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Phytochemicals with antifungal properties: Cure from nature

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ABSTRACT

Aims: The exploration of natural products with innovative uses is dynamic and expanding rapidly. Medicinal plants have fascinated many researchers that subsequently lead to research publications highlighting plant extracts with wide range of secondary metabolites such as flavonoids, alkaloids, glycosides, quinones, terpenoids, tannins and saponins that exhibit antimicrobial activities and disease control. The concentration of these bioactive compounds in each plant species varies based on the pathosystem and environmental conditions. This study aims to uncover the various types of phytochemicals with antifungal properties.
Methodology and results: Seven categories of plant-based antifungal compounds were reviewed, which are terpenoids, saponins, phenolic compounds, coumarins, alkaloids, essential oils and peptides, with examples and structures of some available compounds. The mechanism of action of each category of phytochemical was discussed. Also, the impact of some compounds was explained and elaborated.
Conclusion, significance and impact of study: It is of a great importance to explore natural plant fighters against fungal infection. Those active plant components do not only have antifungal properties, but they also help in the healing process and some even exhibit anticancer activities. The development and knowledge of antifungal activities from plant extracts have the potential for applications in antifungal therapy. Since the exact description of how antifungal compounds function in the human body is still unclear more studies are required to unveil phytochemicals' properties and to elucidate their effects on living cells.

Keywords: Antifungal, phytochemical, secondary metabolite, natural products, minimum inhibitory concentration (MIC)

INTRODUCTION

A fungus belongs to eukaryotic organisms that embrace both multicellular and unicellular microorganisms such as molds and yeasts, as well as mushrooms. Fungi are classified as a kingdom, separated from other eukaryotic kingdoms of animals and plants. Pathogenic fungi cause diseases in humans and other organisms (Baxi *et al.*, 2016). Fungi are ubiquitous microorganisms that can be found everywhere. When airborne, fungi take the form of spores, mycelia and hyphal fragments. When inhaled, such particles contribute to health effects in human, including several diseases. Common fungi include *Cladosporium*, *Epicoccum* and *Alternaria*, to name a few. Moreover, fungi that are associated with decay or indoor water damage include *Aspergillus*, *Penicillium*, *Chaetomium* and *Stachybotrys* (Baxi *et al*., 2016) and few species that are pathogenic to human. *Candida*, *Aspergillus* and *Cryptococcus* are pathogens that normally caused infection to humans (Karkowska-kuleta *et al*., 2009). However, fungi play an important role in an ecosystem and significantly contribute to biological stability such as in the form of insect symbionts, mycorrhizae and lichens. Several types of fungi break down organic biomaterials such as complex carbohydrates and contaminants such as petroleum, xenobiotics and hydrocarbons. Thus, fungi play a vital part in the carbon cycle (Gulis *et al*., 2009).

There are two general groups of fungal pathogens, which are primary and opportunistic pathogens. Primary pathogens usually develop an environmental reservoir and infect individuals that encounter with a large dosage

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of the fungi from the environment. Opportunistic pathogens provoke disease in hosts when their systemic immunity has been innately dysfunctional, damaged, or impaired. Fungal pathogenesis's mechanisms are not well-recognized as bacterial pathogens. Few fungi are proficient pathogens in contrast to bacteria (Burik and Magee, 2001).

Candida albicans is the foremost fungal pathogen to humans. The infections with *Candida* are severe, particularly for individuals with weak immune defense. *C. albicans* can form highly ordered biofilms, microbial colonies that are enclosed by extracellular matrix and they are attached to a solid surface (Chandra *et al*., 2001). The formation of *C. albicans* biofilms is troublesome to medical practitioners, where the biofilm is normally found on an implanted medical device (Uppuluri and Lopez Ribot, 2017). It may act as a reservoir for the pathogenic cells that are resistant to host immune system and drugs that may lead to invasive systemic infections of tissue, organ and infect almost all inner organs, including lungs, kidney, heart, liver, spleen and brain, causing fungemia and life-threatening septicemia (Karkowska-kuleta *et al*., 2009). Over 50% of the central venous catheters are infected by *C. albicans* biofilm with approximately 100,000 death and excess of $6.5 billion in expenditure annually in the United States (Fox and Nobile, 2013).

The development of *C. albicans* biofilm undergoes four distinct phases. The formation of a biofilm begins with the seeding step, where the cells adhere to a solid surface to form a basal layer of anchoring cells. This phase normally takes approximately 60-90 min. Next, is the early-stage of filamentation, where the cells start to proliferate. Following this is the formation of a complex network that consists of several layers of cells such as hyphal cells, pseudohyphal cells and round cells enclosed in an extracellular matrix. This stage is called a biofilm maturation stage, which typically takes 24 h to form. The last stage is the dispersal stage, where the round cells detach from the biofilm to seed a new site (Douglas, 2003).

At present, the exploration of natural products with innovative uses are dynamic and expanding rapidly. Medicinal plants have fascinated many researchers that subsequently lead to research publications highlighting plant extracts with wide range of secondary metabolites such as flavonoids, alkaloids, glycosides, quinones, terpenoids, terpenoids, tannins and saponins that exhibit antimicrobial activities and disease control. Figure 1 shows the number of publications on antifungal, phytochemicals, essential oils and plant extract in the period 2008- 2019. The concentration of these bioactive compounds in each plant species varies based on the pathosystem and environmental conditions. A medicinal plant is in which one or more of its parts, contains functional phytochemical, which can be isolated and applied either for medicinal treatment or as a drug constituent (Sofowora *et al*., 2013). To date, plant species of *Allium sativum* L. (garlic), *Glycyrrhiza glabra* L. (licorice) and *Aloe vera* (L.) Burm f. (aloe) are known to have bioactive natural products with significant antifungal activities (Sales *et al*., 2016). This review paper aimed to uncover various plants with antifungal properties. It also reveals the mechanism of actions of several groups of antifungal compounds and their effect at the cellular level.

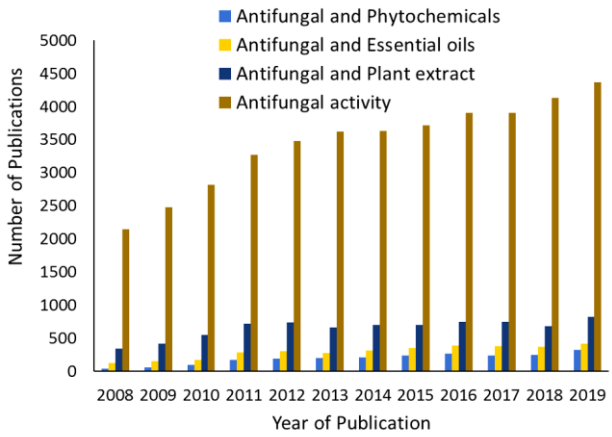

Figure 1: Number of publications in 2008 to 2019 duration through Scopus search, using the keywords: "antifungal + phytochemicals", "antifungal + essential oils", "antifungal + plant extract" and "antifungal activity", as of 21st May 2020.

SECONDARY METABOLITES WITH ANTIFUNGAL ACTIVITY

Terpenoids

What are terpenoids?

Terpenoids are secondary metabolites that can be isolated from natural sources such as plants, microorganisms, animals, insects, marines and endophytes. Terpenoids compose of more than 40,000 variations to date, which are still being explored. Most of the terpenoids variations with biologically active properties are useful as therapeutics and preventive agents for several diseases, including cancer. Terpenoids exhibit antifungal, antimicrobial, antiviral, antiparasitic, antispasmodic, antiallergenic, anti-inflammatory, immunomodulatory and antihyperglycemic properties (Thoppil and Bishayee, 2011). Meanwhile, there are some terpenoids variations that are toxic and have a severe impact on the nervous system and the functional ability of the human body (Mbaveng *et al*., 2014).

Terpenoids are classified based on the number of C_5H_8 isoprene units (2-methylbuta-1,3-diene) and the structural organization of carbons. The single isoprene unit accounts for most classes of terpenoids. Hemiterpenoids contains five carbons and one unit of isoprene. The subsequent classes increase by five carbons at a time and one isoprene unit, starting with monoterpenes. The backbone and structure of terpenoids reported in this review are shown in Figure 2.

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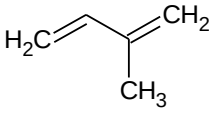
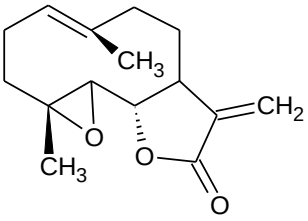
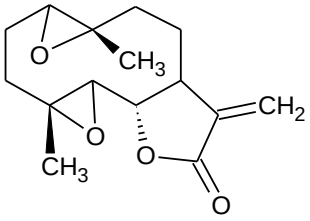


