EXTRACTION, ISOLATION AND CHARACTERIZATION OF ANTIFUNGAL METABOLITES FROM THE FERMENTED BROTH OF *PENICILLIUM PURPUROGENUM* (MF-12)

Moe Moe Aye[1], Yin Yin Mya[2], Nant Si Si Htay[3]

Abstract

In this study, twenty six fungi were isolated from nine different soil samples for the production of antibiotics. The antimicrobial activities of isolated fungi were tested by ten test organisms. The bioactive metabolites of *Penicillium purpurogenum* were extracted by using *n*-BuOH solvent. The antifungal activity of *n*-BuOH extracts from the fermented broth of *P. purpurogenum* against *C. albicans* was examined. Separation of bioactive compounds by fractionations of the crude extract in silica gel column chromatography eluted with co-solvent in gradient was able to separate almost all impurities. From the analysis, four compounds (I, II, III and IV) were obtained. Characterization and classification of four compounds were performed by some chemical reagent tests and some modern spectroscopic techniques such as UV and FTIR. The isolated antifungal compounds could be applied as medicine to treat infectious diseases such as candidiasis, dermatophytosis, meningitis and arthritis which are related to infections of *C. albicans.*

Keywords: microbes, antibiotics, antifungal compounds, *C. albican,* candidiasis

Introduction

Most of the naturally occurring antibiotics have been isolated from soil microorganisms. Secondary metabolites are small molecules that are not directly involved in metabolism and growth of the organism. Both plants and fungi are known for producing a large number of chemically diverse secondary metabolites. It is well known that fungi remain one of the most important resources for the discovery of new bioactive compounds against bacteria, fungi, insects and nematodes as well as antitumor compounds (Pelaez, 2005).

Microorganisms that are able to producing secondary metabolites have a diverse chemical structure and biological activities (Stachelhaus *et al*., 1995). Some soil filamentous fungi such as *Penicilium* produce many bioactive small molecules, or secondary metabolites, that range from beneficial bioactive compounds (antibiotics, anticancer, anti-infective, antimicrobial, and antioxidant) to harmful toxins (Tajick *et al*., 2014). Fungi have provided several bioactive compounds and chemical models currently used as pharmaceuticals, and the soils are traditionally the main source of fungal genetic resources for bio-prospection programs (Adrio and Demain, 2003).

The fungi species of genus *Penicillium* are very attractive organisms for production of useful protein and biologically active secondary metabolites. These organisms have the ability to secrete various compounds with different biological activities, such as antibiotics, antitumor, antifungal, anti-tuberculosis, as well as pesticides. Pharmaceutical industry is now facing a scarcity of new antibiotics; therefore, to ensure the availability of effective drugs in the future, it is important to increase efforts for identification of new antibiotics (Marasabessy *et al.,* 2017). In the last 25 years, a steady increase of microbial resistance to antibiotics has become a serious threat to global public health (Marasabessy *et al.,* 2017).

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

[1] Dr, Lecturer, Department of Chemistry, Hinthada University
[2] Dr, Professor, Department of Botany, Myeik University
[3] Dr, Professor, Department of Chemistry, Myatkyinar University

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*. Systemic candidiasis occurs when *Candida* enters the blood stream and phagocytic host defenses are inadequate to contain the growth and dissemination of the yeast. From the circulation, *Candida* can infect the kidneys, attach to prosthetic heart valves, or produce *Candida* infections almost anywhere (e.g., arthritis, meaningitis, endophthalmitis).

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. 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.

Bioautography is a technique that combines thin layer chromatography (TLC) with bioassay in situ (Shitu *et al*., 2006). It can be used for the screening of separated components of natural product extracts. The purification of bioactive metabolites from fermented broth of microorganisms largely depends upon the physicochemical properties of metabolites.

For the past 50 years, fungal secondary metabolites have revolutionized natural product research, affording drugs and drug leads of enormous medicinal and agricultural potential. For instance, penicillins (e.g. penicillin G, 1.1) and cephalosporins (e.g. cephalotin, 1.2), β-lactam antibiotics firstly isolated from *Penicillium* and *Acremonium* species, are still among the world's blockbuster drugs, representing about 50% of the total antibiotic market in 2009 (Aly *et al.,* 2011).

The antifungal agent griseofulvin (1.3, Fulvicin), that was isolated from the mold *Penicillium griseofulvum* is approved for the treatment of dermatophyte infections of the skin, nails and hair of humans (Aly *et al*., 2011). Certain fungi are of great importance in our daily life. For instance, the metabolic physiology of yeast has been used since ancient times for preparing cheese, bread, and alcoholic beverages. 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.

