# A Study on Bioactive Metabolites of *n*-BuOH Extracts from the Fermented Broth of *Penicillium purpurogenum* (MF-12) Against *Candida albicans*

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**Abstract**

The bioactive metabolites of *Penicillium purpurogenum* (MF-12) were extracted by using *n*-BuOH solvent for the production of antibiotics. The antifungal activity of *n*-BuOH extracts from the fermented broth of *P. purpurogenum* against *C. albicans* was examined. The antioxidant activity of *n*-BuOH extract was studied by DPPH assay. DPPH radical scavenging activity was determined by UV spectrophotometric method. The minimum inhibitory concentrations (MICs) were determined by 2-fold dilution method and paper disc diffusion assay method. The minimum inhibitory concentrations were performed by using different concentrations (0.3125, 0.625, 1.25, 2.5, 5.0, and 10µg /mL). The MICs of *n*-BuOH crude extracts were observed on a 5.0 µg/ mL and 5.0 µg/ disc against on *C. albicans*. The fungicidal effect of *C. albicans* did not grow on metabolites concentrations of 10 and 5.0 µg/mL.

**Keywords:** bioactive metabolites, antibiotics, *Penicillium purpurogenum*, MICs

## Introduction

Soil is a primary source of microorganisms. Most soil fungi are regarded as saprobes, decomposing organic matter and contributing to nutrient ceding, while several species form mycorrhizal associations with plants or are plant pathogens (Pfenning and Abreu, 2006). Also recognized as prolific secondary metabolite producers, fungi have provided several bioactive compounds and chemical models currently used as pharmaceuticals, and soils are traditionally the main source of fungal genetic resources for bioprospection programs (Adrio and Demain, 2003).

Microorganisms that are able to produce secondary metabolites have a diverse chemical structure and biological activities (Stachelhaus *et al*., 1995). The fungi species of genus *Penicillium* are very attractive organisms for production of useful protein and biologically active secondary metabolites. Phenolics or polyphenols, including flavonoids are the main secondary metabolites of medicinal plants, mushrooms and fungi, responsible for their antioxidant, antimutagenic and antitumor activity.

*Penicillium* is a large anamorphic (asexual state) ascomycetous fungal genus with widespread occurrence in most terrestrial environments. This genus comprises more than 200 described species and many are common soil inhabitants, as well as food borne contaminants or food ingredients used in the preparation of cheese and sausages (Frisvad and Samson, 2004).

Thousands of *Penicillium* isolates have probably been screened in bio-prospecting programs since the discovery of penicillin, and new bioactive metabolites continue to be discovered from these fungi nowadays (Ge, *et al*., 2008; Takahashi and Lucas, 2008), indicating their current importance as sources of high amounts of novel bioactive molecules to be used by pharmaceutical industry.

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Fermentation procedures have to be developed for the cultivation of microorganisms under optimal condition and for the production of desired metabolites or enzymes by the microorganisms. The proper cultivation and transfer of inoculum are essential for the production of both primary and secondary metabolites. The pre-culture (seed culture) media and culture condition often have to be designed for optimal yields. However, the kinetics of product formation is not necessary correlated with the length of the lag phase (Yamane, 1984).

The constituents of a medium must satisfy the elemental requirements for cell biomass and metabolite production (Stanbury *et al.*, 1997). Biomass was separation of fermentation broth. Fermentation processes are a central process step in industrial biotechnology. The chemical, pharmaceutical, and food industries rely on fermentation to transform bacteria, yeasts or molds into valuable products and materials.

Paper chromatography and thin layer chromatography have become tools in the screening of antimicrobial agents through bio-autography. Three bio-autographic methods namely (i) agar diffusion or contact bio-autography, (ii) direct TLC bio-autographic detection and (iii) immersion or agar overlay bio-autography are used to detect antimicrobial agents in a mixture of compounds (Tomita, 1988).

Agar diffusion or contact bio-autography; antimicrobial agents diffuse from a developed TLC plate or paper to an inoculated agar plate. The chromatogram is placed face down onto the inoculated agar layer for specific period to enable diffusion. Then the chromatogram is removed and the agar layer is incubated. The zones of inhibition on the agar surface, corresponding to the spots in chromatographic plates are the indicative of antimicrobial substances. The disadvantages of contact bio-autography are difficulties in obtaining complete contact between the agar and the plate and adherence of the adsorbent to the agar surface (Tomita, 1988).

