Antibacterial Activity of Extracts from *Phyllanthus emblica* Gaertn., *Chromolaena odorata* L. and *Syzygium aromaticum* (L.) Merr.

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ABSTRACT

The dried leaves, fruits and barks of *Phyllanthus emblica* Gaertn. (Zi-byu), the dried leaves of *Chromolaena odorata* L. (Bi-zat) and the dried flowers of *Syzygium aromaticum* (L.) Merr. (Lay-hnyin) were extracted with ethanol, 50% ethanol and water. The antibacterial activity on the growth of different pathogenic bacteria was tested by treating then with various soluble extracts. The paper disc diffusion technique with the surface swab plately method was used on 18 different types of bacterial isolates. The bacterial species included five strains of *Escherichia coli*, four species of *Shigella*, three strains of *Vibrio cholerae* and one strain each of *Klebsiella, Plesiomonae, Pseudomonas, Proteus, Salmonella* and *Staphylococcus*. Among a total of 15 different extracts from the leaves, fruits and barks of *Phyllanthus emblica* Gaertn. (Zi-byu), the leaves of *Chromolaena odorata* L. (Bi-zat) and the flowers of *Syzygium aromaticum* (L.) Merr. (Lay-hnyin), the watery extract and 50% ethanol extract of the barks from *Phyllanthus emblica* Gaertn. showed the best antibacterial activity (22 mm and 19 mm of inhibitory zones) on *Staphylococcus aureus.* Then, 50% ethanol extract of the leaves from *Phyllanthus emblica* Gaertn. had good antibacterial activity (18 mm of inhibitory zone) on *Staphylococcus aureus* and ethanol extract of the flowers from *Syzygium aromaticum* (L.) Merr. had good antibacterial activity (18 mm of inhibitory zone) on *Staphylococcus aureus.*

Key words – plant part extracts, antibacterial activity, inhibitory zone

INTRODUCTION

There is evidence of increasing interest in the search of higher plants which would be sources of active antibacterial substances as is seen from the large number of references to work on plate antibiotics in recent years. It is thus obvious that due attention has to be given to the exploitation of indigenous medicinal plants for potential antibiotic substances (Osborn, 1943 and Dhar *et al.*, 1968).

The basic evaluation of a new antimicrobial agent begins with an *in vitro* study of the agent in the microbiology laboratory. Such studies not only help characterize an agent but indicate with surprising accuracy of its potential clinical effectiveness. In certain special situatuins, this may not be true for certain agents with considerable activity *in vitro* against a given organism which may prove clinically to be ineffective in the therapy of the infection produced by the organism. However, the repeated studies on various new antimicrobial agents as they have been discovered and characterized have shown a high correlation between *in vitro* and *in vivo.* This is indeed fortunate in view of the difficulty today in collecting a sufficient number of clinical cases of a specific infection for therapeutic evaluation of a new agent (Bondi, 1964).

As an effort of microbiologists, antibiotics have revolutionized the treatment of bacterial and rickettsial diseases. Chemists have also synthesized potent drugs such as the arsenical and antimalarial compounds which have proved effective in the treatment of protozoal diseases and the sulphonamides useful in the treatment of bacterial diseases. A variety of plant extracts have been employed for the treatment of numerous diseases including those of microbial origin. In Myanmar, there are a number of plant species which may have antimicrobial potential through experience. In recent year, some workers have also researched on antimocrobial action of plant extracts (Win, Saw-Kyaw, 1976).

The objective of this study is to investigate the antibacterial properties of three plant origins namely *Phyllanthus emblica* (Zi-byu), *Chromolaena odorata* (Bi-zat) and

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Syzygium aromaticum (Lay-hynin) by the paper disc diffusion method with 15 different extracts.

MATERIALS AND METHODS

Three selected plants including three different families were collected from Pathein for testing the antibacterial activity by extraction. These plants are *Phyllanthus emblica* Gaertn. (Zi-byu) belonging to Phyllanthaceae, *Chromolaena odorata* L. (Bi-zat) belonging to Asteraceae and *Syzygium aromaticum* (L.) Merr. (Lay-hynin) belonging to Myrtaceae by investigation of the outstanding characters of the literature at Department of Botany, Pathein University.

