

IN VITRO SCREENING OF ANTIMICROBIAL ACTIVITY OF SELECTED MYANMAR MEDICINAL PLANTS

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Abstract

The global burden of microbial infection is very high and antibiotic resistance leads to failed treatment of infections, which can ultimately lead to death. To overcome antibiotic resistance, it is necessary to find new antimicrobial agents. In this study, twenty medicinal plants which are traditionally used for the treatment of various diseases in Kalonehtar village tract, Yebyu Township were selected to examine their antimicrobial potential against five pathogenic microorganisms such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans*. This research was performed at Department of Botany at University of Yangon in 2019. The extraction of phytopharmaceuticals from twenty medicinal plants was performed by using Ultrasound assisted solvent extraction technique. The polar solvent, methanol (80%) was employed for extraction process. The result showed that phytopharmaceuticals from *Symphorema involucratum* (20.6%) and *Portulaca oleracea* (20.2%) were the most soluble in methanol where as *Peperomia pellucida* (3.2%) was the least soluble in methanol. The antimicrobial activity of selected medicinal plants was conducted by paper disc diffusion method. It was revealed that the methanol extract of selected medicinal plants such as *Piper cubeba*, *Amomum subulatum* showed the higher antimicrobial activity on *Escherichia coli* (20 mm), *Dracaena angustifolia*, *Emilia sonchifolia*, *Pittosporum glabratum* showed the highest inhibitions (22mm-32mm) on *Pseudomonas aeruginosa* whereas *Melastoma malabathricum* exhibited the significant activity (32mm) on *Staphylococcus aureus*, the plants *Amomum subulatum*, *Glycosmis pentaphylla*, *Portulaca oleracea*, *Homonoia riparia* showed the activity (20 mm-28 mm) on *Bacillus subtilis*. Moreover, the result showed that *Homonoia riparia* Lour. posses antifungal activity (30mm) on *Candida albicans*. The present findings indicated that the active chemical compound responsible for the antimicrobial action must be a polar soluble compound.

Keywords: antimicrobial activity, methanol extract, *Pittosporum glabratum* Lindl.

Introduction

Medicinal plants have long been used to treat many infectious diseases (Mohanta *et al.*, 2014). The use of medicinal plants as complementary and alternative medicine has increased dramatically in the last 20-25 years (Rios & Recio, 2005). According to a WHO report, 80% of the world's inhabitants depend on traditional medicines as their main source of health care (Ballabhet *et al.*, 2007).

Worldwide research for antimicrobial agents continued to focus on flowering plants, non-flowering plants, fungi and bacteria (Fabry *et al.*, 1998). Myanmar is one of richest countries in the world with regards to the genetic resource of medicinal plants. The country has a wide range of topography and climate which influences its vegetation and floristic composition (DeFlippis, 2018). Although a great potential of the plants to be studied and developed into commercial products there are many species are unknown and their useful functions are still undiscovered (Thet Thet Mar Win, *et al.*, 2019).

The secondary metabolites can be identified through random, ethno (including ethnobotanical, ethnomedical and ethnopharmacological) and ecological searches. (Fabricant *et al.*, 2001). The random collection of plant samples from certain habitats (eg. Tropical rain forest) can be very useful for identification of novel chemical entities. However, this method is time consuming and labor intensive (Vuorela *et al.*, 2004).

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Previous studies have provided evidence that antimicrobial compounds isolated from different solvent extracts never provided the expected final output based on the activity of crude extracts and fractions (Eloff *et al.*, 2008). This is probably because different plant metabolites often work in combination with other compounds to regulate microbial infections and may therefore not be effective alone (Lewis *et al.*, 2006). The solvent (extraction agent) used to prepare phytopharmaceuticals must be able to dissolve all key phytoconstituents (Pauw *et al.*, 2014).

In this study, methanol (80%) extract was used in the preliminary screening (paper disc diffusion method). It is believed that the organic solvent methanol could efficiently penetrate the cell membranes, permitting the extraction of high amounts of endocellular components in contrast to low polarity solvents which can only extract extracellular material. Methanol (methyl alcohol) primarily dissolves polar constituents together with median and low polarity compounds extracted by cosolubilization. Therefore, the present investigation was conducted to evaluate methanol (80%) extracts of selected plants belonging to a wide range of families based on random sampling.

