

Biological Activities by Chemistry Substances Isolated from Mycorrhizal Fungi of *Pinus* Sp.

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ABSTRACT

The genus *Pinus* is represented for woody plants which has big economic importance. There are reports that some mycorrhizal fungi associated with this plant produce chemistry compounds with biological activities. The aim of this study was to evaluate the association between genus of fungi found in *Pinus*, biological activities detected and substances isolated. In this way, it was realized a systematic review of mycorrhizal fungi found in *Pinus*. The articles were selected in the databases Lilacs, Scielo, Pubmed, Scopus, Science direct and Web of Science, using the program Endnote® for the data storage. As keywords we used the terms: mycorrhizal fungi and biological activity and *Pinus*, mycorrhizal fungi and chemical components and *Pinus*. It was found 2322 articles, these, 1478 had the abstracts evaluated, and, 13 were selected to be read in full, leaving 7 for analysis of the data. In the articles evaluated were found 23 genres of mycorrhizal fungi present in *Pinus* species. The activity most reported was the antioxidant activity and the tocopherol was the isolated substance with more prevalence. This review can contribute to research in the humanities, agricultural and veterinary due to the scarcity of research in this theme.

INTRODUCTION

The genus *Pinus* comprises woody plants that belong to Pinaceae family. Usually arborescent, it is part of the Kingdom Plantae, it has a high variety of species (more than 100) being a potential species to be searched ^[1]. The taxonomic classification of the genus family the genus *Pinus* was described by Schultz ^[2] according to **Table 1**.

About 105 species of the genus *Pinus* were identified. It can be found from the region of the poles to the tropics, in the European, Asian and the Americas continents, but in South America its occurrence is not natural. This genus is resistant to drought, supporting great variation of temperature (- 65°C until 50°C) ^[3].

The *Pinus* plantations proliferating rapidly in open environments, of high insolation, adapting to low fertility soils ^[4]. The crops possibility the supplies wood industries, pulp and MDF. This practice becomes increasingly important for the maintenance of natural forests ^[4].

Table 1. Scale taxonomic of *Pinus* sp. Authorship: SCHULTZ (1968).

Kingdom	Plantae
Division	Embryophytae siphonogamae (Spermatophytae) Subdivision
Sub Division	Gymnospermae
Class	Coniferopsida
Order	Coniferae
Family	Pinaceae
Subfamily	Pinoideae
Genus	<i>Pinus</i>
Specie	<i>Pinus</i> sp.

The first report of fungi symbiosis with roots of *Pinus* species occurred in 1964, it was observed the o growth of the fungi *Thelephora terrestris* in the same environments *Pinus elliottii* var. *elliottii* crops [6].

The first description in the literature about the association between fungi and roots of trees was made by the botanical Albert Bernard Frank in 1885, and it was called mycorrhizal (term of Greek origin, “myco”: fungi “rrhizal”: roots). To Frank this association would be sourced from the union between the mycelium of fungi and the roots of trees, with physiological dependence between them, the mycorrhizal fungi would be responsible for absorbing nutrients, minerals and water of the soil, and transferring them to the host, without causing injures to the root, and in return receives simple sugars necessities for its metabolism. The formation of the mycorrhizae allows plants to capture nutrients from a more extensive soil region that it could get without them. This phenomenon is view in the most part of the vascular plants [7,8].

There are several cases reports and the attempt to introduce species of the genus *Pinus* in the most diverse environments, which has not occurred satisfactorily. The seedlings did not grow enough, did not capture any nutrients of the soil, and even it was observed plants' death, due to lack of the association with mycorrhizal fungi. The mycorrhizal increases the roots absorption surface, enables the selective uptake of soil substances, protects the roots against the pathogenic attacks through different mechanisms such as the formation of a physical barrier around the roots, production of substances with antibiotic action, production of inhibitory compounds [6].

It is observed that substances from fungi are generally simple molecules and of low complexity, but may present biological activities with pharmacological or toxic action. As activities of pharmacological interest can be cited antioxidants, antitumor, antimicrobial among others [8,9].

The aim of this study is investigate a possible correlation with *Pinus*, mycorrhizal fungi, biological activities and chemical substances isolated. In this way, it was realized a systematic review.

