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Correlation between Plant Secondary Metabolites and Their Antifungal Mechanisms–A Review

Sara Holm Freiesleben and Anna K Jäger*

Department of Drug Design and Pharmacology, Faculty of Health and Medicnal Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark

Abstract

The search for new antifungal drugs often involves secondary metabolites from plants because of their pharmacological activity against foreign pathogens. Among the modern drugs in use today about 40% are of natural origin. To distinguish the secondary metabolites they can be divided into groups based on their structure or biosynthetic origin.

When searching for new antifungal agents it is crucial to search for a mechanism of action for which unwanted side effects can be avoided. This can be done if the mechanism of action only involves fungal cells and not mammalian cells. For that reason it is interesting to investigate a relation between the biosynthetic origin of the antifungal compound and it's mode of action against fungi. This correlation could limit the search to those natural compounds, which only interferes with the target of interest.

This review is based on a comprehensive literature search for existing knowledge about antifungal mechanisms of different secondary metabolites from plants. The secondary metabolites have been grouped into three major groups according to their biosynthetic origin, and into subgroups according to their structure.

There seems to be a correlation between the biosynthetic group of terpenes and their antifungal mechanisms of action, all of them exhibiting their antifungal action through cell membrane disruption, although some of the terpenes also seemed to work through mitochondrial dysfunction. A clear correlation has not been demonstrated between the two other biosynthetic groups of secondary metabolites; the phenolic compounds and the nitrogen containing compounds. Despite this there are correlations between some of the subgroups and their antifungal mechanism of actions.

Keywords: Antifungal mechanisms; Mode of action; Plants; Secondary metabolites; Biosynthetic origin.

Introduction

In the search for new antifungal drugs many plants have been tested for their antifungal activity and mode of action. Many of the human fungal pathogens are developing resistance to already existing antifungal drugs, such as fluconazole and amphotericin B [1]. Therefore there is an urgent need for new antifungal agents [1]. A place to search for this could be in the plant kingdom, because higher plants develop secondary metabolites, which are pharmacologically active [2]. The secondary metabolites in plants are often a part of their own protective mechanism against phytopathogens [3]. The development of secondary metabolites can either be a part of the plant's normal program of growth (*preformed antifungal compounds*), or it can happen in response to pathogenic attack (*induced antifungal compounds or phytoalexins*) [3].

The secondary metabolites in plants can be divided into different categories according to their biosynthetic principles [2,4]. A simple classification includes 3 main groups [4]:

1. Terpenes such as mono-, di-, tri-, sesqui- and tetraterpenes, saponins, steroids, cardiac glycosides and sterols
2. Phenolics such as phenolic acids, coumarins, lignans, stilbenes, flavonoids, tannins and lignins
3. Nitrogen containing compounds such as alkaloids and glucosinolates

These groups contain compounds with similar biosynthetic properties, and the compounds within the groups do also have some similarities in their structures.

The purpose for this review is to investigate any type of correlation between the biosynthetic origin of the antifungal compound and it's mode of action against fungi. This correlation could limit the search to only those natural compounds, which interferes with the target of interest.

Biochemical Basis of Antifungal Mechanisms of Action

In the following section the composition and the functions of fungal cells will be described, in order to understand the pharmacology of antifungal agents. Furthermore some antifungal mechanisms of action will be described.

Composition of the fungal cell

Fungal cells are eukaryotic and have a lot of similarities with mammalian cells, including DNA within the cell nucleus, mitochondria, endoplasmic reticulum and the Golgi apparatus. A point where mammalian and fungal cells differ is in the cell membrane. Both cells contain sterols in the cell membrane, but the content of sterols differs [5,6].

***Corresponding author:** Anna K Jäger, Department of Drug Design and Pharmacology, Faculty of Health and Medicinal Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark, Tel: +45 35336339; Fax: +45 3533641; E-mail: anna.jager@sund.ku.dk.

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Mammalian cells contain mainly cholesterol, while the fungal cells contain mainly ergosterol [5,6]. This difference in the sterol content has been a major drug target of interest in the search for antifungal agents [5,6]. The fungal cell wall is composed of different layers, mannoproteins being the external part of the cell wall (Figure 1). The mannoproteins are supported by a matrix of β-glucan consisting of β-(1,3)-glucan and β-(1,6)-glucan and chitin mixed within the β-glucans [6]. The fungal plasma membrane consists of a phospholipid bilayer in which ergosterol is the main content [5,6].

