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# *IN -VITRO* ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF ETHANOLIC AND WATER EXTRACTS OF *MIMOSA PIGRA* AND *MIMOSA DIPLOTRICHA*

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**ABSTRACT**

*Mimosa pigra* and *Mimosa diplotricha* are known as invasive plant and noxious weeds. These plants are spread throughout all areas of Thailand, causing problems in agriculture and transportation. This study aims to evaluate the antimicrobial activity of the water and ethanolic extracts of these plants. Stems, roots, and leaves crude extracts of *M.pigra* and *M.diplotricha* were investigated the antimicrobial activity by agar well diffusion. Seven different strains of gram-positive bacteria, gram-negative bacteria and yeast, i.e. *Escherichia coli* TISTR073, *Pseudomanas aeruginosa* TISTR781, *Staphylococcus epidermidis* TISTR518, *Staphylococcus aureus* TISTR2329 *Propionibacterium acnes* DMST 41283, *Streptococcus mutans* DMST14916 and *Candida albicans* TISTR5554 were used as test microbes. MIC and MBC of the crude extracts against those pathogens were also evaluated. The results revealed that the ethanolic extracts of both plant species had high antimicrobial activity against all test microbe and greater efficiency than the water extracts. The water extracts of *M.diplotricha* have inhibition activity against only *S.aureus* and *S.epidermidis* whereas the water extracts of *M.pigra* against only *S.epidermidis*. The MIC of the ethanolic extract of both plants varied between 1.56-100 mg/ml while MBC ranged from 6.25-50 mg/ml. The MIC and MBC of water extract ranged from 6.25-100 mg/ml and 25-100 mg/ml, respectively. Consequently, the ethanolic extracts of stem, root and leaves of *M.pigra* and *M.diplotricha* could be considered as a source of antimicrobial agents to be used as alternatives for a pharmaceutical application.

## INTRODUCTION

At present, medicinal plants are currently used and studied extensively in various fields such as pharmaceuticals, cosmetics, nutraceuticals and healthcare. Various medicinal plants have been used for years in daily life to treat disease all over the world. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness [1, 2]. WHO reported that in developing countries, more than 80% of people use of folk medicine is used in primary care [3]. During the past decade, Thailand's medicinal plant has also received interest as novel source of alternative Medications. Le Anh Dao et al. [4] reported that more than 2500 species of medicinal plants in Southeast Asia have been used as a traditional medicine for various conditions, such as diuretic, antioxidant, cytotoxicity and anti-microorganism. The beneficial medicinal effects of plant material typically result from the secondary metabolites such as alkaloids, flavonoids, steroids, tannins, phenol, etc. which capable for producing definite physiological action on the body [5]. Besides, compounds extracted from different parts of plants along with various kinds of solvent extraction methods for antimicrobial activity had been

investigated by many researchers [6-8]. For example, La-ongthitirat et al. [9] tested the ethanolic extract from stem and leave of *Hiptage candicans* Hook f. against human pathogens such as *S.epidermidis*, *S.aureus*, *S.mutans*, *P.acne*, *E.coli*, *P.vulgaris* and *C.albicans*. Barku et al. [10] investigated six wound healing medicinal plants which were known as Africa folk medicine i.e. *Amaranthus spinosus*, *Anogeissus leiocarpus*, *Spondia monbin*, *Corchorus olitorius* and *Mallotus oppositifolia* against *S.aureus*, *E.coli*, *K.pneumoniae* and *Citrobacter* sp. However, the extraction of important substances from medicinal plants had been carried out in various literatures but the extraction from weeds to bring benefits in pharmaceutical remains to be seen few. *Mimosa pigra* commonly known as giant sensitive tree or in Thailand calls Maiyaraab yak is belong to subfamily Mimosoideae in the larger family Fabaceae of legumes (Leguminosae). It is a native plant in tropical America, Asia, Africa and Oceania. *Mimosa pigra* has been described as an environmentally invasive weed in Thailand and also several parts of the world. With a large shrub and upright growth habit, *M.pigra* has the potential to spread through the ecosystems, grassland, flood plains and pasture then turn that area into a scrubland resulting that the biodiversity in those area was destroyed. In Thailand, *M. pigra* blocks irrigation systems that supply rice fields, reducing crop yield and harming farming livelihoods. In Vietnam it has invaded unique ecosystems in protected areas, threatening the biodiversity of seasonally inundated grasslands [11]. *M.pigra* is recorded by IUCN Invasive species specialist group as one of one hundred of the World's Worst invasive alien species. It is also included in the USA Federal Noxious Weeds List and has been listed as a noxious weed in Australia [12,13]. Rosado-Vallado et al. [14] and Koodkaew et al. [15] reported that the phytochemical profile of *M.pigra* was presence of flavonoids, alkaloid, quinones, saponins, sterols, and tannins. The methanolic extract of *M.pigra* leaf provided the phytotoxic compounds which inhibited the early seedling plant growth and it also had a potential for weed control [13, 15].

