Section 4

18

Pharmacology

From the Traditional Recipe to the Modern Remedy: The antitumoural activity of the small burr parsley (*Caucalis platycarpos*)

Croatia

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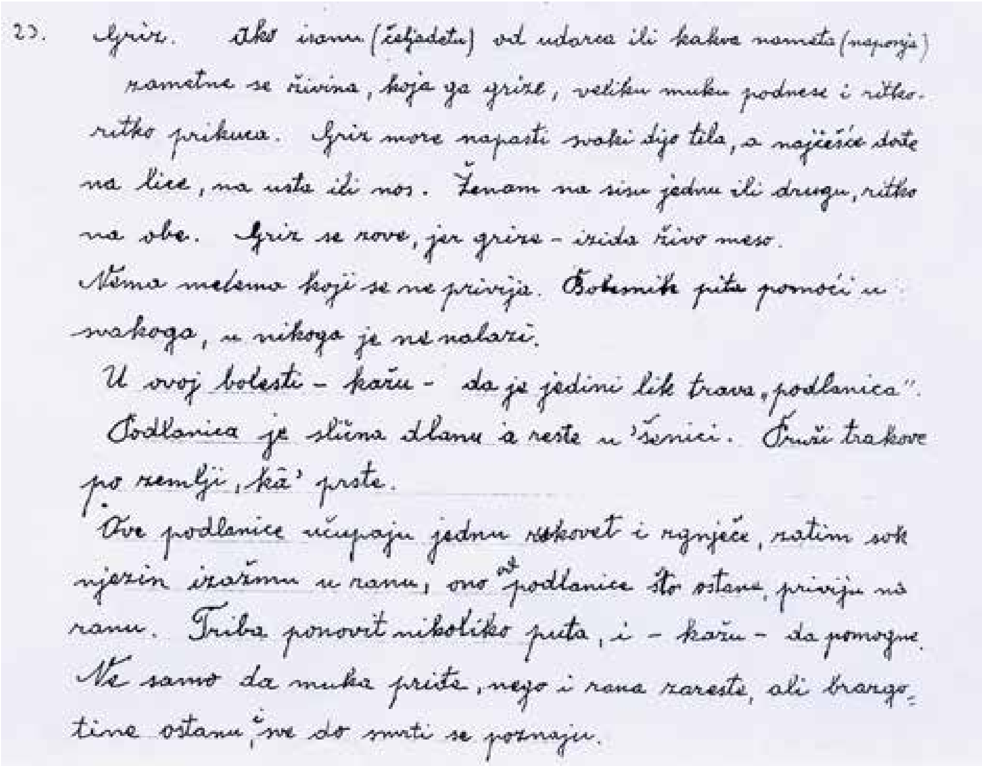
Summary

While studying old manuscripts of folk pharmacopoeias for the treatment of diseases, written mostly by Croatian priests in past centuries, we found a recipe for the preparation of a remedy used in cancer treatment. It included instructions for its use. The basis of the remedy was a plant which we identified as small burr parsley *Caucalis platycarpos* L. The results of initial experiments, performed at the M.D Anderson Hospital and Tumor Institute, Houston, Texas, USA, demonstrated an antimetastatic effect as a result of the stimulation of the host's immunological system. During the last ten years many additional experiments have been performed. While some results were published, most experiments were performed for the purpose of obtaining the documentation needed to meet the registration procedure and EU Pharmacopoeia requirements. Under the trade name Primus®, the product passed the standard legal procedure in Croatia, a license was obtained and the drug was put on the market. A company, Fitofarmacija d.o.o., based in Zagreb, was founded with the intention of developing and producing drugs based on the plant. The firm grows *C. platycarpos* a rare plant, and develops and produces Primus®, a herbal medicine. Primus® is recommended as an adjuvant therapy for patients whose immune system has been weakened by chemotherapy and radiotherapy for tumoural diseases. It is also recommended for persons liable to recurrent infections. Its active compounds strengthen the non-specific resistance of the body by increasing the number and activity of the cells of the immune system. Recently the remedy has been registered and classified as a food supplement.

Background and Justification

Our experience of developing the plant remedy based upon the old recipe books is unique in Croatia, there being no similar experience based upon such sources so far, although the potential for such investigations is strong. Before the beginning of the project dealing with the antiproliferative properties of the small burr parsley we had been exploring many old traditional pharmacopoeias (called "*ljekaruše*" in the Croatian language) written in Croatia between the 14th and 19th centuries. During this screening process we noticed and singled out some which we considered worth testing (Fig. 1).