Another problem may arise due to the differential diffusion of components, especially water-insoluble, from the chromatogram to the agar plate. The principle of the method was the same and antimicrobials had to be transferred from the chromatographic plates to agar causing their loss and dilution. This is a technique familiar to the microbiologists in the search for antibiotics from microorganisms and different procedures have been used to improve its performance. TLC contact bio-autographic assay was introduced for the detection of antibiotic resistance reversal agents.

Antibiotic is a drug used to treat infections caused by bacteria that can cause illness to humans and animals. Antibiotic functions to inhibit or destroy the bacterial cells that cause certain disease. Soil microorganisms have continually been screened for their useful biological active metabolites, such as antibiotics since long ago. Therefore, this study was an attempt to discover novel antibiotics from microbes in soil samples.

Free radicals are formed naturally within the cells. At high concentrations, they can damage major components of cells including DNA, proteins, and cell membranes. The damage leads to the development of health condition. Antioxidant is a molecule that inhibits the oxidation of other molecules. Antioxidant also has many industrial uses, such as preservative in food and cosmetics and prevention of the degradation of rubber and gasoline.

The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in colour. The colour turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 1660 to 1640 when the odd electron of DPPH paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-

H. DPPH method can be used for solid or liquid sample. DPPH is a reactive free radical that acts as an electron acceptor (oxidant/ oxidizing agent) and causes oxidation other substances.

On the other hand, antioxidants act as electron donors (reluctant/ reducing agent). Antioxidants neutralize DPPH by being oxidized themselves. DPPH is found as dark-colored crystalline powder composed of stable free-radical molecules and forms deep violet color in solution. The scavenging of DPPH free radical (neutralization) is indicated by the deep violet color being turned into pale yellow or colorless.

Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations (MICs) are considered the 'gold standard' for determining the susceptibility of organisms to antimicrobials and are therefore used to judge the performance of all other methods of susceptibility testing. MICs are used in diagnostic laboratories to confirm unusual resistance, to give a definitive answer when a borderline result is obtained by other methods of testing or when disc diffusion methods are not appropriate (Jannifer, 2001). Rodriguez *et al*., 2002, state that dilution methods are used to determine the minimum inhibitory concentrations of antimicrobial agents and are the reference methods for antimicrobial susceptibility testing. In dilution tests, microorganisms are tested for their ability to produce visible growth in micro-titration plate wells of broth (broth micro-dilution) containing serial dilutions of the antimicrobial (Rodriguez *et al*., 2002).

The lowest concentration of antimicrobial agent (in mg/L) that, under defined in *vitro* conditions, prevents the appearance of visible growth of a microorganism within a defined period of time, known as the MICs agents. The range of antibiotic concentrations used for determining MICs is universally accepted to be in doubling dilution steps up and down from 1 mg/L as required. The MIC is defined as the lowest concentration of a drug that will inhibit the visible growth of an organism after overnight incubation (this period is extended for organisms such as anaerobes, which require prolonged incubation for growth) (Jannifer, 2001). According to Rios and Recio, 2005, in the research for substances of natural origin with antimicrobial activity, those that present concentrations higher than 1 mg/mL for extracts or 0.1 mg/mL for isolated compounds should be avoided. However, the evaluation of activity is very interesting in the case of concentrations below 100 µg/mL for extracts and 10 µg/mL for isolated compounds.

In vitro antimicrobial susceptibility testing (AST) is performed routinely in most clinical microbiology laboratories to assess the relative susceptibility microbial pathogens to selected therapeutic agents as an aid in optimizing the treatment of infectious diseases. AST has been performed with antibacterial agents for decades, and recently these methods have been adapted, standardized, and validated for testing antifungal agents against yeasts (National Committee for Clinical Laboratory Standards [NCCLS] M27-A2) and filamentous fungi (NCCLS M38-A) AST methods include agar and broth dilution and agar diffusion tests (disc and E test), all of which measure the inhibitory activity of the tested antimicrobial agents. Efforts at developing standardized methods for performing time-kill and minimal fungicidal concentration (MFC) determinations for various antifungal agents have begun and have demonstrated that by following a common procedure, various laboratories may reliably perform and interpret both types of studies with *Candida* and *Aspergillus* (Pfaller *et al*., 2004)

*Candida albicans*, a dimorphic fungus which causes a variety of superficial and deep-seated mycoses, exists in two easily identifiable morphologies, namely yeast and hypha forms. There is some evidence indicating that the hypha form may play an important role in the pathogenesis of *C. albicans,* estaparticularly with respect to the ability of the organisms to

penetrate host cells and tissues. Similarly, the hypha form has often been considered to be important in the ability of the fungus to adhere to cell surface (Hawser and Islam, 1999).

