

## Isolation and Antimicrobial Activities of Endophytic Bacteria from the Leaves of *Madhuca longifolia* L. (Myitzu-thaka-nat-pan)

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### Abstract

In the present investigation, the endophytic bacteria were isolated from the leaves of *Madhuca longifolia* L. This plant belongs to the family Sapotaceae. The plant samples were collected from Pyay University Campus, Pyay Township, Bago Region. Six endophytic bacterial strains were isolated from the leaves of *Madhuca longifolia* L. The cell morphologies were described by observing colony characters, shape of cells, gram staining reaction and starch hydrolysis test. Antimicrobial activities of fermented broth of isolated strains were carried out by using paper disc diffusion assay with eight pathogenic microorganisms such as *Agrobacterium tumefaciens*, *Bacillus pumilus*, *Candida albicans*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas fluorescens*, *Staphylococcus aureus* and *Malassezia furfur*. This study showed that fermented broth of EMT 2, EMT 3 and EMT 4 established the most highly activity on *Micrococcus luteus* and followed by *Agrobacterium tumefaciens*, EMT 1 showed the highly activity on *Agrobacterium tumefaciens* and followed by *Micrococcus luteus*. EMT 5 and EMT 6 showed activity on *Agrobacterium tumefaciens*. According to the results of present study, it can be inferred that the leaves may have therapeutic potential as remedy by virtue of its antimicrobial activities.

Keynote: Antimicrobial Activity, Endophytes, *Madhuca longifolia* L.

### Introduction

*Madhuca longifolia* L. commonly known as mahwa or mahua belonging to family Sapotaceae, is a large deciduous tree growing widely under dry tropical and sub-tropical climate conditions. It was fast-growing tree that grows to approximately 20 meters in height, possesses evergreen or semi-evergreen. Mahuca tree was adapted to arid environment, being a prominent tree in tropical mixed deciduous forests. The tree was valued for its oil-bearing seeds and flowers, which are used for alcoholic beverage production. Mahua seeds are of economic importance as they are a good source of edible fats (Ramadan et al., 2006). Leaves extract is used for wound healing activity, rheumatism, hemorrhoids, chronic bronchitis and Cushing disease.

The leaves of Mahua tree contain saponin, an alkaloid glucoside. *Micrococcus luteus* possibly causes infections such as hepatic, brain abscess, septic arthritis and bacteremia (Minghui Zhu and et.al., 2021). Endophytes have a symbiotic relationship with their plant host and function as the biological defense for the plant against plant pathogens, insects and nematodes (Alvin et. al., 2014). In this investigation, the isolated endophytic bacteria from the leaves *Madhuca longifolia* L. were investigated the antimicrobial activities with eight test organisms.

### Materials and Methods

#### Collection of samples

The leaves samples were collected from *Madhuca Longifolia* L. plant (Myitzu-Tha-Ka) in Pyay University Campus. The identification of *Madhuca longifolia* L. was carried out at Botany department in Pyay University.

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### Isolation of Endophytic Microorganisms

The collection of Endophytic Microorganisms was carried out by Suto's method (1999) in Figure.1. The isolated endophytes were carried out their morphological and microscopical characters, requirements of oxygen and starch hydrolysis test (Dubey and Maheshwari, 2002, Alexander and Strete, 2001).

