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Review

A review on bioactive compounds isolated from plants against plant pathogenic fungi

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Plant-derived compounds are regarded as a substantial source for novel lead structures to develop medicines and biocides natural products. Concurrent with greater awareness towards the use of synthetic chemicals in agricultural practice, the application of integrated pest management programs has also increased. In recent years, there has been considerable public pressure to reduce the use of synthetic fungicides in agriculture. Although, the use of synthetic fungicides in plant disease control has been successful in improving agricultural output, several of these have been found to exhibit side-effects in the form of carcinogenicity, detrimental effects and other residual toxicities. The alternative choice therefore would be the use of botanical fungicides, which are found to be largely non-phytotoxic, systematic and easily biodegradable in nature. The present study is a summary of review literature during past decades which focused on bioactive compounds isolated from plants against plant pathogenic fungi.

Key words: Bioactive compounds, secondary metabolites, plant pathogenic fungi, plant extracts.

INTRODUCTION

Bioactive compounds or plant secondary metabolites (SMs) consist of low-molecular weight compounds that are regarded as not essential for sustaining life, but as crucial for the survival of the producing organism (Hadacek, 2002). More than 50,000 structures have been identified in plants by NMR, MS and X-ray analysis. However, as only less than 20% of all plants have been studied, it is very likely that the actual numbers of secondary metabolites (SMs) or bioactive compounds in the plant kingdom would exceed 100,000 structures (Wink, 2006). SMs are produced in specific pathway and sites of synthesis can differ between types of compounds and between plant species. Furthermore, some compounds can be produced by all tissues, whereas others are produced in a tissue or even cell-specific fashion.

The site of synthesis for SMs is not necessarily the site of accumulation. Hydrophilic compounds (such as alkaloids, flavonoids, tannins, and saponins) are stored in the vacuole while the lipophilic SMs (such as terpenoids) are sequestered in resin ducts, laticifers, oil cells, trichomes, or in the cuticle. Bioactive compounds affect the fungi via interference with molecular targets in their organs, tissues and cells. The major targets include: Biomembrane, proteins and nucleic acids. Bioactive compounds are still regarded as a valuable pool for discovering novel mode of action (Engelmeier and Hadacek, 2006).

The cost for the development of a biocide as well as the formulation of their application is currently estimated to be about USD150. Despite good plant protection practices, the use of fungicides is still regarded as crucial to maximize yields (Hewitt, 2000). To lower biocide input, attention has shifted from discovering novel fungicides with known mode of actions to the identification of novel modes of fungicidal activities (Engelmeier and Hadacek, 2006).

In recent years, public pressure to reduce the use of

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synthetic fungicides in agriculture has increased. Concerns have been raised about both the environmental impact and the potential health risks related to the use of these compounds. Hence, there is a great demand for novel antifungals belonging to a wide range of structural classes, selectively acting on new targets with fewer side effects (Abad et al., 2007). Every year substantial crop damage is caused by various diseases and among them fungal diseases is very common. According to current estimates, about 10 to 20% of staple foods and cash crops are destroyed by plant pathogens (Hewitt, 2000).

MAJOR GROUPS OF ANTIMICROBIAL COMPOUNDS

Plants produce a wide variety of bioactive metabolites which serve as plant defense mechanisms against pests. Some SMs give plants their odors (terpenoides), some are responsible for plant pigments (quinines and tannins) and others (e.g., some of terpenoids) are responsible for plant flavor. These antimicrobial bioactive compounds are divided by Cowan (1999) into 5 main classes consisting: Terpenoids and essential oils; phenolics and polyphenols; alkaloids; polypeptides and mixtures (crude extract).

Essential oils

Essential oils, volatile oils or simply the "oil" of the plant from which they were extracted, such as “oil of lemongrass” are hydrophobic liquids containing volatile aroma compounds extracted from vegetal materials using steam or hydro distillation techniques. Most of these volatile natural products belong to monoterpenoids compounds (Hanson, 2003). The essential oils are important because of their antibacterial, antifungal, antioxidant and anti-carcinogenic properties (Tzortzakis, 2007).

