ANTIMICROBIAL ACTIVITY OF ENDOPHYTIC FUNGI FROM FLOWER OF *CATHARANTHUS ROSEUS* (L.) G. DON

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Abstract

In this research, various kinds of fungi were screened from flowers of *Catharanthu sroseus* (L.) G. Don. The plant specimen of *C. roseus* was collected from Hlaing Township, Yangon Region, near the Oakkyin bus-stop, on the Insein Road. The direct inoculation method was used to isolate different strains of fungi on Potato Glucose Agar medium. Five strains of fungi were found out in this experiment. These strains of fungi were designated as F1, F2, F3, F4 and F5. The morphological and microscopically characters, colony appearances, hyphae, spore shape, appearance of conidiophores, characteristics of spore head, starch hydrolysis test and urease test were carried out at Microbiology Laboratory, Department of Botany, University of Yangon. Isolated fungi F2 from flower of *Catharanthus roseus* was assumed to be the genus *Mucor* sp. and F3 was also to be the genus *Aspergillus* sp. based on their morphological and microscopic characters. Starch hydrolysis and urease tests were also investigated F2, F3 and F5 are positive result of starch hydrolysis. Except F2, all strains were possessed urease activity. All of fungal isolates were cultured on six different agar such as Czapek dox agar (CZA), Potato dextrose agar (PDA), Potato sucrose agar (PSA), Sucrose yeast agar (SY), Yeast malt extract agar (YMA), and Sabouraud's dextrose agar (SDA) respectively and their reverse color and observed color were recorded. These isolated fungal strains did not grow well on Sabouraud agar medium. Among them potato sucrose medium is the best for growth of these isolates. These media were applied as fermented broth for antimicrobial activities. The antimicrobial activity of fermented broths from all isolated strains were examined on ten test organisms by paper disc diffusion method and fermentation were carried out for 1 day to 10 days. The fermented broths of all isolated strains F1 to F5 selectively showed good antimicrobial activity from 4 to 6 days on eight tests organisms. Secondary metabolite of activity strains were extracted with ethyl acetate (1:1Vol/Vol) and their antimicrobial activity was also investigated on ten tests organism. The crude of five strains showed excellent antimicrobial activity on eight test organisms.

Keywords *Catharanthus roseus*, fungi, media, *Mucor*, *Aspergillus*, incubate, isolate, Antimicrobial activities

Introduction

Endophytes are microorganisms that inhabit in living tissues of various plants. Endophytes are mainly colonize vegetative parts but are also found in reproductive organs. These endophytes are organisms that colonize internal plant tissues without causing apparent harm to their host. Fungi, bacteria, actinomycetes and mycoplasma are groups of microorganisms and these are reported as endophytes of plants (Arnold and Lutzoni, 2007).

Endophytes protect plants against herbivores, insect attacks or tissue invading pathogens and they are mutualistic and commensalis relationship with its host (Marcellano *et al*., 2017). Filamentous fungi are organisms that can break down organic matter, releasing phosphorus, oxygen, nitrogen and carbon into the atmosphere and the soil (Svahn, 2015). *Catharanthus roseus* (L.) G. Don is important to explore endophytic microflora in the medicinal plant.

It belongs to the family Apocynaceae. Medagascar periwinkle is also known as Thinbaw-ma-hyno-pan. Two common cultivars of *Catharanthus* which is named on the basis of their flower color that is the pink flowered "Rosea" and the white flowers "Alba" Monika and Sharma (2013).

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In this study, the effective endophytic fungi were screened from flower of *Catharanthus roseus* (L.) G. Don that was grown on Potato glucose agar medium by direct plate method (Atlas, 1993). The growth effect of these isolates were studied on six media such as Czapek dox agar (CZA), Potato dextrose agar (PDA), Potato sucrose agar (PSA), Sucrose yeast agar (SYA), Yeast malt extract agar (YMA), and Sabouraud's dextrose agar (SDA), respectively. Their potential of fermented broth extracts and ethyl acetate solvent extracts were preliminary carried out using the ten tested organisms. The Aim of this research is to isolate and identify the endophytic fungi from flower of *Catharanthus roseus* (L.) G. Don and to find out their potential of antimicrobial activities from fermented broth extracts and ethyl acetate solvent extracts against test organisms.

Materials and Methods

Samples collection and surface sterilization (Arnold *et al*., 2007)

The flower specimens were collected in early January of 2018 from Hlaing Township,Yangon Region, near the Oakkyin Bus-stop, on the Insein Road. This plant was identified as *Catharanthus roseus* according to Dassanayake and Fosberg (1981). So, this plant was identified into *Catharanthus roseus* (L.) G. Don by using available literature in the Department of Botany. Endophytic fungi were isolated from flower of *Catharanthus roseus* (corolla lobe F1, corolla lobe F2, corolla tube F3, anther F4 and calyx F5). Each 0.5 cm of sterilized segments were directly cultured on Potato glucose agar medium. Single colonies were picked up and sub cultured on this medium respectively. Morphological characters of isolated fungi were visually checked and microscopic characters of these fungi were examined under microscope.

Isolation of endophytic fungi from flower of *Catharanthus roseus* (Atlas, 1993)

Endophytic fungi were isolated from flower of *Catharanthus roseus* (corolla lobe F1, corolla lobe F2, corolla tube F3, anther F4 and calyx F5). Each 0.5 cm of sterilized segments were directly cultured on Potato glucose agar medium. Single colonies were picked up and sub cultured on this medium respectively. Morphological characters of isolated fungi were visually checked and microscopic characters of these fungi were examined under microscope. Procedure for isolation of Fungi from flower of *Catharanthus roseus* (L.) G. Don.

The effect of isolated fungi F1, F2, F3, F4 and F5 on different medium

To obtain the optimum growth and production of fungal metabolites (F1, F2, F3, F4 and F5), the culture were grown in different media namely Czapak dox agar (CZA), Potato sucrose agar (PSA), Sucrose yeast agar (SYA), and Sabouraud's dextrose agar (SDA), respectively. Their observed and reversed color and sizes of colonies were examined and recorded.

