

**Antimicrobial Activity of Leaves Extracts of *Tamarindus indica* L. (Magyee)**

*Khin Yadanar Kyaw*<sup>1\*</sup>, *Cho Yi Myint*<sup>2</sup> & *Khine Khine Lwin*<sup>3</sup>

<sup>1</sup>Department of Pharmacology, University of Pharmacy, Yangon

<sup>2</sup>Department of Pharmaceutics, University of Pharmacy, Mandalay

<sup>3</sup>Pharmacology Research Division, Department of Medical Research, (Yangon)

This study aimed to determine antimicrobial activity of extracts of *Tamarindus indica* L. leaves (Magyee). It was the laboratory-based quasi experimental study design. Study sites were the University of Pharmacy (Mandalay and Yangon) and the Department of Medical Research (Yangon). The leaves of *Tamarindus indica* L. were extracted with petroleum ether, ethanol and distilled water by successive Soxhlet extraction method. Physicochemical and phytochemical studies were conducted according to reference analytical procedures. *In vitro* antimicrobial activity of three extracts of *Tamarindus indica* L. leaves were studied on control strains of bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae*) by agar disc diffusion method. Phytochemical analysis of *Tamarindus indica* L. leaves showed the presence of alkaloids, amino acids, carbohydrates, flavonoids, glycosides, polyphenol, starches, steroids/terpenoids, tannins, reducing sugars and anthroquinone. Ethanolic and aqueous extracts exhibited significant antibacterial activities against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Both aqueous and ethanolic extracts possessed promising antibacterial activity on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This was an important finding upon pathogenic organisms causing food-borne diseases, skin and soft tissue infection. The petroleum ether extract did not show antibacterial activity against all tested organisms. With ethanolic extracts, the minimum inhibitory concentrations (MIC) results were 6.25 mg/ml for *P. aeruginosa*, *S. aureus* and 25 mg/ml for *E. coli*. For aqueous extracts, the MIC of *P. aeruginosa* and *S. aureus* were 12.5 mg/ml and *K. pneumoniae* was 25 mg/ml, respectively. Oral acute administration of 5000 mg/kg mice body weight of ethanolic and aqueous extracts of *Tamarindus indica* L. leaves resulted in no mortality. Therefore, the crude extracts from *Tamarindus indica* L. leaves had antimicrobial activity against *K. pneumoniae*, *P. aeruginosa*, *S. aureus* and *E. coli*.

**Keywords:** *Tamarindus indica* L., Petroleum ether, Ethanol, Soxhlet apparatus, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*

## INTRODUCTION

Herbal medicine is an important part of both traditional and modern medicine. In Myanmar, the plant *Tamarindus indica* L. (Tamarind) is widely distributed throughout the whole country. Each and every part of the Tamarind has been used as traditional foodstuff and medicine since many years ago. In this study, antimicrobial activity of *Tamarindus indica* leaves was performed against the standard strains such as *E. coli*, *P. aeruginosa*, *S. aureus* and *K. pneumoniae* which are involved in enteric disturbance, food borne diseases, skin and soft

tissue infections, the diseases of concern in the tropics. *Tamarindus indica* L. has broad spectrum antibacterial activity and a potential source of new classes of antibiotics that could be useful for infectious disease chemotherapy and control.<sup>1</sup>

The general antimicrobial activity of leaves extract of *Tamarindus indica* L. was compared with ciprofloxacin which was used as a positive control for bacterial strains.<sup>2</sup>

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\*To whom correspondence should be addressed.

Tel: +95-9974465135

E-mail: yadanark11@gmail.com

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## MATERIALS AND METHODS

Study period was from February 2016 to December 2016. It was a laboratory-based quasi experimental study design.

### *Sample collection*

The fresh leaves of *Tamarindus indica* L. were collected from Amarapura Township, Mandalay Division, Myanmar, in February. The leaves were washed with water and then shade-dried at room temperature at the University of Pharmacy, Mandalay. These dried leaves were crushed to smaller granules and stored in air-tight container.

