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THE RESEARCH IN MERISTEMOTHERAPY – PAST AND PERSPECTIVES

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ABSTRACT
The mersitemotherapy is one of the newest branch of the phytotherapy, its development being performed at the border between classic phytotherapy and homeopathy. It was founded at the begin of 20th century, being developed from the 60'. For this reason the researches are poor and those according to the modern requirements are at the beginning. Because this natural therapy uses those parts of the plants that contain meristematic tissues, the botanic and phytochemical data are poor or almost inexistent. Even if at this moment there are used almost 100 different extracts, just around 5-7 have some pharmacological studies. All recommendations of these extracts are mostly based on the clinical observations of the physicians, according to the procedures used in homeopathy. A smaller part of the physicians has used these extracts based on analogy with their growing place and the special phytosociological characters of the plants. The aim of this paper is to present the existing results on the meristematic extracts and to propose new possible directions to explore in future, to found a solid scientific basis of this new branch of the phytotherapy.

KEYWORDS: meristemotherapy, meristematic tissue, phyochemistry

INTRODUCTION

The phytotherapy is the therapy based on the use of natural resources, plants and, some time, also animal raw materials. Its main goal is to use the simplest processing of the plants, to can express the therapeutic value of the complex mixture of the plants' biocompounds. It is the most ancient therapeutic way, the plants and their extracts being the first used medicines by the people.

The phytotherapy shows a very important development during the time and more evident in last 3-4 decades, by the intensive study from phytochemical, pharmacological and, not least, from clinical points of view. During the time there were formed different branches in phytotherapy, one of the youngest being the gemmotherapy, or most correctly, the meristemotherapy.

If during the time the medicinal plants were used in dry form and, excepting the *Populi gemma*, the parts of the plants used in therapeutic scopes were mature parts, as: leaves, seeds, fruits, bark, roots, flowers or herb, the meristemotherapy is corresponding to the new trend in using the plants in their fresh state respectively uses just those parts that contains meristematic, non differenciated tissues, like: buds, young shoots, young bark, rootlets or fresh seeds.

This new branch of the phytotherapy was developed beginning from first half of the last century, based on the consideration that these young parts of the plants contains "the plants stem cells" that can be powerful therapeutic tools, if they act similarly with the animal stem cells.

The short history of meristemotherapy

The people observed from ancient times that animals, like goats and lambs, use instinctively the buds and young shoots in different illness state, but they had not known why and how to use them in proper way. For this reason the use of the buds was reduced to a few species.

The people from India have used a few buds according to the Ayurveda concept, as is described in the 7th book, Atharvaveda. Galen was the first physician that prepared an ointment or balsam from *Populi gemma*, named Acopon. This balsam was used during the time because of its cicatrizing effect. The alchemists used the buds for springtime tonic, a tonic that can give the power of an entire life cycle. But Paracelsus was the physician that recognized that each part of a plant has different therapeutic power.

In last century a visionary Belgian physician Pol Henry put a simple question. If the animal stem cells have the power to heal, why the vegetal stem cells does not? Based on its homeopathic formation, as physician, and on an analogy between the plants' life and the human's life, he begun to use and study the therapeutic effect of meristematic tissues' extracts. He dedicated all his life to study the plants and humans analogy, to confirm the theory of biological analogy. In this way he put the basis of

phytoembriotherapy, named later gemmotherapy and, today meristemotherapy.

The studies were continued by French, Belgian and Italian physicians – Dr. Julian, Dr. Bergeret, Dr. Tetau, Dr. Lernout, Dr. Pitera, but the indications where established on typical homeopathic clinical observations. Other physicians, like Dr. Nebel, Dr. Vannier, Dr. Roy, observed and based the meristemotherapic extract usage for drainage (Ledoux et al., 2014; Pitera, 2007; Pitera et al., 2016; Rozencwajg, 2008; Steingassner, 2005).

The aim of this paper is to present the actual stage of knowledge regarding the meristemotherapy through the modern requirements and to propose possible studies for the future.

MATERIALS AND METHODS

There were consulted all possible references regarding the gemmotherapy or meristemotherapy: books, articles, websites, to show a clearest possible image of the actual stage of researches in this field.

