ABSTRACT

Fungi have been reported to be active producers of secondary metabolites such as antibiotics, cytotoxic compounds, insecticides etc with high therapeutic value. Antimicrobial compounds from fungi are getting wide attention because of increasing resistance and need for developing new and safe antimicrobial compounds which could be used against various diseases. After the discovery of Penicillin by Alexander Fleming (1928), a lot of fungi have been listed in the literature with various bioactive metabolites, but as still lot remain embedded in the nature which has to be explored for the isolation and production of novel bioactive metabolites. Keeping this in mind a study was carried out to isolate fungi from soil from neighboring areas of Amritsar, Punjab and to screen their antimicrobial potential. The study was further extended for the purification and characterization of some novel compounds from the selected fungi.

Sixty five soil samples were collected from neighboring areas of Amritsar, Punjab (30° 4’ N 75° 5’ E) and from it 113 fungal isolates were obtained. Among 113 fungal isolates, 48.6 % belonged to Aspergillus group while 28.3% were from Penicillium, 6% belonged to Rhizopus and rest 13.2% belonged to different systematic groups. All these isolates were screened for antimicrobial potential against some reference microbial strains of clinical importance including MRSA. Fifty fungal isolates (44.2%) showed antimicrobial activity against at least one or more of the tested microorganisms. On the basis of antimicrobial activity and different morphology, four fungal isolates i.e. HT28, HT 46, HT 113 and HT 66 were short listed for detailed studies. These were identified to be Penicillium sp. close to expansum Link, Penicillium citrinum, Aspergillus sp. close to A. wentii, Aspergillus terreus by Agharkar research institute and have been deposited there vide accession no NFCCI 2554, NFCCI 2555, NFCCI 2565, NFCCI 2556 respectively. Different physiochemical parameters were optimized for selected four fungal isolates to enhance their antimicrobial potential. Yeast Peptone dextrose (YPDS) medium with incubation period of 5-7 days, at 25°C was found to be the optimum for the four selected fungi. The pH optima for antimicrobial activity of both the Penicillium spp. was in the range of pH 6-9, whereas the pH optima for both the Aspergillus spp lies between pH 5-7. Static conditions were found to be best for growth and antimicrobial activity of Penicillium expansum, Penicillium citrinum and Aspergillus wentii whereas Aspergillus terreus showed best antimicrobial activity under shaking conditions at 200 RPM. Starch was found to the
best carbon source for all the four selected fungi and similarly, all the fungi were grown in media containing different nitrogen sources and yeast extract was the best among organic and inorganic nitrogen sources for all the selected fungi. Statistical optimization by Box-Behnken design for the selected four fungi resulted in 1.1 to 1.8 folds increase in antimicrobial activity. Two phase aqueous extraction of culture broth obtained from all the fungi revealed butanol to be the best solvent to elute the antimicrobial components from *Penicillium expansum*, *Penicillium citrinum* and *Aspergillus wentii* whereas ethyl acetate was found to be the best solvent for *Aspergillus terreus*. Purification resulted in four apparently novel compounds viz. 4-(2-Methyl-octyloxy)benzoic acid ethyl ester (KB 1) from *Aspergillus terreus*, 3-(4-Hydroxy-phenyl)-N-[2-(4-hydroxy-phenyl)-propenyl]-acrylamide (KB 2) was isolated from *Aspergillus wentii*. 6-[1,2-Dimethyl-6-(2-methyl-allyloxy)-hexyl]-3-(2-methoxy-phenyl)-chromen-4-one (KB 3) and 7-Methoxy-2, 2-dimethyl-4-octa-4′, 6′-dienyl-2H-napthalene-1-one (KB 4) isolated from *Penicillium expansum* and *Penicillium citrinum* respectively with broad spectrum antimicrobial activity against all the tested microorganisms. MIC of the purified compounds was found to be better or comparable with the standard antibiotics gentamicin and amphotericin B. Time kill studies showed the microbicidal effect of the solvents extracts and the purified compounds. All the purified compounds showed effective microbicidal effect and took lesser time for complete killing of microorganisms than their solvent extracts and the standard antibiotics gentamicin and amphotericin B. The solvent extracts of the four fungi and the purified compounds further demonstrated their effectiveness with longer post antibiotic effect. The purified compounds were found to be relatively thermo stable. All the solvent extracts as well as the purified compounds were found to be neither mutagenic nor cytotoxic by Ames mutagenicity test and MTT assay. The purified compounds isolated from *Penicillium citrinum* (KB 4) and *Aspergillus terreus* (KB 1) were also endowed with valuable cytotoxic potential against the tested human cancer cell lines viz. leukemia cancer cell line (THP-1), lung cancer cell line (A549), prostate cancer cell line (PC-3) and breast cancer cell line (MCF-7) and further showed these compounds (KB1 and KB4) to induce apoptosis in lung cancer (A549) cells. Therefore, all the four purified compounds were found to be potential antimicrobial agents which could further be perused as lead drug molecules and tested in *vivo* before commercialization as safer and natural antimicrobial compounds. The additional potential anticancerous property shown by these compounds further highlights their importance.