Antimicrobial Activity of Isolated Soil Fungi

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Abstract

In this study, the two soil samples were collected from the two different depths (20 cm and 40 cm) near Bago River in (94) Ward, Dagon Seikkan Township, Yangon Region. The twelve soil fungi were isolated from the collected samples on three different media by the serial dilution method. Isolated strains were *Cephalosporium* sp., *Aspergillus* spp., *Penicillium* spp., *Cladosporium* sp., *Madurella* sp., *Paecilomyces* sp., *Hormiscium* sp. and *Gonatobotrys* sp. Antimicrobial activity of the fermented broths from all isolated strains was examined on five test organisms by paper disc diffusion method and fermentation was carried out from 4 to 10 days. The fermented broths of all isolated strains (HAN 1 to HAN 12) showed antimicrobial activity on *Agrobacterium tumefaciens* and *Malassezia furfur* from fourth day to ten day fermentations. Crude metabolites of twelve active strains were extracted with ethyl acetate, and their antimicrobial activity was also examined on five test organisms. The crude extracts of twelve strains (HAN 1 to HAN 12) indicated excellent antimicrobial activity on *Agrobacterium tumefaciens*, *Candida albicans*, *Escherichia coli* and *Malassezia furfur* at day 7 fermentation. The most active strains should be chosen to produce the bioactive compounds to protect *Agrobacterium tumefaciens* causing crown gall disease on plants, *Candida albicans* causing alimentary tract and vaginal infections, *Escherichia coli* causing urinary tract infection and *Malassezia furfur* causing dandruff and skin infections on humans.

Keywords: Antimicrobial activity, Fermented broth, Soil fungi

Introduction

Soil microorganisms are classified into bacteria, actinomycetes, fungi, algae and protozoa. Each of these groups has characteristic functions in soils (Mendes *et al*., 2013). Fungi benefit most plants by suppressing plant root diseases and fungi promote healthier plants by attacking plant pathogens with fungal enzymes (Lowenfels & Lewis, 2006; Sylvia *et al*., 2005).

The various species of *Penicillium* can colonise many different environments: in soils, in air, in foods, etc. (Banke *et al*., 1997). Chemical and biological investigations on the ethyl acetate extract of *Penicillium* were tested with *Candida albicans* (Petit *et al*., 2009). *Cladosporium* species are found in soil and on decayed plant material (Revankar and Sutton, 2010).

Aspergillus has proven to be a prolific source of secondary metabolites with interesting biological activities, including antibacterial activity. The antibacterial metabolites or extracts were described from terrestrial *Aspergillus* spp. which may have pharmaceutical importance as antibacterial agents (Xu *et al*., 2015).

The objectives of present research work are to collect the soil samples of two different depths, to isolate fungal strains from soil samples, to investigate antimicrobial activity of fermented broths of all isolated strain and to evaluate antimicrobial activity of all the extracts of isolated strains.

Materials and Methods

Collection of Soil Samples

The two soil samples were collected from the two different depths near Bago River in Dagon Seikkan Township, Ward (94),Yangon Region. The soil samples (10 g for each sample) were taken between twenty centimeter depth and forty centimeter depth and put them into the clean plastic bags. Soil samples were recorded by using the Global Positioning System (GPS) in Table (1). The pH of each sample measured with the pH paper.

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Table (1) Location of the collected soil samples

Soil sample	Location	Depth
Sample 1	N 16° 49' 34"	Twenty centimeter depth
Sample 2	N 16° 49' 34"	Forty centimeter depth

Composition of culture media (Atlas, 1993)

Medium 1

Nutrient Agar Medium (NA)

Nutrient Agar 3.5 g, Distilled Water 100 ml, Agar 1.0 g, pH 6.8

Medium 2

Sucrose/Yeast Extract Agar Medium (SYA)

Sucrose 1.0 g, Yeast 0.3 g, Distilled Water 100 ml, Agar 2.5 g, pH 6.8

Medium 3

Lactose/Yeast Extract Agar Medium (LYA)

Lactose 1.0 g, Yeast 0.3 g, Distilled Water 100 ml, Agar 2.5 g, pH 6.8

Antimicrobial Activities of Isolated Fungal Strains

Fermentation

The isolated twelve fungal strains grown on 5 days old slant cultures were inoculated into 12 conical flasks (50 ml) containing 20 ml of sucrose/yeast extract medium in each for three day as seed culture. After three days, the seed cultures of twelve strains (1 ml of each) were transferred to twelve fermentation flasks at 30℃, 100 rpm on shaker for 10 days. Inhibitory zones were measured to examine antimicrobial activity of isolated strains (Strobel and Sullivan, 1999).