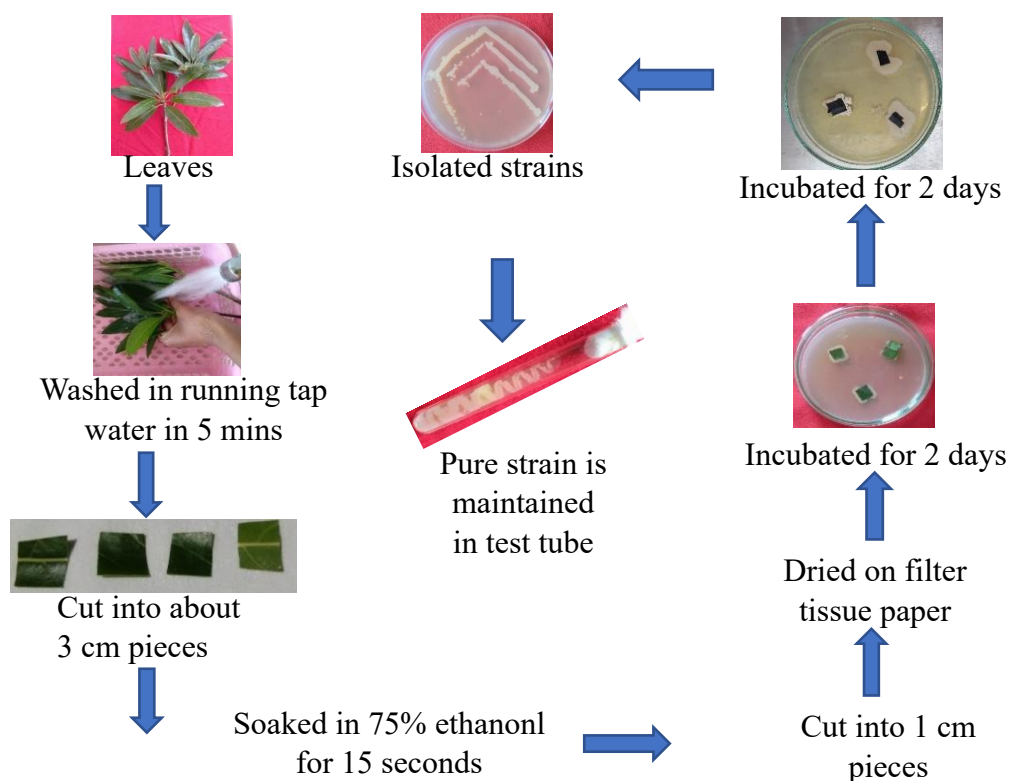


Figure 1. Isolation procedure of endophytes

### Antimicrobial activities of isolated strain

The isolated bacteria were grown at room temperature for 1 day on nutrient agar (NA) medium. The isolated bacteria were inoculated into seed medium and incubated at room temperature for 3 days. Five ml of seed culture was transferred into the fermentation medium. The isolated fermentation was carried out for seven days. After the end of fermentation, the fermented broth was used to examine the antimicrobial activity against test organisms by paper disc diffusion assay. Paper disc having six-millimeter diameter were utilized for antimicrobial assays. The assay medium was used for the antimicrobial activity test. One percent (about 20 $\mu$ L of spore suspension) of test organism was added to assay medium, then poured into plates. After solidification, paper discs impregnated with samples (fermented broth) were applied on the agar plates and the plates were incubated 24-36 hours at 28-30°C. Clear zone (inhibitory zones) surrounding the test discs indicate the presence of bioactive metabolites which inhibit the growth of test organisms in Figure.2.

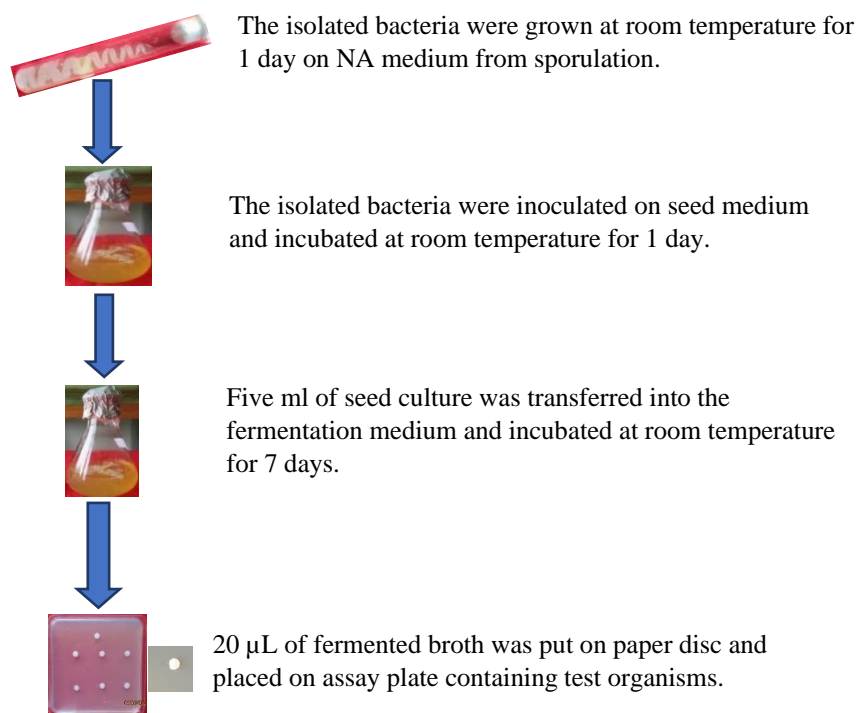


Figure 2. Antimicrobial activities by paper disc diffusion assay

## Results

Morphological characters of *Madhuca longifolia* L.

Scientific name -*Madhuca longifolia* (J.Koenig ex L.) J.F.Macbr. Contr. Gray Herb.,

Local name - Myitzu-thaka-natpan, Me-ze

Common name - Indian Butter Tree, Mahua

Family - Sapotaceae

Flowering and fruiting time - March to August

Outstanding characters of *Madhuca longifolia* L.