### *Phytochemical analysis*

In order to establish the various chemical constituents of the leaves of *Tamarindus indica*, the preliminary phytochemical tests were performed according to the Harborne J.B. phytochemical methods for plant analysis.<sup>3</sup>

### *Determination of physicochemical characterization*

The physicochemical properties of leaves of *Tamarindus indica* L. were determined according to quality control methods for medicinal plant materials, WHO.<sup>4</sup>

### *Soxhlet successive extraction*

The leaves of *Tamarindus indica* L. were washed thoroughly three times with running water. The leaves were air-dried in shadow at room temperature for 14 days. The leaves were crushed into coarse powder using an electric grinder. The leaves samples were successively extracted by using soxhlet apparatus. The filtrate was evaporated by rotary evaporator. Three portions were carried out to obtain successive petroleum ether (40-60°C), ethanol and aqueous fractions.

### Fraction 1 (petroleum ether)

Two-hundred grams of *Tamarindus indica* L. leaves powder sample were extracted with petroleum ether (40-60°C) by Soxhlet apparatus for 3 hours and the filtrate was evaporated by rotary evaporator at 60°C.

### Fraction 2 (ethanol)

The residue of defatted leaves powder was extracted with ethanol by Soxhlet apparatus for

3 hours. The filtrate was evaporated by rotary evaporator at 80°C.

### Fraction 3 (distilled water)

The residue of fraction 2 was extracted with distilled water at 80°C for 2 hours. The filtrate was evaporated in water bath at 100°C.<sup>3</sup>

### *Determination of antimicrobial activity of different extracts from the leaves of Tamarindus indica L.*

The antimicrobial activity of different extracts of the leaves of *Tamarindus indica* L. was determined by standard disc diffusion technique according to modified Kirby-Bauer method<sup>5</sup> using Mueller-Hinton agar recommended by Clinical Laboratory Standards Institute.<sup>6</sup>

Agar plates were inoculated with *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, respectively. The dried discs impregnated with different crude extracts were placed on the agar. A disc impregnated with solvent only was also used as negative control. Commercial drug ciprofloxacin discs were also used as positive controls. The plates were incubated at 35°C for 30 minutes.

After overnight incubation, diameters of zone of inhibition (including 8 mm discs) were measured with the thin plastic transparent ruler. For antibiotic discs, the results were interpreted according to zone-size interpretative table of Clinical and Laboratory Standards Institute.<sup>6</sup>

The complete inhibition of growth as determined by the naked eyes was recorded as "sensitive." The crude extracts of the plant showing antimicrobial activity by agar disc diffusion technique were further tested to find out minimum inhibitory concentration and minimum bactericidal concentration. The experiment was repeated three times.

### *Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanolic and aqueous extracts of the leaves of Tamarindus indica L.*

In this study, MIC and MBC of ethanolic and aqueous extracts were determined by Broth Dilution Method.<sup>7</sup> One milliliter of extract dilution and one milliliter of nutrient broth containing bacteria were transferred to the six sterile tubes. Tubes containing interval dilutions of extracts were inoculated with broth culture

containing test organisms and incubated at 35°C for 18-24 hours. A positive control (culture of test organism without drugs) and negative control (nutrient broth only) were also included in this experiment. After incubation, the tube with the lowest concentration showing no visible turbidity was taken as MIC.

From above tubes dilutions, one loopful from each tube was inoculated on the Mueller-Hinton agar plate and incubated at 35°C for 18-24 hours. Minimum bactericidal concentration end point was determined as the lowest concentration of test antimicrobial agent which killed at least 99.9% of original inoculums (i.e., not more than 1 or 2 colonies in a sub-culture agar plate).<sup>7</sup> The experiments were repeated three times. The minimum bactericidal concentration of extracts of leaves was noted in terms of mg/ml.

#### Determination of acute toxicity of ethanolic and aqueous extracts of leaves of *Tamarindus indica* on albino mice

Acute toxicity of doses of ethanolic and aqueous extracts of *Tamarindus indica* L. leaves were evaluated by the methods of OECD Guidelines for the Testing of Chemicals 425.<sup>8</sup> Total number of 9 adult female albino mice weighing 25-30 g were selected and divided into three groups. Each group contained three animals. They were fasted for 18 hours before giving the extracts. Group (1) mice were orally administrated with 5000 mg/kg of ethanolic extract. Group (2) mice were given orally with 5000 mg/kg of aqueous extract. As a control group Group (3) mice were administered with distilled water and normal animal food. All groups of mice were kept in the three mouse cages in the separated room at the room temperature of 26±1°C. After administration of extracts, each group of animals were observed first 4 hours continuously for mortality and behavior changes. Then, the animals were checked 24 hours for fourteen days. The mortality during this period was noted (nil or present death). The LD<sub>50</sub> data were assessed by the AOT 425 statistical program (Version: 1.0).