Potato Glucose Agar (Atlas, 1993)	**Composition per Liter**
Peeled potato	200 g
Glucose	20 g
Agar	20 g
Distilled water	1000 ml
pH	5.6

Potato Sucrose Agar Medium (Atlas, 1993)	
Peeled potato	200.0 g
Sucrose	20.0 g
Agar	20.0 g
pH	5.6

Biochemical Tests

Urease test (Christensen, 1946)

Starch hydrolysis test (Dubey and Maherwari, 2002)

Extraction of crude compounds from the fungal isolates F1, F2, F3, F4 and F5 (Collado and Pelaez, 1996)

Pieces of fungal mycelium (3mm) were transferred to 5 petridishes containing 30 ml of potato sucrose agar medium and were stand cultured for 7 days at room temperature. The 3mm of 7 days old culture inoculum were incubated in 250 ml flask containing 100ml of Potato sucrose broth for 21 days at room temperature. The fungal mat was separated from the fermented broth using filter paper and this broth was thoroughly mixed with ethyl acetate 1:1. The organic layer containing metabolites was separated from broth layer using funnel separating method. The separated organic layer containing the metabolites was concentrated to dryness at the temperature of 70°C to evaporate the solvent. After completing solvent evaporation, the crude extracts were weighed and recorded.

Extraction of Bioactive Metabolites from Selected Fungal Strains (Strobel and Sullivan, 1999)

Selected fungal strains F-1 to F-5 were inoculated into five conical flasks containing PSB fermentation medium. 100 ml of the fermented broths in 250 ml of the flasks were incubated for 7 days at room temperature as shown in Figure.

The fermented broth 100 ml from each of the fermented flasks was separately taken out at seven days fermentation. Each separated broth (35 ml) was extracted with ethyl acetate (35 ml) by shaking in the separating funnel, and collected the solvent layer from the aqueous phase. Then, the collected solvent layer was concentrated to obtain an organic solvent extract. Ethyl acetate (1.0 ml) was added in each organic extract, and stirred with a glass rod to get the uniform extract, then the extract (60µl) was applied for each disc. The paper disc size was 10.0 mm. The test plate size was 11.5 cm. The paper disc diffusion assay was done according to the method of (Davis and Stout, 1971).

Results

The outstanding characters of *Catharanthus roseus* (L.) G. Don

ScientificName : *Catharanthus roseus* (L.) G. Don
Family Name : Apocynaceae
Elglish Name : Cape periwinkle, Rose periwinkle, Rosy periwinkle and "Old-Maid".
Myanmar Name : Thinbaw-mahnyo

Perennial shrubs. Leaves opposite and decussate, simple, laminae oblong, margin entire, apex obtuse, base cuneate both surfaces pubescent, petiolate, exstipulate. Inflorescences terminal and axillarycymose. Flowers bracteate, ebrateolate, pedicellate bisexual complete,pink; sepal 5, aposepalous, green;petals 5, sympetalous, corolla lobe (salver shape), pink with a long corolla tube, the throat of the corolla tube hairy. Stamen 5, epipetalous, anther sagittate, dithecous, dorxifixed. Ovary, bicarpellary, stigma capitate, style long, filiform, marginal placentation, superior. Fruits follicle. Seeds small, numerous. The flowering and fruiting period throughout the years.

a. Habit b. Inflorescence

Figure 1 *Catharanthus roseus* (L.) G. Don

A total of five strains of fungi were isolated from flowers of *Catharanthus roseus* (L.) G. Don that grown on Potato glucose agar medium (PGA) during 7 days by direct inoculation method. Their culturing periods of fungal isolates were significantly different. These isolates were denoted as F1 (corolla lobe), F2 (corolla lobe), F3 (corolla tube), F4 (anther) and F5 (calyx), respectively. The colony of fungal isolates F1, F2 and F3 were grown on PGA medium during 3 days but isolate F4 and F5 were well grown during 7 days. The individual colonies of filamentous fungi were picked up and kept on PGA medium to get pure colony. Their morphological and microscopic properties of fungal isolates showed differences in colony observe and reverse color. The observe color of colonies on PGA of isolate F1 and F5 were white margin and center black, F2 was white and F3 was cottony white and F4 was margin pale yellow and center black. The reverse color of fungal isolates had F1, F3 and F5 were white, pale orange in F2 and yellow in F4 respectively. The sizes of colony on PGA medium were all a like (30x30 mm) except F3 (40x40 mm). The colony characters and microscopic characters of isolated fungi from flower of *Catharanthus roseus* shown Figures 2 to 7.

Microscopic Character of fungal isolates F1, F2, F3, F4 and F5

Fungal isolate F1

Hyphae were septate, hyaline and conidiophore were long, erect, and conidia head round. Conidia were 1- celled and round.

Fungal isolate F2

Hyphae were septate, hyaline. Sporangiophores were hyaline, short and often banched and bear terminal round spore-filled sporangia, brown. The sporangial walls were dissolved. Spores were round or oblong, numerous spores present in sporangia.

Fungal isolate F3

Hyphae were septate, hyaline and conidiophores were long, erect, globose and dark. Vesicle was globose. Phialide were biseriate, covering the entire surface of the vesicle. Conidia were 1- celled, chain, mutulae present, round, black, wall with spine.

Fungal isolate F4

Hyphae were septate and hyaline. Sporangiophores were long, erect and sporangium head globose, round and radiate. The spores were round.

Fungal isolate F5

Hyphae were septate, hyaline and conidiophore were long, erect, and conidia head round. Conidia were 1- celled and round.

