9. Natural products: Anti-fungal agents derived from plants

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Abstract. As new spectrums of human fungal infections are increasing due to increased cancer and AIDS patients. The increased use of antifungal agents also resulted in the development of resistance to these drugs. It makes necessary to discover new classes of antifungal compounds to treat fungal infections. The research on natural products and natural products derived compounds has accelerated in recent years due to their importance in drug discovery. Plants are rich source of bioactive secondary metabolites of wide variety such as tannins, terpenoids, alkaloids, and flavonoids, reported to have in vitro antifungal properties. A series of molecules with antifungal activity against different strains of fungus have been found in plants, which are of great importance to humans and plants. These molecules may be used directly or considered as a model for developing better molecules. This review attempts to summarize the current status of reported antifungal compounds from plants.

1. Introduction

The prevalence of resistance to antifungal agents significantly increased in the past decade. Resistance to antifungal agents has important implications for morbidity, mortality and health care in the community. Until recently,
fungi were not recognized as important pathogens because the annual death rate due to candidiasis was steady from 1950 to 1970 [1, 2]. Since 1970, this rate increased significantly due to more widespread use of immunosuppressive therapies, indiscriminate use of broad-spectrum antibacterial agents, the common use of indwelling intravenous devices and immunosuppressive viral infections such as AIDS. These developments and the associated increase in fungal infections [3] necessitated the search for new, safer, and more potent agents to combat serious fungal infections. For nearly 30 years, amphotericin B, which causes significant nephrotoxicity, was the sole drug available to treat serious fungal infections. The imidazoles and the triazoles in late 1980s and early 1990s were major advances in safe and effective treatment of local and systemic fungal infections. The high safety profile of triazoles, in particular fluconazole, has led to their extensive use. Fluconazole has been used to treat in excess of 16 million patients, including over 300,000 AIDS patients, in the United States alone since the launch of this drug [4]. Due to selective pressure and widespread use of these few antifungal drugs, there have been increasing reports of antifungal resistance [5].

Medicinal plants have been a source of wide variety of biologically active compounds for many centuries and used extensively as crude material or as pure compounds for treating various disease conditions. Relatively 1-10 % of plants are used by humans out of estimated 250,000 to 500,000 species of plants on Earth [6]. The plants are relatively cheap source of biological material having a vast variety of metabolites, primary or secondary, available in them for selecting the molecule of desired biological activity. Mainstream medicine is increasingly receptive to the use of antimicrobial and other drugs derived from plants, as traditional antibiotics become ineffective. Another driving factor for the renewed interest in plant antimicrobials in the past 20 years is due to the rapid extinction rate of (plant) species [7]. The scientific discipline, ethno botany, is utilizing the impressive array of knowledge assembled by indigenous peoples about the plant and animal products they have used to maintain health [8,9]. Lastly, the ascendancy of the human immunodeficiency virus (HIV) has spurred intensive investigation into the plant derivatives, which may be effective, especially for use in underdeveloped nations. Few of the compounds isolated from plants such as 2-decanone, hydroxydihydrocornin-aglycones [10], various indole derivatives [11] and isoflavanone are reported to have antifungal activities. However, development of useful antifungal drugs from these compounds has not yet been possible.
2. Major groups of antifungal compounds from plants

Plants have an almost limitless ability to synthesize aromatic substances of different functional groups, most of which are phenols or their oxygen-substituted derivatives [12]. Most are secondary metabolites, of which at least 13,000 have been isolated that is less than 10% of the total [13]. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some plants used for their odors (terpenoids), pigment, (quinones and tannins) and flavor (terpenoid capsaicin from chili peppers) were found to be endowed with medicinal properties. Some of the herbs and spices used by humans as season food yield useful medicinal compounds.

2.1. Simple phenols and phenolic acids

In recent years, numbers of studies have been reported on the antifungal activity of phenolic compounds from natural sources. Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring (Fig 1). The common herbs tarragon and thyme both contain caffeic acid, a representative of a wide group of phenylpropane-derived compounds which is effective against fungi [14]. The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity [12]. In addition, it was also reported that more highly oxidized phenols are more inhibitory [15, 16]. The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins [17].
Tannins and salicylic acid are polyphenol compounds extracted from Gaullher procumbens, Rhammus purshian and Anacardum pulsatilla showed antifungal activity [18,19]. Piper crassinervium, Piper aduncum, Piper hostmannianum and Piper gaudichaudianum, contain phenolic acid derivatives crassinervic acid (1), aduncumene, hostmaniane and gaudichaudanic acid, respectively, were reported for fungitoxic activity [20]. Phenolic compound Eriosemaones A–D (2, MIC ¼ 20 mg/mL) are reported to have good antifungal activities [21]. Phenolic compound from Croton hutchinsonianus [22], and pinosylvin (3), a constituent of pine, showed growth-inhibitory activity against C. albicans and Saccharomyces cerevisiae [23].