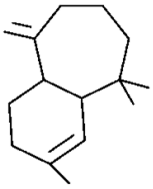
Isoprene (unit)
Building block



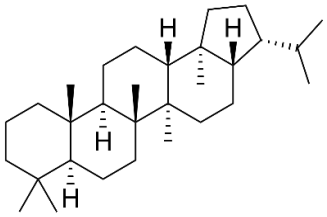
Parthenolide



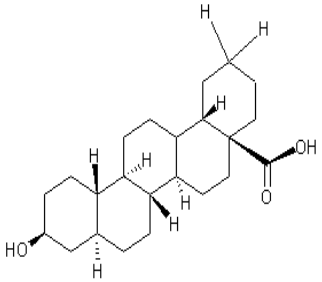
1,10-Epoxyparthenolide



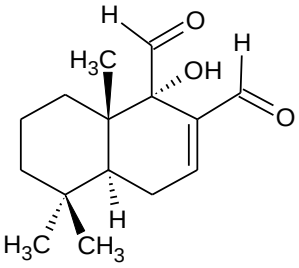
Sesquiterpenes



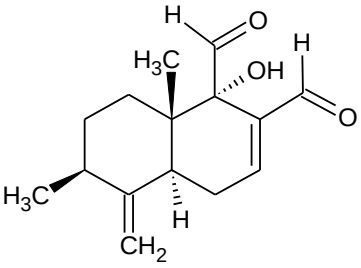
Triterpinoid



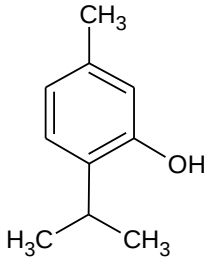
Oleanolic acid



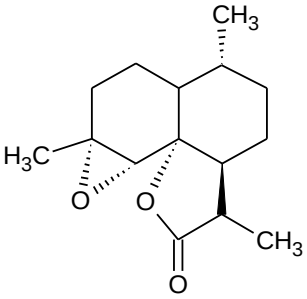
Warburganal



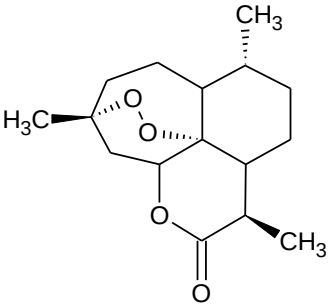
Muzigadial



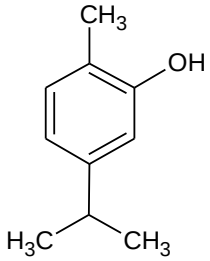
Thymol



Arteannuin B



Artemisinin



Carvacrol

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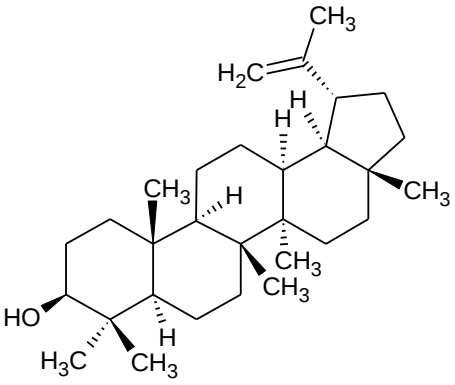
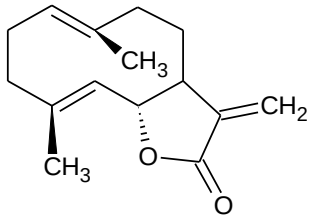
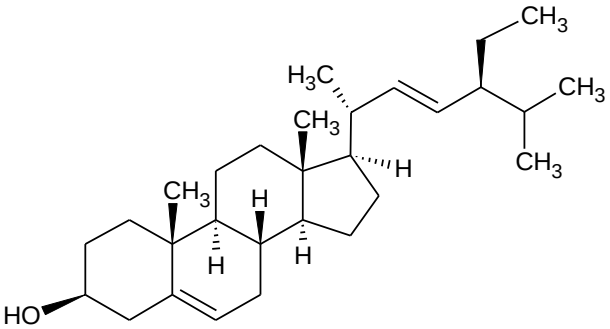


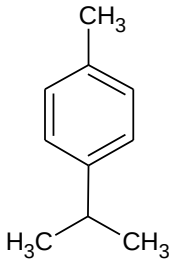
Lupeol



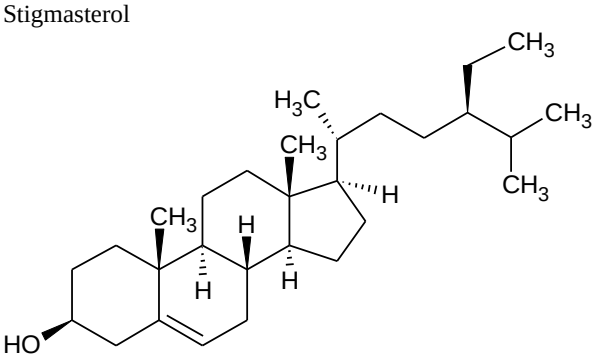
Costunolide



Stigmasterol



P-cymene



β-sitosterol

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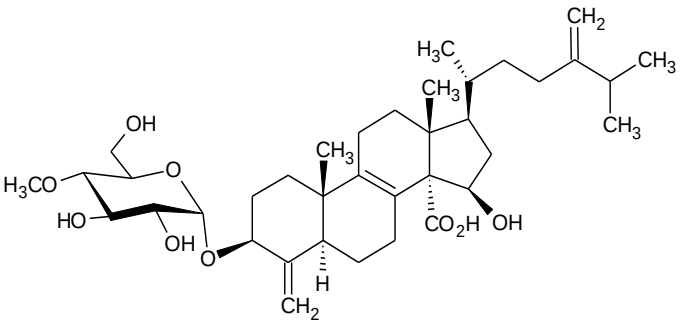
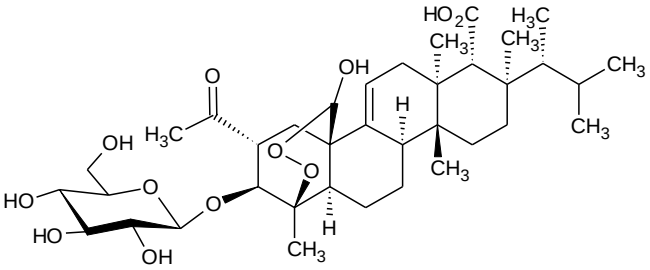
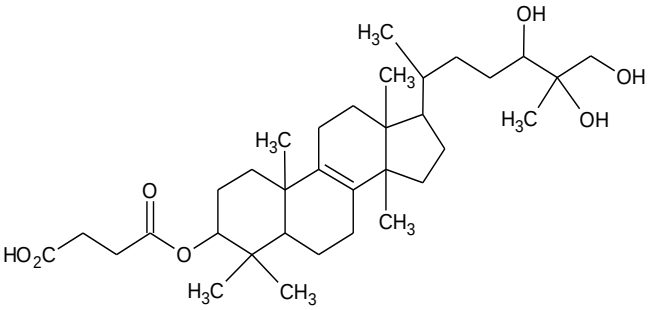


Ascosteroside



Enfumafungin



Arundifungin

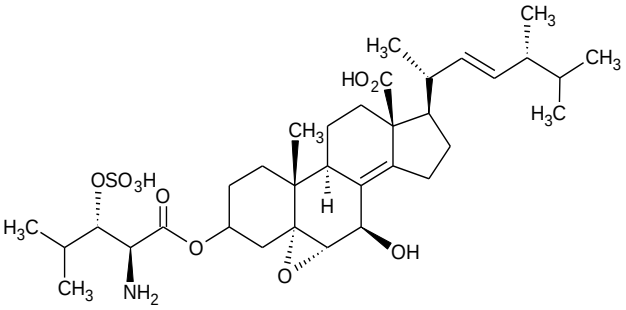


Figure 2: The isoprene building block of terpenoids and chemical structures of some terpenoids.

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Most sesquiterpenes ($C_{25}H_{40}$) have anti-plasmodial and antimicrobial activities while some have antifeedant, anticancer, anti-mycotoxigenic, antioxidant, anti-inflammatory, anti-protozoal and cytotoxic activities. Costunolide, parthenolide and its derivative (1,10-epoxyparthenolide) from sesquiterpenes family were recognized for their antimicrobial activities against *Helminthosporium* spp. Significant antifungal activities were also detected by warburganal and muzigadial, against *Sclerotinia libertiana*, *Candida utilis*, and *Saccharomyces cerevisiae* (Mbaveng *et al*., 2014).

Mechanism of action

The main key phytochemicals to determine the reactivity of terpenoids are thymol and carvacrol that are abundant in thyme oil. Thymol normally hinders the formation and viability of hyphae and promotes morphological alterations in the envelope of *C. albicans*. Furthermore, in a dose-dependent manner, thymol express anti-inflammatory effects by depriving the expression of the pro-inflammatory mediators' gene. Terpenoids also target mitochondria resulting in significant death of *S. cerevisiae,* especially in the case of lupeol. Data clearly shows that terpenoids reduced the mitochondrial content, thus modified the level of reactive oxygen species (ROS) and ATP generation. It is also reported that triterpenoid possesses more potent antifungal activity as compared to the tetraterpenoid (Haque *et al*., 2016).

The hydrophobic nature of terpenoid phenols allow infusion into the lipid membrane. However, *p*-cymene, a precursor of carvacrol, has a higher partition coefficient for lipid membranes; therefore, hydrophobicity alone does not ensure its antifungal action. Instead, the hydroxyl group contributes to the function. The delocalized electron in carvacrol facilitates the dissociation of H^+ from the OH group that resulted in H^+ and monovalent cations such as K^+ migrate across membranes by carvacrol and eliminate pH and K^+ gradients across cell membranes. Besides, carvacrol also depolarizes bacterial cell membranes. However, that mechanism does not explain the transient Ca^{2+} bursts associated with carvacrol. It might, therefore, be that the effects on membrane expansion and fluidity that cause the opening of ion channels followed by their rapid desensitization (Rao *et al*., 2010). To sum up, terpenoids may act in four ways; (1) the formation of hyphae (2) reducing gene expression (3) mitochondrial dysfunction (Ludwiczuk *et al*., 2017), (Haque *et al*., 2016) and (4) depolarization of membranes and calcium ion stress (Rao *et al*., 2010).

