

Physically-chemical characteristics of birch sap

In this study the physically-chemical characteristics during the preservation of birch sap and efficiency of preservatives is investigated.

Birch sap is obtained, frozen and prepared for preservation according to definite procedure. There are 4 preservatives used in preservation – single and mixed in various proportions (Ethyl Alcohol, Phenethyl Alcohol, mix of Sodium Levulinate and Sodium Anisate, mix of Benzyl Alcohol and Potassium Sorbate). During the study all changes in samples are measured and noted in test protocols.

Microbiological purity is tested for samples with best results. The challenge tests are realized to check survival ability of selected microorganisms that are purposely introduced into a preserved test product system.

All results are evaluated in accordance with the tabulated acceptance criteria of the Pharmacopoeia 2011:5.1.3 (DAB 10: VIII.14) protocols.

The best results are shown by sample with Ethyl alcohol and mix of Sodium Levulinate and Sodium Anisate.

Effects of birch sap on skin cells *in vitro*

Birch sap is a complex mix of organic and inorganic substances. Health promoting and regenerative properties have long been attributed to it by Northern traditional agriculture and medicine. There have been previous observations of birch sap's positive effects both in cases of nutritional and topical use; however no known proven mechanisms of action on different regenerative processes have been characterized.

This study focused on elucidation of birch sap's effects on skin cells *in vitro*. Sap's potency to stimulate dermal and epidermal cell proliferation, delay of cell senescence, *in vitro* protection against reactive oxygen species, and promotion of growth factor secretion were tested.

Morphology of dermal and epidermal cells remained unchanged during cultivation in presence of 5% and 50% (concentrated by lyophilization) sap in cultivation media. Concentrated sap decreased population doubling time in dermal cell cultures for more than 20%; effect on proliferation of keratinocytes was less pronounced. Sap's effect on proliferation was confirmed also by cell cycle analysis – in presence of concentrated birch sap number of dermal cells in resting phase decreased for 8.2% and percentage of cells in synthesis and mitotic phase increases for 4.8%. Induction of oxidative stress in dermal cells incubated in presence of 10 and 50% birch sap showed that there was significantly lower accumulation of reactive oxygen species in sap treated cells – this indicates on birch sap's protective characteristics. Cell senescence was assessed after cultivation of cells in presence of concentrated birch sap. Compared to control, birch sap treated cells showed lower accumulation of senescence marker; low accumulation was maintained even after induction of oxidative stress. Most pronounced effect on senescence marker accumulation was observed in case of concentrated 50% sap. Birch sap at all tested concentrations did not show effect on fibroblast growth factor 2 secretion in dermal cell cultures, however concentrated (50%) sap induced fibroblast growth factor 7 secretion.

Current results show that low concentrations of birch sap – 5% and 10% - have little effect on skin cell cultures *in vitro*. Concentrated 50% sap promotes cell proliferation, protects cells from oxidative stress, and stimulates senescent cell cultures. However in case of protection against oxidative stress, sap even at concentration of 10% showed positive effect. As positive effects of sap on skin cells *in vitro* were most observed at high concentrations it is recommended either to use it in a concentrated form or as a substitute for cosmetic base. Observed effects indicate that birch sap could be included in anti-ageing cosmetic formulations.

In vitro testing was done in Laboratory of Bioanalytical and Biodosimetry Methods, University of Latvia

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Effects of birch sap on skin cells in vivo

The aim of *in vivo* research was to observe impact towards skin rejuvenating features of active substance with birch hydrolate versus placebo substance. From the ancient times birch has been known for its cellular repair properties.

50 healthy persons aged 18 to 51 with no previous allergic reactions were included in the research. *In vivo* research was performed by observation of probates within 4 weeks. Both active and placebo substances were used twice a day, in the morning and evening.

Active substance is safe for use and did not indicate any early or late irritant reactions. Active ingredients regenerate superficial layers of skin and minimize pigmentation. It has excellent moisturizing features and did not showed comedogenic potential. Research showed positive effects on deeper layers of skin: increased elasticity of skin due to formation of new collagen.