Antifungal activity of volatile components extracted from leaves, stems and flowers of *Lantana camara, Malvaviscus arboreus* and *Hibiscus rosa-sinensis* were tested against *Alternaria solani, Botrytis cinerea, Fusarium solani f. sp. cucurbitae, F. oxysporum f. sp. niveum, Pythium ultimum, Rhizoctonia solani* and *Verticillium dahlia* (Boughalleb et al., 2005). The results demonstrated that volatile components from flowers have stronger antifungal activity than extracts from stems or leaves against all fungi tested, except for *P. ultimum.* Volatile components extracted from the flowers of *L. camara* at concentration of 100 mg/ml, showed the strongest antifungal effect (38%) against tested fungi. However, *P. ultimum* was not affected by the extracts of any of the four plants tested (Boughalleb et al., 2005).

Although *pythium* is classified as a fungus, it is not regarded as a true fungus in the modern classifications systems. Oomycete belongs to the heterokonts including diatoms and brown algae and share similar characteristics with plants (Engelmeier, 2006). As the target of many classic fungicides is the biosynthesis of ergosterol, they fail to affect Oomycete fungal pathogens because their cell walls are made of cellulose, such as those of higher plants (Deacon, 1997).

Tzortzakis and Economakis (2007) investigated the antifungal activity of lemongrass (*Cymbopogon citratus*) oil against *Colletotrichum coccodes, Botrytis cinerea, Cladosporium herbarum, Rhizopus stolonifer* and *Aspergillus niger*. The results showed that fungal spore production was inhibited up to 70 to 100% at 25 to 500 ppm of lemongrass oil concentration. However, lemongrass oil (up to 100 ppm) accelerated spore germination for *A. niger*.

Ranasinghe et al. (2002) reported that essential oils of *Cinnamomum zeylanicum* and *Syzygium aromaticum* at concentration of 0.03 to 0.11% (*v/v*) exhibited strong antifungal activity against *F. proliferatum, Lasiodiplodia theobromae* and *Colletotrichum musae*, the causal agents responsible for crown rot and anthracnose of banana.

Screening of essential oil from 30 species of higher plants against *Penicillium italicum* causing blue mould rot of mandarins was carried out by Dixit et al. (1995). The essential oil of *Ageratum conyzoides* exhibited the strongest effect against mycelial growth of *P. italicum*. The MIC of the oil was found to be 0.2% at which level the oil exhibited fungistatic nature.

Chang et al. (2008) investigated the antifungal activity of essential oil and its constituents from *Calocedrus macrolepis* var. *formosana* on the growth of plant pathogenic fungi. Their experiments showed that sesquiterpenoid components were more effective than monoterpenoid components of the leaf oil. These results revealed that T-muurolol and a-cadinol possess antifungal activities against a broad spectrum of tested plant pathogenic fungi. These two compounds strongly inhibited the growth of *Rhizoctonia solani* and *Fusarium oxysporum*, with the IC50 values < 50 µg / ml. These compounds also efficiently inhibited the mycelial growths of *Colletotrichum gloeosporioides*, *Pestalotiopsis funerea*, *Ganoderma australe* and *F. solani* (Chang et al., 2008).

The inhibitory effect of essential oil from *Satureja hortensis* against *Aspergillus parasiticus* as an aflatoxins producer was investigated by Razzaghi-Abyaneh et al. (2008). They found that both carvacrol and thymol compounds were able to significantly inhibit fungal growth and AFB1 and AFG1 production at concentrations from 0.041 to 1.32 mM. The IC50 values for growth inhibition were 0.79 and 0.86 mM in methanol for carvacrol and thymol, while for AFB1 and AFG1, it was 0.50 and 0.06 mM for carvacrol and 0.69 and 0.55 mM for thymol (Razzaghi-Abyaneh et al., 2008).

In another experiment reported by Dikbas et al. (2008),

antifungal activity of essential oil from *Satureja hortensis* were also tested against *Aspergillus flavus*. The results of *in vitro* assay indicated that the oil of *S. hortensis* at 6.25 µl/mL had fungicidal effect against *A. flavus*. The results of *in vivo* assay on lemon fruits under storage conditions showed, the concentrations of 6.25 µl/mL applied before 8 days of pathogen inoculation had significant antifungal activity even at the end of the 20^{th} days (Dikbas et al., 2008).

