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Identification and analysis of flax fiber components with biomedical properties.

Abstract

Flax plants (*Linum usitatissimum* L.), with rich traditions of cultivation in moderate climate zone, serving as a source of fiber and seeds, are used especially in food, paint, paper, textile and cosmetic industry. The last years of flax products, mainly oil and seeds components investigations, have brought a lot of information about their different health-beneficial properties. The positive influence has also been noticed in preclinical trials of flax fiber application as a chronic wound dressing. These results and the lack of precise data on the flax fiber hydrophobic compounds and their activities, prompted the identification and properties investigations of chosen flax fiber hydrophobic components.

With the use of chromatographic methods, fatty acids, including polyunsaturated linoleic and linolenic acids, many steroids, with β-sitosterol comprising of more than half of their content, were identified as the main compounds of flax fiber hydrophobic extract. Precise chromatographic, spectrophotometric and mass spectroscopy analysis revealed also the presence of very interesting and unique component in the flax fiber - cannabidiol. The presence of this kind of molecule was never previously reported in any other than *Cannabis* plant, but the experiments included in this work indicate their presence in flax plants.

The extract, containing mentioned potentially biologically active molecules, was analyzed to investigate their influence on the processes connected mainly to chronic wound healing. The experiments especially included the role of cannabidiol, taking in consideration its characteristic biological activity and unique nature, but also phytosterols - the molecules of known valuable health-beneficial properties. The influence of flax fiber hydrophobic components on skin fibroblasts and keratinocytes proliferation potential was analyzed, where only highest extract concentration of 0,2 μg CBD/ml showed to lower the keratinocytes proliferation. In all other cases positive or no effect was observed in this parameter. The extract have also accelerated the process of wound closure of investigated

skin cells *in vitro*, in which the cells migration was the key element. The influence was not connected to the presence of cannabidiol, and according to the literature, can be associated with both sterols and unsaturated fatty acids content.

The analysis of the flax fiber extract's influence on the fibroblasts' transcriptome revealed that probably, despite of the quantitative advantage of fatty acids and sterols, the main bioactive component of the extract is the identified cannabidiol. This conclusion is suggested by both quantitative and qualitative analysis, in which many characteristic for cannabinoids actions on genes expression were noticed, including connected to signaling cascade and inflammation state inhibition. Also many observed changes implicate the activity of sterols, especially related to steroids' metabolism, extracellular matrix and cell cycle regulation.

The more specified analysis of chosen inflammation- and extracellular matrix-related genes expression indicates inhibition of inflammatory responses and matrix reorganization, when flax fiber hydrophobic extract was used. Cannabidiol was shown to be the main component responsible for the anti-inflammatory activity, but the sterols can act in synergy causing similar changes to the ones observed for CBD. In the case of extracellular matrix remodeling, not including changes in collagens expression, other than cannabinoids and sterols extracts' components may be involved.

The experiments performed in this work confirmed also antibacterial properties of the cannabinoid containing extract. Demonstrated inhibition of *Staphylococcus aureus* and *Pseudomonas aeurginosa* proliferation was correlated with the CBD concentration in the extract. The extract concentrations capable of bacterial growth inhibition were at least twice as high as investigated in the experiments with human skin cells. The cytotoxic effect therefore cannot be excluded in the case of these cells.

Separate part of the work focuses on the cannabidiol's presence in flax plants itself. Performed measurements confirm the presence of the molecule in all parts of the plant, but not in the oil produced from seeds, and suggest changes of the CBD production in dependence of stress conditions and plant hormones. The potential flax cannabidiolic acid synthase gene was also identified, along with the promotor sequence, which characterizes the presence of many regulatory elements connected to stress of drought, cold or infection,

and also to hormones, mainly giberelins and abscisic acid. *In silico* analysis indirectly explains the observed changes of cannabinoids levels in stress conditions and suggests the role of these molecules in the plant's defense reactions.

The work describes also obtained transgenic flax plants, expressing cannabidiolic acid synthase from *Cannabis*. The plants showed increased cannabidiol level, what indicates the presence of active CBD synthesis pathway in flax, similar to the one described for hemp plants.

Obtained results show the presence of unique, biologically active cannabidiol, as the main component responsible for anti-inflammatory and anti-bacterial properties of the flax fiber hydrophobic extract. Synergistic activity of the other extract's components, especially sterols, but probably also fatty acids, causing activation of skin cells proliferation and migration, is very important in the aspect of flax fiber application as a chronic wound dressing. Preliminary data confirm also possibility of cannabinoid's content manipulation with transgenesis, that can determine plants characteristic and stress resistance.