Antimicrobial, Anti-diabetic and Antioxidant Activities of the Leaf of *Morinda citrifolia* Linn

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Abstract

In this study, one of the Myanmar indigenous medicinal plants, Morinda citrifolia Linn., Myanmar named Ye-yo was chosen for investigation of antimicrobial, anti-diabetic and antioxidant activities. The leaf of Morinda citrifolia Linn. was collected from Magway Township, Magway Region in May, 2019. Antimicrobial activity was determined by agar well diffusion method and tested on six microorganisms such as Bacillus subtilis, Staphylococcus aureus, Bacillus pumilus, Pseudomonas aeruginosa, Candida albicans and Escherichia coli. All extracts responded against effective antimicrobial activity on all tested organisms except chloroform extract. Moreover, a potent anti-diabetic activity of the leaf of this plant was examined by adrenaline induced diabetic mice model method. Glibenclamide was used as standard drug. This plant showed remarkable inhibitory activity. In addition, antioxidant activity of ethanol extract was determined by DPPH assay method. IC_{50} value of the ethanol extract of the selected sample was found to be 3.26μg/mL compared with standard ascorbic acid (IC_{50}=0.91μg/mL).

Keywords *–Morinda citrifolia* Linn., antimicrobial activity, anti-diabetic activity, antioxidant activity

1. Introduction

Myanmar is rich in natural resources. Among them, plants are essential for human being. Most of the people in Myanmar depend on traditional medicinal plants and herbal medicines for the treatment of various diseases. *Morinda citrifolia* Linn., Myanmar named Ye-yo is one of the useful medicinal plants in Myanmar traditional medicine. Several medicinal properties have been attributed to *Morinda citrifolia* Linn.. *Morinda citrifolia* Linn. is a fruit-bearing tree in the coffee family, Rubiaceae. Its native range extends through Southeast Asia and Australasia. English name of *Morinda citrifolia* Linn. is noni or Indian mulberry [2]. The leaf and fruit of this plant has been used as food, medicine, colorful dye, cosmetic purpose and has a high demand in medicines for different kinds of illnesses like diabetes, arthritis, cancer, gastric ulcer, mental depression, poor digestion and regulate blood circulation [3]. Hence the leaf of *Morinda citrifolia* Linn. is selected for chemical and pharmaceutical investigation. Effective bioactivities play very important role in medicinal plants. In this research work, antimicrobial activity of various solvent (methanol, ethanol, acetone, ethyl acetate and chloroform) extracts, anti-diabetic and antioxidant activities of the ethanol extract from leaf of *Morinda citrifolia* Linn. were evaluated.

1.1. Botanical Description

Figure 1. Plant, leaf and fruits of *morinda citrifolia* linn.

Family name	-	Rubiaceae
Genus	-	*Morinda*
Species	-	*M. citrifolia*
Common name	-	Noni or Indian Mulberry
Botanical name	-	*Morinda citrifolia* Linn.
Myanmar name	-	Ye-yo
Part used	-	Leaf

2. Materials and Methods

2.1. Sample Collection and Preparation

The leaf of *Morinda citrifolia* Linn., Myanmar named Ye-yo was collected from Magway Township, Magway Region, Myanmar in May, 2019. They were cut into small pieces and air dried at room temperature for about two weeks.

2.2. Extraction

The air dried leaf of the plant was percolated with ethanol. After two weeks, the mixture solution were filtered, filtrate was evaporated with rotary evaporator. Ethanol extract was obtained. This ethanol extract was used for the determination of antioxidant and anti-diabetic activities.

2.3. Preliminary Phytochemical Screening

Phytochemical evaluation for major phytochemicals was done using standard qualitative method [1], [4]. Tests for presence of alkaloid, carbohydrate, flavonoid, glycoside, phenolic compound, polyphenol, reducing sugar, saponin, tannin, steroid and terpene were carried out.

2.4. Determination of Antimicrobial Activity

Antimicrobial activity of various solvent extracts from the leaf of *Morinda citrifolia* Linn. was performed by agar well diffusion method and tested on six microorganisms such as *Bacillus subtilis,*

Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans and *Escherichia coli* respectively.

2.4.1. Sample

The crude extracts of the sample were prepared by extracting the sample with different solvents like chloroform, methanol, ethyl acetate, ethanol and acetone by percolating methods. The extracts (1g each) were introduced into sterile petri-dishes and 1mL of their respective solvent.