Among them was small burr parsley, *Caucalis platycarpos* L., an annual plant that grows in the Mediterranean region and central Europe (Fig. 2).



23. Griz. Ako izamu (čeljadetu) od udorca ili kakva nameta (napornja) zametne se živina, koja ga grize, veliku muku podnese i ritko-ritko prikuca. Griz more napasti svaki dijo tila, a najčešće dote na lice, na usta ili nos. Ženam na sisu jednu ili drugu, ritko na obe. Griz se zove, jer grize – izida živo meso.
Nima melema koji se ne privija. Bolesnik pita pomoći u svakoga, u nikoga je ne nalazi.
U ovoj bolesti – kažu – da je jedini lik trava „podlanica".
Podlanica je slična dlanu a reste u šenici. Puži trakove po zemlji, kā' prste.
Ove podlanice učupaju jednu rukovet i zgnječe, zatim sok njezin izažmu u ranu, ono od podlanice što ostane, privije na ranu. Triba ponovit nikoliko puta, i – kažu – da pomogne.
Ne samo da muka prite, nego i rana zareste, ali brazgotine ostanu, i ne do smrti se poznaju.

Figure 1: Old recipe recommending small burr parsley as remedy against cancer.

Figure 2: *Caucalis platycarpos* L, according to Thome, 1885.

A range of traditional pharmacopoeias from 14th to late 19th centuries are preserved in Croatia. Some of them have been published as facsimile editions while many more are preserved as manuscripts within various institutions or private collections. The unique aspect of this corpus is the fact that preserved sources were written in the Latin, Glagolitic and Cyrillic alphabets. The oldest pharmacopoeias, written in Glagolitic script, are preserved within the Croatian Academy of Sciences and Arts and date from the 14th and 15th centuries. The Croatian Academy of Sciences and Arts pays a lot of attention to their preservation as well as to the editing and publishing of manuscripts of traditional pharmacopoeias, particularly those which were written in scripts are no longer in use. Part of this corpus presents a rich source of data which inspires the need for further investigations and scientific testing of the healing properties described. Here we present a case which illustrates such an approach. The small burr parsley tested in our investigation was identified in the old recipe collection from the manuscript written by a Franciscan priest, Silvester Kutleša, and preserved in the archives of the Croatian Academy of Sciences and Arts. In the recipe the plant was proposed for use in the treatment of certain tumours. Tracing this information, we performed a number of experiments which demonstrated the experience described in the pharmacopoeia in question.

The first issue involved was to prove that a water extract from small burr parsley has an antiproliferative effect. The next was to explore the mechanism of its activity. The third element was to make a detailed analysis of its active constituents. As the plant is relatively rare and poorly described in the literature, some of its morphological, anatomical and growth characteristics had to be studied with an aim to its correct cultivation. For the purpose of the registration of the plant remedy all the demands of the European pharmacopoeia (a monograph of the plant, methods for its qualitative and quantitative analysis, elements for microscopy of powdered material, stability, toxicology, etc.) had to be answered.

Description

Small burr parsley contains several chemical groups known as substances with immune-stimulating properties. About 30 phenolic compounds (flavonoids and phenolic acids) were structurally identified and characterized. Their activity is based upon enhancing a non-specific immune response by increasing the number and activity of certain immune system cells. Various experiments were performed on different animal tumour models which resulted in the demonstration of this anti-tumoural activity. A set of experiments proposed by the European pharmacopoeia were performed to meet the demands for the registration of the remedy. Finally, the remedy, named Primus®, was registered and

distributed throughout the Croatian pharmaceutical market. The remedy is used as a tea and recommended for persons who are liable to recurrent infections, and as an adjuvant therapy in patients whose immune system is weakened by chemotherapy and radiotherapy for tumoural diseases.

The steps taken during the investigation and implementation process are outlined as follows:

The small burr parsley extract was tested for anti-tumoural activity using the method of artificial lung metastases. The studies used fibrosarcoma induced in mice with 3-methylcholantrene, a spontaneously induced fibrosarcoma. The extract strongly decreased the number of metastases of both tumours (Fig. 3).

