Antiviral, Cytotoxic and Antioxidant Effects of Tanacetum Vulgare L. Crude Extract In Vitro

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Abstract

Introduction: Due to the high prevalence of viral infections having no specific treatment and the constant emergence of resistant viral strains, searching for effective antiviral compounds is crucial. The present study explores in vitro the antiviral activity of ethanolic extract from aerial parts of Tanacetum vulgare L. against viral strains of three taxonomic groups, including agents that cause socially significant diseases in humans for which antiviral chemotherapy is indicated, namely coxsackievirus B1 (family Picornaviridae), herpes simplex virus type 1 (family Herpesviridae) and influenza A virus (family Orthomyxoviridae).

Aim: The aim of the current study was to evaluate antiviral activity of ethanolic extract from herbaceous plant Tanacetum vulgare L. against some important human viruses for which antiviral chemotherapy is needed and to characterize extract for its antioxidant activity in vitro.

Materials and methods: The crude aqueous ethanolic extract from aerial parts of Tanacetum vulgare L. contained flavonoids determined as apigenin, coumarins determined as aesculin, tannic compounds determined as tannin, and others. Antiviral activity of ethanolic extract from herbaceous plant Tanacetum vulgare L. against coxsackievirus B1, influenza A and herpes simplex virus type 1 was evaluated by viral yield reduction technique. The total antioxidant activity was determined by measuring the capacity of the sample to inhibit the generation of thiobarbituric acid reactive substances (TBARS).

Results: The results show that the extract has the lowest toxicity on the MDBK cell line and similar cytotoxicity in Hep-2, whereas in the MDCK cells it has more than twice the highest toxicity. Testing the antiviral activity of Tanacetum vulgare L. extract revealed a slight inhibition of replication of HSV-1 with a selective index of 7.07 and IAV/H3N2 (SI = 3.69) but no specific antiviral effect against CVB1 replication was found. The evaluation of the antioxidant activity showed great antioxidant activity of the ethanolic extract from T. vulgare – 26 mmol/l for the applied 20 mg/ml extract.

Conclusion: The crude extract from aerial parts of the medicinal plant Tanacetum vulgare L. demonstrated low cytotoxicity in Hep-2, MDBK and moderate cytotoxic effects in MDCK cells. It exerted significant antiviral activity against HSV-1 as determined by the recorded inhibition of viral replication, the blockage of virus entry - absorption stage and direct virucidal effects on extracellular virions. The observed effect when testing Tanacetum’s extract on influenza A H3N2 virus infection in vitro was milder, which probably resulted from the interference with the cellular pathways involved in the replication cycle. The presence of virucidal and adsorption-suppressing activity but the absence of viral replication inhibitory effects against CBV-1 suggests a possible interaction of the extract’s components with viral capsid proteins or related cell receptors.

Keywords
antioxidant activity, CVB1, H3N2, HSV-1, Tanacetum vulgare L.
INTRODUCTION

Tanacetum vulgare L. (Tansy) is a herbaceous plant growing in the temperate regions of Europe, Asia, and North Africa. For many years, Tanacetum vulgare L. (the leaves and flowering tops) has been used in the traditional Asian and North African medicine as an antihelminthic, carminative, antispasmodic, stimulant to abdominal viscera, tonic, emmenagogue, anti diabetic, diuretic, and it is also antihypertensive. It has also been used in treating hysteria, migraines, neuralgia, rheumatism, kidney weakness, stomach problems, and fever. It is worth noting that the plant can be toxic and poisonous even in small doses, and toxicity can also be observed when it is administered externally. The herb should not be used for self-medication and should only be used as prescribed and under medical supervision.

Many biological activities, such as antibacterial, antiviral, antifungal, anti-inflammatory, immunomodulatory, antihypertensive, antioxidant, antitumor and many others have been described for extracts and active compounds isolated from Tanacetum vulgare. The antioxidant activity of extracts from various representatives of genus Tanacetum has been established using different techniques. It is supportive to the traditional medicinal application of the plant for conditions such as wound healing, rheumatic arthritis and other inflammatory conditions. Currently, some pharmacopoeias have described the use of tansy in anti-inflammatory drugs and in drugs used for treatment of colds and fever.