Four phenolic amides, dihydro-N-caffeoyltyramine, trans-N-feruloyloctopamine, trans-N-caffeoyltyramine, and cis-N-cafeoyltyramine isolated from Lyctum chinense reported to have anti-fungal activity in a range of 5-10 μg/mL [24a]. Three phenolic compounds, 1-galloyl-β-D-glucopyranosyl-(1-→4)-β-D-galactopyranoside, 2-methoxy-5-(1’,2’,3’-trihydroxypropyl)-phenyl-1-O-(6”-galloyl)-β-D-glucopyranoside and 2-methoxy-5-hydroxymethyl-phenyl-1-O-(6”-galloyl)-β-D-glucopyranoside together with the known compounds from the leaves of Baseonema acuminatum were reported for antifungal activity against Candida albican strains with inhibitory concentration to 50% microorganism (IC50) values in the range of 25-100 μg/mL [24b].

2.2. Flavonoids

Flavones are phenolic structures containing one carbonyl group and the addition of a 3-hydroxyl group yields a flavonol (Fig 2). Flavonoids are hydroxylated phenolic substances synthesized by plants in response to microbial infection. They have been found to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with fungal cell walls. More lipophilic nature of flavonoids may also disrupt fungal membranes [25].

Flavonoids isolated from the stem bark of Erythrina burtii [26] were reported for antifungal activity. 4-methoxy-5,7-dihydroxyflavone 6-C-glucoside (isocytisoside) from the leaves and stems of Aquilegia vulgaris showed activity against the mould A. niger [27]. Pelalostemumol (4) from Pelalostemium had strong antifungal activity against many pathogenic fungi [28]. Galangin (5), derived from the perennial herb Helichrysum aureonitens, seems to be a particularly useful compound, since it has shown activity against wide range of fungi [29]. A flavonoid from rhizome of Alpinia officinarum had strong antifungal activity against variety of pathogenic fungi. Minimum inhibitory concentration (MIC) against the fungi varied from
3 μg/mL [30,31]. A flavon 3,4',5,7-tetraacetyl quercetin isolated from heartwood of Adina cordifolia exhibited moderate antifungal activity against A. fumigatus and Cryptococcus neoformans [32]. Flavonoid derivative phloretin from Malus sylvestris have antifungal properties [33]. Isopiscerythrone (6), allolicoisoflavone A (7), piscisoflavones A (8) and B (9) from different plants were reported to be endowed with antifungal activity [34].

Heartwood extracts of Acasia auriculiformis and Acasia mangium were reported to have antifungal activity due to the compounds 3,4',7,8-tetrahydroxyflavanone and tettracidin [35]. The four compounds eupomatenoid-3, eupomatenoid-5, conocarpan and orientin, from Piper solmsianum exhibited antifungal action against all the dermatophytes tested, with MIC values in range of 2 to 60 μg/mL and with a potency as high as the standard antifungal drug ketoconazole [36]. Flavonoids, azulenes, sesquiterpenes and essential oils from Inula viscosa were proved to have a significant antifungal activity against dermatophytes even at low concentrations (0.01 mg/mL). The high concentration of the sesquiterpene
(carboxyeudesmadiene), occurring in the leaf extracts, is reported to have greater antifungal activity [37].

The 95% ethanol extract of the bark of Swartzia polyphylla afforded the flavonoids biochanin A and dihydrobiochanin A as antifungal constituent and another plant from the Fabaceae family Teramnus labialis was also reported for antifungal flavonoid [38]. The antifungal activity of a series of prenylated flavonoids purified from five different medicinal plants of moraceae family was reported antifungal against C. albicans and S. cerevisiae [39]. These results also support the use of prenylated flavonoids in traditional medicine to treat fungal infections. A. nobilis furnished several flavonoids which showed good fungicidal activity against C. cladosporioides [40]. Amentoflavone from Selaginella tamariscina exhibited potent antifungal activity against several pathogenic fungal strains but had a very low hemolytic effect on human erythrocytes [41].

2.3. Coumarins

Coumarins have been reported to stimulate macrophages which could have an indirect negative effect on infections. Coumarins are phenolic substances made of fused benzene and α-pyrene rings (Fig 3). Their fame has come mainly from their antithrombotic50, anti-inflammatory [42] and vasodilatory [43] activities and their use to prevent recurrences of cold sores caused by HSV-1 in humans 48. Hydroxycoumarin scopoletin (10) was isolated from seed kernels of Melia azedarach [44] reported to be antifungal against Fusarium verticillioides.
Tithoniamarin is a new isocoumarin dimer isolated from *Tithonia diversifolia* [45] showed antifungal and herbicidal activities. Deng and Nicholson [46] reported the antifungal properties of surangin B (11), a coumarin from *Mammea longifolia*. Phytoalexins, which are hydroxylated derivatives of coumarins, are produced in carrots in response to fungal infection and can be presumed to have antifungal activity [47]. A coumarin namely, 6,7-dimethoxycoumarin (12), isolated from *P. digitatum*-infected Valencia fruit confers resistance against the mycotoxigenic fungi *A. parasiticus* [48]. Clausenidin (13), dentatin, nor-dentatin, and carbazole alkaloid clauszoline J (14) isolated from *Clausena excavata* showed antimycotic activity (MIC 50 μg/mL). Methylated clausenidin (MIC 50 μg/mL), a synthetic coumarin, also exhibited moderate antimycotic activity [50]. Data about specific antibiotic properties of coumarins are scarce, although many reports give reason to believe that some utility may reside in these phytochemicals [51].

