Natural Phenolic Compounds: A Potential Antifungal Agent

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Fungi are one of the most common microbes living around us in the environment which could be pathogenic and sometimes life threatening. Lots of antifungal agents have been discovered so far and many are still under the procedure to be defined as an effective fungicide. But ever increasing phenomenon of multi drug resistance is a major obstacle associated with the treatment strategies thereby limiting the number of effective therapeutic drugs and prompting us to identify novel antifungal agents. Since, compounds based on combinatorial chemistry and synthetic procedures could be much expensive and have toxicity effects, natural compounds that can be used as antifungal agents have become a renewed source of interest. The following chapter will examine the antifungal potential of natural phenolic compounds, a class of natural products, possessing a diverse range of pharmacological properties and how it holds a good promise against human fungal pathogens thereby considerably improving our therapeutic strategies.

1. Introduction

Fungal infections are one of the major problems associated with our daily life. But unfortunately despite having knowledge that it could be life threatening they are still one of the most neglected pathogens. It can be seen by the fact that amphotericin B despite being discovered as long as in 1956 is still in use as “Gold Standard” for antifungal therapy [1]. During the last few years the visual increment in the life threatening fungal infections have came into notice. As it is a well known fact that there are many opportunistic fungal infections that could affect the immunodeficient people (eg. AIDS, Cancer, transplant patients etc.), they are becoming resistant by the use of the surplus amount of antibiotics by the phenomenon known as Multidrug resistance (MDR). Antimicrobial resistance is associated with high mortality rates, high medical costs and has a significant impact on the effectiveness of antimicrobial agents due to which there comes a challenge in front of scientific community to diminish the pathogenic effect of the fungus (eg. Candida albicans, Cryptococcus neoformans, Rhizopus oryzae and Aspergillus spp. etc.). Identification of natural compounds capable of circumventing MDR with minimal adverse side effects is an attractive goal. Hence there is a great need to find the novel drug from the natural source and its mode of action which could selectively act on the fresh target with lesser side effects. The uses of natural products such as phenols as an antifungal drug are of great importance both economically and for human health as well. A lot of natural compound such as bisbibenzyls, a group of phenolic compound exclusively occurred in liverwort [2], Ajugol which is extracted from Spathodea campanulata [3] has been reported to have antifungal activities. Extract of some of these plants that are traditionally used (eg. Oleuropein, a phenolic compound extracted from unripe olive fruits responsible for the bitterness of olive fruit and is capable to show antifungal activity) can be used efficiently as antifungal compounds. Therefore, exploiting the potential of natural phenolics against the fungal infections should facilitate the development of better antimicrobial strategies which could efficiently control the human infectious diseases.

2. Natural Phenolic Compounds

Natural phenolic compounds are low molecular weight naturally occurring organic compounds which contains one or more phenolic group. They are naturally produced by plants and microorganisms [1]. They are the secondary metabolites synthesized by the plant during there normal development or because of the stressed conditions such as wounding, infection or exposure to UV radiation [4]. Phenolic acids, flavonoids, tannins, stilbenes, curcuminoinds, coumarins, lignans, quinines etc. are some of the phenolic compounds extracted from the medicinal herbs and dietary plants [5]. Natural phenols also play many significant roles in human health as evident from their antifungal, antioxidiant and anti-cancerous activities. For example, four Piper sp. namely Piper crassinervium, Piper aduncum L., Piper hostmannianum and Piper gaudichaudianum shows antifungal activity because of the phenolic compounds present in them viz. crassinervic acid, aduncumene, hostmaniane and gaudichaudanlic acid, respectively [1]. Hydroxytyrosol and its derivatives found in olive oil shows antioxidant activity [6]. Similarly, various compounds showing anticancerous activity such as gingerol, capsaicin, ellagic acid etc. have also been reported [7]. Furthermore, there are many other reports about the beneficial effects of different phenolic compounds (Table 1) and lots of works are still going on to explore their more benefits.
3. Natural Occurrences

In broad senses natural phenol occurs in most of the organisms no matter whether they are microbes, plants or animals but they are abundantly known to be found in plants as a natural source. Plants are much more attractive source of natural phenols because there are enormous numbers of plants which are known to have many natural phenols which can act as fungicides. Nevertheless, there do exists some microbes and animals as well that could be source of important phenolic compounds [8].

