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PLANT EXTRACTS FROM MERISTEMATIC TISSUES (FOLIAR BUDS AND SHOOTS): ANTIOXIDANT AND THERAPEUTIC ACTION

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ABSTRACT. Foliar buds form a new category of vegetal product, being used in modern phytotherapy, also known as gemmotherapy. Rheumatoid arthritis, often called RA, is a chronic (long-standing) disease that damages and eventually destroys the joints of the body. It has been used three types of bud extracts (Ribes nigrum, Buxus sempervirens, Vitis vinifera) with proved antirheumatic effects, during a period of 3 months. The success of treatment is checked by regular physical examinations and interviews. In all the patients we observed a clinical amelioration, permitting a slight reduction of the dose of the NSAIDs and the DMARDs.

Keywords: foliar buds, juvenile rheumatoid arthritis, polyphenols, antioxidant activity

INTRODUCTION

Wild black currant, *Ribes nigrum L. (Grossulariaceae)*, can be found in wet forests and alder swamps, but the plant is more commonly cultivated in gardens (Ph.Eur., 2005). Foliar buds form a new category of vegetal product, being used in modern phytotherapy, also known as gemmotherapy. In the classical phytotherapy, leaves *Ribis nigri folium* and fruits *Ribis nigri fructus* are used; modern phytotherapy, gemmotherapy, started to use foliar buds (Ph.Eur., 2005, Heinrich, 2004). The black currant foliar buds contain diterpenic acids, volatile oil, vitamin C (107 mg/g), amino acids (arginine, proline, alanine), plant hormones (auxins, cytoquinines), mineral salts, carotenoids, polyphenol structures and are known for their diuretic action and as urinary antispastics (Hyun A.j., 2008, Neacsu M., 2002). Its profile as very good systemic antiinflammatory, suprarenal and immunity stimulant, with a natural origin, and without secondary and adverse reactions. Its action is directed specially to the suprarenal glands, stimulating the cortex. It behaves as a natural cortisone (cortisone- like action), without having its toxicity. The black currant extracts are recommended for a vast aria of affections and varied range of ages, from children to old people. It is administrated as drops, 50-150 drops-day, between meals; it can be recommended as singular remedy or in association with other drugs (Peev C., 2006, Pitera, 2000). The antioxidant activity and the poly-phenol content are correlated parameters in case of vegetal product analysis (Hyun A.J., 2008). *Buxus sempervirens* and *Vitis vinifera* are two types of extracts that are recommended in association in rheumatoid arthritis treatment. The hydro-glycero-alcoholic extract of *Buxus sempervirens (Buxaceae)* is prepared from fresh shoots, and that of *Vitis vinifera* (*Vitaceae*) is prepared from foliar buds and from fresh shoots (Pitera, 2000, Peev C., 2006). Rheumatoid arthritis, often called RA, is a chronic (long-standing) disease that damages and eventually destroys the joints of the body. Juvenile rheumatoid arthritis, (JRA) is not a single disease, but a group of diseases with symptoms and signs developed in children younger than 16 years. What they all have in common is chronic joint inflammation (Pitera, 2000). There are three major forms: particular disease (affects only a few joints, fewer than 5), poly-articular disease (affects 5 joints or more), and systemic disease (affects many systems of the body). The aim of treatment is to stop or slow down the progress of inflammation, thus relieving symptoms, improving function, and preventing joint damage and other complications. The most useful drugs are:

1) Non-steroidal anti-inflammatory drugs (NSAIDs) reduce inflammation, swelling, and pain,

2) Disease modifying antirheumatic drugs (DMARDs), in children Methotrexate is the most used drug, it interferes in the immune processes that cause inflammation and JRA.

3) Biologic response modifiers are a newer, specialized type of immunosuppressive drugs, carefully designed to block the actions of natural substances that are part of the immune response, such as tumour necrosis factor. A variety of complementary approaches can be very effective in relieving pain and improving the outcome of the disease. One of these complementary therapy is gemmotherapy, a scientific use of a special glycerin extracts from plant buds (Pitera, 2000).

MATERIALS AND METHODS

The foliar buds were harvested in spring 2007, just before opening, from culture. They were positively identified at the Department of Pharmaceutical Botany of the Faculty of Pharmacy, Timisoara. Voucher samples were deposed in the *Herbarium* of the mentioned faculty. Extracts were realized with a

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mixture from the fresh buds and solvent water – ethanol 99%- pharmaceutical glycerin 98% in a 1:1:1 ratio, 5% dried vegetal product, according to French Pharmacopoeia Xth ed.; the extraction time was 10 days.

Total poly-phenol quantitative determination

Poly-phenol compounds were quantified in sample extracts using Folin-Ciocâlteu method (phospho-molybdeno-wolframic reagent), with a pyrogallol solution as standard, which permits spectro-photometry at λ = 760 nm. The method followed a technique from the European Pharmacopoeia Vth ed.

Antioxidant activity determination

The hydro-glycero-alcoholic bud extracts were screened for their antioxidant capacity using an adapted DPPH technique. Trolox was used as the positive control. In its radical form, DPPH absorbs at 520 nm, but upon its reduction by an antioxidant or a radical species, the absorption disappears.

