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EPICOCCUM SP., AN EMERGING SOURCE OF UNIQUE BIOACTIVE METABOLITES

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Abstract: Fungi are playing a vital role for producing natural products, most productive source of lead compounds in far reaching endeavor of new drug discovery. *Epicoccum* fungus is known for its potential to produce diverse classes of biologically active secondary metabolites. The intent of this review is to provide detailed information about biology and chemistry of *Epicoccum* fungus. Most of the fungus metabolites showed cytotoxic, anticancer, antimicrobial and anti-diabetic activities. The literature given encompases the details of isolation of different unusual and unique secondary metabolites, their chemical nature and biological activities find out *Epicoccum* spp., a potential source of lead molecules.

Keywords: anticancer, biocontrol, *Epicoccum,* epicorazines, epicoccamides

In the food, cosmetics and pharmaceutical industries, the fungi are important for their role in different biotechnological processes like fermentation, synthesis and production of bioactive metabolites (1). Out of 1.5 million known fungal species, only 15% have been described for their relevance to and very few of them have actually been explored for bioactive metabolites production (2). The endophytes are fungi which might complete their life cycle partly or wholly inside a plant. This process may occur inter- and/or intra-cellularly (3). These endophytic fungi are rich sources of biologically active compounds (4). Among the endophytes, the most common endophytic fungi are Ascomycota fungus in nature with highly diverse polyphyletic group. Functionally their occurrence are in asymptomatic tissues of plants.

Epicoccum nigrum (*E. purpurascens*) is an anamorphic Ascomycota having worldwide distribution. It makes its colonies in different types of plants as hosts and soils. *E. nigrum* is primarily connected with decay of plant tissues (5) and sometimes has been stated as a weak plant pathogen (6). Like other ubiquitous mould, this fungus genus can display an endophytic lifestyle and is usually found in inner tissues of several plant species (7, 8). In plant pest *E. nigrum* can be used as a biological control (9-12). Many scientists had focused on study of wide variety of anticancer, antimicrobial and anti-diabetic metabolites from *E. nigrum* (13-17).

Source of bioactive metabolite

The fungal strain *Epicoccum nigrum* is a unique source of different bioactive metabolites which are summarized in Figure 1.

Microbiology and morphology

General growth appearance of *Epicoccum* colonies, on fungal Sabouraud dextrose agar media showed typical orange pigments releasing mold specially when observed from reverse sides (Fig. 2). Colonies are often bright, red, orange, brown and yellow in color. It can grow between -3 and 45^{o}C at pH 3.0-4.5. The conidiophores of *Epicoccum* are smaller in size (ranges from 15-25 microns), unremarkable and grouped in clusters. Spores are globose, dark brown and muriform (septa in both directions, like a soccer ball). Spores are often found as little black dots on the growing colonies of cultures. *E. nigrum* belongs to fungi Ascomycota (Table 1)

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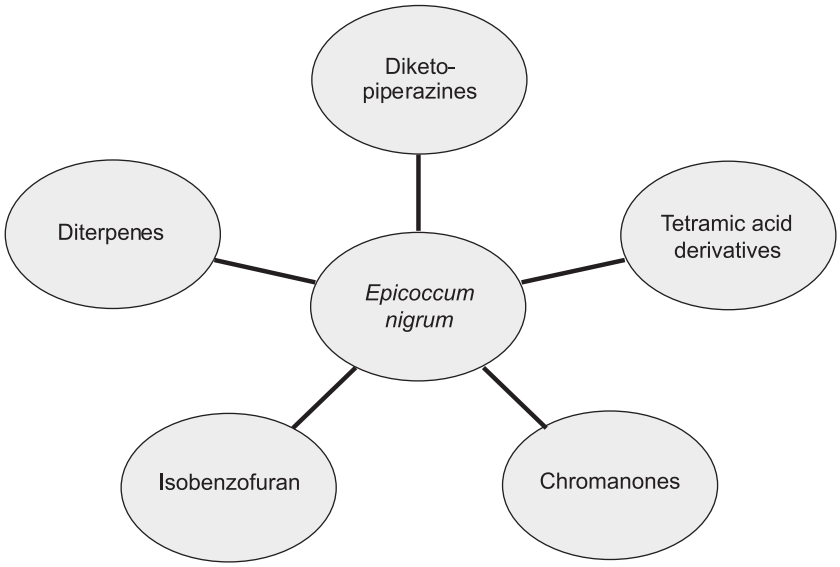


