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RESEARCH

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THE CHEMISTRY BEHIND NATURAL COLORANTS SOURCED FROM MUSHROOMS

ARVIND NEGI, RIIKKA RÄISÄNEN

Most of us have seen mushrooms at grocery stores or stalls in shopping plazas and malls, such as Arabia Mall and metro stations within the Helsinki metropolitan area, as well as in other locations across Finnish cities. Mushrooms are recommended for various cuisines, and, in Finland, they are common ingredients in several dishes, including pizzas. These edible mushrooms are known to be rich sources of protein, vitamin B, and vitamin D. However, the nutritional value can vary depending on the type of mushroom. The trend of consuming and collecting mushrooms, known as mushroom foraging, is emphasized in many publications, reflecting Finnish society's close relationship with nature and inclination towards organic products. Nevertheless, Finland is also home to some poisonous mushrooms. Therefore, organizations, including the Finnish Food Authority and other regulatory bodies, provide timely guidance and updates about the necessary precautions to take during mushroom foraging and consumption. Similar notifications are continuously updated in other European countries as well, particularly in regions where historical instances of mushroom poisoning are more common. The ideal approach to mushroom picking is to be certain of the species; if there is any doubt, it is best not to pick them. Working in Professor Riikka Räisänen's research group, which includes myself (Arvind Negi) and my colleague Peppi Toukola (who is currently a doctoral student in the Chemistry Department), has involved characterizing Finnish mushrooms. In our project work, while we found that some mushrooms can be poisonous if consumed in large quantities, others contain beautiful color compounds, known as biocolorants or biocolors. In our work, we collect mushrooms from various parts of Finland and process them in our lab to remove unwanted parts. For instance, mushrooms typically contain about 90% water and 10% solid mass. To illustrate, if 100 kg of mushroom material is used, it would yield approximately 10 kg of solid mass. Importantly, only 6% of this solid mass can be extracted as colorants. Our studies indicate that the extracted color mass, which represents the total amount of biocolorants, is significantly influenced by environmental factors beyond human control, such as rain, humidity, temperature, and other ambient conditions. While decision-making during the extraction process can lead to variations in the yields obtained from mushrooms, the impact is relatively minor.

Mushroom based colorants

When searching for natural colorants, a helpful guideline for identifying *Cortinarius* mushrooms for our research is to look for species with prominent colored parts, such as the cap, gills, or stalk, as illustrated in **Figure 1A**. This characteristic makes it easier to locate them in their natural habitat compared to other mushroom species. However, due to the close resemblance among species within the *Cortinarius* genus, we specifically focus on those mushrooms that have red gills. These red-gilled mushrooms are well-known members of the *Dermocybe* subgroup within the *Cortinarius* genus and are recognized for containing biocolorants in the form of anthraquinones. Using HPLC-MS/MS methodology, we found that species within the *Dermocybe* group exhibit significant variation in their expressions of anthraquinone-based biocolorants, as reported in our recent publication [1]. These anthraquinones are polyphenolic compounds, which are secondary metabolites. They can be classified into two categories: those that contain a carboxylic group and those that do not. We observed considerable chemical diversity among these anthraquinones, attributed to the presence of different organic groups such as phenolic -OH, chloro, methyl, and methoxy. Some examples of these variations are presented in the accompanying figures (**Figure 1** and **Figure 2**).

Applications to textiles

As our lab specializes in performing coloration experiments for different types of materials (cellulose, polyester, polyamide, cellulose acetate, EVA polymer

