

Summary of the PhD Thesis: PHYTOCHEMICAL STUDIES ON FRUITS OF *PHYSALIS ALKEKENGII* L (winter cherry)

PhD candidate: ZÖLD ESZTER ERIKA

University of Medicine and Pharmacy Târgu Mureş

Scientific Coordinator: Prof. Emeritus Dr. Carol Csedő

The vegetable product *Alkekengi fructus* was mentioned in elder editions of the French and Venezuelan Pharmacopoeia and was an ingredient of the „Chicoree compose” syrup officinal in *Pharmacopée français* (1937).

In Romania as well as in other European countries traditional medicine recommends these fruits for their diuretic effect in urolithiasis and urinary bladder pathology, in rheumatologic conditions as an anti-inflammatory agent and a source of vitamins. They were also used to eliminate the excess of uric acid in gout (e.g. *Pilules antigoutteuses de Laville*). Nowadays, on dietary supplements market there are many products for external use containing winter cherry fruits. On homeopathic medicines market there are a number of products containing *Physalis alkekengi* indicated in urolithiasis, dysuria with urinary hesitancy, frequent urination and urinary retention. Despite this, there are very few modern phytochemical and pharmacological studies of these fruits

The aim of the thesis was to bring new phytochemical and pharmacological informations about this forgotten ancient medicinal plant, hoping that in the future its fruits - winter cherry - will be currently used in phytotherapy.

The General Part presents a synthesis of botanical, phytochemical and pharmacological data from literature concerning *Physalis* species, especially *Physalis alkekengi* L.

The Experimental Part comprises researches concerning pharmacognostic and pharmacological characterization of the vegetable product *Alkekengi fructus*.

Macro- and microscopic description of *Alkekengi fructus* was illustrated with original images.

The phytochemical study started with a preliminary qualitative analysis by CC and TLC of a petroleum ether extract obtained in Soxhlet apparatus. Sterolic esters and free sterols, tri- and diacylglycerols, tocopherols, sitosterol and carotenoids were identified. Phytochemical study continued with fatty acids identification by GC and tocopherols and carotenoids analysis by HPLC.

The composition of fatty oil extracted from whole fruits, seeds and pulp was determined by GC. Linolic, oleic, palmitic, stearic and linoleic acids were identified. Linolic acid is quantitatively the most important (62,79%, 69,18%, 64,04%), followed by oleic acid in seeds and whole fruits (16,97%, 15,77%, 11,97%) and palmitic acid in fruit pulp (15,8%).

HPLC analysis completed literature data concerning qualitative and quantitative analysis of tocopherols. α -, β -, γ - and δ - tocopherol and tocotrienols were identified in winter cherry fruits and the total amount of tocopherols was between 275-290 mg%.

Qualitative analyses of carotenoids by HPLC confirmed literature data that *Alkekengi fructus* contains mostly hydroxylated carotenoids (xanthophylls), the most important being zeaxanthin (over 50%) and cryptoxanthin. For the first time a comparative study of carotenoid content in fresh and dried fruits was made. In dried fruits most of zeaxanthin is diesterified while in frozen fruits the quantity of mono and diesterified compounds of zeaxanthin is quite equal.

Quantitative determination of carotenoids was performed using spectrophotometric and HPLC methods. The results demonstrate that *Alkekengi fructus* is rich in carotenoids in fresh/frozen, as well as in dried condition. Comparing the quantity of carotenoids extracted in water to the total carotenoid content (79 mg/100 g dried fruits), extractability was established

at 36%. This high water extractability is due to the high amount of water soluble carotenoids (xanthophylls).

