Analele Universităţii din Oradea, Fascicula: Ecotoxicologie, Zootehnie si Tehnologii de Industrie Alimentara, Vol. XV/B, 2016

THEORETICAL CONSIDERATIONS REGARDING THE THERAPEUTIC EFFECTS OF *AESCULUS HIPPOCASTANUM* L. GLYCEROL MACERATE AND MOTHER TINCTURE ACCORDING WITH THE IDENTIFIED BIOACTIVE COMPOUNDS AND THEIR POTENTIAL USE FOR ATHLETES

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Abstract

The main differences between the glycerol macerates and mother tinctures are regarded the solvent used for extraction respectively the development stage of the used vegetal raw material, both extracts being mainly obtained from fresh plants. Due by the fact that the development stage of the used part of plant is different the chemical composition profile of these extracts could be very different. In this paper have made a comparison, by TLC, of the phytochemical profile of the Aesculus hippocastanum L. mother tincture respectively glycerol macerate and based on the scientific data from literature correlates these profiles with the active compounds pharmacological effects respectively with the observed clinical efficacy. It was observed that the Aesculus mother tinctures contains mainly the saponoside escin, while the glycerol macerates is more concentrated in esculetine, a coumarin. These compounds sustains the indication of these extracts in venous circulatory disturbances. At athletes, the risk of venous problems' occuring is high, the glycerol macerates of Aesculus could be potential food supplements for sustaining the healthy venous circulation in a mild and natural way.

Keywords: *Aesculus hippocastanum* L., glycerol macerates, mother tinctures, TLC, athletes venous problems, food supplements

INTRODUCTION

The phytotherapy is the oldest therapy used by people from ancient times. The use of different plants or part from plants show the high therapeutic value that can be obtained from natural sources. The teas, different alcoholic and oily extracts were used for long times being obtained from different mature parts of plants in dry form (Petrovska, 2012).

The new trend in this field is the use of freshly processed plants, in this way being possible to maintain more accurately the bioactive compounds profile of the plants. The

mother tinctures are obtained mostly from fresh mature parts of the plants. They were been used in last 200 years in homeopathy, but today are more and more used as a better substitute of the classical tinctures obtained from dry parts of the plants.

A new branch of the modern phytotherapy is the gemmotherapy or, more exactly, the meristemotherapy. This uses the fresh meristematic tissues to obtain some special extracts named glycerol macerates. Due by the use of special parts of the plants, like the buds, young shoots, sap, etc., rich in meristematic tissues, by the processing in fresh state and the special phytochemical profile of the obtained extracts, these will show powerful therapeutic effects (Pitera & Nicoletti, 2016).

To know the phytochemical profile of a natural plant extract is the same to know the chemical composition of a classical drug. These information can lead us to the product pharmacological activity and sustain the explanation of its mechanism of action. The studies made on plants and their extracts show that not only one compounds is responsible for their efficacy, but the entire phytocomplex is involved in the pharmacological effects (Donno et al., 2016; Wagner, 1999; Carmona & Pereira, 2013). For this reason the determination of the phytochemical profile, as detailed as possible, is very important.

The natural compounds can be useful also for people that perform different sports. These people can develop different diseases with higher risk, due by the sustained physical effort that must be performed. In case of the athletes the venous problems can occur due by the overpressure on the legs (Jones, 2014). Because in case of performant sport the use of the drugs must be reduced at minimum, some natural products, that can sustain the normal and healthy venous circulation and prevent the possible damages, can be helpful (Kundrat, 2005).

This paper present a study of glycerol macerate and mother tincture from Horse chestnut (*Aesculus hippocastanum* L.). It will be presented below the comparative determination of the phytochemical profile by TLC and based on the identified biocompounds will be discussed the possible pharmacological effects and mechanism of action in comparison with the already made clinical observations.

MATERIAL AND METHODS

The *Aesculus hippocastanum* L. (Horse chestnut) mother tincture was obtained from fresh, shelled seeds, harvested from culture, from Cluj county, in September 2014. The glycerol macerate was obtained from fresh buds harvested in March 2015 from the same place.

The plant materials were identified by the biologist from the SC PlantExtrakt SRL Quality Control Laboratory. There were retained also voucher specimens from all checked vegetal materials.

The extracts were obtained by standardized processes, on a GMP certified production flow at SC PlantExtrakt SRL, Cluj, Romania.

The mother tincture was obtained according to German Homeopathic Pharmacopoeia respectively the European Pharmacopoeia, by cold extraction (maceration) during 10 days. In these 10 days the mixture of the cut plant material and 90 % vol. ethanol (1 part of plant material to 1.4 parts of solvent) was daily mixed, twice time, 10 minutes each time. After 10 days the extract was decanted from the plant material, the plant material was pressed and the obtained extract was mixed with the decanted one. The obtained tincture was maintained in dark for 5 days and filtered.

The glycerol macerate was obtained according to French Pharmacopoeia respectively the European Pharmacopoeia, by cold extraction (maceration) during 20 days. In these 20 days the mixture of the cut plant material and a 1:1 mixture of 96 % vol. ethanol and glycerol (1 part of plant material, dry part, to 20 parts of solvent) was daily mixed, twice

time, 10 minutes each time. After 20 days the extract was decanted from the plant material, the plant material was pressed and the obtained extract was mixed with the decanted one.