In plants, phenolic compounds could be present both in vascular (monocot and dicot) as well as non-vascular plants. In vascular plants they are abundantly found to be present in lycopodiophyta [9], pteridophyta, angiosperm and gymnosperm [10]. Ferns, in particular serves as an important source of some phenolic compounds such as kaempherol which is known to be extracted from Phegopteris connectilis [11] and kaempherol-3-O-rutinoside, extracted from Selliguea feei [12]. Similarly, Salvinia molesta a fresh water fern is source for lots of phenolics such as hypogallic acid, caffeic acid, paoniflorin and pikuroside [13]. Likewise in monocots, alkyl resorcinol are found in cereals and 2,4-Bis(+hydroxybenzyl) phenol is known to be found in orchids like Gastrodia elata and Galeola faberi. In non vascular plants there are various phenolic compounds found like rosmarinic acid found in the hornwort Anthoceros agrestis. Green algae such as Spongiochloris spongiosa is also source of phenolic compounds viz. protocatechuic, p-hydroxybenzoic, 2,3-dihydroxybenzoic, chlorogenic, vanillic, caffèic, p-coumaric, salicylic acid and cinnamic acid and hydroxybenzaldehydes such as p hydroxybenzaldehyde, 3,4-dihydroxybenzaldehyde. Phenolic compounds as mentioned above are not restricted to the plants and they are widely present in animal as well. Guaiacol is a phenolic compound which is present in a gut of desert locust Schistocerca gregaria [14]. Nevertheless, for some of the above mentioned phenolic compounds anti fungal activity are not known, deciphering the antifungal activities of such compounds will remain an open area of research.

4. Antifungal Activity and there Mode of Action

There are many pathogenic microorganisms living around the environment among which fungus is one of the most common pathogen causing various diseases and infection. There are many agents which are known of having antifungal properties. The following sections describe some of the common fungal diseases, phenolic compound used against them and their antifungal mechanism of action.

4.1. Mode of action against Candida albicans

*C. albicans* is a dimorphic pathogen found commonly as a commensal organism inside the human body. But sometimes in the immune deficient cases such as in the case of AIDS, cancer, burn or transplant patients it shows its pathogenic effect. It resides in the various anatomical sites in the body due its dimorphic nature. While being in its non pathogenic phase it remains in circular shape i.e. yeast form but while transforming into a pathogen it converts itself into elongated structures i.e. hyphal form and causes disease which is better adapted to penetrate the epithelium and helps it to evade the immune response [15]. Since the dimorphic nature plays a crucial role for the survival of this pathogen inside the host so it could be the better target to evade the pathogenic effect of this fungus. There are various phenolic compounds which affect this pathogen by affecting its dimorphic transition. For instance three new phenolic compounds isolated from the leaves of *Baseonema acuminatum* P. Choux (Asclepiadaceae) has been known to show antifungal activity against two different strains of *C. albicans* [16]. Similarly, four different phenolic acid derivatives were isolated from ethyl acetate extract of the root bark of *Lycium chinense* Miller [17] which belongs to solanaceae and all of them had the antifungal effect on *C. albicans*. [17]. One of the effective phenolic compounds curcumin (CUR) produced by *Curcuma longa* has been showed to exert toxic affect against *C. albicans* as well as non *albicans* species by increasing reactive oxygen species (ROS) levels and inducing early apoptosis (Fig. 1) that could be reversed by adding antioxidant [18]. CUR is an important spice in Asia and has several important pharmacological properties such as antioxidant, antimutagenic, antitumour activity [19]. CUR also shows other mode of action to activate the apoptotic pathway. It activates the CaMCA1 gene (Metacaspase 1) which is the homologue of mammalian caspasdes and induces apoptosis in *C. albicans* [20]. CUR is also known to act on farnesol, a quorum sensing molecule which play an important role in raising the level of ROS and inhibiting hyphal development by targeting the TUP1 gene which is involved in the hyphal development [21].
Another type of fungicidal phenolic compound is bisbibenzyl which is a new type of antifungal agent that inhibits the growth of *C. albicans* by inhibiting the morphogenetic switch and by inhibiting biofilm formation due to up regulation of DPP3 gene [2]. DPP3 gene is known to perform an important role in the farnesol synthesis as it codes for the an enzyme that converts farnesy1 pyrophosphate to farnesol. As already mentioned farnesol, being a very attractive target to the antifungal agents, one of the targets of bisbibenzyl is also farnesol. Many of the bisbibenzyls acts on the DPP3 and up regulate its activity which apparently increases the farnesol production and inhibits the hyphae formation by enhancing the ROS and subsequently decreasing the biofilm formation [2].