## RESULTS

### Phytochemical analysis

The phytochemical investigation of *Tamarindus indica* L. leaves indicated the presence of alkaloids, amino acids, carbohydrate, flavonoids,

glycosides, polyphenol, starches, steroids/terpenes, tannins, reducing sugars and anthroquinone. There were no cyanogenic glycosides and saponins.

### Determination of physicochemical characterization

Physicochemical characterization of *Tamarindus indica* L. leaves showed moisture content (7.90%), total ash values (3.54%), acid-insoluble ash (0.93%), water-soluble ash (0.9285%), foaming index (<1 cm), extract values (petroleum ether extract 7.22%, ethanolic extract 18.29%, aqueous extract 30.07%) and swelling index (7 ml).

### Yield of extracts

After defatting with petroleum ether, the ethanolic extraction yielded 17% and the aqueous extraction yielded 13%.

Table 1. Zone of inhibition of leaves extracts of *Tamarindus indica* L. by agar disc diffusion method (disc diameter is 6 mm)

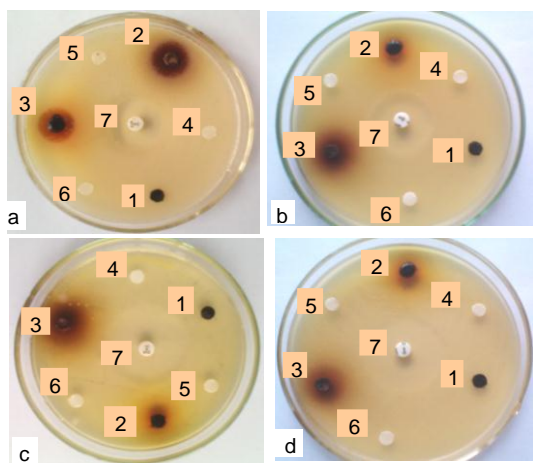
Tested organisms	Mean diameter of zone of inhibition (mm)						
	A	B	C	D	E	F	G
<i>Escherichia coli</i>	0	12	9	0	0	0	30
<i>Klebsiella pneumoniae</i>	0	0	12	0	0	0	21
<i>Pseudomonas aeruginosa</i>	0	15	18	0	0	0	32
<i>Staphylococcus aureus</i>	0	12	15	0	0	0	30

0=no zone of inhibition

A=Petroleum ether extract (30mg)      E=Control (ethanol solvent)  
 B=EtOH extract (30mg)      F=Control (aqua solvent)  
 C=Aqua extract (30mg)      G=Control (ciprofloxacin, 5 µg)  
 D=Control (petroleum ether solvent)

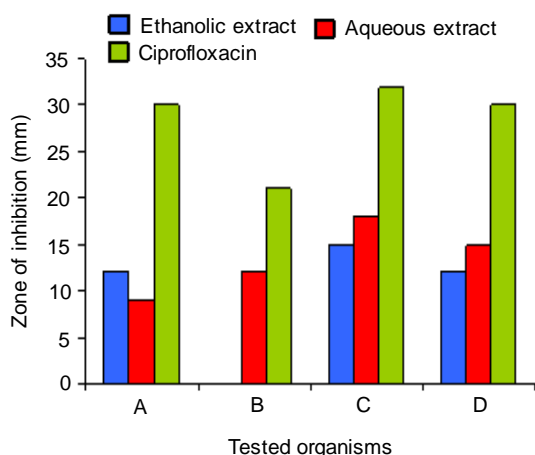
### Determination of zone of inhibition for antibacterial activity

Zones of inhibition with aqueous extracts against each bacterial species were 12 mm for *K. pneumoniae*, 18 mm for *P. aeruginosa*, 15 mm for *S. aureus* and 9 mm for *E. coli*, respectively. With ethanolic extract, the zones of inhibition were 12 mm for *E. coli*, 15 mm for *P. aeruginosa*, 12 mm for *S. aureus* and no action against *K. pneumoniae*. Inhibition zones of control drugs were larger than those of aqueous and ethanolic extracts. The zones of inhibition against each species tested with petroleum ether extract and all control solvents were not detected (Table 1, Fig. 1 & 2).