Sucrose/Yeast Extract Medium (SY)

Sucrose 1.0 g, Yeast extract 0.3 g, NaCl 0.3 g, $CaCO_3$ 0.01 g, Distilled Water 100 ml, pH 6.8

Test agar plates

There are five test organisms: three bacterial test organisms (*Agrobacterium tumefaciens, Bacillus subtilis* and *Escherichia coli*) and two fungal test organisms (*Candida albicans* and *Malassezia furfur*) in Table (2). Broth culture (0.3 ml) of each test organism is added into 100 ml nutrient agar medium, and then poured into plates.

Paper disc diffusion method

After solidification, the paper discs impregnated with fermented broth samples were applied on the test plates. These plates were incubated at 30°C for 24 hr. After 24 to 48 hr, clear zones (inhibitory zones) surrounding the test discs were measured. These zones indicate the presence of the bioactive compounds which inhibit the growth of test organisms (Davis and Stout, 1971).

Table (2) Test organisms and diseases

Test organisms	Diseases
Agrobacterium tumefaciens	Crown gall on plant
Bacillus subtilis	It can cause dysentery, but at the first sign of diarrhoea
Candida albicans	Skin infections, vaginal candidasis, alimentary tract infections
Escherichia coli	Cholera, diarrhoea and vomiting, urinary tract infection
Malassezia furfur	Dandruff, skin infections

Antimicrobial activity of crude extracts of fungal strains

The fungal strains were inoculated into the twelve conical flasks containing SY seed medium. Each flask contained 20 ml of medium. After three day, seed cultures (1 ml of each) were transferred to the twelve fermentation flask at 30℃ for 7 days on the shaker at 100 rpm. Each fermentation flask contained 20 ml of medium. At day 7 fermentation, the fermented broth of each fungal strain was extracted with ethyl acetate (10 ml) at pH 4.5. Then, the ethyl acetate extracts were dried in the incubator at 40℃. Then, each dried extract was added 0.5 ml of ethyl acetate and mixed thoroughly with glass rod. The extract (20 µl/disc) of each strain was applied for their antimicrobial activity.

Results

Isolation of Soil Samples

Twelve fungal strains were isolated from two different soil samples. These strains were given as temporary names HAN 1 to HAN 12 as shown in Table (3) and Fig (1).

Table (3) Isolation of soil fungal strains

Strain	Soil sample	Depth
HAN 1 to HAN 5	Sample 1	Twenty centimeter depth
HAN 6 to HAN 12	Sample 2	Forty centimeter depth

Surface view of HAN 1 to HAN 12 Reverse view of HAN 1 to HAN 12

Fig. (1) Isolated fungal strains on slant cultures (HAN 1 to HAN 12)

Antimicrobial activity of fermented broths (day 4 and day 5) of isolated strains

The fermented broths of all strains showed weakly antibacterial activity on *Agrobacterium tumefaciens* at day 4 and day 5 fermentation. All isolated strains showed

weakly antifungal activity on *Malassezia furfur* at day 4 fermentation. Five strains showed weakly antibacterial activity on *Escherichia coli* at day 4 fermentation in Table (4). At day 5 fermentation three strains (HANs 5, 10 & 12) showed moderately antifungal activity but other strains weak activity on *Malassezia furfur*. Eight strains showed weak activity on *E. coli* at day 5 fermentation in Table (5).

Table (4) Inhibitory zones of day 4 fermented broths of isolated strains

Test org. Strain	*Agrobacterium tumefaciens*	*Bacillus subtilis*	*Candida albicans*	*Escherichia coli*	*Malassezia furfur*
HAN 1	10	-	-	9	9
HAN 2	10	-	-	-	10
HAN 3	11	-	-	-	10
HAN 4	12	-	-	-	11
HAN 5	11	-	-	-	12
HAN 6	10	-	-	-	10
HAN 7	11	-	-	10	9
HAN 8	10	-	-	-	11
HAN 9	9	-	-	10	9
HAN 10	10	-	-	-	10
HAN 11	10	-	-	9	12
HAN 12	11	-	-	10	12