Combining different chromatographic methods (CPC, TLC, HPLC) physalin D was isolated and identified (using $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data). Physalin D is a sterolic lactone with 28 carbon atoms, a specific class of substances of the Solanaceae family, which present antiproliferative action on cancer cells. The quantity of physalin D was evaluated in fruits and calyx and cytotoxic activity was tested on three cancer cell lines (HeLa, MCF7, A431). From the studied samples, immature calyx has the highest content of physalin D (0,78 g%), while mature calyx contains 4 times less (0,20 g%). Fruit content of physalin D is much lower; immature fruits contain 0,09 g% physalin D and mature fruits 0,025 g%. Decreased cytotoxic activity of physalin D is in concordance with literature data. Because physalin D content of *Alkekengi fructus* was not yet reported in literature, this determination is an innovative one.

TLC analysis of the extracts obtained from fruits, calyx and leaves of *Physalis alkekengi* using different mobile phases shows the presence of alkaloids in calyx and leaves and their absence in fruits.

Recent literature data mention the presence of nortropanic alkaloids – calystegines - in *Physalis* species; their presence/absence in winter cherry fruits was verified. For this purpose extracts purified on ion exchange column were analyzed by TLC using different mobile phases and visualization reagents. Because of the lack of specific reactions for identification of calystegines and the impossibility to obtain reference substances in time, these compounds could not be identified in plant extracts.

TLC analysis allowed us to identify 4 amino acids: serine, lysine, valine and tyrosine. These results could not be found in literature for *Alkekengi fructus* and bring up a new approach to the phytochemical knowledge of this vegetable product.

The content of glucose, fructose and sugar was determined, as well as the effect of preservation (by drying and freezing) on the content of these compounds. The highest amount of sugars has been found in frozen fruits (11.64 g% glucose, 12.45 g% fructose and 3.54 g% sugar).

TLC and HPLC analysis of methanolic extract of fruits showed the presence of a single flavonoid: luteolin-7-O-glucoside. Literature data mention the presence of this compound only in leaves of *Physalis alkekengi*. TLC analysis of polyphenolic acids shows the presence of many compounds, but HPLC analysis confirmed only the presence of caffeic acid.

Macro- and microelements study by ICP-AES showed a high content of K in winter cherry fruits which can explain the diuretic effect. Determinations made on different fruit extracts show that they are better extracted in water than in hydroalcoholic solution. Determination of macro- and microelements from *Physalis alkekengi* fruits represents a personal contribution to the knowledge of this plant.

Pharmacological researches were made on two directions: determination of antioxidant activity and determination of diuretic effect, these being the first studies made on winter cherry fruits.

Antioxidant activity of aqueous freeze-dried extracts obtained from dried and frozen fruits was determined by DPPH method and by inhibiting the lipid peroxidation as well as determination of ascorbic acid and total polyphenols content highlighting the relationship between active principles content and antioxidant activity. The ability to trap free DPPH radicals of frozen fruits is approximately 10 times higher than in dried fruits. The same difference can be observed in ascorbic acid; the decrease of antioxidant activity is probably due to the decomposing of ascorbic acid during drying. The total polyphenols content is not significantly influenced by preservation method (drying or freezing). The ability to inhibit lipid peroxidation of these extracts is lower than DPPH radicals trapping and it also tends to

decrease by drying the fruits. In conclusion, antioxidant activity of *Physalis alkekengi* fruits is strongly correlated with ascorbic acid content and decreases significantly by drying.

After the pharmacologic study it can be concluded that winter cherry fruits do not present a diuretic and uricosuric effect in rats after acute administration. Subacute treatment for 3 days demonstrated a remarkable diuretic effect and a significant increase in uric acid elimination after the second day of administration.

These results confirm the diuretic effect of the fruits of *Physalis alkekengi* L., which for they are used in traditional medicine of many European countries, as well as their efficacy in eliminating uric acid, a property exploited in the past to control rheumatism.

FAAS determinations show a high content of potassium in fruits (2.6 g %) and in decoction (1.7 g %) and a low sodium content. As winter cherry fruits do not contain important amounts of saponosides, flavonoids and volatile oil, which are considered responsible for the diuretic effect of vegetable products, in this case the diuretic effect confirmed by pharmacologic studies is probably due to potassium.