- 1=Petroleum ether extract 30 µg/disc
- 2=Ethanol extract 30 µg/disc
- 3=Aqueous extract 30 µg/disc
- 4=Petroleum ether solvent (control)
- 5=Ethanol solvent (control)
- 6=Aqueous solvent (control)
- 7=Ciprofloxacin 5 µg/disc (control)

Fig. 1. Zone of inhibition of extracts on (a) *Escherichia coli*, (b) *Klebsiella pneumoniae*, (c) *Pseudomonas aeruginosa*, (d) *Staphylococcus aureus*



- A= *Escherichia coli*      C=*Pseudomonas aeruginosa*
- B= *Klebsiella pneumoniae*      D=*Staphylococcus aureus*

Fig. 2. Comparison of zone of inhibition against four bacteria species by ethanol and aqueous leaves extracts of *Tamarindus indica* with that of control drugs ciprofloxacin

#### Determination of minimum inhibitory concentration

In this study, different concentrations (1.56, 3.125, 6.25, 12.5, 25 mg/ml) of ethanol and aqueous extracts were tested against *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus* by tube dilution method.

Table 2. Minimum inhibitory concentration (MIC) of ethanol and aqueous leaves extracts of *Tamarindus indica* L.

Extracts	Concentration (mg/ml)	Bacteria growth			
		<i>Pseudo- monas aeruginosa</i>	<i>Staphy- lococcus aureus</i>	<i>Esche- richia coli</i>	<i>Kleb- siella pneumoniae</i>
Ethanol extract	25.0	NBG	NBG	NBG	BG
	12.5	NBG	NBG	BG	BG
	6.25	NBG	NBG	BG	BG
	3.13	BG	BG	BG	BG
	1.56	BG	BG	BG	BG
Aqueous extract	25.0	NBG	NBG	BG	NBG
	12.5	NBG	NBG	BG	BG
	6.25	BG	BG	BG	BG
	3.13	BG	BG	BG	BG
	1.56	BG	BG	BG	BG

NBG=No bacteria growth

BG=Bacteria growth

#### MIC for ethanol extract

There was no bacteria growth of *P. aeruginosa* and *S. aureus* with concentrations of 6.25, 12.5, 25 mg/ml and *E. coli* with 25 mg/ml. Bacteria growth of *P. aeruginosa* and *S. aureus* was seen with concentrations of 1.56, 3.125 mg/ml and of *E. coli* with 1.56, 3.125, 6.25, 12.5 mg/ml. The results are shown in Table 2. Therefore, minimum inhibitory concentrations of ethanol leaves extract of *Tamarindus indica* L. were 6.25, 12.5, 25 mg/ml for *P. aeruginosa* and *S. aureus*, and 25 mg/ml for *E. coli*.

#### MIC for aqueous extract

There was no bacteria growth of *P. aeruginosa* and *S. aureus* with concentrations of 12.5, 25 mg/ml and of *K. pneumoniae* with 25 mg/ml concentration. Bacteria growth of *P. aeruginosa* and *S. aureus* was seen with concentrations of 1.56, 3.125, 6.25 mg/ml and of *K. pneumoniae* with concentrations of 1.56, 3.125, 6.25, 12.5 mg/ml. The results are shown in Table 2.

Therefore, minimum inhibitory concentrations of aqueous leaves extract of *Tamarindus indica* were 12.5, 25 mg/ml for *P. aeruginosa* and *S. aureus*, and 25 mg/ml for *K. pneumoniae*.

#### Acute toxicity study of ethanol and aqueous leaves extracts of *Tamarindus indica* L.

Acute toxicity study of ethanol and aqueous leaves extracts of *Tamarindus indica* L. was done with the dosage of 5000 mg/kg body weight in albino mice. The conditions of mice were recorded after being administered for 14 days. No mortality of the mice was observed up to fourteen days after administration. Each group of animals was also observed while alive

and did not show any visible symptoms of toxicity like restlessness, respiratory disorders, convulsion, aggressive activities, coma and death.