Figure 1. Bioactive metabolites obtained from *Epicoccum nigrum* (21, 27-29, 34, 36)

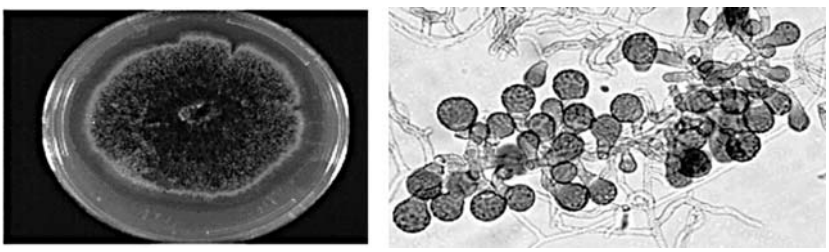


Figure 2. Morphological features of *Epicoccum nigrum* (culture and conidia)

Table 1. Taxonomy of genus *Epicoccum*.

Kingdom	Fungi	Order	Pleosporales
Phylum	Ascomycota	Family	Leptosphaeriaceae
Class	Dothideomycetes	Genus	*Epicoccum*

due to absence of known sexual state. It is also called dematiaceous fungus due to it dark color and melanin in their cell walls (18).

Although more than 70 species were reported in this genus but recently all they are classified in one variable species with distinct morphological and physiological types i.e., *E. nigrum* (19, 20).

Epicoccum **prevalence**

Epicoccum species are saprophytic in nature. They are considered the moulds of environment. *Epicoccum* sp. also colonizes plants and resides as an endophyte. *E. nigrum* is also reported as endophyte in many marine plants for unique metabolites production of these plants. It has worldwide distribution and one of most common invader of many different plants. It also infects seeds from barley, oats, wheat, and corn. The mutual relationship of *E. nigrum* with host plant might be responsible for using host metabolic machinery reprogramming, production and accumulation of unique classes of secondary metabolites produced by the host plants.

Many scientists have isolated *E. nigrum* from different hosts, plants, animals and marine organisms. Marine organisms are considered as promising source of bioactive metabolites. *E. purpurascens* was isolated from inner tissue of jellyfish *Aurelia aurita* and then investigated for its secondary metabolites (21). Abel-Latef and coworkers (14) also reported *Epicoccum* sp., from Pheophyta as a source of peptide antioxidant bioactive metabolites. This fungus was also found in fruiting bodies of other fungi. *Epicoccum* sp. had been isolated from fruiting bodies of two tree fungus i.e., *Pholiota squarrosa* and *Cordyceps sinensis* (a Chinese caterpillar fungus) (15, 16). *Epicoccum* sp. was also isolated from marine sponge *Tethya aurantium* (22). Studies reported isolation and metabolic investigation of *Epicoccum* sp., from *Lysidice rhodostegia*, *Saccharum officinarum* (sugarcane) and *Mentha suaveolens* (leaves) (23, 24).

Secondary metabolites and their significance

The *Epicoccum* species became the focus of many studies in mycologist circles for their production of biologically active secondary metabolites (Fig. 1 and Table 1). Following are few of important classes of metabolites reported.

Diketopiperazines

Diketopiperazines (DKP) are the class of organic compounds which contains the two nitrogen atoms of a piperazine, 6-membered ring, which are apart by amide linkages. They were biologically synthesized from amino acids by a large of organisms, including mammals.

