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Factors Affecting the Efficiency of Two-Phase Extraction of Flavonoids and Carotenoids from *Hypericum maculatum L.*

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Abstract:
Current problem in pharmacy is the creation of complex drugs based on *Hypericum maculatum L.*, which have anti-inflammatory, astringent, antispasmodic and tonic effects. *Hypericum* herb contains a complex of biologically active substances of different polarity: flavonoids, hypericin, tannins, vitamins, carotenoids. Biphasic extraction solvents system allows, by one cycle, to simultaneously obtain two extracts, alcohol and oil, containing a complex of hydrophilic and lipophilic compounds. The dependence of the ratio of the polar and non-polar phases on the biologically active substances extraction from the *Hypericum* herb was studied. The effectiveness of two-phase extraction was determined by the content of flavonoids in the water-alcohol phases and carotenoids in oil phases. It was determined that a two-phase solvent system extracts a complex of biologically active substances of *Hypericum maculatum L.* more effectively than a sequential extraction with separate solvents of different polarities. The polar phase enhances the processes of desorption, diffusion and phase transfer of not only hydrophilic, but also lipophilic *Hypericum* herb substances. The optimal ratio of raw materials: 70% ethanol: oil, which provided the maximum yield of flavonoids and carotenoids from raw materials, was 1:10:10.
Keywords: ***two-phase extraction; Hypericum; flavonoids; carotenoids.***

Inroduction

One of the actual problems of modern pharmacy is the creation of complex drugs and dietary supplements based on *Hypericum maculatum L.*, which have anti-inflammatory, astringent, antispasmodic, and tonic effects.

A rational plant materials use requires the development of modern technologies of the extraction process, ensuring maximum yield of biologically active substances.

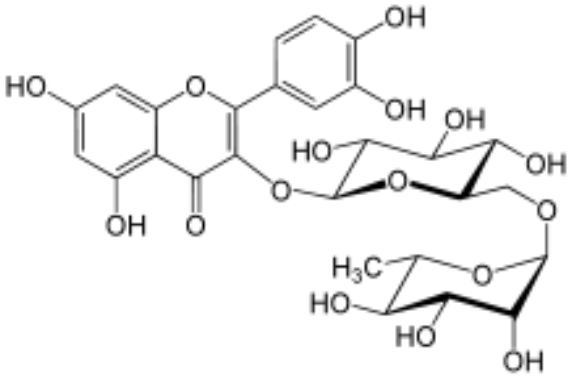
The dependence of the ratio of the polar and non-polar phases on the biologically active substances extraction from the *Hypericum* herb was studied.

The chemical composition of the herb *Hypericum maculatum L.*

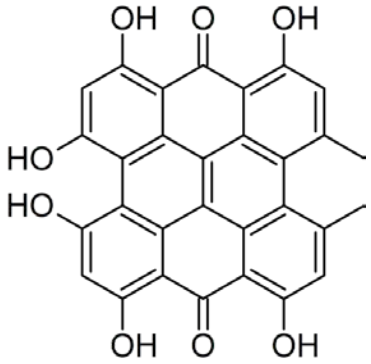
Hypericum herb contains a complex of biologically active substances of different polarity:

flavonoids: hyperoside, rutin, quercetin up to 7%;
hypericin (up to 0.4%),
pigments (anthocyanins),
tannins (8-10%),
water-soluble vitamins,
choline, saponins,
carotenoids (about 50 mg%),
essential oil (up to 3%)

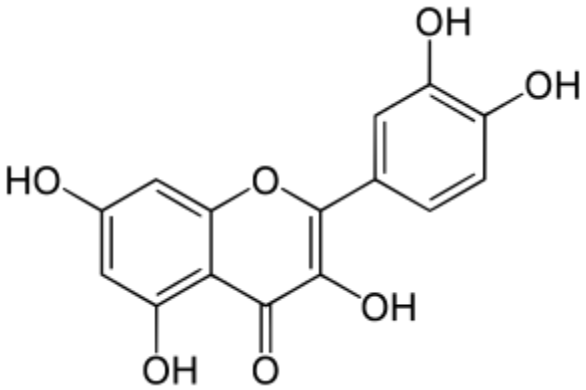
Structural formulas of bioactive substances of *Hypericum*



