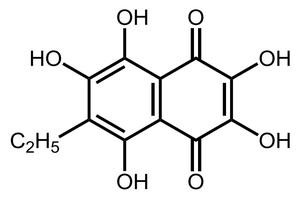
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Development of composition based on echinochrome A with ascorbic acid and α-tocopherol for oral administration

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Echinochrome A

Secondary metabolites specific to sea urchins are known as spinochromes. The most well-known sea urchin pigment echinochrome A exhibit a wide range of pharmacological activities, for example antioxidant, anti-diabetic [1], anti-allergic [2], cardioprotective [3], and also protects mitochondrial functions against cardiotoxic drugs [4]. In addition, echinochrome A is the active substance in the cardioprotective and ophthalmic drug Histochrome, produced in Russia from the sand dollar *Scaphechinus mirabilis* [5].



Histochrome

One of the main obstacles to the wide use of echinochrome A is its insolubility in water. Histochrome is available only in ampoules in the form of echinochrome A sodium salts for intravenous injections. To expand area of echinochrome A application, it is very important to investigate abilities of echinochrome A to form complexes with substances that can increase its solubility, protect its hydroxyl groups from oxidation, preserving or enhancing its pharmacological properties.

Very recently, we obtained a powder composition of echinochrome A (Ech) with other well-known antioxidants – ascorbic acid (Asc) and alpha-tocopherol (Toc). Ratio of components was chosen based on the results of lipids peroxidation inhibition assay (Fig. 1, Table 1). The most strong antioxidant effect demonstrated composition Ech-Asc-Toc 5:5:1 [6].

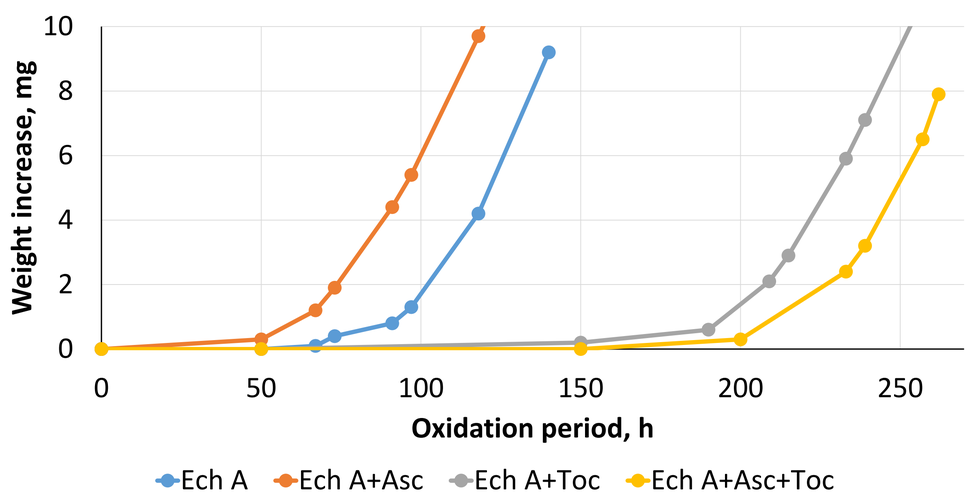
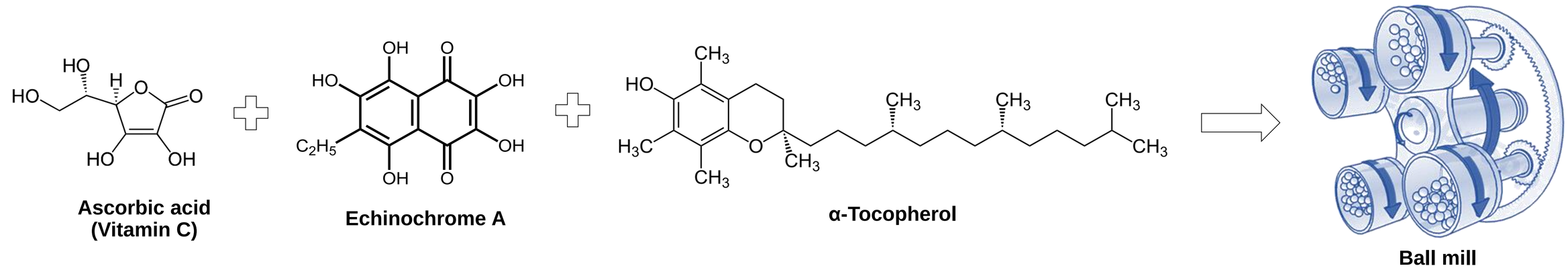


Figure 1. Periods of induction of the oxidation reaction of linetol in the presence of echinochrome (Ech) and its mixtures with ascorbic acid (Asc) and alpha-tocopherol (Toc).