*Mimosa diplotricha* (synonym: *Mimosa invisa*) is commonly known as giant sensitive weed, creeping sensitive plant, nila grass or the local name in Thai calls Maiyaraap thao. It is broadly found in South-East Asia, Pacific Islands, Northern Australia, South and Central America, Hawaiian Islands and part of Africa [16]. *M. diplotricha* is an upright shrub, creeper or climbing plant that has much-branched leaves. *M. diplotricha* is well known for its rapid plant movement like invasive alien species. It gave a big problem for forest ecosystem, agricultural land and pastures in many countries include Thailand. Naima et al. [16] reported that the phytochemical analysis of the methanolic extract of *M. diplotricha* leaves presence of alkaloids, carbohydrates, saponins, glycosides, phytosterols, flavonoids, proteins and lipids. Also, Uyi et al. [17] reported that the phytochemical constituents of *M.dilplotricha* had the efficacy of insecticide against cowpea beetle. Chitra et al. [17] indicated that the methanol extract of *M.invisa* had antimicrobial activity against *B.subtilis*, *S.aureus*, *K. pneumoniae* and *P.fluorescens*. Giant sensitive weed (*Mimosa diplotricha*) is very similar to giant sensitive tree (*Mimosa pigra*) and common sensitive plant (*Mimosa pudica*). With the same genus, *M.pudica* had been reported by the researchers worldwide for its pharmacological activities such as antibacterial, antifungal, antioxidant, anti-inflammatory, antidiarrheal, antimalarial, antiparasitic, antinociceptive, anticonvulsant, cytotoxicity etc. from its different parts [1,18-20]. For example, Balsaraf et al. [21] investigated the antibacterial efficacy of aqueous extract of *M. pudica* against *S.mutans*. Sunil et al. [22] evaluated the antibacterial activity of ethanolic extracts of *M.pudica* leaves against *E.coli*, *P.aeruginosa*, *S.pyrogenes* and *S.aureus*. However, a few studies have made reference to *M.pigra* and *M.diplotricha*. Therefore, this study was conducted to examine the in-vitro antimicrobial activity of ethanolic and water extract from roots, stems and leaves of *M.pigra* and *M.diplotricha* in order to detect new sources of antimicrobial agents for medicinal use. Not only weeding, this research may also support a value added of the plant species causing the

biological circular economy to the local community in Thailand.

## MATERIALS AND METHODS

The plant materials were collected from the local region of Thailand, *M.pigra* and *M.diplotricha* were collected from Petchaboon and Pathumtani province, respectively. The leaves, stem and root samples were dried separately in an oven at 50 ℃ for 5-7 hr then separately ground to fine powders and kept in a desiccator until use.

### *Preparation of crude extracts*

The extractions were prepared by soaking powder samples in two different solvents: distilled water and ethanol 95%. For water extraction according to method of Srisook et.al. [23], powdered samples were added to boiling distilled water (1:5) and then heated for 30 min and filtered through Whatman No.1.

The filtrate then was evaporated at 40 ℃ by rotary evaporator and freeze-dried to give crude water extracts. The ethanol extraction was done by mercelation extraction according to a modified method of La-ongthitirat et.al. [24]. Powdered samples of leaves, stem and root of *M. pigra* and *M. diplotricha* were separately soaked in 95% Ethanol (1:5) for 5 days at room temperature. The solvent containing extracts were then decanted and filtered. The ground samples were further extracted 3 times with solvent. The filtrated were combined and then were evaporated under reduced pressure using a rotary evaporator and freeze dried to give the crude ethanolic extracts.