Antifungal mechanisms of action

Antifungal activity can be obtained by destroying the pathogenic fungal cell. From looking at the composition of the fungal cell, at least 6 different antifungal mechanisms can be suggested [5,6].

Inhibition of cell wall formation: The fungal cell wall primarily consists of β-glucans. If the synthesis of these compounds is inhibited, the cell wall integrity will disrupt [5,6].

Cell membrane disruption: The ergosterols are essential for the cell membrane. If these sterols are bound by antifungal drugs, or the synthesis of them are inhibited by ergosterol biosynthesis inhibitors, the cell membrane's integrity will disrupt. Thereby the membrane becomes leaky [5,6].

Dysfunction of the fungal mitochondria: Inhibition of the mitochondrial electron transport will result in reduction in mitochondrial membrane potential. The inhibition can occur via inhibition of the proton pumps in the respiratory chain, leading to reduction in ATP production and subsequent cell death [5-7].

Inhibition of cell division: Inhibition of cell division can happen via inhibition of microtubule polymerization, and thereby inhibiting the formation of the mitotic spindle [5,6].

Inhibition of RNA/DNA synthesis or protein synthesis: If the antifungal agent enters the cell, for instance via active transport on ATPases, and interferes with the RNA, it can cause faulty RNA synthesis and inhibition of DNA transcription. Inhibition of protein synthesis is also a known antifungal target [6].

Inhibition of efflux pumps: Efflux pumps are present in all living cells and their function is to transport toxic substances out of the cell [8]. This transport often includes transport of accumulated drug out of the fungal cell. Overexpression of efflux pumps can lead to drug-resistance. By inhibiting the efflux pumps it is believed that drug resistance can be reduced [8].

An important objective when targeting fungi is to ensure that the mammalian cells are not affected by the antifungal drug, thereby causing side effects. If the antifungal drug is not specific for fungal cells, the drug will inhibit or destroy the mammalian cells as well as the fungal cells. As mentioned above fungal cells mainly contain ergosterols, while mammalian cells mainly contain cholesterol. So by inhibiting the synthesis of ergosterol or binding of ergosterol to the antifungal drug, mammalian cells may not be affected to the same extent because they have cholesterol instead of ergosterol [9].

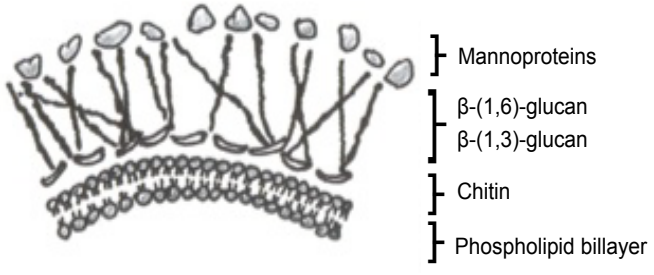

Figure 1: Composition of the fungal cell membrane and cell wall.

Materials and Methods

In this literature review, the search for previous studies about secondary metabolites in plants, and their antifungal mechanisms of action has been conducted with library databases such as EMBASE and PubMed. The search for literature was performed from September to December 2013.

"*Antifungal, mechanism of action, mode of action, plants, and secondary metabolites*" were used as keywords in the literature search. Furthermore, original literature cited in different reviews and studies concerning antifungal activity in plants were used.

Results

In the following, the results obtained in this review about the antifungal mechanisms of action of plant material from previous studies, will be presented.

Plants have been tested for their antifungal activity and mode of action in several studies. This review tries to give an overview of some secondary metabolites and their mode of antifungal action. The secondary metabolites are examined for the antifungal mechanisms of action mentioned above. The results of this can be seen in Table 1.

The secondary metabolites covered in this review have been tested on different fungal strains. Some of them are human pathogens and some of them are plant pathogens, or both. The human pathogenic fungal strains include: *Aspergillus flavus, Candida strains, dermatophyte strains* and *S. aureus*, *P. aeruginosa*. The non-human pathogenic fungal strains include: *Neurospora crassa, Phytophthora megasperma, botrytis cinerea, Fusarium lateritium, Pichia membranifaciens.* One microorganism, *S. cerevisiae*, is not a human pathogen in healthy individuals but is increasingly being isolated from immunocompromised patients [10], though it is assumed to be an orportunistic pathogen, albeit of relatively low virulence [11]. In some of the studies mentioned in this review the pathogenicity of the test organisms were not described [12-15]. The terpenes mentioned in this review were tested on mainly human pathogens. The phenolic compounds have all been tested on human pathogenic fungi, whereas the nitrogen containing compounds have mainly been tested on plant pathogenic fungi, such as *Phytophthora megasperma* [15,16] and *Botrytis cinerea* [15].

