Impact of the preparation process of European spruce (*Picea abies*) homogenates and Common marigold (*Calendula officinalis*) isolates on total phenolic content

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OUTLINE

Plants have an incredible ability to synthesize many of the active ingredients on which today's medicines and therapies are based. They are able to defend themselves against external influences (enemies) by releasing the appropriate molecules into individual parts of plants[1]. The spruce tree (*Picea abies*) and common marigold (*Calendula officinalis*) (**Figure 1**) contain different composition of biologically active and complex substances. The composition of these substances is based on several factors, e.g. geology, climate, time of harvesting, availability of nutrients, etc.

Polyphenols are compounds with one or more hydroxyl groups attached to the aromatic ring, which give them the ability to capture free radicals, moreover it gives them a stronger acidic character in comparison to other alcohol groups[1]. This chemical reactivity is responsible for the antioxidant character of polyphenols. Nisca *et al.* (2021) have found a strong correlation between the antioxidant capacity and content of total polyphenols using ultrasound assisted extraction and microwave assisted extraction[2]. The results also suggest that higher polyphenolic content may lead to a stronger antioxidant activity[1].

The focus of this work was determination of polyphenolic content in homogenates and/or isolates of spruce needles and common marigold, that are a source of cellular components and active compounds. The focus was put on different methods of preparation of homogenates and drug isolates preparation.

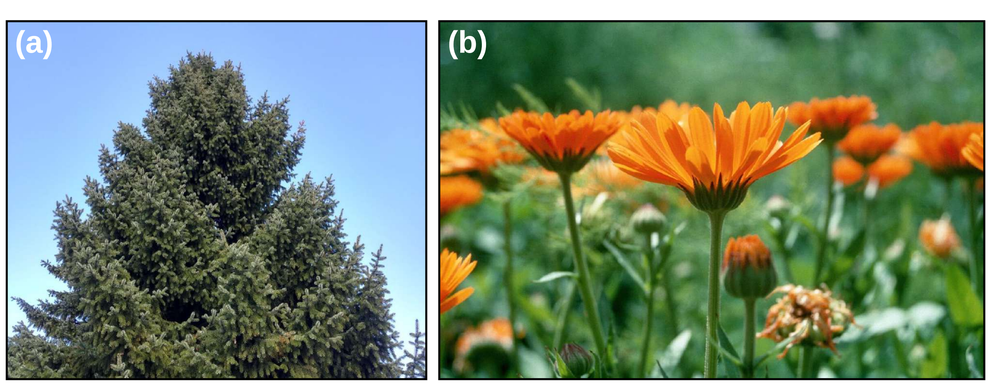

Figure 1: Plants: (**a**) European spruce (*Picea abies*) and (**b**) Common marigold (*Calendula officinalis*).

EXPERIMENTAL METHODS

An **European spruce** homogenates were prepared from the freshly collected needles in distilled water and physiological solution. For each individual experiment, 10.0 g of needles were weighed into a 100 mL Erlenmeyer flask and 20 mL of the medium – distilled water or saline (0.9% sodium chloride solution) was added. So prepared samples were exposed to various experimental conditions (**Figure 2**). Prior to the analysis, the contents were homogenized and the remaining needles were quantitatively filtered through a white ribbon paper (pore size 4.0–12.0 µm, Whatman) into a 50 mL falcon tube.

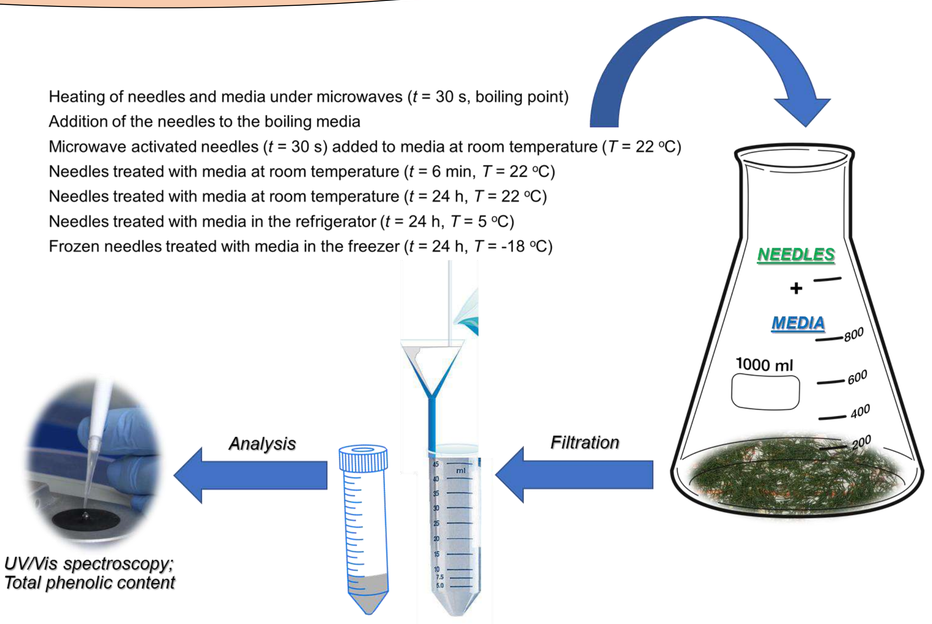

Figure 2: Flow chart of the experimental design for the preparation of homogenates from European spruce needles.