### 2.4. Quinones

Quinones are aromatic rings with two ketone substitutions and characteristically highly reactive. Fig 4 shows some of the important antifungal quinones. They can switch between diphenol (or hydroquinone) and diketone (or quinone) easily through oxidation and reduction reactions. These compounds, being colored, are responsible for the browning reaction in cut or injured fruits and vegetables [52]. In addition to providing a source of stable free radicals, quinones are known to complex irreversibly with nucleophilic amino acids in proteins [53]. Therefore the quinone inactivate the protein and impair there function. Quinones bind with surface-exposed adhesins, cell wall polypeptides, membrane-bound enzymes and form complex which inactivate the enzymes.

In the anthraquinone group, there are only a few reports concerning their antifungal activity. Schmidt *et al.* [54] reported the antifungal activity of the major anthraquinone aglycones, alizarin (15) and emodin (16) of *Rubia tinctorum* and *Rhamnus frangula*. Hypericin (17), from *Hypericum perforatum*, known as an antidepressant and Duke reported in 1985 that it had general antimicrobial properties [14]. Examples of other antifungal anthraquinones from medicinal species also included a new 1,3-dihydroxy-2-methyl-5,6-dimethoxyanthraquinone (18) from the roots of *Prismatomeris fragrans* [55]. The naphthoquinones kigelinone (19), isopinnatal, dehydro-alpha-lapachone, and lapachol from *Kigelia pinnata* were reported for antifungal activity [56].
A novel compound 11-hydroxy-16-hentriacontanone isolated from *Annona squamosa* was reported for its antifungal potential [57]. Hypericum, an anthraquinone extracted from *Hypericum perforatum* showed antifungal activity [58]. The 2-hydroxy-1,4-naphthoquinone (Lawson) (20) from *Lawsonia inermis* were found to exhibit strong fungitoxicity [59]. Emodin, physcion and rheins were isolated from *Cassia tora* showed strong fungicidal activity against the microorganism tested [60]. Hopeanolin MIC value range 0.1-22.5 μg/mL, an unusual resveratral trimer with an O-quinone nucleus, from the stem bark of *Hopea exalata* is reported to have antifungal activity [61].

### 2.5. Saponins

Saponins are secondary metabolites that occur in wide range of plant species (Fig 5). They are stored in plant cells as inactive precursors but are readily converted into biological active antibiotics by enzymes in response to pathogen attack. Saponins are glycosylated compounds widely distributed in plant kindom and can be divided into three major groups, a triterpenoid, a steroid or a steroidal glycoalkaloid. CAY-1, a triterpene saponin from the *Capsicum frutescens* was found to be active against sixteen different fungal strains, including *Candida* spp, *A. fumigatus* and *C. neoformans* [62]. Importantly, CAY-1 appears to act by disrupting the membrane integrity of fungal cells.

Recently, steroidal saponins ypsilandroside B, ypsilandroside A, iso ypsilandroside A, iso ypsilandroside B and isoypsilandrogaine isolated from *Ypsilandra thebetica* were reported for antimicrobial activities by [63]. Two new spirostanol saponins were isolated from the roots of *Smilax medica*,
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Together with the known smilagenin 3-O-β-D-glucopyranoside (21) exhibited antifungal activity against the human pathogenic yeasts C. albicans, C. glabrata and C. tropicalis in a range of 6.25-50 μg/mL [64]. Mollugo pentaphylla, a tropical herb, contains an antifungal saponin, mollugogenol-A (22) [65]. Phytolaccosides B (23) and E (24) from Phytolacca tetrameria showed antifungal activities against a panel of human pathogenic opportunistic fungi [66]. Novel spirostan saponins together with three known saponins were reported for antimycotic activity. The most active compound was found to be 6α-O-[β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl]-(25,S)-5α-spirostan-3β-ol, with IC₅₀ values of 25 μg/mL against T. mentagrophytes and T. rubrum [67]. From Solanum species, Solanum chrysotrichum five new spirostan saponins showed antimycotic activity against T. mentagrophytes, T. rubrum, A. niger and C. albicans. Another compound isolated from same plant, 6-α-O-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl-(25R)-5α-spirostan-3β,23α-ol was reported to be active in a rage of 12.5 to 200 μg/mL against T. mentagrophytes, T. rubrum, A. niger and C. albicans [68]. Recently, saponins isolated from Alternanthera tenella is reported to have strong antifungal in the range varied from (MIC) 50-500 μg/mL [69].