Several species of the yeast genus *Candida* are capable of causing candidiasis. They are members of the normal flora of the skin, mucous membranes and gastrointestinal tract. Superficial candidiasis is established by an increase in the local census of *Candida* and damage to the skin or epithelium that permits local invasion by the yeast and *pseudophae*. In this current study, *C. albicans* was selected and tested for the antifungal activity of secondary metabolites in *P. purpurogenum* (MF-12).

The aim of the present study is to screen the bioactive metabolites of *n*-BuOH extracts from the fermented broth of *Penicillium purpurogenum* (MF-12) against *C. albicans* and to screen of their antifungal activities.

## Materials and Methods

### Collection of soil samples and isolation of soil microorganisms

Nine different soil samples were collected from Mawlamyinegyun Township, Ayeyarwady Region during June, 2015. The soil samples were analyzed by laboratory method and seven bacteria and twenty six fungi were isolated from soil samples by using physical and chemical treatment methods (Phay and Yamamura, 2005) and soil dilution method (Ando, 2014).

### Antimicrobial activities of isolated soil microorganisms

The isolated soil microorganisms were inoculated into seed medium and incubated for 3 days at room temperature. Five percent of seed medium were inoculated into the fermentation medium and incubated for 4 days at room temperature. The fermented broth was used by paper disc diffusion assay method (NITE, 2005) to check the antimicrobial activity against ten test organisms, namely *Agrobacterium tumefaciens* NITE 09678, *Aspergillus parasciticus* IFO5123, *Bacillus subtilis* IFO90571, *Candida albicans* NITE09542, *Micrococcus luteus* NITE83297, *Salmonella typhi* AHU9743,*Escherichia coli* AHU5436, *Saccharomyces cerevisae* NITE52847, *Pseudomonas fluorescens* IFO94307 *and Staphylococcus aureus* AHU8465 which are provided by NITE & Kyowa Hakko Co. Ltd.

One percent of test organism was added to assay medium, then poured into plates. After solidification, paper discs impregnated with samples (fermented broth) were applied on the agar plates incubated at room temperature for 24-36 hours.

Clear zones (inhibitory zones) surrounding the paper discs indicate the presence of bioactive metabolites which inhibit the growth of test organisms. Paper disc having eight millimeter diameter were utilized for antimicrobial activities. Inhibition diameter zone was determined by digital caliper.

### Studies on antifungal metabolites by using fermentation process

Selected Fungus *P. purpurogenum* (MF-12) was grown at room temperature for (3) days on PGA medium. 6 mm disk of the fungus inoculum was grown on seed medium and incubated at room temperature for 3 days. After 3 days, fermentation medium was prepared for the antimicrobial metabolites production. 85 mL of fermentation medium was prepared in distilled water containing glucose (0.7g), yeast extract (0.6g), polypeptone (0.3g), $K_2HPO_4$ (0.01g), $KNO_3$ (0.1g), $CaCO_3$ (0.1g), pH (6.0) was taken in 250 mL Erlenmeyer flasks. These flasks were autoclaved at 121°C, for 15 minutes. 25% of seed medium was transferred into the fermentation medium and incubated at room temperature for 6 days under shaken

condition. The fermented broth was filtered through sterilized Whatman filter paper no.1. 10 µL of fermented broth was put on paper disc and placed on assay medium containing test organisms and incubated at room temperature for 24-36 hours. Clear zones (inhibitory zones) surrounding the paper discs indicate the presence of bioactive metabolites which inhibit the growth of test organisms. Once the incubation period was complete, mycelia were filtered by using sand column.

**Study on the solvent systems for the extraction of antifungal metabolites by using bio-autography method**

The purified fermented broth was extracted with four different organic solvents, watery saturated ethyl acetate, watery saturated *n*-Butanol, 20% of ammonium chloride, *n*-Butanol: acetic acid: water (3:1:1 v/v) was used for extraction of antimicrobial metabolites from fermented broth of *P. purpurogenum*. Antifungal activity of each extract extracted with different solvents was determined out by using bioassay.