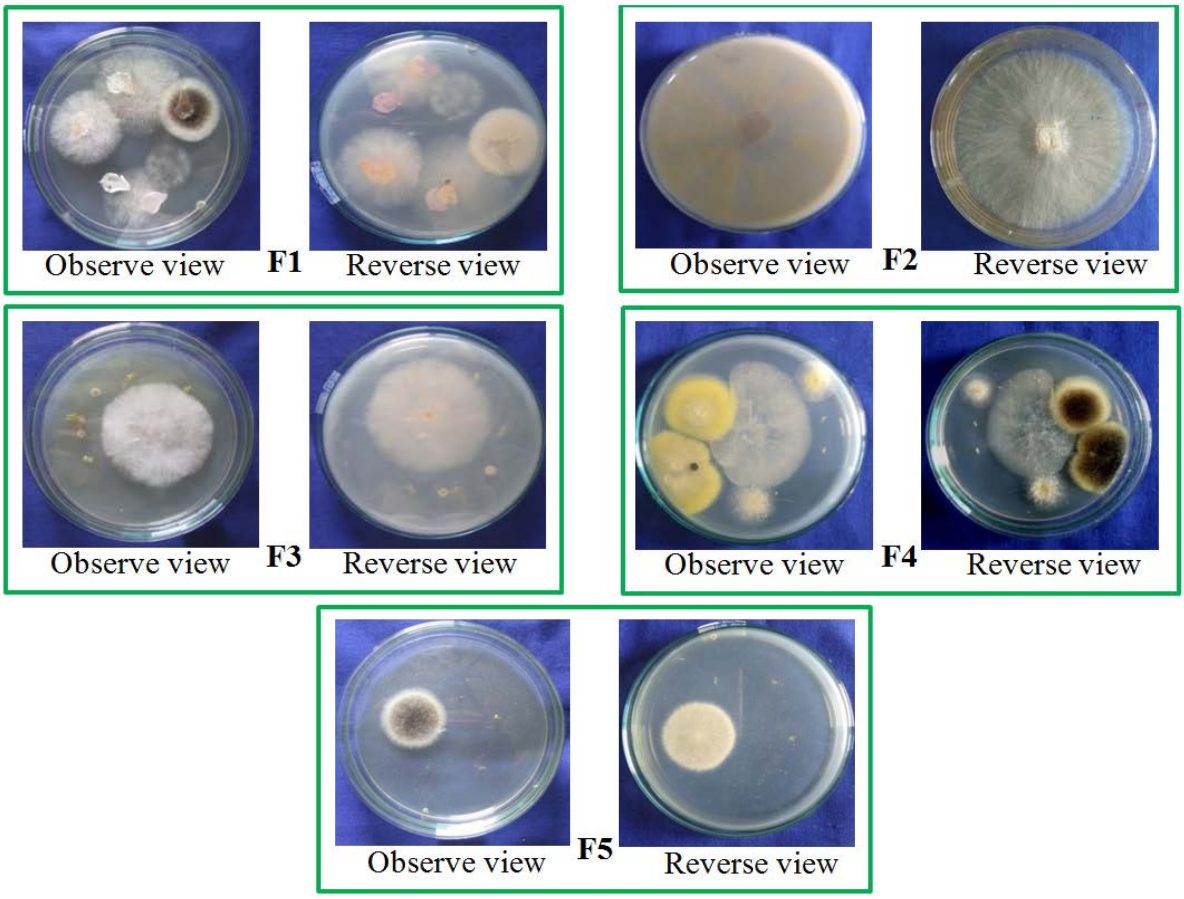

Figure 2 Isolated fungi F1, F2, F3, F4 and F5 from flower of *Catharanthus roseus* (L.) G. Don. grown on Potato Glucose Agar medium

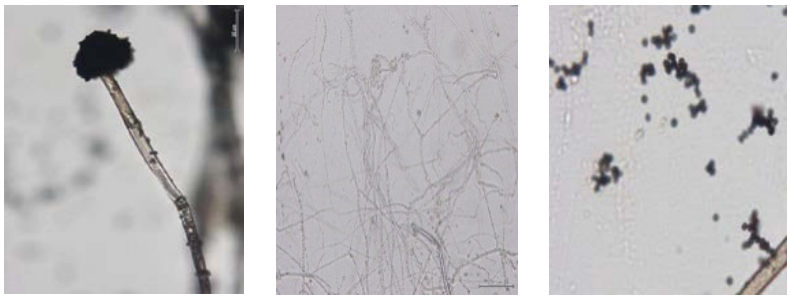

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Figure 3 Microscopic character of isolated strain F1

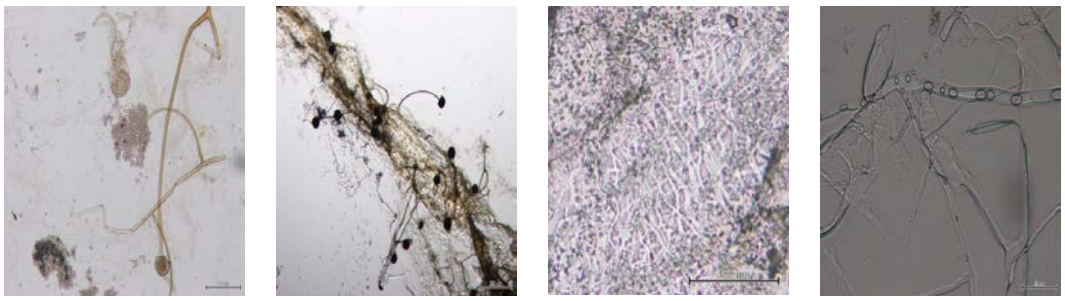

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Figure 4 Microscopic character of isolated strain F2

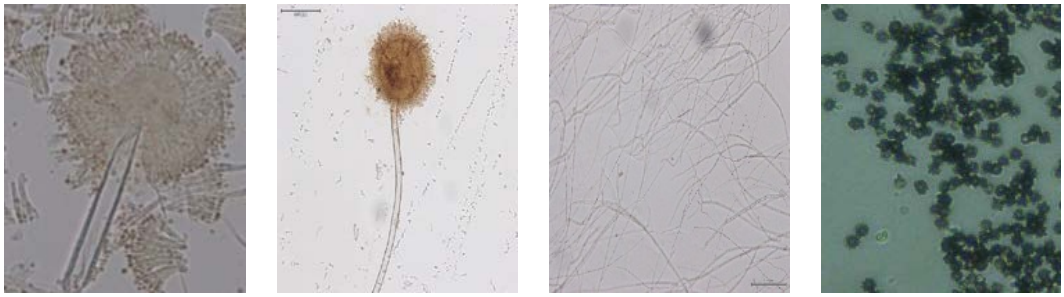

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Figure 5 Microscopic character of isolated strain F3

