Antimicrobial Activity of Endophytic Fungi Isolated From *Zingiber Officinale* Rosc.

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Abstract

In the present study, leaves and rhizomes of *Zingiber officinale* Rosc. were collected from officer housing, Dagon University Campus, East Dagon Township in Yangon Region. Ten endophytic strains (SYH 1 to SYH 10) were isolated from leaves and rhizomes of *Zingiber officinale* Rosc. on three different media at Microbiology Laboratory, Department of Botany, Dagon University. Fungal strain SYH 1 was identified as *Dendryphiella* sp., strains SYH 2, SYH 5, SYH 6 and SYH 7 were identified as *Aspergillus* spp., strains SYH 3 and SYH 4 were identified as *Cephalosporium* spp., strain SYH 9 was identified as *Madurella* 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 2 to 11 days. The fermented broths of all isolated strains showed antimicrobial activity on *Agrobacterium tumefaciens*, *Candida albicans*, *Escherichia coli*, *Malassezia furfur* from day 2 to day 11 fermentation. Crude metabolites of all strains were extracted with ethyl acetate, and their antimicrobial activity was also examined on five test organisms. The crude extracts of ten strains indicated excellent antimicrobial activity on *Agrobacterium tumefaciens*, *Candida albicans*, *Escherichia coli* and *Malassezia furfur* at day 6 fermentation. Therefore, the most active strains (SYH 1 to SYH 8) should be chosen to produce the bioactive compounds to inhibit *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, Endophytic fungi, *Zingiber officinale* Rosc.

Introduction

Zingiberaceae is a family of herbs that grow abundantly in tropical to subtropical region with center of divergence located in Southeast Asia (Pandey, 2001) and ginger grows all over Myanmar. *Zingiber officinale* Rosc. (ginger) is also used in a variety of food and beverage applications, providing specific functional properties due to their bioactive compounds (Srinivasan, 2017). Endophytes are defined as organisms isolated from surface-sterlisied explants or from within the plant tissue and produce no harm to the host plant (Hallman *et al*., 2011). The endophytic fungi in the host cells have shown that the plant can live for long life because the endophytic fungi possess some various type of bioactivity such as antibacterial, antifungal, antitumor, antibiotic, antioxidant and anti-inflammatory activities (Maheshwari *et al*., 2006). Endophytic fungus such as *Dendryphiella* isolated from rice seed micro-flora in India (Sethi *et al*., 2018).

Endophytic fungi *Aspergillus* strains have a wide range of bioactivities such as antimicrobial, antitumor, etc. (Wang *et al*., 2018). Endophytic *Aspergillus* was isolated from healthy *Moringa oleifera* leaves and identified morphologically, genetically and fungal extract contains 16 major bioactive compounds with extensive pharmaceutical activities (Hashem *et al*., 2022). Endophytic *Madurella* to the *Anogeissus leiocarpus* leaf extracts showed the potent antifungal activity of the extracts against mycetoma causing pathogen and justifying its traditional uses as a medicinal plant for treatment of skin infections (Elsiddig *et al*., 2015). Endophytic fungus such as *Cephalosporium* have a wide range of novel antimicrobial compounds, which could be used in drug development and agrochemical production for protecting agricultural crops from plant root diseases (Farhat *et al*., 2019).

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The objectives of present research work are to isolate endophytic fungal strains from *Zingiber officinale* Rosc., to investigate antimicrobial activity of fermented broths of all isolated strains and to evaluate antimicrobial activity of all the extracts of isolated strains.

Materials and Methods

Collection of Plant Samples

The plant samples (leaves and rhizomes) of *Zingiber officinale* Rosc. (Ginger) were collected from office housing, Dagon University Campus, East Dagon Township in Yangon Region.

Isolation of Endophytic Fungal Strains from *Zingiber officinale* Rosc.

Endophytic strains were isolated from the leaves and rhizomes of *Zingiber officinale* Rosc. (Ginger) on three different media. Isolation of endophytic strains was carried out by the following procedure (Lee *et al*., 1996).