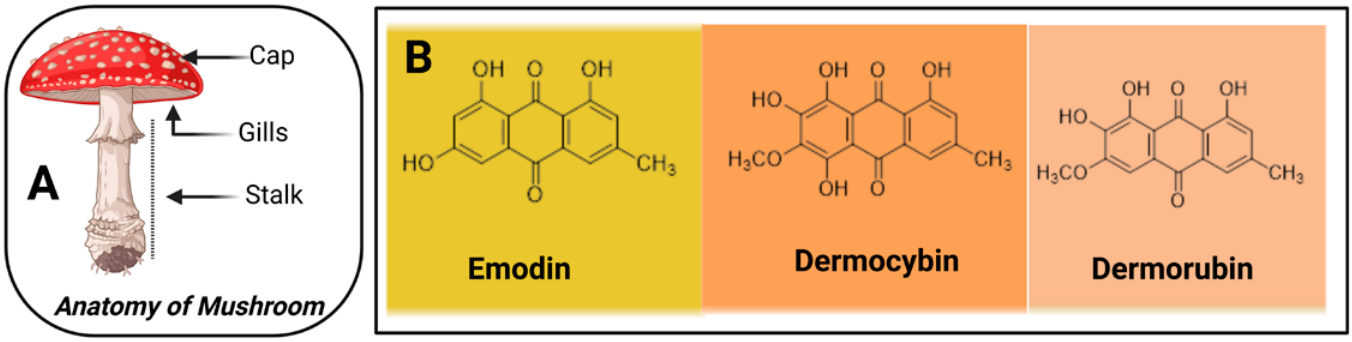


Figure 1. Illustration of the practical applications of Finnish mushrooms: (**A**) Depiction of mushroom anatomy. (**B**) Color of polyester material dyed with natural anthraquinones using the supercritical carbon dioxide dyeing process [3]. *Reproduced in Biorender.*

etc.), we apply extracted anthraquinones as colorants to a wide variety of textile fibers. Interestingly, the requirements for colorants and dyes differ based on the type of fabric (fiber type). Consequently, dyes are categorized based on their applications. Cellulose fibers (such as cotton and viscose) hold a significant proportion in global textile production. Other common fibers in the textile industry include polyester, followed by polyamide, wool, and silk. Wool and silk are protein fibers and are more expensive. In contrast, polyester is a synthetic fiber that can be produced on demand; therefore the process matures with time and becomes cost efficient, making it the second most used textile material after cellulose fibers. Cellulose fibers , like cotton, are widely available due to the natural abundance of cellulose. Since these fibers differ from one another, their dyeing processes also vary. Comparatively, dyeing cellulose fibers (such as cotton and viscose) is more complex than dyeing other fibers. For instance, reactive dyes are used to dye cellulose fibers. They are water soluble and designed to form a covalent bond with the fiber. Reactive dyes consist of three components: water-soluble groups (like sulfates and carboxylic acids), a chromophore (which imparts color), and reactive groups (such as vinyl sulfonyl and triazine). On the other hand, polyester fibers are dyed using disperse dyes. These dyes are characterized by their smaller molecular size and lack of water-soluble or reactive groups. As a result, the dyeing process for polyester is more of a physical phenomenon, where small dye molecules are entrapped within the microfibers when cooled after being heated to high temperatures. Wool and silk, being protein fibers, feature peptide backbones that readily form bonds with dyes. Wool and silk are dyed with acidic dyes. Interestingly, since we understand which dye molecules are needed for each type of fiber, therefore the extracted biocolorants are specifically designed for wool, polyester, and other synthetic fibers, as illustrated in **Figure 1B**.

Scope of natural colorants

A paradigm shift in the global market for colorants, especially in the food, textile, and other industries, emphasizes the need for sustainable yet affordable solutions. Therefore, use of natural colorants for such industries not only meets consumer demand for natural and sustainable products but also aligns with global trends toward green practices. Importantly, natural colorants often have a favorable regulatory status, making them easier to use in various applications while complying with safety standards set by regulatory bodies like the FDA and EFSA. Unlike synthetic dyes, natural colorants often possess additional functional properties, such as antimicrobial activity, UV protection, and antioxidant properties, and are known as biodegradable and have a lower environmental impact. These benefits can enhance their performance and value to commercial industries. In this context, natural anthraquinones, particularly those studied by our research group, have shown potential applications in industrial settings. Evidence suggests that anthraquinones and colorants with similar structures are commonly used across various industries due to their sustainability and health benefits. Additionally, natural dyes are being investigated for packaging materials, contributing to a lower environmental footprint in product packaging. Other than finding applications in food and textile industries, anthraquinone-based biocolorants can be found for therapeutic purposes, as anthraquinone-based scaffolds are common to the pharmaceutical industries. In pharmaceuticals, natural colorants are used to color tablets, capsules, and syrups due to their non-toxic nature and potential health benefits. Natural products are thus important for drug industries as nearly 60% of drugs are found to be derived from natural compounds approved by the FDA in the last decades. Therefore, various natural compounds serve different purposes in various stages of drug development.