The TLC analysis was performed on silicagel plates with fluorescence indicator at 254 nm (Merck, Germany). As mobile phase were used ethyl acetate – ethyl-methyl-ketone – formic acid – water (50:30:10:10, vol.) on 10 cm, and after the plates were dried on 15 cm with toluol – ethyl acetate (93:7, vol.). The mother tincture was applied without any sample preparation, 40 µl, as band. The glycerol from glycerol macerate was separated by SPE on silicagel-C18 columns (Supelco, Germany). This extract was concentrated from 3 ml to 0,5 ml, being finaly solved in methanol and applied as band, 40 µl. As standards were used escin (10 mg/ml in ethanol) and esculetine (1 mg/ml in methanol). From each standard solution was applied as band 10 µl. The esculetine was identified in fluorescence at 365 nm. The escin was identified in daylight after spraying with anisaldehyde reagent at 100-105^{0}C for 10 minutes (Wagner, 1983). All solvents used for TLC analysis are of analytical grade and purchased from Merck, Germany.

There were consulted different medical databases to find scientific papers regarding the proof of pharmacological effects of the different compounds identified in the studied extracts.

RESULTS AND DISCUSSIONS

In figures 1 and 2 are presented the obtained TLC chromatograms. The chromatograms of Aesculus extracts show that the seeds contains mainly the saponoside named escin and the buds mainly the coumarins, the esculetine being clearly identified. The buds contain also small quantity of escin.

The scientific references contain information regarding the presence of triterpenic saponin escin in the Horse chestnut seeds (Hagers Handbook, 1992), but are no information about the phytochemical profile of the buds extract. The Aesculus tincture efficacy in internal and mainly external administration in case of chronic venous insufficiency is well known (EMEA, 2009). Regarding the Aesculus buds extract are just a few information based on the physicians observation, being recommended also for venous circulation disturbances at legs, haemorrhoids and lungs levels (Pitera & Nicoletti, 2016).

The studies demonstrate the escin anti-inflammatory, anti-oedematous and venotonic properties with recommendation in the treatment of venous circulatory disturbances. These properties are possible due by a deep molecular mechanism involving selective vascular permeabilization, sensitization of calcium channels to ions and 5-HT, leading to the tonification of vessels walls (Sirtori, 2001; Pearson & Vanhoutte, 1993).

Escin can prevent the forming the oedema in the first, exudative phase. This together with the calcium channels sensitization can lead to the sealing of small, capilary vessels determining their reduced permeability to water and being related also to a reduced hypoxia-induced activation of human endothelial cells respectively with the increase of endothelial nitric oxid synthetase activity (Arnould et al., 1996; Pierrakos et al., 2013).

Figure 1. The TLC-chromatogram in fluorescence

The same escin can antagonize the ATP reduction and increase the A2 phospholipase responsible by the release of pro-inflammatory prostaglandins lead to vein protection and reduced oedema (Bazzoni et al., 1991; Guillaume & Padioleau, 1994). Escin seems to enhancing the effect of already existing glucocorticoids by A2 phospholipase suppression, NF-kB inhibition and reduction of cell adhesion factors. It have also selective antagonist effect on 5-HT due by selective activation of 5-HT2 receptors and a relative antagonism for the pro-inflammatory 5-HT1 receptors, explaining both the anti-inflammatory and anti-oedematous effects (Hiai et al., 1981; Hu et al., 2004; Matsuda et al., 2000).

The increased venous tone could be associated with the presence of prostaglandins $PGF\alpha_2$ that depends on some enzymes activation: phospholipases, acylhydrolases and α-keto-reductase (Longiave et al., 1978).

Esculetine have been demonstrated to inhibit 5-lypooxygenase and the B4 leucotriens, having antinociceptive and anti-inflammatory effects (Rzodkiewicz et al., 2015). It reduces the nitric oxid secretion in endothelium and inhibit the secretion of soluble intercellular adhesion molecules lead to reduce the inflammation. It can also reduce significantly the nitric oxid and E2 prostaglandin levels in synovial fluid being helpful also in osteoarthritis (Mao et al., 2015). Esculetine being a coumarin will be shown also antivitamin K effect, being a good anticoagulant, blood thinner. This effect could be helpful in case of venous disturbances or stasis, when the clotting can occur with high risk (Jain & Joshi, 2012).

Figure 2. The TLC-chromatogram in vizible light

At oral administration the extracts from Aesculus seeds, with high concentration of escin, can be less safe than the buds extract, due by the potencial toxic effect of the saponosides (EMEA, 2009). Even that the phytochemical profile of the two studied Aesculus extract are very different, both will have anti-inflammatory and anti-oedematous effects due both by escin and esculetine, as the above presented studies demonstrated. Due by the same pharmacological effects, but the higher safety of the buds extract this could be proposed as efficacy food supplement for people and athletes with high risk of venous disturbances, mainly at legs, and the seeds extract for external use.

CONCLUSIONS

The correlation of the identified active compounds pharmacological effects with the observed clinical efficacy lead us to conclude that the Aesculus extracts, both from seeds or buds, can exhibit positive effect in case of venous disturbances at legs. The buds extract being more safe at oral administration can be proposed as food supplement for people and athletes with high risk to develop venous disturbances at legs.

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