10-12 mm = weak activity, 13-17 mm = moderate activity, > 18 mm = high activity

Table (5) Inhibitory zones of day 5 fermented broths of isolated strains

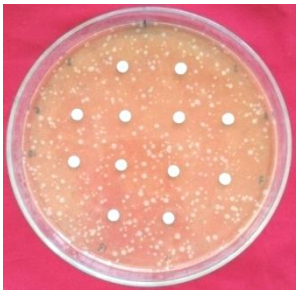
	A .tumefaciens	*B. subtilis*	*C. albicans*	*E. coli*	*M. furfur*
HAN 1	10	-	-	10	12
HAN 2	10	-	-	-	11
HAN 3	10	-	-	-	11
HAN 4	9	-	-	10	12
HAN 5	11	-	-	-	13
HAN 6	10	-	-	9	11
HAN 7	9	-	-	9	12
HAN 8	10	-	-	9	12
HAN 9	9	-	-	-	12
HAN 10	10	-	-	10	13
HAN 11	11	-	-	10	11
HAN 12	11	-	-	10	14

Antimicrobial activity of fermented broths (day 6 and day 7) of isolated strains

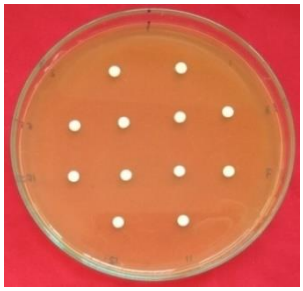
The fermented broths of four strains (HANs 2, 4, 5 & 11) showed moderately activity but other strains weak activity on *A. tumefaciens* at day 6 fermentation. Eleven strains showed weak activity on *E. coli.* At day 6 fermentation, two strains (HANs 1 & 4) showed moderately activity but other strains weak activity on *M. furfur* in Table (6) and Fig. (2). The fermented broths of all twelve strains showed moderately activity on *A. tumefaciens* at day 7 fermentation. Six strains (HANs 1, 2, 6, 9, 10 & 11) indicated better antimicrobial activity than other strains at day 7 fermentation. All strains showed moderate activity on *E. coli.* At day 7 fermentation, six strains (HANs 2, 6, 8, 10, 11 &12) showed moderately antifungal activity but other strains weak activity on *M. furfur* in Table (7) and Fig. (3).

Table (6) Inhibitory zones of day 6 fermented broths of isolated strains

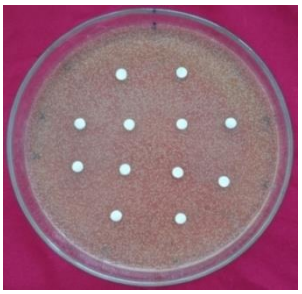
Strains	*A .tumefaciens*	*B. subtilis*	*C. albicans*	*E. coli*	*M. furfur*
HAN 1	12	-	-	11	14
HAN 2	13	-	-	9	12
HAN 3	12	-	-	9	11
HAN 4	14	-	-	10	13
HAN 5	13	-	-	9	11
HAN 6	12	-	-	9	12
HAN 7	11	-	-	9	10
HAN 8	10	-	-	9	11
HAN 9	10	-	-	-	9
HAN 10	11	-	-	10	11
HAN 11	14	-	-	11	11
HAN 12	12	-	-	11	10



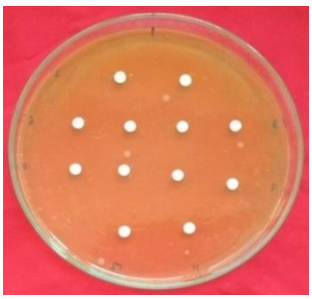
Agrobacterium tumefaciens



Bacillus subtilis



Candida albicans



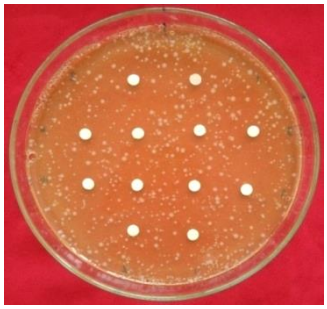
Escherichia coli

Malassezia furfur

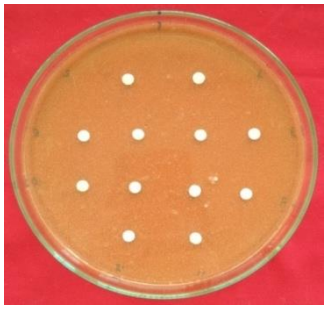
Fig. (2) Inhibitory zones of 6 day fermented broths of isolated twelve strains

