

Review

## Phytochemical, pharmacological and clinical studies of an extract prepared from silver fir branches (*Abies alba*)

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**Summary.** *Abies alba* (silver fir) is a tree native to the mountains of Europe, that has rarely been used in traditional phytotherapy. Its bark, wood and particular branches are rich in antioxidative polyphenols among which lignans (taxiresinol, 7-(2-methyl-3,4-dihydroxytetrahydropyran-5-yloxy)-taxiresinol, secoisolariciresinol, laricinresinol, hydroxymatairesinol, isolariciresinol, matairesinol and pinoresinol) are the most characteristic. In our group, we optimised an extraction procedure under the name Belinal. In several pharmacological, toxicological and clinical studies the extract has been shown to prevent atherosclerosis in guinea pigs and to have cardioprotective effect in isolated rat hearts. It was found to reduce the post-prandial glycaemic response in healthy volunteers. Its topical use improved skin appearance and function. We found no influence on liver and kidney function. The production of this extract and its use has been patented and in collaboration with industrial partners was put on the market as a food supplement.

**Keywords:** *Abies alba*, lignans, polyphenols, silver fir.

### INTRODUCTION

The use of medicinal plants is usually based on tradition and experiences collected from past generations. However, medicinal plants can also be the subject of modern in-depth research. Our group have been investigating extracts from silver fir (*Abies alba*) for many years. The silver fir is the only type of fir that grows in Slovenia and has only occasionally been used in folk medicine.

Our research is divided into several areas: ethnopharmacological research of traditional and folk remedies; botanical studies of the influence of plant species, plant part and growing sites; phytochemical research of biologically active substances; pharmaceutical-technological research aimed to optimize the extraction process; pharmacological research to determine the mechanism of biological effects;

clinical research to find therapeutic effects and the safety of medicinal plants in humans.

### ETHNOPHARMACOLOGY AND ETHNOBOTANY

In a review of old records, we found only a few references for the therapeutic use of silver fir. Mostly, essential oil and resin were used in a manner similar to spruce. Beer was produced from the branches of this plant centuries ago (London Medical Gazette 1837). Unfortunately, it is not clear from these old records whether the fir served as the (only) source of carbohydrates for fermentation in this brewing, or if it was added as a flavouring and/or preservative.

## BOTANY

Only one species of fir grows in Slovenia. For comparison we did our initial research with other conifers such as spruce and pine, but the silver fir contained the highest proportion of low molecular polyphenols (non-tannins). We also compared different parts of the fir: wood, knots, bark, and different parts of the branches (right next to the trunk and further away from the trunk). According to the amount and variety of antioxidant phenols contained, we found that the branches, namely the part closest to the trunk, were the most promising for further research. Most of these substances are in the heartwood (inner dark part of the branch) and less in the sapwood (outer pale part) (Fig. 1).

## PHYTOCHEMISTRY

Silver fir branch extract contains a complex mixture of antioxidant polyphenols (Tavčar Benkovič et al. 2017) with known biological, pharmacological and clinical activities that contribute to human health: with a range of effects such as antioxidant, antidiabetic and anti-inflammatory activity, as well as cancer and cardiovascular disease prevention. The three main types of polyphenols in this extract are: lignans, flavonoids and phenolic acids. The main flavonoids are catechin and epicatechin. The main phenolic acids are protocatechuic acid, *p*-coumaric acid and gallic acid. Lignans in this extract are derivatives of lariciresinol (Fig. 2), of which the most abundant is secoisolariciresinol (5.5% in dry extract), followed by izolariciresinol, hydroxymatairesinol, lariciresinol, matairesinol and pinoresinol. Some of these lignans are also found in flax and sesame seed, as well as in other foods (Gerstenmeyer et al. 2013). In addition to polyphenols, the extract also contains some water-soluble wood polysaccharides.



Fig. 1. Transverse and longitudinal section of a fir branch with visible dark heartwood (photo: Tadej Rejc).

## PHARMACEUTICAL TECHNOLOGY

Antioxidant polyphenols are effectively extracted from the branches with water, so it was not necessary to use more expensive, flammable and less environmentally-friendly solvents such as ethanol, ether or hexane. However, it was necessary to optimize the temperature and extraction times, because at higher temperatures and during longer extraction

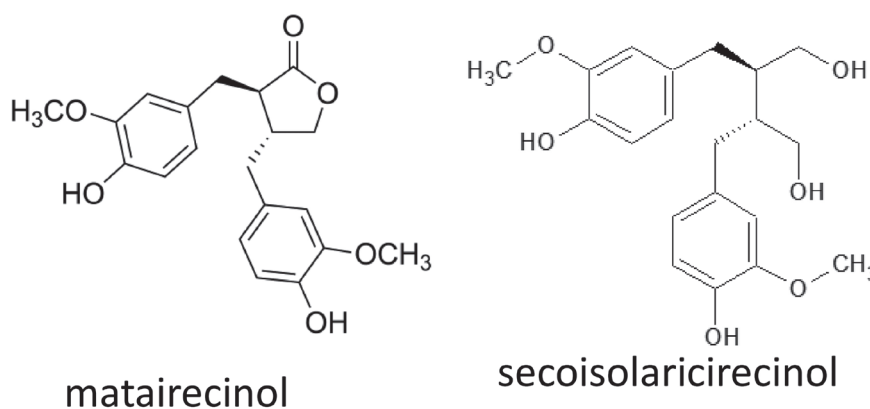


Fig. 2. Chemical structure of the two most characteristic polyphenols in fir branch extract.