Terpenoid phenols such as carvacrol which is present in oregano and some of the other plants essential oils act as a potent antifungal agents and acts on the wide range of pathogens such as *C. albicans*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* [22]. Major components of oregano extract, which includes the terpenoid phenols carvacrol, thymol, and eugenol, have been shown to posses potent antifungal activities of their own, however, carvacrol showed the strongest antifungal activity against *C. albicans* biofilms. The study demonstrated that carvacrol is responsible for the disruption of both Ca$^{2+}$ and H$^{+}$ homeostasis in yeast and that these disruptions likely lead to loss of cell viability. According to very recent study, gallic acid and 3,3',4'-tri-O-methylellagic acid-4-O-β-D-glucopyranoside (TMEG) was also found to have the antifungal activity which are the main constituents of phenolic compounds found in plants [23].

4.2. Mode of action against *Aspergillus*

Some fungus show less effect directly but can affect by many indirect means. But *Aspergillus* is one such fungus that affects human being by both of them. It not only causes the disease in us but also affects the human diet by poisoning the fruits and vegetables. Some species such as *Aspergillus flavus* are toxigenic and can contaminate food and produce mycotoxins among others [24]. Phenolic compounds are very potent to show the antifungal activity against such pathogens. The structures of the phenolic compounds is such that they can diffuse through the microbial membrane and can penetrate into the cell, where they can interfere in the metabolic pathways by interfering with the synthesis of ergosterol, glucan, chitin, proteins and glucosamine in fungi [25]. *Spirulina (Arthrospira)* a cyanobacterium is widely used as a food among human being and is source of high quality and quantity of phenolic compounds such as caffeic, chlorogenic, salicylic, synaptic and trans-cinnamic acids. There has been evidences suggesting that some of the phenolic extracts from *Spirulina* shows antifungal activity by inhibiting ergosterol which is the component of fungal cell membrane [26], glucosamine, a growth indicator present only in the fungal cells of some genera [27] and some other proteins. Some of them can reverse the effect of MDR when given in the combination with cytotoxic agents. For instance, some of the phenolic compounds like phenolic acids, flavonoids, catechins, chalcones, xanthones, stilbenes, anthocyanins, tannins, anthraquinones, and naphthoquinones being lipophilic in nature are able to to inhibit the activity of ABC transporters [28, 29]. Polyphenols can also bind directly with the proteins hence hindering the tertiary structure of proteins and thus effectively inhibit the function of ABC transporters that makes the fungal pathogens resistant to the drug administered [30-32]. It has also seen that some of the essential oils such as oregano, thyme, rosemary and clove essential oils and some of their main constituents such as eugenol, carvacrol and thymol show efficient antifungal activity against *Aspergillus niger* among which thymol and carvacrol proved to have better anti-*Aspergillus* effect than eugenol [33].

Fig. 1 Shows the action of CUR as antifungal agent against *Candida albicans* by increasing the Reactive Oxygen Species (ROS) and the expression of CaMCA1 gene leading to apoptosis.
4.3. Mode of action against Cryptococcus neoformans

The infection caused by Cryptococcus was very rare until 1880 [34], occurring mostly in the people suffering from immune deficient conditions. But now cryptococcal meningitis has become the second most common opportunistic infection associated with AIDS in sub-Saharan Africa and in South Asia [35]. Cryptococcus neoformans is the only pathogenic species found among all the cryptococcal species [36]. Many antifungal agents have been identified but because of their severe side effects it can be said that safe and effective drug has still not developed because of the similarity between the fungi and the mammalian cells. For instance, Amphotericin B show side effect by causing immediate hypersensitivity reaction, fever, hypotension, nausea and vomiting during administration, hypokalemia, and nephrotoxicity. Similarly, azoles despite of having weak side effects still show nephrotoxicity and hepatotoxicity and cause vomiting and impotence [36]. Four antifungal phenols isolated from Cudrania cochinchinensis; cudraxanthone S and cudraflavanone B, toxyloxanthone C and wighteone shows selective activity against several fungi such as C. neoformans, C. albicans and Aspergillus fumigates [37]. Benzophenones are considered to be biosynthetic precursor of xanthone, benzophenone such as cudraphenone extracted from C. cochinchinensis, is found to have an antifungal activity against C. neoformans [38]. The extracts of C. cochinchinensis do not have any side effects, but is not recommended for prescribing to the pregnant women [39].

Many other studies have demonstrated that the phenolic compounds can also show synergistic effect with present antifungal agents. 2,5 Dihydroxybenzaldehyde has shown synergistic affect with itraconazole, and amphotericin B against C. neoformans. Similarly, thymol also shows synergistic effect with azoles such as fluconazole [40]. Synergistic activity by thymol could be attributed to either disruption of the cell wall/membrane integrity [41] or by creating lesions in the plasma membrane in addition to inhibition of ergosterol biosynthetic pathway by fluconazole [42]. Thus phenolic compound can not only themselves act as antifungal agents but can also enhance the performance of present antifungal agents.