Table (7) Inhibitory zones of day 7 fermented broths of isolated strains

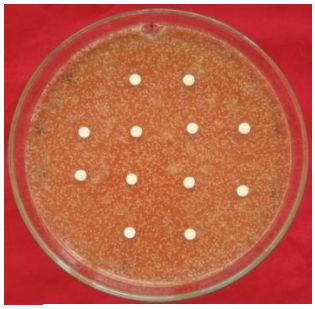
Strains	*A .tumefaciens*	*B. subtilis*	*C. albicans*	*E. coli*	*M. furfur*
HAN 1	15	-	-	13	11
HAN 2	16	-	-	13	13
HAN 3	13	-	-	16	11
HAN 4	14	-	-	16	10
HAN 5	13	-	-	15	12
HAN 6	15	-	-	15	13
HAN 7	14	-	-	16	10
HAN 8	13	-	-	13	13
HAN 9	15	-	-	14	9
HAN 10	15	-	-	15	13
HAN 11	16	-	-	15	13
HAN 12	13	-	-	16	14



Agrobacterium tumefaciens

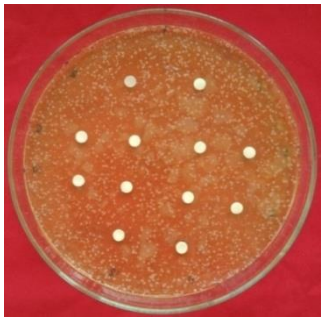


Bacillus subtilis



Candida albicans

Escherichia coli



Malassezia furfur

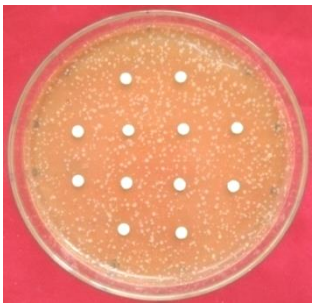
Fig. (3) Inhibitory zones of 7 day fermented broths of isolated twelve strains

Antimicrobial activity of fermented broths (day 8 and day 9) of isolated strains

At day 8 fermentation, the fermented broths of five strains (HANs 4, 6, 9, 10 & 11) showed moderately antimicrobial activity but other strains weak activity on *A. tumefaciens* and *M. furfur* and five strains showed moderate activity on *E. coli* in Table (8) and Fig. (4). At day 9 fermentation, two strains (HANs 9 & 10) indicated moderately antibacterial activity but other strains weak activity on *A. tumefaciens*. Seven strains (HANs 1, 2, 3, 4, 5, 6 & 10) indicated moderately antimicrobial activity but other strains weak activity on *M. furfur* in Table (9) and Fig. (5)

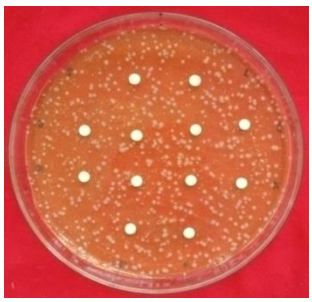
Table (8) Inhibitory zones of day 8 fermented broths of isolated strains

Strains	*A .tumefaciens*	*B. subtilis*	*C. albicans*	*E. coli*	*M. furfur*
HAN 1	11	-	-	12	10
HAN 2	12	-	-	13	12
HAN 3	11	-	-	13	12
HAN 4	13	-	-	15	14
HAN 5	11	-	-	12	11
HAN 6	14	-	-	11	13
HAN 7	12	-	-	14	12
HAN 8	10	-	-	10	11
HAN 9	14	-	-	10	13
HAN 10	15	-	-	12	13
HAN 11	13	-	-	14	15
HAN 12	12	-	-	12	11