The chemical composition of this plant includes flavonoids - surface flavonoids (methyl esters of flavones scutellarin, 6-hydroxyluteolin), vacular flavonoids (apigenin, luteolin 7-glucorinides), caffeic acid, glycosides, sterols (β-sitosterol, stigmasterol, cholesterol, campesterol), triterpenes (α-amyrin, β-amyrin, taraxasterol), camphor, α-thyon, β-thyon, borneol, 1,8-syneol, chrysanthenone, camphene, sabine, boryl acetate and others have been identified as major constituents of the essential oil. It is characterized by a specific amphipod-like myrrh. The biological activities of the herb are largely related to the chemical composition of the essential oil. Important group of biologically active compounds found in T. vulgare essential oil are sesquiterpene lactones (STLs), which include parthenolide and tanacetin. A study reported a small plant population of T. vulgare native to Bulgaria, presented with three pure chemotypes, depending on the class of STLs detected: germacranolids, endemanesolides, and no STLs-free. There is strong intraspecific variability between individual populations of T. vulgare occurring in different habitats due to the adaptation of the plant to the specific environmental conditions regarding to the chemical composition of the essential oil. The chemical composition also includes methoxyflavones (eupatorin, chrysoriol, diosmetin), alkaloids, tannins, organic acids, carotenoids, vitamin C, and others.

Onozato et al. (2009) obtained data for the presence of antiviral activity against HSV-1 (herpes simplex virus 1) of ethyl acetate extract from aboveground parts of tansy followed by parthenolide isolation. Alvarez et al. (2011) showed the antiviral properties of methanol extract of T. vulgare aerial parts and five fractions finding that the ethyl acetate fraction and petroleum ethers are active against HSV-1 and HSV-2. This data revealed that active compounds of the plant other than parthenolide (3,5-DCQA-3,5-dicaffeoylquinic acid, axylarine) are probably responsible for the antiviral activity of T. vulgare. Methanol extract from tansy’s blossom is known for its antitumor activity against cucumber mosaic virus (CMV) and potato virus Y (PVY).

The present work explores in vitro the antiviral activity of ethanolic extract from aerial parts of Tanacetum vulgare L. against viral strains of three taxonomic groups, including agents that cause socially significant diseases in humans for which antiviral chemotherapy is indicated, namely coxsackievirus B1 (family Picornaviridae), herpes simplex virus type 1 (family Herpesviridae) and influenza A virus (family Orthomyxoviridae).

AIM

The aim of the present study was to evaluate the antiviral activity of ethanolic extract from herbaceous plant Tanacetum vulgare L. against some important human viruses for which antiviral chemotherapy is needed and to characterize the extract for its antioxidant activity in vitro. The antiviral activity of the extract was characterized by evaluating the effect on pretreatment, the virucidal activity and the effect on the adsorption of the viral cycle.

MATERIALS AND METHODS

Viruses

Coxsackieviruses B1 (Connecticut 5 strain, CVB1), from the collection of the Stephen Angeloff Institute of Microbiology, BAS (Soﬁa, Bulgaria), grown on HEp-2 cells (maintenance solution DMEM (Gibco, BRL) with 10 mmol/l HEPES, 0.5% fetal calf serum (Gibco), penicillin 100 IU/ml and streptomycin 100 mg/ml); infectious titer 10^6.5 CCID50/ml. Influenza A virus Panama/2007/99/H3N2 - IAV (H3N2) represented a laboratory-adapted strain from the collection of the Stephen Angeloff Institute of Microbiology, BAS (Soﬁa, Bulgaria); the virus stock was obtained from allantoic fluids of virus-inoculated 10-days-embryonated eggs, incubated at 37°C; infectious virus titer 10^6.0 CCID50/ml. Herpes simplex virus type 1, Victoria strain (HSV-1) was received from Prof. S. Dundarov, National Center of Infectious and Parasitic Diseases, Soﬁa. The virus was repli cated in monolayer MDBK cells in a maintenance solution DMEM Gibco BRL, Paisley, Scotland, UK, plus 0.5% fetal
bovine serum Gibco BRL, Scotland, UK. The infectious titer of stock virus was $10^{6.75}$ CCID$_{50}$/ml.

