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TILIA TOMENTOSA FOLIAR BUD EXTRACT: PHYTOCHEMICAL ANALYSIS AND DERMATOLOGICAL TESTING

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ABSTRACT. Foliar buds are a new category of vegetal products which are used in gemmotherapy. The present study has highlighted the average content of water, carotenoides, total polyphenols, chlorophyll, vegetal hormones and hemolytic saponins, contained in the foliar buds of Silver Lime or Silver Linden.
The study consists of a comparative bio-test on *Lepidium sativum* and of the evaluation of the balance between efficacy and noxiousness of a topic Linden bud preparation, on human skin by determining the level of hydration and that of transepidermal water loss (TEWL). The chemical analysis of the Linden bud extracts conducted to the following results: total polyphenols - 380 mg/100 g buds; the detected phyto-hormones were: auxin, cytokinin and gibberellin; the total hemolytic saponin concentration was 5.2%.
According to the comparative test of *Lepidium sativum*, the sample showed an inhibition rate of 83.33%. The balanced composition of the hydro-glycero-alcoholic extract of Linden foliar buds, along with the positive results of the dermatologic hydration analysis, and with the possible anti-proliferative activity, conduct to a more clear hypothesis that these extracts may become ingredients in chemo-protective and anti-aging preparations.

Keywords: Silver Linden, buds, cosmetic, hydration, vegetal extracts

INTRODUCTION

Tilia tomentosa Moench. (Silver Lime or Silver Linden) is a species of *Tiliaceae family* native to southeastern Europe and southwestern Asia, from Hungary and the Balkans east to western Turkey, occurring at moderate altitudes. It is a deciduous tree growing to 20-35 m tall, having rounded to triangular-ovate, broad leaves, recognizable upon the densely white tomentose hairs on the inferior side of the limb. The yellow flowers groped in cymes, with or without their connected pale green subtending leafy bract are frequently used in traditional phytotherapy. In the European Pharmacopoeia (EP), the inflorescence of *Tilia platyphyllos* Scop., and *T. cordata* Miller are considered offcinal (Ciocârlan V., 2000; Toker G et al., 2004).

They are stated to possess expectorant, diuretic, diaphoretic, antispasmodic, stomachic and sedative activities and have been used for the treatment of flu, cough, migraine, nervous tension, ingestion problems, various types of spasms and liver and gall bladder disorders. The medicinal properties claimed for the drug have been attributed to its flavonoids, volatile oil and mucilage components. The use of the leaves as a remedy is not as common as that of the flowers, but they have been suggested to be employed as a diaphoretic; however, the effect has not been evaluated experimentally so far (Toker G et al., 2004).

Foliar buds form a new category of vegetal product, being used in modern phytotherapy, also known as gemotherapy (Hunnius, 1998; Pitera F., 2000). They contain growth phyto-hormones (auxins, cytokinins, and gibberellins), free aminoacids, proteins, mineral salts, essential oils with terpenes like pinen and limonen (Pitera F., 2000; Peev C., 2007). They are used for hidro-glycero-alcoholic extracts and recommended with action anxiolitic, antispastic, sedatif in case of distonie neurovegetativ, spasmophilia (Pitera F., 2000).

Cosmetology is nowadays more and more focused on vegetal extracts as active ingredients for different kind of topic preparations. This new trend is based on a more natural approach of the anti-aging therapy of the skin in which anti-oxidants and phyto-hormones contained in the vegetal extracts act as hydrating and regenerating agents (Man-Jau Chang et al., 2008).

Water is necessary for the maintenance of normal skin physiology. Water retention in *stratum corneum* plays an important role in the regulation of skin function and depends on two major factors: the presence of natural moisturizing factor within the corneocytes and the intercellular lipids from *stratum corneum*, arranged orderly to form a barrier to transepidermal water loss (TEWL) from the epidermis (Man-Jau Chang et al., 2008).

The present study brings on one hand new data regarding the chemical composition of Silver Linden buds, concerning the average content of water, chlorophyll, carotenoids, polyphenols and hemolytic saponins; while on the other hand the phyto-biological research and the dermatologic test on human skin comes up with an evaluation of the balance between efficiency and toxicity of a semisolid preparation based on the extract of Silver Linden foliar buds.

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MATERIALS AND METHODS

The buds of Silver Linden (*Tilia tomentosa* Moench.) were harvested from Cluj's Botanical Garden, in spring 2006, just before opening, and were positively identified at the Department of Pharmaceutical Botany of the Faculty of Pharmacy, Timişoara. Voucher samples were deposed in the *Herbarium* of the mentioned faculty.

The determination of water content in fresh foliar buds, was done in conformity with the monograph from the Romanian Pharmacopoeia - X th ed., on batches of buds harvested in different moments, that is at the end of winter-time and at the beginning of spring-time(Farmacopeea Română, ed. a X-a, 1993; Peev C., 2006).

Chlorophyll A, B and the carotenoids were spectrophotometrically determined, using the Jasco V-500 device.

Total polyphenols were spectrophotometrically determined as well, according to the Folin Ciocalteu technique.

