Abstract

Background: Antibiotic resistance is becoming a major concern as a result of the widespread usage of antibiotics. This has been increasingly apparent in recent coronavirus pandemic. Fungi have been shown to produce a variety of novel bioactive metabolites with distinct structures and considerable biological activity. Flavipin, a metabolite produced by Aspergillus species, was reported to be a potent antibiotic, antifungal, and nematicidal agent. Flavipin also exhibits anti-oxidant and anti-tumor properties, as well as mild cytotoxicity against normal cell types compared to cancerous cell lines.

Objective: Evaluation of cytotoxic and antimicrobial activity (antifungal and antibacterial activities) of the extracted flavipin (secondary metabolite) from Aspergillus species (especially *Aspergillus flavus, and Aspergillus terreus*).

Materials and Methods: In this study, 80 Aspergillus isolates were evaluated for their production ability of flavipin. These fungi were collected from various locations and laboratories. The extracted flavipin was evaluated against the following isolates: *Acinetobacter baumannii*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Candida albicans*. These microorganisms were previously obtained from clinical samples and identified as multi-drug resistant at the Laboratories of Medical Microbiology Department / College of Medicine/ Al Nahrain University. This study was carried out in several laboratories from the first of October 2020 to the first of June 2022 to detect the presence of flavipin using a standard curve, then purify it using silica gel chromatography, followed by characterization of the extracted flavipin using Fourier-Transform Infrared Spectroscopy (FT-IR), thin layer chromatography (TLC), and High Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectroscopy (GC/MS), and Nuclear magnetic resonance (^{1}H-NMR). The antimicrobial activities, Minimum Inhibitory Concentrations (MIC), Minimum Bactericidal Concentrations (MBC), and Minimum Fungicidal Concentration (MFC) of flavipin were determined using

the resazurin microtiter plate assay at the Central Health Laboratory/Maysan Health Directorate. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay was also used to determine cytotoxic activities of the extracted flavipin.

Results: Out of eighty isolates of Aspergillus species, two species were identified as producers of flavipin. They were *Aspergillus terreus* and *Aspergillus flavus*. According to HPLC analysis, the retention times of flavipin and standard flavipin were 7.7 minutes and 7.6 minutes, respectively. By using the TLC technique, the relative flow (Rf) value was 0.55 cm for both standard flavipin and flavipin. The melting point of flavipin ranged from 230 ºC to 231 ºC. The presence of absorption bands at 3126 cm-1 due to (C-H) aromatic group, 2956 cm-1 due to (CH3) methyl group, 1703 cm-1 due to the (C=O) aldehyde group, 1641 cm-1 due to the (C=C) aromatic group, and 3360 cm-1 due to the (OH) hydroxyl group , were observed in the FT-IR spectra of flavipin.

In 1H-NMR spectrum, six singlets were observed. The singlet at 2.80 ppm showed the integral value of CH3, the sharp singlets appeared at 6.96 to 7.77 ppm for CH aromatic, the sharp singlets appeared at 8.61 to 8.69 ppm, the broad singlets were OH protons, and the singlet at 14.43 ppm showed the integral value of CHO protons. The optimization of growth conditions and production of flavipin were studied. It is revealed that optimum conditions were as follows: pH 7 in day 16, temperature of 25ºC for 12 days, volume of broth (50 ml) on the 16th day, shaking speed of 150 rpm on the day 12, inoculum size of 8 fungal agar disc on the 12th day, optimal incubation period of 14 days, and Potato Dextrose Broth as the optimal culture media.

The results of the antibacterial effect of flavipin against two bacterial isolates (MRSA and *Acinetobacter baumannii*) showed a wide zone of inhibition. It was 35 mm and 30 mm, respectively. Flavipin exhibited the highest antifungal activity against *C. albicans,* with inhibition zones of 44 mm in diameter. The MICs of flavipin for both *Acinetobacter baumannii* and MRSA

were 31.2 µg /mL, while the MIC of flavipin for *C. albicans* was 62.5 µg /mL. The MBC values of flavipin for MRSA and *A. baumannii* were 62.5 µg /mL and 125 µg /mL respectively, while the MFC of flavipin against *Candida albicans* was 125 µg /mL. The inhibitory concentration 50% (IC50) of a cancerous cell line (MCF-7) was 52.34 µg/mL, while the IC50 of a normal cell line (WRL-68) was 298.1 µg/mL.

Conclusions: Flavipin has a potent antimicrobial activity against multidrug resistant (MDR) bacteria and fungi such as MRSA, *A. baumannii*, and *Candida albicans*. The cytotoxic activity of flavipin against cancerous cell lines (MCF-7) was significantly higher than its cytotoxicity against normal cell lines (WRL-68). Secondary metabolites produced by fungi (such as flavipin) could be the safest and most appropriate solution to the problem of antimicrobial resistance that raises alarms in the present and future, and as anticancer agent.