Materials and Methods

Study area

Ye-Byu Township is located in the district of Dawei. The region is extended over 2447.0 sq.km between 97° 50' 00" E to 98° 20' 00" E and 14° 15' 0" N to 15° 0' 0" N. The region is known to have a rich floral diversity with many of them having medicinal values Fig. (1). The tribal communities residing around the study area are Htar wai, Kayen, and Mon. This place offers unique opportunities to study indigenous medicinal plants used by populations. It is estimated that more than hundred plant species are collected from that area during the flowering period of 2017-2018. In this research, twenty medicinal plants were randomly selected for antimicrobial activity screening. The authors samples mostly leaf materials, unless ethnomedicinal information was available regarding other parts in Table (1), because leaves are renewable and resource and it is easier to recollect leaves from the same plant for follow-up work.



Figure 1 Location Map of Study Area in Yebyu Township in Dawei District

Processing

The leaves, roots the whole plant were collected separately during field trips to different places of Ye-Phyu Township. Healthy leaves were plucked from the large plant and the herbs were washed with distilled water. Following collection, the sample were dried at room temperature for three weeks. Then, the dried sample were powdered separately and stored in airtight containers to prevent from moisture and air-borne contamination.

Preparation of plant extract

A hundred gram of each powdered sample were added to a round bottom flask (1000ml) then moistened with 80% methanol (600 ml) and the sample was sonicated in an ultrasonic bath (Power Sonic 410) for 30 min at room temperature. The obtained extract was reduced by Rotary evaporator (at 50°C, 40 rpm, for 45 min) to get methanol soluble matter. Yield of extract was calculated in mg/g and converted into percentage (Azwanida, 2015).

$$\text{Yield (\%)} \text{ of extract} = \frac{\text{Weight of dry extract} \times 100}{\text{Weight of dry powdered sample}}$$

Antimicrobial assay

The antimicrobial activity was assessed against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans*. using the paper disc diffusion assay (Cruickshank *et al.*, 1975; Madigan *et al.*, 2005). In brief, the isolated microbial strains grown on nutrient agar were inoculated into conical flasks containing 10 ml of sterile growth medium. They were incubated at 30°C for 72 hours on a reciprocal shaker at 200 rpm. Then, test organisms (0.3ml) was added to assay medium, then poured into plates. The solutions of 50mg/ml plant extract were prepared by dissolving 80% methanol. Sterile 6mm paper discs (Whatman) were impregnated with 100µL of the plant extract solutions, dried at 40°C. Then, all disc was added to the agar surface. After 48-hour incubation at 42°C, the diameters of the zones of inhibition were measured. The tests were conducted at Microbiology Laboratory, Department of Botany, University of Yangon.

Table 2 List of plant species screened for antimicrobial activity

Scientific Name	Family	Local name	Part of use
<i>Hydrolea zeylanica</i> (L.) Vahl	Hydroleaceae	Le-hgin tha	the whole plant
<i>Bulbophyllum careyanum</i> Spreng.	Orchidaceae	Kaung-say-thi	the whole plant
<i>Cryptocoryne retrospiralis</i> (Roxb.) Kunth	Araceae	Unknown	The whole plant
<i>Portulaca oleracea</i> L.	Portulacaceae	Mya-Pyit	the whole plant
<i>Glycosmis pentaphylla</i> (Retz.) DC.	Rutaceae	Taw-shauk	leaves
<i>Sympheorema involucreatum</i> Roxb.	Lamiaceae	Daung-ttalaung	leaves
<i>Peperomia pellucida</i> (L.) Kunth	Piperaceae	Thit-yay-kyi	the whole plant
<i>Dracaena angustifolia</i> (Medik) Roxb.	Asparagaceae	Ein-lone-chan-thar	leaves
<i>Melastoma malabathricum</i> L.	Melastomataceae	Say-oh-pok	leaves
<i>Pterospermum semisagittatum</i> Buch-Ham	Malvaceae	Nwa-labyin	leaves
<i>Buchanania arborescens</i> (Blume) Blume	Anacardiaceae	Che-ti	leave
<i>Piper cubeba</i> L. f.	Piperaceae	Peik-chin	leaves
<i>Hellenia speciosa</i> (J. Koeing) SR	Costaceae	Phalan-taung-hmwe	the whole plant
<i>Homonoia riparia</i> (Lour.)	Euphorbiaceae	Yeta - kyi	the whole plant
<i>Amomum subulatum</i> Roxb.	Zingiberaceae	Chin-baung-phar-lar	the whole plant
<i>Emilia sonchifolia</i> (L.) DC. ex Wight	Asteraceae	Unknown	the whole plant
<i>Getonia floribunda</i> Roxb.	Combretaceae	Bu-nwe	leaves
<i>Syzygium lineatum</i> (DC.) Merr & L. M. Perry	Myrtaceae	Tha-bye	leaves
<i>Ochna integerrima</i> (Lour.) Merr.	Ochnaceae	Indaing – say-ni	leaves
<i>Pittosporum glabratum</i> Lindl.	Pittosporaceae	Hin- cho- pin	leaves