Bioassay-guided fractionation of the ethanol extracts of the aerial parts of the Tibetan medicinal herb Clematides tangutica led to the isolation of two new antifungal triterpene saponins 3-O-α-L-arabinopyranosyl hederagenin 28- O-α- L-rhamnopyranosyl ester and 3-O-β-D-glucopyranosyl-(1→4)-α-L-arabinopyranosyl hederagenin 28-O-α-L-rhamnopyranosyl ester (MIA 2.5 μg/disc) [70]. Two dammarane saponins from the stems of Anomospermum grandifolium jujubogenin 3-O-α-l-arabinofuranosyl(1→2)-[β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl(1→3)]-α-l-arabinopyranoside, ujubogenin 3-O-α-l-arabinofuranosyl(1→2)-[6-O-[3-hydroxy-3-methylglutaryl]-β-D-glucopyranosyl(1→3)]-α-l-arabinopyranoside and a new lupane saponin, 3β-hydroxylup-20(29)-ene-27,28-dioic acid 28-O-β-D-glucopyranosyl(1→2)-[β-D-xylopyranosyl(1→3)]-β-D-xylopyranosyl(1→2)-β-D-glucopyranoside ester, jujubogenin 3-O-α-l-arabinofuranosyl(1→2)-[β-D-glucopyranosyl(1→3)]-α-l-arabinopyranoside and 3β-hydroxylup-20(29)-ene-27,28-dioic acid revealed antifungal properties against C. albicans [71].

From the rhizomes of Dioscorea cayenensis, the dioscin (25) exhibited antifungal activity against the human pathogenic yeasts C. albicans, C. glabrata and C. tropicalis [72]. Three antifungal steroidal saponins were isolated from the root of Smilax medica [73, 74]. Saponins, named minutoside A, minutoside B, minutoside C, sapogenins, alliogenin and neoagigenin, were isolated from the bulbs of Allium minutiflorum showed promised antifungal activity [75].
Nineteen saponins from *Medicago sativa*, *M. murex*, *M. arabica* and *M. hybrida*, were reported to be active against three dermatophytic fungi *Microsporum gypseum*, *T. interdigitale* and *T. tonsurans* [76].
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Two saponins from *Tribulus terrestris* were reported for promised antifungal activity against fluconazole resistant *Candida* strains (MIC 0.15 mg/mL) [77,78]. Tigogenin-3-O-β-D-xylopyranosyl (1-->2)-[β-D-xylopyranosyl (1-->3)]-β-D-glucopyranosyl (1-->4)-[α-L-rhamnopyranosyl (1-->2)]-β-D-galactopyranoside was reported to have *in vivo* activity in *C. albicans* vaginal infection model. Another saponin from same plant, tigogenin-3-O-β-D-glucopyranosyl (1-->2)-[β-D-xylopyranosyl (1-->3)]-β-D-glucopyranosyl (1-->4)-β-D-galactopyranoside was found to be *in vitro* very effective against several pathogenic *Candida* species (*MIC*80 = 4.4, 9.4 μg/mL), *C. neoformans* (*MIC*80 =10.7, 18.7 μg/mL) and inherently resistant *C. krusei* (*MIC*80 = 8.8, 18.4 μg/mL [79]. The Avenacin obtained from the *Avena sativa* showed varying degree of *in vitro* antifungal activity [80]. Other antifungal saponins from medicinal plants also included *Astragalus verrucosus* [81], *A. suberi* [82], *A. auriculiformis* [83] and *Hedera taurica* which possessed *in vitro* antifungal activity against *C. albicans*, *C. krusei* and *C. tropicalis* [84]. Saponins from several plants i.e *Hedera colchica* [85], *Kalopanax pictus* [86], *Dracaena mannii* and *D. arborea* [87], *Trillium grandiflorum* [88] and *Solidago virgaurea* [89] were reported for antifungal activity.

### 2.6. Xanthones

Xanthones are a restricted group of plant polyphenols, biosynthetically related to the flavonoids. These are planar-six carbon molecules in a conjugated ring system consisting of a backbone molecule and various chemical groups attached to it. Xanthone backbone consists of two benzene rings attached through a carbonyl group and oxygen not allowing free rotation about the carbon-Zcarbon bonds. The unique backbone along with type and position of the attached chemical groups defines specific properties of xanthones. Xanthones possess numerous bioactive capability including antifungal properties (Figure 6).

Caledonixanthone E (26) isolated from the stem bark of *Calophyllum caledonicum* was reported for strong antifungal activity (MIC80 ¼ 8 mg/mL) [90]. Isoprenylated xanthones, toxyloxanthone C (27), and wighteone (28) showed antifungal activity against *C. albicans* with MIC values of 25 and 12.5 mg/mL, respectively [91]. The dichloromethane extract of *Securidaca longipedunculata* yielded 1,7-dihydroxy-4-methoxyxanthone (29) which exhibited antibacterial activity against Staphylococcus aureus and antifungal activity against *A. niger, A. fumigatus*, and a *Penicillium* species [92]. 1,3,6-Trihydroxy-2,5-dimethoxyxanthone (30) isolated from the aerial part of *Monmina obtusifolia* was reported to have antifungal potential [93]. Seven xanthanolides from *Xanthium macrocarpum* were reported to be effective
against *C. albicans*, *C. glabrata*, and *A. fumigatus* [94,95]. Two new 2-hydroxy-3-methylbut-3-enyl-substituted xanthones, -caledol and -dicaledol, were isolated from a dichloromethane extract of the leaves of *Calophyllum caledonicum* and have been reported for antifungal activity against *A. fumigatus* [96]. Xanthones from the green fruits of *Garcinia mangostana* were reported to have strong antifungal activities [97]. *Cudrania fruticosa* yielded an isoprenylated xanthone, cudrafrutixanthone which showed antifungal activity against *C. albicans* [98, 98]. Xanthone analogues bearing the basic chain of butenafine were reported for significant activity against *C. neoformans* (1.5 mg/mL) [100].

