

ANTIVIRAL ACTIVITY OF DRIED EXTRACT OF *STEVIA*

S. A. Kedik,¹ E. I. Yartsev,¹ and I. E. Stanishevskaya¹

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The biological (antiviral) activity of a dried purified extract of *Stevia* was evaluated in vitro. Tests were performed using Teschen disease virus, infectious rhinotracheitis virus, and human coronavirus.

Key words: *Stevia*, dried extract, diterpene glycosides, antiviral activity.

Extracts and isolated diterpene glycosides are currently widely used in the food industry as sugar substitutes and sweeteners, because of their unique organoleptic properties, namely their sweetness and the virtual absence of bitterness and adventitious flavors, as well as their extremely low caloric value [1].

At the same time, there is great interest in published data on the antiviral and antibacterial activities of *Stevia* extracts. Thus, in in vitro experiments, *Stevia* extracts effectively suppressed the activity of human retroviruses (HRV) by blocking the binding of virus with susceptible cells. Another study presented data on the antibacterial activity of aqueous extracts of *Stevia* in relation to enterohemorrhagic *Escherichia coli* [2 – 5].

The limited amount of information available on this question led us to study the antiviral properties of a dried extract of *Stevia*.

EXPERIMENTAL SECTION

The biological activity of a dried, purified extract of *Stevia* leaves was assessed using a modified method [6, 7] to test antiviral activity in collaboration with the All-Russian Science Research Institute of Veterinary Virology and Microbiology (VNIIBiM).

VIRUSES

Tests were performed using the RNA-containing Teschen disease virus (*porcine teschovirus*) and the DNA-containing infectious rhinotracheitis (IRT) virus (*bovine herpesvirus 1*).

Experiments also used the RNA-containing human coronavirus (*human coronavirus* (Hco V-229E)).

METHODS

Studies were performed using a model based on transformed green monkey kidney (Vero) cells. The virustatic and virucidal actions of the dried extract were tested in three repeats on monolayer cultures of Vero cells, which were incubated at 37°C in an atmosphere of 5% CO₂ using 96-well plates (Costar, UK). The antiviral activity of the dried extract from *Stevia* leaves was assessed using standard methods in terms of the ability of the substance to prevent the cytopathic effect of the virus on cells as compared with controls after 72 h of incubation [8]. Virus titers were measured as lg TCD₅₀ (50% tissue cytopathic doses) values as described by Reid and Mench [9].

Controls consisted of cell cultures infected with virus at the experimental dose without addition of test substance (virus controls) and intact cell cultures supplemented with Eagle-M maintenance medium instead of test solution (cell controls).

Virustatic (inhibitory) actions were determined by infecting Vero cell cultures with virus-containing material at a multiplicity of infection of 0.0001 – 0.001 TCD₅₀/cell. After virus infection, cells were incubated at 37°C for 1 – 1.5 h (for virus adsorption), after which cell cultures were supplemented with solutions of the dried, purified *Stevia* extract at different concentrations in an incubator at 37°C until the clear appearance of the cytopathic effect (CPE) in the virus control. After appearance of the CPE in the virus control, the experimental and control samples were titrated in cell cultures. The virustatic action of the substance was assessed in terms of the difference in virus titers in the experimental and

¹ M. V. Lomonosov Moscow State Academy of Fine Chemical Technology, Moscow, Russia.

TABLE 1. Virustatic Activity of Dried, Purified Extract of *Stevia* Leaves

Virus	Dose, $\mu\text{g/ml}$	IRT virus		Difference in virus titers in control and experiment, lg TCD ₅₀
		experiment, lg TCD ₅₀	control, lg TCD ₅₀	
Teschen disease virus	2000.0	3.25	3.75	0.5
Coronavirus	2000.0	3.00	3.33	0.33
Virus titer in:	2000.0	4.50	4.75	0.25

control tests, expressed in lg TCD₅₀ (tissue cytopathic dose) units.

The virucidal (inactivating) activity of solutions of the dried, purified *Stevia* extract was assessed by mixing doses of 5.0 – 5000.0 $\mu\text{g/ml}$ with equal volumes of virus-containing material and incubating at 37°C for 18 – 20 h. The maximum tolerable dose was 5000.0 $\mu\text{g/ml}$. Controls consisted of virus-containing material mixed with Eagle-M maintenance medium instead of test substance solution and intact cell cultures. After contact, mixtures were titrated in parallel with controls. Virucidal activity was determined in terms of the difference in virus titers between the experimental and control tests and was expressed in lg TCD₅₀ units. Results were assessed after 72 – 144 h of incubation at 37°C, after the clear appearance of the CPE in the virus controls.

RESULTS AND DISCUSSION

These studies showed that the dried, purified extract obtained from *Stevia* leaves at a dose of 2000 $\mu\text{g/ml}$ inhibited the reproduction of Teschen disease virus by 0.5 lg TCD₅₀, IRT virus by 0.25 lg TCD₅₀, and coronavirus by 0.33 lg TCD₅₀ (Table 1).

The dried, purified extract at a dose of 4000 $\mu\text{g/ml}$ inactivated Teschen disease virus by 0.75 lg TCD₅₀, IRT virus by 0.5 lg TCD₅₀, and coronavirus by 0.66 lg TCD₅₀ (Table 2).

The commonly used antiviral agent remantadine is known [10] to have high virustatic action but virtually no

TABLE 2. Virucidal Activity of Dried, Purified Extract of *Stevia* Leaves

Virus	Dose, $\mu\text{g/ml}$	Virus titer in:		Difference in virus titers in control and experiment, lg TCD ₅₀
		experiment, lg TCD ₅₀	control, lg TCD ₅₀	
Teschen disease virus	4000.0	3.00	3.75	0.75
Coronavirus	4000.0	2.66	3.33	0.66
IRT virus	4000.0	4.25	4.75	0.5

virucidal activity, i.e., it does not suppress viruses in the intercellular space or in blood vessels. Unlike remantadine, solutions of the dried, purified *Stevia* extract had marked virustatic and virucidal properties. Until recently, dried *Stevia* extracts (steviosides) were regarded mainly as sweeteners. The demonstration of antiviral properties of *Stevia* extract makes it attractive for further investigation as a potential medicinal agent.

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