**Cells**

Human epithelial type 2 (HEp-2) cells originated from human laryngeal carcinoma (used for cultivation of CVB1) and Madin-Darby bovine kidney (MDBK) cells (for HSV-1 propagation) were obtained from the National Bank of Industrial Microorganisms and Cell Cultures, Sofia. Madin-Darby canine kidney (MDCK) cells (NBL-2; CCL-34) were purchased from ATCC, Manassas, USA and were used for cultivation of IAV (H3N2). The three cell lines were grown in DMEM medium containing 10% fetal bovine serum (Gibco BRL, USA), supplemented by 10 mM HEPES buffer (Merck, Germany) and antibiotics (penicillin 100 IU/ml, streptomycin 100 μg/ml) in CO$_2$ incubator (HERA cell 150, Heraeus, Germany) at 37°C/5% CO$_2$.

**Plant extract**

The crude aqueous ethanolic extract from aerial parts of Tanacetum vulgare L. was provided by Vemo 99 Ltd (Sofia, Bulgaria). The extract contained (in percent of dry matter): flavonoids, determined as apigenin (no less than 18.0%); coumarins, determined as aesculin (no less than 7.5%); tannic compounds, determined as tannin (no less than 7.5%) and others.

**Cytotoxicity assay**

Inoculation of monolayer cells in 96-well plates (Costar®, Corning Inc., Kennebunk, ME, USA) was performed with 0.1 mL/well-containing concentrations of the compounds diluted in a maintenance medium. Cells were incubated in a humidified atmosphere at 37°C and 5% CO$_2$ for 48 h. After microscopic evaluation, the maintenance medium containing the test compound was removed, cells were washed, and 0.1 mL of maintenance medium supplemented with 0.005% neutral red dye was added to each well, and cells were incubated at 37°C for 3 h. After incubation, the neutral red day was removed, and cells were washed once with PBS, and 0.15 mL/well desorb solution (1% glacial acetic acid and 49% ethanol in distilled water) was added. The optical density (OD) of each well was read at 540 nm in a microplate reader (Biotek Organon, West Chester, PA, USA). The 50% cytotoxic concentration (CC$_{50}$) was defined as the material concentration that reduced the cell viability by 50% when compared to untreated control.

**Antiviral activity assay**

Antiviral screening was based on the viral yield reduction technique. Cytopathic effect (CPE) inhibition test used confluent cell monolayer in 96-well plates infected with 100 CCID$_{50}$ in 0.1 mL. After 1 h of virus adsorption, extract were added in various concentrations and cells were incubated for 48 h at 37°C. The viable cells were stained according to the neutral red uptake procedure and the percentage of CPE inhibition for each concentration of the test sample was calculated using the following formula: % CPE = (OD$_{virus control}$ – OD$_{test sample}$) × 100. The selectivity index (SI) was calculated from the ratio CC$_{50}$/IC$_{50}$.

**Virucidal assay**

Samples of 1 ml containing CBV1, HSV-1 and IAV (H3N2) (10$^4$ CCID$_{50}$), and tested compound in its maximum tolerable concentration (MTC) were contacted in a 1:1 ratio and subsequently stored at room temperature for different time intervals (15, 30, 60, 90 and 120 min). Then, the residual infectious virus content in each sample was determined by the endpoint dilution method, and Δlgs as compared to the untreated controls were evaluated.

**Virus attachment assay**

The cells monolayers in 24-well cell culture plates (pre-chilled at 4°C) were inoculated with $10^4$ CCID$_{50}$ of CVB1, IAV (H3N2) and HSV-1 for adsorption at 4°C and treated in parallel with the maximum tolerable concentration of the extract. At particular time intervals (15, 30, 45 and 60 min) cells were washed with PBS in order to remove both the compound and the unattached virus, then overlaid with maintenance medium and incubated at 37°C for 24 hours. Following triple freezing and thawing, the infectious virus titer of each sample was determined by the end-point dilution method. Each sample was prepared in triplicate.

