Investigation on the Phytochemical Constituent and Antimicrobial Activity of Ethylacetate Extract of Leaves of *Morinda Angustifolia* Roxb.

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Abstract

The phytochemical screening and antibacterial activity test were done on leaves of *Morinda angustifolia* Roxb., Myanmar name Ye′yo which is one of the well-known medicinal plants. It was collected from Mahaaungmyay Township, Mandalay, Myanmar. Firstly the bacterial used were *Bacillussubtilis*, *Staphylococcus aureus* and *Pseudomonous aeruginos*a in four solvents such as ethylaceate, ethanol, acetone and water respectively. Among then the highest activities of leaves of EtOAC extract was examined on six strains of bacterial which are *Bacillus subtilis, Staphylococcus aureus, Pseudomonus aeruginosa, Bacillus pumilus, Candida albicans,* and *Mycobacterium* species by employing agar well diffusion method, utilizing antibiotic tetracycline as a standard reference compound. It was found that this extract showed more remarkable zone of inhibition on *P. aeruginosa, B. pumalis, S. aureus,* and *C. albicans*than on the remaining bacterial species. As the resultant calibration curves are approximately straight lines, leave extract is considered to have many bioactive properties.

***Keywords*:** Phytochemical, antibacterial activity,quantitative,antibiotic tetracycline

Introduction

In most of the countries of the world, many plants, largely based on the use of roots, leaves, barks, seeds, fruits and flowers, are used in traditional medicine. These plants may save many lives if they are used correctly.Also in Myanmar, the study of traditional medicinal plants and this usage in therapy play a very important role. These plants may have biologically active principles. To fulfill the primary health needs of all the world's inhabitants, it will be necessary to utilize both the western and the traditional medical system. Thus,the present scientific investigation will assist the potent formulation of traditional medicines to compete with that of the modern western medicines in the fight against dreadful diseases such as AIDS, heart disease, typhoid, tuberculosis, cancer and tumor. *Morinda angustifolia* Roxb, belongs to the family Rubiaceae, locally known as Ye′yo, which is one of the well-known medicinal plants. Its native range extends across southeast Asia, southwest China, northeast India, Nepal, Bangladesh, Myanmar, Thailand and Laos.The species is now cultivated throughout the tropics and widely naturalized. (Ozorio, 1979; Squarrosa, 1984) (Web:wiki/Morinda)

In this research work, leaves of Ye`yo were selected and its antimicrobial activities were studied quantitatively.

Botanical description

Morinda angustifolia Roxb is a flowering and fruiting tree in coffee family, Rubiaceae. It is an evergreen shrub or a tree with few, erect branches; it can grow up to 6 metres tall.(Web:wiki/Morinda)

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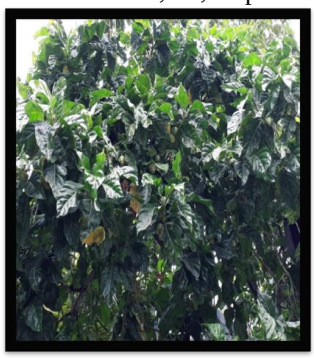

Figure 1. Tree of *Morinda angustifolia* Roxb.

Figure 2. Leaves of *Morinda angustifolia* Roxb.

Kingdom	Plantae
Family	Rubiaceae
Genus	Morinda
Botanical name	*Morinda angustifolia* Roxb.
Habit	Evergreen Tree /Small tree /Tropical plant
English name	Noni
Myanmar name	Ye`yo ; Ni-pah-saye
Part of used	Leaves

Therapeutic properties and uses

The plant has several medicinal uses. These are likely to include as ananemia, typhoid, febrifuge, heart-disease, tuberculosis, agalaxy, asthma, abdominal disease, hemorrhage, and cancer. In Myanmar, the leaves of *Morinda angustifolia* Roxb can be used not only for traditional diet but also for indigenous medicine. (Nagathein, 1976) (Web.wiki/Morinda)

Experiment

Material and Methods

Collection and preparation of sample

The leaves of *M.angustifolia* Roxb (Ye′yo) were collected from Mahaaungmyay Township, Mandalay. Firstly, it was cut into small pieces and dried in the shade for about two weeks. Then, air dried sample was ground into powder. The raw material was kept in the glass bottle with stopper and used throughout the experiment.

Phytochemical method

The phytochemical constituents of leaves of *M.angustifolia* Roxb were tested by test tube method. The results are shown in Figure 6.

Agar well diffusion method

Antimicrobial activities of the leaves of ethylacetate, ethanol, acetone and water extracts were performed by agar well diffusion method qualitatively.