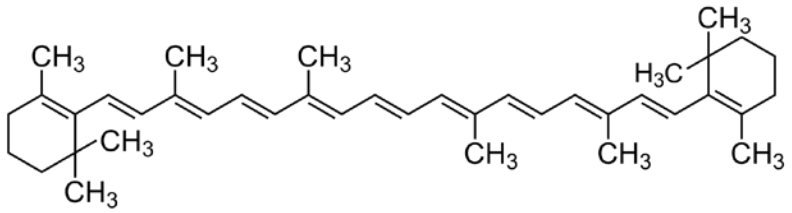
rutin



hypericin



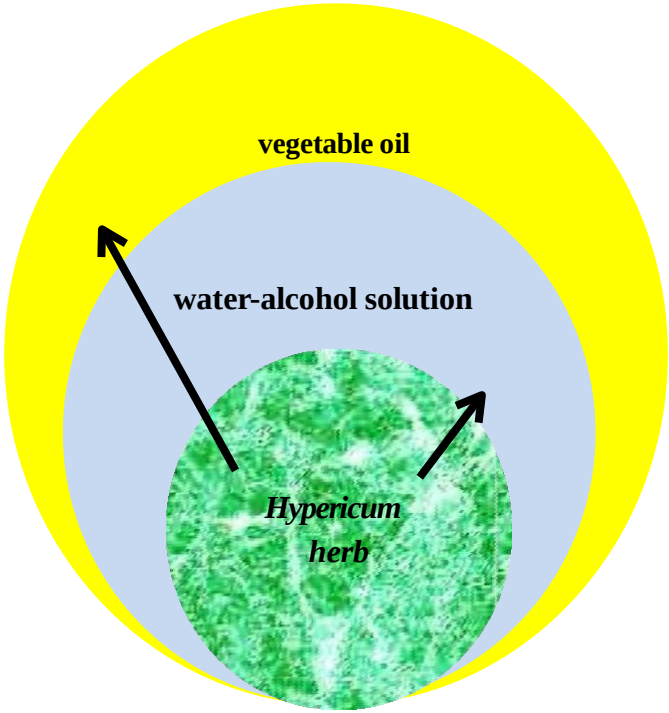
quercetin



β-carotin

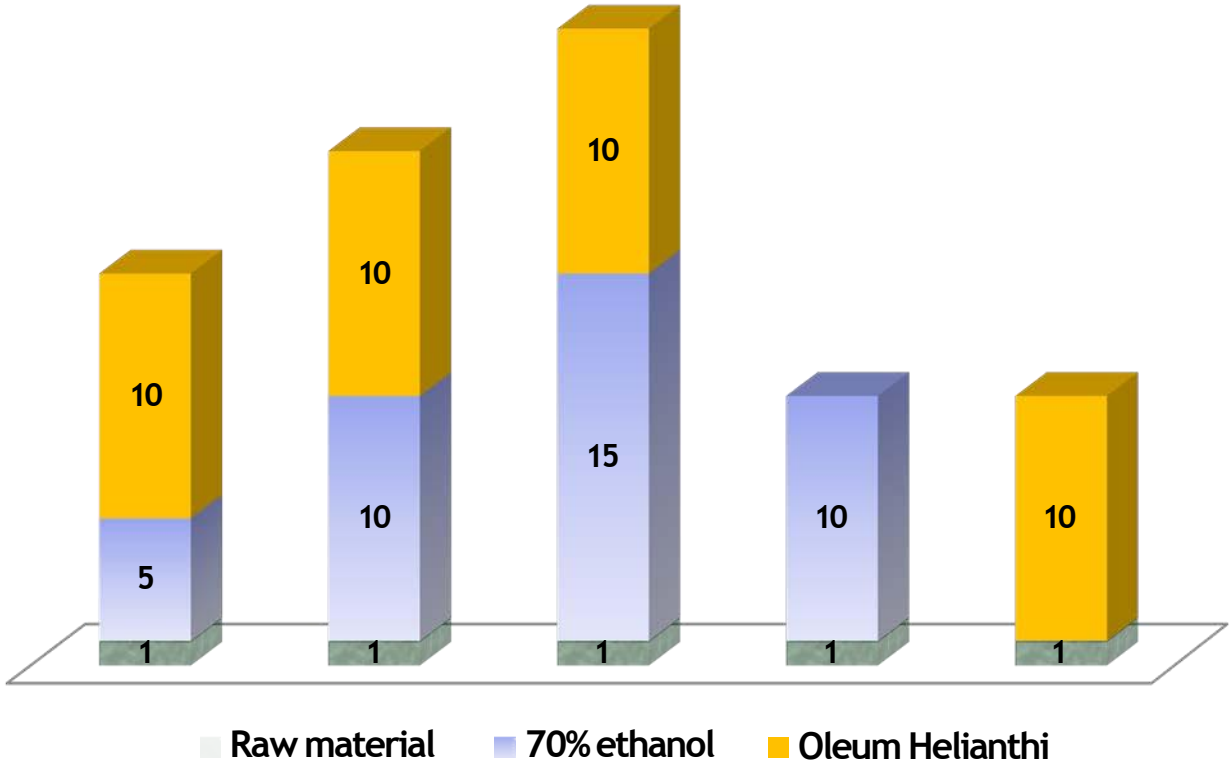
The principle of two-phase extraction from plant raw materials

- Biphasic extraction solvents system of different polarities, such as a water-alcohol solution and vegetable oil.
- This method allows, by one cycle, to simultaneously obtain two extracts, alcohol and oil, containing a complex of various hydrophilic and lipophilic compounds.



