50	+	_	+	+	_	+	+	_	+
25	+	_	+	+	_	+	+	_	+
12.5	_	_	+	+	_	+	+	_	+

Table 3. The results of anti-adenovirus assays for *Thymus vulgaris* essential oil.

Tested compound concentration (µg/ml)	*Thymus vulgaris* essential oil cytopathic effect inhibition		
	Simultaneous treatment	Pre-treatment	Post-treatment
1000	+	_	+
500	+	_	+
200	+	_	+
100	+	_	+
50	+	_	+

by these results.

Conclusions

Since current chemotherapy agents for adenovirus infections have low efficiency, there is thus a need to search for new and more effective antiviral agents. According to the results of this study, *Z.multiflora*, *T.daenensis* and *T. vulgaris* can serve as a potential resource in the development of new anti-adenovirus drugs in the future. More studies for determination of the mode of action and quality standards are also recommended.

REFERENCES

Ali MS, Saleem M, Ali Z, Ahmad VU (2000). Chemistry of *Zataria multiflora* (Lamiaceae). Phytoch., 55(8): 933-936.

Echavarría M (2008). Adenoviruses in immunocompromised hosts. Clin. Microbiol. Rev., 21(4): 704-715.

Gandomi H, Misaghi A, Basti AA, Bokaei S, Khosravi A, Abbasifar A, Javan AJ (2009). Effect of *Zataria multiflora* Boiss. essential oil on growth and aflatoxin formation by *Aspergillus flavus* in culture media and cheese. Food Chem. Toxicol., 47(10): 2397-2400.

Gordon YJ, Araullo-Cruz TP, Johnson YF, Romanowski EG, Kinchington PR (1996). Isolation of human adenovirus type 5 variants resistant to the antiviral cidofovir. Invest Ophthalmol Vis Sci., 37(13): 2774-2778.

Ismaili H, Sosa S, Brkic D, Fkih-Tetouani S, Ilidrissi A, Touati D, Aquino RP, Tubaro A (2002). Topical anti-inflammatory activity of extracts and compounds from *Thymus broussonettii*. J Pharm Pharmacol., 54: 1137-1140.

Ismaili H, Milella L, Fkih-Tetouani S, Ilidrissi A, Camporese A, Sosa S, Altinier G, Della Loggia R, Aquino R (2004). In vivo topical anti-inflammatory and in vitro antioxidant activities of two extracts of *Thymus satureioides* leaves. J. Ethnopharmacol., 91: 31-36.

Koch C, Reichling J, Schneele J, Schnitzler P (2008). Inhibitory effect of essential oils against herpes simplex virus type 2. Phytomed., 15(1-2): 71-78.

Lenaerts L, De Clercq E, Naesens L (2008). Clinical features and treatment of adenovirus infections. Rev. Med. Virol., 18(6): 357-374.
Mahboubi M, Bidgoli FG (2010). Antistaphylococcal activity of *Zataria multiflora* essential oil and its synergy with vancomycin. Phytomed., 17(7): 548-550
Miura K, Nakatami N (1989). Antioxidative activity of flavonoids from thyme (*Thymus vulgaris* L.). Agric. Biol. Chem., 53: 3043-3045.
Mojab F, Poursaeed M, Mehrgan H, Pakdaman S (2008). Antibacterial activity of *Thymus daenensis* methanolic extract. Pak. J. Pharm Sci., 21(3): 210-213.
Nickavar B, Mojab F, Dolat-Abadi R (2005). Analysis of the essential oils of two *Thymus* species from Iran. Food Chem., 90(4): 609-611.
Nolkemper S, Reichling J, Stintzing FC, Carle R, Schnitzler P (2006). Antiviral effect of aqueous extracts from species of the Lamiaceae family against Herpes simplex virus type 1 and type 2 in vitro. Planta Med., 72(15): 1378-1382.
Parsaeimehr M, Basti AA, Radmehr B, Misaghi A, Abbasifar A, Karim G, Rokni N, Motlagh MS, Gandomi H, Noori N, Khanjari A (2010). Effect of *Zataria multiflora* Boiss. Essential oil, nisin, and their combination on the production of enterotoxin C and alpha-hemolysin by *Staphylococcus aureus*. Foodborne Pathog. Dis., 7(3): 299-305.
Porte A, Godoy RLO (2008). Chemical composition of *Thymus vulgaris* L. (thyme) essential oil from the Rio de Janeiro State (Brazil). J. serb. Chem. Soc., 73(3): 307-310.
Rajbhandari M, Mentel R, Jha PK, Chaudhary RP, Bhattarai S, Gewali MB, Karmacharya N, Hipper M, Lindequist U (2009). Antiviral activity of some plants used in nepalese traditional medicine. Evid. Based Complement Alternat. Med., 6(4): 517-522.
Reed LJ, Muench H (1938). A simple method for estimating fifty percent end points. Am. J. Hyg., 27: 493-497.
Reichling J, Nolkemper S, Stintzing FC, Schnitzler P (2008). Impact of ethanolic lamiaceae extracts on herpesvirus infectivity in cell culture. Forsch Komplementmed, 15(6):313-320.
Saei-Dehkordi SS, Tajik H, Moradi M, Khalighi-Sigaroodi F (2010). Chemical composition of essential oils in *Zataria multiflora* Boiss. from different parts of Iran and their radical scavenging and antimicrobial activity. Food Chem. Toxicol., 48(6): 1562-1567.
Shafiee A, Javidnia K (1997). Composition of essential oil of *Zataria multiflora*. Planta Med., 63(4): 371-372.
Stahl-Biskup E, Saez F (2002). Thyme. Taylor & Francis, London.
Wild R (1994). The complete book of natural and medical cures. Rodale Press, Inc, Emmaus, PA.
Zandi K, Fouladvand M, Pakdel P, Sartavi K (2007). Evaluation of in vitro antiviral activity of a brown alga (*Cystoseira myrica*) from the Persian Gulf against herpes simplex virus type 1. Afr. J. Biotech., 6 (22): 2511-2514.
Zargari A (1990). Medicinal plants. Tehran University Press, Tehran.