**Pretreatment of MDBK cells**

MDBK cell monolayers in 24-well cell culture plates (CELLSTAR, Greiner Bio-One) (2×10$^6$ cells per well) were pretreated for 15, 30, 60, 90 and 120 min at concentration of MTC of extract in the maintenance medium (1 ml per well). Then, the cell culture media containing substance were removed, the cells were washed twice with phosphate-buffered saline (PBS) and inoculated with HSV-1 (1000 CCID$_{50}$ in 1 ml per well). After a 60-min absorption the virus was removed and cells were covered with maintenance medium. The culture plates were incubated at 37°C for 24 hours and following triple freezing and thawing the infectious virus titers were determined by the end-point
dilution method. Δlogs were evaluated as compared to the viral control (untreated by compounds).

**Evaluation of the antioxidant activity (AOA)**

The total antioxidant activity was determined by the method of Koracevic D, et al. (2001). The method measures the capacity of the sample to inhibit the generation of thiobarbituric acid reactive substances (TBARS). The reaction indicator is sodium benzoate and the inducer for the production of free oxygen radicals is the Fenton’s reagent. 1 mmol/l uric acid is used as a reference solution. The oxidation reaction is initiated by the interaction of the Fe²⁺-EDTA complex with hydrogen peroxide, which results in the generation of hydroxyl radicals. They react with sodium benzoate as a result of which TBARS are formed. The antioxidants contained in the sample under study inhibit the production of TBARS. The interaction is measured spectrophotometrically and the antioxidant activity is determined at 532 nm.

Reagents: 1) 100 mM Na₂HPO₄ – citric acid buffer, pH 7.4; 2) 10 mM of sodium benzoate; 3) 50 mM NaOH; 4) 2 mM EDTA dissolved in phosphate buffer; 5) 2 mM of FeSO₄; 6) Fe²⁺-EDTA complex; 7) 10 mM of H₂O₂; 8) 20% acetic acid; 9) 0.8% (w/v) thiobarbituric acid (TBA) in 50 mM NaOH; 10) 1 mM uric acid in 5 mM of NaOH. Solutions 4-9 are prepared immediately before the experiment, the phosphate buffer and sodium benzoate are stored at (0-4°C) and the uric acid solution – (at -20°C to -30°C).

The preparation and examination of the samples was performed according to the scheme presented in Table 1.

**RESULTS**

The cytotoxicity of extract from *Tanacetum vulgare* was determined by three cell lines, MDBK, MDCK, and HEp-2, which are then the basis for conducting antiviral experiments (Table 1). The results show the lowest toxicity of the extract on the MDBK cell line and close cytotoxicity in Hep-2, whereas in the MDCK cells it has more than twice the highest toxicity.

Testing the antiviral activity of *Tanacetum vulgare* extract revealed a slight inhibition of replication of HSV-1 with a selective index of 7.07 and IAV/H3N2 (SI = 3.69) but no specific antiviral effect against CVB1 replication was found (Table 2).

The extract also showed activity against extracellular virions of all three viruses tested. As shown in Table 3, the effect was the strongest in the HSV-1 virions, with the effect still accounting for 15 min of exposure (Δlog = 1.5) maintained with this value until 90 min and with a slight increase of 120 min (Δlog = 1.75). The effect on CVB1 virions was less pronounced, with a known effect observed at 30 min (Δlog = 1) and significant after 60 min (Δlog = 1.5).

No effect was observed upon the interaction of the extract with the extracellular virions of IAV/H3N2 at 15 and 30 minutes. Low activity manifested after 60 minutes (Δlog = 1.17 and a slight increase at 90 and 120 min (Δlog = 1.33).

After determining the effect of *Tanacetum vulgare* L extract on extracellular virions and replication of intracellular viruses, we monitored its effect on the stage of adsorption of the virus to the respective sensitive cells. The inhibition of adsorption on all three viruses occurs with approximately the same decrease in viral titers. At 15 min the effect was weak in all three viruses, at 30 min it became significant in HSV-1 (Δlog = 1.75), while in the other two viruses it still had relatively low values and after 45 min the adsorption of all three viruses was significantly suppressed, with the strongest effect observed at SVB1 at 60 min (Δlog = 2) (Table 4).