They are secondary metabolites made up of smallest cyclic peptides. They had variety of pharmacological properties reported in different literature likewise antimicrobial, antiviral, antitumor and immunosuppressive activities (25, 26). Naturally occurring diketopiperazines were commonly isolated from fungus.

Diffieux and Filleau (27) isolated two diketopiperazines, epicorazines A and B (**1**) from chloroform extract of culture broth of *E. nigrum* (strain 751-5). These two compounds were isomers. Triornicin **(2)** and isotriornicin **(3)** are members of coprogens in which diketopiperazine ring is formed by condensation of two N-hydroxy-N-acyl-L-ornithine units (Fig. 3). These tumor inhibitory factors produced by *E. purpurascens* indicated that they were of alike structure to the known siderophore desferricoprogen, which was also created by the fungi (28). *E. nigrum* colonized *Cordyceps sinensis* and produced four unique epipolythiodioxo-piperazines epicoccins A-D (**4**-**7**), with unusual sulfur bridges (29). Epicoccin A (**4**) had moderate antimicrobial activity. Similarly in 2009, Guo and his coworker (16) had reported six new diketopiperazines, along with three known epicoccins A, B and D from endophytic *E. nigrum* of *Cordyceps sinensis*. The new compound isolated by them were epicoccins E-H (**8**-**11**) and diphenylalazines A (**12**), B (**13**). Compounds **9**-**12** showed inhibitory effects on HIV-1 replication in C8166 cells. *Epicoccum* sp. isolated from roots of Chinese medicinal herb (*Lysidice rhodostegia*) was also studied for its metabolites. From *Epicoccum* species of Chinese medicinal herb 13 new diketopiperazines, epicoccin I (**14**), ent-epicoccin G (**15**), and epicoccins J-T (**16**-**26**) were isolated (Fig. 3). Compunds **15**, **19** and **25** showed remarkable results aligned with the discharge of enzyme β-glucuronidase in rat with 3.07, 4.16 and 4.95 mM IC_{50} values, respectively, against polymorphonuclear leukocytes induced by platelet-activating factor (23).

Tetramic acid derivatives and pyridine alkaloids

Tetramic acid ring are one of the important classes of pharmacological active natural products system. A novel and unique tertramic acid derivative, epicoccamide A (**26**), was isolated from *E. purpurascens,* an endophyte colonizing inner tissue of jellyfish *Aurelia aurita* (21). Epicoccamide was biosynthetically made up of three different subunits i.e., glycosidic acid, fatty acids and tetramic acid. Three more epicoccamides B-D (**28**-**30**) were isolated from endophytic *Epicoccum* sp., of mushroom *Pholiota squarrosa* (15). The cytotoxic effect of epicoccamide D against Hela cell lines was weak to moderate (CC_{50} 17.0 μM) while against mouse fibroblast and human leukemia cell lines the antiproliferative effects result in growth inhibition (GI_{50}) of 5.05 and 33.3 μm, respectively. Epicoccarins A and B (**31, 32**) another type of novel tetramic acids derivatives were also isolated from this fungus (Fig. 4). The MIC value of epicoccarin A against *Mycobacterium vaccae* was 6.251 g/mL while epicoccarin B showed moderate effects. A new pyridone alkaloid epipyridone (**33**) was also isolated and showed moderate antibacterial activity (30).

Chromanone and isobenzofuran derivatives

Chromanones are the derivatives of benzopyran containing substituted pyran ring. Many complex compounds of this chemical moiety are responsible for diverse biological activities including potent anti-inflammatory actions. Diphenolic chromone derivatives are the latest division of anti-inflammatory lead compound when they were studied