The habit was deciduous tree, the leaves are alternate, simple, clustered at the end of the branches. The flowers are bisexual, regular and actinomorphic. The fruits are berry, ovoid and greenish in Figure.3.



Figure 3. Morphological characters of *Madhuca longifolia* L.

### Morphological and Microscopical characters of isolated strains

Isolated all strains are large and creamy white color on nutrient agar medium. The different elevation umbonate, flat, raised and convex are found on EMT 1, EMT2, EMT 3, EMT 4, EMT 5 and EMT 6 respectively. The colony form of all isolated strains was irregular, circular and the margin lobate and entire. EMT 1 and EMT 4 are aerobic and other four strains are facultative. EMT 1 is cocci and other five strains are rod shape and gram negative in Table 1 and Figure 4 to 6.

Table 1. Morphological and Microscopical characters of isolated strains from *Madhuca longifolia* L.

	EMT 1	EMT 2	EMT 3	EMT 4	EMT 5	EMT 6
Margin	Lobate	Lobate	Entire	Lobate	Entire	Entire
Form and texture	Irregular, dry	Circular, wet	Circular, mucoid	Irregular, mucoid	Circular, mucoid	Circular, wet
Color	Creamy-white	Creamy-white	Creamy-white	Creamy-white	Creamy-white	Creamy-white
Elevation	Umbonate	Flat	Convex	Pulvinate	Convex	Convex
Cell Morphology	Cocci	Rod-shape	Rod-shape	Rod-shape	Rod-shape	Rod-shape
Gram reaction	Gram-Positive	Gram-Negative	Gram-Negative	Gram-Negative	Gram-Negative	Gram-Negative
Starch hydrolysis test	-	-	-	+	-	-
Oxygen requirement	Aerobic	Facultative	Facultative	Aerobic	Facultative	Facultative

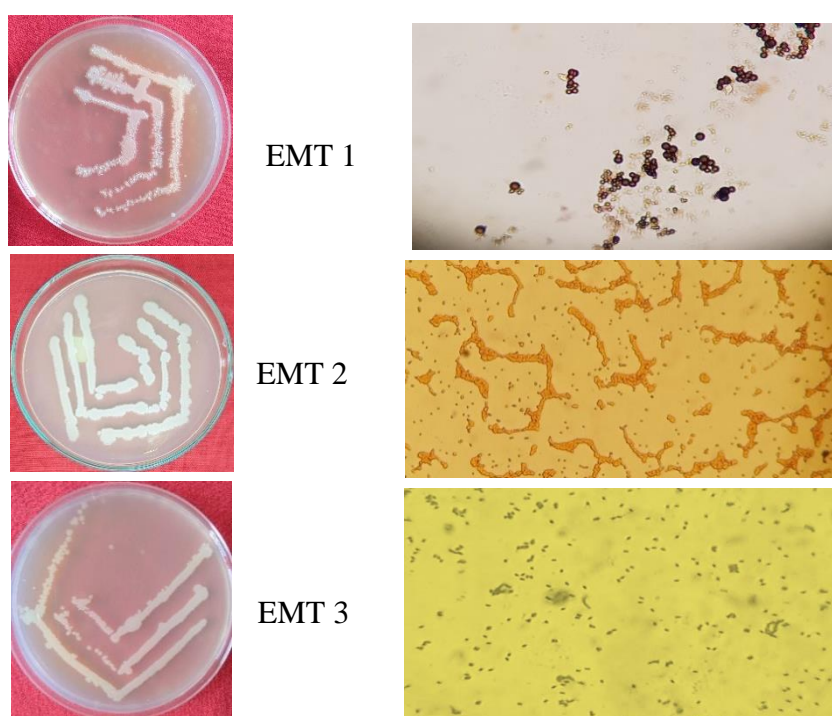


Figure 4. Morphological and microscopical characters of isolated strains EMT 1, EMT 2 and EMT 3