### *Test microorganisms*

Microbial cultures of seven different strains of gram-positive bacteria, gram-negative bacteria and yeast were used for determination of antimicrobial activity. Four bacterial strains; *Escherichia coli*TISTR073, *Pseudomanas aeruginosa*TISTR781, *Staphylococcus epidermidis*TISTR518, *Staphylococcus aureus* TISTR2329 and one yeast strain*; Candida albicans*TISTR5554 were obtained from Thailand Institute of Science and Technological Research, Thailand.

Bacterial strains were maintained on Muller Hinton Agar (MHA) and yeast strain was maintained on Sabouraud Dextrose Agar (SDA) under aerobic condition at 37 ℃ for 18-24 hr. Two clinical strains; *Propionibacterium acnes*DMST41283 and *Streptococcus mutans* DMST14916 were obtained from Department of Medical Sciences, Ministry of Public Health, Thailand. Both bacterial strains were maintained on Brain Heart Infusion (BHI) and incubated under anaerobic conditions at 37 ℃ for 18-24 hr.

### *Antimicrobial activity*

In-vitro antimicrobial activities were examined for water and ethanolic extracts of *M.pigra* and *M.diplotricha* against seven pathogens were investigated by agar well diffusion according to a modified method of Le Thoa et al. [25]. The suspensions of the test microbes were made in sterile normal saline and adjusted to $1\times10^8$ CFU/ ml or 0.5 McFarland's standard.

Small volumes of microbial suspensions were swabbed by using a sterile cotton swab on the agar plate surface. Then the agar wells were prepared by using a sterilized cork borer with 5 mm diameter. 30 µl of the water and ethanolic crude extracts solutions (100 µg/ml) were carefully added to the respective wells in the plate media. The plant crude extracts then were allowed to diffuse for about 30 min before incubation and then the plates were incubated at 37℃ for 24 hrs. The diameters of inhibition zones were measured in mm. The experiments were carried out in triplicates.

### *Minimum Inhibitory Concentration (MIC)*

*M.pigra* and *M.diplotricha* crude extracts were investigated for their MIC against seven strains microbe by using the standard broth dilution method according to La-ongthitirat et.al. [24]. The MIC was determined in Mueller Hinton broth for bacteria and YM broth for yeast.

The serial two-fold dilutions of plant crude extracts were done at concentrations ranging from 0.132 mg/ml to 10 mg/ml and adjusted concentration of test microbes by 0.5 McFarland. The inoculated tubes were overnight incubated at 37℃. The highest dilution of the plant extracts to inhibit growth (no turbidity in the tube) was considered as the MIC value. The MIC is the lowest concentration of antimicrobial agents that visually inhibits 99% growth of microorganisms.

| **Table 1** Antimicrobial activity of the ethanolic extracts of *M.pigra* and *M.diplotricha* | | | | | | |
|---|---|---|---|---|---|---|
| **Microorganisms** | **Zone of inhibition (mm)** | | | | | |
| | ***M.pigra*** | | | ***M.diplotricha*** | | |
| | **Leaves** | **Stems** | **Roots** | **Leaves** | **Stems** | **Roots** |
| *C. albicans* TISTR5554 | 22.46±0.53 | 16.56±0.98 | 16.82±0.37 | 14.50±0.53 | 15.18±0.45 | 15.55±0.46 |
| *E.coli* TISTR073 | 14.23±0.71 | 13.83±0.70 | 13.83±1.46 | 13.23±0.35 | 12.70±1.39 | 12.97±1.10 |
| *P.aeruginosa* TISTR781 | 17.30 ±0.70 | 19.75±0.26 | 18.70±0.45 | 17.70±1.54 | 16.57±0.63 | 16.07±0.64 |
| *P. acnes* DMST 41283 | 14.87±1.75 | 15.03±1.33 | 14.40±1.47 | 14.30±1.31 | 12.40±0.66 | 14.23±1.98 |
| *S.aureus* TISTR2329 | 16.07±0.19 | 17.13±1.01 | 17.57±0.45 | 12.90±0.25 | 13.10±0.36 | 13.13±0.35 |
| *S.epidermidis* TISTR518 | 11.43±0.60 | 12.25±0.17 | 16.45±0.42 | 17.27±0.85 | 19.05±0.92 | 19.10±0.41 |
| *S.mutans* DMST14916 | 13.17±0.75 | 12.60±0.17 | 13.37±0.06 | 16.30±1.25 | 15.20±1.54 | 15.87±1.14 |
| Values are expressed as mean ± SD (n=3) | | | | | | |