The aim of the present study is to isolate the soil microorganisms from different soil samples, to study the antimicrobial activities of isolated soil fungi, to extract, isolate and characterize antifungal metabolites from the fermented broth of selected fungus *P. purpurogenum* and to screen some of their biological 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 & Yamamure, 2005) and soil dilution method (Ando, 2014).

Antimicrobial activities of isolated soil microorganisms

The antimicrobial activities of isolated soil microorganisms were tested by paper disc diffusion assay method (NITE, 2005). 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. The fermented broth was used by paper disc diffusion assay 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* HU9743*,Escherichia coli* AHU5436*, Saccharomyces cerevisae* NITE52847*, Pseudomonas fluorescens* IFO94307 *and Staphylococcus aureus* AHU8465 which are provided by NITE & Kyowa Hakko Co. Ltd. Paper disc having eight millimeter diameter were utilized for antimicrobial assays.

Study on the solvent systems for the extraction of antifungal metabolites by bioautograph method

The purified fermented broth were 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) were 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

Selected soil fungi *P. purpurogenum* were inoculated in a fermentation medium 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).The fermented broth (1L) was incubated agitation for 6 days at 25 °C. Once the incubation period was complete, mycelia were filtered by using sand column. The pure fermented broth was submitted to a liquid-liquid extraction (1:1) 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 phase. 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.

Separation and purification of the active antifungal metabolites from *n*-BuOH crude extract

A glass chromatographic column (50×3 cm) with a tap attached was clamped so that it was perfectly vertical. The column was packed by the wet method, using PE (only). The column was plugged by pushing a small piece of cotton wool through the solvent with a glass rod. Care was taken so that no air bubbles were trapped in the cotton wool. Silica gel (ca.50 g) was measured and placed in a beaker and made into slurry by mixing with pet ether and the suspension was thoroughly stirred. A portion of the slurry was poured into the column and at the same time the tap was opened so that the solvents flowed at a slow but constant rate. As the column material slowly settled to the bottom, the column was lightly tapped with a rubber tubing around the outside wall so as to achieve an air bubble free, uniform packing. Column materials sticking to the upper walls of the column were washed down with the solvent. When the level of solvent had fallen to a few millimettres above the top of the silica gel column, the tap was closed.

A 4.0 g of *n*-BuOH crude extract was dissolved in *n*-BuOH and mixed with a little amount of silica gel. The mixture was allowed to evaporate with continuous agitation so that a free flowing dry silica gel on which the sample was uniformly adsorbed. By careful pouring of the adsorbed gel down the small funnel and adjusting the position of the lower end of the tube, a uniformed layer of

adsorbed gel placed onn the top of the column. The top of the layer was wet with solvent. A piece of cotton wool was placed between the solvent and the column gel. The column was then completely filled with the solvent system and fraction was started; flow rate was adjusted to about one drop per five seconds. Gradient elution was performed successively with increasing polarity (PE only, PE:EtOAc, 10:1; 10:2; 10:3; 10:4; 2:1; 1:1 and followed by PE: EtOAc: MeOH in ratio 2:3:1 v/v). Successive fractions obtained were combined on the basic of their behaviour on TLC. Finally 29 main fractions (F_1 to F_{29}) were collected. Fraction F-21 was occurred as compound mixture, fraction F-23 and F-26 were occured as solid materials. Other fractions occurred as mixtures.

Antifungal activity of each fractions were examined in bioassays to determine the fractions containing the active compound. Fractions F-21, F-23 and F-26 were found to have antifungal activity against *C. albicans*. Other fractions were found to be inactive against the tested *C. albicans* and so were disposed.

Successive fraction F-21 containing compound mixtures was further chromatographed (column size 30×1.5 cm) on small scale as the above procedure using eluted solvent PE:EtOAc in ratio (5:1 and 3:1) and PE:EtOAc:MeOH (2:3:1) finally provided two compounds I and II. Fraction F-23 was evaporated, washed with PE only and then PE:EtOAc (2:1 v/v) and then crystallized from MeOH, yielded 0.1625 %, (6.5mg) of compound III as colourless needle shape crystals. Fraction F-26 was evaporated, washed with PE (only) and then PE:EtOAc (2:1 v/v) and then crystallized from MeOH, yielded 0.1125 %, (4.5 mg) of compound IV as colourless solids.

Bioassay for antifungal activity

Fractions of 1-29, each fraction was re-dissolved with 2 ml methanol and put on paper disc. A 20 ml GPA assay medium (Goucose, peptone and agar) was suspended with 0.1 ml *C. albicans* spores suspension in water and shaken gently, then poured into a sterile petri dish and allowed to solidify. Bioassay for measuring the antifungal activity was carried out by the so-called paper disc diffusion method, done according to the following procedures.