2.4.2. Procedure

The antimicrobial activity of the crude extracts from the selected sample was determined against six strain microorganisms by the agar well diffusion method. The extract 1g was introduced into sterile petri-dish and dissolved in 1mL or with least amount of its respective solvent till it was dissolved. The bacteria suspension from trypticase soy broth was done evenly onto the surface of the trypticase soy agar slants immediately after hardening of the agar-well were made with a 10 mm sterile cork borer from each extract agar. After inoculums had dried for 5 minutes, the agar discs were removed and the wells were filled with sample to be tested. And then, the plates were incubated at 37°C. After overnight incubation at 37°C the diameter of inhibition zone including 10mm wells was measured. This method was used to test antimicrobial action of the extracts on 24 hours broth culture of the organism used. The extracts from sample were tested with six microorganisms. The observation was done the inhibition zone diameters and the measurements were recorded.

2.5. Determination of Anti-diabetic Activity

The anti-diabetic activity of the ethanol extract from the leaf of Ye-yo was determined by adrenaline induced diabetic mice model method [5], [6], [7],[8].

2.5.1. Selection of the mice

The strain of ICR albino mice was used in this study. The mice with 60-65 g of body weight and age of 10-12 weeks were selected to use and kept separately.

2.5.2. Induction of the diabetes to mice

The selected mice were prepared to cause hyperglycemic effect by using adrenaline injection. For giving adrenaline injection, the selected mice were fasted overnight. The animals were given intraperitoneally with adrenaline 0.2 mL/kg body weight in distilled water. They were stared for 4 hours after injection and then they were given 0.5 mL of glucose solution orally at hourly interval to prevent hypoglycemic shock. They were offered unlimited amounts of standard laboratory diet food and water. After one week, the mice were used to test the anti-diabetic activity.

2.5.3. Determination of normal fasting blood glucose level

In order to determine the normal blood glucose level, the mice were fasted over night before the commencement of the experiment, to ensure stable blood sugar resulting blood drops were tested by Glucometer and test strips.

2.5.4. Experimental design of groups of selected mice

The selected mice were divided into 4 groups for the determination of anti-diabetic activity. They are tested plant sample, positive and negative control and normal group. Each group contained five mice and gave them markers by using sodium picrate solution.

2.5.5. Administration of the selected plant extract, standard drug and water as treatment

A total of 15 fasted mice were used to give orally for the determination of anti-diabetic activity. Group I mice were administered with the plant sample extracts of 1 g/kg of body weight. Group II mice were kept as a positive control, the standard drug, Glibenclamide was administered 0.5 mg/kg of body weight. The remaining group was given orally with water 0.2 mL to each mouse. No fasting and no adrenaline injection group of mice were used as normal group.

2.5.6. Second induction of diabetes to mice

After administration of the tested sample and the controls, these mice were used to get further anti-diabetic effect as mentioned above.

2.5.7. Screening of blood glucose level

During the experimental procedure, three observations were performed at five times of 45 minutes interval after injection of the adrenaline by using Glucometer. The results were collected from each group for the data analysis.

2.6. Determination of Antioxidant Activity

Antioxidant activity of the ethanol extract from the selected sample was determined by DPPH assay method by using APEL UV/Vis spectrophotometer.

2.6.1. Preparation of reagents

In this experiment three solutions were prepared. They are DPPH solution, standard solution and various concentrations of sample solution.

2.6.2. Preparation of 100 µM DPPH Solution

DPPH powder 0.004 g (4 mg) was weighed and it was thoroughly and gently dissolved in 100 mL of ethanol and stored in brown colored volumetric flask. It must be kept in the fridge for no longer than 24 hours before use.

2.6.3. Preparation of standard ascorbic acid solution

2 mg of ascorbic acid was dissolved in 20 mL of ethanol (analar grade). This solution was thoroughly mixed at room temperature to obtain 100 µg/mL of standard solution. Various concentrations of standard solution were determined by using parallel dilution method. 1 mL of ascorbic acid and 3 mL of DPPH solutions were thoroughly mixed for about 15 minutes at room temperature. The absorbance of the mixture was measured at 517nm.

2.6.4. Preparation of test sample solution

0.01 g of sample was dissolved in 20 mL ethanol (analar grade). This solution was thoroughly mixed at room temperature for 15 minutes to obtain 500 µg/mL of sample solution. The various concentrations of sample solution were determined by using two fold dilution method.1 mL of sample solution and 3 mL of DPPH solution were thoroughly mixed for about 15 minutes at room temperature. The absorbance of the mixture was measured at 517 nm.