1. The plant samples were washed under running tap water for ten minutes.
2. The plant parts (leaves and rhizomes) were cut into about 1.5 cm - 2.0 cm pieces.
3. These pieces were sterilized by soaking in 75% alcohol for 1 to 2 minutes.
4. These parts were dried on sterilized paper and then they were placed on agar plates containing different media.
5. Then, the plates were incubated for 3-7 days at room temperature.

Composition of Culture Media for Isolation (Atlas, 1993)

Medium 1

Nutrient Agar Medium (NA)

Nutrient Agar 3.9 g, Distilled Water 100 ml, pH 6.8

Medium 2

Sucrose Yeast Extract Agar Medium (SY)

Sucrose 1.0 g, Yeast Extract 0.3 g, Distilled Water 100 ml, Agar Powder 2.25 g, pH 6.8

Medium 3

Lactose Yeast Extract Agar Medium (LY)

Lactose 1.0 g, Yeast Extract 0.3 g, Distilled Water 100 ml, Agar Powder 2.25 g, pH 6.8

Antimicrobial Activity of Isolated Fungal Strains

Fermentation

Isolated ten fungal strains grown on 5 days old slant cultures were inoculated into 10 conical flasks (50 ml) containing 20 ml of sucrose/yeast extract medium in each for three day at 100 rpm as seed culture. After three day, seed cultures were transferred to ten fermentation flasks at 30°C on shaker for 10 days at 100 rpm. Every day 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 7.

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 1. 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 assay

After solidification, paper discs impregnated with fermented broth samples were applied on the test plates. These plates were incubated at 30°C for 24 to 48 hrs. After 24 to 48 hrs, 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 1. Test organisms and diseases

Test organisms	Diseases
Agrobacterium tumefaciens	Crown gall diseases.
Bacillus subtilis	It can cause dysentery, but at the first sign of diarrhea.
Candida albicans	Vaginal candidasis, urogenital infection, alimentary tract infection.
Escherichia coli	Cholera, diarrhea and vomiting, urinary tract infections.
Malassezia furfur	Dandruff.

Antimicrobial activity of crude extracts of fungal strains

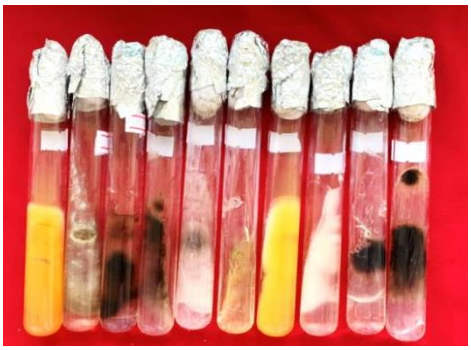
The fungal strains were inoculated into the ten conical flasks containing SY seed medium. Each flask contained 20 ml of medium. After three day, seed cultures (1 ml of each) were transferred to the ten fermentation flasks at 30ºC for 6 days on the shaker at 100 rpm. Each flask contained 20 ml of medium. At day 6 fermentation, the fermented broth of each fungal strain was extracted with ethyl acetate (10 ml) at pH 4.5. Then, the extracts were dried in the incubator at 40ºC. 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 Endophytic Fungi

Ten fungal strains were isolated from leaves and rhizomes of *Zingiber officinale* Rosc. Six fungal strains were isolated from leaves and four strains from rhizomes of *Zingiber officinale* Rosc. The strains were given as temporary names SYH 1 to SYH 10 as shown in Figures 1.

Surface view of strains SYHs 1-10



Reverse view of strains SYHs 1-10

Fig. 1. Isolated fungal strains on slant cultures (SYH 1 to SYH 10)

Antimicrobial Activity of Selected Fungal Strains

Antimicrobial activity of day 2 fermentation

Ten endophytic fungal strains showed antimicrobial activity on four test organisms. At day 2 fermentation all strains did not show antibacterial activity on *Agrobacterium tumefaciens* and *Bacillus subtilis.* Strain SYH 8 showed moderately antifungal activity whereas the other strains indicated weak activity on *Candida albicans*. Strains SYHs 2, 6 and 7 showed moderately antibacterial activity while the other strains indicated weak activity on *Escherichia coli*. Strains SYHs 7 and 8 showed highly antifungal activity, strains SYHs 2 and 6 indicated moderately activity but the other strains showed weak activity on *Malassezia furfur* in Table 2.