**Table 2** Antimicrobial activity of the water extracts of *M.pigra* and *M.diplotricha*

| **Microorganisms** | **Zone of inhibition (mm)** | | | | | |
|---|---|---|---|---|---|---|
| | ***M.pigra*** | | | ***M.diplotricha*** | | |
| | **Leaves** | **Stems** | **Roots** | **Leaves** | **Stems** | **Roots** |
| C. albicans TISTR5554 | - | - | - | - | - | - |
| *E.coli* TISTR073 | - | - | - | - | - | - |
| P.aeruginosa TISTR781 | - | - | - | - | - | - |
| *P. acnes* DMST 41283 | - | - | - | - | - | - |
| *S.aureus* TISTR2329 | - | - | - | 11.97±0.10 | **1**2.17±0.98 | **1**2.40±0.00 |
| *S.epidermidis* TISTR518 | 11.43±0.51 | 12.13±0.15 | 12.50±0.98 | 10.63±0.57 | 10.80±0.46 | 10.77±0.35 |
| *S.mutans* DMST14916 | - | - | - | - | - | - |
| Values are expressed as mean + SD (n=3) | | | | | | |

**Table 3:** MIC, MBC and MFC of the ethanolic extracts of *M.pigra* and *M.diplotricha* against test microbes

| Microorganisms | | Concentration (mg/ml) | | | | | |
|---|---|---|---|---|---|---|---|
| | | *M.pigra* | | | *M.diplotricha* | | |
| | | Leaves | Stems | Roots | Leaves | Stems | Roots |
| *C. albicans* TISTR5554 | MIC | **3.12** | **6.25** | **50** | **50** | **50** | **50** |
| | MFC | - | - | - | - | - | - |
| *E.coli* TISTR073 | MIC | **12.5** | **12.5** | **25** | **50** | **100** | **100** |
| | MBC | - | - | - | - | - | - |
| *P.aeruginosa* TISTR781 | MIC | **6.25** | **6.25** | **6.25** | **12.5** | **12.5** | **6.25** |
| | MBC | 12.5 | 12.5 | 12.5 | 25 | 25 | 25 |
| *P. acnes* DMST 41283 | MIC | **12.5** | **6.25** | **50** | **50** | **50** | **50** |
| | MBC | - | - | - | - | - | - |
| *S.aureus* TISTR2329 | MIC | **25** | **25** | **50** | **3.12** | **25** | **50** |
| | MBC | **25** | **25** | 50 | 50 | 50 | **50** |
| *S.epidermidis* TISTR518 | MIC | **1.56** | **1.56** | **6.25** | **3.12** | 1.56 | **6.25** |
| | MBC | 25 | 6.25 | 12.5 | 25 | 25 | 25 |
| *S.mutans* DMST14916 | MIC | **12.5** | **1.56** | **12.5** | 12.5 | **12.5** | **12.5** |
| | MBC | 25 | 25 | **12.5** | 25 | 50 | 25 |
| Values are expressed as mean + SD (n=3) | | | | | | | |

**Table 4: MIC and MBC of the water extracts of *M.pigra* and *M.diplotricha* against test microbe**

| Microorganisms | | Concentration of the extracts (mg/ml( | | | | | |
|---|---|---|---|---|---|---|---|
| | | *M. pigra* | | | *M. diplotricha* | | |
| | | leaves | Stems | Roots | Leaves | Stems | Roots |
| *S.aureus* TISTR2329 | MIC | No activity | | | 12.5 | 100 | 25 |
| | MBC | | | | - | - | - |
| *S.epidermidis* TISTR518 | MIC | 12.5 | 6.25 | 12.5 | 12.5 | 12.5 | 12.5 |
| | MBC | 50 | 25 | 50 | 100 | 50 | 100 |

***Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)***

From all tubes showed no visible signs of growth/turbidity (MIC), loopfuls were inoculated onto sterile MHA (for bacteria) and SDA (for yeast) by streak plate method. The plates were then overnight incubated at 37°C. The least concentration that did not show any growth of tested bacteria was considered as the MBC value. MFC is defined as the lowest concentration of that did not show growth of yeast cell. The MBC and MFC endpoint are defined as the lowest concentration of antimicrobial agent that kills 99.9% of the initial microbial population.

***Statistical analysis:*** *Data* of the zone of inhibition were expressed as mean ± standard deviation by Microsoft Excel Software. Analysis of variance (ANOVA) was performed by using SPSS 16.0 (SPSS Inc., Chicago, USA). Results were considered statistically significant when P>0.05 level.