Discussion

In this section the results seen in Table 1 will be further described and discussed. Active compounds are seen in Figure 2.

Terpenes

In the group of terpenes all the tested compounds disrupt the cell membrane and some of them are also destroying the fungal mitochondria. The monoterpenes and the sesquiterpenes are shown to act through both mechanisms of action [17-20], while the saponins have a clear tendency to act as detergents through cell membrane disruption [3,12, 21-24].

The monoterpenes mainly affect the quantity of ergosterol in the

			Antifungal mechanisms of action						
Biosynthetic groups	Type of secondary metabolite (subgroup)	Reference	**I.** inhibition of cell wall formation	**II.** Cell membrane disruption	**III.** mitochondria dysfunction	**IV.** inhibition of cell division	**V.** inhibition of RNA-, DNA- or protein synthesis	**VI.** Inhibition of efflux pumps	**VII.** Other mechanisms
Terpenes	Monoterpenes	[18]		X	X				
		[17,43,44]		X					
	Sesquiterpenes	[19,20]		X	X				
	Terpenoid alchohols	[17,26]		X					
		[26,27]							X*
	Steryl glycosides	[12]		X					
	Polyacetylenes	[12]		X					
	Saponins	[3,4,21-24]		X					
Phenolics	Flavonoids	[34]		X					
		[1]			X				
	Phenylpropanoids	[18]		X	X				
		[28]		X					
	Polyphenols	[35]				X			
		[36,38]	X		X				
Nitrogen containing compounds	Alkaloids	[13,39]		X					
	Lectins	[15]	X						
Other compounds	Quinones	[8,40]	X		X		X	X	
	Sulfur compounds	[41]		X	X		X		
	Defensins	[14]		X	X				
		[42]		X					

*Activation of specific signaling pathways. Calcium stress. Inhibition of the TOR pathway.

Table 1: Overview of secondary metabolites and their antifungal mechanisms of action.

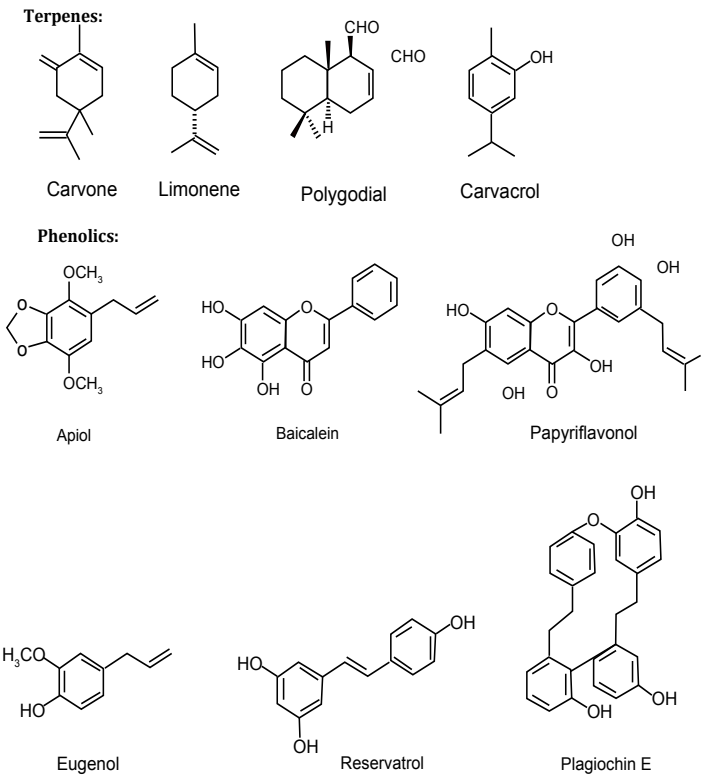

Figure 2: Structures of active compounds.

fungi [17,18]. Though in a study of essential oil from dill, containing mainly carvone, limonene (both monoterpenes) and apiol (a phenylpropanoid), despite plasma membrane disruption, another mechanism of antifungal action was also reported [18]; mitochondrial dysfunction seen as ROS accumulation, inhibition of electron transport (inhibition of proton pump) and inhibition of ATPase in the mitochondria [18]. These mechanisms of action of the essential oil from dill were suggested to be attributed to its major components, but it is not possible to conclude if the mechanisms are attributed to the monoterpenes or to the phenylpropanoid.