Wilson et al. (1997) evaluated 49 essential oils for their antifungal activity against *B. cinerea.* Of all the essential oils tested, *Cymbopogon martini*, *Thymus zygis*, *Cinnamomum zeylanicum* and *Eugenia caryophyllata* demonstrated the most antifungal activity against *B. cinerea.*

Mixture (crude extract)

Initial screenings of plants for possible antimicrobial activities usually begin with crude aqueous or alcohol extractions, followed by various organic fractionation methods. The choice of extraction procedure depends on the nature of the source material and the compounds to be isolated (Hanson, 2003). Since most of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction (Cowan, 1999). The present review on plant secondary metabolites against plant pathogenic fungi showed that most investigations were performed by crude extract instead of specific fractions. Advantages of using crude extract are the additive or synergetic effect of the mixtures, the increase in the antimicrobial spectrum of the extract and the decreased risk for pathogen resistance to mixture.

With regard to the foregoing, Tahany et al. (2010) investigated antimicrobial activity among combination mixtures of six active constituents isolated from *Moringa pregrina, Achillea fragrantissima* and *Coleome droserifolia*. In this experiment synergistic combination mixtures were detected against different pathogens, indicating the high efficacy of combination mixtures over monotherapy treatments.

Ethanolic extract of 40 higher plants representing 23 families were studied by Begum et al. (2007) for antifungal activity against 6 phytopathogenic fungi (*Alternaria alternata*; *Curvularia lunata*; *Fusarium equiseti*; *Macrophomina phaseolina*; *Botryodiplodia theobromae* and *Colletotrichum corchori).* The results revealed that the two most active plants extract with antifungal potential were *Acorus calamus* and *Piper betel*. The rhizome extract of *A. calamus* at 1 mg/ml concentration exhibited the highest antifungal activity, inhibiting mycelial growth (100%) of 6 tested pathogens.

P. betel extract with same concentration exhibited more than 50% inhibition against most of the fungi tested.

The antifungal activity of *Aloe vera* (syn: *A. barbadensis*) leaf pulp (gel) and its liquid fraction were evaluated for the effect on mycellium growth of *Rhizoctonia solani*, *Fusarium oxysporum*, and *C. coccodes* (Rodriguez et al., 2005). The results showed an inhibitory effect of both pulp and liquid fraction of *A. vera* on *F. oxysporum* at 10^4 µg/l. Further the liquid fraction reduced the rate of colony growth at a concentration of 10^5 µg/l in *R. solani*, *F. oxysporum*, and *C. coccodes*.

Another investigation for antifungal activity of *A. vera* against postharvest fruit pathogens was undertaken by Saks and Barkai-Golan (1995). They assayed the antifungal activity of *A. vera* leaf *pulp* at 1 to 10^5 µl/l on four postharvest fruit pathogens*: Penicillium digitatum, Penicillium. expansum, Botrytis cinerea,* and *Alternaria alternate*. The inhibitory effect was calculated based on the suppression of spore germination and mycelial growth. The spore survival of all tested fungi was reduced by 15 to 20% at 1 to 10^3 µl/l of leaf pulp. The *P. digitatum* and *A. alternata* species were the most sensitive against *A. vera* leaf pulp (Saks and Barkai-Golan, 1995).

Screening for antifungal compounds was done on Chinese medicinal plants against growth of some filamentous fungi (Lee et al., 2007). They reported the effect of aqueous, methanol and acetone fractions of some Chines medicinal plants against some food borne pathogens. The results showed that acetone extracts of *Cinnamomum cassia* against *Borytis cinerea* and *Glomerella cingulata* had MIC values of 8.3 and 10 mg/ml, respectively. The hot water extracts of *C. cassia* inhibited significantly the growth of *A. niger*, *B. cinerea*, *Fusarium moniliforme*, and *Phyllosticta caricae* with MIC values at 10, 11.7, 5, and 6.7 mg/ml, respectively. The acetone extracts of *Curcuma longa* inhibited effectively *P. caricae* with the MIC value at 6.7 mg/ml (Lee et al., 2007).