**Extraction of antifungal metabolites by using separation method**

The pure fermented broth was submitted to a liquid-liquid extraction (1:1 v/v) with *n*-BuOH by using (250 mL) separation funnel and shaken vigorously for 15 minutes and kept without any disturbance for another 10 minutes to separate the solvent from aqueous phases. The upper (organic phase) and lower layer (aqueous phase) were separated and tested bioassay. The organic phase was evaporated with a vacuum rotary evaporator at a temperature of 50-55 ºC until the organic solvent completely evaporated, leaving a dried crude extract in a rotary evaporator flask.

***In vitro* screening of antioxidant activity of *n*-BuOH extract from the fermented broth of *P. purpurogenum* by DPPH Assay**

DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging assay was chosen to assess the antioxidant activity of the *n*-BuOH extract of the fermented broth of *P. purpurogenum* metabolites. Accurately weighed 2 mg of test sample was dissolved into 10 mL of 95% EtOH under vigorous shaking. The mixture solution was filtered and the stock solution was obtained. Desired concentrations (10 µg/mL, 5.0 µg/mL, 2.5 µg/mL, 1.25 µg/mL and 0.625 µg/mL) of sample solutions were prepared from this stock solution by serial dilution with appropriate amount of 95% EtOH. Then, $IC_{50}$ (50% oxidative inhibitory concentration values) were also calculated by linear regressive excel programme.

**MICs of *n*-BuOH extract by 2-fold dilution method**

Minimal inhibitory concentration of *n*-BuOH extracts were carried out by 2-fold dilution method using different concentrations (0.3125 µg/mL, 0.625 µg/mL, 1.25 µg/mL, 2.5 µg/mL, 5 µg/mL and 10 µg/mL). The suspension of test fungus was prepared by scrapping the spores from 2-day-old PDA culture plate with fresh PDB. 0.1 mL of *C. albicans* suspension ($1.6\times10^9$ CFU/mL of Test Organism) was mixed with metabolites of different concentrations. Control was also prepared. These tubes were incubated at room temperature. After 24 hour incubation, different concentrations and control were put on assay medium by using Streak culture method on fresh PDA plates and were incubated at room temperature for 24-36 hours. MIC values were determined and compared to control.

**MICs of *n*-BuOH extract by paper disc diffusion assay method**

Minimal inhibitory concentration of *n*-BuOH extracts was determined by paper disc diffusion assay method. 10 µL of different concentrations (0.3125 µg/mL, 0.625 µg/mL, 1.25 µg/mL, 2.5 µg/mL, 5 µg/mL and 10 µg/mL) of *P. purpurogenum* metabolites were

impregnated on paper disc, dry and placed on assay medium containing test. The plates were then incubated at room temperature until growth was visible in the control subculture. The MICs will be that lowest concentration where no visible growth will be observed on plate. MIC values were determined by digital caliper and compared to control.

**Studies on MFCs and dry cell weight of *n*-BuOH extract**

In this study, fungicidal effect and the dry cell weight (g/mL) were determined in 6 hours intervals. 0.1 mL of broth of *C. albicans* was inoculated into 250 mL of each conical flasks containing 100 mL PDB medium and incubated for 66 hours. After 24 hour, it was introduced with the treatment of different concentrations of metabolites (0.3125 µg/mL, 0.625 µg/mL, 1.25 µg/mL, 2.5 µg/mL, 5 µg/mL and 10 µg/mL) into 6 flasks (Fig. 1). The dry cell weight (DCW) was determined in 6 hours intervals and calculated the following standard equation.

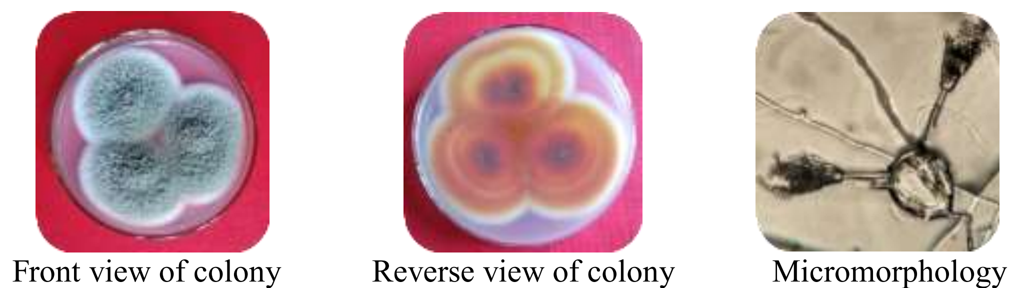
$$\text{DCW (g/mL)} = \frac{\text{weight of filtered+dry residue (mg)} - \text{weight of filtered} \times 1000\text{mL}}{\text{sample volune (mL)}}$$