MATERIAL AND METHODS

A research was made during July 2015. During the survey, it was conducted in the databases that it was not stipulated publication dates for the studies, which were found from 1922 to 2015 articles. It was used the keywords: mycorrhizal fungi and biological activity and *Pinus*; mycorrhizal fungi and chemical components and *Pinus*. The databases consulted were: Lilacs, Scielo, Pubmed, Scopus, Science direct and Web of Science. To the storage of the selected articles was used the program Endnote®.

As inclusion criteria it was considered articles containing species of fungi from *Pinus* and articles that showed biological activity and/or isolated substance of these fungi.

As exclusion criteria were books, systematic reviews, articles that the fungi species were obtained commercially, articles about primary metabolites, fungi species isolated directly from the soil and articles about isolation and biological activity evaluation of substances that were not from the fungi.

These exclusion criteria were used because the main objective of the article was to identify substances produced by mycorrhizal fungi isolated directly from *Pinus*. Duplicated articles were excluded by the Endnote® Program and manually.

The titles e abstracts were evaluated and read by two reviewers, in case of not concordance a third reviewer was consulted road. The selected articles which were analyzed in fuel were treated in the same way.

RESULTS AND DISCUSSION

The search in the databases revealed 2322 articles, all of them in English. From this due to duplication with previously located studies 616, trials were digitally excluded by the Endnote® program and 228 manually, leaving 1478 articles to abstract examination. After that, 13 articles were selected to be read in full. From these, 6 articles were excluded because presented at least one of the criteria exclusion, leaving 7 articles which data were analyzed and included in this review (**Figure 1**).

The articles included in the study were evaluated in accordance with the next aspects: specie studied, association, the country where the study is from, it was made extraction method, technique of isolation and/or identification, biological activity, isolated

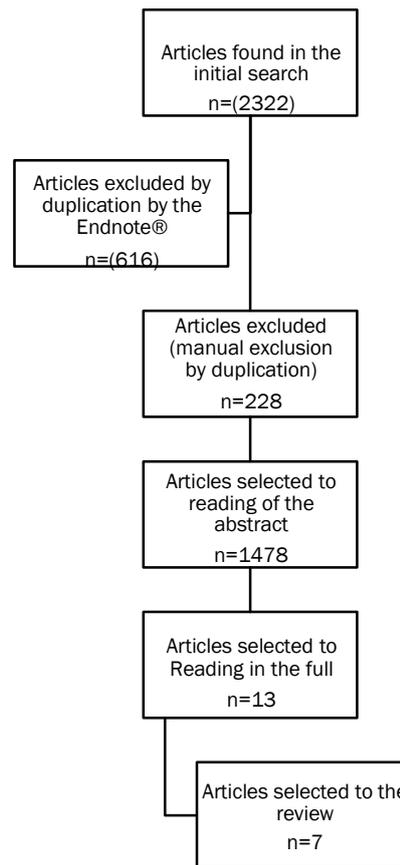


Figure 1. Flowchart demonstrating database searches and selection of included studies.

chemistry compounds (name, molecular formula), and the results of the research are shown in **Supplementary Table 2**.

In the selected articles were described 23 genus with 29 species described and 4 did not mention mycorrhizal fungi, in association with *Pinus sylvestris* in the most part of the study (6 articles). Among this genus, the genus *Suillus* showed the highest number of species: *Suillus collinitus*, *Suillus mediterraneensis*, *Suillus variegatus*, *Suillus bovinus*, *Suillus luteus*.

It is known about 50 species in the genus *Suillus*. This mycorrhizal fungus is found in boreal regions, temperate and Mediterranean ^[10]. To be able to form ectomycorrhizas fungi is important in reforestation ecosystems, in addition to its use as Environmental quality indicator ^[11]. it is given the variability of the genre species and their geographical distribution, it can be said that there is a dearth of research involving this fungi.

The studies were realized in 5 different countries, 2 of them in Germany, 2 in Portugal, 1 in Finland, 1 in Poland and 1 in Sweden.