## DISCUSSION

It was revealed from previous studies that *Tamarindus indica* L. is a potential antimicrobial agent.<sup>2, 9, 10</sup> Ethanolic extract of leaf and stem of *Tamarindus indica* L. shows antibacterial activity against some gram-negative bacteria.<sup>11</sup>

*Tamarindus indica* L. extract has antibacterial properties against *Burkholderia pseudomallei*, *K. pneumoniae*, *S. paratyphi*, *Bacillus subtilis*, *Salmonella typhi*, *E. coli* and *S. aureus*. Antibacterial effect of this plant is linked with its lupeol content.<sup>12</sup>

In this study, *Tamarindus indica* L. leaves are rich in secondary metabolites such as alkaloids, polyphenols, tannins, flavonoids and terpenes which have antibacterial action.

The extraction of *Tamarindus indica* L. leaves was made by leaves tissue homogenization with pet ether, water and ethanol as solvents. Water soluble polar compounds were obtained in aqueous extract. Less polarity compounds were contained in petroleum ether defatting process.

More polar compounds were soluble in ethanolic extract. Therefore, defatting with petroleum ether was done before going for ethanolic extraction. Selection of appropriate solvent is important for obtaining best yield. The extraction yield depends on solvent, time and temperature of extraction as well as the chemical nature of sample. In this study, yield percent of ethanolic extract of *Tamarindus indica* L. leaves was better than aqueous and petroleum ether extracts. In this study, the agar disc diffusion technique was used because this method is reliable to search for antibacterial activity.

*In vitro* antibacterial activities of three extracts of *Tamarindus indica* L. leaves on pathogenic microorganisms such as *E. coli*, *P. aeruginosa*, *S. aureus* and *K. pneumoniae* were investigated. The zones of inhibition of extracts were compared with zone of inhibition formed by the antibiotic discs ciprofloxacin used as positive control.

The diameters of zones of inhibition with aqueous leaves extracts against each species ranged from 21 to 30 mm for *E. coli*, *P. aeruginosa*, *S. aureus* and *K. pneumoniae*.<sup>2</sup> In the present study, aqueous extracts showed large zones size against *S. aureus*. Ethanolic and aqueous extracts exhibited significantly large zones against *P. aeruginosa* and *S. aureus*. Gumgumjee, *et al.* reported that with ethanolic leaves extracts, the zones of inhibition ranged from 35 to 41 mm for *E. coli*, *P. aeruginosa*, *S. aureus* and *K. pneumoniae*.<sup>2</sup> These findings were comparable with the present study, although different plants, extraction methods, isolation of organism and concentrations were used.

Zones of inhibition with aqueous extracts against *K. pneumoniae* and with ethanolic extract against *E. coli* were 12 mm, respectively. Petroleum ether extracts of leaves did not show activity against all tested bacteria and there was no action with ethanolic extract against *K. pneumoniae*. Ciprofloxacin is a pure drug and more active than the crude extracts of *Tamarindus indica* L. leaves containing several components in which some pigments and phenolics have been reported to interfere with the antibacterial activity of the extracts.<sup>13</sup>

In the study of Gumgumjee, *et al.* the MIC of ethanolic extracts were recorded as 15 mg/ml for *S. aureus* and *E. coli*, 10 mg/ml for *pneumoniae* and 25 mg/ml for *P. aeruginosa*.<sup>2</sup> Ugoh, *et al.* reported the results of MIC of aqueous extracts showed ranging from 50 to 500 mg/ml for *S. aureus*, *E. coli* and *P. aeruginosa*.<sup>13</sup> These findings differed from those of the present study against *S. aureus*, *E. coli* and *P. aeruginosa*.

In the present study, LD<sub>50</sub> value was greater than 5000 mg/kg body weight that was considered safe according to the recommendation of Organization for Economic and Cultural Development (OECD) guideline.<sup>8</sup>

## Conclusion

According to the results obtained from this study, the aqueous extract of leaves of *Tamarindus indica* L. had antibacterial activity against *P. aeruginosa*, *S. aureus* and *K. pneumoniae*, whilst the ethanolic extract of leaves of *Tamarindus indica* L. had antibacterial activity against *P. aeruginosa*, *S. aureus* and *E. coli*.

Before clinical trial in human is performed, subacute and chronic toxicity in animal model should be studied. *In vivo* antibacterial activity of extracts of *Tamarindus indica* L. leaves against other potential pathogens should be studied. Formulation of bioactive compound of *Tamarindus indica* L. leaves as antibacterial agent in suitable dosage form should be studied.

#### Competing interests

The authors declare that there is no competing interest.

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