Figure (1) Treatment of different concentrations of metabolites

## Results and Discussion

In this study, the soil samples were taken near the tree by digging under 4 cm depth from surface of soil. Tangjang, *et al*., (2009) they found out that there were greater amounts of bacterial and fungal populations in the top soil (0-10 cm) if compared to that of other depths. Alexander, 1997 and Arunachala., *et.al*. 1997 said that physicochemical analysis of soil showed that pH range of soil conditions ranged from 5.1 to 7.5 and soil textures determined the fungal population. In this study, the most soil samples indicated that acid range 5.15 to 6.41. The soil textures were clay, silt clay and silt clay loam. During the investigation period seven bacteria and twenty six fungi (MF-1 to MF-26) were isolated from the nine soil samples. Seven bacteria were not used in this study.

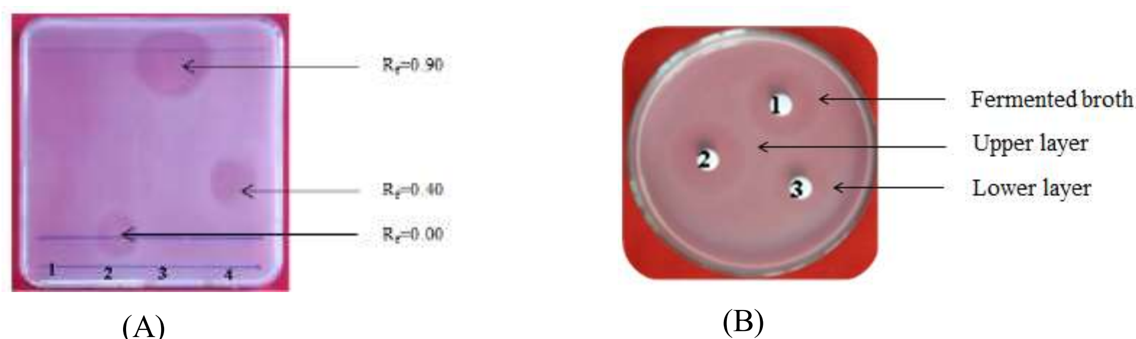
In this study, antimicrobial activity of fungal isolates was screened for research and selection of new antimicrobial metabolites with using paper disc diffusion assay method. Twenty-six soil fungi were tested by using ten test organisms, namely *A. tumefaciens*, *A. paraciticus*, *B. subtilis, C. albicans*, *M. luteus*, *S. typhi, E. coli, S. cerevisae, P. fluorescens and S. aureus*. Mostly soil fungi were against *A. tumefaciens*, *B. subtilis, C. albicans*, *M. luteus*, *S. typhi*, *E. coil, S. cerevisae, and S. aureus*. In this study, soil fungi MF-12 was selected for further investigation depending on the experimental data of antifungal activities. Isolated fungal MF-12 was identified as *Penicillium purpurogenum* based on the distinct characters (Fig. 2). In this research, several clinically important yeasts *C. albicans* was selected for the antifungal activity of *P. purpurogenum*.



Front view of colony Reverse view of colony Micromorphology

Figure (2) Colony characters (7 days) and micromorphology of *P. purpurogenum*

The antifungal secondary metabolites were detected from the fermented broth of *P. purpurogenum* by using bio-autography method. Agar overlay bio-autography and paper chromatography were used in detect antimicrobial agents in a mixture of compounds. In the paper chromatography, by using (1) $NH_4Cl$, (2) EtOAc, (3) *n*-BuOH and (4) *n*-BuOH: $CH_3COOH$: $H_2O$ (3:1:1). An overall view of bio-autography has been depicted in figure 3 (A). According to the $R_f$ values of bio-autography, it was suitable for the extraction of antifungal compound of *P. purpurogenum* metabolites by using *n*-BuOH solvent.




(A) (B)

Figure (3) Extraction of antifungal metabolites by (A) bio-autography, (B) bioassay

Antifungal metabolites from the fermented broth of *P. purpurogenum* were extracted by using separation method. In the preparation, the filtrate of fermented culture, upper and lower layer were tested by using assay medium containing test organisms. In this study, the filtrate of fermented broth (ID=26.70 mm) showed that upper layer (ID=31.85 mm) and lower layer (ID=16.34 mm) were optimized as described in figure (3.B). Therefore, the upper metabolites were collected for the separation and purification of antifungal metabolites. From the extraction, 0.8 g/L of dry reddish brown crude extract was obtained. The crude extract was stored in a refrigerator if not immediately used for the separation. Before the separation, the metabolites in the *n*-BuOH crude extracts were studied by antioxidant activity, minimum inhibitory concentrations, minimum fungicidal concentrations and dry cell weight.