We also examined the protective effect of the extract on virus-free cells and determined the extent to which it could protect cells from viral invasion. We chose the model to use MDBK cells in which the extract showed the lowest cytotoxicity. The cells treated with the extract had a significant decrease in infectious viral titers. At the first studied time interval - 15 min this decrease was with Δlog = 2.0 and remained with such a value for up to 60 minutes. At 90 minutes it increased (Δlog = 2.5) and with increasing time of exposure to the cells the protective effect increased too such as at 120 min reached the value of Δlog = 4.

The evaluation of the antioxidant activity showed great antioxidant activity of the researched ethanolic extract from *T. vulgare* – 26 mmol/l for the applied 20 mg/ml extract. It is very important to note that ethanol does not affect to great extent the observed antioxidant effect: the antioxidant activity of ethanol itself was 5 mmol/l, meaning that the activity of *T. vulgare* is more than 5 times higher. In comparison to standard antioxidants, *T. vulgare* also shows extremely high antioxidant activity: the measured antioxidant activity of vitamin C (20 mg/ml) was 0.86 mmol/l and the one of vitamin E (20 mg/ml) - 2 mmol/l. All activities were measured and compared to a standard solution of uric acid - 1 mmol/l.

**DISCUSSION**

*Tanacetum vulgare* L. extract is a mixture of many biologically active substances. There is a wealth of data in the literature on the biological activity of related components. Many are reported to have antiviral activity, affecting certain stages of the virus's multiplication. In some, the extracellular virions are targeted, in others - the stage of virus adsorption to the cell, in still others, it is the blocking at different stages of the internal replicative cycle of the virus affecting its essential structures.

Extract of *Tanacetum vulgare* L. influences to a certain extent specifically the stage of the intracellular replicative cycle of the HSV-1 and IAV (H3N2). The multicomponent structure of the extract contributes to the ability of its con-
constituents to interact with the capsid and supercapsid proteins of the virus, to alter their structure, thereby reducing the virulence of the virus. Most likely, such an interaction is due to the virucidal effect we observed. Extract of *Tanacetum vulgare* L. influences to a certain extent specifically the stage of the intracellular replicative cycle of the HSV-1 and HSV-2.

28,29 The multicomponent structure of the extract contributes to the ability of its constituents to interact with the capsid and supercapsid proteins of the virus, i.e. HSV-1 gC1 and HSV-2 gG which leads to block of virus entry. 30 By a similar mechanism, some of the components of the extract probably bind to the cell receptors recognizing the viral particle or to other parts of the cell membrane responsible for attaching the virus to the cell, which explains its effect on pre-treatment of cells and its effect on the adsorption of HSV-1, CVB1 and IAV (H3N2). The use of such preparations before viral infection could protect the cells from subsequent infection.

The mild antiviral activity of *Tanacetum vulgare* L. crude extract against IAV (H3N2) could be partially explained by the polyphenolic compounds and particularly by their flavonoid fraction. Flavonoids extracted from various plant species have been demonstrated to exert antiviral effect against influenza viruses most likely by interacting with the viral neuraminidase, which is an enzyme responsible for the release of viral progeny, by up-regulation of P38 and JNK.

### Table 1. Scheme of samples for testing of antioxidant activity

<table>
<thead>
<tr>
<th>[ml]</th>
<th>$A_1$</th>
<th>$A_0$</th>
<th>$K_1$</th>
<th>$K_0$</th>
<th>$UA_1$</th>
<th>$UA_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>sample</td>
<td>0.01</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>uric acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>buffer</td>
<td>0.49</td>
<td>0.49</td>
<td>0.50</td>
<td>0.50</td>
<td>0.49</td>
<td>0.49</td>
</tr>
<tr>
<td>sodium benzoate</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>acetic acid</td>
<td>-</td>
<td>1.00</td>
<td>-</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Fe$^{2+}$-EDTA complex</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>$H_2O_2$</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>incubation 37°C/60 min</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>acetic acid</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>TBA</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

All samples were incubated at 100°C/10 min, cooled with cold water and measured spectrophotometrically against a cuvette with deionized water.