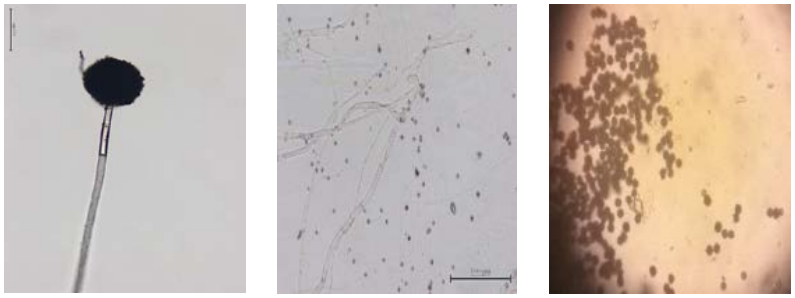

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Figure 6 Microscopic character of isolated strain F4

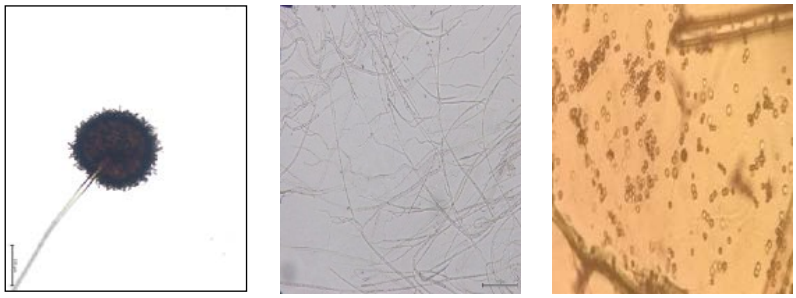

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Figure 7 Microscopic character of isolated strain F5

Effect of different media for antimicrobial metabolite production

These fungal isolates F1, F2, F3, F4 and F5 were cultured on Czapek dox agar (CZA), Potato dextrose agar (PDA), Potato sucrose agar (PSA), Sucrose yeast agar (SYA), Yeast malt extract agar (YMA) and Sabouraud's dextrose agar (SBA). Among these media, all fungal isolates were well grown on potato sucrose medium. The largest size of colony was observed in F2 on Potato sucrose agar (20×20 mm) followed by the second largest size 14×12mm in F5, 11×12 mm in F3, 18×18 mm in F1 and 16×20 mm in F4. So, potato sucrose media were also used as fermentation medium for antimicrobial activities.

Antimicrobial activities of potato sucrose fermented broth extracts from fungal isolates F1, F2, F3, F4 and F5

In Potato sucrose broth, the effective activity of antimicrobial test showed at 4-5 day. The potato sucrose broth was suitable for the production of antimicrobial metabolites from these endophytic fermented broth extracts as F3 showed maximum diameter zone of growth inhibition was observed by against *E. coli* (16.4 mm) followed by *S. aureus* (15.2 mm) and *B. Subtilis* (15.3 mm). Similarly, the maximum zone of inhibition was produced by F5 towards *B. subtilis*,

(15.5 mm) diameter zone of inhibition, *X. oryzae* (15.7mm) and *S. cerevisiae* (15.0 mm). It is therefore this medium should be further used for extraction of secondary metabolites from isolated endophytic fungi F1, F2, F3, F4 and F5 from the flower of *Catharanthus roseus* (L.) G. Don.

Table 1 Antimicrobial activities of fermented broth extract from isolate F1 in PSB

Test Organisms	measurement of inhibitory zones(mm)									
	D1/ pH6	D2/ pH5	D3/ pH4	D4/ pH3	D5/ pH2	D6/ pH2	D7/ pH2	D8/ pH2	D9/ pH1	D10/ pH1
E.coli	-	-	-	-	10.0	9.2	7.2	9.6	10.6	-
S.aureus	-	-	-	7.4	8.2	9.2	-	-	-	-
B.subtilis	-	-	-	8.4	11.2	9.4	8.0	-	-	-
X.oryzae	-	-	-	-	-	-	7.2	10.6	10.0	-
A.tumefaciens	-	-	-	7.4	11.2	12.0	7.0	7.0	7.0	-
S.typhi	-	-	-	-	7.0	8.6	9.8	9.8	9.2	-
P.aureginosa	-	-	-	-	11.6	8.2	8.0	8.2	8.0	-
S.cerevisiae	-	-	-	-	8.2	8.0	8.0	8.0	7.0	-
A.flavus	-	-	-	-		-	-	-	-	-
C.albicans	-	-	-	-	-	-	-	-	-	-

10-12mm = weak activity,
13-18mm = high activity,
>18mm= very high activity
Size of Paper disc = 6mm/D=Day C = Control with potato glucose broth
Test organisms
Escherichia coli,
Staphylococcus aureus,
Bacillus subtilis,
Xanthomonas oryzae,
Agrobacterium tumefaciens,
Salmonella typhi,
Pseudomonas auregiosa,
*Saccharomyces cerevis*iae,
Aspergillus flavus,
Candida albican

Table 2 Antimicrobial activities of fermented broth extract from isolate F2 in PSB

