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The Merit of Medicinal Mushrooms from a Pharmaceutical Point of View

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ABSTRACT: Whereas pharmaceuticals prepared by extraction of medicinal plants constitute an important part of evidence-based medicine also in the Western Hemisphere, medicinal mushrooms are mainly used as dietary supplements without declaration of a medical indication. Scientific investigations and case studies from Asian medicine show that fungi have very promising pharmacological potential. This article provides an overview of the principles of authorization and market access of herbal drugs in Europe, with special reference to Germany. The current status regarding mushrooms is reported, with an aim toward supporting the development of legalized pharmaceutical preparations of medicinal mushrooms in Europe.

KEY WORDS: medicinal mushrooms, drug authorization, market access, Europe

ABBREVIATIONS: BfArM, Bundesinstitut für Arzneimittel und Medizinprodukte (Federal Institute for Drugs and Medical Devices); **DAB,** Deutsches Arzneibuch (German Pharmacopoeia); **DAC,** Deutscher Arzneimittelcodex (German Drug Codex); **EMA,** European Medical Ageny; **EU,** European Union; **HAB**, Homöopathisches Arzneibuch (Homoeopathic Pharmacopoeia); **Ph. Eur.,** European Pharmacopoeia.

I. INTRODUCTION

In many countries, herbal medicines constitute an important part not only of traditional but also of evidence-based medicine. Normally, herbal medicines are obtained by extraction of dried medicinal plants (herbal drugs) or other materials. The special status of such medicines is due to their complex composition. They consist of many different compounds: main active components, components that support the main activity, substances that are valuable for nutrition (e.g., vitamins, amino acids or minerals), tasting or smelling compounds, and desired or undesired accompanying substances. This complex composition has advantages and disadvantages in comparison to isolated compounds. Advantages include the possible combination of desired activities of several compounds, a combination of medical and nutritional properties, better taste and smell, and/or better bioavailability. Otherwise, this complexity poses significant challenges for analytical methodologies and activity tests. Next to the classical tea drugs, the importance of phytopharmaceuticals, which are labeled as defined or standardized extracts, is increasing.[1] In Germany, approximately 2000 licensed phytopharmaceuticals are on the market. They can be considered equal to synthetic drugs in terms of their quality, efficacy, and safety.

Why have mushroom products previously been excluded from these licensed phytopharmaceuticals? On the European markets, products containing medicinal mushrooms are sold as food or dietary supplements and not as licensed drugs, meaning that the declaration of medicinal indication is not allowed.[2] Medicinal mushrooms are regulated by food law and not by pharmaceutical law. What must be done to sanction the evidence-based medicinal use of mushrooms in the Western Hemisphere as it is in Asia? In this paper, I explore these questions and explain the processes of drug authorization in the European Union, with special reference to Germany.

II. DEFINITIONS

"Medicinal mushrooms" are comparable to "medicinal plants" and can be defined as macroscopic fungi,

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mostly higher *Basidiomycetes*, which are used in the form of extracts or powder for prevention, alleviation, or healing of diseases and/or to complete nutrition. "Herbal drugs" are dried fruit bodies, mycelia or spores are "mushroom drugs" or "fungal drugs." Analogous to "phytopharmaceuticals" or "herbal preparations," medicinal mushroom preparations should be considered as "mushroom pharmaceuticals" or "mushroom preparations."

Although biologically incorrect, the EU directive 2004/24/EC includes fungi in the definition of herbal substances.[3] **Herbal medicinal products** are defined as "Any medicinal product, exclusively containing as active ingredients one or more herbal substances or one or more herbal preparations, or one or more such herbal substances in combination with one or more such herbal preparations." **Herbal substances** are defined as "All mainly whole, fragmented or cut plants, plant parts, algae, **fungi**, lichen in an unprocessed, usually dried, form, but sometimes fresh. Certain exudates that have not been subjected to a specific treatment are also considered to be herbal substances. Herbal substances are precisely defined by the plant part used and the botanical name according to the binomial system (genus, species, variety, author)." **Herbal preparations** are defined as "Preparations obtained by subjecting herbal substances to treatments such as extraction, distillation, expression, fractionation, purification, concentration or fermentation. These include comminuted or powdered herbal substances, tinctures, extracts, essential oils, expressed juices, and processed exudates."[3] Therefore, in the sense of the European Medical Agency (EMA), "mushroom pharmaceuticals" are considered "herbal preparations." However, pure substances obtained by isolation from plants or other organisms or by synthesis do not belong to the herbal preparation category. In peppermint, *Mentha piperita* L., for example, this would mean that *Mentha piperita* folium is the herbal substance and an extract of peppermint leaves is the herbal preparation. But isolated or synthesized menthol does not belong to this category.