A **Common marigold** isolates were prepared by supercritical extraction and ethanol/water maceration. A 18 kg of marigold flowers were added into the reactor. The process was carried out under supercritical CO_2 environment, and the yield was 1 kg of the final isolate. The final isolate was stored in a dark place. For the ethanol/water maceration process, a 1 kg of plant leaves was treated overnight at room temperature in a 20% ethanol solution. The plant debris was filtered off, and the filtrate (1 kg) was stored in a dark place.

Total **phenolic contents** were determined according to Jeran *et al*. (2023) using the Folin-Ciocalteu method[4] using the 96-well. A 12.5 µL of 10-times diluted Folin-Ciocalteu reagent (Sigma) was added to 2.5 µL aliquot of the sample and mixed. A 10 µL of 7.5% solution of sodium carbonate (≥ 99.5 %, Sigma-Aldrich) was added and allowed to stand (t = 30 min) in dark at room temperature. The absorbance was assessed spectrophotometrically at 760 nm (Nanodrop One C, Thermo Scientific). A mixture of a 0.9% solution of sodium chloride (p.a., Sigma-Aldrich), and reagents was used as a blank. The calibration curve was prepared using gallic acid standard solutions (2–90 µg/mL). The results were expressed as mg of gallic acid per plant weight.

Particles in the water-based European spruce homogenates were heterogeneous in size thus they were **visualized** using **light microscopy** using a inverted light microscope (Nikon EM CCD Eclipse, TE2000-S, Nikon) with a digital camera system: spot boost (Visitron Systems).

RESULTS

The highest total phenolic content was detected in the direct microwave processing of the **spruce needle samples** in media to the boiling point (**Figure 3**). In this case 55.0 µg/g and 32.8 mg/g of polyphenols were found in distilled water and saline. The process was slightly slowed down after activation of the common extract by boiling media or at room temperature (T = 22 °C). Active compounds leached at low temperatures (refrigerator or freezer). The experimental tendency can be explained by the theory of Brownian particle motion – particles divide at higher temperature, move faster, and the higher temperature contributes to the opening of the pores of the plant tissue.

The technique of direct freezing of needles with media proved to be useless, as higher temperature appears to have a greater effect on leaching than deformation of the plant tissue by freezing and subsequent thawing. This again confirms the theory of Brownian particle motion that changes occur much more slowly at low temperatures than at higher temperatures.

Following the process of excretion of total phenolic compounds from plant tissues at different time periods (t = 6 min and 24 h), it was found that leaching into distilled water over a longer period of time is more effective; an increase from 15.2 to 28.2 µg/g was observed. In saline solution, the amount of total phenolic compounds decreased over time from 28.1 (t = 6 min) to 12.6 µg/g (t = 24 h). The reason for this could be the sensitivity of substances in the saline medium, as the *p*H of the medium may change with prolonged exposure to the needles, resulting in a decrease in total phenolic compounds due to formation of phenolates or decomposition. Another reason is a sensitivity to a *p*H. After an addition of the base (1 M NaOH) to the yellow colour sample the solution turned to a purplish brown. Conversely, the addition of an equal volume of a 1 M HCl solution to an alkaline solution turned the colour back to yellow. Since these are compounds with at least one aromatic ring containing one or more hydroxyl groups, the colour transition can be interpreted similarly to the natural dyes/indicators anthocyanins, which also belong to the polyphenols.

The highest total phenolic content was found in the water/ethanol extraction of **Common marigold** leaves (14.3 mg gallic acid/kg leaves), and the lowest content in the supercritical extract of flowers (0.0357 mg gallic acid/kg flowers). The reason is supercritical CO_2 extraction is used for extraction of lipophilic components of the sample, while the mixture of ethanol and water macerates mainly polar substances.