The leaves, fruits and barks of *Phyllanthus emblica* Gaertn. (Zi-byu), the leaves of *Chromolaena odorata* L. (Bi-zat) and the flowers of *Syzygium aromaticum* (L.) Merr. (Lay-hnyin) were air-dried and crushed. 25 mg of crushed powder were extracted with 150 mL of ethanol, 50% ethanol and water for 6 hrs. at 80°C and filtered. The filtrate was dried on water-bath to get the extract residue.

The sterilized paper disc (8 mm in diameter) was soaked in 1 mg of the respective extract and allowed to dry at room temperature. The antimicrobial activity on the growth of different pathogenic bacteria was tested by treating then with various soluble extracts. The paper disc diffusion technique with surface swab plately method by Kirby Bauer method (Bauer, *et al.*, 1966) was used in 18 different types of bacterial isolates on the nutrient medium (dextrose 3 g, lactose 10 g, peptone 3 g, yeast extract 5 g, agar 15 g per litre) by Department of Medical Research (DMR). The bacterial species included five strains of *Escherichia coli*, four species of *Shigella*, three strains of *Vibrio cholerae* and one strain each of *Klebsiella, Plesiomonas, Pseudomonas, Proteus, Salmonella, Staphylococcus*. Most of them were from clinical infection specimens. The diameter of the inhibitory zone around the paper disc was measured after 24 hrs. at room temperature.

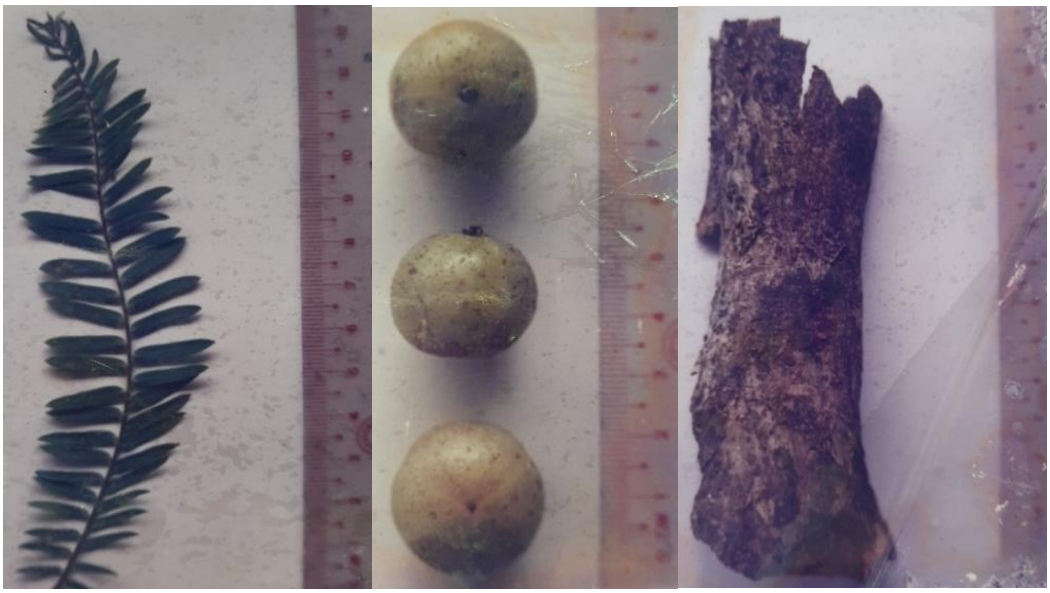

Fig. 1. Leaves, fruits and bark of *Phyllanthus emblica* Gaertn. (Zi-byu)

Fig. 2. Leaf of *Chromolaena odorata* L. (Bi-zat) and flowers of *Syzygium aromaticum* (L.) Merr. (Lay-hnyin)