Of the 50 plant species investigated, two leaf extracts, that is, from *Nerium oleander* and *Pithecellobium dulce* showed the higher inhibition against mycelial growth (77.4 and 75.1%) and spore germination (80.3 and 80.0%) of *Bipolaris oryzae* (=*Cochliobolus miyabeanus*) causal agent of Rice brown spot (Harish et al., 2007).

Satish et al. (2007) reported on the antifungal potential of aqueous and solvent extract of fifty-two plants from different families against eight important species of *Aspergillus*. Among the fifty two plants screened, the aqueous extract of *Acacia nilotica*, *Achras zapota*, *Datura stramonium*, *Emblica officinalis*, *Eucalyptus globulus*, *Lawsonia inermis*, *Mimusops elengi*, *Peltophorum pterocarpum, Polyalthia longifolia*, *Prosopis juliflora*, *Punica granatum* and *Syzygium cumini* showed significant antifungal activity at 25% concentration in media culture against one or the other *Aspergillus* species tested. *A. flavus* demonstrated high susceptibility

against most of the extracts. The results indicated that the most tested *Aspergillus*, which exhibited more susceptibility to aqueous extracts also showed high susceptibility to solvent extracts at 500 µL concentration of all tested plants. Among the solvent extracts tested, methanol was more effective than the other tested solvens (Satish et al., 2007).

The *in vitro* activity of a novel alkaloid, 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1-methylethyl pentanoate isolated from *Datura metel* was evaluated by Dabur et al. (2005) against *Aspergillus* species. The compound was found to be active against all the *Aspergillus* species tested. The MIC at which more than 90% of growth was inhibited (MIC90) ranged from 21.87 to 43.75 µg/ml against *A. fumigatus*, *A. flavus* and *A. niger*.

Pinto et al. (2010) screened the methanol extract from 200 plant species against *Colletotrichum lindemuthianum*, causal agent of bean anthracnose in Brazil. The experiments showed that the extract of *Miconia argyrophylla* was most promising for preventing both mycelia growth and conidial germination of *C. lindemuthianum.* Under greenhouse conditions, the extract of *M. argyrophylla* and *Origanum vulgare* had the biggest effect in reducing the disease severity in the local effect assay by 41.82 and 37.65%, respectively. In the systemic effect assay, the extract of *Inga marginata*, *M. argyrophylla, Myrica fallax, Malvaa sylvestris, Ocimum gratissimum, O.vulgare* and *Siparuna arianeae* showed the best reduction in disease severity to value below 35% (Pinto et al., 2010).

In another screening, antifungal effects of 66 medicinal plants belonging to 41 families were evaluated against *Pythium aphanidermatum*, the causal agent of chilli damping-off (Muthukumar et al., 2010). The Zimmu (*Allium sativum* L. × *Allium cepa* L.) leaf extract at 10% concentration had the highest inhibitory effect (13.7 mm) against mycelial growth of *P. aphanidermatum.*

The efficacy of fresh garlic extract (*Allium sativum*) prepared by juicer against a range of plant pathogenic fungi: *Alternaria brassisicola*, *Botrytis cinerea*, *Fusarium tabacinum*, *Magnaporthe grisea*, and *Phytophthora infestans* was investigated by Curtis et al. (2004). The results showed that growth of *A. brassisicola*, *B. cinerea*, *M. grisea* and *P. cucumerina* were inhibited by garlic extract at 20 µl by *in vitro* test. In the experiment performed on plant diseases a reduction in disease was observed for rice blast caused by *M. grisea*, downy mildew of *Arabidopsis* caused by *Hyaloperonospora parasitica* and tuber blight caused by *P. infestans*. In all the cases, the reduction in disease confirmed the inhibition observed by *in vitro* experiment. In the experiments with *M. grisea* and *H. parasitica,* the highest reduction in disease occurred when plants were treated 24 h before infection (Curtis et al., 2004).