The phyto-hormones were identified through TLC, the mobile phase being represented by the mixture: ethyl acetate – methanol – water: 100:16,5:13,5 and UV- visualization done at 254 nm.

The saponin content determination was done in conformity with the Romanian Pharmacopoeia - X th ed. procedure. The University of Agricultural and Veterinary Sciences of Banat provided the ox blood. The hemolytic activity of the sample was compared to the standard solution of a hemolytic saponin (Merck). The concentration of hemolytic saponins was expressed in grams of saponin per 100 grams of dried vegetal material (Farmacopeea Română, ed. a X-a, 1993).

Extracts were realized with a mixture of water – ethanol 99%- pharmaceutical glycerin 98% in a 1:1:1 ratio, according to French Pharmacopoeia X th ed.; the extraction time was 10 days. The solvent / dry vegetal product ratio was 100 / 5. The ethanol was removed from the extractive solution by a rotavapor, at 45-50 ^{o}C and 150-160 mbars.

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 Linden bud extract solutions was added, in different concentrations; some seeds were treated with water and others 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 antiproliferative activity (Peev C., 2007; Peev C. et al., 2006).

The semisolid preparation formula consisted of: sodium lauryl sulfate (SLS) 1%, cetyl alcohol 10%, cocoa butter 9%, vaseline 15%, paraffin oil 5%, carbopol 940 0,3%, triethanolamine 0,3%, glycerin 10%, ethanol 10% and a preservative solution -38,4%. The vegetal bud liquid extract (corresponding to 1% dried material) was incorporated in this cosmetic semisolid form.

Ethic statement

We examined 15 healthy female volunteers (aged 20-30) with healthy skin. All subjects were informed about the details of the study and each signed an informed consent form, approved by the Human Experiment and Ethics Committe of Biotehnos S.A.- Bucuresti.

Skin irritation

Skin irritation was analyzed by a patch test and observed visually. Any kind of changes of the skin surface was recorded.

Skin hydration and TEWL

The study was performed on 15 healthy female volunteers, aged 35-55, clinically healthy. The hydration process was monitored with the Corneometer CM 820, while the transepidermal water loss was measured with the Tewameter TM 210. All measurements were performed under constant environmental parameters (humidity and temperature) and the monitoring time was 6 hours. Before treating the forearm with the Silver Linden bud preparations, the skin was exposed to a 10% solution of SDS (sodium dodecyl sulfate), a surfactant ingredient which imitates physiologic stress conditions for the skin.

RESULTS AND DISCUSSIONS

The water content of the buds varies in accordance with the harvesting period: winter 33.90%, and the beginning of spring - just before opening, 57.93%.

The results for the chemical quantitative analysis are: chlorophyll A - 6%, chlorophyll B - 4%, total carotenoids - 1,99 mg/100 g buds, and provitamin A - 0,77 mg/100 g buds; total polyphenols - 380 mg/100 g buds; the detected phyto-hormones were: auxin Rf = 0,62; cytokinin Rf = 0,42 and gibberellin Rf = 0,34; the total hemolytic saponin concentration was 5,21%.

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 83,33% , 1% dilution – inhibition rate = 43,33%.

The values for the hydration process, initially, after SDS and after the 6 hour treatment with the semisolid Linden bud dermo- cosmetic preparation, are presented in table no 1.

The values for TEWL, initially, after SDS and after the 6 hour treatment with the semisolid Linden bud dermo-cosmetic preparation, are presented in table no.2.

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Table 1

Hydration values (U.H.) for the semisolid analyzed preparation

Hydration (U.H.)	Cream	Extract
Initially	66,5	62,6
SDS	56,8	53,6
Silver Linden 6h	92,2	96,2

Table 2

TEWL values (g/ hm2) for the semisolid analyzed preparation

TEWL g/ hm2	Cream	Extract
Initially	14,08	12,11
SDS	15,2	16,16
Silver Linden 6h	11,09	13,18

The present study highlights the fact that the Silver Linden buds accumulate 24.03 percent more water during their biological development process, from the sleeping stage to the opening moment.

Linden foliar buds contain chlorophyll, polyphenols, carotenoids, phyto-hormones (auxins, cytokinins, and gibberellins), and hemolytic saponins in a percentage of 5.21%. These facts encourage future studies and the use of Linden foliar buds as ingredients for dermo-cosmetic products with non-invasive properties, sustaining normal skin functions and preventing skin damage.

The semisolid preparations with Linden foliar buds were well tolerated by all the volunteers, and did not induce any visible skin irritation.

CONCLUSIONS

The semisolid preparations with Silver Linden foliar buds were well tolerated by all the volunteers, and did not induce any visual skin irritation. They showed positive effects on skin hydration and TEWL.

The complex composition of the hydro-glycero-alcoholic extract of Linden foliar buds, along with the positive results of the dermatologic hydration analysis, and with the possible antiproliferative activity, conduct to a more clear hypothesis that these extracts may become important ingredients in chemo-protective and anti-aging topic preparations.

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