Antifungal activity of terpenoids

Terpenoids in *Cannabis* have a variety of effects, such as antifungal and antimalarial activity. Terpenoids from hash oil obtained from drug cultivars of *Cannabis* displayed an antimicrobial effect that was greater than essential oil derived from fiber cultivars (Hazekamp *et al*., 2010). Terpenoid phenols carvacrol, thymol, and eugenol, which are the major components of oregano extract, have a potent antifungal activity of their own. Besides, terpenoid phenols are efficacious not only on planktonic cells but also on biofilms of *C. albicans* that are resistant to many antifungal drugs (Rao *et al*., 2010). Among all terpenoid phenols, carvacrol exhibited the strongest antifungal activity against *C. albicans* biofilms, with a minimum inhibitory concentration, MIC of <0.03%. Aside from *C. albicans*, the terpenoid phenols were also able to inhibit biofilms of several other strains of *Candida*, including *C. glabrata*, and *C. parapsilosis*. (Abdel-Massih *et al*., 2010). Antifungal fractions derived from the chloroform extract of *Artemisia annua* afforded two cadinane derivatives (arteannuin B and artemisinin), oleanolic acid, β-sitosterol, stigmasterol. arteannuin B, a main sesquiterpenoid in *A. annua*, showed antifungal activity against one human (*C. albicans*, MIC: 100 µg/mL) and four plant pathogenic fungi (*Gaeumannomyces graminis* var. tritici, *Rhizoctonia cerealis*, *Gerlachia nivalis* and *Verticillium dahliae*, MICs: 150, 100, 150 and 100 µg/mL, respectively) (Tang *et al*., 2000). Terpenoids isolated from sunflower have also shown antifungal activities towards *V. dahliae* and *S. sclerotiorum* as they inhibited the hyphal growth (Picman *et al*., 1990). Ascosteroside, ergokonin A, arundifungin, and enfumafungin were reported as novel natural products. There is a possibility to develop an oral antifungal agent by the action that inhibits (1,3)-β-D-glucan synthase.

The promising antifungal properties of terpenoids were comparable to the commercial compounds, MK-0991 and L-733560 (antifungal agents). Moreover, the four terpenoids (ascosteroside, ergokonin A, arundifungin and enfumafungin) preferentially inhibited various pathogenic *Candida* and *Aspergillus* strains. The antifungal spectrum, the change in morphology, and the antagonism of antifungal activity by sorbitol, for instance, suggested that terpenoids disrupted the construction of glucan fungal cell wall (Onishi *et al*., 2000).

Saponins

What are saponins?

Saponins are phytochemicals that produce foam (soap properties) when dissolved in water. Saponins are glycosides comprising of an aglycone (sterol or common triterpene) unit linked to one or more carbohydrate chains (sugar) as shown in Figure 3. The most common saponins are triterpenoid saponins that contain a hydrophilic part at one end separated from the lipophilic or hydrophobic part at the other. Steroidal saponins are normally found in the monocotyledons. Natural sources of saponins are oats, asparagus, spinach, soy legumes, beans and peanuts, and tea (Bone and Mills, 2013). The fundamental role of saponins in plants is protection against the attack of pathogens and pets. These molecules have high commercial value that has been used as drugs, foaming agents, sweeteners, taste modifiers and cosmetics (Mert-Türk, 2006).

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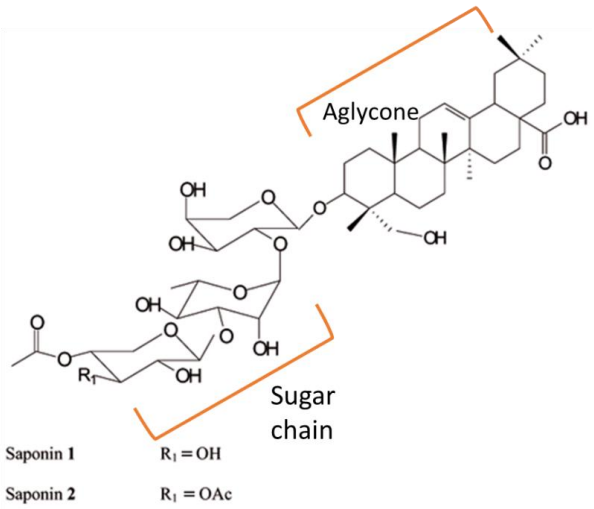
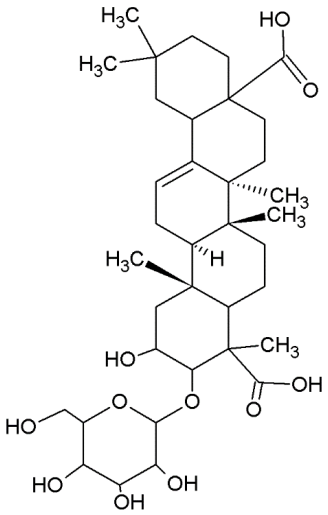
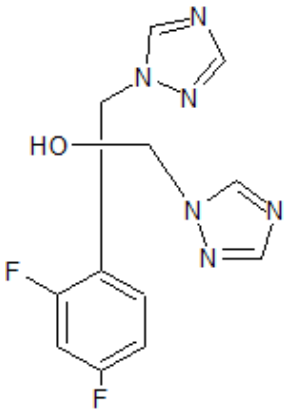


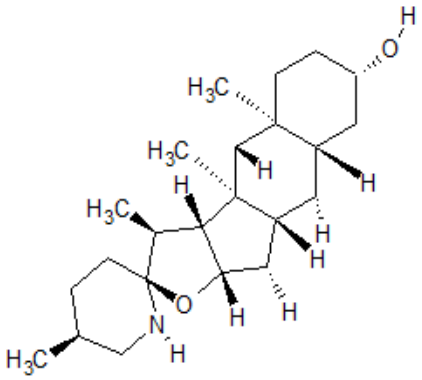
Saponin



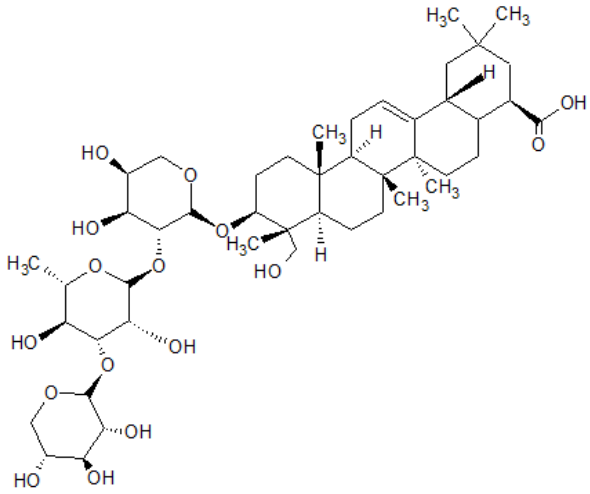
Medicagenic acid 3-O-beta-D-glucopyranoside



Fluconazole



Tomatidine



Sapindoside B

Figure 3: Structure of saponins reported in this review. Reproduced with permission from (Tsuzuki *et al*., 2007).

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Mechanism of action

Based on the literature, spirostanol framework and the number of oligosaccharide residue attached at C-3 of aglycone appear closely associated with antifungal effects of steroid saponins (Zhang *et al*., 2006). Saponins may damage the cell membrane and cause cellular materials to leach out, which ultimately leads to cell death (Mshvildadze *et al*., 2006). For example, an antimycotic saponin from alfalfa root (medicagenic acid 3-O-beta-D-glucopyranoside), formed stable complexes with ergosterol, which resulted in lethal leakage of ions out of the yeast cells (Polacheck *et al*., 1991). The cell membrane of *C. albicans* was severely destroyed by fluconazole (saponin) from *Tribulus terrestris* L (Zhang *et al*., 2006). Tea polyphenols (TP) and tea saponin (TS) and their combination were investigated against *Rhizopus stolonifer*. The two compounds significantly induced the production of H_2O_2, leading to membrane lipid peroxidation, which resulted in increment of the cell membrane permeability and leakage of soluble sugar, soluble protein and K^+. Saponins have also destroyed the structure of the hyphal cell. It is concluded that TP, TS and their combination inhibited the *R. stolonifer* growth through inducing the production of H_2O_2, resulting in the oxidative damage of the cell membrane and leakage of intracellular materials (Jiang *et al*., 2015). The antifungal and antiparasitic activities of tomatidine (a saponin produced by tomato; *Solanum lycopersicum*) against *Saccharomyces cerevisiae* and some parasites, such as *Leishmania amazonensis* and *Phytomonas serpens*, have been reported. It was revealed that tomatidine induced a perturbation of ergosterol biosynthesis through the inhibition of Erg6 (C-24 sterol methyltransferase) activity and Erg4 (C-24 sterol reductase) activity (Dorsaz *et al*., 2017).

Antifungal activity of saponin

Saponin extract from rhizomes of *Dioscorea panthaica* Prain et Burk (Huangshanyao saponin extract, HSE) was tested against *C. albicans*. HSE inhibits the planktonic growth and biofilm formation and development of *C. albicans* at a concentration of 16–64 µg/mL. Furthermore, inhibitory activities against extracellular exopolysaccharide (EPS) production and ROS production in preformed biofilms could be inhibited by 64–256 µg/mL of HSE. Cytotoxicity against human was also tested with Chang's liver cells, but the cytotoxicity was low with a half-maximal inhibitory concentration (IC_{50}) of about 256 µg/mL (Yang *et al*., 2018).

The antifungal activity of *Sapindus mukorossi* extract was tested against *Venturia inaequalis* and *Botrytis cinerea* – two important fungal pathogens worldwide. The spray extract with a concentration of (1% v/v) significantly reduced *V. inaequalis* symptoms and sporulation (99%) on apple seedling leaves ($P \leq 0.05$). The applications of 1% v/v of the extract reduced the disease severity *of B. cinerea* on grapes on average by 63%. The saponins identified were sapindoside B (hederagenin-pentosylhexoside), and oleanolic acid- hexosyl-deoxyhexosyl-hexoside (Porsche *et al*., 2017).