times, oxidation and polymerization take place, and thus reduce the content and/or activity of the antioxidants. For easier storage, better stability and more appropriate incorporation into tablets and capsules, the aqueous extract was evaporated to form a dry extract in the form of a powder (Fig. 3). The amount of extract obtained is quite small, only about 4% of the mass of the branches used in the extraction. The process of obtaining the extract and its effects were patented, and the extract was named Belinal. Belinal was named after Belin, the ancient god of the Veneti and Slovenes. Statues of Belin and altars dedicated to him were found during excavations at Ljubljana Castle, Celje, Tolmin, Klagenfurt and the Veneto region.

## PHARMACOLOGY

Aqueous extracts from the branches of the silver fir tree display strong antioxidant effects in different *in vitro* tests. In a model system simulating conditions in the gastrointestinal tract, most polyphenolic substances are preserved, as well as most of their antioxidant activity (Tavčar Benković et al. 2017). Antioxidative substances were shown to be able to pass through the membrane of eukaryotic cells. This was found by measuring the antioxidant activity inside the cells



**Fig. 3.** Dried water extract from the branches of silver fir called Belinal (photo: Tadej Rejc).

when the extract was added to the growth medium outside the cells. In further studies, the extract effectively protected rat heart muscle cells from damage due to ischemia and reperfusion (Drevenšek et al. 2016). In addition, we observed that in hearts protected with fir extract after undergoing ischemia, there were also less arrhythmias than in controls. In guinea pigs, which received extract of fir tree branches added to their food for two months (Fig. 4), we found significantly



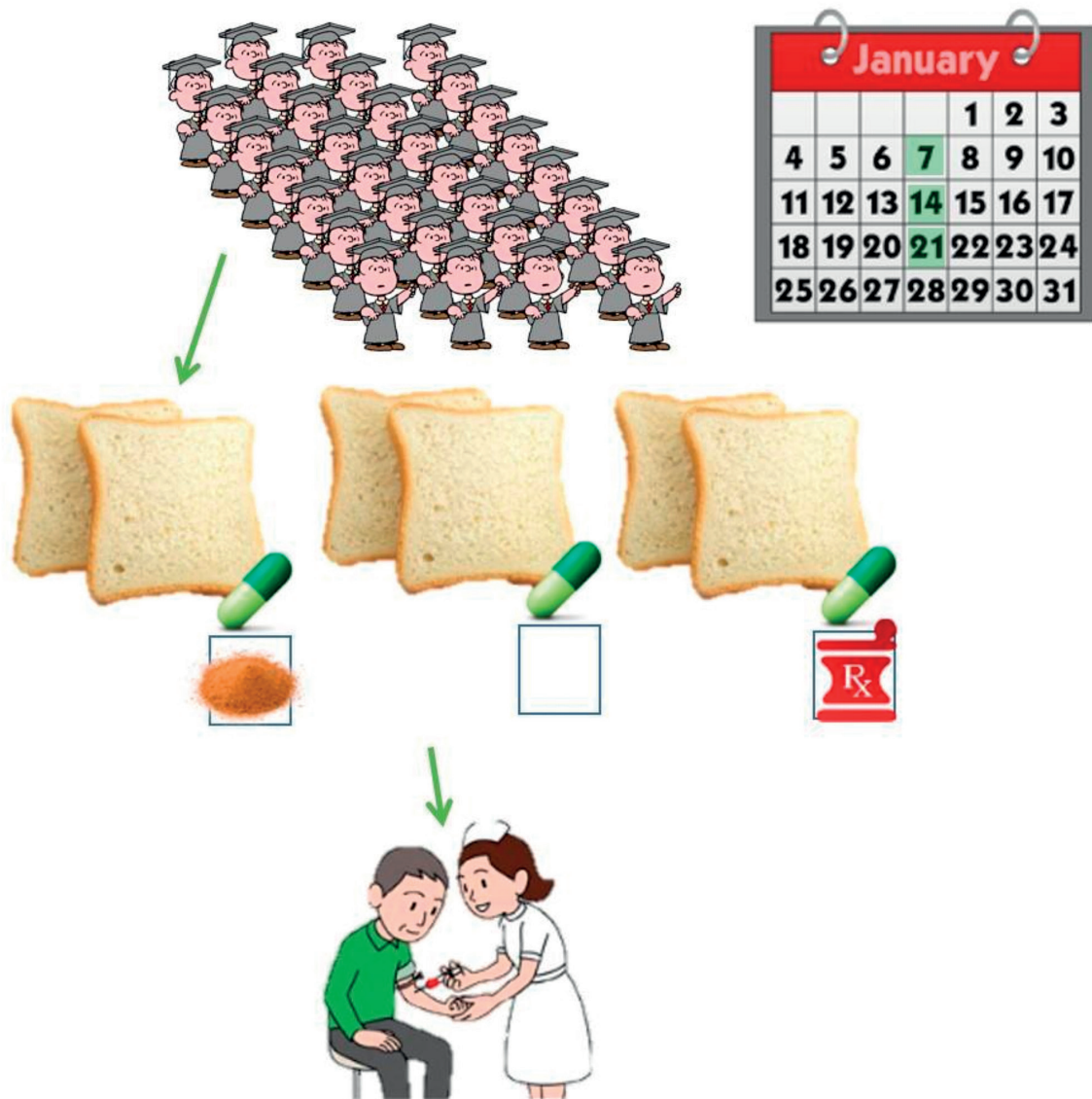
**Fig. 4.** Three groups of guinea pigs were fed three different diets for two months (from above: atherogenic diet, non-atherogenic diet, atherogenic diet + silver fir branch extract). After three months, functional examinations (elasticity and contractility) and morphological examinations (presence of atherosclerosis) were performed.