The antioxidant activity of *n*-BuOH extract was studied by *in vitro* DPPH free radical scavenging assay method. This method is based on the reduction of coloured free radical DPPH in ethanolic solution by different concentrations of each sample. The antioxidant activity was expressed as 50 % oxidative inhibitory concentration ($IC_{50}$). The lower the $IC_{50}$ value, the higher the antioxidant activity of the sample. In this experiment, ascorbic acid was used as a standard. The antioxidant activity of the crude extracts of *n*-BuOH extract was determined for five different concentrations: 0.625 μg/mL, 1.25 μg/mL, 2.5 μg/mL, 5 μg/mL and 10μg/mL of each samples in EtOH solvent. The absorbance of control solution (DPPH in

EtOH solvent), blank solution (sample in EtOH) and sample solution (sample + DPPH in EtOH) were measured at wavelength 517 nm using UV spectrophotometer.

Antioxidant activity of *n*-BuOH extract from the fermented broth of *P. purpurogenum* was expressed in terms of percent inhibition. From, the average values of percent inhibition, $IC_{50}$ values (50 % inhibition concentration) were calculated by linear regressive excel programme. The percent oxidative inhibition and $IC_{50}$ values of crude extract from the fermented broth of *P. purpurogenum* are summarized in table (1). Plot of percent oxidative inhibition and concentration of crude extracts and standard ascorbic acid is illustrated in figure (4). From these experimental results, it was found that as the concentrations increased, the absorbance values decreased and the antioxidant activity increased. In the *vitro* antioxidant activity screening by using DPPH free radical scavenging assay method, *n*-BuOH extract ($IC_{50}$=1.64 µg/mL) was found to have slightly lower antioxidant activity than that of standard ascorbic acid ($IC_{50}$=1.17 µg/mL).

From the results, it can be inferred that due to the high antioxidant potential, *n*-BuOH crude extract from fermented broth of *P. purpurogenum* contains active metabolites which may be used in prevention of diseases related to oxidative stress such as coronary heart disease, neurodegenerative diseases such as Alzheimer′s disease, Parkinson′s disease and even in various types of cancer.

Table (1) Percent oxidative inhibition and $IC_{50}$ values of *n*-BuOH extract and standard ascorbic acid

| Sample | Percent Oxidative Inhibition (%) (mean ± SD) In different concentration (µg/mL) | | | | | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|---|---|
| | 0.625 | 1.25 | 2.5 | 5 | 10 | |
| *n*-BuOH extract | 43.41±3.23 | 48.30±2.43 | 55.05±4.31 | 69.57±2.33 | 90.04±3.13 | 1.64 |
| Ascorbic acid | 14.04±4.13 | 54.83±3.13 | 72.44±3.26 | 77.13±3.23 | 87.40±3.23 | 1.17 |

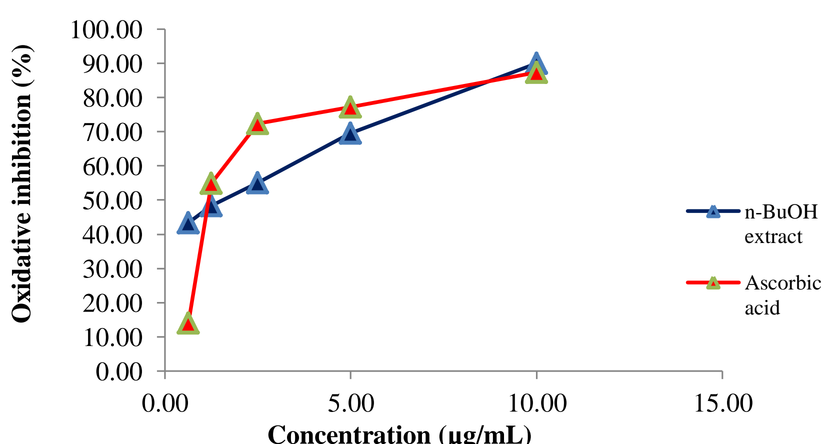



Figure (4) Plot of oxidative inhibition percent Vs concentration (µg/mL) of *n*-BuOH extract and standard ascorbic acid