Quantitative determination of antimicrobial activity

The Apparatus Included

The apparatus employed in this experiment were Automatic High Speed Autoclave (Tomy Seiko Co.ltd. Tokyo, Japan.), Hot Air sterilizer (Hirasawa Works ltd. Japan.), Yamato

Sater bath(Japan), Clean Bench(Hitachi ltd. Japan.), Steam drying oven(Hitachi ltd. Japan.), Yamato Agar well cutter(Japan), Direct reading Balance L-200 SM(Shimadzu, Japan).

Preparation for antimicrobial activity test

The study of antimicrobial activity was performed by agar-well diffusion method. Nutrient agar was prepared according to the method described by Cruick Shank. Nutrient agar was boiled and 20-25ml of the medium was poured into the test tube and plugged with cotton wool and sterilized at 121°C for 15minutes in autoclave. After autoclaving, the tubes were cooled down to 30-35°C and poured into sterilized petri dishes and 0.2ml of test organism was also added into dishes. They were allowed to set the agar for 2-3hours. After the agar was set, 10mm agar well was made by the help of sterilized agar well cutter. Then about 0.2ml of sample was introduced into the agar well and incubated at 37°C for (24-48) hours. Candida organism was set at 30°C for 24 hour. (Cruick, 1970)

Study onantimicrobial activity tests for ethylacetate extract of leaves of *Morinda angustifolia* Roxb by agar well diffusion technique

In this study, ethylacetate extract of leaves was tested on six strains of microbial which were *Bacillus subtilis, Staphylococcus aureus, Pseudomonus aeruginosa, Bacillus pumilus, Candidaalbicans,* and *Mycobacterium* species using antibiotic tetracycline as standard.

Results and Discussion

The results of leave extracts in four solvents and their antimicrobial activity tests are shown in Table 1.

Table 1. Antimicrobial activity tests for leave extracts of *Morinda angustifolia* Roxb.

No	Sample	solvent	Type of microbial		
			B. subtilis	*S. aureus*	*P.aeruginosa*
1	Ye-yo leave	Ethyl acetate(EtOAc)	+++	+++	+++
		Ethanol	+	++	++
		Acetone	+	+	++
		Water	–	–	–

Low activity = (+) Medium activity = (++) High activity = (+++)

It was observed that the leaves of ethylacetate extract gave rise to be higher activity that compared to other solvent extracts. The high activities of ethylacetate extract on three strains of microbial which are *B.subtilis, S.aureus,* and *P.aeruginosa* were shown in figure 3,4 and 5.

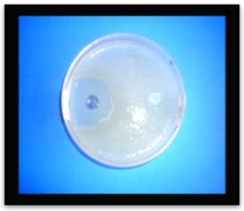

Figure 3.
Solvent = Ethylacetate
Organism = *B.sublilis*
Agar well = 35mm(+++)

Figure 4.
Solvent = Ethylacetate
Organism = *S. aureus*
Agar well = 34mm(+++)

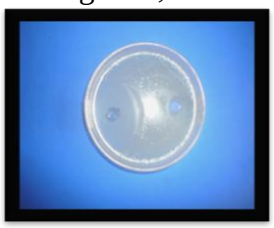

Figure 5.
Solvent = Ethylacetate
Organism = *P.aeruginosa*
Agar well = 40mm (+++)

Photochemical tests for the leaves of *Morinda angustifolia* Roxb

The leave of *Morinda angustifolia* Roxb (Ye`yo) was tested by phytochemical method. The constituents of Ye`yo, showed the positive response toward flavanoids, terpenoids,

carbohydrates, alkaloids, steroids, polyphenols, tannins, saponins, glycosides and resin, the negative response to amino acids,were shown in Figure 6.

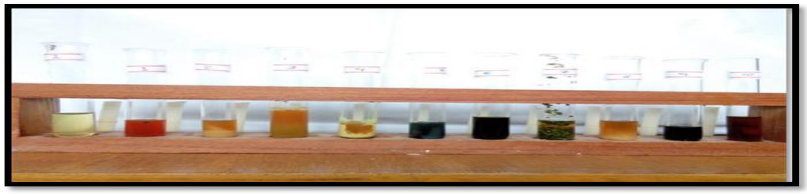

Figure.6 Photochemical tests for the leaves of *Morinda angustifolia* Roxb.

Determination of diameters of zones of inhibition for ethylacetate extract of leaves of *Morinda angustifolia* Roxb with standard tetracycline

The antimicrobial activities of tetracycline and ethylacetate extract of leaves were also determined by agar well diffusion method. The diameters of zones of inhibition were measured for various concentrations of the standard tetracycline and ethylacetate extract. The diameters and the relevant concentrations are described in Table 2.