Results

. In this research, twenty medicinal plants were randomly selected for antimicrobial screening. The scientific name, local name, family, plant parts used and its medicinal values are mentioned precisely in Table (1), (2) and Figure (2).



Figure 2 Twenty selected medicinal plants from Ye-phyu Township namely (A) *Hydrolea zeylanica*(L.) Vahl. (B) *Bulbophyllum careyanum* Spreng. (C) *Cryptocoryne retrospiralis* (Roxb.)Kunth (D) *Portulaca oleracea* L. (E) *Glycosmis pentaphylla* (Retz.) DC. (F) *Symphorema involucratum* Roxb. (G) *Peperomia pellucida* (L.)Kunth (H) *Dracaena angustifolia* (Medik) Roxb. (I) *Melastoma malabathricum* L.(J) *Pterospermum semisagittatum* Buch-Ham. (K) *Buchanania arborescens* (Blume) Blume (L) *Piper cubeba* L. f. (M) *Hellenia speciosa* (J. Koeing) SR (N) *Homonoia riparia* (Lour.) (O) *Amomum subulatum* Roxb. (P) *Emilia sonchifolia* (Q) *Getonia floribunda* Roxb. (R) *Syzygium lineatum* (DC).Merr & L.M.Perry (S) *Ochna integerrima* (Lour.) Merr. (T) *Pittosporum glabratum* Lindl.

Extraction yield of selected medicinal plants

In this study, Table (2) represents the results for 80% methanol soluble matter content of selected medicinal plants. It was observed that phytoconstituents in *Peperomia pellucida* (L.) Kunth were least soluble in 80 % methanol as succulent herbs.

Table 3 Yield of the methanol (80%) of twenty selected medicinal plants using Sonication method

Scientific Name	Part of use	Yield (%)
<i>Hydrolea zeylanica</i> (L.) Vahl	the whole plant	10.3
<i>Bulbophyllum careyanum</i> Spreng.	the whole plant	7.5
<i>Cryptocoryne retrospiralis</i> (Roxb.) Kunth	the whole plant	12.5
<i>Portulaca oleracea</i> L.	the whole plant	20.2
<i>Glycosmis pentaphylla</i> (Retz.) DC.	leaves	9.4
<i>Symphorema involucreatum</i> Roxb.	leaves	20.6
<i>Peperomia pellucida</i> (L.) Kunth	the whole plant	3.2
<i>Dracaena angustifolia</i> (Medik) Roxb.	leaves	8.5
<i>Melastoma malabathricum</i> L.	leaves	15.4
<i>Pterospermum semisagittatum</i> Buch-Ham	leaves	8.1
<i>Buchanania arborescens</i> (Blume) Blume	leave	6.4
<i>Piper cubeba</i> L. f.	leaves	10.2
<i>Hellenia speciosa</i> (J. Koeing) SR	the whole plant	18.6
<i>Homonium riparia</i> (Lour.)	the whole plant	10.3
<i>Amomum subulatum</i> Roxb.	the whole plant	15.6
<i>Emilia sonchifolia</i> (L.) DC. ex Wight	the whole plant	7.4
<i>Getonia floribunda</i> Roxb.	leaves	17.5
<i>Syzygium lineatum</i> (DC.) Merr & L.M. Perry	leaves	18.3
<i>Ochna integerrima</i> (Lour.) Merr.	leaves	8.4
<i>Pittosporum glabratum</i> Lindl.	leaves	10.1