Test Organisms	measurement of inhibitory zones(mm)									
	D1/ pH6	D2/ pH6	D3/ pH5	D4/ pH4	D5/ pH3	D6/ pH2	D7/ pH2	D8/ pH2	D9/ pH1	D10/ pH1
E.coli	-	-	-	7.0	9.0	10.0	9.0	12.0	7.0	7.0
S.aureus	-	-	-	7.0	8.0	7.0	8.0	-	-	-
B.subtilis	-	-	-	11.0	7.0	10.0	-	-	-	
X.oryza	-	-	-	7.0	7.0	-	-	-	-	-
A.tumefaciens	-	-	-	7.0	7.0	9.0	8.0	-	-	-
S.typhi	-	-	-	7.0	7.0	10.0	-	-	-	-
P.aureginosa	-	-	-	7.0	7.0	-	-	-	-	-
S.cerevisiae	-	-	-	8.0	7.0	9.0	-	-	-	-
A.flavus	-	-	-	-	-	-	-	-	-	-
C.albicans	-	-	-	-	-	-	-	-	-	-

10-12mm = weak activity,
13-18mm = high activity,
>18mm= very high activity
Size of Paper disc = 6mm/D=Day C = Control with potato glucose broth
Test organisms
Escherichia coli,
Staphylococcus aureus,
Bacillus subtilis,
Xanthomonas oryzae,
Agrobacterium tumefaciens,
Salmonella typhi,
Pseudomonas auregiosa,
*Saccharomyces cerevis*iae,
Aspergillus flavus,
Candida albican

Table 3 Antimicrobial activities of fermented broth extract from isolate F3 in PSB

Test Organisms	measurement of inhibitory zones(mm)									
	D1/ pH6	D2/ pH6	D3/ pH5	D4/ pH4	D5/ pH3	D6/ pH2	D7/ pH2	D8/ pH2	D9/ pH1	D10/ pH1
E.coli	-	-	-	12.0	16.4	11.4	9.8	11.2	10.0	10.0
S.aureus	-	-	-	8.4	11.8	15.2	10.2	9.6	9.6	10.0
B.subtilis	-	-	-	13.0	15.3	10.0	9.6	9.6	10.2	10.2
X.oryza	-	-	-	7.0	11.5	11.2	15.0	12.2	9.4	9.2
A.tumefaciens	-	-	-	10.0	12.2	13.8	10.0	8.2	8.2	8.2
S.typhi	-	-	-	9.4	9.0	11.2	10.0	9.2	9.2	9.6
P.aureginosa	-	-	-	8.0	15.0	10.0	10.4	9.8	9.2	9.2
S.cerevisiae	-	-	-	9.4	9.0	9.0	8.4	8.2	7.2	7.0
A.flavus	-	-	-	-	-	8.0	-	-	-	-
C.albicans	-	-	-	-	-	-	-	-	-	-

Table 4 Antimicrobial activities of fermented broth extract from isolate F4 in PSB

Test Organisms	measurement of inhibitory zones(mm)									
	D1/ pH6	D2/ pH6	D3/ pH5	D4/ pH4	D5/ pH3	D6/ pH2	D7/ pH2	D8/ pH2	D9/ pH1	D10/ pH1
E.coli	-	-	-	7.2	7.8	6.4	-	-	-	-
S.aureus	-	-	-	7.0	9.2	-	-	-	-	-
B.subtilis	-	-	-	9.6	9.6	8.8	-	-	-	-
X.oryza	-	-	-	-	-	-	-	7.8	7.0	7.0
A.tumefaciens	-	-	-	10.2	8.6	-	-	-	-	-
S.typhi	-	-	-	-	-	-	-	-	8.0	7.4
P.aureginosa	-	-	-	9.2	-	-	9.2	-	-	-
S.cerevisiae	-	-	-	9.0	-	-	-	-	-	-
A.flavus	-	-	-	-	-	-	-	-	-	-
C.albicans	-	-	-	-	-	-	-	-	-	-

Table 5 Antimicrobial activities of fermented broth extract from isolate F5 in PSB

Test Organisms	measurement of inhibitory zones(mm)									
	D1/ pH6	D2/ pH 5	D3/ pH4	D/4 pH3	D5/ pH3	D6/ pH2	D7/ pH2	D8/ pH2	D9/ pH 1	D10/ pH 1
E.coli	-	-	-	7.6	10.2	11.4	10.0	10.0	10.0	-
S.aureus	-	-	-	8.2	15.0	15.0	11.2	11.0	9.8	7.0
B.subtilis	-	-	-	7.0	15.5	10.0	10.4	10.6	-	-
X.oryzae	-	-	-	8.0	15.7	11.2	9.2	9	9.0	-
A.tumefaciens	-	-	-	10.6	10.8	13.8	12.0	12.0	-	-
S.typhi	-	-	-	9.8	12.4	11.2	12.0	10.2	7.2	-
P.aureginosa	-	-	-	-	11.4	10.0	9.4	9.8	9.5	-
S.cerevisiae	-	-	-	9.4	13.2	15.0	8.2	10.0	7.0	-
A.flavus	-	-	-	-	-	-	-	-	-	-
C.albicans	-	-	-	-	-	-	-	-	-	-

10-12mm = weak activity,
13-18mm = high activity,
>18mm= very high activity
Size of Paper disc = 6mm/D=Day C = Control with potato glucose broth
Test organisms
Escherichia coli,
Staphylococcus aureus,
Bacillus subtilis,
Xanthomonas oryzae,
Agrobacterium tumefaciens,
Salmonella typhi,
Pseudomonas auregiosa,
*Saccharomyces cerevis*iae,
Aspergillus flavus,
Candida albican

Extraction of secondary metabolites from F1, F2, F3, F4 and F5 with ethyl acetate

The crude extracts of all isolated fungal strains was studied for test organisms. Among isolates F1, F2, F3, F4 and F5 showed the highest yield of crude extracts obtained from isolate F1, F3 and F4 (0.2 g) followed by F2 and F5 (0.1 g). All extracts gave brown color except F2 (yellow). The highest fresh weight was obtained from F2 (9.6 g) followed by F4 (8.4 g), F3 (8.2 g), F5 (7.2 g) and the least fresh weight was obtained from F1 (5.2 g). The maximum amount of mycelium biomass was obtained from F3 (2.2 g), followed by F4 (1.9 g). The biomass of mycelium was observed by F2 (1.7 g) and (1.5 g). The 30µl of all of these fungal secondary metabolites extract the showed effectively against tested organisms. The isolates F1 and F3 showed maximum activity against *A. tumefaciens* with a diameter of inhibition zone 46 mm followed by *S. aureus* with a diameter 42 mm. Among these fungal isolates, the F3 and F5 showed the highest activity because the zone of inhibition of test organisms ranges between 30-40 mm and 28-40 mm. The results of extraction of antimicrobial secondary metabolites from fungal isolates F1, F2, F3, F4 and F5 with ethyl acetate indicated in Table 6, Figures 8 to 12.