Phenolics

What are phenolic compounds?

Natural phenolic compounds are secreted by plants and microorganisms. They are characterized by their low molecular weight with at least one phenolic group. They are the secondary metabolites produced by the plant within their ordinary development and they are reproduced as well under pressure conditions, for example, ultraviolet and incision (Shi *et al*., 2003). Phenolic compounds are standout amongst the most famous phytochemicals; they are of extensively significant in terms of physiology and morphology in plants. These compounds assume a vital job in development and generation, giving insurance against pathogens and predators, other than contributing towards the taste and the color of the plant fruits. These compounds are derived from the pentose phosphate, shikimate, and phenylpropanoid tracks in plants (Balasundram *et al*., 2006). Phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinines and so forth are phenolic compounds isolated from the herbs of medical benefits and dietary plants (Huang *et al*., 2009).

Mechanism of action

One of the proposed mechanisms for antifungal agents is their binding to membrane ergosterol. To determine whether the phenolic extract of ethyl acetate fraction from *Cochlospermum regium* (mart. Et. Schr.) Pilger roots bind to the fungal membrane sterol, the MIC was determined with and without the addition of exogenous ergosterol. MICs values will be higher with the addition of fungal sterol if the activity was caused by binding to ergosterol. An increase at MIC of *Candida krusei* was induced, but no change was detected with *C. glabrata*, *C. albicans* and *C. tropicalis*. This is a piece of evidence of the binding of this phenolic compound to the membrane (Carvalho *et al*., 2018). Two mechanisms could be involved in this process: (i) binding to membrane ergosterol forming pores in this structure (Campoy and Adrio, 2017), or (ii) inhibition of enzymes involved in the synthesis of ergosterol, which reduces the content of that macromolecule (Ahmad *et al*., 2015). Phenolic acids, such as ferulic and gallic acids, are known to affect the cell membrane of bacteria, which results in changes in the hydrophobicity and charge of the cell surface, causing leakage of cytoplasmic content (Borges *et al*., 2013). A similar effect has been proposed for the caffeic acid derivatives on *Candida* cytoplasmatic membrane. Mode of action of several phenolic compounds have provided some clues to infer the mechanism of phenolic acids. For instance, curcumin, isoquercetin and lariciresinol can damage the *C. albicans* cell membrane. However, methyleugenol and eugenol significantly reduced the biosynthesis of ergosterol in

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Candida and thus, affected the cell membrane (Ahmad *et al*., 2015). Few studies have explained about other possible pathways of phenolic acid mechanism against *Candida*. For instance, isocitrate lyase was inhibited in *C. albicans* after treatment with caffeic acid, rosmarinic acid and apigenin (Cheah *et al*., 2014), (Figures 4 and 5).

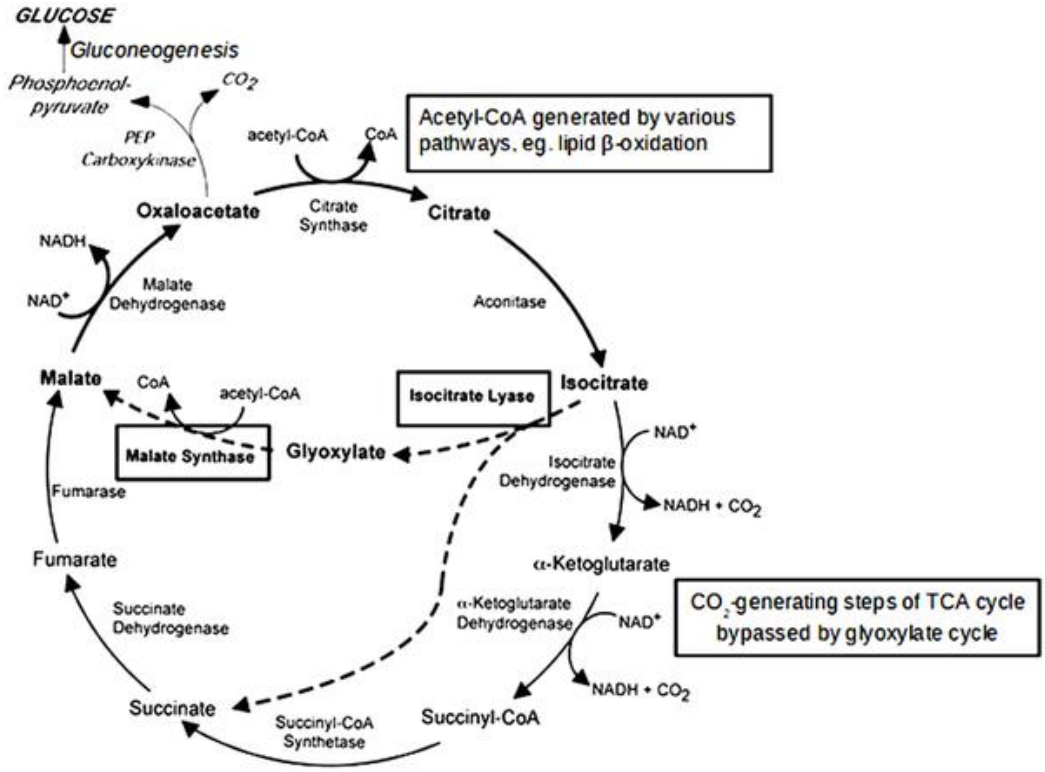
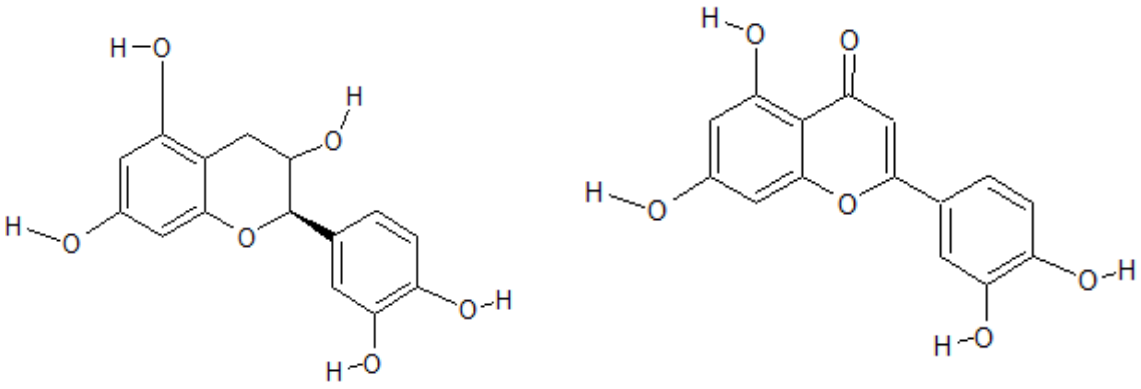


Figure 4: Tricarboxylic acid (TCA) cycle (black arrows) and glyoxylate cycle (dashed arrows). Adapted from Cheah *et al.* (2014). Creative Commons Attribution License.



Catechin Luteolin

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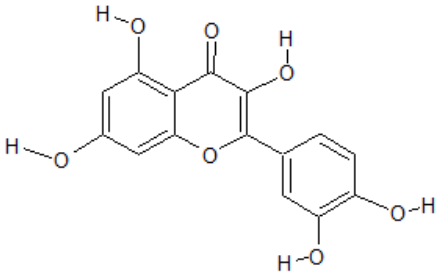
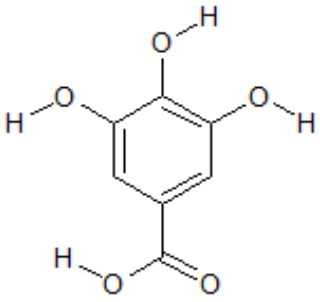
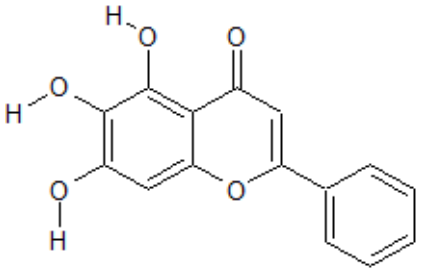