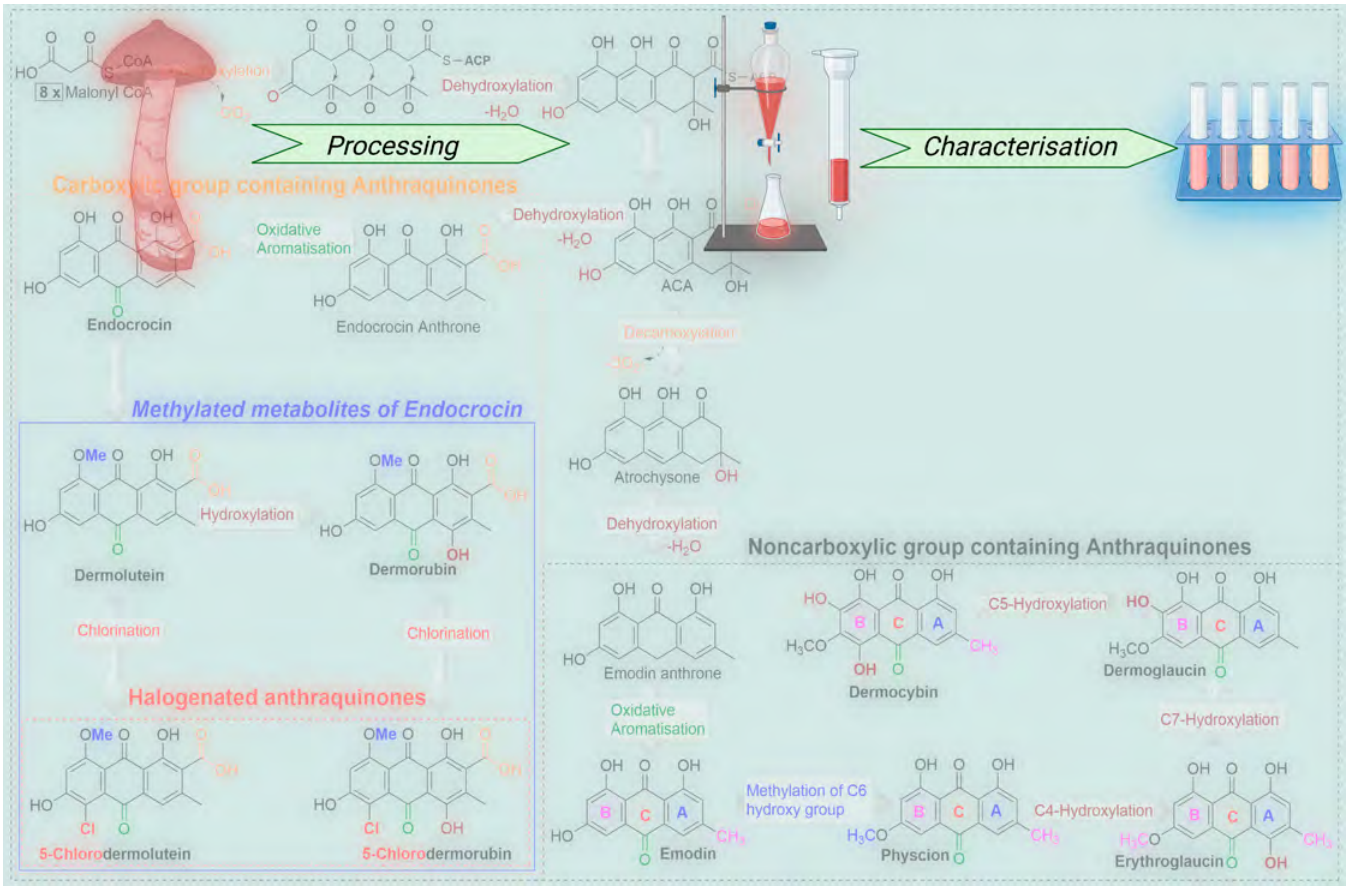


Figure 2. Chemical diversity of natural anthraquinone biocolorants.

Current challenges and solutions

However, as the saying goes, "*All that glitters is not gold*," which aptly describes the challenges associated with using natural anthraquinones as biocolorants. Despite their advantages, natural dyes/colorants face obstacles such as variability in color yield, lower color fastness compared to synthetic dyes, and the necessity for large quantities of raw materials. Advancements in dyeing technologies and the adoption of eco-friendly mordants are helping to address some of these challenges. However, the use of biocolorants in the food and cosmetic industries also presents issues such as color stability, a limited color spectrum, and the need for additional treatments to enhance color fastness. These limitations restrict the widespread use and application of natural dyes, prompting researchers to seek new colorants that offer greater stability and a broader color spectrum. One approach to overcoming the limitations of extracting anthraquinones from natural sources is to mitigate the variability caused by non-human factors. For instance, at VTT, a key partner in this project, biotechnological methods using microbes have been applied to produce specific anthraquinone-based biocolorants. Microbial production is more sustainable, requiring fewer resources and generating less waste compared to traditional methods. Additionally, it supports the scalability of these natural colorants, as microbes can be cultured on a large scale. Moreover, microbial production ensures improved consistency since the process can be tightly controlled, resulting in uniform quality and color of the pigments produced. Ensuring the quality and consistency of bio-based dyes and pigments is essential for meeting the demands of sustainable colorant production.

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