Table 6 Antimicrobial activities of fermented broth extract from isolated strains

Isolated strains / Test organisms	F1	F2	F3	F4	F5
Escherichia coli	35	35	35	35	30.5
Staphylococcus aureus	42	30	30	30	28
Bacillus subtilis	40	27	35	30	28
Xanthomonas oryzae	22	30	40	35	33
Agrobacterium tumefaciens	46	35	35	30	40
Salmonella typhi	35	42	40	30	30
Pseudomonas aeruginosa	40	38	38	30	32
Saccharomyces cerevisiae	40	30	36	35	30.5
Aspergillus flavus	35	22	35	27	40
Candida albicans	30	37	35	35	*40*

Size of paper disc = 10mm, Control = 95% ethyl acetate

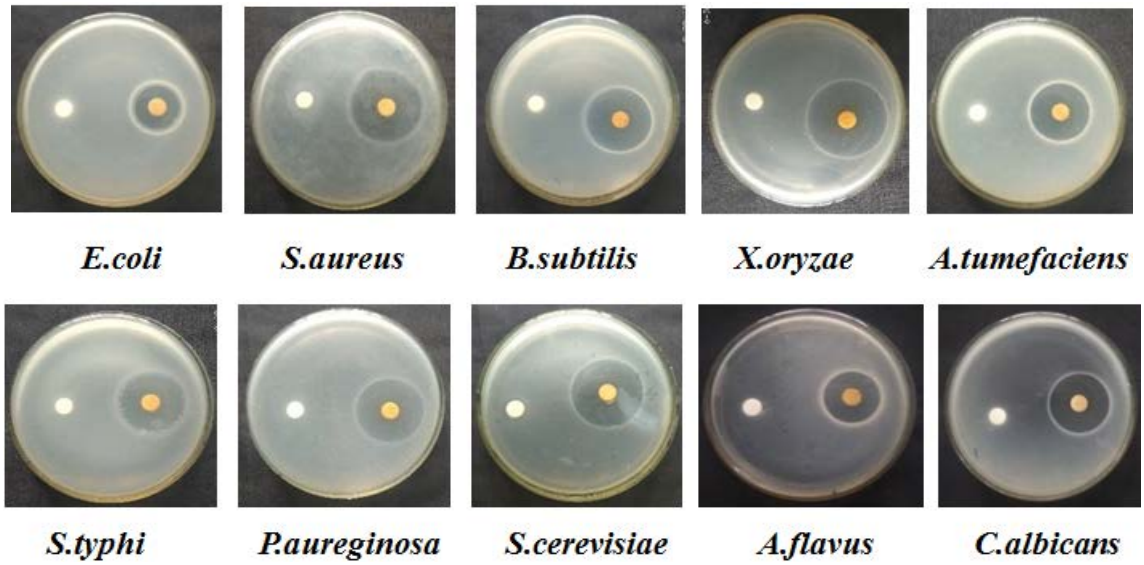

Figure 8 Inhibitory zones of Isolated strain F1 from crude extract

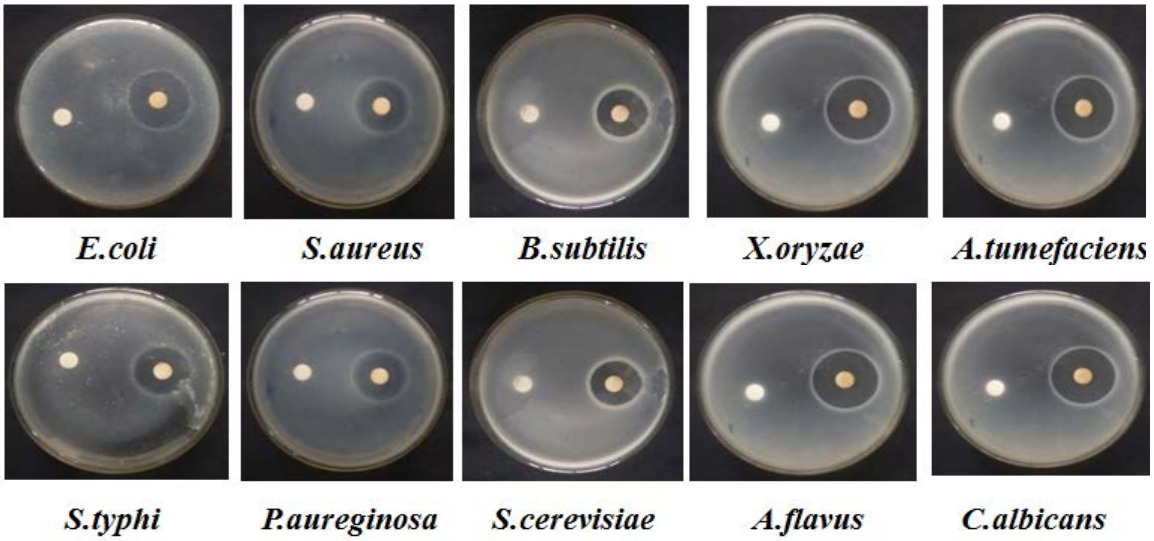

Figure 9 Inhibitory zones of Isolated strain F2 from crude extract

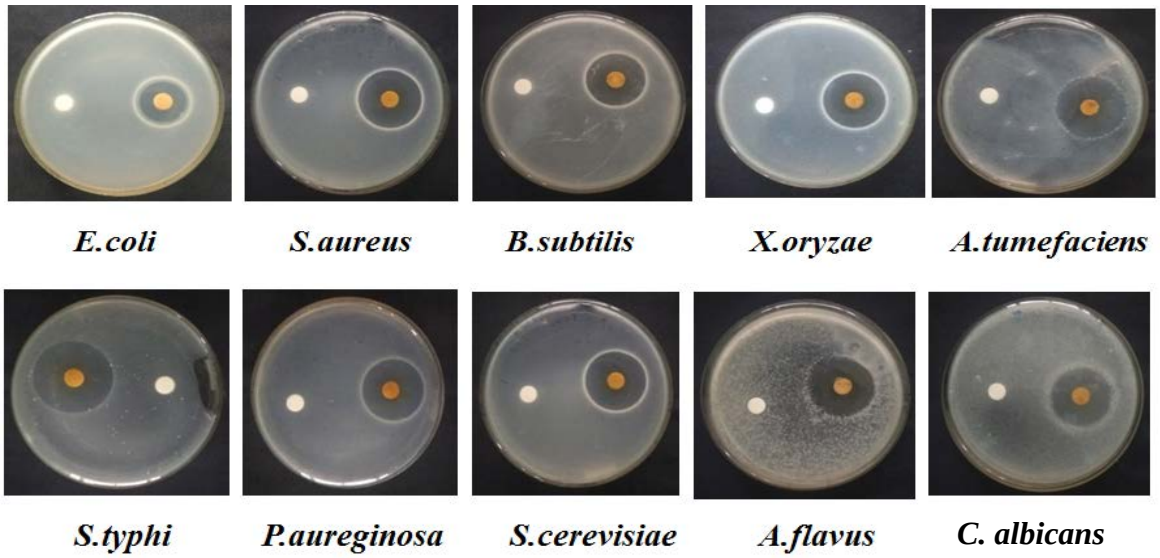

Figure 10 Inhibitory zones of Isolated strain F3 from crude extract

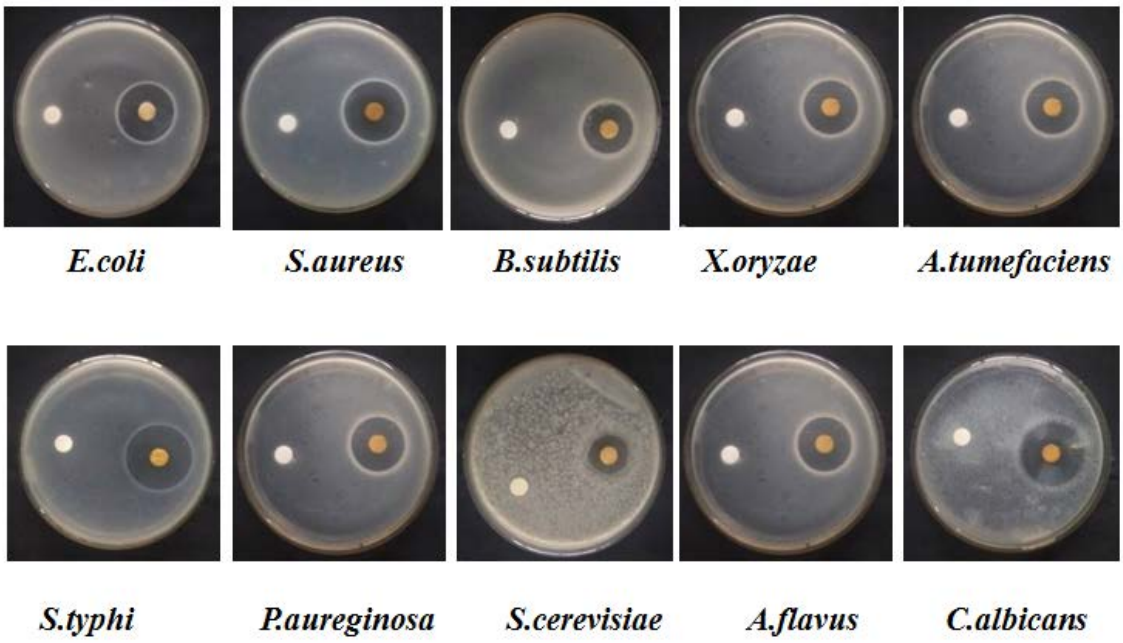

Figure 11 Inhibitory zones of Isolated strain F4 from crude extract

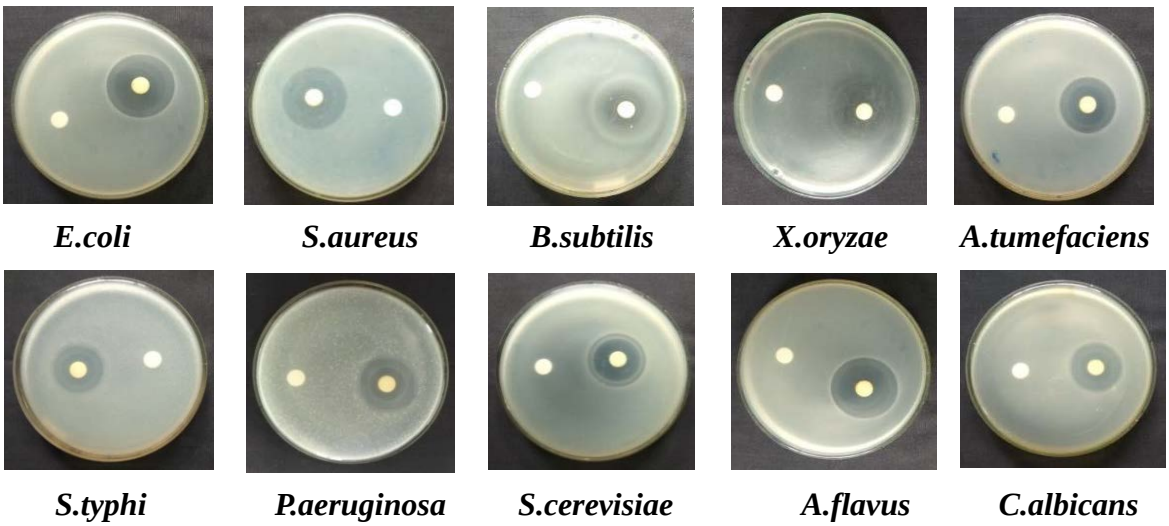

Figure 12 Inhibitory zones of Isolated strain F5 from crude extract

*E.coli = Escherichia coli, S.aureus = Staphylococcus aureus, B.subtilis = Bacillus subtilis, X.oryzae = Xanthomonas oryzae, A.tumefaciens = Agrobacterium tumefaciens, S.tyhi = Salmonella typhi, P.aeruginosa = Pseudomonas aeruginosa, S.cerevisiae = Saccharomyces cerevis*iae, *A.flavus =Aspergillus flavus, C.albicans = Candida albican*

Discussion and Conclusion

The flower specimens of *Catharanthus roseus* (L.) G. Don was collected from Hlaing, Township,Yangon Region. It belongs to the family Aypocynaceae. Their morphological character of this plant was confirmed the scientific name with the literature of Dassanayake and Fosberg (1981) and Soe Myint Aye (2002).The five strains of fungi were isolated from flower of *Catharanthus roseus* (L.) G. Don. that grown on Potato glucose agar medium. These isolated

fungi were denoted as F1, F2, F3, F4 and F5. Based on their morphological and microscopic character, these isolated fungi F2 and F3 were identified into Genus Level

(*Mucor* and *Aspergillu*s).