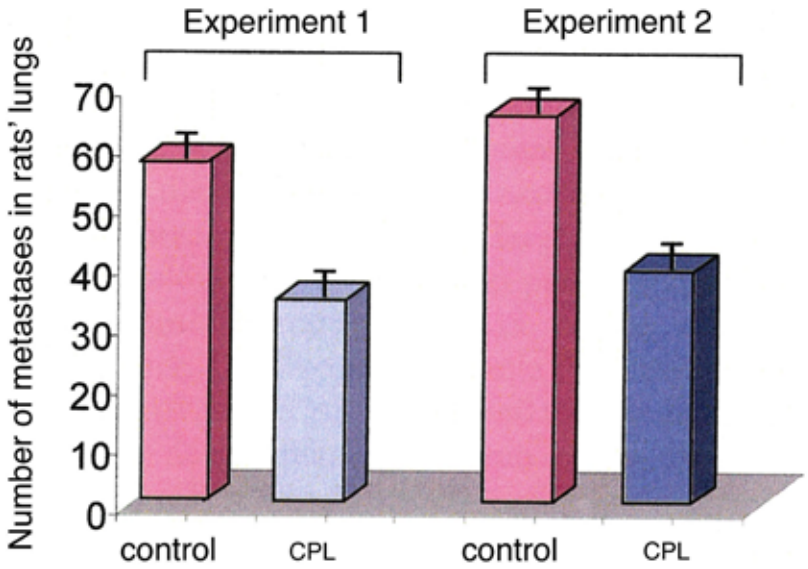


Figure 3: Experiment 1: Fibrosarcoma tumour cells were injected. The second and the fifth day after that, 10 mg of small burr parsley extract Caucalis platycarpos L. (CPL) was injected. Control group: experiment no CPL treatment. Experiment 2: The same conditions as in experiment 1 only with double the dose (20 mg) of CPL.

The substance was even more effective if injected before the intravenous injection of tumour cells. These data suggest that the anti-metastatic effect is probably the result of the stimulation of the host's immune system. In addition, no anti-tumoural activity of small burr parsley was observed in mice that had been immunosuppressed by irradiation.

When small burr parsley extract was injected before irradiating the mice, the facilitation of the metastases induced by the immunosuppression was completely abolished. Since one of the two tumours was non-immunogenic, it seems that the mechanism of action of the small burr parsley involved natural killer (NK) cells and/or macrophages in that similar effects were observed. The same *modus operandi* for small burr parsley chemoprotective

effects is proposed. The extract increases spleen weight and cellularity as well as NK activity. It is also a stimulator of the haematopoietic system in general. Antioxidant activities of small burr parsley were also monitored (Fig. 4).

In addition to these experiments, antitumoural activity of small burr parsley was demonstrated on the models which included rats with artificial liver metastases of colorectal cancer, when a comparison was made with the anti-cancer drug levamisole (Fig. 5), and with tumor metastases in the lungs (Fig. 6).

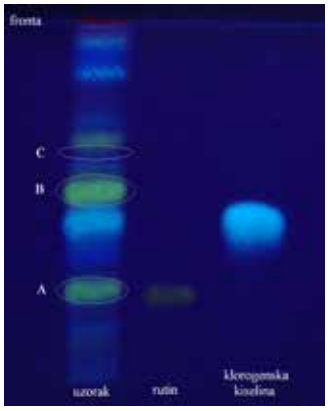


Figure 4: High performance thin layer chromatography (HPTLC) of small burr parsley showing the presence of antioxidants: flavonoids and phenolic acids (left side track).

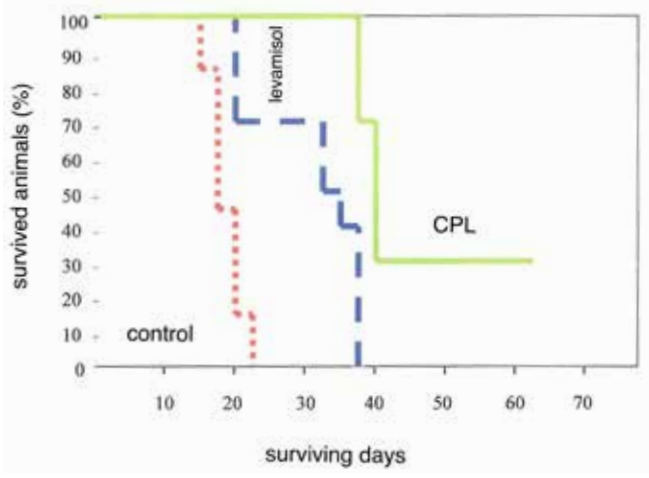


Figure 5: The effect of *Caucalis platycarpos* L. (CPL) and levamisole on the survival of rats with colorectal cancer metastases of the liver.