After solidification, paper discs impregnated on the agar plates. The test plates were incubated at 27 ºC for 24-48 hours. Clear zones (inhibitory zones) were observed surrounding the paper disc indicated the presence of bioactive metabolites which inhibit the growth of *C. albicans*. The inhibition zone diameter was measured with (digital caliper) three times in three different directions, and then averaged. No inhibitory diameter zone of other fractions were deposited.

Physicochemical characterization of isolated compounds

The isolated compounds were subjected to TLC analysis and their R_f values were determined. GF_{254} silica gel precoated aluminium plate (Merck) was empolyed and the chromatogram was developed in the appropriate solvent system. After the TLC plate was dried, the R_f values of isolated compounds were measured. Localization of spot was made by viewing directy under UV 254 nm and 365 nm light or by treating with visualizing agents.

Determination of solubility of isolated compounds

A 0.5 mg each of isolated compounds was subjected to 0.5 mL of polar and non-polar solvents such as H_2O, EtOAc, Hexane, $CHCl_3$, MeOH, EtOH, Acetone and PE in order to know their solubilities.

Determination of some chemical properties of isolated compounds

The isolated compounds were subjected to TLC analysis and then treated with some coloured reagents such as Vanaline sulphuric acid, Anisaldehyde sulphuric acid, Lieberman Burchard,, 5% H_2SO_4, 5% $FeCl_3$, and I_2 vapour to study their behaviour on TLC. Isolated

compounds were also treated with Hydroxyl ammonium chloride to examine their type of compounds.

Classification and identification of isolated compounds

The isolated compounds were structurally identified by modren spectroscopic techniques such as UV-visible and FTIR spectrometry.

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, where they found out that there was greater amounts of bacterial and fungal populations in the top soil (0-10 cm) if compared to that of other depths. Alexander, 1997, said that physicochemical analysis of soil showed that pH range of soil conditions ranging from 5.1 to 7.5 and soil textures were determined the fungal population. In this study, the most soil samples were 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.

The selected fungus MF-12 was identified to the genus level and to the species when possible on the basis of macro-morphology and micromorphology characteristics on LCA medium, according to references keys of (Ando, 2016). Colonies on PGA medium (7 days, room temperature) was 47.45 mm in diameter. Mycelium was white to pale at the margins and greyish in the center. Sporulation was heavy with greyish green colors, reverse was orange with cream to white margins and deep orange in the center. The vegetative characters of genus *Penicillium* sp is a mycelium, which consists of freely branched, hyaline coloured and septate hyphae.

The mycelium may grow superficially forming a weft upon the substrate. The hyphae cells contain usually a single nucleus. The conidiophores are unbranched. At the ends of the unbranched conidiophores, clusters of finger-shaped metulae are developed two stage branched (Biverticillate-asymmetrical). Each metullae finally terminated in a tuft of uninucleated flask-shaped branches, which are called sterigmata or phialides. The conidiophore along with its branches (metullae), sterigmata or phialides and conidia in basipetal chains, looks like a brush-like structure, known as the *penicillus* (brush).

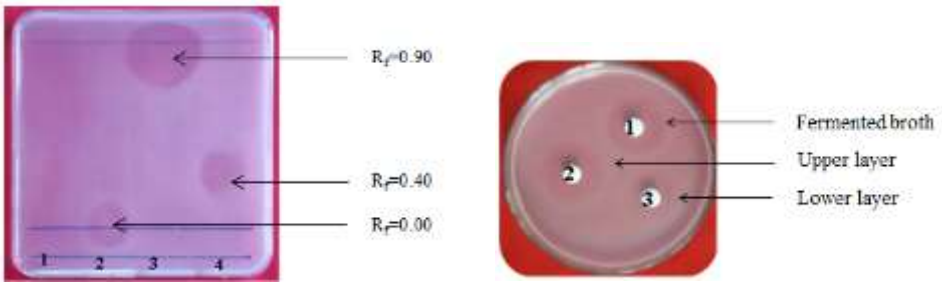
Based on the morphologically and microscopically characters and according to Barnett, 1956; Domsh 1980, Watanabe, 2002 and Ando, 2016, the selected fungus MF-12 was identified as *Penicillium purpurogenum* Stoll 1904 (Figure.1).

Front view of colony Reverse view of colony Micromorphology (10 × X 40)

Figure 1 Colony characters (7 days) and micromorphology of *P. purpurogenum*

Andriy, *et al*., 2016 describe that the antimicrobial activity of the fungal extracts derived from their mycelia. Geweely, *et al*., 2011 said that *P. purpurogenum* inhibits 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. In this research, several clinically important yeasts *C. albicans* was selected for the antifungal activity of *P. purpurogenum*.