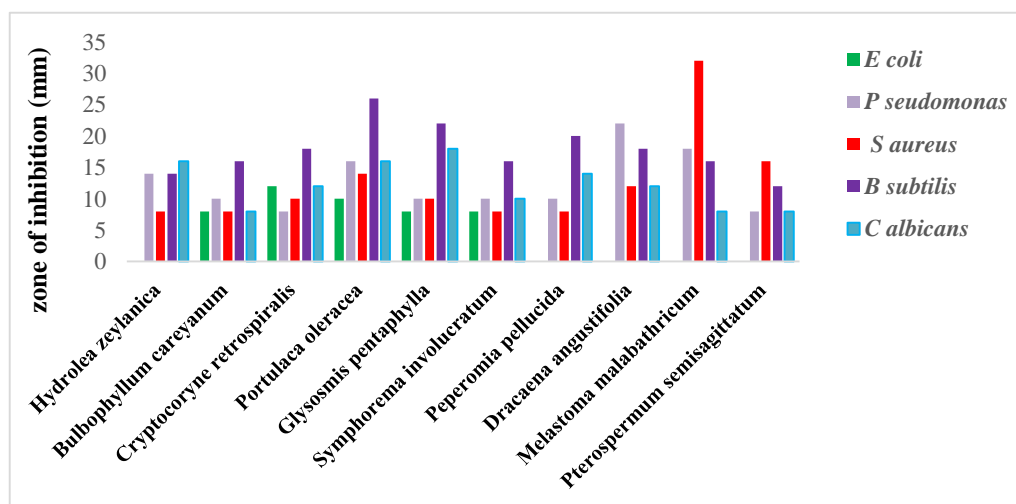
Antimicrobial activity of twenty selected medicinal plants

The inhibitory activities of methanol extract of twenty medicinal plants obtained using paper disc diffusion assay against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans*. It was found that the methanol extract of selected medicinal plants such as., *Piper cubeba* L.f., *Amomum subulatum* Roxb., showed the higher antibacterial activity on *Escherichia coli* (20 mm), *Dracaena angustifolia* (Medik.) Roxb, *Emilia sonchifolia* (L.) DC., *Pittosporum glabratum* Lindl showed the highest inhibitions (zone of inhibition between 22mm-32mm) on *Pseudomonas aeruginosa* whereas *Melastoma malabathricum* exhibited the significant activity (32mm) on *Staphylococcus aureus*, the plants *Amomum subulatum* Roxb., *Glycosmis pentaphylla* (Retz.) DC., *Portulaca oleracea* L., *Homonium riparia* (Lour.) showed the activity (zone of inhibition between 20mm-28mm) on *Bacillus subtilis*. Moreover, the result indicated that *Homonium riparia* (Lour.) possess antifungal activity (30 mm) on *Candida albicans* The results are shown in Table (3) and Fig. (3), (4) and (5).