Calculations: $AOA$ [mmol/l] = $(C_{UA}) \cdot (K - A) / (K - UA)$, where:
- $K$ - control absorption ($K_1$-$K_0$)
- $A$ - sample absorption ($A_1$-$A_0$)
- $UA$ - absorption of uric acid solution ($UA_1$-$UA_0$)
- $C_{UA}$ - uric acid concentration (mmol/l)

### Table 2. *In vitro* antiviral activity of ethanolic extract from aerial parts of *Tanacetum vulgare* L.

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>Tanacetum vulgare</em> extract</th>
<th>CVB1</th>
<th>HSV-1</th>
<th>IAV (H3N2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HEP-2</td>
<td>MDBK</td>
<td>MDCK</td>
<td>IC$_{50}$ (μg/ml)</td>
</tr>
<tr>
<td><em>Tanacetum vulgare</em> extract</td>
<td>1428</td>
<td>1635</td>
<td>687</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 3. Virucidal activity of ethanolic extract from aerial parts of *Tanacetum vulgare* L.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Δlog 15 min</th>
<th>Δlog 30 min</th>
<th>Δlog 60 min</th>
<th>Δlog 90 min</th>
<th>Δlog 120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.75</td>
</tr>
<tr>
<td>CVB1</td>
<td>0.5</td>
<td>1.0</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>IAV (H3N2)</td>
<td>0</td>
<td>0.16</td>
<td>1.17</td>
<td>1.33</td>
<td>1.33</td>
</tr>
</tbody>
</table>

### Table 4. Effect of ethanolic extract from aerial parts of *Tanacetum vulgare* L. on viral adsorption

<table>
<thead>
<tr>
<th>Virus</th>
<th>Δlog 15 min</th>
<th>Δlog 30 min</th>
<th>Δlog 45 min</th>
<th>Δlog 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1</td>
<td>1</td>
<td>1.75</td>
<td>1.75</td>
<td>1.75</td>
</tr>
<tr>
<td>CVB1</td>
<td>1</td>
<td>1</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td>IAV (H3N2)</td>
<td>0.83</td>
<td>1.33</td>
<td>1.67</td>
<td>1.67</td>
</tr>
</tbody>
</table>
expression (modulation of MAP-kinase pathway) resulting in enhanced cell-immunity, by restriction of RNPs export from the nucleus (hesperidin). Studies performed with polyphenolic extract from popular Bulgarian medicinal plant *Geranium sanguineum* revealed strong anti-herpes, anti-influenza (virecidal effect including) and antioxidant effects in model systems, cell cultures and in mice. The registered antioxidant effect of *T. vulgare* could be due to the presence of polyphenolic compounds such as 3,5-O-dicaffeoylquinic acid (3,5-DCQA), axillarin and luteolin and vitamin C. Phenolic compounds have been reported to have multiple biological effects, including antioxidant activity by scavenging reactive oxygen, nitrogen, and chlorine species, such as superoxide anion, hydroxyl radical, peroxyl radicals, hypochlorous acid and peroxynitrous acid. Flavonoids possess more hydroxyl groups and higher antioxidant activity.

Cooperation of all various antioxidants provides greater protection against oxidative stress, than any single compound alone. Thus, the overall antioxidant activity may give more relevant biological information compared to that obtained by the measurement of individual components, as it considers the cumulative effect of all antioxidants present. The displayed antioxidant activity of the extract is in support of the reported results in other studies and of the traditional medicinal uses of the plant for various conditions such as injury recovery, arthritis, inflammatory processes, etc.

The antiviral effects are very likely to be a result of reactions of the extract's components with cellular components involved in viral replication cycle. Another aspect refers to the indirect efficacy associated with the potent antioxidant activity of the extract. Further detailed analysis of the extract's composition biological activities and the particular effects of each constituent would be advantageous in the elucidation of its complex mechanism of action.