The extraction technique most used was the maceration using methanol or ethyl acetate as solvent. Among the methods of isolation and identification prevails the use of the HPLC in 5 studies, and in 3 of them was used the CG- MS after.

From the 7 studies evaluated, only 3 tested the biological activity of the fungi, in 2 of them was tested the antioxidant activity and in 1 the antifungal activity.

Among the isolated substances (16 different substances), the most prevalent was the tocopherol (22 fungi), indole 3-acetic acid (6 fungi), chitin (5 fungi) and ergosterol (5 fungi).

It gives the name to four tocopherol isomers belonging to the family of vitamin E they are alpha, beta, gama and delta tocopherol. The human body does not possess the ability to synthesis tocopherols, which are found primarily in vegetable oils do not processed. The alpha tocopherol is considered the most active among the four isomers, but all of them have the primary duty to antioxidant action. Studies indicate the tocopherol efficacy in some kinds of neoplasia, benefits in blood flow, prevention of coronary heart disease, increased HDL, improves resistance to infection, improved functioning of the nervous system, anticoagulant properties reducing the risk of arterial thrombosis and ischemic disease atherosclerosis, among others ^[12].

The 3-indole acetic acid (IAA) is an auxin, which can be found in plants and microorganisms at low concentrations, responsible for regulating growth and development of plants ^[13].

Chitin is a long chain linear polysaccharide found in the cell wall of fungi and in the exoskeleton of arthropods, which can

be found in the forms alpha, beta and gama chitin. Partial deacetylation of chitin leads to chitosan obtained which has several biological activities such as hemostatic, analgetic, anti-inflammatory, antimicrobial, antioxidant, anti-cholesterolemic, anti-tumor and immunostimulatory [13,14].

The ergosterol is the type of sterol present in higher amounts in mycelial membranes of most fungi, found in free and esterified forms [15]. The ergosterol is known as vitamin D2 it has an important action on the calcium fixation in bones, with other activities such as antioxidant, antitumor, antimicrobiana, reducing inflammatory and cardiovascular diseases [16].

The isolated substances can be classified according with its chemistry structure in: terpene (7), steroids (5), alkaloids (2), polysaccharides (1) and polyketide (1). The chemistry structure of these substances can be observed in the **Figure 2**.