Table 4 Antimicrobial activity of methanol (80%) extract of selected plants

Scientific Name	Part Use	Zone of Inhibition in mm				
		<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Candida albicans</i>
<i>Hydrolea zeylanica</i> (L.) Vahl	WP	-	14	8	14	16
<i>Bulbophyllum careyanum</i> Spreng.	WP	8	10	8	16	8
<i>Cryptocoryne retorspiralis</i> (Roxb.) Kunth	WP	12	8	10	18	12
<i>Portulaca oleracea</i> L.	WP	10	16	14	26	16
<i>Glycosmis pentaphylla</i> (Retz.) DC.	L	8	10	10	22	18
<i>Symphorema involucratum</i> Roxb.	L	8	10	8	16	10
<i>Peperomia pellucida</i> (L.) Kunth	WP	-	10	8	20	14
<i>Dracaena angustifolia</i> (Medik) Roxb.	L	-	22	12	18	12
<i>Melastoma malabathricum</i> L.	L	-	18	32	16	8
<i>Pterospermum semisagittatum</i> Buch-Ham	L	-	8	16	12	8
<i>Buchanania arborescens</i> (Blume) Blume	L	8	8	-	14	14
<i>Piper cubeba</i> L. f.	L	20	8	8	14	12
<i>Hellenia speciosa</i> (J. Koeing) SR	WP	10	12	8	20	8
<i>Homonia riparia</i> (Lour.)	WP	8	12	-	28	30
<i>Amomum subulatum</i> Roxb.	WP	20	8	8	20	12
<i>Emilia sonchifolia</i> (L.) DC. ex Wight	WP	8	22	8	16	8
<i>Getonia floribunda</i> Roxb.	L	8	8	-	14	12
<i>Syzygium lineatum</i> (DC.) Merr & L.M. Perry	L	12	12	8	18	10
<i>Ochna integerrima</i> (Lour.) Merr.	L	-	8	-	8	12
<i>Pittosporum glabratum</i> Lindl.	L	-	32	-	-	8

Paper disc - 6mm
 WP - The Whole Plan
 L - Leaves

**Figure 3** Effects of selected plants 80% methanol extract on Microbial growth

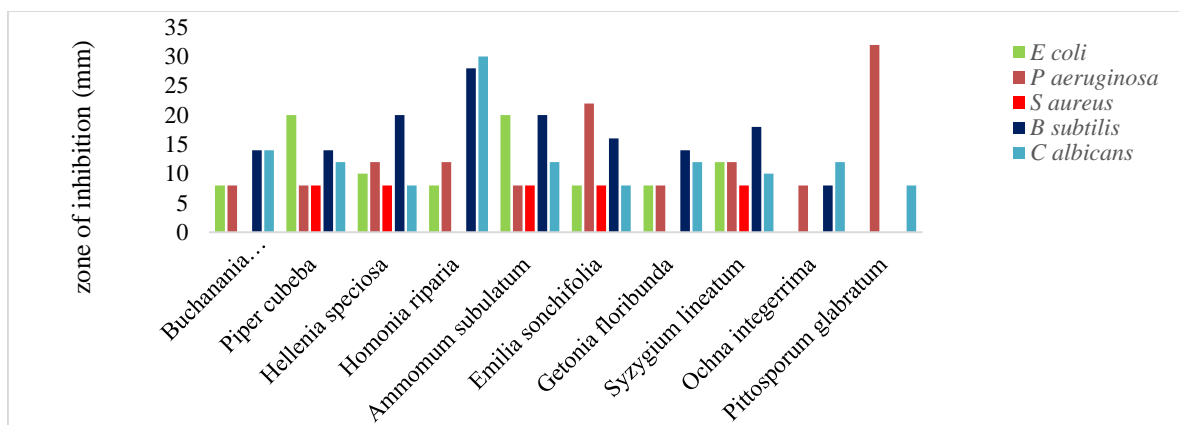
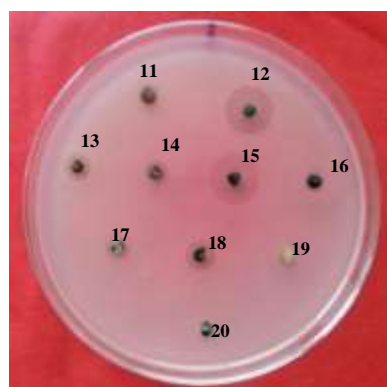
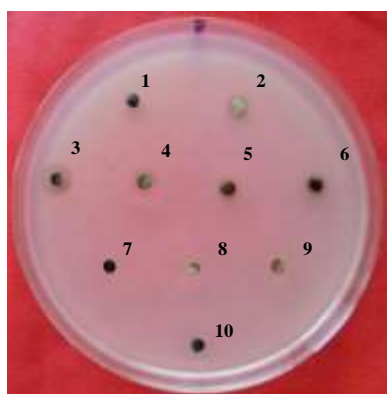
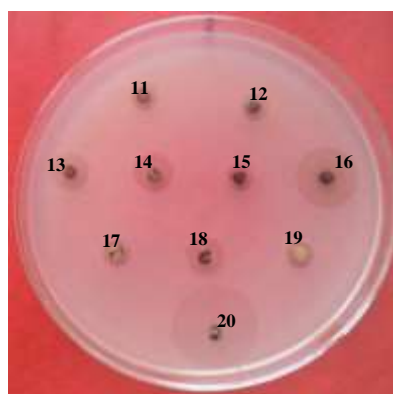
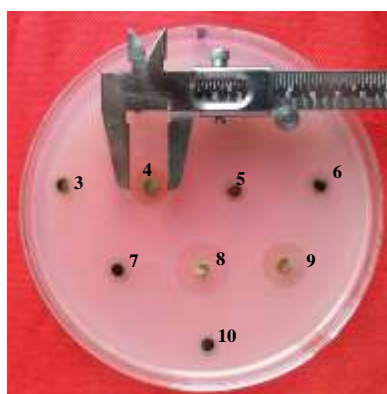