Table 2. Inhibitory zones of day 2 fermented broths of isolated ten strains

Strain / Test organisms	SYH 1	SYH 2	SYH 3	SYH 4	SYH 5	SYH 6	SYH 7	SYH 8	SYH 9	SYH 10
Agrobacterium tumefaciens	-	-	-	-	-	-	-	-	-	-
Bacillus subtilis	-	-	-	-	-	-	-	-	-	-
Candida albicans	11	10	10	11	10	11	10	13	+	+
Escherichia coli	12	14	11	12	11	13	14	10	10	9
Malassezia furfur	12	15	11	12	10	14	19	18	10	10

8-12 mm = weak activity, 13-17 mm = moderate activity, > 18 mm = high activity

Antimicrobial activity of day 3 fermentation

At day 3 fermentation strains SYHs 2, 4, 7 and 8 showed moderately antibacterial activity while the other strains showed weak activity on *A. tumefaciens*. All strains did not show antibacterial activity on *Bacillus subtilis.* All strains showed weak antifungal activity on *C. albicans*. Strains SYHs 2 and 7 showed moderately antibacterial activity while the other strains indicated weak activity on *E. coli*. Strains SYHs 2, 6, 7 and 8 showed moderately antifungal activity but the other strains showed weak activity on *M. furfur* in Table 3.

Table 3. Inhibitory zones of day 3 fermented broths of isolated ten strains

Strain / Test organisms	SYH 1	SYH 2	SYH 3	SYH 4	SYH 5	SYH 6	SYH 7	SYH 8	SYH 9	SYH 10
A. tumefaciens	10	13	10	13	10	12	17	15	8	8
B. subtilis	-	-	-	-	-	-	-	-	-	-
C. albicans	11	10	10	10	9	11	10	12	+	+
E. coli	11	13	11	12	12	11	14	11	10	10
M. furfur	12	13	12	12	10	14	15	15	10	10

Antimicrobial activity of day 4 fermentation

At day 4 fermentation all strains showed weak antibacterial activity on *A. tumefaciens*. All strains did not show antibacterial activity on *Bacillus subtilis.* Strains SYHs 3, 4, 7, 8 and 9 showed moderately antifungal activity whereas the other strains showed weak activity on *C. albicans*. Strains SYHs 3, 5, 7 and 8 showed moderately antibacterial activity while the other strains indicated weak activity on *E. coli*. Strains SYHs 1, 3, 5 and 7 showed moderately antifungal activity but the other strains showed weak activity on *M. furfur* in Table 4.

Table 4. Inhibitory zones of day 4 fermented broths of isolated ten strains

Strain / Test organisms	SYH 1	SYH 2	SYH 3	SYH 4	SYH 5	SYH 6	SYH 7	SYH 8	SYH 9	SYH 10
A. tumefaciens	+	+	+	+	12	+	+	+	+	12
B. subtilis	-	-	-	-	-	-	-	-	-	-
C. albicans	10	12	15	14	11	12	13	15	14	9
E. coli	10	12	13	12	14	11	15	13	12	11
M. furfur	14	10	14	12	14	10	14	12	11	11

Antimicrobial activity of day 5 fermentation

At day 5 fermentation strains SYHs 2, 4, 7 and 8 showed moderately antibacterial activity while the other strains indicated weak activity on *A. tumefaciens*. Strains SYHs 2, 3, 4, 7, 8 and 9 showed moderately antifungal activity whereas the other strains showed weak antifungal activity on *C. albicans*. Strains SYHs 6 and 7 showed moderately antibacterial activity while the other strains indicated weak activity on *E. coli*. Strains SYHs 4, 6 and 7 showed highly antifungal activity but the other strains indicated moderately activity on *M. furfur* in Table 5.