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). The antifungal secondary metabolites were detected from the fermented broth of *P. purpurogenum* by using bio-autography method. An overall view of bio-autography has been depicted in Figure 2(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. 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.



(A) (B)

Figure 2 (A) Extraction of antifungal metabolites by bio-autography, (B) bioassay

In this study, the filtrate of fermented broth showed that (ID=26.70 mm) and upper layer (ID=31.85mm) and lower layer (ID=16.34mm) were optimized and described in Figure 1 (B). Therefore, the upper of metabolites were collected for the separation and purification of antifungal compounds. 0.8 g/L of dry reddish brown crude extract was obtained. The reddish brown crude extract obtained after *n*-BuOH extraction was subjected to thin layer chromatography to detect the various components present in the crude extract of *P. purpurogenum*.

The various metabolites present in the crude extract were detected by thin layer chromatography. By using the solvent system, PE (only), PE: EtOAc (10:1; 10:2; 10:3; 10:4; 2:1; 1:1) and PE: EtOAc: MeOH (1:1.5:0.5) for silica gel column chromatography, 29 fractions (F_1-F_{29}) were obtained. Bioassay for measuring the antifungal activities of fractions 1 to 29 were carried out by bioassay method and the results are shown in Table 1 and Figure 3. Isolation of some bioactive compounds from fraction $F_{21.1}$ to $F_{21.11}$ was done out by paper disc diffusion method. Among the fractions, F_1-F_{20}, F_{22}, $F_{24\text{-}25}$, $F_{27\text{-}29}$ were discarded because these fractions did not show the antifungal activity. Fractions F_{23} and F_{26}, compounds III and IV and fraction $F_{21.11}$, compounds I and II were obtained. Compound I (colourless needle shape crystals, 0.1517 %,), compound II (pale yellow solids, 0.12%), compound III (colourless needle shape crystals, 0.1625 %) and compound IV (colourless solids, 0.1125 %,) were isolated from *n*-BuOH extract of *P. purpurogenum* metabolites.

Table 1 The average diameter of the inhibition zone of fractions F_1-F_{29}

Fraction	Inhibition diameter zone (mm)	Fraction	Inhibition diameter zone (mm)
F_1 to F_2	-	F_{21}	18.43
F_3	15.47	F_{22}	-
F_4	-	F_{23}	22.21
F_5	14.56	F_{24}	-
F_6 to F_{15}	-	F_{25}	-
F_{16}	+	F_{26}	18.21
F_{17} to F_{20}	-	F_{27} to F_{29}	-

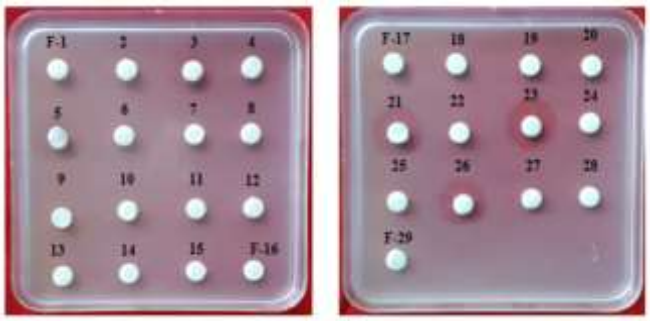


Figure 3 The inhibition diameter zone of fraction F_1 to F_{29}

The isolated compound I was classified by chemical reagent tests and UV, FT IR spectroscopy. Its R_f values was found to be 0.33 in PE:EtOAc (3:1 v/v) solvent system. It gave a yellow with iodine vapour, violet spot with anisaldehyde-sulphuric acid followed by heating and greenish blue colouration with Liebermann Burchard reagent on TLC and gave deep red colouration on testing with ethanolic hydroxylamine hydrochloride reagent which is the test for ester and so it could be classified as a steroid which contained an ester group. No absorption was observed in readily accessible UV region.

The FT IR spectrum at compound I showed the absorption bands at 3323 cm^{-1} for –OH stretching vibration of alcohol. Absorption band at 2921 and 2853 cm^{-1} were due to asymmetric and symmetric C-H stretching vibration of $–CH_2$ and $–CH_3$ groups. The corresponding C=O stretching vibration of cyclic ketone was observed at 1717 cm^{-1}. The C=C stretching vibration of olefinic group was found at 1642 cm^{-1}. The corresponding –CH bending vibrations of $–CH_2$ and $–CH_3$ were shown at 1450 and 1375 cm^{-1}. The corresponding C–O–C stretching vibration of ester was observed at 1106 cm^{-1}. The corresponding –C–O stretching vibration of alcohol was observed in 1044 cm^{-1}. The corresponding =C–H bending vibration was observed at 877 cm^{-1}. All the above information inferred the isolated compound I as a steroid that contained an ester group.