The potential of *Tulbaghia violacea* and *Agapanthus africanus* crud extracts to control leaf rust (*Puccinia triticina*) in wheat was investigated (Cawood et al., 2010). In symptom development study, *A. africanus* extract reduced significantly (43%) the percentage pustules formed on the leaves of susceptible wheat. The study on direct effect of extracts on germination of spores showed that both the *T. violacea* and *A. africanus* extracts significantly inhibited the germination of *P. triticina* spores and prevented further germ tube development. Quantification activities of the apoplastic pathogenesis-related proteins in wheat lines under both non-infected and infected conditions showed only treatment with the *A. africanus* crude extract increased the *in vitro* β-1,3-glucanase, chitinase and peroxidase activities significantly in both susceptible and resistant wheat lines, whether uninfected or infected. This strongly indicated that the *A. africanus* extract showed the highest potential to induce resistance towards leaf rust in wheat (Cawood et al., 2010).

Antifungal activity of plant extracts from *Larrea tridentata*, *Flourensia cernua*, *Agave lechuguilla*, *Opuntia* sp. and *Yucca* sp., obtained with alternative organic solvents (lanolin and cocoa butter) and water against the *Rhizoctonia solani* were studied by Castillo et al. (2010).Their results showed that extracts of *F. cernua* and *L. tridentate* using lanolin and cocoa butter at 2000 and 1000 ppm of total tannins inhibited 100% the *R. solani* growth. The authors concluded that lanolin and cocoa butter solvents allowed high recovery of polyphenolic molecules with strong antifungal activity against *R. solani*.

Storage solutions of black ripe olives (*Olea europaea*) with 1.2% concentration of acetic acid, salt free and pH around 4.0 showed a potent antifungal activity in the culture medium against phytopathogenic fungi (Brenes et al., 2011). They found that mycelial growth of *Alternaria* spp. and *Phytophthora cactorum* was completely inhibited by the olive storage solutions diluted up to 50%; although they were less effective against *Botrytis cinerea*, *Colletotrichum acutatum* and *Pestalotiopsis dyospiri*. Among the phenolic and oleosidic compounds detected in the storage solutions, HyEDA (dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol) and EDA (dialdehydic form of decarboxymethyl elenolic acid) were more concentrated in the antifungal storage solutions of black olives. Thus, the antifungal activity in the solutions might be attributed to these two strong anti-bacterial compounds (Brenes et al., 2011).

Abdel-Monaim et al. (2011) studies the effect of water extract and organic solvents from some plant species against *F. oxysporum* f. sp. *Lupine* casual agent of damping-off and wilt diseases of lupine plants. Their experiments revealed that solvent extracts of *Eugenia jambalaya*, *Nerium oleander* and *Citrullus colocynthis* are most effective against *F. oxysporum* f. sp. *lupini*. Amongst the tested organic solvents, the butanolic and ethereal extracts were highly effective in reducing

diseases than the other tested extracts. Under field conditions, ethereal and butanolic extracts of *N. oleander* and *E. jambolana* leaves and *C. colocynthis* fruits significantly reduced the percentage of wilt severity as well as improved plant growth parameters and increased seed index that is total seed yield/hectare compared with control treatment, while protein content in seeds was not affected.

Methanol extracts of 57 plants species were screened for their antifungal activity against rice blast (caused by *Magnaporthe grisea*); rice sheath blight (caused by *Corticium sasaki*); tomato gray mold (caused by *Botrytis cinerea*); tomato late blight (caused by *Phytophthora infestans*); wheat leaf rust (caused by *Puccinia recondita*) and barley powdery mildew (caused by *Blumeria graminis* f. sp. *hordei*) by Choi et al. (2004). The results indicated that none of the plant extracts was highly active against tomato gray mold. The methanol extracts of *Chloranthus japonicas* and *Paulownia coreana* displayed the highest antifungal activity. The *C. japonicus* extract controlled the development of rice blast, rice sheath blight, and wheat leaf rust more than 90%, and tomato gray mold and tomato late blight more than 80%. The *P. coreana* extract displayed control values of more than 90% against rice blast, wheat leaf rust, and barley powdery mildew and more than 80% against tomato gray mold. The extract of *Rumex acetosella* roots reduced specifically the development of barley powdery mildew (Choi et al., 2004).