Table 5. Inhibitory zones of day 5 fermented broths of isolated ten strains

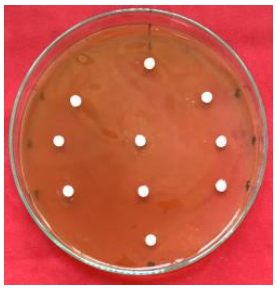
Strain / Test organisms	SYH 1	SYH 2	SYH 3	SYH 4	SYH 5	SYH 6	SYH 7	SYH 8	SYH 9	SYH 10
A. tumefaciens	11	14	12	15	11	12	14	14	10	10
B. subtilis	-	-	-	-	-	-	-	-	-	-
C. albicans	10	14	16	16	12	12	15	17	16	10
E. coli	11	12	10	12	10	14	15	12	10	11
M. furfur	15	15	14	20	14	20	20	16	14	16

Antimicrobial activity of day 6 fermentation

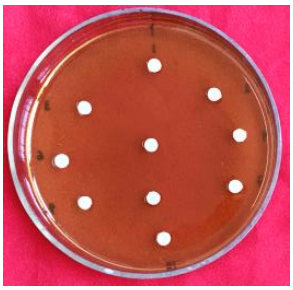
At day 6 fermentation strains SYHs 2, 4, 6 and 7 showed moderately antibacterial activity while the other strains indicated weak activity on *A. tumefaciens*. Strains SYHs 2, 3, 4, 7, 8 and 9 showed highly antifungal activity, strains SYHs 5 and 6 indicated moderately activity whereas the other strains showed weak activity on *C. albicans*. Strains SYHs 6 and 7 showed highly antibacterial activity, strains SYHs 2, 4, 5 and 8 indicated moderately activity while the other strains showed weak activity on *E. coli*. Strain SYH 9 indicated moderately antifungal activity while the other strains showed highly activity on *M. furfur* in Table 6 and Figure 2.

Table 6. Inhibitory zones of day 6 fermented broths of isolated ten strains

Strain / Test organisms	SYH 1	SYH 2	SYH 3	SYH 4	SYH 5	SYH 6	SYH 7	SYH 8	SYH 9	SYH 10
A. tumefaciens	12	14	12	15	10	14	13	11	10	10
B. subtilis	-	-	-	-	-	-	-	-	-	-
C. albicans	10	18	20	18	13	13	18	20	21	10
E. coli	+	13	11	14	14	18	18	13	12	10
M. furfur	18	19	18	20	20	22	24	18	15	20

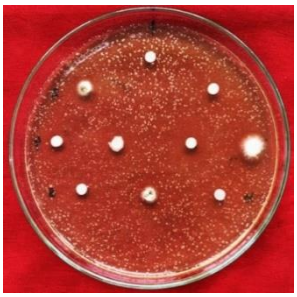


Agrobacterium tumefaciens

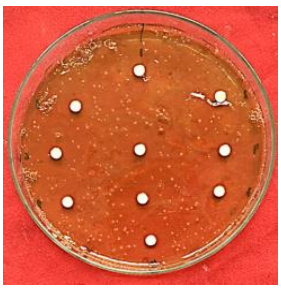


Bacillus subtilis

Candida albicans



Escherichia coli



Malassezia furfur

Fig. 2. Inhibitory zones of day 6 fermented broths of isolated ten strains

Antimicrobial activity of day 7 fermentation

At day 7 fermentation strains SYHs 2, 4 and 6 showed moderately antibacterial activity while the other strains indicated weak activity on *A. tumefaciens*. All strains did not show antibacterial activity on *Bacillus subtilis.* Strains SYHs 2, 3, 4, 7, 8 and 9 showed moderately antifungal activity whereas the other strains showed weak activity on *C. albicans*. Strains SYHs 1, 4, 6, 8 and 9 showed moderately antibacterial activity while the other strains showed weak activity on *E. coli*. Strain SYH 7 showed highly antifungal activity but the other strains showed moderately activity on *M. furfur* in Table 7 and Figure 3.