RESULTS AND DISSCUSIONS

The studies on vegetal raw materials morphology

In meristemotherapy are used mainly the buds and young shoots from the trees and shrubs (Ledoux et al., 2014; Pitera, 2007; Pitera et al., 2016; Rozencwajg, 2008; Steingassner, 2005). There are no special studies regarding the buds morphology. Recently a group of scientists from Vasile Goldis Western University from Arad have begun the study of microscopic and ultramicroscopic characteristics of some buds used in meristemotherapy. These studies can reveal the special morphological characteristics of the meristematic tissues and could give valuable data for a better control of harvesting time. Such of studies can offer the data to monographs that establish the quality conditions for vegetal raw materials. A future direction for studies could be this field of optical and electronical microscopy, orientated to determine those special characters that can lead to cover the lack of information from standards.

The formulation of the extracts used in meristemotherapy

The French Pharmacopoeia from 1965 describes at the preparation of homeopathic stocks also the preparation of the extracts from buds, establishing the extraction ratio of 1:20 for stocks and the use of first decimal dilution as final product. This monograph establishes also the processing of buds in their fresh state, without any drying, the use of a mixture of 96 % vol. ethanol and glycerol (1:1) as extraction solvent respectively the extraction procedure by cold extraction or maceration. This methodology for the preparation of the meristematic extracts, named also glycerol macerates, was maintained also today and was included also in the new European Pharmacopoeia.

A lot of time the meristematic tissues extracts were used only in D1 form, meaning a final extraction ratio of 1 part vegetal material (dry content) to 200 parts of solvent mixture. Today, near the classical extracts, exist on the market also the stocks (extraction ratio 1 part of plant to 20 parts of solvent mixture). The different meristematic extracts are also used in complex pharmaceutical formulations (Ledoux et al., 2014; www.plantextrakt.ro, www.hofigal.ro, www.miaerboristeria.it).

In future, could be made studies to establish, from scientific point of view, the reason of use the compendial solvent mixture and extraction ratio. Could be studied also the use of other edible solvents or other extraction techniques that can lead us to better meristematic extracts.

Phytochemical studies on meristems and meristematic extracts

The buds chemical composition was very slightly studied in last decades when the phytochemical studies of the plants exploded. But in last 5-10 years, when the meristemotherapy gained an important place next to phytotherapy and natural therapies, the studies of chemical composition of the glycerol macerates were begin.

As a main characteristic of the extracts obtained from meristematic tissues is the content in growing phytohormones like gibberelins, cytokines, auxines, etc. The gibberelins were first time detected in these tissues about 50 years ago by the Netien and Combet, but there were used just from agricultural point of view, not from therapeutic one (Pitera, 2007; Pitera et al., 2016).

Today are phytochemical studies that determine different secondary metabolites from buds. In Italy are studies regarding the phytochemical fingerprint of some buds extracts obtained from *Ribes* and *Rubus spp.*, determining the polyphenols, monoterpens and vitamin C using chromatographic methods (Donno et al., 2016; Donno et al., 2013). The polyphenols, like flavonoids and hydroxycinnamic acids, were identified also in *Ribes nigrum* L., *Tilia tomentosa* M. respectively *Rosa canina* L. meristematic extracts. The Blackcurrant extract was found to be rich in quercetine derivatives and p-coumaric acid, the Dog rose extract in kaempferol and ellagitannins and the Linden extract in quercetine, kaempferol respectively apigenine derivatives (Ieri et al., 2015).

Other scientists identified the monoterpenes from *Ribes nigrum* L. buds essential oil, finding to be rich in carenes and sabinene (Ethordevic et al., 2014; Dvaranauskaite et al., 2008).

Even that are different parts of plant, with different stage of differentiation of the cells, that can translate in the accumulation of different

primary and secondary metabolites, long time and a lot of specialists extended the phytochemical composition of classically used part of plants to buds and young tissues. But the studies demonstrate that in glycerol macerates exist similar but also specific biocompounds in comparison with the leaves, flowers or fruits.