Resuts and discussion

The dependence of the ratio of the polar and non-polar phases on the biologically active substances extraction from the *Hypericum* herb was studied



Resuts and discussion

Single phase extraction

Single phase flavonoid extraction

The ratio of raw materials:

extract 1:10

(extractant - 70% ethanol).

Raw materials were soaked in the extractant for 60 minutes, extracted for 30 minutes (t =100ºC).

Extract was cooled and filtered under vacuum.

Single phase oil extraction

The ratio of raw materials:

extract 1:10

extractant - *Oleum Helianthi*

Extracted for 120 min (t = 100ºC).

Extract was cooled and filtered under vacuum.

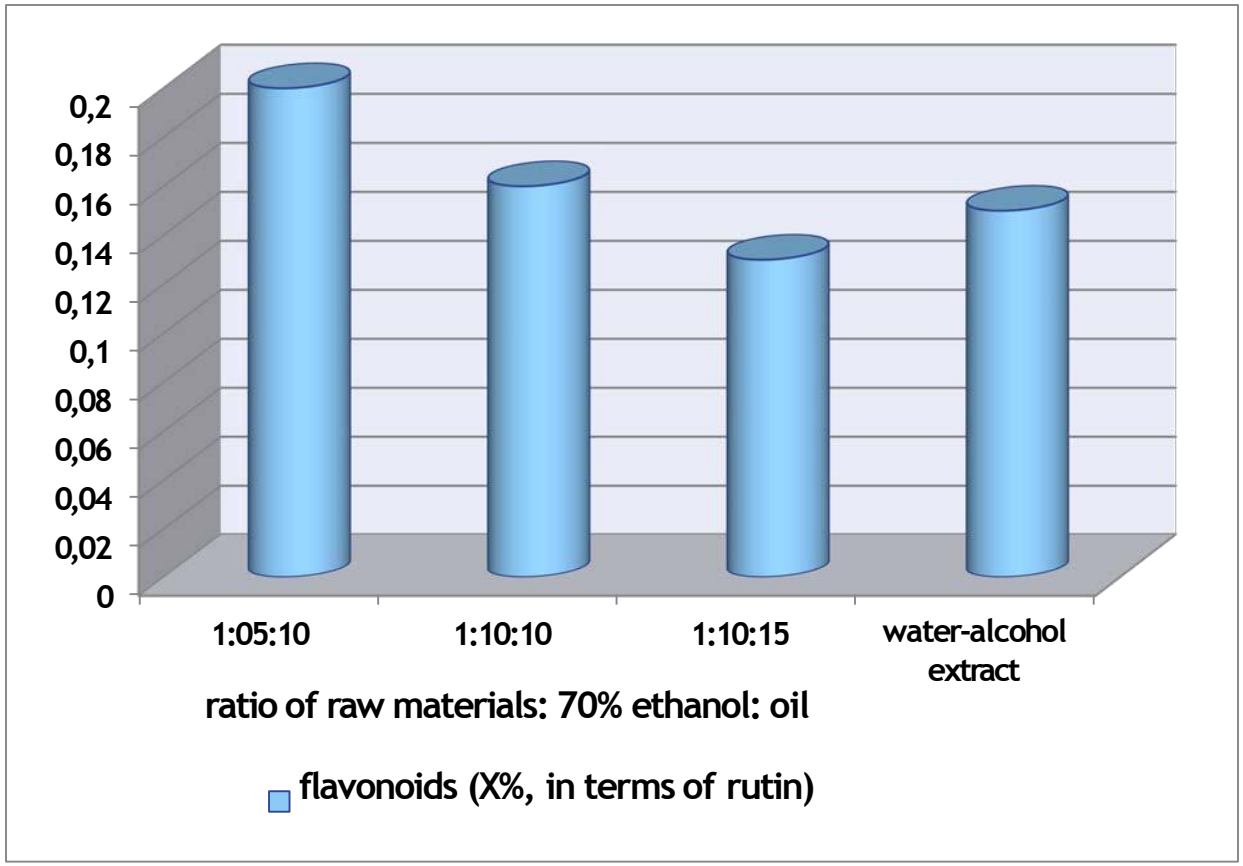
Stages of DF extraction from Hypericum

Raw material preparation
Hypericum herb was crushed to particles 2-4 mm in size, then was soaked in 70% ethanol in accordance with specified ratios (1:10; 1:20, 1:30) for 60 minutes (t = 20ºC).