III. AUTHORIZATION AND REGISTRATION OF MEDICINAL PRODUCTS IN THE EUROPEAN UNION WITH SPECIAL REFERENCE TO GERMANY

In Germany, finished medicinal products have been required to undergo an authorization procedure before they can be placed on the market (Section 21 subsection 1 German Medicines Act, AMG).[4] The authority for human medicinal products in Germany is the Federal Institute for Drugs and Medical Devices (BfArM) and is seated in Bonn. It is an independent higher federal authority within the portfolio of the Federal Ministry of Health of Germany.[5] Apart from the **national authorization procedures, a centralized procedure** effecting a license for the whole European Union (EU) can be obtained. In this type of procedure, authorization is granted by the European Commission in Brussels, Belgium. The organizational process is handled by the EMA. Alternatively, a **decentralized authorization process** or **mutual recognition procedure** can be done. This means that the marketing authorization already granted by one EU member state is recognized by the licensing agencies of other Member states within a definite time, unless there are major objectives against doing so. Five years after licensing, marketing authorization has to be renewed by the BfArM.[5,6]

The main criteria that must be provided by pharmaceutical companies to the authorities before authorization are **pharmaceutical quality, efficacy,** and **safety** of the product. In the following sections, these criteria are further explained in the context of medicinal mushrooms.[5,6]

A. Pharmaceutical Quality

The most important aspects of pharmaceutical quality are **identity, purity,** and **amount of active constituents** (or lead components if the active constituents are unknown). Identity means verification of the declared material. Purity means that only limited amounts of ash, sand, or other impurities are allowed and that adulterant materials are forbidden. The amount of active (or lead) constituents

means the percentage of a declared compound in the dried plant or fungal material. The definition of acceptable ranges of active components or upper content limits for possible negative markers is very challenging.[7] Suitable analytical methods include microscopy, thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC), spectroscopy, and other methods. Suitable reference compounds (pure compounds typical for the declared herbal drug) are necessary.

For many plants, quality specifications and methods of verification are collected in the form of monographs in so-called pharmacopoeias. In Germany, the most important pharmacopoeias are the European (Ph. Eur.), the German (DAB), and the Homoeopathic Pharmacopoeias (HAB). The European Pharmacopoeia contributes considerably to the international harmonization of quality specifications across the European Union. It is continuously updated. In addition, quality specifications can be found also in other collections, such as the Deutscher Arzneimittel Codex (DAC).

European and German pharmacopoeia do presently not contain monographs for medicinal mushrooms. But there are monographs concerning *Ganoderma lucidum* (W. Curt.:Fr.) P.Karst., the Lingzhi or Reishi mushroom, in the Pharmacopoeia of the People's Republic of China[8] and in the American Herbal Pharmacopoeia.[9] They include microscopic identification and chromatographic investigation for a limited number of components. Specifications regarding the content of total and of defined triterpenes and the content of specific carbohydrates, etc., are not available. The latest edition of the Chinese Pharmacopoeia from 2010 includes some further medicinal mushrooms.

On the European markets, products with medicinal mushrooms are sold as food or as dietary supplements and not as licensed drugs.[2] Quality parameters for this category include nutritional values indicated by content of energy, carbohydrates, proteins, lipids, vitamins and minerals and the absence of undesired microorganisms, pesticides, heavy metals, or radioactivity. In contrast to a licensed drug, it is forbidden to declare pharmacological activities, bioactive compounds responsible for the pharmacological activities, and therapeutic uses.[6]

Obviously, the spectrum and content of bioactive substances depend on the origin of the mushroom (i.e., wild collection or cultivation, cultivation conditions), on the mushroom part used (i.e., fruiting body, mycelium, spores), and on the mode of preparation. The composition of the extract can be influenced by solvent type and mode of extraction (e.g., time, temperature, extraction cycles, quantitative relation between mushroom and solvent). In my group, we have determined the content of β-glucans in different medicinal mushroom species and in different preparations of the same species. Our results show considerable differences between the β-glucan content of the different samples. In addition, these results depend on the analytical method used (manuscript in preparation). But which β-glucan concentration is necessary to achieve the desired pharmacological effect in humans? This question has to remain unanswered. Only a recommended dose for isolated lentinan[10] is available, and it cannot be applied to a complex mushroom preparation. The situation for small-molecular-weight bioactive compounds in medicinal mushroom like triterpenes, sesquiterpenes, or eritadenin is similarly complicated. My group has established fingerprint chromatograms for different samples; we have determined the content of total and selected single compounds; and we have revealed large differences among different samples of the same mushroom species and in small molecular compounds (manuscript in preparation). Considering that *Ganoderma lucidum* contains approximately 150 different triterpenes with different biological activities,[11,12] it is highly probable that distinct Reishi products possess distinct medicinal properties. Therefore, it is essential to know the indications for which a Reishi preparation should be used, that is, as hypotensive, hepatoprotective, antiallergic drugs, or for other indications. Knowledge about structure–activity relationships and bioavailability is also needed. Depending on this information, bioactive compound(s) must be investigated to determine their appropriate concentration(s) in

the preparation.