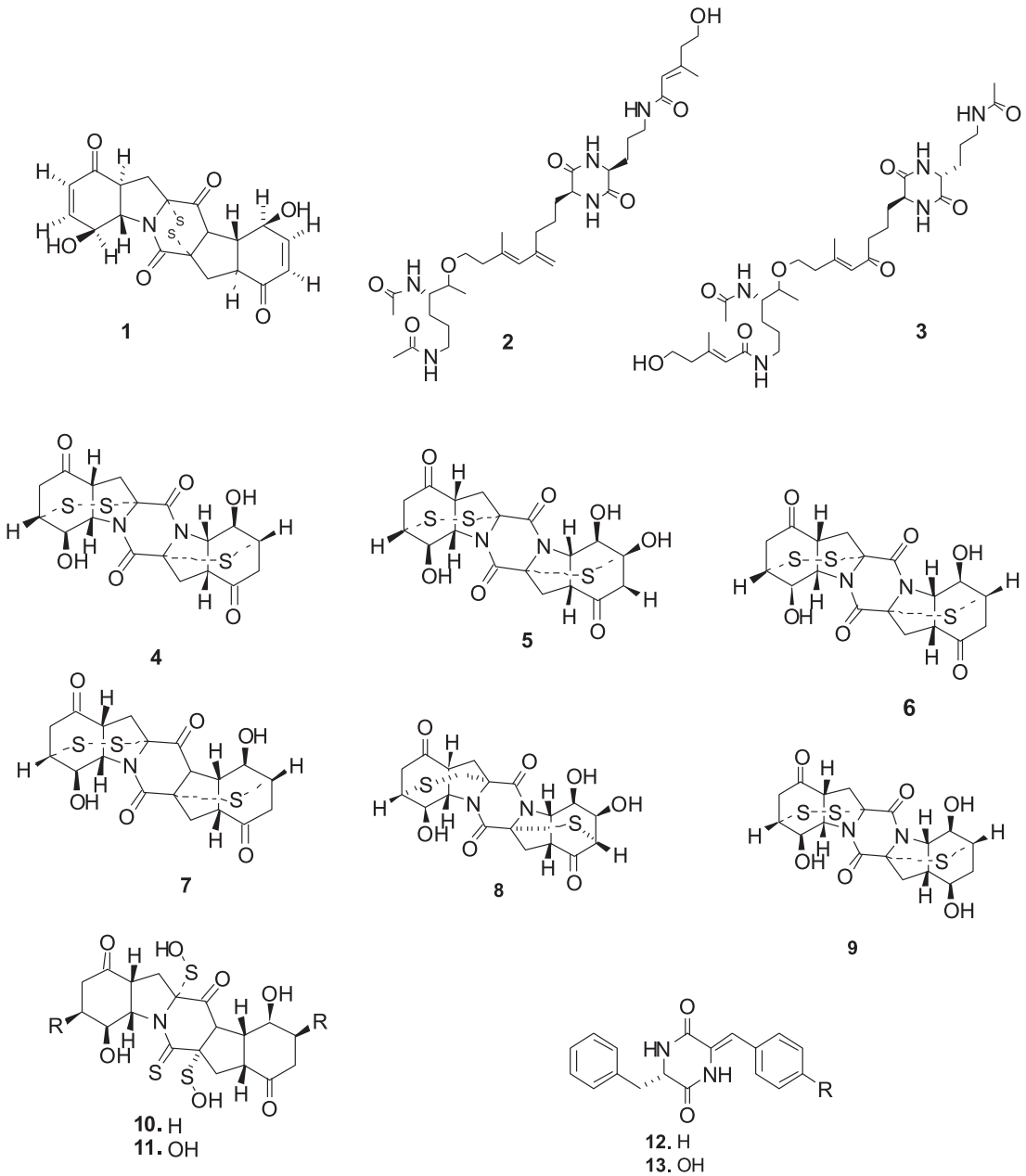
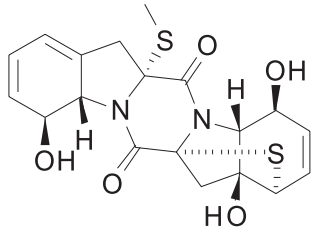


Figure 3. Diketopiperazines isolated from *Epicoccum nigrum*

showed effective inhibitory activity in opposition to nitric oxide (NO) production in RAW264.7 cells and mouse primary peritoneal macrophages (31, 32). Isobenzofuran contains benzofuran nucleus and compounds containing benzofuran moiety showed wide range of therapeutic uses like antibacterial, antidiabetic, analgesic, antifungal, anti-inflammatory, antidepressant, antitumor, imaging, anti-HIV, antitubercular and antioxidant activities (33).