Figure 6: The effect of small burr parsley on the number of tumour metastases in a rat's lung. Left: Lung section of rat from the control group showing metastases. Right: Lung section of rat from the group treated with small burr parsley with reduced number of metastases.

On the basis of these results, herbal remedy was developed. In order to meet the requirements of commercialization, the plant had to be domesticated for horticultural production (Fig. 7). The product, Primus®, is now available on the market in Croatia (Fig. 8).

The target market includes patients whose immune system has been compromised by chemotherapy and radiotherapy for tumourous diseases, as well as persons liable to recurrent infections..

Figure 7: Cultivating small burr parsley for commercial production.

Fig. 8: The final product, Primus®, now available on the market in Croatia.

Technical data

Toxicological studies on mice showed that the doses proposed for human use are safe. There are now 15 years of experience with the product and no side effects have been noted.

Results

The results therefore correspond to the observations and description in the old traditional pharmacopoeia. Three PhD theses were completed during the research. The results were presented at various scientific meetings and published in the proceedings book (*N. Kujundžić, L.Milas, H. Ito. Antimetastatic effects of the plant extract P-VE-A. Proceedings of 8th Medical Days of Sombor, Sombor, 16-18 June 1985 p. 155*). Many of the results have also been published in scientific journals (see below).

Recently the remedy has been registered and classified as a food supplement.

Partnerships

Those who helped research and developed this successful experience included:

- Nikola Kujundzic, PhD, with expertise in pharmacy (herbal medicine, history of pharmacy), the chemistry of metal complexes of pharmaceutical importance and analytical chemistry. He is retired as Professor of Chemistry and former head of the Department of Analytical Chemistry at the Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia.
- Luka Milas, MD, PhD, is an expert in tumoural immunotherapy and radiotherapy. He was head of the Department of Radiotherapy, M.D. Anderson Hospital and Tumor Institute, Houston, TX, USA.
- Ivo Basic, PhD, is an expert in tumour immunology. He was Professor of Animal Physiology and head of the Department of Animal Physiology at the Faculty of Sciences at the University of Zagreb.
- Stella Fatovic-Ferencic, MD, PhD, medical historian, head of the Department of the History of Medicine of the Croatian Academy of Sciences and Arts.

The company Fitofarmacija d.o.o. was established exclusively for the production, development and marketing of this plant remedy. It is still operative. The small-scale and expensive production has always made the drug's development difficult.

Impact

Prizes for this study include The Prize of the City of Zagreb in 2008; The Croatian Chamber of Economy has granted Primus® tea product with the title "Croatian Creation" as a mark of high standard of quality (Fig. 9). The product also won a diploma and bronze medal at the Eco World Fest 2009 held in Opatija, Croatia (Fig. 10).

Figure 9: Croatian Chamber of Economy Prize for Primus® tea, 2009.

Figure 10: Diploma presented to Fitofarmacija d.o.o. for Primus® tea, Eco World Fest 2009.

Sustainability

The potential for growing the plant on a wider scale has been demonstrated. Its larger scale cultivation is now planned which may provide a basis for the development of the local community.

The knowledge and experience gained from exploring old pharmaceutical manuscripts (pharmacopoeias) proved the main trigger for further exploration. The experience of traditional medicine, particularly old recipes noted in various manuscripts has strong potential to aid the search for new remedies always subject to testing using modern scientific methods.

Patents

The product is not protected by patent, since it is beyond the financial potential of the company and only the logo is protected (legally named *seal* – it is the visual identity, marked with®).

Lessons Learned

The 1991-95 war affecting Croatia and the very modest financial funds available were the main obstacles in conducting effective research. On the other hand, the prejudices of Western medicine towards traditional medicine were among difficulties the research faced from time to time. In addition, legislation and registration remain limiting factors for the marketing of herbal remedies.

Apart from scientific papers, targeting a technical audience, information about the project was presented to the public through the media, especially regarding the prizes the project was awarded.

Future Plans

Although preclinical studies were sufficient for marketing the product, the clinical proof of the concept is planned, as well as the patenting of the product. Furthermore, the large scale planting of the small burr parsley is planned. Greater and more reliable availability of the source material will mean that export can be considered.

Publications

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