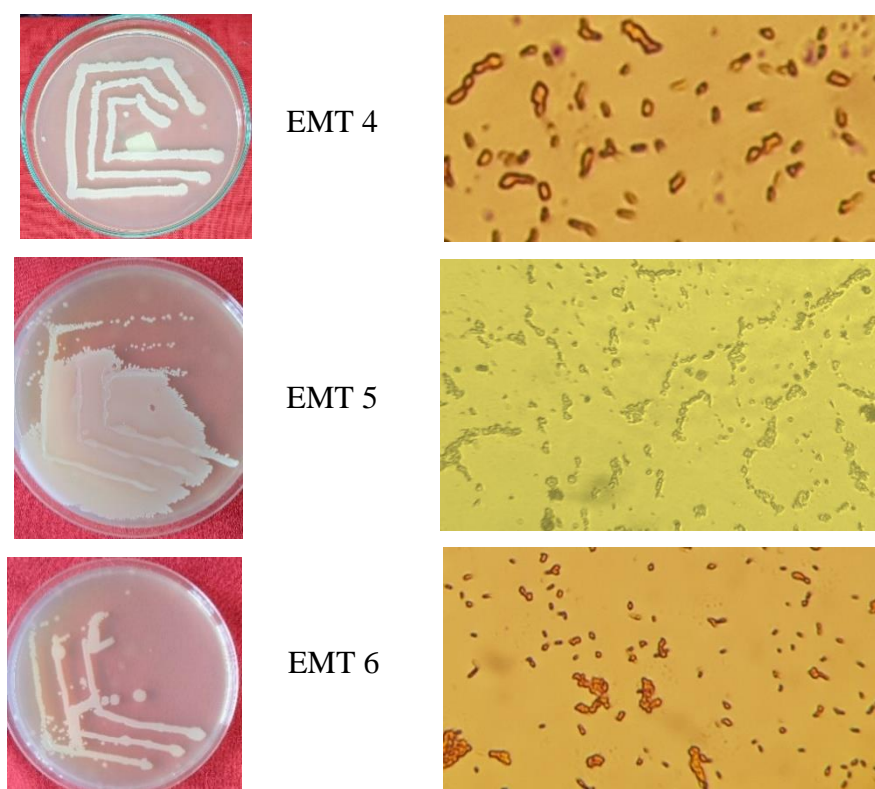
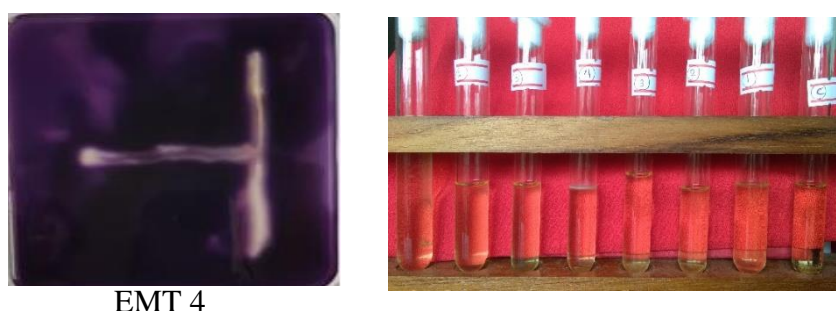


Figure 5. Morphological and microscopical characters of isolated strains EMT 4, EMT 5 and EMT 6



EMT 4  
Figure 6. Starch hydrolysis test and Influence of oxygen on microbial growth of isolated strains

#### Antimicrobial Activities of isolated bacteria

Endophytic bacteria strains were isolated from the leaves of *Madhuca longifolia* L. (Myitzu-thaka-nat-pan) and symbolized as EMT. Antimicrobial activities of isolated strains were carried out one day to seven days old culture, isolated strains EMT 2 fermented broths showed the highest activities 39.7mm on *Micrococcus luteus* and EMT 1 fermented broth showed the highest activity 36.9 mm on *Agrobacterium tumefaciens*. EMT 3 and EMT 4 fermented broth also showed 39.1, 33.2 mm on *Micrococcus luteus*. EMT 5 and EMT 6 fermented broth showed the highest activities 33.2, 33.1 mm on *Agrobacterium tumefaciens*. Except EMT 4 fermented broth, all strains showed activities on activity on *Pseudomonas fluorescences*. These results were shown in Table 2 to 6 and Figure 7 to 11.

Table 2. Antimicrobial activities of EMT metabolites (1- day old culture)

Test Organisms	EMT	EMT	EMT	EMT	EMT	EMT
	1	2	3	4	5	6
	mm	mm	mm	mm	mm	mm
<i>Agrobacterium tumefaciens</i>	36.9	<b>39.0</b>	30.8	33.2	33.2	33.1
<i>Bacillus pumilus</i>	25.4	<b>32.6</b>	29.6	14.2	21.5	15.0
<i>Candida albicans</i>	28.1	<b>35.4</b>	28.6	25.4	17.6	19.5
<i>Escherichia coli</i>	28.8	<b>32.1</b>	29.7	29.0	20.7	-
<i>Micrococcus luteus</i>	31.7	<b>39.7</b>	39.1	37.6	10.7	13.4
<i>Pseudomonas fluorescens</i>	-	-	-	<b>31.2</b>	-	-
<i>Staphylococcus luteus</i>	15.9	-	-	14.3	16.6	<b>17.1</b>
<i>Malassezia furfur</i>	17.0	-	-	16.6	18.8	<b>19.7</b>