Lee and coworkers in 2007 (34) reported isolation of one chromanone (**34**) and three benzofurans (**35**-**37**) and their derivatives from an organic extract of cultures of *E. purpurascens* MYC 1097 (Fig. 5). Chromanone was isolated from least polar fraction by using normal phase silica gel column chromatography while furan derivatives were obtained from polar fraction by using HPLC. Compound **34** (7-methoxy-4-oxo-chroman-5-carboxylic acid methyl ester) and **35** (1,3-dihydro-5-methoxy-7-methyl-isobenzofuran) were new while other were already known.



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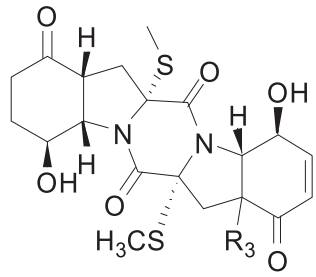

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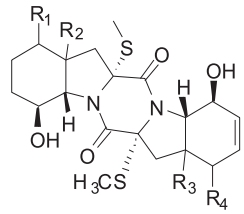
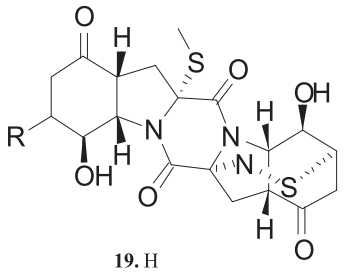
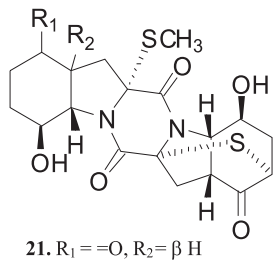


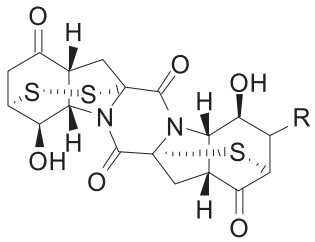
15. $R_1 = R_4 = =O$, $R_2 = R_3 = \alpha$ H
17. $R_1 = R_4 = \alpha$ OH, $R_2 = R_3 = \alpha$ H
18. $R_1 = =O$, $R_2 = \beta$ H, $R_3 = \alpha$ H, $R_4 = \alpha$ OH



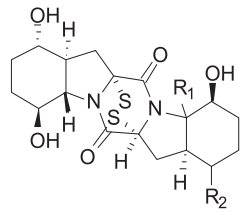
19. H
20. β OH



21. $R_1 = =O$, $R_2 = \beta$ H
22. $R_1 = \alpha$ OH, $R_2 = \alpha$H



23. R = αOH
24. R = H
25. R = βOCH_2CH_3



26. $R_1 = \alpha$H, $R_2 = =O$

Figure 3. Continued

Terpene metabolites

Terpenes are a large class of naturally occurring hydrocabons produced by plants and some other organisms. Terpenes consists of multiples isoprene units. Fungi produce terpenes by mevalonic acid pathway. Mevalonate, an intermediate in terpene biosynthesis, leads to variety of secondary metabolites during cyclization reactions. These metabolites might be triterpenes, steroidal lanosterol, diterpenes, and other sterol derivatives and can provide as the ancestor for a number of biologically key compounds (35).