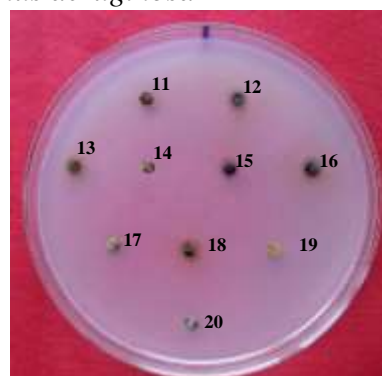
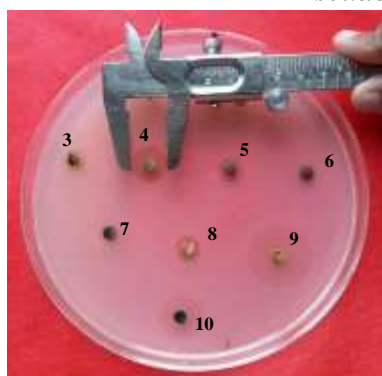
Figure 4 Effects of selected plants 80% methanol extract on Microbial growth



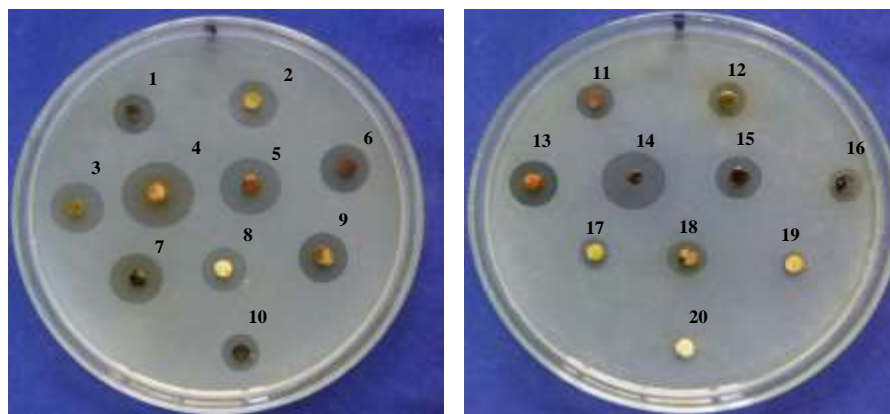
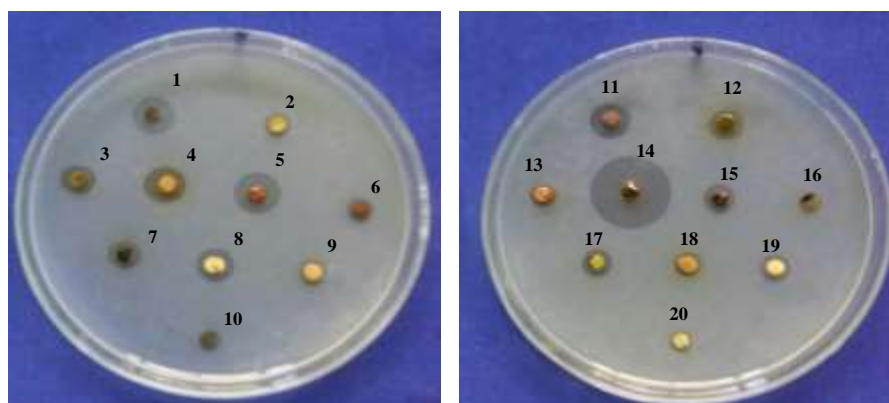
Escherichia coli