**CONCLUSION**

The crude extract from aerial parts of the medicinal plant *Tanacetum vulgare* L. demonstrated low cytotoxicity in Hep-2, MDBK and moderate cytotoxic effects in MDCK cells. It exerted significant antiviral activity against HSV-1 as determined by the recorded inhibition of viral replication, the blockage of virus entry - absorption stage and direct virucidal effects on extracellular virions. Comparable as an overall assessment, but noticeably milder inhibitory properties we observed when testing Tanacetum's extract on influenza A H3N2 virus infection *in vitro*, which probably resulted from the interference with cellular pathways involved in the replication cycle. The presence of virucidal and adsorption-suppressing activity but the absence of viral replication inhibitory effects against CBV-1 suggests a possible interaction of the extract's components with the viral capsid proteins or related cell receptors. The pronounced antioxidant potentials tested in model systems were likely due to the rich polyphenol contents of the extract. Further analysis *in vivo* and a deeper insight in the individual constituents’ antiviral and antioxidant effects would elucidate the complete efficacy and the particular mechanisms of action of *Tanacetum vulgare* L. crude ethanol extract.

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Antiviral, cytotoxic and antioxidant effects of \textit{Oba oblonga} L. crude extract in vitro.


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**Резюме**

**Введение:** В связи с высокой распространенностью вирусных инфекций, которые не получают специфического лечения, и постоянным появлением устойчивых штаммов вируса, поиск противовирусных ингредиентов имеет решающее значение. В этом исследовании рассматриваются противовирусные свойства in vitro этанолового экстракта надземных частей Tanacetum vulgare L. против вирусных штаммов трех таксономических групп, в том числе агентов, вызывающих социально значимые заболевания, для которых назначена противовирусная химиотерапия, а именно коксакивирус В1 (семейство Picornaviridae) вирус простого герпеса типа 1 (семейство Herpesviridae) и вирус гриппа А (семейство Orthomyxoviridae).

**Цель:** Цель этого исследования состояла в том, чтобы определить противовирусную активность этанольного экстракта травы Tanacetum vulgare L. в отношении некоторых важных вирусов человека, нуждающихся в противовирусной химиотерапии, и охарактеризовать антиоксидантную активность экстракта in vitro.

**Материалы и методы:** Неочищенный водный этанольный экстракт надземных частей Tanacetum vulgare L. содержал флавоноиды, идентифицированные как апигенин, кумарины, такие как эскулин, соединения танина, определённые как танин, и другие. Противовирусную активность этанольного экстракта травы Tanacetum vulgare L. в отношении вируса Коксаки B1, вируса простого герпеса типа 1 и вируса гриппа A оценивали методом снижения выхода вируса (viral yield reduction technique). Общая антиоксидантная активность была определена путём измерения способности образца ингибировать образование ре-активной тиобарбитуровой кислоты (TBARS).

**Результаты:** Результаты показали, что экстракт имел самую низкую токсичность в клеточной линии MDBK (Madin-Darby Bovine Kidney) и аналогичную цитотоксичность в отношении Hep-2, тогда как в клетках MDCK (Madin-Darby Canine Kidney) их концентрация была более чем в два раза выше. В исследовании противовирусной активности экстракта Tanacetum vulgare L. наблюдалось незначительное увеличение репликации HSV-1 с селективным индексом 7,07 и IAV / H3N2 (SI = 3,69), но никакого специфического противовирусного эффекта в отношении CVB1 обнаружено не было. Оценка антиоксидантной активности показала высокую антиоксидантную активность этанольного экстракта T. vulgare - 26 mmol/l для используемого экстракта 20 mg / мл.

**Выводы:** Неочищенный надземный экстракт лекарственного растения Tanacetum vulgare L. показал низкую цитотоксичность в отношении Hep-2, MDBK и умеренный цитотоксический эффект в клетках MDCK. Он проявлял значительную противовирусную активность против HSV-1, определяемую по зарегистрированному ингибированию репликации вируса, блокированию проникновения вируса в стадию абсорбции и прямому вирулицидному воздействию на внеклеточные вирусы. Наблюдаемый эффект в исследовании экстракта Tanacetum на заражение вирусом H3N2 типа A in vitro был более слабым, возможно, в результате иммунитета в клеточные пути, вовлечённые в цикл репликации. Наличие вирулицидной и абсорбционно-подавляющей активности и отсутствие ингибиторных эффектов на репликацию вируса против CBV-1 позволяют предположить вероятное взаимодействие компонентов экстракта с вирусными капсидными белками или родственными клеточными рецепторами.

**Ключевые слова**

Tanacetum vulgare L., CVB1, H3N2, HSV-1, антиоксидантная активность