### 2.7. Terpenoids and essential oils

A large number of studies have been done in recent years on the antifungal activity of terpenoids of natural origin (Fig 7). These reports concern mainly sesquiterpenes and sesquiterpene lactones. The fragrance of plants is carried in essential oil fraction. These oils are secondary metabolites that are highly enriched in compounds based on an isoprene structure. They are called terpenes, their general chemical structure is C$_{10}$H$_{16}$, and they occur
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as diterpenes, triterpenes, and tetraterpenes (C\(_{20}\), C\(_{30}\), and C\(_{40}\)), as well as hemiterpenes (C\(_{5}\)) and sesquiterpenes (C\(_{15}\)). The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic nature. Mendoza et al. [101] found that increasing the hydrophilicity of kaurene diterpenoids by addition of a methyl group drastically reduced their antimicrobial activity.

A number of terpenes or terpenoids are reported active against fungi [102-104]. In 1977, it was reported that 60% of essential oil derivatives examined to date were inhibitory to fungi while 30% inhibited bacteria [105]. The antifungal activities of the essential oil from *Agastache rugosa* and its main component, estragole (31), combined with ketoconazole, were reported to show significant synergistic effects [106]. A known sesquiterpene lactone, encelin (32), isolated from the Mexican species *Montanoa speciosa* has a determining action on growth and the morphogenetic process of fungal cells [107]. The roots of *Delphinium denudatum* have yielded 8-acetylheterophyllisine, panicutine, and 3-hydroxy-2-methyl-4H-pyran-4-one (33) has shown antifungal activity against a number of human pathogenic fungi [108]. *Khaya ivorensis* afforded methyl angolensate and 1,3,7-trideacetylkhivorin displayed antifungal activity, with 62.8 and 64% mycelial growth inhibition at 1000 mg/L, respectively [109]. Estragole and the essential oil of *A. rugosa* exhibited strong activities against the tested fungi and showed synergism with ketoconazole against *B. capitatus* [110]. From other *Centauraea* species, *C. thessala* and *C. attica*, two eudesmanolides, 4-epi-sonchucarpolide, 8-(3-hydroxy-4-acetoxy-2-methylene-butanoyloxy) derivative and eudesmane derivative named atticin (35) showed a considerable antifungal effect against nine fungal species [111]. Two new dammarane type triterpenes, ailexcelone and ailexcelol from *Ailantus excelsa* were reported to be endowed with antifungal activities [112]. Amesterol, isolated from *Amaranthus viridis* strongly inhibited a growth of pathogenic fungus [113]. Two diterpenes isolated by Batista et al. [114] were found to be active against *Candida* spp. Terpenoid isolated from *Atrus sinensis*, *Armoracia rusticana*, *Metha peperita* [115] and grapefruit showed antifungal activity [116]. *A. sativum* oil exhibited the strongest inhibition of growth of *T. rubrum*, *T. erinacei*, and *T. soudanense* with MIC of 4.0 μg/mL, while the activities of *A. cepa* and *A. fistulosum* were relatively mild [117]. The bark extract of *Drimys brasiliensis* led to the isolation of the sesquiterpene polygodial, 1-β-(p-methoxycinnamoyl)-polygodial (36), drimanial and 1-β-(p-cumaroyloxy)-polygodial which were selectively active against fungi [118]. An antimicrobial diterpene 8β-17-epoxylabd-12-ene-15, 16-dial from *Alpinia galanga* synergistically enhanced the antifungal activity
of quercetin and chalcone against *C. albicans* [119]. Triterpenoid glycosides obtained from *Solidago virgaurea* and *Bellis perennis* inhibit the growth of human-pathogenic yeasts (*Candida* and *Cryptococcus* species) [120].

The oil from leaves of *J. oxycedrus* spp. oxycedrus was reported antifungal with MIC and MLC values ranging from 0.08-0.16 μM/L and 0.08-0.32 μM/L, respectively. The chemical constituents of the essential oil extracted from the fruits of *Lindera glauca* have epishybunol acetate, caryophyllene oxide and 3,6,6-trimethyl-2-norpinene exhibited more manifest antifungal properties with MIC between 0.03-0.5 mL/L for pathogenic fungi species [121]. The essential oil from the leaves of *Litsea cubeba* have α-cis-ocimene,3,7-dimethyl-1,6-octadien-3-ol and n-transnerolidol had manifest antifungal activities with MIC between 0.03-0.4 μL/mL for utilized pathogenic fungi and 1.0-2.0 μL/mL for moulds [122,]. The oil of the leaves, clemateol, and the alcohol from *Calea clematidea* showed a moderate antifungal activity [123]. *Daucus carota* (Apiaceae), afforded four sesquiterpene daucane esters [124] found to contain a range of low antifungal activity against *Fusarium oxysporum* and *A. niger*. Carotol, which was observed to be the main constituent of carrot seed, inhibited the radial growth of fungi by 65%. The *Vernonanthura tweedieana* afforded one
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antifungal active sesquiterpene, 6-cinnamoyloxy-1-hydroxyeudesm-4-en-3-one [125]. Barrero et al. [126] investigated six Centaurea species: C. bombycina, C. granatensis, C. monticola, C. incana, C. maroccana and C. sulphurea of Asteraceae family. The sesquiterpene lactones costunolide and dehydrocostunolide showed noticeable IC_{50} values. Other antifungal sesquiterpene lactones from the Asteraceae family also included those isolated from Ajania fruticulosa [127].