Table 7. Inhibitory zones of day 7 fermented broths of isolated ten strains

Strain / Test organisms	SYH 1	SYH 2	SYH 3	SYH 4	SYH 5	SYH 6	SYH 7	SYH 8	SYH 9	SYH 10
A. tumefaciens	12	15	12	16	+	16	12	11	10	10
B. subtilis	-	-	-	-	-	-	-	-	-	-
C. albicans	11	14	15	13	10	11	13	15	15	9
E. coli	15	12	12	13	12	15	10	15	15	11
M. furfur	16	16	15	17	16	17	18	16	13	15

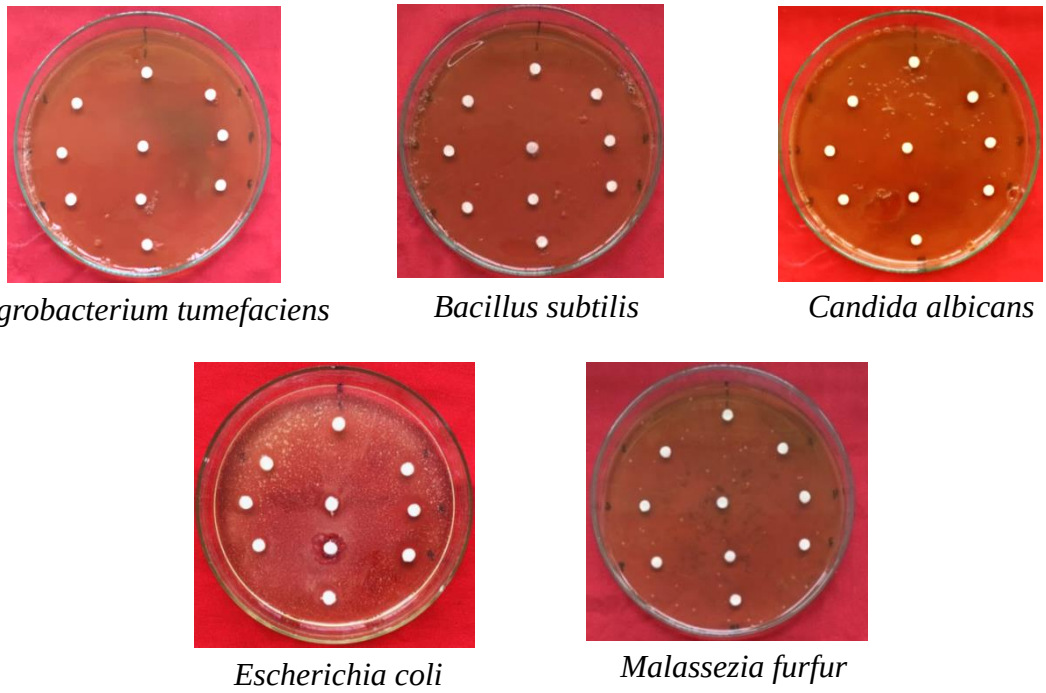


Fig. 3. Inhibitory zones of day 7 fermented broths of isolated ten strains

Antimicrobial activity of day 8 fermentation

At day 8 fermentation strains SYHs 1, 2, 4 and 6 showed moderately antibacterial activity while the other strains indicated weak activity on *A. tumefaciens*. All strains did not show antibacterial activity on *Bacillus subtilis.* Strain SYH 8 showed moderately antifungal activity whereas the other strains showed weak activity on *C. albicans*. Strains SYH 1, 4, 6, 7 and 8 showed moderately antibacterial activity while the other strains showed weak activity on *E. coli*. Strains SYHs 1, 2, 4, 6, 7 and 8 showed moderately antifungal activity but the other strains showed weak activity on *M. furfur* in Table 8.

Table 8. Inhibitory zones of day 8 fermented broths of isolated ten strains

Strain / Test organisms	SYH 1	SYH 2	SYH 3	SYH 4	SYH 5	SYH 6	SYH 7	SYH 8	SYH 9	SYH 10
A. tumefaciens	14	16	12	16	+	14	12	12	10	10
B. subtilis	-	-	-	-	-	-	-	-	-	-
C. albicans	10	11	9	12	8	8	10	13	+	+
E. coli	13	12	10	14	9	13	13	14	10	10
M. furfur	16	15	12	17	12	14	15	16	12	11

Antimicrobial activity of day 9 fermentation

At day 9 fermentation strains SYHs 2 and 4 showed highly antibacterial activity, strains SYHs 1, 3, 5, 7 and 8 showed moderately activity while the other strains indicated weak activity on *A. tumefaciens*. All strains did not show antibacterial activity on *Bacillus subtilis.* All strains showed weak activity against *Candida albicans*. Strains SYHs 7, 9 and 10 showed weak activity whereas the other strains showed moderately antibacterial activity on *E. coli*. Strains SYHs 5, 7 and 8 showed highly antifungal activity, strain SYH 3 indicated weak activity but the other strains showed moderately activity on *M. furfur* in Table 9.