In the biological properties, the minimum inhibitory concentrations (MICs) of *n*-BuOH extracts were determined by streak culture method and paper disc diffusion assay method by using the concentration of 0.3125 µg/mL, 0.625 µg/mL, 1.25 µg/mL, 2.5 µg/mL, 5 µg/mL and 10 µg/mL and control solution. The *n*-BuOH extract showed MIC values of 5.0µg/mL and 5µg/disc in streak culture and paper disc diffusion methods. According to this research data, antifungal metabolite of *P. purpurogenum* may give beneficial effects on the diseases caused by *C. albicans.* MICs of *n*-BuOH against *C. albicans* by streak culture and paper disc diffusion assay are shown in figures (5) and (6).

In the fungicidal effects, the metabolites of *n*-BuOH crude extract suppressed the growth of *C. albicans* at the concentration of 0.1525 µg/mL. The concentration of *n*-BuOH extract metabolites 10 and 5.0 µg/ mL were not growth on *C. albicans.* The fungicidal effect was directly proportional to the dry cell weight (µg /mL). The data of fungicidal effects and dry cell weight are shown in table (2) and figure (7).

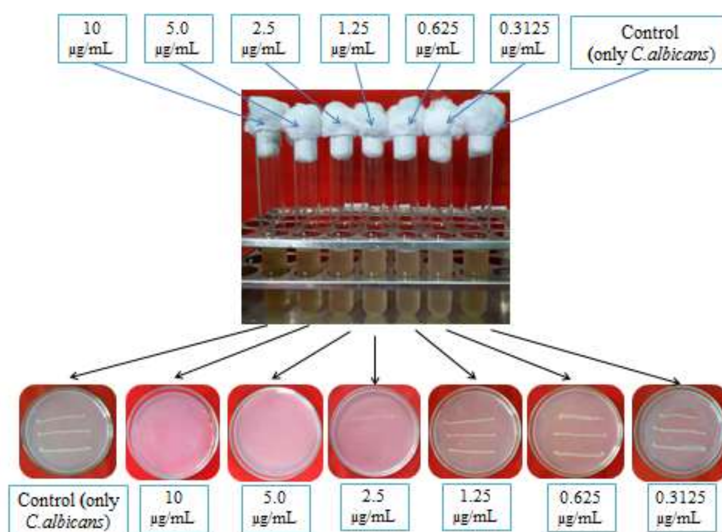



Figure (5) MICs of *n*-BuOH extract against *C. albicans* by streak culture method

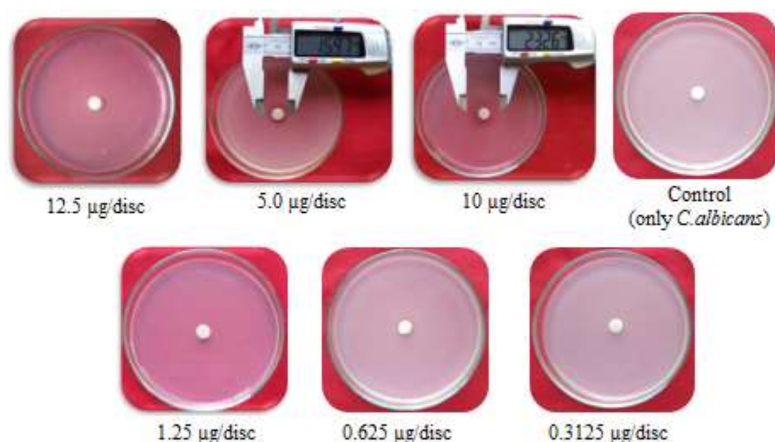



Figure (6) MICs of *n*-BuOH extract against *C. albicans* by paper disc diffusion assay

Table (2) Fungicidal effects and dry cell weight (DCW) of *n*-BuOH extract on *C. albicans*

| Conc: ( µg/mL) | DCW (g/mL) of Test Organism and Culture Time (hr) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 24 hr | 30 hr | 36 hr | 42 hr | 48 hr | 54 hr | 60 hr | 66 hr |
| *Control | 2.0 | 3.5 | 4.8 | 6.0 | 7.2 | 8.6 | 9.8 | 11 |
| 0.1527 | 1.5 | 2.5 | 3.3 | 4.7 | 5.4 | 6.6 | 7.4 | 8.2 |
| 0.3125 | 1.0 | 2.0 | 2.6 | 3.0 | 3.8 | 4.4 | 5.0 | 5.6 |
| 0.625 | 1.0 | 1.5 | 2.0 | 2.4 | 2.6 | 3.0 | 3.5 | 4.0 |
| 1.25 | 1.0 | 1.5 | 1.4 | 1.5 | 1.6 | 1.7 | 1.9 | 2.0 |
| 2.5 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 5 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |