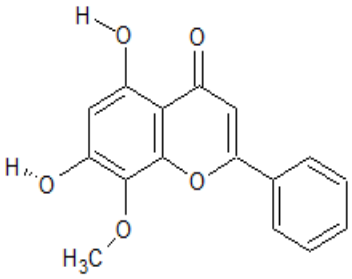
Quercetin



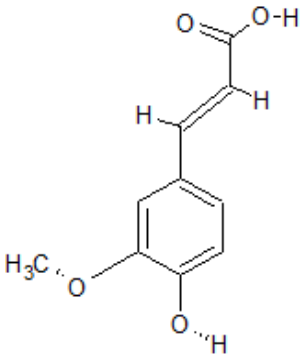
Gallic acid



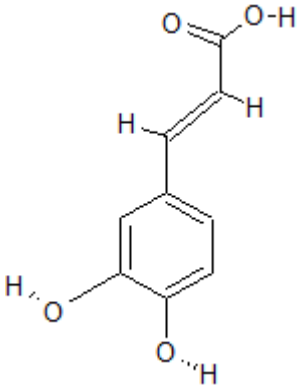
Baicalein



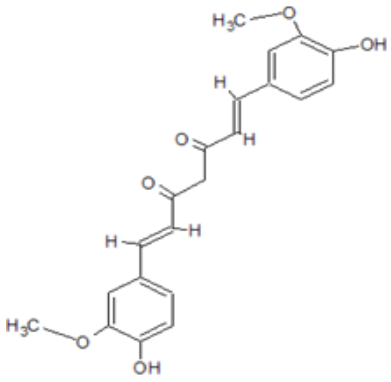
Wogonin



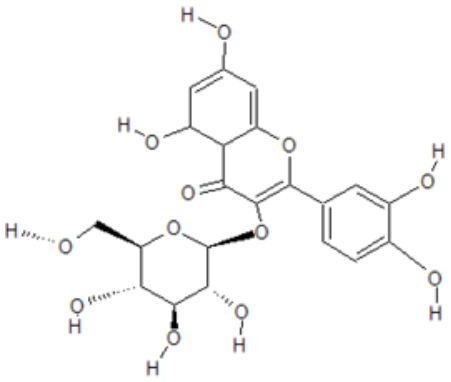
Ferulic acid



Caffeic acid



Curcumin



Isoquercetin

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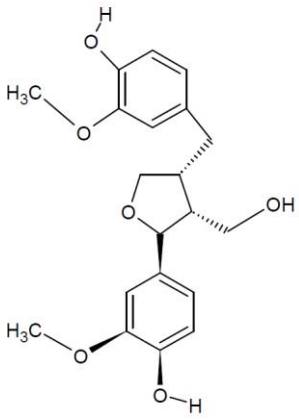
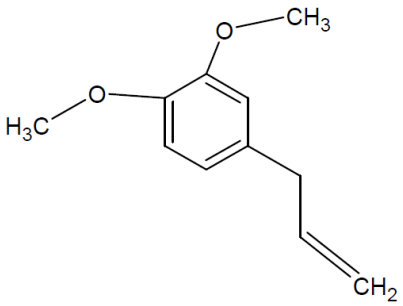
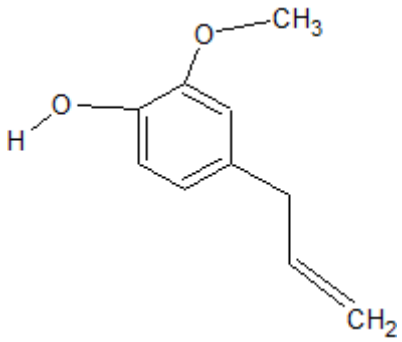


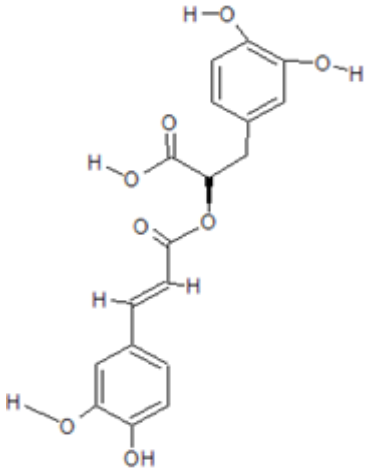
Lariciresinol



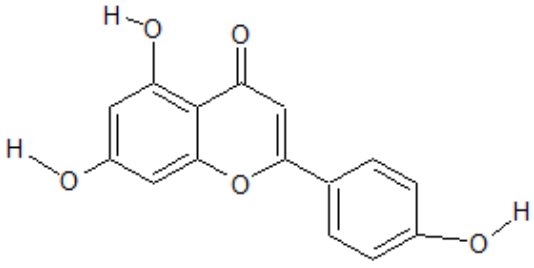
Methyleugenol



Eugenol



Rosmarinic acid



Apigenin

Figure 5: Structures of phenolic compounds reported in this review.

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Antifungal activity of phenolic compounds

Several studies have been performed in order to evaluate the antifungal potential of polar extracts from plant origin, which are enriched in phenolic compounds, such as aqueous, ethanolic, methanolic, hydroalcoholic, acetone and dimethyl sulfoxide (DMSO). The most studied preparations are the aqueous extracts, followed by methanolic and ethanolic extracts (Martins *et al*., 2015). The aqueous phenolic extract from *Fragaria virginiana* Duchesne, *Epilobium angustifolium* L. and *Potentilla simplex* Michx. demonstrated strong antifungal potential. *Fragaria virginiana* had some degree of activity against all of the fungal pathogens; *Alnus viridis* DC., *Betula alleghaniensis* Britt. and *Solidago gigantea* Ait. (Webster *et al*., 2008). Alves *et al*. (2014) evaluated the antifungal effect of catechin, luteolin, quercetin, and gallic acid, phenolic compounds identified from flowers of North-Eastern Portugal, against *Candida* planktonic and biofilm cells. MIC values demonstrated that gallic acid presented the highest effect against all *Candida* species. Catechin showed a similar effect against *C. albicans* American Type Culture Collection (ATCC) 90028 cells. In addition, gallic acid and quercetin had demonstrated only a minimal effect against *Candida* species biofilms (Alves *et al*., 2014). Zabka and Pavela tested the antifungal efficacy of 21 phenolic components of essential oils and plant substances against these filamentous fungi; *Fusarium*, *Aspergillus* and *Penicillium* genera. Thymol and carvacrol were evaluated as the most effective. The MIC_{50} values for thymol ranged from 30 to 52 µg/mL. The MIC_{100} values for thymol ranged from 76 to 255 µg/mL, respectively. For carvacrol, the MIC_{50} values ranged from 37 to 76 µg/mL, and the MIC_{100} ranged from 131 to 262 µg/mL (Zabka and Pavela, 2013). Fungal culture in the presence of various concentrations of baicalein extracted from Ou-gon extracts showed antifungal activity against *T. rubrum*, *Trichophyton mentagrophytes*, *Aspergillus fumigatus*, and *Candida albicans*. Wogonin obtained from the same plant exhibited antifungal activity towards all fungi except *C. albicans* (Da *et al*., 2019).

Coumarins

What is coumarins?

From their name, coumarins are derived from *Coumarouna odorata* plant; they are one of the benzopyrones which consist of benzene fused to *α*-pyrone ring. Coumarins are numerous; around one thousand coumarins are present with abundance in angiosperms. They are found in over 150 different species of plants belonging to almost 30 different families. They are mainly secondary metabolites of plants and present in some microbes. In addition to their activity as an antioxidant, antiinflammatory, antifilarial, antiulcerogenic, trypanocidal, antibacterial, antitumor and antiHIV, coumarins have shown a strong antifungal activity (Montagner *et al*., 2008).

Coumarins are classified based on their structural diversity into four groups. Coumarin derivatives or called simple coumarins, consist of two rings and substituted on their C7, C6 and C3 positions by benzopyrone, hydroxyl, methoxy and aliphatic groups. The second group of coumarins is isocoumarin derivatives, formed by benzene rings and α-isopirone and they have substituents in positions C3, C6, C7 and C8. They are isolated mainly from fungi: *Artemisia*, *Aspergillus*, *Fusarium*, *Penicillium*, *Streptomyces* and the few plants belonging to families: *Compositae*, *Leguminosae* and *Myrtaceae*. The third group is furanocoumarin derivatives, consist of coumarin ring coupled with the furan ring at the C6-C7 position (psoralen type) or in the C7-C8 position (angelicin type). Finally, the fourth group is pyrancoumarin derivatives, where the coumarin ring is condensed with pyran ring to form xanthyletin-type if the condensation is at the C6-C7 position, or seselin-type if at the position C7-C8 (Tomasz Kubrak *et al*., 2017).

Antifungal activity of Coumarins

Osthole is a coumarin isolated from plants with therapeutic capacities like *Angelica pubescens*, *Cnidium monnieri*, and *Peucedanum ostruthium*. *Rhizoctonia solani*, *Phytophtora capsici*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, and *Fusarium graminearum* are highly pathogenic fungi and osthole is used as a treatment against them due to its high antifungal activity spectrum. Psoralen, imperatorin, and ostruthin have the highest antifungal effectiveness of all the coumarins (Venugopala *et al*., 2013). In addition to the examples cited in Table 1, byosthol is a derivative of coumarin isolated from celery plants, which demonstrates activity against *R. solani, P. capsici, B. cinerea, S. sclerotiorum* and *F. graminearum* (Tomasz Kubrak *et al*., 2017). In Table 1, the two types of coumarins with antifungal activity are cited.

In Srinivasan and Sarada (2012), the antifungal activity of pyranocoumarin (PDP) available in *Psoralea corylifolia* was established. In addition to their capacity as an antifungal agent against *Fusarium* sp., the molecular docking analysis performed with the X-ray crystal structures of Tri101, trichothecene 3-O-acetyltransferase available in the Protein Data Bank proposed a hypothetical mechanism for antifungal activity against plant pathogen *Fusarium* sp. The minimum inhibitory concentration of 1 mg/mL was detected for the PDP against *F. oxysporum*, *F. moniliforme*, and *F. graminearum.* The molecular docking has shown the affinity towards the target protein and by binding to the protein, it will inhibit the acetylation mechanism ending by fungi death. *C. albicans* exposition to coumarin within twenty-four hours has shown cell membrane and cell wall alteration, and

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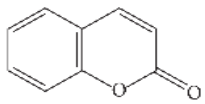
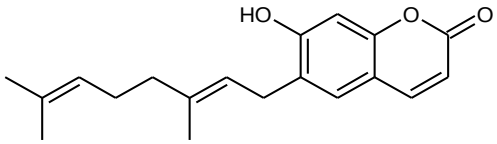
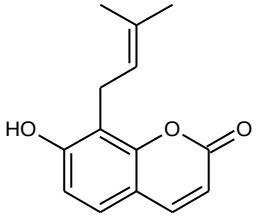
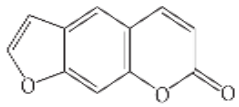
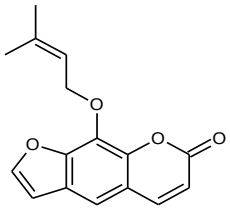
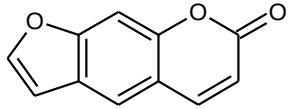
Table 1: Most effective coumarins with antifungal activity.