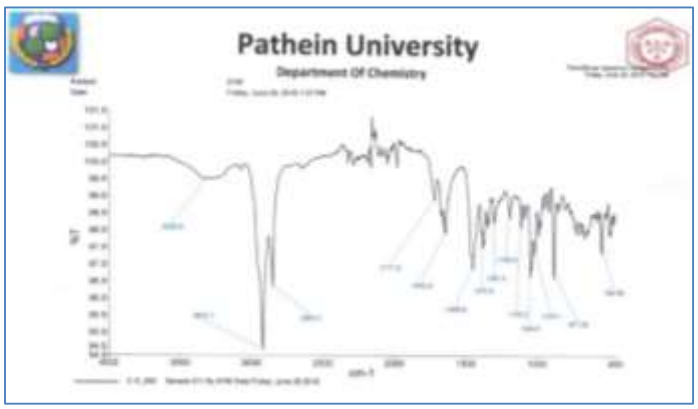


Figure 4 FT IR spectrum of isolated compound-I from $F_{21.11}$

The R_f value of compound II was found to be 0.35 in the solvent system of PE: EtOAC (2:1 v/v). According to the result obtained from chemical test compound II developed into cherry red on TLC while spraying with 5%H_2SO_4 followed by heating, yellow with iodine vapour, violet with anisaldehyde-sulphuric acid followed by heating and green colouration with Liebermann Burchard reagent and so it could be classified as a steroid. It was UV active indicating the presence of conjugated double bond.

The FT IR spectrum at compound II showed the bands at 3373 cm^{-1} due to stretching vibration of O–H. Absorption band at 2960 cm^{-1} and 2874 cm^{-1} were due to asymmetric and symmetric C–H stretching vibration of -CH_2 and -CH_3 groups. The corresponding C=C stretching vibration was observed at 1660 cm^{-1}. The banding vibration of methyl parts and methylene parts were noticed by the medium intense bands at 1450 and 1306 cm^{-1}. The corresponding C–O stretching vibration of cyclic alcohol was shown as weak intense band at 1037 cm^{-1}. From the physicochemical properties, melting point, R_f values and FT IR spectral data, isolated compound II was classified as a steroid.

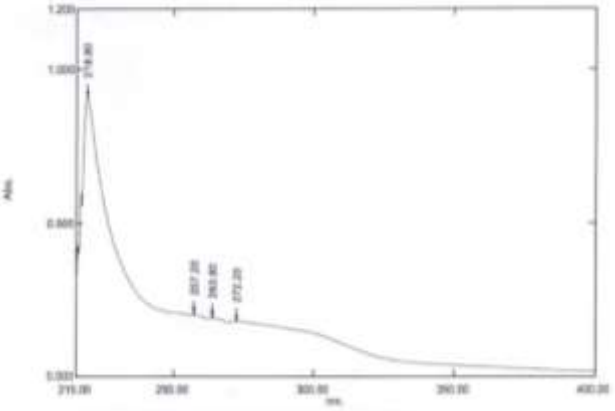


Figure 5 UV-Vis spectrum of isolated compound-II from $F_{21.11}$

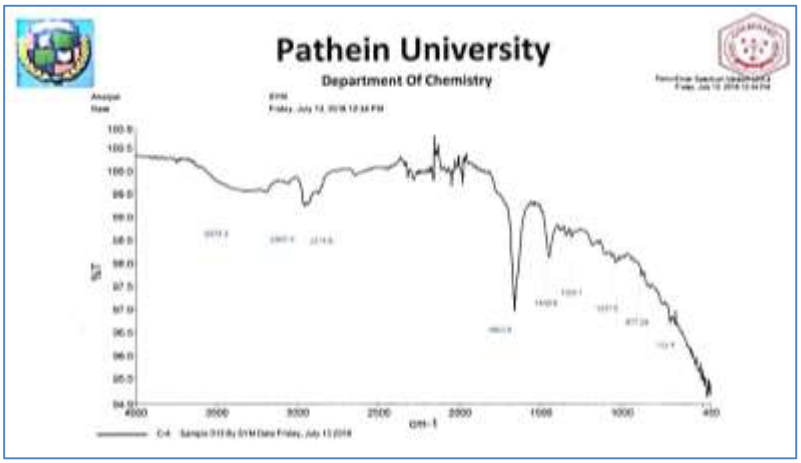


Figure 6 FT IR spectrum of isolated compound-II from $F_{21.11}$

The R_f values of compound III was found to be 0.86 in PE:EtOAC (1:2 v/v) solvent system. According to the result obtained from chemical test, compound III developed into yellow on TLC with iodine vapour and it decolourized 1% $KMnO_4$. It was UV active indicating the presence of conjugated double bond.