*Control = Only *C. albicans*

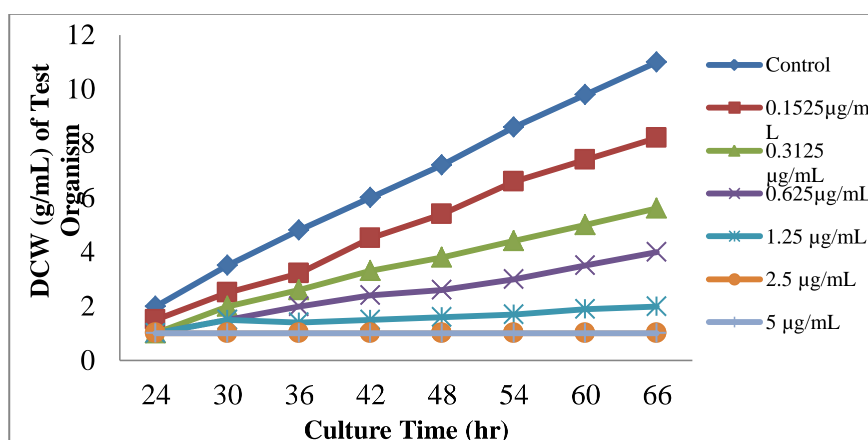



Figure (7) Fungicidal effects and dry cell weight of *n*-BuOH extract on *C. albicans*

Andriy, *et al*., (2016) described that the antimicrobial activity of the fungal extracts derived from their mycelia. Geweely, *et al*., (2011) said that *P. purpurogenum* inhibited the growth of all bacterial species tested. Amna *et al*., (2009) reported that the species of *Penicillium* sp. was the most potent fungal producers of antibacterial compounds. Moreover, fungus *Penicillium* is one of the antifungal against all fungi tested. Amna *et al*., (2009) reported that the species of *Penicillum* showed marked antibacterial activity against all test bacterial species in their study. *Penicillium* sp. was the most potent fungal producers of antibacterial compounds (Arishi., 2015).

*Penicillium* is a genus of ascomycetous fungi of major importance in the environment, food and drug production. Fungi produce a wide range of secondary metabolites such as antibiotics, toxins, alkaloids, fatty acids, ketones and alcohols during active cell growth. Nose, *et al.,* (2000) purified two novel antifungal antibiotics, PF 1163 A (1) and B (2) from the fermentation broth of *Penicillium* sp. which showed potent growth inhibitory effect on *C. albicans*. Therefore, *Pencillium* sp. may be used to treat bacterial and fungal infections including respiratory infection, pneumonia and bacterial throat infections, treatment of typhoid fever and other intestinal infections like dysentery, tuberculosis, sexually transmitted diseases like gonorrhoea and syphilis (Tiwari, 2016).

Antibiotics are one of the important pillars of modern medicines. Antibiotic producing microbes are abundant in soil, water, sewage and compost that serve source for isolation. Soil bacteria and fungi have played a significant and an important role in antibiotic discovery

(Ball *et al*., 2004). Therefore, the extraction of secondary metabolites from the fermented broth of *P. purpurogenum* was performed by using the paper chromatography method and the bio-autography method for the production of antibiotics. The crude extracts 8g/10L were obtained. The *n*-BuOH crude extracts were applied on antioxidant activity, minimum inhibitory concentrations, fungicidal effects and dry cell weight. Based on the results from this study, bioactive metabolites of *P. purpurogenum* showed antifungal activities against on the diseases of *C. albicans.*

## Conclusion

The bioactive metabolites could be applied as medicine to treat infectious diseases such as candidiasis, dematophytisis, meningitis and arthritis which are related to infections of *C.albicans.* The results proved that antifungal metabolites of *P. purpurogenum* contain the potential antifungal component that may be used for the medicine and pharmaceutical industry. Further research is needed for the separation of antifungal metabolites in the extracts from the fermented broth of *P. purpurogenum*, classification and identification of the antifungal compounds.

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