Three new pimarane diterpenes (**38**-**40**), together with one known compound diaporthins B (**41**) were cut off from the extract of *Epicoccum* sp. HS-1 an endophyte of *Apostichopus japonicas* (Fig. 6). All isolated compounds were experienced for cytotoxicity in opposition to human epidermoid carcinoma KB and KBv200 cells. Compounds **38**, **39** and **41** inhibited the growth of KB and KBv200 with

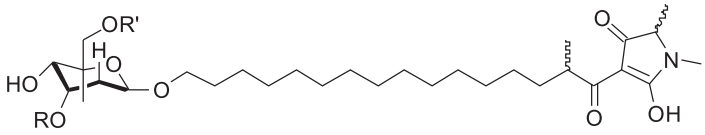
IC_{50} values ranging from 2.34 µg/mL to 20.74 µg/mL (36).

Miscellaneous compounds

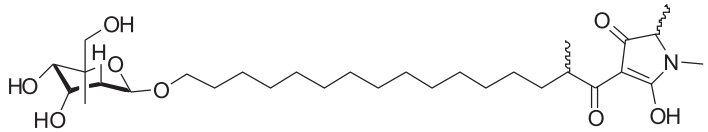
Different pigments have also been reported from *Epicoccum* fungus. Two amorphous red photosensitive pigments, epirodins A and B were isolated from *E. nigrum*. These pigments represent a new class of compounds appearing to be carbonyl-conjugated octaenes which possess some of the structural features and activities of the polyene macrolides of Streptomycetes origin (37). A new natural fluorescent probe epicocconone (**42**) based on polyketide skeleton was isolated from *E. nigrum* (38), which is useful for biotechnological applications. High yield of carotenoids and flavonoids was produced from *E. nigrum* in solid state fermentation. These pigments were easily extracted with alcoholic solutions or other polar solvents. These extracts showed potent antioxidant activity (39-42). Cretu and his coworkers studied physicochemical properties of carotenoids synthesized by *E. nigrum*, which also showed antioxidant activities (43).

An antifungal substance flavipin (3,4,5-trihydroxy-6-methylphthalaldehyde) (**43**) was from *E. nigrum* (44). It might be responsible for biocontrol effects of *E. nigrum* because it could inhibit attack of pathogens to plants (45). In another study, a novel oxopolyene orevactaene (**44**) was isolated from *E. nigrum* WCA7880, which displayed inhibitory activity against the HIV 1 Rev/RRE binding, at an IC_{50} value of 3.6 µM (13). A new natural compounds with unusual carbon skeleton, i.e., epicolactone and mullein derivatives (**45**-**48**) were isolated from ethyl acetate extract obtained from endophytic *E. nigrum* of sugarcane (14, 17).

In another study, five new polyketides, epicocconigrones A and B (**49**, **50**), 3-methoxyepicoccone B (**51**), 3-methoxyepicoccone (**52**), and 2,3,4-trihydroxy-6-(methoxymethyl)-5-methylbenzaldehyde (**53**), together with five known compounds were isolated from an endophytic fungus *E. nigrum* of *Mentha suaveolens* (24). All isolated compounds were experienced for their reticence in opposition to a panel of 16 protein kinases. As positive controls staurosporine and quercetin were used, both known



27. R = R' = H
28. R = Ac, R' = H
29. R = H, R' = Ac



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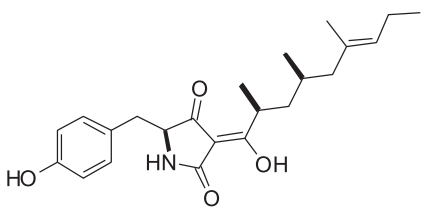
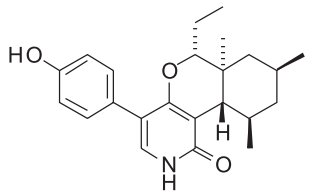
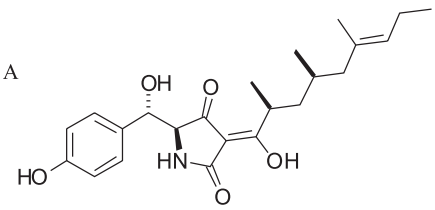