In summary, many unresolved issues remain regarding quality aspects of medicinal mushroom preparations (see also[13]). Valid quality prescriptions and methods of controlling these criteria are necessary. Any evaluation of clinical results requires precisely defined and declared test samples.

B. Efficacy

Investigations of the efficacy and safety (see below) of possible drug candidates have to be conducted first in preclinical assays. These include *in vitro* investigations and, afterward, suitable animal assays. Information about the spectrum of activities, quality and quantity of main activity, pharmacological target and mode of action, selectivity of action, compatibility, pharmacokinetics, and bioavailability must be obtained. When positive results of preclinical assays and official permission is obtained, clinical trials of a promising drug candidate can be planned and begin. The first stage of clinical trials is done with a small group of healthy adult people (10–50 people) to test safety and pharmacokinetics, to detect expected effects, and to reveal unexpected effects. In the second stage, the preparation is given to a small group of patients with the relevant indication (100–300 people). Only after a successful second clinical trial can a higher number of patients be included in the third stage. The third stage of testing should take the form of controlled (in comparison to a reference drug or a placebo), double-blinded, randomized, and multicentric studies. The fourth level of clinical trials is performed after licensing. In this phase, the effects of the new drug are observed in the patients. In the case of sufficient efficacy and safety, the product authorization must be confirmed 5 years after licensing. [6]

What is the situation with respect to medicinal mushrooms? The most well-investigated medicinal mushroom species are *Ganoderma lucidum*, *Trametes versicolor* (L.:Fr.) Pilát (=*Coriolus versicolor* (L.:Fr.) Quél.), *Lentinus edodes* (Berk.) Singer, *Agaricus brasiliensis* Wasser et al., *Cordyceps sinensis* (Berk.) Sacc., *Grifola frondosa* (Dicks.:Fr.) Gray, *Hericium erinaceus* (Bull.:Fr.) Pers., and some others. For these mushrooms, a great deal of data are available regarding *in vitro* activities, effects in animal assays, traditional experience, and mode of action.[12–16] For example, the initial studies concerning the pharmacokinetics and mode of action of β-glucans have been published.[17,18] Human studies report "single case observations" or clinical studies with mostly small groups of patients. Nearly all originate in Asian countries.

Meanwhile, some promising meta-analyses have been published. One study analyzed eight randomized controlled trials including 8,009 patients with gastric cancer. The patients were treated with PSK, a polysaccharide-protein complex from *Trametes versicolor* (purified substance, not a typical mushroom pharmaceutical) in combination with chemotherapy. The results of this study indicate that adjuvant immunochemotherapy improved the survival of patients after curative gastric cancer resection in comparison to chemotherapy alone.[19] A meta-analysis of the effects of the ß-glucan lentinan, purified from *Lentinus edodes*, in adjuvant tumor therapy evaluated five studies with 650 patients with non-resectable or recurrent stomach cancer. A significant prolongation of life span in the groups treated with chemotherapy and lentinan could be shown in comparison to patients treated only with chemotherapy.[20] A Cochrane review analyzed the use of *Ganoderma lucidum* in tumor therapy. Only 5 of 257 studies fulfilled the analysis criteria. In total, 373 patients, mainly with lung cancer, were included. Improvement of life quality, measured by the Karnovsky Index, and positive effects on proliferation of lymphocytes were detected. Severe side effects did not occur. A significant prolongation of life span was not detected. The authors recommend better studies with higher numbers of patients and clear randomization.[21] The results of biological and pharmacological investigations can only be compared when standardized and exact, comparable samples are investigated. Thus far, this had not been the case, making the evaluation of clinical results obtained with medicinal mushrooms more difficult.

C. Safety

As mentioned, safety tests are an important part of preclinical assays. First, investigations are done using *in vitro* methods. These are followed by animal assays with different animal species (at least one rodent and one non-rodent). Tests have to be done for possible acute, subacute, and chronic toxicity, as well as for mutagenicity and teratogenicity. Important parameters include the so-called "no-effect level," which means the highest dose without any biological effect, and the lethal dose 50 (LD 50), or the dose at which 50% of animal subjects die.