**RESULTS**

The antimicrobial activities of the *M.pigra* and *M.diplotricha* crude extracts from leaves, stems and roots in term of zone of inhibition are shown in Table 1 and 2. Data in Table 1 shown that all parts of the ethanolic crude extracts of both plants had antimicrobial activity against all seven microorganisms. The highest zone of inhibition was found in the leaves extract of *M.pigra* against *C. albicans* (22mm). The stem and root crude extracts of *M. pigra* showed the highest inhibition zone against *P.aeruginosa* at 19 and 18 mm,

respectively. The leaves extract of *M.diplotricha* showed high inhibition against *P.aeruginosa* (17 mm) and *S.epidermidis* (17 mm) while stem and root extracts expressed the most sensitive against *S.epidemidis* (19 mm). The water extracts from leaves, stem and root of *M.pigra* were observed against only *S. epidermidis* whereas the extracts of *M.diplotricha* were effective against *S.aureus* and *S.epidermidis* (Table 2). There were found less inhibition from leaves extract of both plant species with zone of inhibition 10-11 mm and the high inhibition zone of the water extracts were found from stem and root (12 mm). This study also showed that the water extracts had inhibition against gram-positive bacteria but not effective against gram-negative and yeast. Minimum Inhibitory Concentration (MIC) is defined as the highest dilution or least concentration of the plant extracts that inhibit growth of test microorganisms. The MBC and MFC were determined by sub-culturing the test dilution (MIC) on the fresh agar plate and incubated further for 24 hr. The concentration of plant extracts that completely killed bacteria and fungi was taken as MBC and MFC, respectively. The MIC and MBC of the ethanolic extracts are presented in Table 3. The MIC of the *M.pigra* and *M.diplotricha* crude extracts from leaves, stems and roots ranged from 1.56-100 mg/ml. While the MBC ranged from 6.25-50 mg/ml and there were not seen to have a minimum fungicidal concentration (MFC). The ethanolic crude extracts of both plant species had only inhibition activity against *C.albicans*, *E.coli* and *P.acne* (no MBC) while *P.aeruginosa*, *S.aureus*, *S.epidermidis* and *S.mutans* effective as an inhibitory and bactericidal activity. *S.epidermidis* had the highest minimum inhibitory concentration of 1.56 mg/ml from leaves and stems crude extracts of *M.pigra* and stem crude extract of *M.diplotricha*. Similarly for leaves crude extract of *M.pigra*, *C. albicans* TISTR5554 had the minimum inhibitory concentration of 3.12 mg/ml as same as leaves crude extract of *M.diplotricha* against *S.aureus* and *S.epidermidis*. For stem and root extracts of *M.diplotricha*, *E.coli* showed the lowest minimum inhibitory effects at 100 mg/ml. MIC of the water extracts of *M.pigra* and *M.diplotricha* against *S.epidermidis* ranged from 6.25-12.5 mg/ml while MBC ranged from 25-100 mg/ml (Table 4). The least MBC value 25 mg/ml was observed in stem crude extract of *M. pigra*. For stem extracts of *M.diplotricha*, *S.aureus* showed the lowest minimum inhibitory effects at 100 mg/ml and there was no MBC from water extracts of all parts of *M.diplotricha*.

## DISCUSSION

In this present study, *M. pigra* and *M.diplotricha* extract from leaves, stem and root exhibited the antimicrobial activity against all the seven microorganisms. Screening of antimicrobial activity of the plant extracts was done by agar well diffusion. Data of the zone of inhibition (Table 1&2) could be classified into 3 groups; highly sensitive >17 mm, moderate 14-17 mm and < 14 mm low sensitive. The result from zone of inhibition revealed that the ethanolic extracts exhibited higher antimicrobial property than water extract. The ethanolic crude extract of leaves, stem and root of *M.pigra* expressed high sensitivity against *P.aeruginosa* and less sensitive against *E.coli* and *S.mutans*. While leaves extract showed the highest inhibitory activity against *C.albican* and stem and root extracts were exhibit the highest sensitive against *S.aureus*. On the other hand, the ethanolic extracts from *M.diplotricha* showed highly sensitive against *S.epidermidis* and less sensitive against *E.coli* and *S.aureus*.