less atherosclerotic lesions and loss of functionality in their arteries in comparison to guinea pigs with the same atherogenic food without the extract (Drevenšek et al. 2015).

With respect to diabetes, in one of our studies we measured inhibition of the enzyme alpha-amylase and beta-glucosidase, the two enzymes responsible for degradation of starch in the intestine. The extract from silver fir branches strongly inhibited the action of these two enzymes (Lunder et al. 2018), which is useful in diabetes because it slows down the passage of glucose into the blood. Some inhibitors of these two enzymes (e.g. acarbose) are already in routine use as antidiabetic drugs.

## CLINICAL RESEARCH

Antidiabetic activity of the extract was also confirmed in a double blind, placebo and active-controlled clinical study (Debeljak et al. 2016). We tested the ability of the silver fir branch extract to reduce postprandial blood glucose peaks. Thirty-one healthy participants consumed a test meal (100 g of white bread) 3 times in 1-week intervals (Fig. 5). With the meal, they consumed a capsule containing: either silver fir branch extract, a placebo or acarbose (active control).



**Fig. 5.** Study scheme in which 31 volunteers (medical students from Maribor) consumed a test meal with a capsule of the test substance, and blood samples were taken for glucose and insulin measurements.

After the meal blood samples were taken to measure glucose and insulin concentrations (Fig. 6) and the Glycaemic index was calculated. The standard meal consumed with the extract and acarbose resulted in 35% and 43% lower glycaemic index respectively. Insulin concentrations in the blood of volunteers taking the extract were also significantly lower than in controls.

Another clinical study (Tavčar et al. 2016) aimed to determine the effects of silver fir branch extract on the skin. Ten volunteers, aged 45 to 65 years, used a topical preparation on their face twice a day for 12 weeks. They applied a cream with 2% of this extract on one half of their face, and a cream without the extract (placebo) on the other half of their face. The condition of the skin was monitored by measuring skin moisture, barrier function, dermis density and thickness, skin elasticity, and wrinkles. All the parameters except for dermis thickness and skin elasticity improved significantly.

## CONCLUSION

Investigations into an extract prepared from silver fir branches were performed at the Faculty of Pharmacy in Ljubljana on several different levels: ethnopharmacological, botanical, phytochemical, technological, pharmacological and clinical. Our results show that the extract has promising antioxidant and anti-diabetic properties, as well as protective effects against cardiovascular ailments and positive properties when applied on the skin. We are happy to have good collaboration with industry, which produced the food supplements containing this extract (Fig. 7). Additional clinical studies will be needed to enable registration of the extract as a medicinal product.

## Literature

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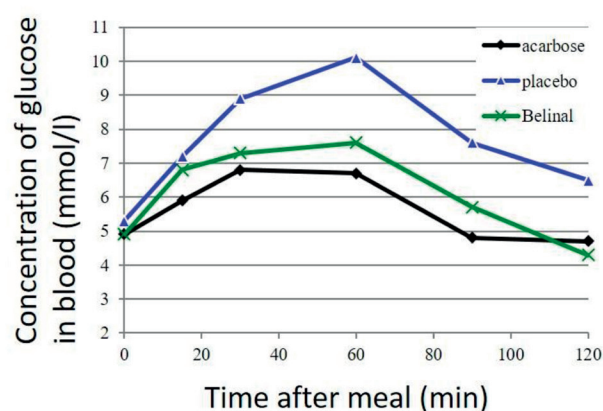


Fig. 6. Postprandial glucose levels were significantly lower if the meal was consumed with the extract from the branches of a silver fir tree (in the figure marked as Belinal), compared to placebo.



Fig. 7. Example of some Belinal products that contain an extract from silver fir branches developed and investigated at the Faculty of Pharmacy in Ljubljana. Belinal superior is used to reduce fatigue and Belinal gluco is used to maintain normal blood glucose levels.

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