Thus DPPH reduction was followed by monitoring the decrease in absorbance at a characteristic wavelength during the reaction (Peev et al., 2006).

Phytobiological study

The comparative biotest of *Lepidium sativum* was performed consistent with Tanase's technique. The extract was dissolved in water, resulting solutions of different concentrations: 2%, 1%, 0.5% and 0.25%. The seeds of *Lepidium sativum* were set to germinate; after 24 hours, the length of the radicle was measured. The water was removed and 10 ml of the solutions was added, in different concentrations; some seeds were treated with water and other only with the mixture used as solvent (water – ethanol 99%- pharmaceutical glycerin 98% in a 1:1:1 ratio), as witnesses. The inhibition coefficient was calculated, and when higher than 50%, the product was assumed to have anti-proliferating activity (Peev C., 2006).

Therapeutic use

We studied 30 cases of patients diagnosed with JRA and that were already treated with antirheumatic drugs NSAIDs and the DMARDs. We administered for a month the gemmotherapeutic preparation from *Ribes nigrum*, with a slow decrease of the doses of NSAIDs and the DMARDs. We introduced in the treatment scheme the gemmotherapeutic preparation from *Buxus sempervirens* and *Vitis vinifera*, for three months (Pitera, 2000).

RESULTS AND DISCUSSIONS

Antioxidant activity of hydro-glycero-alcoholic foliar bud extracts is presented as mM trolox/400 µl while the total polyphenol concentration, as mg pyrogallol/100 dry product. (Table 1).

Table 1

Species	Antioxidant activity (DPPH) mM trolox/400 µl	Tot poly-phenol content mg/100g
Ribes nigrum buds	0.9749	506
Buxus sempervirens shoots	0.8038	421
Vitis vinifera buds	0.7436	384

Table 2

Species	Inhibition rate Conc.2%	Inhibition rate Conc.1%	Inhibition rate Conc.0.5%	Inhibition rate Conc.0.25%
Ribes nigrum buds	62%	46%	23%	11%
Buxus sempervirens shoots	89%	72%	53%	38%
Vitis vinifera buds	61%	43%	25%	12%

The values for inhibition index are presented in Table 2 for each type of studied sample. According to the comparative test of *Lepidium sativum*, values of more than 50% were found in the solutions with the following dilutions: 2% dilution - where the inhibition rate was estimated at 62%, 1% dilution – inhibition rate = 46% and the 0.5% dilution – inhibition rate = 23%.

From the 3 types of extracts, *Buxus sempervirens* shoots shown the higher value of inhibition index comparing to other studied samples. These results can be considered as important for future studies to establish the possible antiproliferative activity on *in vitro* (tumour cell lines) and *in vivo* (animal model). In the first month of treatment with the gemmotherapeutic preparation from *Ribes nigrum* there were no symptom improvements. During the three months of treatment associated with the three preparations, we could note improvements of the clinical aspect in 27 patients. It has been used three types of bud extracts (*Ribes nigrum, Buxus sempervirens, Vitis vinifera*) with proved antirheumatic effects, during a period of 3 months. The success of treatment is checked by regular physical examinations and interviews. In all the patients we observed a clinical amelioration, permitting

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a slight reduction of the dose of the NSAIDs and the DMARDs. The fact that upon administering the unique extract of *Ribes nigrum* there were no improvements can be explained either by the short period of time (one month) or by the fact that its therapeutic action was not enough. The phenomenon of synergism is well-known (two to three natural remedies potentate their therapeutic action). Within the study, over the three months we could note the improvement of chronic disease symptoms. This is the proof of the fact that gemmotherapy is a direction to follow in rheumatoid arthritis conditions. Gradually implementing this treatment in time will lead to total removal of administration of NSAIDs and DMARDs.

The favourable results obtained after the treatment with natural remedies allows the diminution of chemical medicine doses – synthesis ones – and, in time, through an efficient cooperation between doctor and patient and the monitoring of the general condition of the body and of the disease, and even its gradual removal.

CONCLUSIONS

The antioxidant action of a plant preparation is strictly linked to the content of poly-phenol substances. Of the three plant extracts we studied, blackcurrant buds extract had the highest antioxidant action. The phyto-biological test on plant extracts showed no obvious anti-proliferating action for the studied concentration (2%), linked to the anti-tumour active compounds contained by the analyzed vegetal products. The present study designs the validity of the Gemmotherapy system and its applicability at young ages (up to 16 years). Hydro-glycerol-alcoholic extracts used in gemmotherapy have no side effects in either short or long duration administration. Gemmotherapy acts through the biological drainage. Biological drainage at cortical suprarenal and suprarenal levels (*Ribes nigrum*) and the effect of immunitary and lymphatic biological drainage (*Vitis vinifera*) prove to be efficient in improving JRA symptomatology.

We promote the idea of expanding biological culture of the studied plants – *Ribes nigrum L. (Grossulariaceae), Buxus sempervirens (Buxaceae), Vitis vinifera* (*Vitaceae)* – in order to efficiently solve the source of high-quality plant products necessary to prepare pharmaceutical extracts.

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