Table 9. Inhibitory zones of day 9 fermented broths of isolated ten strains

Strain / Test organisms	SYH 1	SYH 2	SYH 3	SYH 4	SYH 5	SYH 6	SYH 7	SYH 8	SYH 9	SYH 10
A. tumefaciens	16	18	13	18	14	12	15	13	10	10
B. subtilis	-	-	-	-	-	-	-	-	-	-
C. albicans	10	11	10	10	+	+	10	9	+	+
E. coli	14	15	14	15	14	14	12	15	11	10
M. furfur	15	16	12	16	18	17	20	18	15	17

Antimicrobial activity of day 10 fermentation

At day 10 fermentation strains SYHs 1, 2, 3, 4, 6 and 7 showed moderately antibacterial activity while the other strains indicated weak activity on *A. tumefaciens*. All strains did not show antibacterial activity on *Bacillus subtilis.* All strains showed weak activity against *C. albicans*. Strains SYHs 1, 2, 3 and 4 showed moderately antibacterial activity whereas the other strains showed weak activity on *E. coli*. Strains SYHs 2 and 4 showed moderately antifungal activity but the other strains showed weak activity on *M. furfur* in Table 10.

Table 10. Inhibitory zones of day 10 fermented broths of isolated ten strains

Strain / Test organisms	SYH 1	SYH 2	SYH 3	SYH 4	SYH 5	SYH 6	SYH 7	SYH 8	SYH 9	SYH 10
A. tumefaciens	15	15	13	16	10	14	13	12	9	+
B. subtilis	-	-	-	-	-	-	-	-	-	-
C. albicans	+	+	+	+	+	+	+	+	+	+
E. coli	13	14	13	14	10	10	12	12	11	11
M. furfur	11	13	+	13	+	11	12	11	10	10

Antimicrobial activity of day 11 fermentation

At day 11 fermentation strains SYHs 6 and 8 showed moderately antibacterial activity while the other strains indicated weak activity on *A. tumefaciens*. All strains did not show antibacterial activity on *Bacillus subtilis.* All strains showed weak activity against *Candida albicans* and *Escherichia coli*. Strains SYHs 3 and 5 showed weak antifungal activity but the other strains showed moderately activity on *M. furfur*.

Antimicrobial Activity of Crude Extracts of Fungal Strains

Among all strains, the extracts of day 9 fermented broth of all fungal strains showed highly antimicrobial activity on four test organisms. The extracts of all strains indicated highly activity against *Agrobacterium tumefaciens* (21 mm - 33 mm), *Candida albicans* (21 mm – 30 mm), *Escherichia coli* (26 mm – 31 mm) and *Malassezia furfur* (30 mm – 40 mm). But, all strains did not show antibacterial activity on *Bacillus subtilis* in Table 11 and Figure 4.

Table 11. Antimicrobial activity of crude extracts of all strains

Strain / Test organisms	SYH 1	SYH 2	SYH 3	SYH 4	SYH 5	SYH 6	SYH 7	SYH 8	SYH 9	SYH 10
A. tumefaciens	30	30	26	30	26	33	30	29	21	29
B. subtilis	-	-	-	-	-	-	-	-	-	-
C. albicans	30	26	27	27	21	28	30	28	24	23
E. coli	30	31	27	30	26	31	27	29	26	26
M. furfur	40	40	39	40	40	38	40	39	30	30