Table 1. Bacterial test organisms for antibacterial activity

No.	Test organisms	Source
1	*Escherichia coli* ETEC	H1-3, DMR
2	*Escherichia coli* EPEC	N10/83, DMR
3	*Escherichia coli* EAEC	N3/83, DMR
4	*Escherichia coli* VTEC	Sumon 10-1, DMR
5	*Escherichia coli* ATCC	25922, DMR
6	*Klebsiella aeruginosa* NCTC	418, DMR
7	*Plesiomonas shigelloides*	wt 8, DMR
8	*Proteus morganii*	Hayashi, DMR
9	*Pseudomonas pyocyanea*	DTW, DMR
10	*Salmonella typhi*	Biken, DMR
11	*Shigella* boydii	N136-7, DMR
12	*Shigella dysenteriae*	sd 4, DMR
13	*Shigella flexnerii*	N186-6, DMR
14	*Shigella sonnei*	N398-2, DMR
15	*Staphylococcus aureus*	ws 1, DMR
16	*Vibrio cholerae* Inaba	KSA, DMR
17	*Vibrio cholerae* 0139	A535-1, DMR
18	*Vibrio cholerae* Ogawa	A537-1, DMR

RESULTS

The outstanding characters of *Phyllanthus emblica* Gaertn. (Zi-byu) (Phyllanthaceae)

A deciduous small tree. Leaves alternate, simple, linear-obling, margin entire, the base obtuse, both surfaces glabrous, unicostate, petiole subsessile, stipule ovate, pubescent. Inflorescence axillary cyme, peduncle sessile. Flowers monoecious, incomplete, actinomorphic, cyclic, pentamerous, hypogynous, staminate flower greenish-white, bract ovate, pedicel long, glabrous, ebracteolate, sepal free, obovate, imbricate, petal absent, stamens 3, monadelphous, filaments sessile, inserted, anther dithecous, adnate, extrorse, longitudinal dihiscence, pistillate flowers green, pedicel absent, ovary superior, globoid, glabrous, 3-carpels, sybcarpous, 3-loculed, 2 pendulous ovules in each locule, axile placentation, style long, stigma 2-lobed, disc present. Fruits berries, globoid, greenish-white, seeds elliptic-oblingoid, reddish brown.

Fig. 3. Habit and inflorescence of *Phyllanthus emblica* Gaertn. (Zi-byu)

The outstanding characters of *Chromolaena odorata* L. (Bi-zat) (Asteraceae)

Shrubs, pubescent. Leaves opposite and decussate, ovate-lanceolate, unicostate, the tips acuminate, the margins widely crenate-dentate, the bases rhomboidal, both surfaces pubescent, petiole flattened, exstipulate. Inflorescence terminal corymbose heads, bract numerous, involucrate, scaly, lanceolate, glabrous, persistent. Ray florets absent. Disc florets bisporangiate, white, actinomorphic, pappus numerous, long, slender, persistent, corolla 5, tubular, stamens 5, epipetalous, inserted, filaments long, anther syngenesious, dithecous, basifixed, longitudinal dehiscence, introrse, ovary inferior, one locule with one basal ovule, style long, slender, stigma bifid. Fruits one-seeded achenes, seeds ellipsoidal, glabrous.

Fig. 4. Habit and flower of *Chromolaena odorata* L. (Bi-zat)

The outstanding characters of *Syzygium aromaticum* (L.) Merr. (Lay-hynin) (Myrtaceae)

Evergreen tree, branching with distinctly jointed. Leaves entire, smooth, coriaceous and glandular punctate. Inflorescence axillary and terminal racemes of cymes. Flowers red, aromatic, taste, pungent, tetramerous, slightly flattened, 4-sided hypanthium, receptacle terminated by epigynous calyx of four thick, divergent sepals, surmounted by a nearly globular head, petals imbricate, enclosed stamens and style, stamens numerous, 2-celled ovary, inferior, numerous ovules, central placentation.