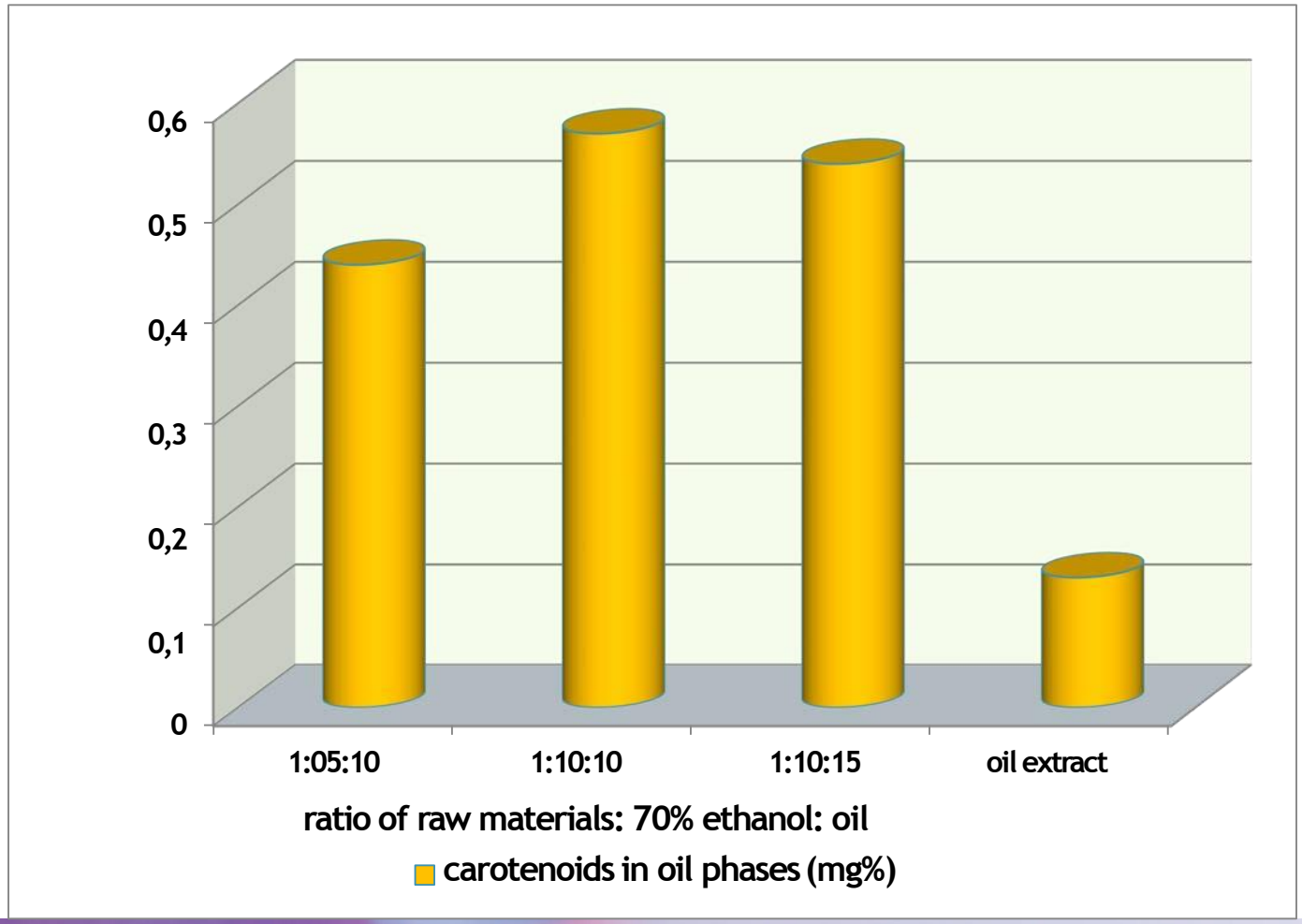
Extraction
An oil phase was added to the plant raw material. Then a two-phase extraction was performed for 120 minutes in the heating mode (t = 100 ° C) and stirring. The extract was cooled and the phases were separated and filtered under vacuum.

Quantitative analysis of extracts
The content of flavonoids (X,%, in terms of rutin) was determined in a water-alcohol extract, and the content of carotenoids (mg %) was determined in the oil extract.

Content of flavonoids in the water-alcohol phases (X%, in terms of rutin)



Content of carotenoids in oil phases (mg%)



Conclusions

- Two-phase solvent system extracts a complex of biologically active substances of *Hypericum maculatum L.* more effectively than a sequential extraction with separate solvents of different polarities.
- The polar phase enhances the processes of desorption, diffusion and phase transfer of not only hydrophilic, but also lipophilic *Hypericum* herb substances.
- The optimal ratio of raw materials: 70% ethanol: oil, which provided the maximum yield of flavonoids and carotenoids from raw materials, was 1:10:10.

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