In two studies of the sesquiterpene polygodial [19,20] the main target for this compound was shown to be the mitochondrial ATP synthase. The studies also report that cell membrane disruption is a mechanism of antifungal action for polygodial, but they do not discuss, how the membrane is disrupted. Another study of polygodial [25] assumes that the main mechanism of polygodial is the cell membrane disruption. Nor does this study discuss how the membrane disruption occurs [25]. Since there is a disagreement about the main mechanism of action in the three studies it can only be concluded that the sesquiterpene, polygodial, has two antifungal mechanisms of action, cell membrane disruption and mitochondrial dysfunction [19,20,25].

The terpenoid alcohols have been shown to exert their antifungal activity by mainly cell membrane disruption [17,26]. One study though claims that the terpenoid alcohol carvacrol exerts its mechanism of action through activation of specific signaling pathways, resulting in calcium stress and possibly inhibition of the TOR (target of rapamycin) pathway, rather than a nonspecific lesion of membranes [27].

Both the steryl glycosides, polyacetylenes and the saponins have been tested in a study of the genus *Bupleurum* [12]. The study reports that the mechanism of antifungal action for the steryl glycosides, polyacetylenes and the saponins happens through a direct action on the biomembrane. Both saponins (saikosaponins) and steryl glycosides act as detergents. They are anchored with their lipophilic moiety in the lipophilic membrane bilayer after complexing with cholesterol, and the hydrophilic moiety outside the cell [12]. Because they are complexing with cholesterol, steryl glycosides and saponins do not seem to be specific for fungi, but could also affect mammalian cells.

In a study of clove essential oil [28], a correlation between essential oils in general, and their antifungal mechanism of action is suggested [28]. The study refers to other studies [29-31], which have tested essential oils for their antifungal mechanism of action, and it is suggested that essential oils exert their antifungal activity through membrane damage. The studies do not attribute the activity of essential oils to their great number of constituents, but rather on the fact that essential oils are typical lipophilic [29]. Therefore the essential oils pass through the cell wall and cytoplasmic membrane of the fungus, and disrupt the structure of the membrane composition. Thereby the essential oils are making the membrane of the fungus more permeable [29].

In two studies of tea tree essential oil, the oil's direct action of membrane damage is considered to be due to lipophilic terpenoid components [31,32], as was the case with the essential oils reviewed [33].

Phenolic compounds

For the group of phenolic compounds the antifungal action happens through cell membrane disruption [17,18,26,28, 34], inhibition of cell wall formation [35] and inhibition of the mitochondria [1,18,36]. It has been concluded by some researchers that phenolic compounds involve many mechanisms of action, and that there may be several targets, which lead to inhibition of microorganisms and fungi [37].

The antifungal activity of the flavonoid baicalein has been tested in a study, where it seemed to happen through perturbation in mitochondrial homeostasis, without causing elevation of the intracellular ROS level, and it did not involve apoptosis [1]. The flavonoid papyriflavonol A has also been tested for its antifungal mechanism of action and shown to disrupt the cell membrane integrity of fungal cells but the mode of action is not yet clear [34].

Phenylpropanoids seem to exceed their mechanism of antifungal action through both cell membrane disruption and mitochondria dysfunction [18,28]. A study of dill essential oil [18] has been discussed in section 5.1. It is not possible to conclude if the mechanisms of action are attributed to the monoterpenes or to the phenylpropanoid.

In the before mentioned study of clove essential oil [28] a high content of the phenylpropanoid eugenol was reported, and the antifungal mechanism of action was specified to be due to the high content of this compound [28]. The fungicidal effect resulted from direct damage to the cell membrane, and was due to a lesion of the cell membrane and a reduction in the quantity of ergosterol. Eugenol was shown to impair the biosynthesis of ergosterol [28].