Inhibitory zones (mm) Paper disc - 6mm

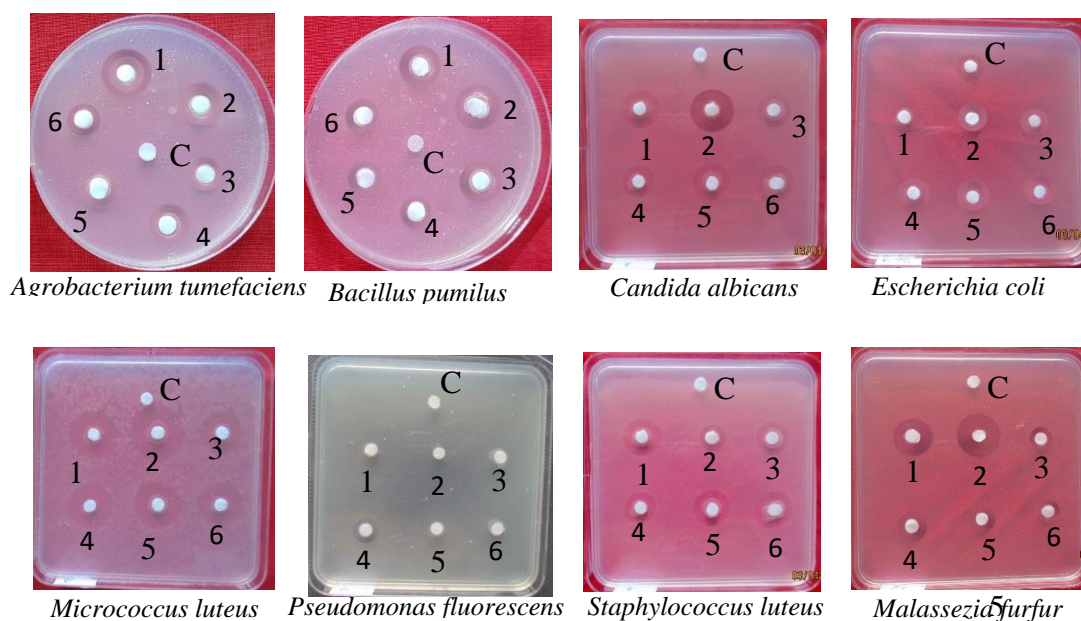


Figure 7. Antimicrobial activities of EMT metabolites (1-day old culture)

Table 3. Antimicrobial activities of EMT metabolites (2-days old culture)

Test Organisms	EMT	EMT	EMT	EMT	EMT	EMT
	1	2	3	4	5	6
	mm	mm	mm	mm	mm	mm
<i>Agrobacterium tumefaciens</i>	<b>29.0</b>	22.2	25.1	26.6	27.0	24.6
<i>Bacillus pumilus</i>	16.1	<b>20.2</b>	16.9	8.1	7.7	10.0
<i>Candida albicans</i>	25.7	22.0	<b>28.0</b>	-	14.5	12.5
<i>Escherichia coli</i>	-	-	-	-	-	-
<i>Micrococcus luteus</i>	11.3	<b>22.3</b>	11.7	12.1	13.5	11.0
<i>Pseudomonas fluorescens</i>	-	-	-	-	-	-
<i>Staphylococcus luteus</i>	12.9	14.0	10.1	-	<b>17.0</b>	9.4
<i>Malassezia furfur</i>	<b>14.4</b>	-	11.9	13.1	-	13.0