Ayob and Simarani, (2016) observed two strains of filamentous fungi from violet color of *Catharanthus roseus* showed hyphae septate and spore rounded shaped. Sreekanth *et al*., (2017) found that twenty-five endophytic fungi were isolated from different tissue of Catharanthus roseus. The 48% from leaves, 44% from stems and 4% from each of the roots and flowers were investigated.

Kidd *et al*., (2016) said that colony of *Mucor were* very fast growing white to yellow with the development of sporangia, sporangiophore hyaline, erect, simple, globose to spherical, multispore present in sporangia. As characters of fungal isolate F2 are in accordance with Kidd *et al*., (2016).

During the starch hydrolysis and urease test, the isolates F2, F3 and F5 were positive effect on starch hydrolysis test as well as only F2 did not produce urease enzyme that bioactive compound were explored from endophytic fungi *Aspergillus fumigatus* strain KARVSO4 from Piper crocatum Ruiz and Pav. More components were observed in ethyl acetate extracts indicating its potential exploration for other bioactive substances having pharmaceutical values. *Aspergillus niger* and *A. flavus, Eurotium amsteldom* and *Fusarium* sp. showed positive effect on starch hydrolysis and urease tests but urease activity was only found in *Aspergillus niger* (Shivanni *et al.,* 2015). The amylase, lipase, pectinase and protease activity was observed in several species of *Muco*r. Alves *et al.,* (2006). The amylase and protease could be produced by *Aspergillus niger* and *Aspergillus flavus* (Ayanda, 2013).

So the characters of isolated fungi F1, F2, F3, F4 and F5 were agreed in Alves *et al*., (2006), Ayanda (2013) and Shivanni *et al.,* (2015).

Barnett and Hunter (1979) observed that the conidiophore of *Aspergillus* sp. were clevate swelling bearing phialides at the apex or rading from the entire surface and conidia are one celled, globose often various color and mass and dry basipetal chains.

Pumphrey and Christran (1996) observed that glucose is the most readily metabolized sugar but most fungi could use sucrose and they require ammonia, nitrate and nitrite for nitrogen sources. Zhang *et al*., (2012) discovered that the 11 strains of endophytic fungi were separated from the healthy stems of *Artemisa annua. Aspergillus* sp. isolated from *Artemisa annua* showed actively against *Escherichia coli and Staphylococcus aureus, Tricoderma rubrum* but *Mucor sp.* indicated against *Rhizotonia cerealis.*