Fig. 4. Habit and inflorescence of *Syzygium aromaticum* (L.) Merr. (Lay-hynin)

The ethanol, 50% ethanol and watery leaf extracts of *Phyllanthus emblica* Gaertn. (Zi-byu) showed the highest antibacterial activity (14 mm, 18 mm and 15 mm of inhibitory zones) on *Staphylococcus aureus.* The ethanol, 50% ethanol and watery fruit extracts of *Phyllanthus emblica* Gaertn. (Zi-byu) gave the highest antibacterial activity (16 mm, 17 mm and 17 mm of inhibitory zones) on *Staphylococcus aureus* and the higher antibacterial activity (14 mm, 14 mm and 13 mm of inhibitory zones) on *Shigella* boydii. The ethanol, 50% ethanol and watery bark extracts of *Phyllanthus emblica* Gaertn. (Zi-byu) showed the highest

antibacterial activity (17 mm, 19 mm and 22 mm of inhibitory zones) on *Staphylococcus aureus.*

The ethanol and 50% ethanol flower extracts of *Syzygium aromaticum* (L.) Merr. (Lay-hynin) exhibited the highest antibacterial activity (18 mm and 16 mm of inhibitory zones) on *Staphylococcus aureus* and the higher antibacterial activity (14 mm and 17 mm of inhibitory zones) on *Shigella* boydii. The watery leaf extracts of *Chromolaena odorata* L. (Bi-zat) showed little antibacterial activity (13 mm and 10 mm of inhibitory zones) on *Staphylococcus aureus* and *Plesiomonas shigelloides.*

Table 2. Antibacterial activity (mm of inhibitory zone) of the extracts of *Phyllanthus emblica* Gaertn. (leaves, fruits and barks)

No.	Testorganisms	Leaf			Fruit			Bark		
		ethanol	50%	water	ethanol	50%	water	ethanol	50%	water
1	*Escherichia coli* ETEC	13	-	13	10	10	13	-	-	-
2	*Escherichia coli* EPEC	12	-	13	12	10	13	-	-	10
3	*Escherichia coli* EAEC	13	-	13	-	13	-	-	-	-
4	*Escherichia coli* VTEC	13	-	-	-	13	13	-	-	10
5	*Escherichia coli* ATCC	-	-	-	-	12	-	-	-	10
6	*Klebsiella aeruginosa* NCTC	-	-	-	-	13	-	-	-	-
7	*Plesiomonas shigelloides*	-	-	10	-	13	13	-	-	11
8	*Proteus morganii*	-	-	10	-	-	-	-	-	10
9	*Pseudomonas pyocyanea*	-	-	-	13	10	-	-	-	-
10	*Salmonella typhi*	13	-	13	15	12	13	-	-	11
11	*Shigella* boydii	13	11	13	**14**	**14**	**13**	-	-	13
12	*Shigella dysenteriae*	10	12	13	-	10	10	12	12	13
13	*Shigella flexnerii*	-	-	-	-	10	13	-	-	-
14	*Shigella sonnei*	13	-	12	-	10	-	-	-	-
15	*Staphylococcus aureus*	**14**	**18**	**15**	**16**	**17**	**17**	**17**	**19**	**22**
16	*Vibrio cholerae* Inaba	-	-	-	-	10	13	-	-	-
17	*Vibrio cholerae* 0139	13	-	13	-	-	10	-	-	-
18	*Vibrio cholerae* Ogawa	-	-	-	-	-	-	-	-	-

Table 3. Antibacterial activity (mm of inhibitory zone) of the leaf extracts of *Chromolaena odorata* L. and the flower extracts of *Syzygium aromaticum* (L.) Merr.

No.	Test organisms	Leaf			Flower		
		ethanol	50%	water	ethanol	50%	water
1	*Escherichia coli* ETEC	-	-	-	13	10	-
2	*Escherichia coli* EPEC	-	-	-	13	-	-
3	*Escherichia coli* EAEC	-	-	-	-	-	-
4	*Escherichia coli* VTEC	-	-	-	13	-	13
5	*Escherichia coli* ATCC	-	-	-	-	-	-
6	*Klebsiella aeruginosa* NCTC	-	-	-	-	13	-
7	*Plesiomonas shigelloides*	-	-	10	-	13	10
8	*Proteus morganii*	-	-	-	10	-	10
9	*Pseudomonas pyocyanea*	-	-	-	13	10	-
10	*Salmonella typhi*	-	-	-	10	13	13
11	*Shigella* boydii	-	-	-	**14**	**17**	10
12	*Shigella dysenteriae*	-	-	-	-	13	10
13	*Shigella flexnerii*	-	-	-	-	13	13
14	*Shigella sonnei*	-	-	-	10	-	-
15	*Staphylococcus aureus*	-	-	**13**	**18**	**16**	12
16	*Vibrio cholerae* Inaba	-	-	-	-	10	10
17	*Vibrio cholerae* 0139	-	-	-	10	-	13
18	*Vibrio cholerae* Ogawa	-	-	-	10	-	-