31. Epicoccarine A

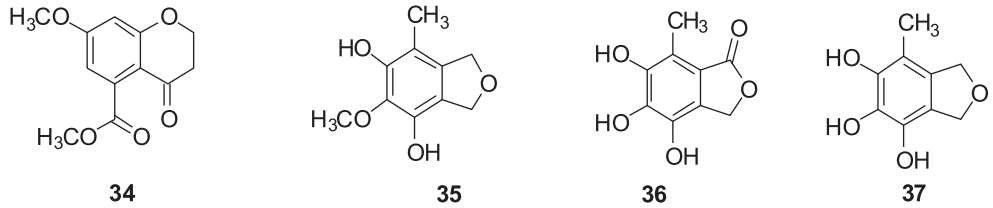


33. Epipyridone



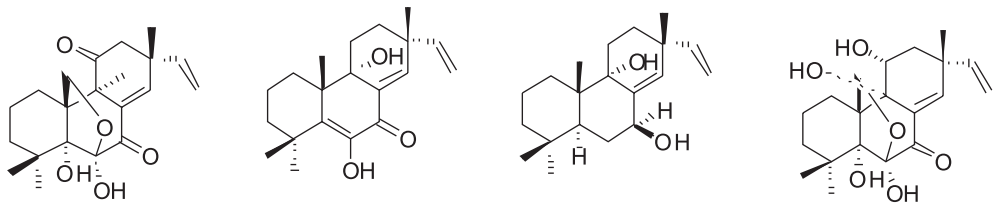
32. Epicoccarine B

Figure 4. Tetramic acid derivatives isolated from *Epicoccum nigrum*



34 35 36 37

Figure 5. Chromanone and isobenzofurans derivatives isolated from *Epicoccum* fungus



38 39 40 41

Figure 6. Terpenes isolated from *Epicoccum* fungus

to inhibit a broad panel of protein kinases. Compound **49** inhibited all tested enzymes while compound **51** and **53** inhibited only some of the tested enzymes (Fig. 7).

In another study organic crude extract obtained from large scale fermentation of *Epicoccum* sp., by using rice as substrate, yielded two polysubstituted polyketides, epicolactone and epicoccolide A (**54**), and epicoccolide B (**55**). These compounds showed antibacterial activity against *B. subtilis*, *S. aureus*, and *E. coli* as well as antifungal activity against plant pathogenic fungi (46).

Nanoparticle production

Safe, cost effective and friendly to environment are pre-requisites for the synthesis of nanoparticles and their development involves greatest interest of modern day biological scientists. Due to their potential application in infection, prevention and wound healing, silver nano-particles (AgNPs) received special attentions (47). Microorganisms especially bacteria and fungi are now considered as potential factories for rapid and environmental friendly production of these AgNPs. The most commonly used bacteria and fungi for the synthesis of silver nanoparticles are: *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas stutzeri*, *Aspergillus niger* and *Fusarium oxysporum* (48, 49).

Qian and his colleagues (50) reported an extracellular synthesis of AgNPs by the endophytic fungus *E. nigrum* QX501, isolated from the cambium of *Phellodendron amurense*. An attempt was made to find the optimum conditions (substrate concentration, pH, and temperature) for biosynthesis of AgNPs. Antifungal potential of AgNPs was evaluated by using nine pathogenic fungi, including *Sporothrix schenckii* and *Cryptococcus neoformans* that have not previously been evaluated. AgNPs displayed much broader antifungal spectrum than itraconazole and fluconazole, two common antifungal agents (50). These results strongly suggested that the AgNPs synthesized by the fungus may be used as a potent antifungal agent especially against *Aspergillus niger*.

Bio-control agent

Phytophthora infestans caused patato late blight and XF1 strain of *E. nigrum* could be used as potential biological pest control agent. The dual culture test showed the hyphae of *P. infestans* growth was reduced and destroyed in circumference of XF1 colony. This occurred due to the degeneration of protoplasm of mycelia of *P. infestans* around the inhibition zone of *E. nigrum* (51).