Quantitative analysis showed that ethylacetate extract of diameters of zones inhibition and corresponding tetracycline concentrations which give the similar inhibition on six antibacterial organisms. The required serial concentration of tetracycline solutions are prepared quantitatively in sterile distilled water and EtOAC extract were made with dilution in EtOAC. Standard calibration curveswere obtained by plotting the various concentrations (0.4, 0.8, 1.6, 2.4, 3.2 and 4 mg/ml) of tetracycline and EtOAC extract against the diameters of the circular areas of inhibition of each microorganism. The resultant curves were approximately straight lines. They are shown in Figure (7),(8), (9),(10), (11), (12), (13), (14), (15), (16), (17), (18). The measurable zone diameter showed the degree of antimicrobial activities. It was observed that this ethylacetate extract showed more remarkable zone of inhibition on *P.aeruginosa*, *B.pumalis*, *S.aureus*, *C. albicans* than on the remaining bacterial species.

Table 2. Determination of diameters of zones of inhibition and concentrations of tetracycline and ethylacetate extract of leaves of *Morinda angustifolia* Roxb.

	ORGANISMS						
	Sample Dilution	*B.subtilis*	*S.aureus*	*P.aeruginosa*	*B.pumalis*	*C.albican*	*Myco. Species*
Tetracycline (standard)	0.4 mg/ml	30mm +++	26mm +++	33mm +++	32mm +++	32mm +++	33mm +++
	0.8 mg/ml	34mm +++	30mm +++	34mm +++	35mm +++	35mm +++	34mm +++
	1.6 mg/ml	36mm +++	32mm +++	35mm +++	37mm +++	40mm +++	35mm +++
	2.4 mg/ml	39mm +++	35mm +++	36mm +++	40mm +++	42mm +++	36mm +++
	3.2 mg/ml	42mm +++	36mm +++	40mm +++	42mm +++	44mm +++	40mm +++
	4.0 mg/ml	44mm +++	37mm +++	42mm +++	44mm +++	45mm +++	42mm +++
	0.4 mg/ml	20mm ++	16mm ++	20mm ++	20mm ++	14mm +	19mm ++
	0.8	22mm	18mm	24mm	25mm	15mm	20mm

	mg/ml	+++	++	+++	+++	+	++
EtOAC extract ofleaves	1.6 mg/ml	24 mm +++	19mm +++	24mm +++	26mm +++	16mm ++	22mm +++
	2.4 mg/ml	25mm +++	20mm ++	25mm +++	27mm +++	18mm ++	24mm +++
	3.2 mg/ml	26mm +++	22mm ++	25mm +++	28mm +++	20mm ++	26mm +++
	4.0 mg/ml	26mm +++	24mm +++	25mm +++	29mm +++	22mm +++	30mm +++

Organisms

(1)*B.sub* = *Bacillus subtilis*
(2)*S.aureus* = *Staphylococcus aureus*
(3)*P.aeruginosa* = *Pseudomonas aeruginosa*
(4)*B.pumilus* = *Bacillus pumilus*
(5)*C.albicans* = *Candida albicans*
(6)*Myco.*Species =*Mycobacterium* species

Agar well ~ 10mm
10mm ~ 14mm (+)
15mm ~ 19mm (++)
20mm ~ above(+++)