In a study of the polyphenol reservatrol it was shown that its antifungal mechanism of action happened through an arrest of cell-cycle processes at the S-phase in the tested fungus [35]. This means that this polyphenol exerts its mechanism of action through inhibition of cell division, and thereby affects fungal cell growth. Another polyphenol, plagiochin E, has been shown to exert its antifungal activity through mitochondrial dysfunction-induced ROS accumulation in one study [36], and through inhibition of cell wall chitin synthesis in another study [38].

Nitrogen containing compounds

The antifungal mechanisms of action for the nitrogen containing compounds are mainly a result of cell membrane disruption via inhibition of ergosterol biosynthesis, or complexing with ergosterols [13,39]. Though, lectins appear to exert their mechanism of action through interference with the biosynthesis of chitin [15]. Chitin is a part of the fungal cell wall [9] and it is therefore reasonable to conclude that these compounds exert their antifungal mechanisms of action through some interference with the cell membrane and/or cell wall.

Other compounds

Some plant compounds, which have been tested for their antifungal mechanism of action, did not fit into the three main groups of the simple classification of secondary metabolites. These compounds were quinones, sulfur compounds and defensins. [8,14,40-42].

The quinones discussed in this review are a naphtoquinone (chimaphilin) and an anthraquinone (purpurin). The naphtoquinone acted through two, possibly three, different mechanisms; interference with fungal cell wall, interference with mRNA transcription and possibly interference with protein synthesis [40]. The anthraquinone acted through two different mechanisms; depolarization of mitochondrial membrane potential and inhibition of energy-dependent efflux pumps [8]. Compounds that inhibit efflux pumps can possibly be used in combination with other antifungal drugs to prevent drug resistance.

The sulfur compounds discussed in this review include compounds

of leaves of ramson (*Allium ursinum*), natural sulfur compounds with different functional groups and sulfur compounds in general [41]. The sulfur compounds of leaves of ramson extract act as uncouplers. They interfere with proton translocation over the mitochondrial membrane and interrupt ADP phosphorylation [41]. The specific sulfur compounds with the functional groups mentioned above interfere with membrane-integrity or associated enzyme proteins, stopping their production or activity [41]. In general the sulfur compounds inhibit synthesis of DNA, RNA, proteins and poly-saccharides in fungal cells [41].

Conclusion

From the results in this review some trends indicating a correlation between the type of natural compound and its antifungal mechanism of action were found.

As a whole biosynthetic group, the terpenes showed a clear tendency to act through cell membrane disruption. Some of the compounds acted through mitochondrial dysfunction as well. For one of the subgroups, the saponins, there was a clear tendency to act through cell membrane disruption.

The findings on the antifungal mechanisms of action of phenolic compounds indicate that these compounds have different modes of action. There is no clear correlation between this biosynthetic group of compounds and their antifungal mechanism of action.

The antifungal mechanisms of action of nitrogen-containing compounds happened through interference with the cell membrane and/or cell wall. In this review only a few nitrogen-containing compounds were included, mostly alkaloids. The alkaloids showed a degree of similarity in their antifungal mechanism of action by interfering with ergosterol in the cell membrane.

The quinones and sulfur compounds had different sorts of mechanisms of actions that none of the other groups possess; inhibition of RNA/DNA synthesis, inhibition of protein synthesis and inhibition of efflux pumps. The inhibition of RNA/DNA synthesis or protein synthesis is a unique mechanism of action and could be used as new drug target for antifungal compounds, provided that there is no interaction with mammal systems. The inhibition against efflux pumps is important in the research of antifungal resistant strains.

To summarize, an overall clear correlation between terpenes and their antifungal mechanism of action has been demonstrated. They seem to act through cell membrane disruption.

Regarding the two other groups; the nitrogen containing compounds and the phenolic compounds, a clear correlation has not been proved. However, within the biosynthetic groups, some subgroups seem to have a consistent antifungal mechanism of action.

The findings in this review show that it is difficult to simplify the mechanism of antifungal action in plant secondary metabolites. There are some correlations between the kind of secondary metabolite and its antifungal mechanism of action, but many of the compounds act through more than one mechanism of action. To be able to see more clearly correlations between types of natural compounds and their antifungal mechanisms of action, it could be necessary to look deeper into subgroups of compounds, instead of grouping the metabolites into biosynthetic groups.

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