Agrobacterium tumefaciens


Bacillus subtilis


Candida albicans


Escherichia coli

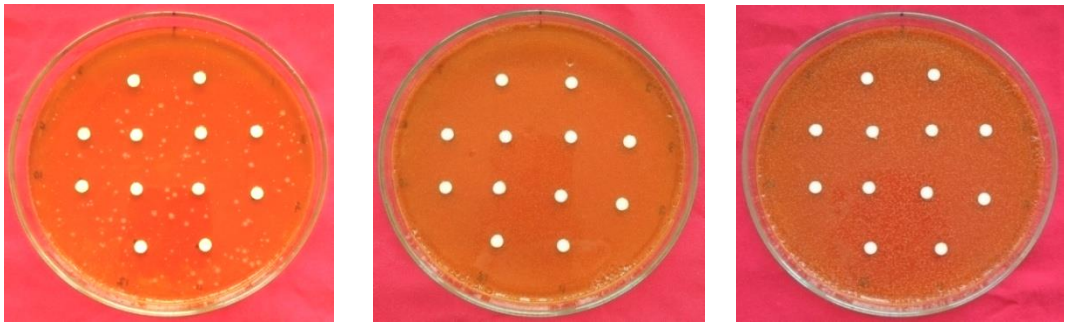

Malassezia furfur

Fig. (4) Inhibitory zones of day 8 fermented broths of isolated twelve strains

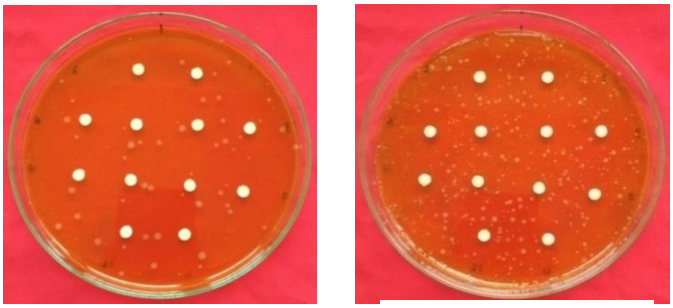
Table (9) Inhibitory zones of 9 days fermented broths of isolated strains

Strains	*A .tumefaciens*	*B. subtilis*	*C. albicans*	*E. coli*	*M. furfur*
HAN 1	11	-	-	11	15
HAN 2	11	-	-	14	16
HAN 3	11	-	-	11	13
HAN 4	12	-	-	12	15
HAN 5	11	-	-	12	13
HAN 6	12	-	-	11	15
HAN 7	11	-	-	12	10
HAN 8	10	-	-	11	10
HAN 9	14	-	-	12	10
HAN 10	14	-	-	12	13
HAN 11	12	-	-	10	10
HAN 12	10	-	-	10	11

10-12 mm = weak activity, 13-17 mm = moderate activity, > 18 mm = high activity



Agrobacterium tumefaciens *Bacillus subtilis* *Candida albicans*



Escherichia coli *Malassezia furfur*

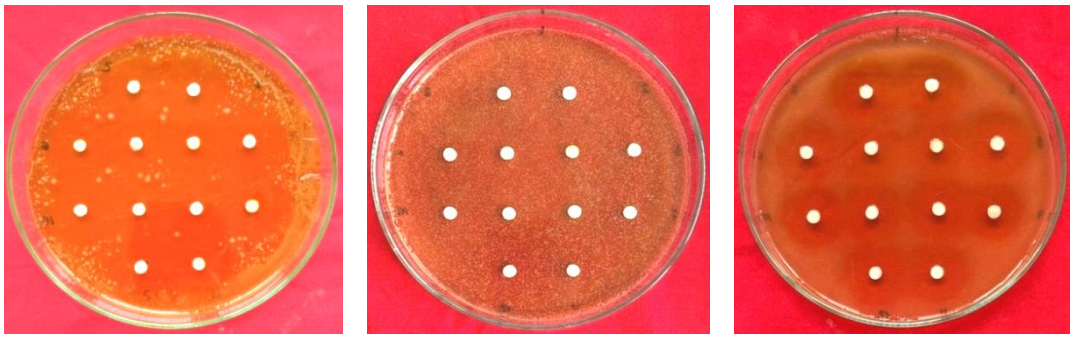
Fig. (5) Inhibitory zones of day 9 fermented broths of isolated twelve strains