For the water extract, the zone of inhibition of *M.pigra* was observed only in *S.epidermidis* with low sensitive inhibition activity whereas water extracts of *M.diplotricha* expressed only *S.aureus* and *S.epidermidis* with low sensitive level. Similar results reported by Sharmin et al. [3], the methanol crude extracts of *M.diplotricha* stem showed the inhibitory activity against gram-positive (*B.megaterium*) and gram-negative bacteria (*E.coli, Shigella boydii*, *S.dysenteriae*) but no activity observed from its water extracts. This result is similar to various reports. Le Thoa et al. [25] explained that ethanol crude extracts have stronger antibacterial activity than than the water crude extracts were due to the total flavonoids content in ethanol extract is higher than in water extract. Because flavonoid compound in *M.pudica* has polarity and chemical characteristics that are much more soluble in ethanol than in water. Jain et al. [26] also

reported that the secondary metabolites such as phenolic compounds, alkaloid and flavonoids which extracted from medicinal plants have antimicrobial properties. The results also showed that the antimicrobial efficacy was different for each part of *M. pigra* and *M.diplotricha*'s crude extracts. Debalke et al. [27] explained that the different level of inhibition even the same species may be due to the plant parts used, extraction protocol, bacterial strains and geographical location of plant collection site. This study also found that the ethanolic extract of both plants gave stronger inhibition to gram-positive, gram-negative and yeast while the water extracts had resistant activity against only gram-positive bacteria. Debalke et al. [27] explained that gram-positive microorganisms are typically more susceptible to antimicrobial agents than gram-negative bacteria. This might be because the outer membrane of gram-negative bacteria is differed from the outer membrane of gram-positive bacteria. This membrane acts as a permeability barrier which limits access of the antimicrobial agents to their targets in the bacterial cell. Another species of the same genus, *M. pudica,* has been reported by various researcher for the antibacterial and antifungal agents [19]. Amengialue et al. [28] indicated that the ethanolic extract (100 mg/ml) of *M.pudica* leaves showed strong antibacterial activity against *K.pneumonia*, *E.coli*, *B.subtilis*, *S.aureus*, *S.pyogenes* and *C.albicans* while its aqueous extract exhibited the least inhibitory activity against *K.pneumonia*, *E.coli* and *S.aureus*. The minimum inhibitory concentrations (MIC) of the different extracts against the test microorganism showed varied results. For ethanolic extract of *M.pigra*, *S.epidermidis* (leaves and stem extract) and *C.albican* (leaves extract) had the highest minimum inhibitory at 1.56 and 3.12 mg/ml, respectively. As well as the *M.diplotricha* ethanolic stem extract had the highest minimum inhibitory effect at 1.56 mg/ml concentration against *S.epidermidis*. While the water extract, *S.epidermidis* had the minimum inhibitory concentration of 6.25 mg/ml from *M.pigra* stem extract. While the minimum bactericidal concentration (MBC) of the ethanolic and water extract were 6.5-50 mg/ml and 25-100 mg/ml, respectively. In the present study, the MIC value of the plant extracts obtained in this study was lower than MBC value 2-5 times. Suggesting that the *M. pigra* and *M.diplotricha* crude extracts were bacteriostatic at lower concentrations but bactericidal at higher concentrations [29].

## CONCLUSION

In conclusion, the ethanolic extract of leaves, stem and root from *M. pigra* and *M.diplotricha* have antimicrobial activity against*E.coli*TISTR073, *P.aeruginosa* TISTR781, *S. epidermidis*TISTR518 , *S. aureus* TISTR232, *P. acnes*DMST 41283, *S. mutans*DMST14916 and *Candida albicans*TISTR5554. The water extracts of *M.diplotricha* have inhibition activity against *S.aureus*TISTR232 and *S.epidermidis*TISTR518 where the water extracts of *M. pigra* have inhibition activity against only *S.epidermidis*TISTR518. The inhibitory effect of ethanolic extracts of stem, root and leaves of both plant species showed broad spectrum activity in preventing the growth of all tested microorganisms including gram-positive, gram-negative and yeast. The results implied that *M.pigra* and *M.diplotricha* extracts can be used for the development of herbal medicine which might be a strong application against disease pathogen. However, further investigation should be done such as the toxicity of purified active compounds to deeply understand bioactive components and in vivo antimicrobial activity of *M. pigra* and *M.diplotricha* also need to study.

**Conflicts of Interest:** There is no conflict of interest to declare.

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