Table 1. Antioxidant activity of the formulations on a model of linetol auto-oxidation. The concentration of Ech, Asc, Toc and their compositions in test medium was of 0.05 mg/mL. * – Statistically significant differences between Ech and antioxidant compositions ($p \leq 0.05$); ** – statistically significant differences between three-component and two-component mixtures of antioxidants ($p \leq 0.05$).

Antioxidants and their compositions	Δτ, h	The efficiency of the composition compared to Ech
Ech	100±5	-
Asc	24±3	-
Toc	125±7	-
Ech+Asc (1:1)	69±4	No effect
Ech+Toc (1:1)	201±8*	Synergism 8%
Asc+Toc (2:1)	195±7*	Synergism 7%
Ech+Asc+Toc (5:5:1)	223±10**	Synergism 40%
Control – linetol	**24±2**	



Ascorbic acid (Vitamin C) + **Echinochrome A** + **α-Tocopherol**

Ball mill

We established that Ech-Asc-Toc composition containing chosen excipients (Table 2) is stable for 1 year and that addition of antioxidants and excipients does not affect echinochrome A permeability through artificial membrane imitating gastrointestinal tract (PAMPA assay, Fig. 2). The developed composition showed *in vitro* antiviral activity against RNA-containing tick-borne encephalitis virus and DNA-containing herpes simplex virus type 1 [6].

Table 2. Selection of excipients to create Ech-Asc-Toc composition.

Exp. No	α-Toc, g	MCC, g	Kaolin, g	Casein, g	Magnesium carbonate, g	Aerosil, %*	Magnesium stearate, %*	Result
1	1	1				10%	1%	**Poor flowability, caking**
2	1	5				10%	1%	**Normal flowability**
3	1	10				10%	1%	**Normal flowability**
4	1		5			10%	1%	**Mixed bad, poor flowability**
5	1		10			10%	1%	**Mixed bad, poor flowability**
6	1			5		10%	1%	**Poor flowability, caking**
7	1			10		10%	1%	**Poor flowability, caking**
8	1				5	10%	1%	**Normal flowability, but worse than in Exp.No 2 and 3**
9	1				10	10%	1%	**Normal flowability, but worse than in Exp.No 2 and 3**
10	**1**	**5**				**5%**	**1%**	**Good flowability**

* Aerosil (sliding excipients) and magnesium stearate (lubricating excipients) were laid at the maximum rates - 10% and 1% of the total mass, respectively.

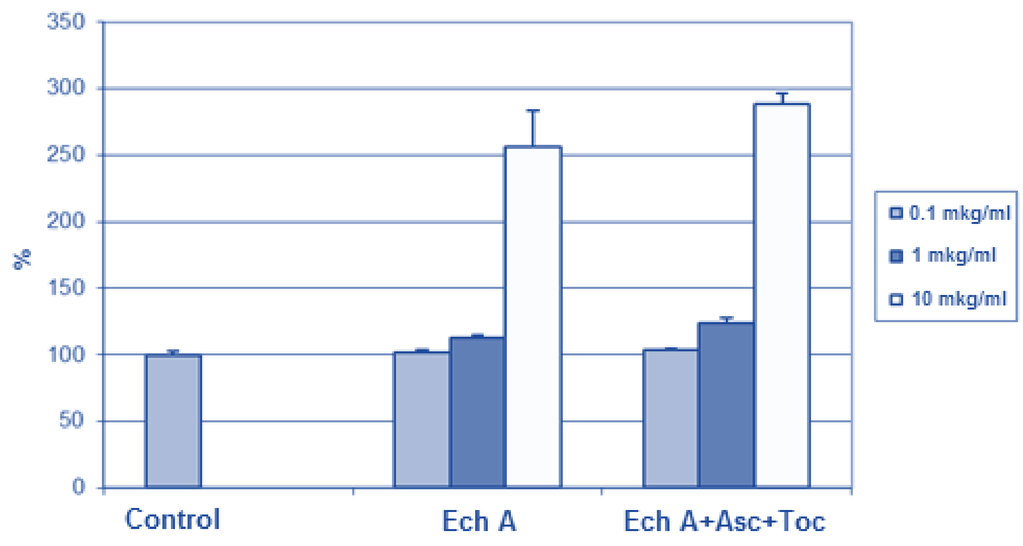


Figure 2. Permeability of echinochrome A from Ech-Asc-Toc composition through the PAMPA membrane that imitates passive gastrointestinal penetration. Control - wells with buffer only.

Conclusions:

- **Ech-Asc-Toc powder composition is stable for 12 months**
- **Ech-Asc-Toc powder composition dissolves at pH 1.2 immediately releasing up to 90% of echinochrome A**
- **Ech-Asc-Toc powder composition exhibits significantly higher antioxidant and antiviral activities than echinochrome A alone**
- **Ech-Asc-Toc powder composition is perspective for oral drug forms development**

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