Antimicrobial Activity of crude extracts of fermented broths

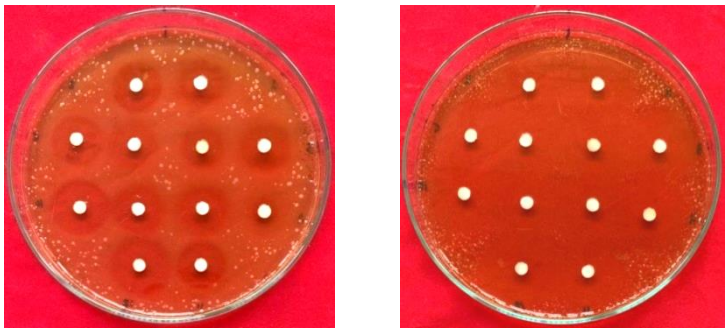
Crude metabolites of twelve strains were extracted with ethyl acetate, and all extracts indicated antimicrobial activity on four test organisms. All crude extracts showed highly antibacterial activity on *A. tumefaciens* (inhibitory zones: 26 mm to 30 mm). All extracts expressed highly antifungal activity on *C. albicans* (inhibitory zones: 22 mm to 29 mm). All extracts indicated highly antibacterial activity on *E. coli* (18 mm to 25 mm). All extracts inhibited highly antifungal activity on *M. furfur* (27 mm to 30 mm) in Table (10) and Fig. (6).

Table (10) Antimicrobial Activity of crude extracts of all strains

Strains	*A .tumefaciens*	*B. subtilis*	*C. albicans*	*E. coli*	*M. furfur*
HAN 1	28	-	27	22	30
HAN 2	28	-	29	22	29
HAN 3	30	-	29	22	30
HAN 4	30	-	25	20	27
HAN 5	28	-	25	20	27
HAN 6	30	-	25	24	29
HAN 7	30	-	22	18	28
HAN 8	26	-	22	22	27
HAN 9	26	-	22	22	27
HAN 10	30	-	25	25	30
HAN 11	28	-	25	21	27
HAN 12	28	-	26	24	29



Agrobacterium tumefaciens *Bacillus subtilis* *Candida albicans*



Escherichia coli *Malassezia furfur*

Fig. (6) Antimicrobial activity of crude extracts of isolated strains

Discussion and Conclusion

In this study, the twelve soil fungi (HAN 1 to HAN 12) were isolated from the two soil samples. Isolated strain HAN 1 was *Cephalosporium* sp., strains HANs 2, 3, 10 were *Aspergillus* spp., strains HANs 4 and 7 were *Penicillium* spp., strain HAN 5 was *Cladosporium* sp., strain HAN 6 was *Madurella* sp., strain HAN 8 was *Paecilomyces* sp., strain HAN 9 was *Hormiscium* sp, strain HAN 11 was *Gonatobotrys* sp. and one strain was unknown. Abraham (2012) has isolated *Cephalosporium* sp. from a sewage outfall in Sardina. Kiroku *et al*., (1981) has isolated *Cephalosporium gregatum* from bean field soil in Adzuki, they reported antimicrobial activity of this fungus.

In this study, the fermented broths of all isolated strains showed antimicrobial activity on *Agrobacterium tumefaciens* and *Malassezia furfur* from fourth day to ten day fermentation. The crude extracts of twelve strains indicated highly antimicrobial activity on *Agrobacterium tumefaciens*, *Candida albicans*, *Escherichia coli* and *Malassezia furfur*. Yee Yee Thu (2006) has isolated *Aspergillus* species from different soil samples for antimicrobial activity of fermented broth on *C. albicans*, *E. coli* and *M. furfur*. Petit *et al*., (2009) produced novel antimicrobial secondary metabolites from a *Penicillium* sp. isolated from Brazilian cerrado soil.

Seyedmousavi *et al*., (2015) stated that *Aspergillus* species are commonly found in soil, decaying vegetation, seeds and grains. Mya Su Maw (2020) isolated fungi from soil samples for antimicrobial activities. Abdulrahman *et al*., (2022) tested antimicrobial activities (on *C. albicans* and *E. coli*) of metabolites from *Cladosporium* species isolated in Cairo, Egypt. Li *et al*., (2020) studied secondary metabolites of *Paecilomyces* species, chemical diversity and biological activity such as anticancer activity and antimicrobial activity.

In conclusion, active strains should be continued to produce the bioactive compounds to protect *Agrobacterium tumefaciens* causing crown gall disease on plants, *Candida albicans* causing alimentary tract and vaginal infections, *Escherichia coli*

causing urinary tract infection and *Malassezia furfur* causing dandruff and skin infections on humans. Therefore, active soil fungi were screened and they possessed good antimicrobial activity in this research work so that this research would b enefit to produce some antibiotic agents.

Acknowledgements

We would like to mention our sincere thanks to Rector, Pro-Rectors from Dagon University, and Professor and Head of Botany Department, DU for their kind permission to summit this paper at 4th Myanmar-Korea Conferenceon Plant Tissue Culture and Genetics (Useful Plants and Life Science) sponsored by Jeonbuk National University, Korea.

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