Type of coumarin	Chemical structure	Example of coumarin	Plant of origin	Fungi target	Reference
Simple coumarins		Ostruthin 	*Peucedanum ostruthium*	*Saccharomyces cerevisiae*	(Feuer, 1974)
		Osthole 	*Angelica pubescens, Cnidium monnieri*, and *Peucedanum ostruthium*	*Rhizoctonia solani, Phytophtora capsici, Botrytis cinerea, Sclerotinia sclerotiorum,* and *Fusarium graminearum*	(Wang *et al*., 2010)
Furano-coumarins		Imperatorin 	*Glehnia littoralis, Prangos pabularia, Clausena ansium,* and *Aegle marmelos*	*Saccharomyces cerevisiae*	(Kozioł and Skalicka-Woźniak, 2016)
		Psoralen 	*Psoralea corylifolia*	*Fusarium oxysporum, Fusarium moniliforme,* and *Fusarium graminearum*	(Srinivasan and Sarada, 2012)

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cytoplasmic volume decreases followed by structural disarrangement. However, the mode of function adopted by coumarin (1,2-benzopyrone) has yet to be depicted. Respiration and ergosterol synthesis dysfunctioning were the strategies adopted by silver (I)-coumarin complexes against *C. albicans* whereby the exact mechanism targeted cytochrome *c* synthesis disruption, which may also cause apoptosis (Srinivasan and Sarada, 2012). Jia *et al*. (2019) have studied the effects of coumarin on cell growth inhibition and strain viability reduction by experimenting and determining yeast apoptosis with phosphatidylserine (PS) externalization, DNA fragmentation, cytochrome *c* release, and metacaspase activation. Their results explored the role of coumarin in PS exposure on the outer leaflet, DNA degradation and nuclear compaction, cytochrome *c* release, and metacaspase activation, suggesting that coumarin induced apoptosis in *C. albicans*. Previous studies have demonstrated that coumarin damages *C. albicans* cells via pore formation in the fungal cell wall and seep of cytoplasmic contents and necrosis are phenomena adopted by coumarins and elaborated previously (Jia *et al*., 2019).

Alkaloids

What are alkaloids?

Deriving from amino acids and with a nitrogen atom in the heterocyclic ring, alkaloids are formed and considered as an important class of structurally diversified compounds. Alkaloid nomenclature is derived from the Arabic term *al-qali,* the plant from which soda was first isolated. Alkaloids cover roughly 20% of plant-based secondary metabolites and they exhibit antimicrobial, anticancer, narcotics, toxic substances and stimulant capacities. Nowadays, around 12,000 alkaloids are confined from various genera of the plant kingdom. This number makes this class of natural products biologically important (Kaur and Arora, 2015).

The examples of alkaloids acting as antifungal are numerous. The mode of their antifungal action is usually pleiotropic, where protein synthesis is inhibited, and the fungal DNA is intercalated or by boosting the development of fungi inhibitors. The first medically useful example of an alkaloid was morphine, isolated in 1805 from the opium poppy *Papaver somniferum* (Arif *et al*., 2009). Phenanthridine, an alkaloid isolated from *Chelidonium majus* exhibits antifungal activity against the clinical drug-resistant yeast isolates. Bis-benzylisoquinoline alkaloids such as cycleanine and cocsoline isolated from *Albertisia villosa* have antibacterial, antifungal, antiplasmodial activities in addition to cytotoxic potential related to these alkaloids (Kaur and Arora, 2015).

Mechanism of action

The two alkaloids; liriodenine methiodide (LMT), a methiodide salt of liriodenine, and eupolauridine 9591 (E9591), a synthetic analog of eupolauridine, exhibit their antifungal activities by interrupting mitochondrial iron-sulfur (Fe-S) cluster synthesis (Tripathi *et al*., 2017). There are several studies lending support to this theory. First, it was shown that both LMT and E9591 provoked a transcriptional response indicating iron imbalance. This induces the genes that are needed for iron uptake and for the maintenance of cellular iron homeostasis. Second, the analysis of a genome-wide fitness profile showed that mutant yeast cells that lack the iron homeostasis-related genes displayed hypersensitivity to LMT and E9591. Third, introducing LMT and E9591 to treat wild-type yeast cells resulted in cellular defects that imitated deficiency in mitochondrial Fe-S cluster synthesis, which include iron levels increment in mitochondria, respiratory function reduction, oxidative stress increment and loss of activity of Fe-S cluster enzymes. Another study by Dhamgaye *et al*. (2014) explored a plant alkaloid berberine (BER) for its antifungal potential. They have found a heat shock factor (*HSF1*) in TF mutant strains of *C. albicans*. The mutant displayed collateral susceptibility towards drugs targeting cell wall (CW) and ergosterol biosynthesis and was highly susceptible to BER. The treatment with BER of *Candida* cells led to dysfunctional mitochondria, proven by the slow growth in non-fermentative carbon source and poor labeling with mitochondrial membrane potential sensitive probe (Dhamgaye *et al*., 2014). In summary, the suggested mechanism of alkaloids is causing a malfunction of the mitochondria, which causes a direct impact on the growth, respiratory activity and enzyme activity, hence, causes cell imbalance. The structures of alkaloids in this review are shown in Figure 6.

Antifungal activity of alkaloids

Singh *et al*. (2007) tested the antifungal properties of allosecurinine, an alkaloid extracted from *Phyllanthus amarus* Linn. (Family: *Euphorbiaceae*). The alkaloid inhibited mild spore germination of *Curvularia lunata*, *Curvularia* sp., *Collectotrichum* sp., *C. musae* and *Heterosporium* sp. at very low concentrations of allosecurinine (Singh *et al*., 2007). The ethanolic extract of the leaves of *Alstonia scholaris* contained seven monoterpenoid indole alkaloids. The isolated compounds were tested in vitro the antifungal potential against five species of fungi. The extract showed antifungal activity against two fungal strains; *Gibberella pulicaris* and *Cercospora nicotianae* (Wang *et al*., 2013). Six different species of *Amaryllidaceae* generated various alkaloids, which were studied by Miroslav *et al*. (2015) with respect to their anti-yeast activity. The analysis showed 25 alkaloids with 16 identified from their retention indexes, retention times and mass spectra. In the antimicrobial assay, isolates of

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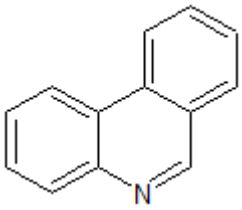
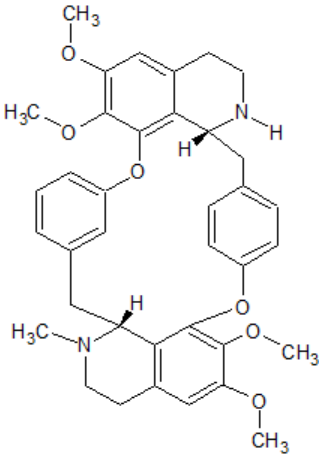
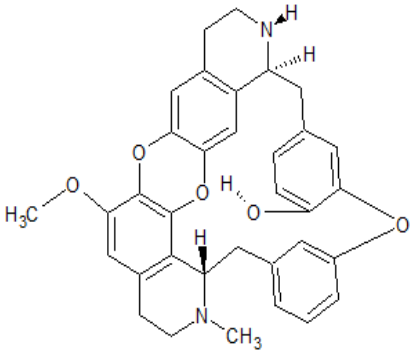


Phenanthridine



Cycleanine



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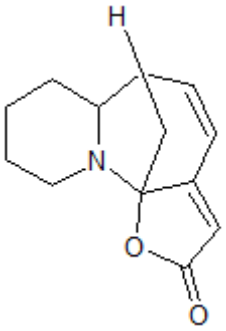

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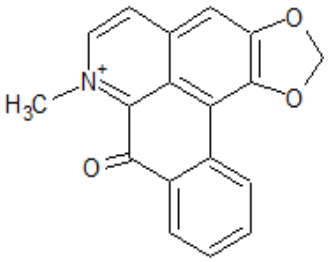
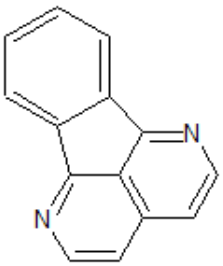
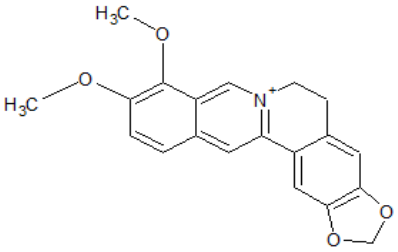


Liriodenine methiodide



Eupolauridine



Berberine

Figure 6: Structures of alkaloids reported in this review.

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the human pathogenic yeasts *Candida albicans*, *C. glabrata, C. dubliniensis* and *Lodderomyces elongiosporus* were tested. The six extracts, together with 19 Amaryllidaceae alkaloids showed promising anti-yeast properties although no antibacterial activity was detected. Among the alkaloidal extracts, *Narcissus jonquilla* cv. Baby Moon had the most effective anti-yeast, with minimal and average MIC values of 128 and 192 µg/mL, respectively, followed by *Leucojum aestivum*, *Narcissus poeticus* var. recurvus and *N. canaliculatus* (Miroslav *et al*., 2015).

Essential oils

What is an essential oil?

Essential oils are a complex mixture of natural compounds obtained mainly from plants or herbs. The physical properties of essential oils are they appear as a colored mixture of several aromatic compounds, liquid and volatile (Macwan *et al*., 2016). The ultimate role of essential oils is to protect plants against any threat from the environment, such as pathogens and insects that act as plug vectors. Therefore, they are well-known for their medicinal properties such as antiseptic, anti-carcinogenic, anti-inflammatory, analgesic, anesthetic and they are mainly used as natural additives in food and food products due to their antioxidant and antimicrobial properties.