Pseudomonas aeruginosa



Scaphylococcus aureus

*Bacillus subtilis**Candida albicans*

- | | |
|---|---|
| 1. <i>Hydrolea zeylanica</i> (L.) Vahl. | 11. <i>Buchanania arborescens</i> (Blume) Blume |
| 2. <i>Bulbophyllum careyanum</i> Spreng. | 12. <i>Piper cubeba</i> L. f. |
| 3. <i>Cryptocoryne retrospiralis</i> (Roxb.) Kunth. | 13. <i>Hellenia speciosa</i> (J. Koeing) SR |
| 4. <i>Portulaca oleracea</i> L. | 14. <i>Homonoia riparia</i> (Lour.) |
| 5. <i>Glycosmis pentaphylla</i> (Retz.) DC. | 15. <i>Amomum subulatum</i> Roxb. |
| 6. <i>Symphorema involucratum</i> Roxb. | 16. <i>Emilia sonchifolia</i> (L.) DC. ex Wight |
| 7. <i>Peperomia pellucida</i> (L.) Kunth. | 17. <i>Getonia floribunda</i> Roxb. |
| 8. <i>Dracaena angustifolia</i> (Medik) Roxb. | 18. <i>Syzygium lineatum</i> (DC). Merr & L. M. Perry |
| 9. <i>Melastoma malabathricum</i> L. | 19. <i>Ochna integerrima</i> (Lour.) Merr. |
| 10. <i>Pterospermum semisagittatum</i> Buch-Ham | 20. <i>Pittosporum glabratum</i> Lindl |

Figure 5 Effect of Antimicrobial activity of twenty selected medicinal plants on six pathogenic microorganisms

Discussion

The total of twenty species (twenty genera, twenty families) were tested on four species of bacteria and one species of fungus mostly involved in common infections such as gastroenteritis, diarrhea, dysentery, skin diseases, food and water contamination.

In total of seven plant species were unable to inhibit the tested organism (*Escheria coli*) namely *Hydrolea zeylanica* (L.) Vahl, *Peperomia pellucida* (L.) Kunth, *Dracaena angustifolia* (Medik) Roxb., *Melastoma malabathricum* L., *Pterospermum semisagittatum* Buch-Ham, *Ochna integerrima* (Lour.) Merr., *Pittosporum glabratum* Lindl. whereas four species namely *Buchanania arborescens*, *Homonoia riparia* (Lour.), *Getonia floribunda* Roxb, *Ochna integerrima*

(Lour.) Merr., did not show any activity on *Staphylococcus aureus* and the plant, *Pittosporum glabratum* Lindl did not exhibit on *S aureus* as well as on *Bacillus subtilis*.

However, nine methanol extracts of selected medicinal plants showed strong inhibition on different test organisms (zones of inhibition ≥ 20 mm). The methanol extract of other plants also showed weakly inhibitions (zone of inhibition in between 8-18 mm). The different results concerning with antimicrobial activity might be due to different secondary metabolites of each plant (Cowan, 1999)