The FT IR spectrum of compound III showed the bands at 3334 cm^{-1} due to stretching vibration of O-H. Absorption band showed at 2914 and 2846 cm^{-1} due to asymmetric and symmetric C-H stretching vibrations of -CH_2 and -CH_3 groups. The aromatic C=O stretching was

observed at 1671 cm^{-1}. The corresponding C=C stretching in aromatic ring was observed at 1575 and 1543 cm^{-1} respectively. The banding vibration of methyl parts and methylene parts were noticed by the medium intense bonds at 1464 cm^{-1} and 1429 cm^{-1} respectively. The C-O stretching vibration of alcohol was observed by 1041 cm^{-1}. The =CH bending vibration was found that 877 cm^{-1}. The corresponding C-H out of plane bending vibration of benzene ring at 720 cm^{-1}. From the physicochemical properties, R_f values and FT IR spectral data, isolated compound III from fraction F_{23} was classified as an aromatic derivative containing carbonyl and alcohol functional group.

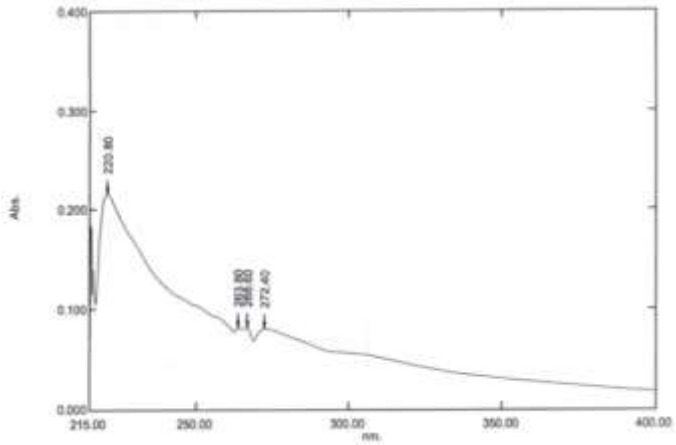


Figure 7 UV-Vis spectrum of isolated compound-III from F_{23}

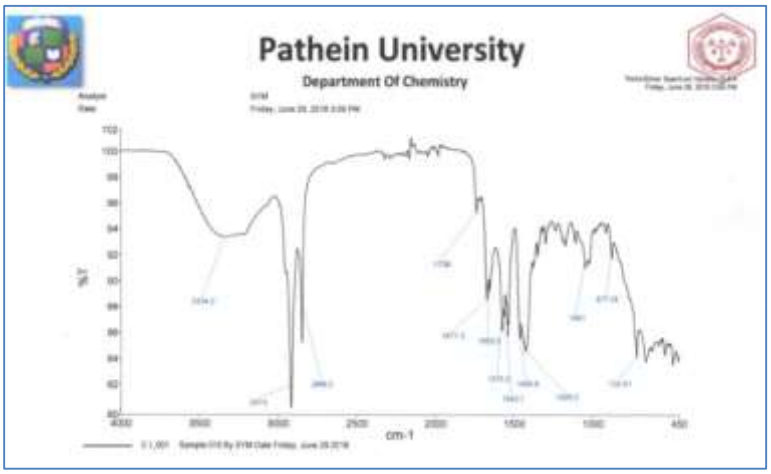


Fig. 8 FT IR spectrum of isolated compound-III from F_{23}

The R_f value of compound IV was found to be 0.6 in the solvent system of PE:EtOAC (1:2 v/v). According to the result obtained from chemical test, compound IV was developed into yellow on TLC chromatogram with shown iodine vapour and did not develop any colour with anisaldehyde-sulphuric acid and libermannburchard test followed by heating. It was UV active indicating the presence of conjugated double bond.

The FT IR spectrum of compound IV showed the bands at 3366 cm^{-1} due to O–H stretching vibration of alcohol. Absorption band at 3089 cm^{-1} was found to be C–H stretching vibration of aromatic compound. The C–H stretching vibration of $–CH_2$ and $–CH_3$ was observed at 2921 and 2849 cm^{-1}. The corresponding C=C stretching in aromatic ring was shown at 1575 and 1511 and 1404 cm^{-1}. The corresponding C–H bending vibration of $–CH_3$ was shown at 1379 cm^{-1}. The C–O stretching vibration of alcohol was observed at 1037 cm^{-1}. From the physicochemical properties, R_f values and FT IR spectral data, isolated compound IV was classified as an aromatic derivative.