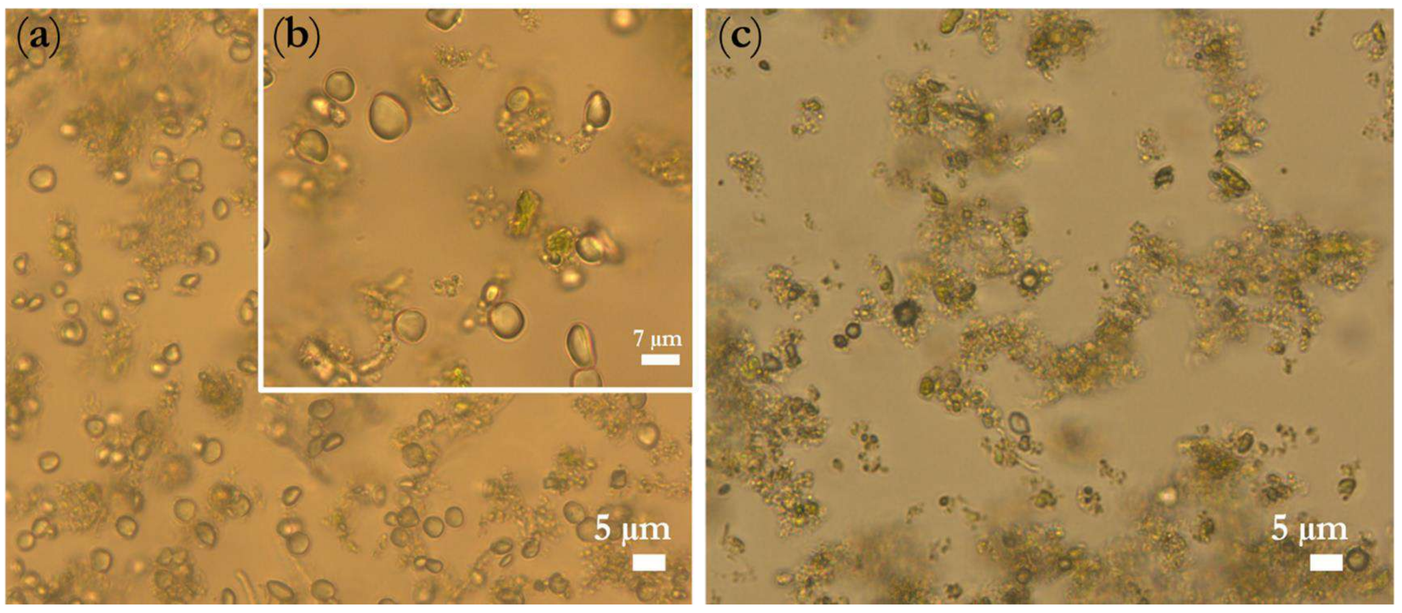

Figure 3: Homogenates in saline (**a**, **b**) and in distilled water (**c**) have the same structural fragments, but the concentration of membrane-enclosed particles is higher in the saline. In the aqueous homogenate (**c**), the particles were located in the clasters, where some deformed plant cells can be seen, among others.

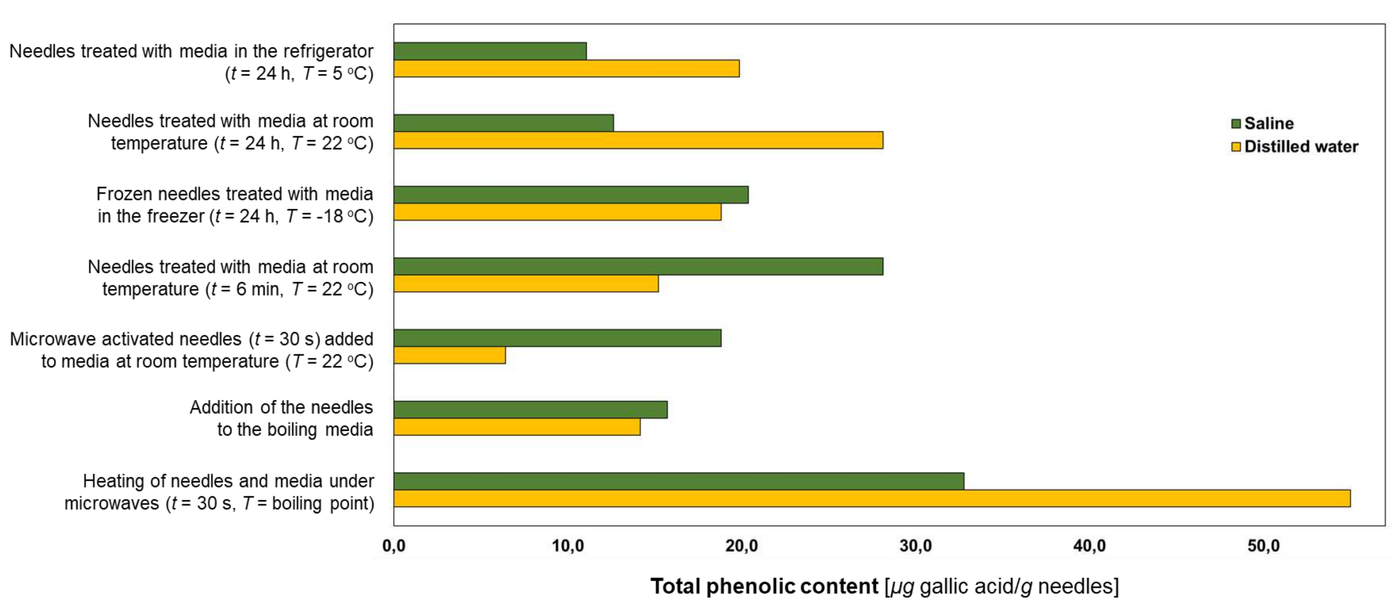

Figure 4: Total phenolic content in homogenates of European spruce (*Picea abies*) needles.

CONCLUSION

Experiments have shown that **factors** such as the choice of isolation method, plant part, temperature, time, and choice of medium have a **significant effect** on the extraction and quantitative **content** of **total phenolic content**.

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