A fruit pulp extract of Detarium microcarpum endowed with four new clerodane diterpenes which showed antifungal activity [128]. The diterpenoids 16α-hydroxy-cleroda-3,13-(14)-Z-diene-15,16-olide and 16-oxo-cleroda-3,13-(14)-E-diene-15-oic acid isolated from the hexane extract of the seeds of Polyalthia longifolia demonstrated significant antifungal activity [129]. Five new diterpenoids from Casimirella namely, humirianthone, 1-hydroxy-humirianthone, 15R-humirianthol, patagonol and patagonal showed activity against pathogenic fungi [130]. Antifungal activity of oxygenated pimarane diterpenes from Kaempferia marginata was reported by Thongnest et al. [131]. The triterpenoids pristimerin and celastrol isolated from the roots of Celastrus hypoleucus exhibited inhibitory effects against diverse pathogenic fungi [132]. Oleanane triterpenoid, triterpenetetrol isolated from the chloroform extract of the aerial parts of Leontodon filii were reported to have antifungal prop [133]. Carvone, dinydrocarvone, limonene, dillapiole and dillapional from Anethum sowa revealed antifungal activity at a concentration of 1:100 and 1:250 [134,135,136]. A derivative of dillapiole, isodilapiole tribromide found to more active [137]. The steam distillate of fresh mature leaves containing odorous oil rich in cyclic tri-and tetra-sulphides of C3, C5 and C9 units exhibited antifungal activity at 125 μg/mL in vitro [139]. The active compounds, 1'-acetoxychavicol acetate from Alpinia galanga strong inhibitory affects at a MIC 0.024 μg/mL against several fungal pathogens [140 a,b]. Most of the species of Oscimum showed in vitro antifungal activities against a broad range of fungi as well as bacteria. An Indian chemotype Ocimum gratissimum, with a high level of ethyl cinnamate, presents, in vitro, an interesting spectrum of antifungal properties [141].

2.8. Alkaloids

Heterocyclic nitrogen compounds are called alkaloids (Fig 8 A-B). The first medically useful example of an alkaloid was morphine; isolated in 1805 from the opium poppy Papaver somniferum [142,]. Codeine and heroin are both derivatives of morphine. Diterpenoid alkaloids, commonly isolated
from the plants of the Ranunculaceae [143, 144] are found to have antimicrobial properties [145]. While alkaloids have been found to have microbiocidal effects including against *Giardia* and *Entamoeba* species [146], the major antidiarrheal effect is probably due to their effects on transit time in the small intestine.

Recently, a novel alkaloid, 2-(3,4-dimethyl-2,5-dihydro-1*H*-pyrrol-2-yl)-1-methylethyl pentanoate (38) was isolated from the plant *Datura metel* showed *in vitro* as well as *in vivo* activity against *Aspergillus* and *Candida* species [147]. Another novel alkaloid, 6,8-didec-(1*Z*)-enyl-5,7-dimethyl-2,3-dihydro-1*H*-indolizinium (37) from *Aniba panurensis* demonstrated the activity against a drug-resistant strain of *C. albicans* [148]. The antifungal alkaloids β-carboline, a tryptamine- and two phenylethylamine-derived alkaloids and *N*-methyl-*N*-formyl-4-hydroxy-beta-phenylethylamine (39) from *Cyathobasis fruticulosa* [149] and haloxylines A and B, new piperidine from *Haloxylon salicornium* displayed antifungal potentials [150]. Jatrorrhizine (40) from *Mahonia aquifolium* was found to be the most effective against all fungal species tested (MIC ranges from 62.5 to 125 μg/mL), while the crude extract, berberine, and palmatine exhibited only marginal activity (MIC 500 to >/= 1000 μg/mL) [151].

Cocsoline (43), a bisbenzylisoquinoline alkaloid from the *Epinetrum villosum* displayed antifungal activities [152]. The alkaloids *N*-methylhydrasteine hydroxylactam and 1-methoxyberberine chloride from *Corydalis longipes* showed high efficacy individually [153]. Four alkaloids, dicentrine (41), glaucine (42), protopine, and alpha-allocryptopin (46) from *Glaucium oxylobum* exhibited good activity against *Microsporum gypseum*, *Microsporum canis*, *T. mentagrophytes* and *Epidermophyton floccosum* [154].
Plants derived anti-fungal agents

Flindersine (45) and haplopine from *Haplophyllum sieversii* were growth-inhibitory compounds against various fungi [155]. Canthin-6-one and 5-methoxy-canthin-6-one of *Zanthoxylum chiloperone* var. angustifolium exhibited antifungal activity against *C. albicans*, *A. fumigatus* and *T. mentagrophytes* [156]. Frangulanine, a cyclic peptide alkaloid and waltherione A, a quinolinone alkaloid from leaves of *Melochia odorata* were reported to exhibit antifungal activities against a broad spectrum of pathogenic fungi [157].