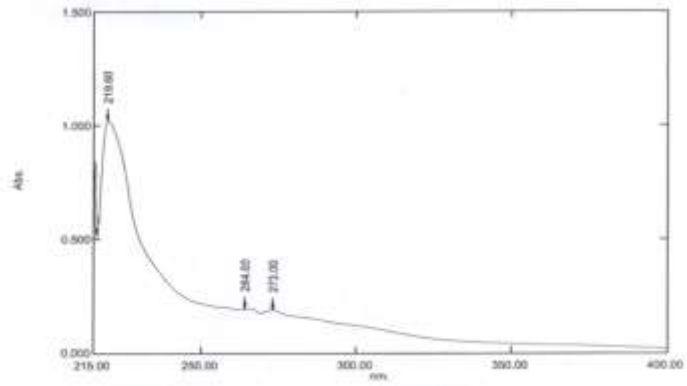


Figure 9 UV-Vis spectrum of isolated compound-IV from F_{26}

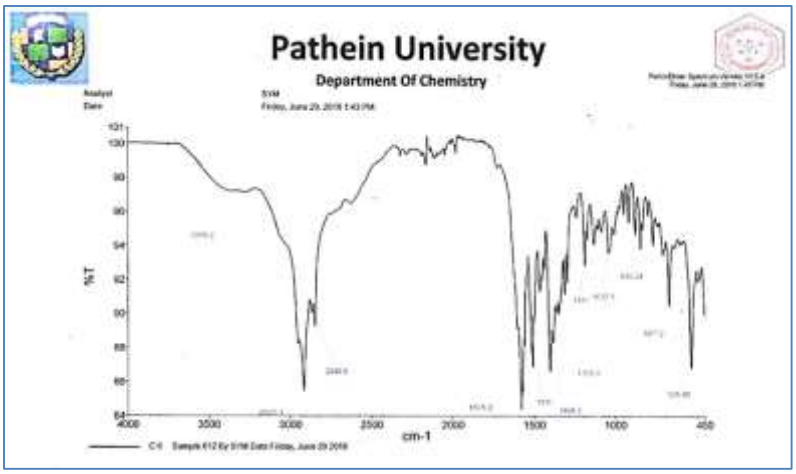


Figure 10 FT IR spectrum of isolated compound-IV from F_{26}

Aromatic steroids are lipids that contain at least one or more aromatic ring (s) in a steroid skeleton. The aromatic steroids can be produced from microorganisms, fungi, and marine invertebrates, and also they were found in plants, animals, marine sediments, and karst deposits. Biologically active aromatic steroids likely have an anti-tumor, anti-inflammatory, and neuron protection activity. This review emphasizes the role of aromatic steroids as an important source and potential leads for drug discovery and they are of great interest to chemists, physicians, biologists, pharmacologists, and the pharmaceutical industry (Valery, *et al.*, 2018). Biological activity of mono, di and tri aromatic steroids isolated from plants, fungi, invertebrates, marine sediments, and oils.

Penicillium is a genus of ascomycetous fungi of major importance in the environment, food and drug production **(**Tiwari, 2011). 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*. The IR spectra of 1 and 2 showed the absorption peaks characteristic for hydroxyl group (3400 cm^{-1}), lactone or ester carbonyl (1740 cm^{-1}) and amide carbonyl (1635cm^{-1}). 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 gonorrhea and syphilis (Tiwari, *et al*., 2011).New bioactive metabolites continue to be discovered from these fungi nowadays indicating their current importance as sources of high amounts of novel bioactive molecules to be used by pharmaceutical industry (Petit*, et al.,* 2009).

Conclusion

Based on the results from this study, metabolites of *P. purpurogenum* showed antifungal activity against on the diseases of *C. albicans.* The isolated antifungal compounds 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 have been proved that 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 determination of chemical structures and identification of the antifungal compounds.

Acknowledgements

The authors would like to thank the Department of Higher Education, Ministry of Education, Myanmar, for the permission of doing this research and also to the Myanmar Academy of Arts and Science for allowing to present this paper. My deepest gratitude is expressed to Dr Nyunt Phay, Director General, Department of Monitoring and Evaluational (Education), Ministry of Education, for his invaluable instruction, suggestion, adivice, guidance and encouragement to do this research work. I would like to tender my thank to Dr Mar Lar, Pro-rector, Hinthada University for her permission to do this research. I am so thank to Dr Ohn Mar Tin , Professor and Head and Dr Thi Thi Aye, Professor, Department of Chemistry, Hinthada University for their encouragements and permission to do this research.

References

Adrio, J. L. and Demain, A. (2003). "Fungal biotechnology". *Int. Microbiol*., 6: 191-199.

Alexander, M., *Introduction to soil Microbiology*, John Wiley &Sons, (1977), New York.