Phytochemical compounds of essential oils

The chemicals composition of essential oils is affected by several factors such as plant species, method of extraction, geography and environment. Different location has a different composition of the chemical compounds of essential oil due to climate differences, humidity which constitute different species of insect or microbial properties that induce the plant to produce its own phytochemicals. Terpenes (*p*-cymene, limonene, terpinene, sabinene and α- and β-pinene) and terpenoids (thymol, carvacrol, linalyl acetate, linalool, piperitone, citronellal, geraniol and menthol Figure 7) are the main categories of compounds in essential oil fairly at high concentration (20-70%) (Niu and Gilbert, 2004).

Mechanisms of action

i. Cell membrane disruption

The fungal cell wall consists of essential elements such as glucan, chitin and mannan for fungal survival. Phytochemicals in essential oils affect fungal cell wall maturation, septum formation and bud ring formation (Wu *et al*., 2008). This leads to the thinning and distortion of the hyphal wall, thus causing the hyphal tip to be divided into bud-like structure. The severity of damage can be up to the level where the cytoplasm leakage inhibits DNA, RNA, protein and peptidoglycan biosynthesis and, lastly, inhibits the ergosterol biosynthesis (Nazzaro *et al*., 2017). Anise oil manifests antifungal activity against filamentous fungus by its trans-anethole that inhibits chitin synthase activity in permeabilized hyphae (Yutani *et al*., 2011). Another example of cell membrane disruption is by the essential oil extracted from *Citrus sinensis* epicarp that contains almost 85% limonene. This essential oil leads to the irreversible deleterious morphological alteration that is capable of inhibiting the growth of *Aspergillus niger* (Sharma and Tripathi, 2008). A similar effect is exhibited by thymoquinone, a component of black cumin seed essential oil that extensively damages the fungal cell wall and cytoplasmic membrane (İşcan *et al*., 2016).

ii. Dysfunction of fungi mitochondria

Essential oils can inhibit mitochondrial dehydrogenase systems responsible for biosynthesis of ATP such as lactate dehydrogenase, malate dehydrogenase and succinate dehydrogenase. For example, *Anethum graveolens* essential oil was capable of inhibiting *Candida albicans's* ATP biosynthesis and at the same time disturbed the citric acid cycle (Chen *et al*., 2013). Other essential oils that were extracted from various plants such as *Origanum compactum, Artemisia herba alba* and *Cinnamomum camphora* also shows mitochondrial damage when treated to *Saccharomyces cerevisiae.* The antifungal activity of essential oil towards mitochondrial damage was comprehended to be the role of terpenoids that give rise to an altered level of reactive oxygen species and ATP generation (Haque *et al*., 2016).

iii. Inhibition of efflux pumps

The physiology of fungi such as the electrochemical proton gradient across the cell membrane for nutrient uptake, intracellular pH, fungal growth, and fungal pathogenicity were all supported by fungal plasma membrane H^+-ATPase (Haque *et al*., 2016). Apart from that, the fungal plasma membrane also regulates nutrient uptake and medium acidification (Perlin *et al*., 1997). Therefore, inhibition of H^+-ATPase leads to intracellular acidification and cell death. Thyme oil exhibits the over-expression of the efflux-pump gene. The chemical components that play a role in the inhibition of the over-expression of efflux-pump genes CDR1 and MDR1 in *C. albicans* are thymol and carvacrol and the reaction is located at the membrane level (Nazzaro *et al*., 2017).

Antifungal activities of essential oils

The presence of fungal infection is more difficult to verify and difficult to treat and eliminate as compared to bacterial infection. The inception and acuteness of fungal infection depend on the host's resistance and inoculum charge. The synthetic antimicrobial treatment of fungal infection is effective, but in the long term, it will generate resistant fungal species and caused side effects on the organ (liver and kidney) functionality (Williams and Lewis, 2011). Due to apprehension on the safety of synthetic antimicrobial agents, the limelight has been diverted to the potential application of essential oil as an alternative

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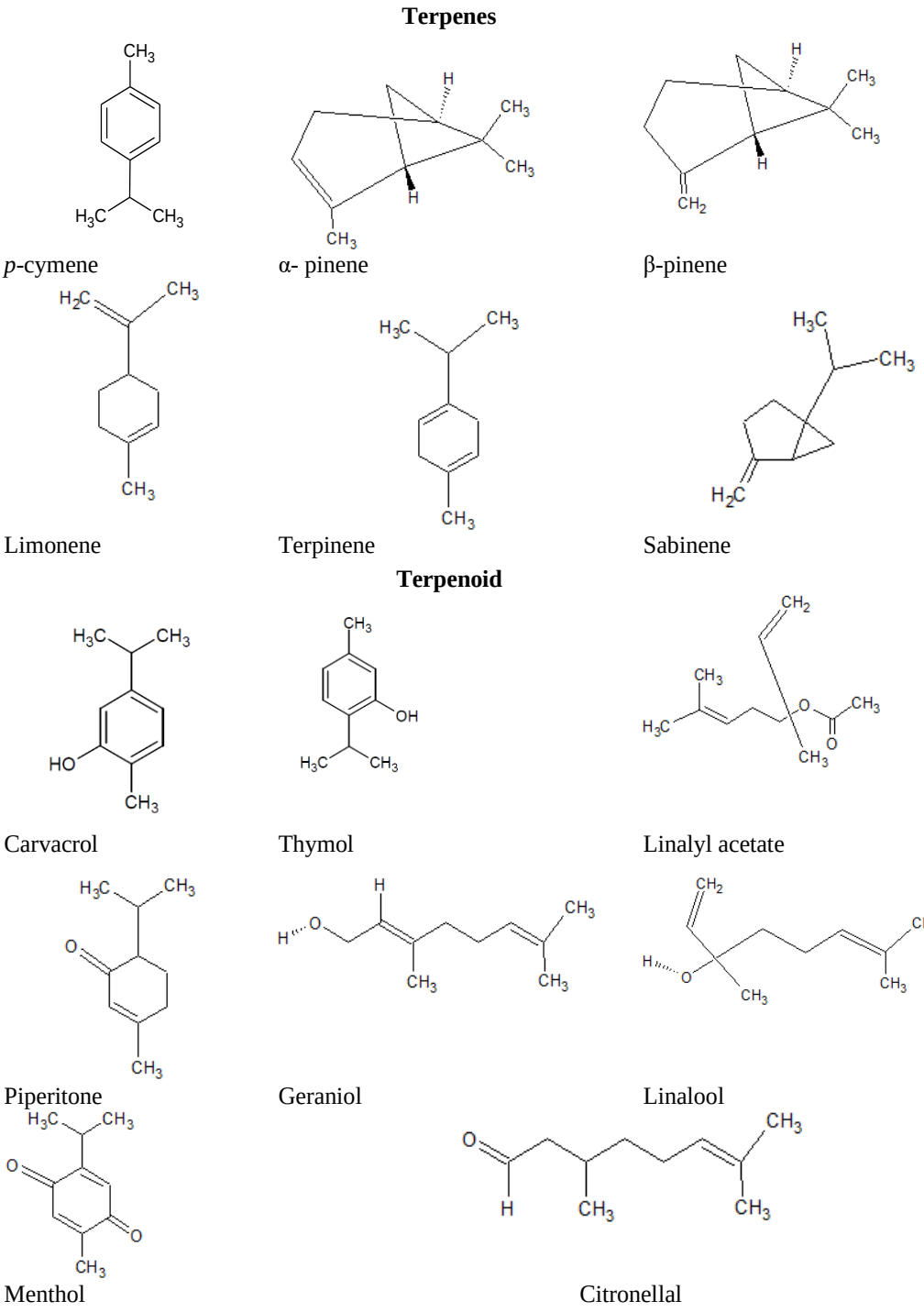

Figure 7: Structures of terpenes and terpenoids reported in this review.

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treatment for fungal infection. Essential oils have a broad spectrum of antifungal properties and are environmentally friendly (no non-toxic residue and by-product). The antifungal activity of essential oil is highly due to the existence of terpenes/terpenoid and the lipophilic properties that enable disruption of the cell wall and membranes and organelles of the fungal cell and/or inhibit nuclear material or protein synthesis that leads to death of fungi (Figure 8) (Tian *et al*., 2011).

List of essential oils with antifungal effect

Many essential oils are now known to contain powerful antifungal properties and the following list is not exhaustive but representative of those commonly believed to be the best treatment for fungal infections and scientifically proven to possess antifungal qualities (Table 2).

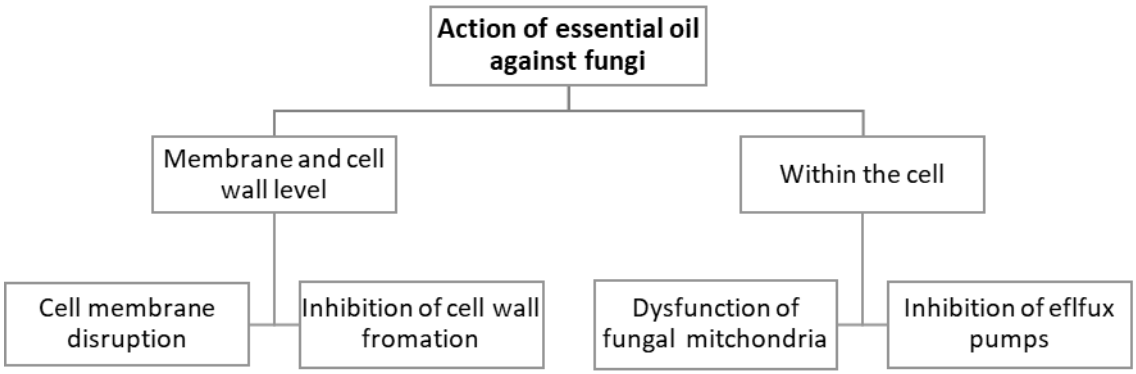

Figure 8: Antifungal activities of essential oils towards fungi.