Potato dextrose broth and ethyl acetate solvent was suitable for secondary metabolites extraction from *Aspergillus* sp. and *Mucor* sp. isolated from *Artemisa annua.* Astuti (2017) observed that bioactive compounds were explored from endophytic fungi *Aspergillus fumigatus* strain KARVSO4 from *Piper crocatum* Ruiz & Pav.

The best bioactive compound producing medium was Potato dextrose broth at 29ºC and pH 5 supplemented with starch or fructose as carbon sources and nitrogen sources. More components were observed in ethyl acetate extracts indicating its potential exploration for other bioactive substances having pharmaceutical values. The result of finding in antimicrobial activity of isolated F1, F2, F3, F4 and F5 were in accordance with previous finding Pumpharey and Christran (1996) and Zhang *et al*., (2012).

To sum up, all isolated fungal secondary metabolite broth extracts exhibited antimicrobial activities in all strains of test organisms. The results showed that among them, Potato sucrose broth was found to be the best for extraction of secondary metabolites and only ethyl acetate

solvent was used to extract secondary metabolites from these fungal strains F1, F2, F3, F4 and F5.

In this experiment, the F1 extract showed highly against *Agrobacterium tumefaciens* with a diameter 46 mm and *Staphylococcus aureus* with a diameter 42 mm but F3 and F5 was the zone of inhibition of tested organisms range between 30-40 mm and 28-40 mm. Isolated F3 and F5 broth extracts showed broad spectrum activity against *Escherichia coli* and *Xanthomonas oryzae* and ethyl acetate extracts showed good activity in antimicrobial tests. These isolated fungal metabolites could be affected on human respiratory tract problems, skin disease, dysentery and phytopathogens. The result of finding showed that ethyl acetate extracts secondary metabolites isolated from flower of *Catharanthus roseus* should be further studied for purification of compounds for medicine and pharmaceutical uses.

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