Metabolomics profile of *E. nigrum* was also studied (52) using solid state fermentation. In this study, they correlated availability of water with metabolites production and it was observed that metabolite production is increased in *E. nigrum* with reduction of water activity. This study described the role of ecophysiological stresses for enhancing natural product discovery.

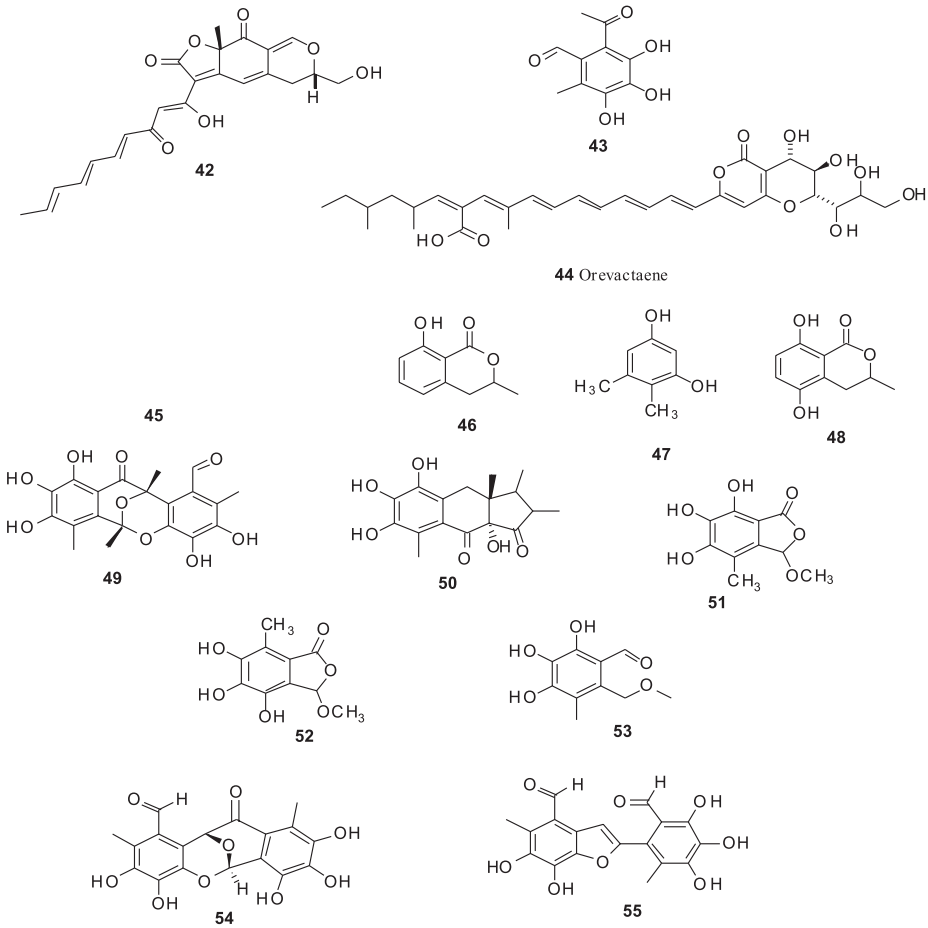


Figure 7. Pigments and antimicrobial metabolites from *Epicoccum* fungus

Future perspectives and conclusion

New bioactive metabolites are a need of time due to ever increasing dilemma of microbial resistance to current therapeutic and control agents along with emergence of new life threatening diseases. These problems have pushed scientists to look for unconventional sources like endophytes for the novel compounds. The fungus capacity to synthesize variety of new bioactive metabolites forced researchers to explore these avenues. *E. nigrum* may be able to prove one of most promising endophytes for the production of chemically structurally and biologically diverse metabolites.

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