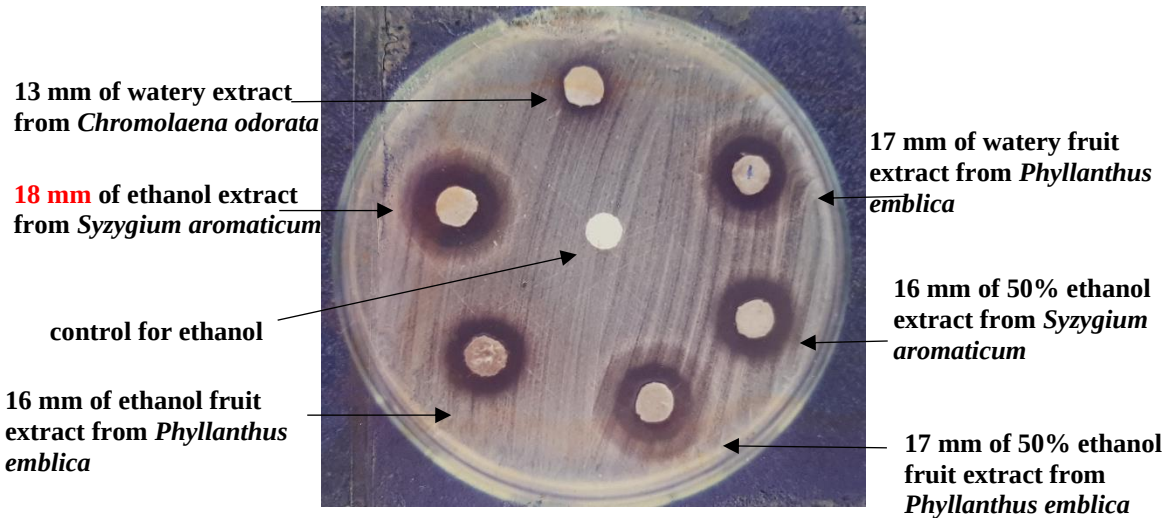

Fig. 5. Antibacterial activity of extracts on *Staphylococcus aureus*

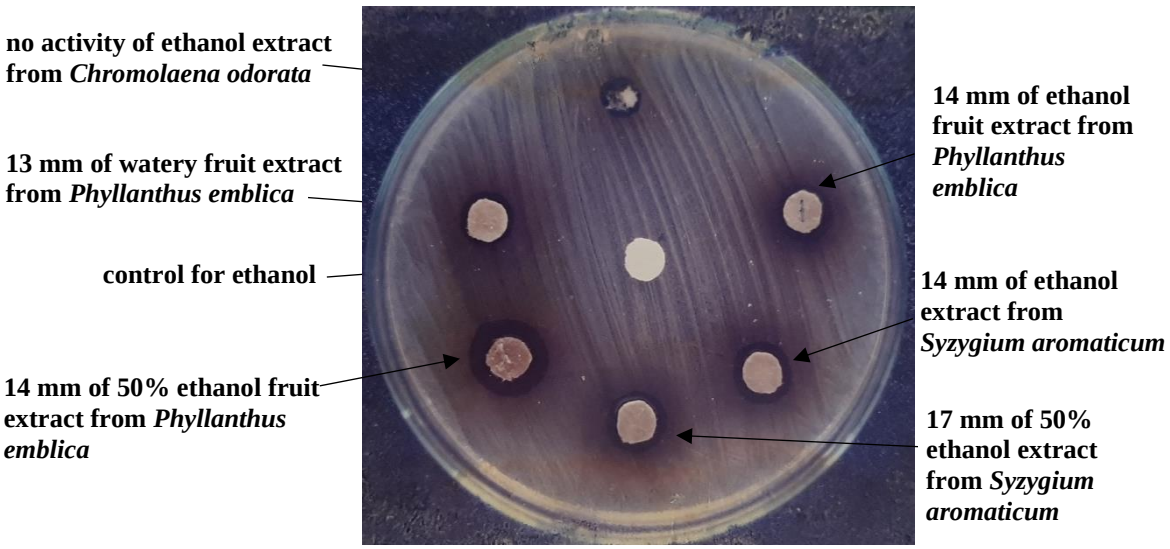

Fig. 6. Antibacterial activity of extracts on *Shigella boydii*

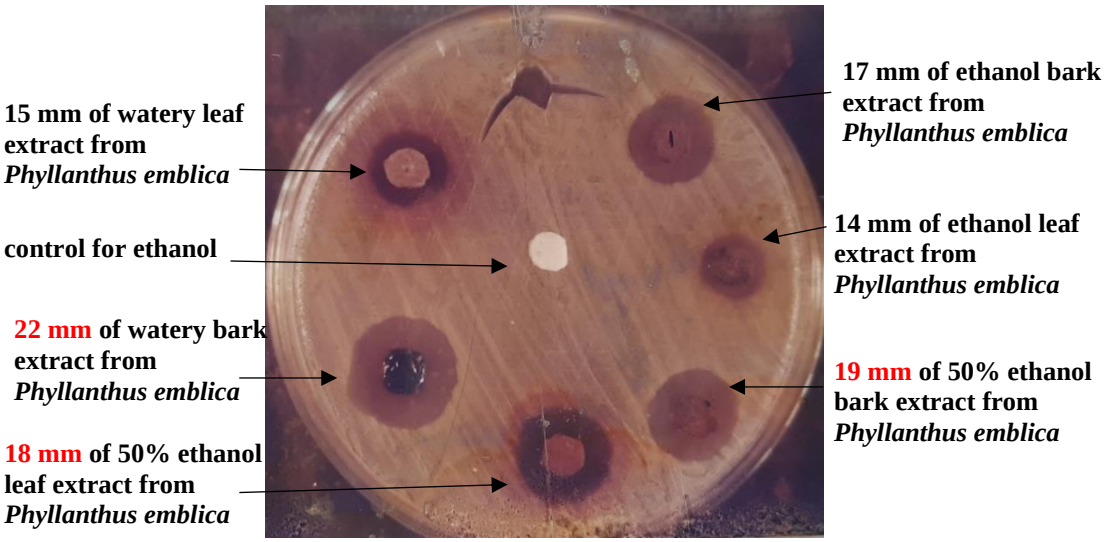

Fig. 6. Antibacterial activity of extracts on *Staphylococcus aureus*

DISCUSSION AND CONCLUSION

In the traditional medicine of Myanmar, the various parts of plants are widely used. They apparently effect on many diseases and infections (Ashin-Narga-Thein, 1968). Scientifically, many workers have also searched for the antimicrobial activities of plant extracts against a number of pathogens (Nyein, Mar-Mar, 1976).

Antibacterial activity of different extracts of *Phyllanthus emblica* leaves and fruits was evaluated by the agar well diffusion method against different pathogenic bacteria viz., *Escherichia coli, Serratia marcescens, Pseudomonas aeruginosa and Bacillus cereus* in the present study. Maximum antibacterial activity was obtained against *E. coli* (ZOI = 17.0±1.0 mm and AI = 0.939) by methanol extract followed by aqueous extract (ZOI = 14.5±0.5 and AI = 0.801) of *Phyllanthus emblica* fruits (Dharajiya *et. al.*, 2015).

The highest antibacterial activity was determined as 20 mm inhibition zone from methanol extracts against *Staphylococcus* sp. MBKK3, *S. aureus* MBKK4 and MBKK5,

Staphylococcus epidermidis MBKK6, MBKK7 and MBKK8. The highest DPPH (2, 2-diphenyl-1-picrylhydrazyl hydrate) scavenging activity was found to be 82% from aqueous extract. *S. aromaticum* extracts have antibacterial, antioxidant and antimutagenic potential (Okmen *et. al,*2018).