The indole alkaloid venenatine exhibited antifungal activity against all the 10 tested fungi, showed an especially high sensitivity towards this compound, exhibiting germination levels below 10% [158]. From the root bark of *Dictamnus dasycarpus* two antifungal furquinoline alkaloids were isolated. 3-methoxysampanagine from *Cleistopholis patens* exhibited significant antifungal activity against *C. albicans*, *A. fumigatus*, and *C. neoformans* [159].

![Chemical structures](image)

2.9. Lectins and polypeptides

Peptides, which are inhibitory to microorganisms, were first reported in 1942 [160]. They are often positively charged and contain disulfide bonds [161]. Their mechanism of action may be the formation of ion channels in the microbial membrane [162] or competitive inhibition of adhesion of microbial
proteins to host polysaccharide receptors [163]. Recent interest has been focused mostly on studying fungi by these macromolecules, such as that from the herbaceous *Amaranthus*, has long been known [164]. Thionins are peptides commonly found in barley and wheat and consist of 47 amino acid residues. Thionins AX1 and AX2 from sugar beet were active against fungi but not bacteria [165, 166].

Raw *Allium Sativum* extract showed antifungal activity beyond 48h in extract stored at room temperature. Antifungal activity was stable upto 8h under these conditions [167]. A novel lectin was isolated from the roots of *Astragalus mongholicus* [168] and a protein with a novel N-terminal sequence from *ginger* rhizomes exerted antifungal activity towards various fungi [169]. An antifungal peptide was reported from fresh fruiting bodies of the mushroom *Agrocybe cylindracea* [170]. Two antifungal peptides from seeds of *Pharbitis nil* exhibited potent antifungal activity against both chitin-containing and non-chitin-containing fungi in the cell wall. Concentrations required for 50% inhibition of fungal growth were ranged from 3 to 26 micrograms/mL and from 0.6 to 75 μg/mL [171].

From *Phaseolus* species, mung bean (Phaseolus mungo) seed, chitinase with antifungal activity was isolated [172]. This species also yielded a novel lysozyme exhibiting antifungal activity toward *Botrytis cinerea* [173]. Another Fabaceae species, *Trigonella foenum-graecum*, yielded defensins, small cysteine rich peptides, which exhibited antifungal activity against the broad host range fungi [174]. A novel antifungal peptide, cucurmoschin, was isolated from the seeds of the black pumpkin inhibited mycelial growth in the fungi [175]. A peptide designated cicerarin from seeds of the green chickpea *Cicer arietinum* showed antifungal activity. The antifungal activity was preserved after exposure to 100 degrees C for 15 min [176]. Two other antifungal peptides cicerin and arietin were reported from seeds of the chickpea (*Cicer arietinum*). Arietin manifested a higher antifungal potency toward *Mycosphaerella arachidicola, Fusarium oxysporum* and *Botrytis cinerea* [177]. An antifungal peptide designated angularin was isolated from the adzuki bean exhibited antifungal activity against a variety of fungal species [178]. Two novel antifungal peptides, designated alpha- and beta-basbrubrins, respectively, were isolated from seeds of the Ceylon spinach *Basella rubra* [179].

An antifungal protein, AFP-J, was purified from potato tubers, *Solanum tuberosum* strongly inhibited yeast fungal strains, including *C. albicans, Trichosporon beigeli*, and *S. cerevisiae* [180]. Pineapple leaf chitinase-B from *Ananas comosus* exhibits strong antifungal activity toward *Trichoderma virida* [181]. Another chitinase with antifungal activity was also purified from the bulbs of the plant *Urginea indica*, known as Indian squill.
The protein was an active growth inhibitor of the fungal pathogens in an \textit{in vitro} assay [182]. A novel protein was isolated from the Chinese herb \textit{Astragalus mongholicus} Bunge. It exerted selective antifungal activity against various fungi [183]. Recently, a new protein from \textit{E. coli}, having anti-\textit{Aspergillus} activity was reported by Yadav et al. [184]. Antifungal peptides and proteins from medicinal species also included two chitin-binding proteins from spindle tree \textit{Evonymus europaeus} [185], a thaumatin-like protein from banana \textit{Musa acuminate} [186], and a protein from ginger rhizomes \textit{Zingiber officinalis} (Zingiberaceae), which exerted antifungal activity toward various fungi [187].