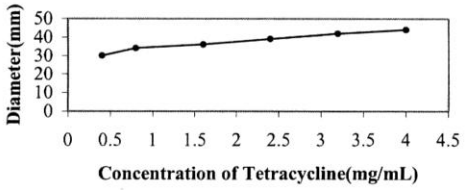

Figure (7) Calibration curve for ***Bacillus subtilis***

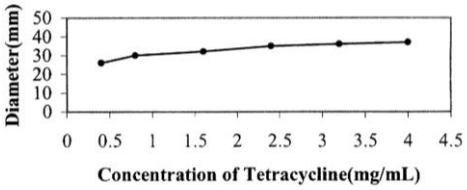

Figure (8) Calibration curve for ***Staphylococcus aureus***

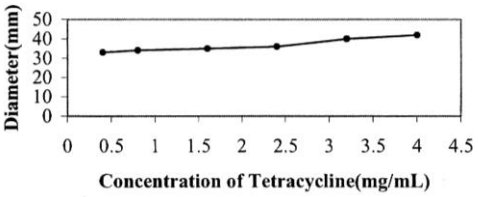

Figure (9) Calibration curve for ***Pseudomonous aeruginosa***

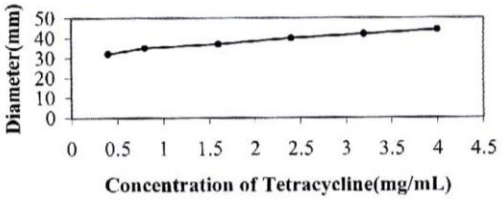

Figure (10) Calibration curve for ***Bacillus pumalis***

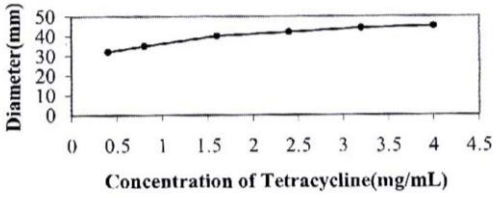

Figure (11) Calibration curve for ***Candida albicans***

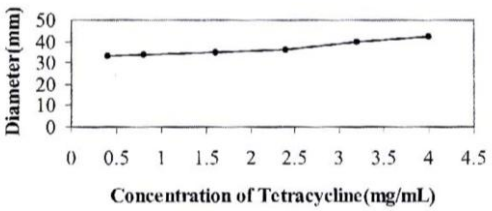

Figure (12) Calibration curve for ***Mycobacterium species***

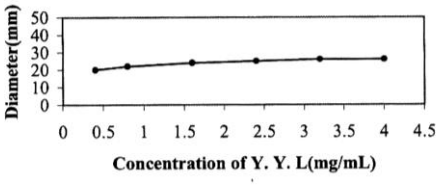

Figure (13) Calibration curve for ***Bacillus subtilis***

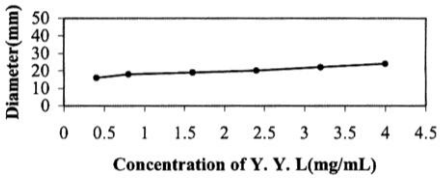

Figure (14) Calibration curve for ***Staphylococcus aureus***

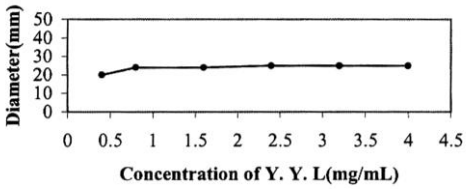

Figure (15) Calibration curve for ***Pseudomonous aeruginosa***

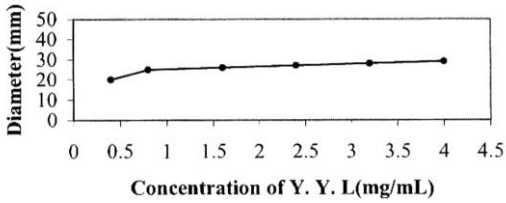

Figure (16) Calibration curve for ***Bacillus pumalis***

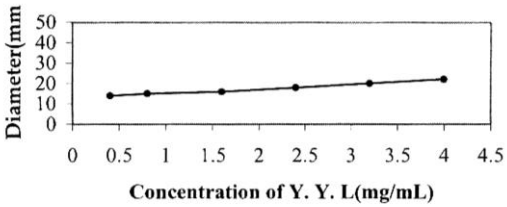

Figure (17) Calibration curve for ***Candida albicans***

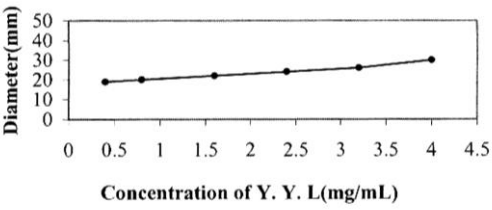

Figure (18) Calibration curve for ***Mycobacterium species***

Conclusion

In this study, one of Myanmar indigenous medicinal plants, *M.angustifolia* Roxb (Ye′yo**),** was selected for the investigation of phytochemical constituent and antimicrobial activity. The highest activities of leaves of EtOAC extract were examined on six strains of bacterial which are *Bacillus subtilis, Staphylococcus aureus, Pseudomonus aeruginosa, Bacillus pumilus, Candida albicans,* and *Mycobacterium* species by employing agar well diffusion method. Quantitative determinations of diameters of zones of inhibition for ethylacetate extract of leaves of *M.angustifolia* Roxb were performed by using antibiotic tetra cycline as a standard reference compound. Standard calibration curves were obtained by plotting the various concentrations (0.4, 0.8, 1.6, 2.4, 3.2 and 4 mg/ml) of tetracycline and EtOAC extract against the diameters of the circular areas of inhibition of each microorganism. The resultant curves were approximately straight lines. Thus, the leave of *M.angustifolia* Roxb is a considerable to have more bioactive compounds that could be continued to study as a future work.

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