Leaf, stem and root of *Chromolaena odorata* were extracted by the maceration extraction method using water, ethanol, methanol and hexane as solvents. Leaf extracts with ethanol, methanol and hexane solvents gave the best inhibitory activity against six gram-positive bacterial strains (*Bacillus cereus* TISTR 687, *Enterococcus faecalis* TISTR 379, *Staphyloc*occus epidermidis TISTR 518, Staphylococcus aureus TISTR 1466, Streptococcus pyogenes ATCC 19615 and *Propionibacterium acnes* DMST 14916) and one gram-negative bacterial strain (*Proteus vulgaris* ATCC 13315). The hexane stem extract showed greater inhibitory activity against *Pseudomonas aeruginosa* ATCC 27853(15.3±0.5 mm), *B. cereus* TISTR 687(14.6±0.8 mm) and *Klebsiella pneumoniae* TISTR 1843 (14.0±1.0 mm), while hexane root extract showed high inhibitory activity against *Enterococcus faecalis* TISTR 379 (14.5±0.9 mm) and *Kleb. pneumoniae* TISTR 1843 (14.7±0.6 mm). The lowest minimum inhibitory concentration (MIC) of the ethanolic leaf extract was 0.81 mg/mL against *Staph. aureus* TISTR 1466, followed by methanolic and hexane leaf extracts with equal MIC of 1.62 mg/mL against both *Staph. aureus* TISTR 1466 and *Strep. pyogenes* ATCC 19615 (Hanphakphoom *et. al.,* 2016).

In this study, the paper disc diffusion technique is simple, reliable and easy to screen the antibacterial properties of plants. Clear zones or inhibitory zones were easily seen if there was an activity with regard to their various extraction methods. The zone size diameters of less than 14 mm were recorded as the intermediate activity and the zone size diameters of more than 14 mm as the sensitive activity. In the present study, the antimicrobial activities of the different plant parts were tested by using 18 strains of pathogenic bacteria. Recently, a number of antibiotics have been discovered by many workers. Moreover, some pathogenic microorganisms have been resistant to antibiotics. Sometimes, the side effects may occur. It is considered that the plant parts can be used in traditional medicine or as extracts for pharmaceuticals.

It was observed that the watery extract and 50% ethanol extract of the bark from *Phyllanthus emblica* Gaertn. showed the best antibacterial activity (22 mm and 19 mm of inhibitory zones) on *Staphylococcus aureus.* Moreover, 50% ethanol extract of the leaves from *Phyllanthus emblica* Gaertn. and ethanol extract of the flowers from *Syzygium aromaticum* (L.) Merr. had good antibacterial activity (18 mm of inhibitory zones) on *Staphylococcus aureus.*

There was no antibacterial activity of all leaf extracts from *Phyllanthus emblica* Gaertn. on *Escherichia coli* ATCC, *Klebsiella aeruginosa* NCTC, *Shigella flexnerii* and 2 strains of *Vibrio cholerae*; all fruit extracts from *Phyllanthus emblica* Gaertn. on *Proteus morganii* and *Vibrio cholerae* Ogawa; all bark extracts from *Phyllanthus emblica* Gaertn. on 2 strains of *Escherichia coli, Klebsiella aeruginosa* NCTC, *Pseudomonas pyocyanea,* 2 strains of *Shigella* and 3 strains of *Vibrio cholerae*; all leaf extracts from *Chromolaena odorata* L. on all 16 pathogenic bacteria except *Staphylococcus aureus* and *Plesiomonas shigelloides* and all flower extracts from *Syzygium aromaticum* (L.) Merr. on only 2 strains of *Escherichia coli.*

From these data, it was clearly seen that *Phyllanthus emblica* Gaertn. and *Syzygium aromaticum* (L.) Merr. possessed more antibacterial activity than *Chromolaena odorata* L. on a variation of the different bacterial pathogens. It was concluded for future work that the plants which are known to have been used in various remedies in traditional medicine need to be exloited scientifically. Different parts of plants used in

Myanmar's traditional medicine should be tested for their antimicrobial activity. The activity of plants from different regions and sesonal variation needs to be experimented with and to be a comparative study from different localities.

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