By simple reactions could be identified in almost all glycerol macerates free aminoacids and glucides, as representatives of the primary metabolites. From the secondary metabolites were identified the flavonoids in Birch species, Hawthorn young shoots, Blackcurrant and Linden buds extracts; caffeic acid derivatives in Rosemary young shoots and Service tree buds extracts; tannins in Sweet chestnut and Oak buds, Dog rose, Blackberry and Red raspberry young shoots extracts; coumarins in Horse chestnut, Common fig respectively Common ash buds extracts; volatile oil terpenic compounds in Silver fir, Mountain pine buds and Juniper respectively Rosemary young shoots extracts; arbutoside in Heather, Bilberry and Red bilberry young shoots extracts. The richest extracts in flavonoids were found to be the Almond buds, the Downy birch buds and catkins, the Walnut buds respectively in polyphenols the Horse chestnut buds, Sweet chestnut buds and Red bilberry young shoots extracts (Olah, 2012; Soescu, 2008).

From some genus are used more similar species and from each species more parts containing meristematic tissues. One of these cases is the *Betula* genus, from this genus being used two species *Betula pubescens* Ehrh. and *Betula verrucosa* Ehrh. A comparative study of the buds and catkins from *Betula pubescens* Ehrh. respectively of the sap, buds, seeds and leaves from *Betula verrucosa* Ehrh. shown a lot of similarities but also differences. The buds contain quercetine and also other 4 flavonoids similary also in catkins. The seeds extract is found to have the lowest polyphenol content. Neither in leaves are so much flavonoids as in buds, where mostly will found aglyca and not glycosides as in leaves, demonstrating the different biochemical process that occurs in young tissues in comparison with adult tissues. In the sap were not identified polyphenols, but is rich in calcium and zinc (Olah, 2012).

The Rosaceae family is also well represented between the glycerol macerates being used the Hawthorn, Dog rose, Blackberry and Red raspberry young shoots respectively the Almond, Blackthorn and Service tree buds. A comparative HPLC analysis regarding the polyphenols from these extracts shown that the Rose dog, Blackberry and Red raspberry extracts have more identical compounds, but between Almond and Blackthorn, belonging to *Prunus* genus, no similarity could be identified (Olah, 2012).

A GC-MS study performed on Rosemary young shoots extract in comparison with Rosemary leaves extract demonstrated that the glycerol macerate from young, fresh tissues have more terpenic compounds, also the esters being better represented as in the dry leaves extract, leading us to conclude that the fresh processing in case of meristematic extracts preserves better the natural biocompound profile of the plants (Olah, 2012).

A comparative HPLC and TLC study on the Blackcurrant buds in different development stages confirmed that the most optimal period for harvesting is the stage in that the buds are developed, but are not opened yet (Olah, 2012).

In order to confirm that the young tissues extracts have different phytochemical profile in comparison with classical tinctures obtained from mature parts of plants, from dry or fresh vegetal raw materials there were performed studies on Juniper and Hawthorn. It is well known that 4-terpineol contained by Juniper berry has kidney irritating effect. A comparative study of dry and fresh berry tinctures and young shoots glycerol macerates shown that the mersitematic extract does not contain 4-terpineol, being more safely for use (Olah, 2013).

The comparative study of buds, young shoots, flowers and leaves respectively fruits of Hawthorn shown that the hyperoside is the flavonoid present in all extracts. It can be observed also differences in biocompounds profile from the same part of plants in fresh respectively dry state, demonstrating that the drying process destroy or transform some components. This difference was observed also at antioxidant capacity, the buds, young shoots and fresh fruit extracts being the most powerful (Olah, 2013).

Similarities and differences were observed also at Oleaceae family representatives. In meristemotherapy are used the Common ash buds and seeds, the Wild privet and Olive young shoots respectively the Lilac buds. In Ash buds, like in leaves, are present coumarins like fraxetin and its derivatives, but the main difference is occurred at triterpenic acids content, the buds containing ursolic acid, but this is missing from leaves. From seeds can be identified just triterpenic compounds. In Wild privet young shoots could be identified oleanolic acid and coumarins, in Lilac buds extract ursolic acid and flavonoids. The Olive young shoots extract contains rutoside, luteolin-7-glucoside, oleanolic acid and oleuropein. The most powerful antioxidant from the Oleaceae family was found to be the Lilac buds extract, having a high flavonoid concentration and also being rich in ursolic acid (Olah, 2014).

Complex evaluation of the phytochemical profile of meristematic tissues' extracts was performed just on few. These studies have importance to prove the therapeutic effects of these extracts, can explain the already observed effects and founded the scientific basis of meristemotherapy. For this reason in future should be awarded more attention for this kind of

studies that can be performed on single extracts or can be made comparison between species belonging to the same genus or family or even comparisons between different plant parts containing meristematic tissues.