\subsection*{2.10. Other compounds}

Many phytochemicals not mentioned above have been found to exert antifungal properties. This review has attempted to focus on reports of chemicals, which are found in multiple instances to be active. There are reports of chemical having antifungal properties associated with several different classes not covered above (Figure 9).
N-trans-feruloyl-4-methyldopamine (47) recently isolated from *Achranthes ferruginea* was reported to be active against a broad range of fungi [188]. Leaves of *Piper aduncum* accumulate the anti-fungal chromenes, methyl 2,2-dimethyl-2H-1-chromene-6-carboxylate and methyl 2,2-dimethyl-8-(3'-methyl-2'-butenyl)-2H-1-chromene-6-carboxylate [189]. Iridodial β-monoenol acetate, from *Nepeta leucophylla*, and actinidine from *N. clarkei* were found to be endowed with antifungal activities [190]. Anofinic acid and fomannoxin acid (48) from *Gentiana alpidea* were found to be active against fungi [191]. The antifungal activity of *Artemisia herba-alba* was found to be associated with two major volatile compounds as carvone and piperitone [192]. The phenylpropanoids p-coumaric acid and ferulic acid from *Kigelia pinnata* were observed to have antifungal activity [193]. *Piper cubeba* afforded the compounds (8R,8'R,9'S)-5-methoxyclusin, (-)-clusin, (-)-yatein, ethoxyclusin, and (-)-dihydroclusin showed very potent and selective inhibitory activity against CYP3A4 with IC$_{50}$ values (0.44-1.0 μM) identical to that of the positive control, ketoconazole (IC$_{50}$, 0.72 μM) [194].

Demethoxyageratochromene (49) from *Ageratum conyzoides* showed antifungal activity [195,196] at a concentration of 2000 ppm. The leaf extract was found to be active and showed strong toxicity against the fungi causing ringworm [197]. 2’,4’-Dihydroxy-3’-methoxychalcone (50) and 2’,4’-dihydroxychalcone (51) in the dichloromethane extracts of *Zuccagnia punetata* was found to have antifungal properties [198]. Plumbagin from *Plumbago zeylanica* was also reported to inhibit the fungel species viz. *Trichophyton*, *Epidermophyton*, *Microsporum* in a range of 30-40 μg/mL [199]. Hexane extracts of the stem bark as well as (-)-kaur-16-en-19-oic acid of *Annona glabra* revealed antifungal activity [200]. Isoalantolactone (52) and alantolactone (53), the major constituents of the roots of *Inula racemosa* showed in vitro antifungal activity against *T. mentagrophytes* and *Microsporum canis* [201]. Withaferin A and amemoneine isolated from *Withania somniferm* and *Anemone pulsatillea*, salicin a phenolic glucoside compound from *Salix alba* showed antifungal activity [202]. The psoralen, 8-methoxypsoralen and imperatorin in extracts of leaf, fruit, stem, bark and root of *Zanthoxylum americanum* demonstrated a broad spectrum of antifungal activity and inhibited fungal species in a disk diffusion assay [203]. *Myrothecium roridum* contains macrocyclic trichothecenes of the verrucarin type, 16-hydroxyverrucarin A and verrucarin X exhibited moderate antifungal activity. Both compounds were reported to preferentially inhibit in vivo protein biosynthesis [204].
3. Conclusion

Phytomedicines are major component of traditional system of healing in developing countries, which have been an integral part of their history and culture. Besides widespread use of botanicals as medicinal products in developing countries, such products are becoming part of the integrative healthcare system of industrialized nations, known as complementary and alternative system of medicines (CAM).

Existing costly therapy is not affordable well for the millions of individuals particularly in the developing world. Plant extracts are the cheap and easily available source to poor people. Plants are a great source of thousands new useful phytochemicals of great diversity, which have inhibitory effects on all types of microorganisms \textit{in vitro}. Till date more than 600 plants have been reported for their antifungal properties, however a few of them were explored for the active components. The current pharmaceutical armoury of antifungal is a clear cause for satisfaction, not from gloom. However, we still do not have agents that fulfill every one of the criteria that a physician would set as desiterata for antifungal drugs. They need to be active against those fungai causing infections which we can’t yet depend on eradacting. They need to be formulated for both oral and parenteral administration, they need to be extremely safe and as cheap as possible. The search for new antifungal agents therefore must go on.

Identification of new chemotypes for drug development remains an urgent need in antifungal therapeutics. Simultaneously, a number of antifungal compounds reported till date, are tested for their \textit{in vitro} activities not for \textit{in vivo} activities. \textit{In vivo} and \textit{in vitro} activities of a compound may be different and a very small number of plants extracts or components have been studied for their \textit{in vivo} activity. Therefore these should be subjected to animal and human studies to determine their effectiveness in whole-organism systems. Also \textit{in vitro} testing and method of extraction should be standardized so that the search could be more systematic.

The current set of clinically available antifungal agents includes three classes of natural product and four classes of synthetic chemicals. We therefore can’t abandon interest in biodiversity as a source of natural antifungal products. Furthermore, the inactive plant extracts may be subjected to chemical diversification of their components to increase the activity. The transformation of chemical groups in natural products into rare chemical groups is possible which are rarely produced by secondary metabolism. Therefore, biosynthesis machinery can be complemented to produce a whole range of new semisynthetic compounds in one step which may become an alternative source of compounds to feed the discovery process for new interesting compounds.
The study of alternative mechanisms of infection prevention and treatment is essential. Plant products furthermore may be structurally modified to increase their in vivo activity. For example isodilapiole tribromide, a derivative of dillapiole was found to more active. Another example include echinacandin type peptide FR901379, chemical modification of which lead toward more active FK463 compound. Therefore, attention toward the plant derived principles, their chemical modification and chemotherapeutic potential is needed.

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