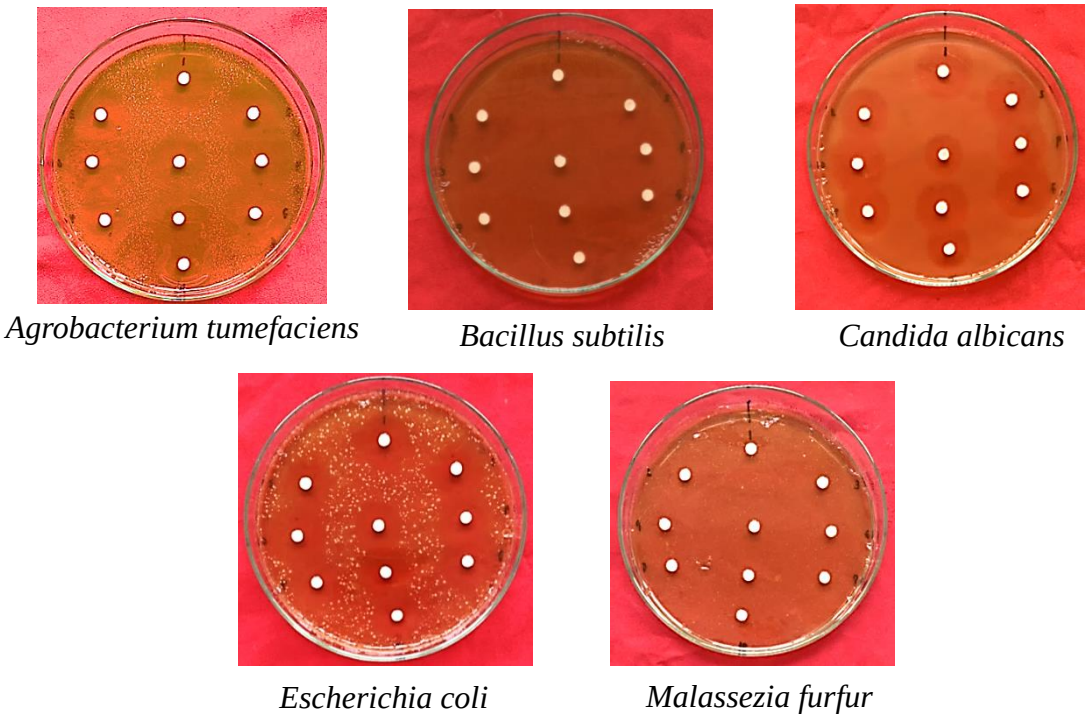


Fig. 4. Antimicrobial activity of crude extracts

Discussion and Conclusion

In this study, ten fungal strains: one *Dendryphiella* sp., four *Aspergillus* spp., two *Cephalosporium* spp., one *Madurella* sp. and two unknown species were isolated from *Zingiber officinale* Rosc. The fermented broths of all isolated strains (SYH 1 to SYH 10) showed antimicrobial activity on *Agrobacterium tumefaciens*, *Candida albicans*, *Escherichia coli* and *Malassezia furfur* from day 2 to day 11 fermentation. The ethyl acetate extracts of day 6 fermented broths of all ten strains indicated antimicrobial activity on four test organisms.

Wang *et al*., (2018) have isolated endophytic *Aspergillus* strains for a wide range of bioactivities such as antimicrobial, antitumor, etc. Yee Yee Thu *et al*., (2016) have reported that endophytic *Aspergillus* spp. was isolated from different plant species to inhibit *Candida albicans*, *Escherichia coli* and *Malassezia furfur*.

Song *et al*., (2005) have isolated endophytic *Cephalosporium* sp. IFB-E001 from *Trachelospermum jasminoides* for antimicrobial and antioxidant activities. Farhat *et al*., (2019) have isolated endophytic *Cephalosporium* that have a wide range of novel

antimicrobial compounds, which could be used in drug development and agrochemical production for protecting agricultural crops from plant root diseases.

Pushpa *et al*., (2018) have isolated endophytic *Madurella* sp. from *Catharanthus roseus* for the production of metabolites in pharmaceutical and food industries. Elsiddig *et al*., (2015) have isolated endophytic *Madurella* to the *Anogeissus leiocarpus* leaf extracts showed the potent antifungal activity of the extracts against mycetoma causing pathogen and justifying its traditional uses as a medicinal plant for treatment of skin infections.

In conclusion, fungi naturally produce antibiotics to kill or inhibit the growth of microbial diseases. The most active strains (SYH 1-SYH 8) should be chosen to produce the bioactive compounds to inhibit *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. This research work would be beneficial to protect some plants and animals diseases because the biological agents (endophytic fungi) were isolated from *Zingiber officinale* Rosc. The discovery of biological agents that possess selective toxicity against human and plant pathogens is an important need nowadays.

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