Inhibitory zones (mm) Paper disc - 6mm

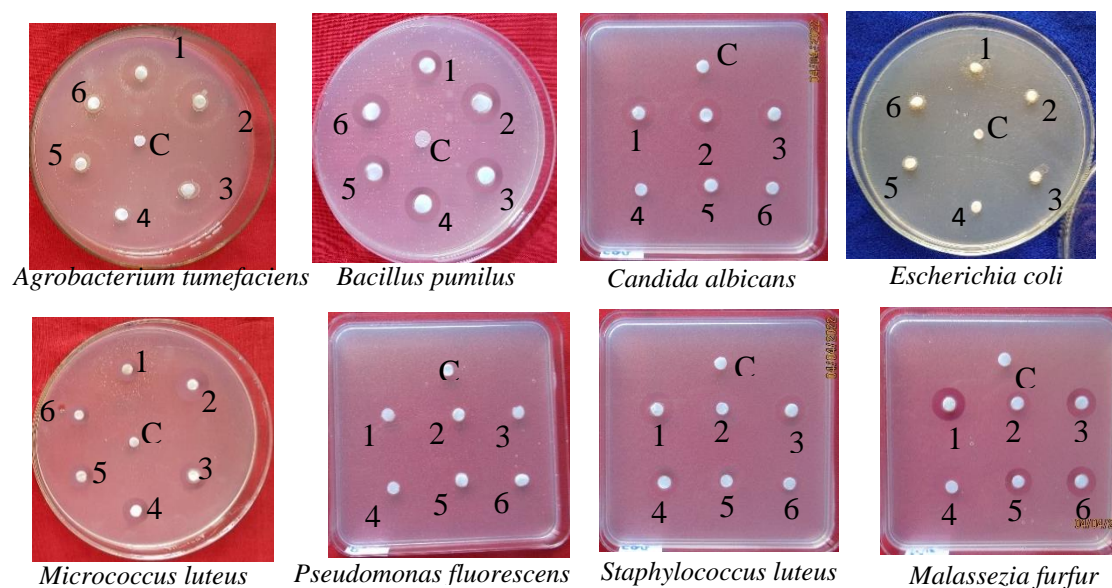


Figure 8. Antimicrobial activities of EMT metabolites (2-days old culture)

Table 4. Antimicrobial activities of EMT metabolites (3-days old culture)

Test Organisms	EMT 1	EMT 2	EMT 3	EMT 4	EMT 5	EMT 6
	mm	mm	mm	mm	mm	mm
<i>Agrobacterium tumefaciens</i>	<b>26.9</b>	19.0	24.3	23.7	24.8	23.8
<i>Bacillus pumilus</i>	<b>15.1</b>	14.8	11.6	13.0	10.5	12.1
<i>Candida albicans</i>	18.1	-	18.5	<b>20.9</b>	17.1	12.7
<i>Escherichia coli</i>	-	-	-	-	-	-
<i>Micrococcus luteus</i>	<b>10.4</b>	-	-	-	-	-
<i>Pseudomonas fluorescens</i>	-	-	-	-	-	-
<i>Staphylococcus luteus</i>	13.2	12.7	11.3	11.9	11.2	<b>13.8</b>
<i>Malassezia furfur</i>	17.6	<b>26.4</b>	18.2	16.0	14.4	19.7

Inhibitory zones (mm)

Paper disc - 6mm

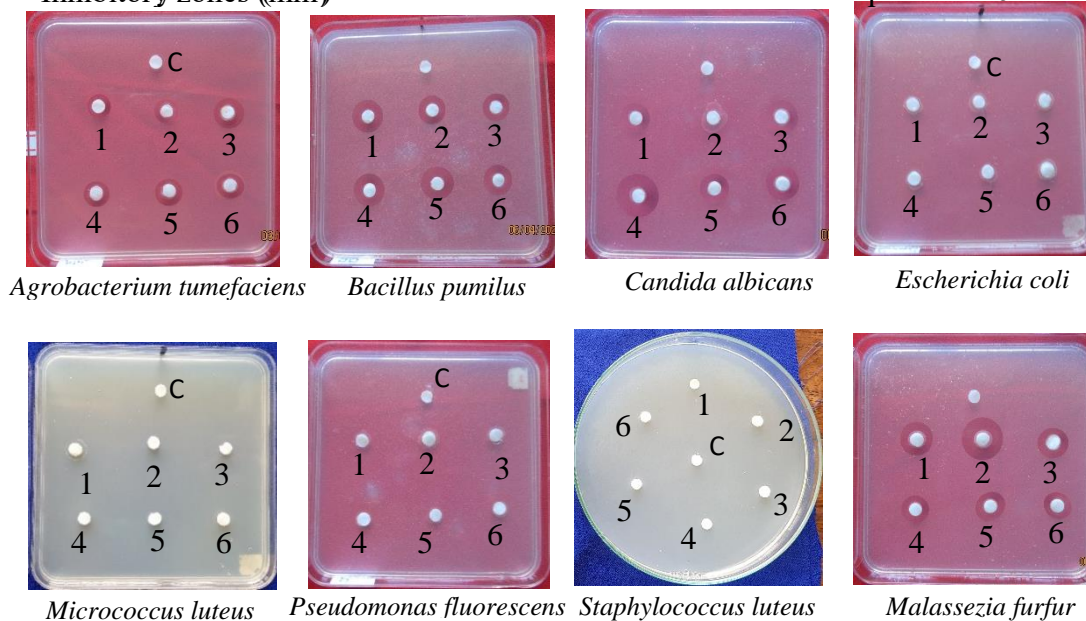


Figure 9. Antimicrobial activities of EMT metabolites (3-days old culture)

Table 5. Antimicrobial activities of EMT metabolites (4-days old culture)