The antimicrobial activities of some of these plants such as *Hydrolea zeylanica* (L.), Vahl. (L.) Vah, *Bulbophyllum careyanum* Spreng., *Cryptocoryne retrospiralis* (Roxb.) Kunth, *Portulaca oleracea*, *Glycosmis pentaphylla* (Retz.) DC., *Symphorema involucreatum* Roxb., *Peperomia pellucida* (L.) Kunth, *Dracaena angustifolia* (Medik) Roxb., *Melastoma malabathricum* L., *Pterospermum semisagittatum* Buch-Ham, *Buchanania arborescens* (Blume) Blume, *Piper cubeba* Lf., *Hellenia speciosa* J Koenig SR, *Homonoia riparia* (Lour.), *Ammomum subulatum* Roxb., *Emilia sonchifolia*, *Getonia floribunda* Roxb., *Syzygium lineatum* (DC.) Merr & L.M. Perry, *Ochna integerrima* (Lour.) Merr., were previously described by other researchers (Qureshi *et al.*, 2017; Manna *et al.*, 2017; Oraibi *et al.*, 2017; Khan *et al.*, 2012; Idris *et al.*; Bapat *et al.*, 2014; Abbas Ali *et al.*, 2011; Saeed *et al.*, 2013; Wadkar *et al.*, 2017; Roy *et al.*, 2019; Pavithra *et al.*, 2009). However, the antimicrobial activity of selected plants such as *Buchanania arborescens* (Blume) Blume and *Pittosporum glabratum* Lindl leaves methanol extract have not mentioned in previous studies.

Conclusion

The twenty selected medicinal plants from Kalonehtar village tract, Ye-Pyu Township showed antimicrobial activity. These plants contained active antimicrobial agents which may use as a good source for antibiotics. Further study is required to identify the active compounds, synergetic affects, toxicity and safety of these plants and eventually clinical evaluations.

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APPENDIX I

Table 1 List of plants and their medicinal uses by indigenous communities in Ye-Phyu Township, Dawei District

Scientific Name	Family	Local name	Part of use	Ethno Medicinal uses
<i>Hydrolea zeylanica</i> (L.) Vahl	Hydroleaceae	Le-hgin tha	leaves	callous ulcers, skin diseases
<i>Bulbophyllum careyanum</i> Spreng.	Orchidaceae	Kaung-say-thi	pseudobulb	tuberculosis, tonic
<i>Cryptocoryne retorspiralis</i> (Roxb.) Kunth	Araceae	Unknown	rhizome	diarrhea, jaundice,
<i>Portulaca oleracea</i> L.	Portulacaceae	Portulacaceae	Whole plant	use as vegetable
<i>Glycosmis pentaphylla</i> (Retz.) DC.	Rutaceae	Taw shauk	leaves	leaves extract possess a healing effect
<i>Symphorema involucreatum</i> Roxb.	Lamiaceae	Daung-ttalaung	Roots	ear diseases, wounds and burns
<i>Peperomia pellucida</i> (L.) Kunth	Piperaceae	Thit-yay-kyi	the whole plant	urinary troubles
<i>Dracaena angustifolia</i> (Medik) Roxb.	Asparagaceae	Ein-lone-chan-thar	leaves	cure for swelling of joints
<i>Melastoma malabathricum</i> L.	Melastomataceae	Say-oh-pok	leaves	skin diseases
<i>Pterospermum semisagittatum</i> Buch-Ham.	Malvaceae	Nwa-labyin	barks	used as a replacement for Areca nut
<i>Buchanania arborescens</i> (Blume) Blume.	Anarcardiaceae	Che-ti	leave	to treat headache
<i>Piper cubeba</i> L.f.	Piperaceae	Peik-chin	leaves	antiseptic, expectorant
<i>Hellenia speciosa</i> (J Koeing) SR	Costaceae	Phalan-taung-hmwe	shoot	Used as vegetable
<i>Homonoia riparia</i> (Lour.)	Euphorbiaceae	Yeta - kyi	the whole plant	cure for pile
<i>Amomum subulatum</i> Roxb.	Zingiberaceae	Chin-baung-phar-lar	seeds	digestive disorders
<i>Emilia sonchifolia</i> (L.) DC.ex Wight	Asteraceae	Unknown	the whole plant	Used for febrifuge, anthelmintic
<i>Getonia floribunda</i> Roxb.	Combretaceae	Bu-nwe	flowers	Cure for abdominal menstruation
<i>Syzygium lineatum</i> (DC.) Merr & L.M.Perry	Myrtaceae	Tha-bye	fruits	edible for tonic
<i>Ochna integerrima</i> (Lour.) Merr.	Ochnaceae	Indaing – say-ni	bark	Used for blood dysentery
<i>Pittosporum glabratum</i> Lindl.	Pittosporaceae	Hin- cho- pin	roots	Cure for arthritis, insomnia

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