The phytochemical studies can help also to establish special monographs that describes the quality requirements for meristematic tissues extracts

Studies regarding the therapeutic effects

According to modern requirements the therapeutic effects of products must be demonstrate by animal models. Also on some glycerol macerates obtained from buds and young shoots were performed pharmacological studies, but they are poor and give information just about 5-7 extracts.

A main contribution to the pharmaceutical studies made on glycerol macerates was those of Dr. Tetau's from France. He gives us the first proves regarding the Blackcurrant anti-inflammatory effect using different animal tests. His tests demonstrate that the mice taking Ribes nigrum buds extract are more resistant to cold, the accumulation of inflammatory liquids and the inflammation size is lower respectively the gamma-globulins and glycoproteins are at normal level (Tetau, 1998).

Tetau demonstrate also that the Downy birch buds extract has depurative effect, stimulates granulopoietic activity and the phagocytosis. These effects were observed by the elimination of carbon suspension administrated to mice (Tetau, 1998).

The sedative effect of Linden buds extract was observed by decrease of spontaneous motility when is administrated to mice. The same Linden buds extract increases the hypnotic response of mice when is administrated together with barbiturics (Tetau, 1998).

The studies performed on rabbit heart demonstrate that the Hawthorn young shoots extract decrease the cardiac frequency, has antiarrythmic and inotrope effects (Tetau, 1998).

The Rosemary young shoots extract hepatoprotective effect was demonstrate on mice intoxicated with carbon tetrachloride. The tests shown also that this meristematic extract increase the biliary flow (Tetau, 1998).

There were performed also *in vitro* microbiological test that evidenced the antimicrobial activity of Dog rose young shoots and Blackcurrant buds extracts against Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, Bacillus subtilis, Proteus vulgaris, Shigella flexneri, Klebsiella pneumonia and Enterococcus faecalis (Mihele et al., 2007).

It can be made also correlations between the clinically observed effects and identified bioactive compounds. These correlations are based on the pharmacological studies performed on separated, single bioactive compounds. Such of correlations can show that the Olive young shoots extract have antioxidant effect due by the content in luteolin-7-glucoside, rutoside and caffeic acid derivatives, that inhibit the superoxide anions release, the production of ROS in mitochondria and LDL oxidation. These effects can lead to the following recommendations: prevent the atheroma formation and decrease the cardio-vascular diseases risk. Due by the content in luteolin-7-glucoside, oleanolic acid and oleuropein it has anti-inflammatory effect by inhibiting the lipopolysaccharides inflammatory pathway. The caffeic acid derivatives from Olive extract can be responsible by the renin, angiotensin II and angiotensin converting enzyme inhibition, explaining by this way the extract anti-hypertensive effect. The same rutoside, luteolin-7-glucoside and oleanolic acid inhibit the alfa-glucosidase, improves the insulin metabolism and resistance, inhibit the polyols metabolism pathway, giving an explanation for Olive young shoots extract antidiabetic, hypoglycemiant and anti-hyperglycemiant effects, with recommendations for cardio-vascular complications in diabetes mellitus (Olah, 2014).

Even that this kind of correlations can explain some effects observed clinically by physicians, it must be continued the pharmacological studies to prove without doubt the beneficial effects of the meristematic tissues extracts. In future these studies must take an important place in the scientists concerns.

Near these the genetic studies can contributes to the elucidation of the mechanism of action and to prove the mild, non-toxic, natural effect of meristematic extracts.

Neither the clinical case studies, nor, eventually, trials should not be forget, for prove the efficacy and safety of these products, that can improve the quality of the people life's.

CONCLUSIONS

This paper shown that this new branch of phytotherapy has great potencial, but the scientific studies to founded the basis are mostly missing. For this reason there is an opportunity for scientists from biology (microscopy, morphology), pharmacy and chemistry (phytochemical profile, pharmacological studies), medicine and genetics to begin detailed studies that can cover the missing information.

The authors suggest that the studies to begin with the vegetal raw material microscopic evaluation, than must determined the detailed phytochemical profile and not at least to perform pharmacological, genetic and clinical studies.

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