Test Organisms	EMT	EMT	EMT	EMT	EMT	EMT
	1	2	3	4	5	6
	mm	mm	mm	mm	mm	mm
<i>Agrobacterium tumefaciens</i>	18.3	15.0	21.3	<b>24.3</b>	21.0	21.8
<i>Bacillus pumilus</i>	<b>12.6</b>	11.2	11	10.5	11.5	11.4
<i>Candida albicans</i>	<b>13.0</b>	-	11.2	10.7	-	10.3
<i>Escherichia coli</i>	-	-	-	-	-	-
<i>Micrococcus luteus</i>	<b>10.0</b>	8.1	-	-	-	-
<i>Pseudomonas fluorescens</i>	-	-	-	-	-	-
<i>Staphylococcus luteus</i>	<b>14.4</b>	-	12.7	12.4	13.2	12.6
<i>Malassezia furfur</i>	17.7	<b>26.2</b>	16	21.3	-	16.5

Inhibitory zones (mm)

Paper disc - 6mm

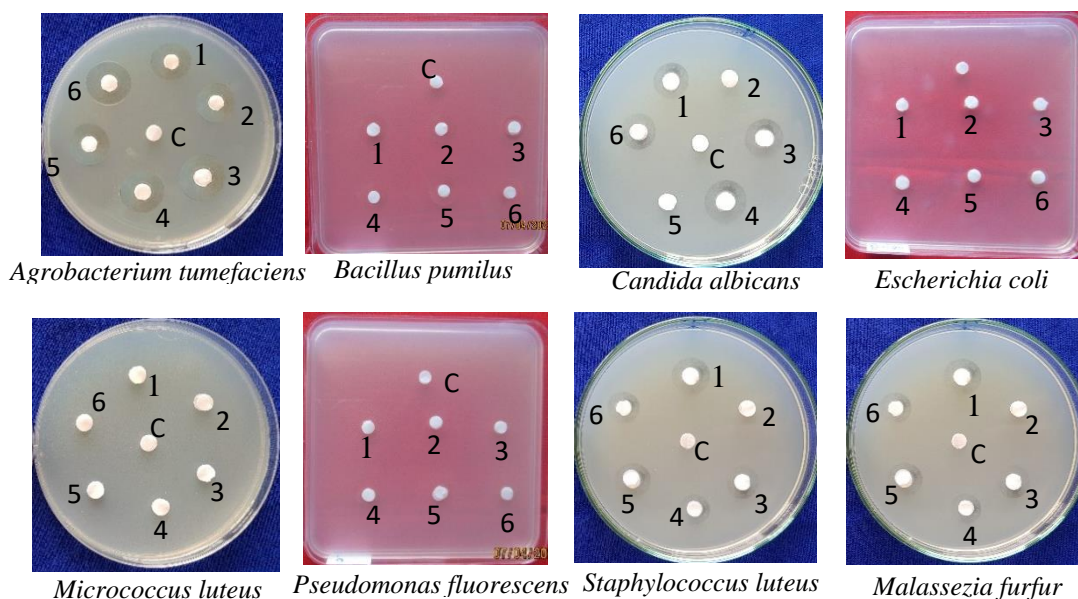


Figure 10. Antimicrobial activities of EMT metabolites (4-days old culture)

Table 6. Antimicrobial activities of EMT metabolites (5-days old culture)

Test Organisms	EMT	EMT	EMT	EMT	EMT	EMT
	1	2	3	4	5	6
	mm	mm	mm	mm	mm	mm
<i>Agrobacterium tumefaciens</i>	12.0	12.7	12.7	-	11.5	-
<i>Bacillus pumilus</i>	-	-	-	-	-	-
<i>Candida albicans</i>	-	10.5	-	-	-	-
<i>Escherichia coli</i>	-	-	-	-	-	-
<i>Micrococcus luteus</i>	-	-	-	-	-	-
<i>Pseudomonas fluorescens</i>	-	-	-	-	-	-
<i>Staphylococcus luteus</i>	-	-	-	-	-	-
<i>Malassezia furfur</i>	-	-	-	-	-	-

Inhibitory zones (mm)

