ANTIVIRAL ACTIVITY OF CUBAN VEGETABLE SPECIES

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Summary

The natural products, fundamentally those based on medicinal plants constitute favourites therapies for the treatment of some ailments and illnesses. In the action against the micro-organisms, the scientific development has propitiated the interest for the medicinal plants and the natural bioactive substances to battle some virus and today it is investigated strongly in this field. We carried out a prospecting of the scientific literature around the use of the medicinal plants for the treatment of viral infection. In the particular thing, it was evaluated the citotoxicity and the antiviral capacity of extracts of several species of the *Erythroxylum*, *Erythrina* and *Zanthoxylum* genus against stumps of banks and clinical isolated. In some cases the activity viruside was evaluated. Extracts of several species of the *Erythroxylum* and *Erythrina* show antiherpetic activity of the type I and II, and they don't turn out to be toxic**.**

KEY WORDS: Antiviral, Antiherpetic, *Erythroxylum*, *Erythrina*, *Zanthoxylum*.

The medicinal plants as part of the traditional medicine have played an important part in the treatment of diverse pathologies (*1,2*).

A great variety of plants in the whole planet and their respective extracts contain different chemical compounds with biological activity that they can produce varied therapeutic effects; for that reason the scientific evidence nowadays is called to validate the popular use (*3,4.*)

The etnomedical properties of the Cuban plants have propitiated an important impulse to satisfy the necessity and to stimulate the convenience to use and to transform, in sustainable way, our flora (*5).*

They are hundred of viruses known by the sciences and every day they are discovered some more than cause a very negative effect for the humanity (*6*).

In this study we intended to evaluate the antiviral activity of several derived vegetable extracts of the species belonging to the goods *Erythroxylum, Erythrina* and *Zanthoxylum* in front of the virus herpes simple types 1 and 2, by means of the determination of the half citotoxic concentration, effective stocking concentration and selective index of each one of the possible candidates.

Methods

The vegetable species were collected in the Pinar del Rio province during the years 2000 and 2005 and identified by the PhD. Armando Urquiola, Director of the Botanical Garden of this province. The vegetable extracts were prepared in a conventional way in form of decoction, infusion or hydroalcoholic maceration.
The citotoxicity of the extracts used was evaluated by means of a assay based on the change of the color that happens for the reduction of the 3-(4,5-dimethyltiazol-2-il)-2,5-diphenyl tetrazolium bromide (MTT) that takes place by mitochondrial enzymes.
For the evaluation of the antiherpetic activity were used kidney cells (VERO) of African green monkey among the passes 140 and 145, grown between 199 supplemented with 10% of inactive serum bovine fetal (SFBI). A stump of reference of the herpes simple virus, type 2, was used (VHS-2), donated kindly by the Institute of Tropical Medicine "Pedro Kourí", Havana, Cuba. The viral title was determined by the method of Reed and Muench, and on the base of this the 100 infectious doses were determined in cell culture of cells (DICC50), used in the antiviral evaluation. To determine the half citotoxicity concentration (CC50), cells VERO was sowed in badges of 96 wells to those that were added different concentrations of the extracts. The badges were incubated at 37^{o}C in atmosphere from CO_2 to 5% for 3 days.
To determine the antiviral effective concentration (CE50) the different quantities were added from the extracts to the sowed badges of cells and were incubated during 1 hour; later on, the 100 DICC of the virus was added. The badges were incubated under the same conditions described previously. All the assays were carried out using the method of the MTT. Later on, the selective index was determined.

Results

Main results obtained in the screening in front of the Virus Herpes Simplex type 1 (VHS-1) of reference (R) and VHS-1 of isolation (A) resistant to Acyclovir, in cells Vero, is presented in the Table 1.

Table 1. Main obtained results by *in vitro* evaluation for extracts vs. *Herpes Simple* Virus, type 1 (VHS-1).