Joseph et al. (2008) reported the efficacy of different plant extracts to control brinjal (*Solanum melongena*) wilt pathogen (*Fusarium solani* f. sp. melongenae). The results showed that 20% concentration of *Azadirachta indica* water extract was most effective, followed by *Rheum emodi, Eucalyptus globulus, Artemessia annua* and *Ocimum sanctum* against *Fusarium solani* f. sp. melongenae.

Wong and Ng (2005) purified an antifungal peptide named vulgarinin from the seeds of *Phaseolus vulgaris*. This peptide displayed antifungal activity against fungal species such as *Fusarium oxysporum*, *Mycosphaerella arachidicola*, *Physalospora piricola* and *Botrytis cinerea*.

In another investigation performed by Sidhu et al. (2009), individual and combined methanolic plant extracts were evaluated for their efficacy against growth and aflatoxin produced by *A. flavus.* The experiments revealed that combined extracts of various plant species have synergistic antifungal and antitoxin activity as compared to their individual extracts. Combined methanolic extract of *Azadiracha indica* and *Pongamia pinnata* oils inhibited 57.32% of fungal growth. However, the combination of *Cymbopogon nardus* (Citronella) essential oil and methanolic extract of *Citrullus colocynthis* roots inhibited 85.67% of fungal growth and more than 90% of aflatoxin produced as compared to that of control (Sidhu et al., 2009). Extracts from 345 fresh plants were evaluated for their antifungal activity against *B. cinerea* (Wilson et al., 1997). Of the plants tested, 10% dilution of *Allium* and *Capsicum* species showed the greatest antifungal activity and completely inhibited spore germination of *B. cinerea* after 24 and 48 h. Methanolic extracts of *Pimpinella anisum* and Illicium *verum* were studied for their potential antifungal activities against some filamentous fungi (Yazdani et al., 2009). The results indicated that methanolic extract of *P. anisum* seeds did not have any inhibitory effect on *A. flavus* mycellial growth, while extracts of *I. verum* fruits at 16 mg/ml concentration was found to be the most active extract against growth of *A. flavus*.

Wang et al. (2005a) reported a chitinase with antifungal activity isolated from *Phaseolus mungo* seeds. This protein exerted antifungal action towards *Fusarium solani*, *Fusarium oxysporum*, *Mycosphaerella arachidicola*, *Pythium aphanidermatum* and *Sclerotium rolfsii*. *Phaseolus mungo* also yielded a novel lysozyme exhibiting antifungal activity toward *Botrytis cinerea* (Wang et al., 2005b)

Defensins (small cysteine rich peptides) produced by *Trigonella foenum-graecum*, exhibited high antifungal activity at 100 µg concentration against the *Rhizoctonia solani* and *Phaeoisariopsis personata* (Olli and kirti, 2006).

Methanol and aqueous extract of *Ocimum gratissimum* and *Aframomum melegueta* on spore germination and mycelial growth of *A. niger* and *Fusarium oxsporium* was studied by Okigbo and Ogbonnaya (2006). The results showed that ethanol extraction was more effective than water extraction. The antifungal activity of *O. gratissimum* leaf extracts was more effective than *A. melegueta* against spore germination and mycellial growth of *A. niger* and *F. oxysporum.*

DISCUSSION

In the reviewed literatures for the present study, 62 genus belonging to 31 plant families were investigated for antifungal activities against plant pathogenic fungi. Of these, Fabaceae (9 species), Liliaceae (8 species), Myrtaceae (7 species), Lamiaceae (5 species) and Solanaceae (4 species) were the predominant families used by researchers.

The present study also indicated that crude extracts were the most common type of extracts used in experiments conducted by researchers and methanol was the best solvent compared to water and other organic solvents. The results also indicated that the largest part (76%) of bioassay experiments performed on fungi belong to Ascomycetes (*Botrytis, Fusarium, Aspergillus, Colletoterichum, Alternaria* and *Rhizoctonia)*. However, not as many studies were conducted on

Oomycetes (9%) and Basidiomycetes (15%).

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