Table 2: List of potential essential oil for antifungal treatment.

Name of plants	Biochemical compound	Antifungal activity	Reference
Stems of *Croton tricolor*	Epiglobulol, α-bisabolol, α-trans-bergamotol and β-caryophyllene	*Candida* species; *C. albicans* (ATCC 90028), *C. albicans* (LM105), *C. tropicalis* (ATCC 13,803), *C. tropicalis* (LM 14), *C. krusei* (ATCC 6538) and *C. krusei* (LM 12)	(Huang *et al*., 2019)
Polyscias fulva	Saponins, tannins, alkaloids, anthraquinone and phenols	*M. audouinii*, *T. rubrum*, *T. ajelloi*, *T. equinum*, *T. mentagrophytes*, *T. terrestre*, *M. gypseum* and *E. floccosum*	(Njateng *et al*., 2013)
Ferulago capillaris	Limonene and α-pinene	*Candida*, *Cryptococcus*, *Aspergillus* and dermatophyte strains	(Pinto *et al*., 2013)
Moringa oleifera Lam	Pentacosane and hexacosane	*T. rubrum*, *T. mentagrophytes*, *E. floccosum* and *M. canis*	(Chuang *et al*., 2007)
Allium sativum	Di-2-propenyl trisulfide and di-2-propenyl disulfide	*T. erinacei*, *T. rubrum*, and *T. soudanense*	(Pyun and Shin, 2006)
Curcuma longa	Turmerone, atlantone, and zingiberone	*T. mentagrophytes*, *T. rubrum*, *E. floccosum*, and *M. gypseum*	(Jankasem *et al*., 2013)
Eugenia cariophyllata	Eugenol	*C. albicans*, *C. tropicalis*, *C. krusei*, *T. rubrum*, *T. mentagrophytes* and *Geotrichum candidum*	(Gayoso *et al*., 2005)
Salvia cryptantha and *S. multicaulis*	α-pinene, eucalyptol, camphor, camphene and borneol	*C. albicans* and *C. krusei*	(Tepe *et al*., 2004)

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Antifungal peptides

What are antifungal peptides?

Antifungal peptides (AFP) are small cationic, amphipathic molecules with less than 50 amino acids isolated from plants, animals, bacteria and fungi (Matejuk *et al*., 2010). AFP is a cysteine-rich protein that encodes various AFP that belong to different classes (Garrigues *et al*., 2018). With confine requirement for commercialization, AFP prominently meets the desired criteria to be a commercial antifungal. Firstly, it is highly stable toward high temperatures, proteolysis, as well as acidic condition (Dorsaz *et al*., 2017). In nature, antimicrobial and antifungal peptides are the first-line defense for any organism against a wide spectrum of microbe infection that has no toxic effect towards the host organism (Matejuk *et al*., 2010). Evidence suggests that the antifungal activity of antimicrobial peptides (AMPs) is multifactorial. The modulation of the immune system and the host immune status determine the efficacy of the peptide likely similar to other antifungal agents (Ben-Ami *et al*., 2008).

Mechanism of action

There are two modes of the mechanism of action of AFP; the first one is through permeabilization of the cell membrane, which may break down into two mode of actions. The first one is the carpet model, where protein molecules insert into the membrane and forming pores. The second one is the pore model, where the protein molecules oligomerize and form a multimeric pore (Brogden, 2005). Both models are based on AFP bacterial interaction, where the cationic character of defensins interacts with the negatively charged plasma membrane of bacteria. What follows is the disintegration of the plasma membrane and cell leakage and cell death (necrosis). Another mode of the mechanism of action of AFP is where the membrane interaction may not primarily damage the plasma membrane, but the interaction with specific lipid or protein components of the plasma membrane caused the formation of a transient pore. The interaction resulted in the protein transport into the host cell and interacted with the intracellular target. This led to an increase in the cellular level of reactive oxygen species (ROS) and triggered programmed cell death (Brogden, 2005).

Classification and antifungal activity of peptides

Antifungal peptides can be classified into two groups based on their mechanism of action, which are lysis and cell wall synthesis interference (Figure 9). Lytic peptides have the characteristic of amphipathic. Amphipathic molecules possess a polar and non-polar region that is hydrophilic and hydrophobic, respectively. The lytic antifungal peptide can bind to the cell membrane and disrupt the membrane structure without traversing, or it is able to traverse the cell membrane and interact with specific intracellular molecules. Some lytic peptides form an aqueous pore allowing transposes of ions and other solutes (De Lucca and Walsh, 1999).

Initial effort to commercialize antimicrobial peptides (AMP) was unsuccessful due to the complex biology and pleiotropic nature of AMP that was not fully understood (Duncan and O'Neil, 2013). Another difficulty for the commercialization of AMP is their biological instability. AMP is more susceptible to proteolytic degradation in the systemic environment that resulted in a shorter half-life of AMP, thus making it unable to maintain plasma concentration needed for their minimal inhibitory concentration (Duncan and O'Neil, 2013). There is a need to develop AMP molecules that retain their positive

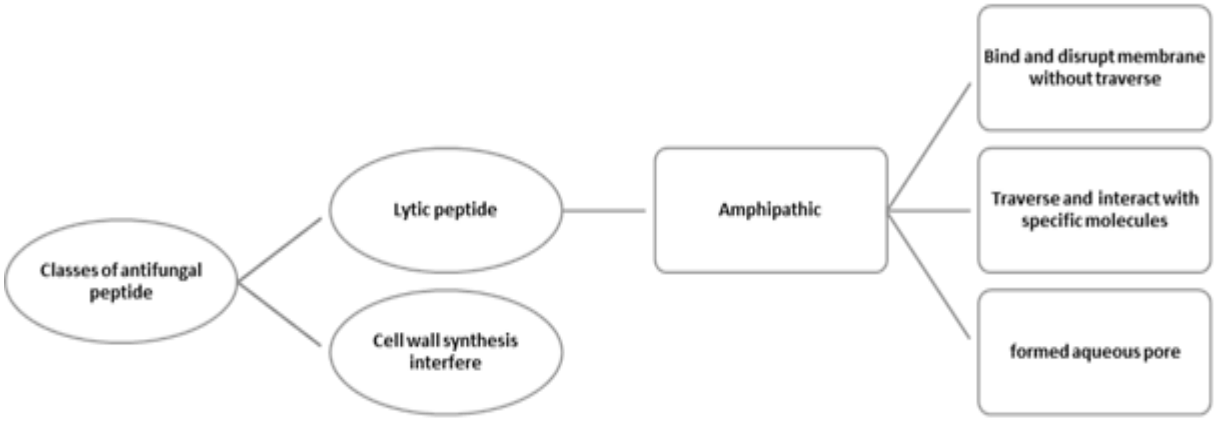

Figure 9: Classification of antifungal peptides.

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physiochemical functions but devoid of the negative features such as hemolytic and inflammatory potential that previously held back their translation into the clinical stage (Ahmad *et al*., 1995). The ability to kill yeast, hyphae, spores and the charge-dependent fungicidal that minimizes the chance of resistance are all attractive therapeutic features of the engineered antifungal peptide (Muralidharan and Bobek, 2005). Meanwhile, the recent technology on control released polymer system that introduces the nanoparticle encapsulation, liposomal delivery and PEGylation are the new ways to tackle the instability problem of AMP that enable it to be commercialized (Duncan and O'Neil, 2013).

Among the wide groups of AMP, defensins are the most outstanding AMP, which have a close structural relationship that generally exists in plants, insects and mammals. Defensins generally contain six to eight cysteine from intramolecular disulfide bond and stabilized with an anti-parallel β-sheet conformation and enclosed with an α-helical segment (Bulet and Stocklin, 2005). The resistance toward extreme conditions like pH, temperature and protease-mediated degradation is due to the compact structure of defensins (Hegedüs and Marx, 2013).

CONCLUSION

A wide number of metabolites from plants and other natural sources have been reported to inhibit pathogenic fungi. These compounds represent a wide variety of structural classes ranging from terpenes, saponins, alkaloids, coumarins to peptides and proteins. The increasing number of multidrug-resistant strains of fungus makes it necessary to discover new classes of antifungal compounds to overcome fungal resistance mechanisms. This has led to a search for therapeutic alternatives, particularly medicinal plants and compounds isolated from them, to be used for antifungal properties. Another challenge is the small number of drugs available in the market due to the strict regulation and complex procedure of clinical trials for potential candidate compounds. From this review, various types of plant-based antifungal compounds against different fungi were identified and discussed. Likewise, some studies demonstrated the correlation between these natural compounds and their antifungal mechanisms of action. There are two major types of mechanisms of action, which are cell membrane disruption mode and interaction with intracellular molecules mode, which lead to programmed cell death. It is somehow challenging to simplify the mechanisms of action in plant secondary metabolites because many compounds exhibit their potency via more than one mechanism. Therefore, it is vital to have an in-depth examination of the compounds subgroups instead of grouping the metabolites into the biosynthetic group. The interference of the cell's nucleic acid and protein synthesis could be used as a new drug target provided that there is no damaging effects and/or interactions with the human system. Moreover, the efflux pump inhibition is foreseen to be significant in antifungal resistant strains in the future.

CONFLICT OF INTEREST

Authors declare no conflict of interest in this project.

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