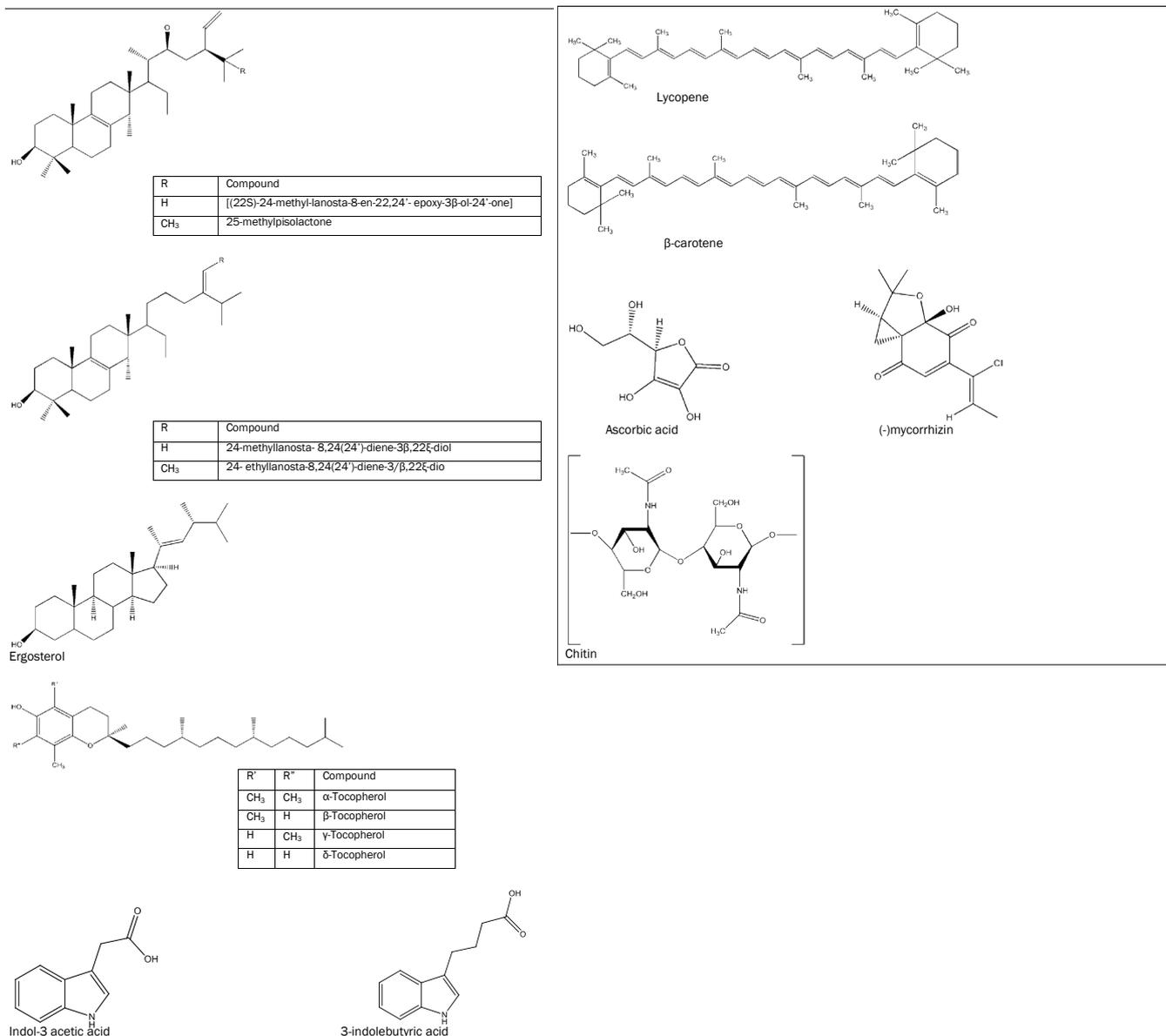


Figure 2. Isolated chemistry structures in the evaluated articles

Terpenes are hydrocarbons found in abundance in plants, fungi and animals. They have antitumor activities, antioxidant, antimicrobial, antifungal, antiviral, antiparasitic and anti-hypoglycemic [17,18].

Modified sterols and triterpenes, coming from secondary metabolism microorganisms, plants and animals [18,19]. The sterols are required for the development of fungi; it has application in the control of fungal diseases in humans and animals, as well as their use in the development of pesticides [20,21].

It is defined as alkaloids and nitrogenous organic bases mainly present in plants, and microorganisms and animals [18]. There are cyclic compounds of natural origin and containing one nitrogen atom in its structure [22]. The alkaloids always have biological and toxic activity when administered to animals [23].

The fungi are able to produce polysaccharides which are natural polymers which consist in one or more types of monosaccharides. They can be applied in the fields of food, pharmaceutical, medical and cosmetic. Introducing a wide range

of biological activities such as antioxidant, antiviral, antitumor and anticoagulant, and the present in some mushrooms act as stimulators of the immune system ^[15].

Due to variety of compounds found in fungi with biological activities, its applicability in industry and as the studies are scarce, it has been keen to increase the field of research in this area.

Polyketides are produced from the condensation of the derivative of thioesterified malonate derivative and an acyl thioester. They may be described as a largest class of structurally diverse products having various biological and pharmacological activities such as anti-cholesterol, antiparasitic, antibacterial, antifungal, and immune suppressant ^[24].

CONCLUSION

From the articles reviewed, the method of extraction most used was the maceration with organic solvent, being that between the methods of identification prevalence the use of the HPLC. The substance most present in the fungi was the terpenes, however for the most part of these compounds was not made biological tests. Only 3 articles showed results of biological activity, being the most described the antioxidant activity. It was observed based on the results found that there is no direct correlation between a genus of fungus, their isolated compounds and biological activity found.

The study made demonstrated that there is few researches about the biological activities and/or isolated substances in mycorrhizal fungi of *Pinus*, demonstrated the necessity of new researches in this area. This review therefore contributes for future studies inside this thematic.

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