Genus	Species	Plant Part	Extract type	CC_{50} (µg/ mL) ±S	VHS-1 Isolation		VHS-1 Reference	
					CE_{50} (µg/mL) ±S	IS	CE_{50} (µg/mL) ±S	IS
ER	*E. fusca* Lour.	Bark	D	> 8000	NE	-	243	> 32.9
				>4000	274.8±2.7	14.6	23.7±0.3	>168.6
	E. poeppigiana	Bark	D	840±2.6	147.6±4.3	5.7	32.6±1.8	25.8
EX	*E. areolatum*	Leaf	HA	>4000	218.5±8.2	>18.3	41.6±6.5	>96.1
	E. armatum	Leaf	HA	>4000	110.9±11.4	>36.1	10.5±0.6	>382.0
	E. confusum	Leaf	HA	480±9.0	Negative	-	12.6±0.2	38.2
	E. minutifolium	Leaf	HA	>4000	107.9±8.4	>37.0	5.9±0.3	>668.9
	E. suave	Leaf	I	> 4000	Negative	-	86.9±10.3	>46.1
			HA	650±5.8	Negative	-	4.3±0.6	149.7
	E. longipes	Leaf	HA	540±6.3	47.9±2.7	11.3	9.4±0.8	57.4
ZA	*Z. cubense*	Bark	HA	431±7.6	126.2±10.0	3.4	22.2±1.8	19.4

ER: *Erythrina*; EX: *Erythroxylum*; ZA: *Zanthoxylum* D: Decoction; I: Infusion; HA: Hydroalcoholic; CC_{100}: Maxim Cytotoxic Concentration; CC_{50}: Half Citotoxic Concentration; CE_{100}: Maxim Effective Concentration; CE_{50}: Half Effective Concentration; IS: Selective Index (Effective: ≥ 10); NE: Not assay S: Standard Deviation

Table 2. Citotoxicity and Antiviral Activity for aqueous or hydroalcoholic extracts vs. *Herpes Simple Virus*, 1 and 2 types.

Species	Plant Part	Extract Type	Citotoxicity	Antiviral Activity µG/Ml ± S		
				VHS-1 (R)	VHS-1 (A)	VHS-2
Erythroxylum areolatum	Leaf	HA	1264.8± 2.7	156.08±5.4	118.01±3.3	59,77±9.5
Erythroxylum minutifolium	Leaf	HA	> 1000	79.53± 4.2	73.91±2.2	107.04±8
Erythroxylum clarensis	Leaf	HA	> 1000	85.94±2.9	75.23±2.5	91.31±5.1
Erythroxylum areolatum	Leaf	HA	> 1000	73.47±4.6	133.92±5.8	68.62±4.4
Erythrina poeppigiana	Bark	D	> 1000	Not have	Not have	Not have
Zanthoxylum elephantiasis	Fruits	HA	> 1000	Not have	Not have	Not have
Zanthoxylum fagara	Fruits	HA	> 1000	Not have	Not have	Not have
Zanthoxylum coriaceum	Fruits	HA	> 1000	Not have	Not have	Not have

HA: Hydroalcoholic; D: Decotion

Discussion

It is feasible to direct the investigations with vegetable species for the search of antiviral extracts, keeping in mind that the etnomedical knowledge constitutes an instrument of very potent information that allows guiding the current scientific investigations in the search of new therapies, in the face of a high diversity of superior plants to explore. In our mark, some genus of plants are promissory at least to find made up with antiviral properties.

Starting from the results showed in the Table 1, it is evident that in general the most promising species for the search of antiviral action are the *Erythrina* and *Erythroxylum* genus. It is prominent the fact that some of the examined extracts had good effectiveness, measured by values of the biggest IS that 10 even in front of the stump of the VHS coming from the clinical isolation.

In other results, the toxic effect of each extract was determined in the cells VERO, where the extracts coming from the species soft *Erythroxylum* (infusion), *Erythroxylum areolatum, Erythroxylum minutifolium, Erythroxylum armatum* didn't show toxicity until a concentration of 4 mg/mL, while the extracts coming from the species soft *Erythroxylum* (hydroalcoholic), *Erythroxylum confusum, Erythroxylum longipes* showed values of toxicity of 650 ± 5,78 µg/mL, 480 ± 8.99 µg/mL and 540±6.33 µg/mL, respectively. Most of the extracts showed antiviral activity in front of the VHS-2 and their selective indexes were superior at 10. We concludes that the extracts that showed antiviral activity in front of the virus of the Herpes simple type 2 and low toxicity could be considered as good candidate of antiherpetic activity.

The floras constitute an important resource for the treatment of viral affections. Diverse extracts coming from vegetable species can diminish or eliminate the undesirable effects of the illnesses caused by virus. Some extracts of the *Erythroxylum* and *Erythrina* goods show antiviral capacity and they could become phytomedicines.

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