Paper disc - 6mm



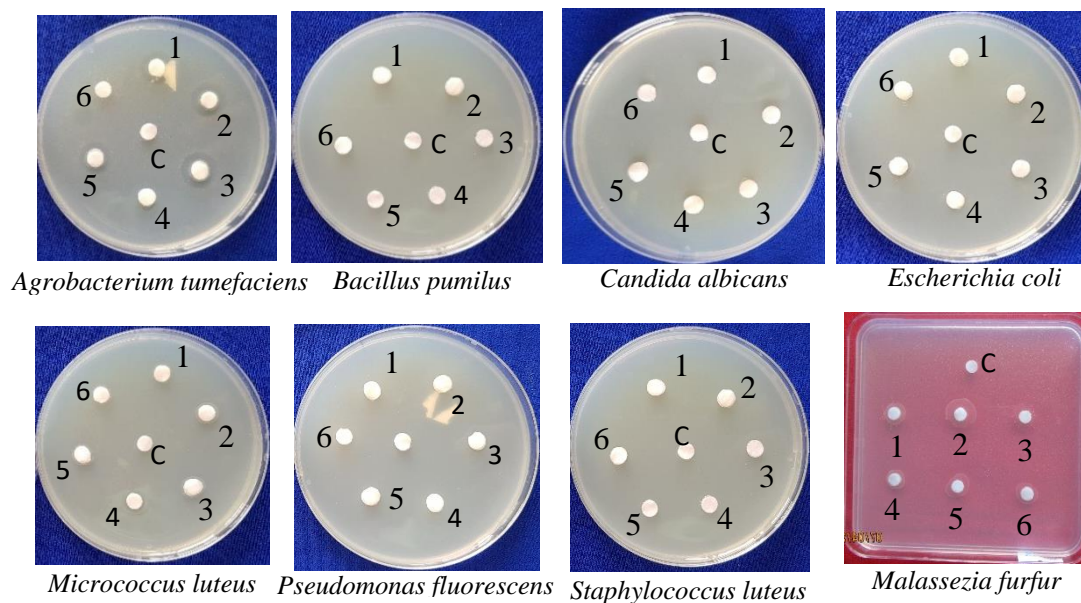


Figure 11. Antimicrobial activities of EMT metabolites (5-days old culture)

### Discussion and Conclusion

Endophytic microorganisms can be applied for biocontrol agents, industrial products such as medicines, enzymes, organic acids, saccharides, alcohols and vitamins. If a plant has the medicinal value, endophytic microorganisms can be isolated from that plant (Scott and Lori 1996).

*Madhuca longifolia* L. (Sapotaceae) commonly known as Butternut tree is an economic plant growing throughout the subtropical region. Extensive studies showed the presence of secondary metabolites like sapogenins, triterpenoids, saponins, flavonoids, glycosides and it has been used to treat infections, wounds, rheumatism, heart disease, diabetes and many other disorders. Experimental studies have demonstrated that Mahua exhibits antimicrobial, antioxidant, anti-inflammatory, antiulcer, cardioprotective, anticarcinogenic, immunomodulant and hypoglycemic properties. The outcome of these studies has emphasized the existing pharmacological profile of Mahua and provided a convincing support to its future clinical use in modern medicine (Devi and Sangeetha, 2016).

Six endophytic bacteria strains were isolated from the leaves of *Madhuca longifolia* L. (Myitzu-thaka-nat-pan) and symbolized as EMT. Isolated all strains are large and creamy white color on nutrient agar medium. The different elevation umbonate, flat, raised and convex are found on EMT 1, EMT2, EMT3, EMT4, EMT 5 and EMT 6 respectively. The colony form of all isolated strains was irregular, circular and the margin lobate and entire. EMT 1 and EMT 4 are aerobic and other four strains are facultative. EMT 1 is cocci and other five strains are rod shape and gram negative.

All isolated strains of fermented broth showed antimicrobial activity. In one-day old culture, EMT 4 fermented broth showed activities against eight test organisms, EMT 1 fermented broth showed highest activities on *Agrobacterium tumefaciens* and *Micrococcus luteus*. EMT 2 fermented broth showed the highest activities on *Micrococcus luteus* and *Agrobacterium tumefaciens*. EMT 3 and EMT 4 fermented broth also showed on *Micrococcus luteus* and *Agrobacterium tumefaciens*. EMT 5 and EMT 6 fermented broth showed the height activities on *Agrobacterium tumefaciens*.

Among them, EMT 1, EMT 2, EMT 3 and EMT 5 does not showed activities on *Pseudomonas fluorescens*. EMT 2 and EMT 3 also does not showed activities on *Staphylococcus luteus* and *Malassezia furfur*. EMT 6 does not showed activities on *Escherichia coli* and *Pseudomonas fluorescens*. Antimicrobial activities of all strains were decreased in fermentation period.

From the results of present research work, EMT 2 fermented broth was the highest activity (39.7mm) against *Micrococcus luteus*. According to Minghui Zhu and *et. al.*, 2021, *Micrococcus luteus* possibly cause infections such as hepatic, brain abscess, septic arthritis and bacteremia. So that, EMT 2 may be utilized to treat various of diseases.

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