Amna, A., Muhammad Saleem Haider, Ibatsam Khokhar, Uzma Bashir, Sobia Mushtaq and Irum Mukhtar. (2011). "Antibacterial activity of culture extracts of *Penicillium* species against soil-borne bacteria", *Mycopath*, 9 (1): 17-20.

Andriy, S. M., Jutamart, B., Roman, M., Anna, R. Tomas., A. Juhee., C. Ekachai. (2016). "Antimicrobial activity of crude extracts prepared from fungal mycelia", *Asian Pacific Journal of Tropical Biomedicine, journal homepage*, **7**: 257-261.

Ando, K. (2014). *Sampling, Isolation, Cultivation and Preservation of Microorganisms*, Biological Resource Center, National Institute of Technology and Evaluation (NITE), Japan.

Ando, K. (2016). *Identification of Mitosporic Fungi***,** Basic Laboratory Workshop**,** Biological Resource Center, National Institute of Technology and Evaluation (NITE), Japan.

Aly, A. H., Debbad and P. Proksch. (2011). "Fungal endophytes: unique plant inhabitants with great promises"**.** *Applied Mocro-biology & Biotechnology*, 90, 1829-45.

Barnett, H. L. (1956): *Illustrated genera of imperfect fungi*, Second Edition, Burgess. Pub. Co. Ltd., US.

Domsch, K. H., Gams, W. and Anderson, T. H. (1980). *Compendium of Soil Fungi.* Academic Press, London.

Geweely, S. and Neveen, S. (2011). "Investigation of the optimum condition and antimicrobial activities of pigments from four potent pigment-producing fungal species", *J. Life Sci*. 5, 201–205.

Marasabessy, A., Rudiyono, R and Diana, D. (2017). "Separation, Purification and Chemical Structure Examination of Antifungal Compound from *Streptomyces herbaricolor* Biomcc-A.RP-131". Agency for the Assessment and Application of Technology (BPPT), Jakarta, Indonesia.

NITE (National Institite of Technology and Evaluation) (2005): *Antifungal activities test*, Japan.

Nose, H., A. Seki, T. Yaguchi, A. Hosoya, T. Sasaki, S. Hoshiko & T. Shomura. (2000). "PF1163A and B,New antifungal antibiotics produced by *Penicillium sp*. I. Taxonomyof producing strain, fermentation, isolation Taxonomy of producing strain, fermentation, isolation and biological activities". *J. Antibiotics* 53: 33-37.

Pelaez, F. (2005). *In Handbook of Industrial Mycology*, 1st Edition, Vol. 22, 49–92, (Marcel Dekker, New York).

Petit, P., Lucus, E. M. F., Abreu, L. M., Pfenning, L. H. and Takahashi, J. A. (2009). "Novel antimicrobial secondary metabolites from *Penicillium* sp. Isolated from Brazilian ce*rrado* soil", *Electron . J. Biotechnology*., 12, 8.9.

Phay & Yamamure, (2005). "Approach method for rare microorganisms from soil sources", *J. Microbial*., 76, 237-239.

Tangjang, S. K. runachalam, A. Arunachalam, A.K. Shukla, (2009). "Research Journal of Soil Biology", 1(1), 1-7.

Shittu, O. B., F.V. Alofe, G.O. Onawunmi, A.O. Ogundaini and T. Tiwalade, (2006). "Bioautographic evaluation of antibacterial metabolite production by wild mushrooms". *Afr. J. Biomed*. Res., 9, 57-62.

Stachelhau, T., Schneide, A and Marahiel, Ma. (1995)**. "**Rational deign of peptide antibiotics by targeted replacement of bacterial and fungal domains", Science, 269, (5220), 69-72.

Stanbury, P.F., Whitaker, A. and Hall, S. J. (1997). *Principles of Fermentation Technology*. Elsevier. London. 269, 69-72.

Tajick, M, A., Hamideh, S, M, K., Valiollah, B. (2014). "Identification of biological secondary metabolites in three *Penicillium* species, *P. goditanum*, *P. moldavicum*,and *P. corylophilum*". Progress in Biological Sciences 4, 53-61.

Tiwari, K L., S.K. Jadhav and Ashish, Kumar. (2011). "Morphological and molecular study of different *Penicillium* species", Middle-East *Journal of Scientific Research* 7 (2): 203-210, 2011.

Valery, M. Dembitsky., N. Savidov., V. V. Poroikov., Tatyana. A. Gloriozova., Andrew, B. Imbs. 2018. "Naturally occurring aromatic steroids and their biological Activities". Applied Microbiology and Biotechnology, 102:4663–4674 8968-7.

Watanabe, T. (2002): *Pictorial Atlas of soil and seed fungi, Morphologies of cultured fungi and key to specie*s, 2nd Edition, CRC Press.