4.4. Mode of action against Rhizopus

The infection caused by the fungi belonging to the class zygomycetes is known as zygomycoses. Under the class zygomycetes there are two orders that are of clinically relevance i.e. Mucorales and Entomophthorales [43], however, majority of human diseases are caused by the order mucorales and hence called as mucormycoses. Mucormycoses stands third as an invasive fungal infection after candidiases and aspergilloses [44, 45]. Some of the common forms of diseases caused by mucormycoses are rhinocerebral, pulmonary, cutaneous, gastrointestinal and disseminated or systemic. The organism commonly found to be the causative agents of zygomycoses are fungi belonging to the genus Rhizopus, Mucor, Rhizomucor and Absidia. But the most common among all is Rhizopus because 70% of its species causes infection by zygomycetes, in which Rhizopus oryzae is the most common etiological species [46, 47]. Most of the R. oryzae strains are resistant to most of the drug such as amphoterin B and azoles such as itraconazole, fluconazole and miconazole, however, thymol, a phenolic extract of an essential oil from Thymus vulgaris, showed an immense activity against fungal growth [48]. Ergosterol not only maintains the fluidity, asymmetry and integrity of the membrane but also involved in the proper functioning of the enzymes bound to the membrane [49]. It has been observed that thymol directly interacts with the ergosterol, the main sterol present in the fungi and is essential for the proper growth and development of it. This interaction of thymol with ergosterol leads to the disruption of the membrane integrity, fluidity and loss of the intracellular content that leads to the mortality of the fungal pathogen [48].

4.5. Mode of action against Fusarium

Fusarium oxysporum is a filamentous ascomycetous fungus having a phylogenetically diverse complex. It is responsible for the localized and dissemination life threatening opportunistic infection in several immune compromised patients [50]. Phenolics also have proved to be an effective antifungal agent even against this fungus. Recently, the effect of various phenols such as salicylic acid, phenol and benzoic acid have been studied against F. oxysporum and found that the phenol and salicylic acid was effective at higher concentration only while benzoic acid was effective at low concentration as well [51].
### Table 1  Various fungal pathogens and natural phenolic compounds used against them.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Fungal Pathogen</th>
<th>Disease</th>
<th>Natural Phenolic compound</th>
<th>Structure of compounds</th>
<th>Mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Candida albicans</em></td>
<td>Candidiasis</td>
<td>Curcumin, Bisbibenzyl, carvacrol and thymol.</td>
<td><img src="image" alt="Curcumin" /></td>
<td>ROS [18], Inhibit Morphogenetic Switch and Biofilm formation [2], Calcium stress [22]</td>
</tr>
<tr>
<td>2.</td>
<td><em>Cryptococcus neoformans</em></td>
<td>Cryptococcosis</td>
<td>Thymol, benzoic acids, and 2,5-dihydroxy benzaldehydes</td>
<td><img src="image" alt="Thymol" /></td>
<td>Synergistic enhancement of fungicide [40, 41, 42]</td>
</tr>
<tr>
<td>3.</td>
<td><em>Rhyzopus sp.</em></td>
<td>Zygomycosis</td>
<td>Thymol</td>
<td><img src="image" alt="Cymene" /></td>
<td>Disrupt fluidity by interacting with ergosterol [49]</td>
</tr>
<tr>
<td>4.</td>
<td><em>Aspergillus sp.</em></td>
<td>Aspergilosis</td>
<td>Eugenol, carvacrol and thymol.</td>
<td><img src="image" alt="Eugenol" /></td>
<td>Disrupt the fluidity of cell membrane and inhibit mycelia growth [33].</td>
</tr>
</tbody>
</table>

**Fig. 2** Different Antifungal phenols and their mode of action in fungi.
5. Conclusion

The fungal infections still remain one of the most common as well as the important problem associated with the day to day life. The efficient, lesser toxic and more effective drug against them is yet to be taken in concern as most of the fungi are developing resistance against the antifungal drugs which are being used extensively and some of them are showing toxic effect on human as well. In such scenario, the natural phenolic compounds have come into noticed as they are proving to be very potent antifungal agents with lesser or no toxic effect. Not only can they themselves be used as antifungal agent but also in synergism with the currently used antifungal agents. They also show their benefits by acting via various modes of actions on different targets in different fungi (Fig. 2). To conclude, it can be said that phenolic compounds are emerging as reasonably effective and efficient antifungal agents that could be used as fungicides.

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