Investigations of the safety of medicinal mushrooms are limited and have been done on the *in vitro* level or in animal models. Systematic safety studies, including those for chronic toxicity/mutagenicity/teratogenicity and fulfilling the requirements of licensing authorities, are not available or have not been published.[22] Clinical observations indicate no or very rare acute severe side effects during the administration of medicinal mushroom preparations. A Cochrane review has confirmed this for *Ganoderma lucidum*.[21] One well-known undesirable side effect is Shiitake dermatitis.[23] Such allergic reactions are individual reactions and cannot be generalized. In such cases, the application has to be stopped immediately. The role of possible mutagenic compounds like agaritine in these reactions remains unclear.[24]

The highest risk in the medicinal use of medicinal mushrooms is probably the waiving of other well-established treatments. Especially in tumor therapy, mushrooms have to be used as an additional (adjuvant) therapy, which could complete with but not substitute for conventional therapies like surgery, chemotherapy, or radiation.

IV. MARKET ACCESS FOT HERBAL MEDICINAL PRODUCTS IN THE EUROPEAN UNION

According to the verification of the above-described criteria and/or to traditional use, market access can fall into one of the following two categories:

A. Well-Established Use

Well-established use is the highest category. It can be demonstrated on the following ways: (1) safety and efficacy data from the company's own development ("stand alone") or a combination of studies and bibliographic data ("mixed application"), and/or (2) scientific literature establishing that the active substances of the medicinal products have been in well-established medicinal use within the European Union for at least 10 years, with recognized efficacy and an acceptable level of safety (bibliographic authorization).

B. Traditional Use

A product is accepted on the basis of sufficient safety data and plausible efficacy resulting from long-term use and experience, i.e., at least 30 years in total and 15 years in Europe (EU guideline 2004/24/EC, 2005). Products are registered and do not need authorization. Special pharmacological trials are not necessary. The declaration must be, "traditionally used for . . . ." The requirements for quality are the same as for well-established products.[1,6]

V. OTHER USES OF MEDICINAL MUSHROOMS IN PHARMACY

Mushrooms are also a source of pure bioactive compounds. Whereas microscopic fungi species of genera *Penicillium* or *Aspergillus* are well-known producers of antibiotics, statins, immunosuppressives, etc., some compounds from mushrooms or their derivatives have also been licensed as drugs. Two examples are the partial synthetic antibiotic retapamulin from *Clitopilus passeckerianus* Pilát and the immunosuppressive compound fingolimod, a chemical modification of myriocin from the ascomycete *Isaria sinclairii* (Berk.) Lloyd. *I. sinclairii* is closely related to the well-known medicinal ascomycete *Cordyceps* and was known previously as *Cordyceps sinclairii* Berk. Fingolimod is the first drug for the oral treatment of multiple sclerosis and has a very interesting mode of action.

Some compounds from poisonous mushrooms, e.g., phallotoxins and amanitins from *Amanita phalloides* (Fr.:Fr.)Link. are important experimental tools in research and diagnostics. Phallotoxin can be used for the detection of the contractile protein actin in cells; α-amanitin can be used for investigation of cellular processes that are connected with RNA polymerase II.

Last but not least, some mushrooms, e.g., *Amanita phalloides, A. muscaria* (L.:Fr.) Pers. or *Russula emetica* (Schaeff.:Fr.) Pers., are used in the form of homeopathic preparations. Corresponding monographs can be found in the HAB. Homoeopathic preparations only need to be registered, provided that no indication claims are made for them and that adequate quality is demonstrated. If indication claims are to be made, the homoeopathic medicinal product has to be authorized at the BfArM.[2]

VI. CONCLUSIONS AND OUTLOOK

Undoubtedly, mushrooms have great potential for pharmaceutical application, and many scientific contributions and case studies in traditional medicine and/or nutritional studies emphasize this potential. To explore this potential, not only in the form of dietary supplements but also in the form of regular drugs, continuous and defined production processes, standardized quality and methods for its control, preclinical and clinical trials according to the regulations, and legal authorization are necessary.[13] These processes require time, money, and close interdisciplinary collaboration. Large companies will invest in this great effort and expenditure only when the developments are protected by patents. New ideas are necessary to allow patent protection or to make the development profitable without patent protection. These ideas could include special formulations, new fields of application and indications, new chemical entities, or new production technologies. In our investigations, we have shown that treatment of mushroom cultures with atmospheric pressure and low-temperature physical plasma (ionized gases that contain different concentrations of low-molecular reactive and UV-emitting atoms or ions) influences the spectrum and content of bioactive substances of cultivated mycelia (manuscript in preparation). In addition, mushrooms from other geographic regions, "forgotten" mushrooms, or mushrooms not previously investigated may also represent promising potentials for drug development.[25,26]

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