2.6.5. Two-fold serial dilutions

A two-fold dilution reduces the concentration of a solution by a factor of two that is reduced the original concentration by one half. A series of two-fold dilutions is described as two-fold serial dilutions.

$$\%\text{inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

This formula is the calculation of percent inhibition of (IC_{50}) value [9, 10].

3. Results and Discussion

3.1. Preliminary Phytochemical Test of the Leaf of *Morinda citrifolia Linn*.

According to qualitative determination of phytochemical tests, the selected sample contained alkaloids, flavonoids, glycosides, steroids, saponins, phenolic compound, polyphenols and tannins resceptively. Among them alkaloids, flavonoids, phenolic compounds and polyphenols are effective medicinal properties.

3.2. Antimicrobial Activity of Crude Extract of the Leaf of *Morinda citrifolia* Linn.

Antimicrobial activity of various solvent extracts of the selected sample was determined by applying agar well diffusion method and tested on six selected organisms. These results are tabulated in Table I and Figure 2. As the results of antimicrobial activities, ethyl acetate extract responded against high activity on all tested organisms. Ethanol extract showed high activity on *Pseudomonas aeruginosa*, *Candida albicans* and medium activity on other tested organisms. Methanol extracts responded high activity on *Pseudomonas aeruginosa* and medium activity on other tested organisms. Acetone extract showed low activity on *Bacillus subtilis, Staphylococcus aureus* and medium activity on other tested organisms. Chloroform extract indicated low activity on *Bacillus pumilus* and *Candida albicans* and no activity on other tested organisms.

Table 1. Antimicrobial activity of the leaf of *morinda citrifolia* linn.

Sample	Solvent extracted	Inhibition zone(mm, diameter)					
		I	II	III	IV	V	VI
Morinda citrifolia	MeOH	17 (++)	16 (++)	20 (+++)	15 (++)	16 (++)	17 (++)
	EtOH	19 (++)	18 (++)	24 (+++)	19 (++)	22 (+++)	18 (++)
	CH_3COCH_3	13 (+)	14 (+)	18 (++)	18 (++)	18 (++)	15 (++)
	EtOAc	40 (+++)	38 (+++)	40 (+++)	38 (+++)	35 (+++)	37 (+++)
	$CHCl_3$	-	-	-	12(+)	12(+)	-

agar well – 10mm
10mm-14mm(+)
(low activity)
15mm - 19mm (++)
(medium activity)
20 mm above (+++)
(high activity)

Organisms
I- *Bacillus subtilis* (N.C.T.C-8236)
II- *Staphylococcus aureus* (N.C.P.C-6371)
III- *Pseudomonas aeruginosa* (6749)
IV- *Bacillus pumilus* (N.C.I.B-8982)
V- Candida albicans
VI- *Escherichia coli* (N.C.I.B-8134)

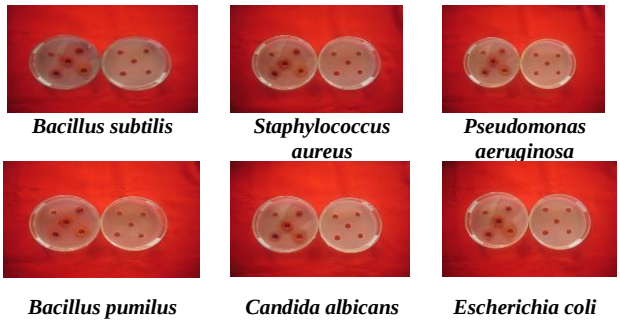

Figure 2. Antimicrobial activities (inhibition zones) of *morinda citrifolia* linn.

3.3. Determination of the Anti-diabetic Activity of Crude Extract on Adrenaline Induced Mice

The blood glucose levels of fasted animals at five times of 45 minutes intervals after adrenaline injection measured. From the measured glucose levels, the mean blood glucose values were calculated as shown in Table 2 and 3 which was also graphically presented in Figure 3. The significant anti-diabetic activity was observed in *Morinda citrifolia* Linn. and compared to diabetic control. According to the results of the experimental

carried out on diabetic mice, it had been shown that the ethanol plant extract has more highly significant activity than the negative control (water) and consistent anti-diabetic effect. Positive control (Glibenclamides) was also more highly significant activity than plant extract and water. The ethanol plant extract has more highly significant activity than the negative control (water) and also effective as positive control (Glibenclamide). Therefore, ethanol plant extract was potential to use as routine drug like the positive control.

Table 2. Anti-diabetic activity of ethanol plant extract, positive control (glibenclamide) and negative control (water)

Group	Dose	Blood Glucose Level (mg/dL) 0 min	45 min	90 min	135 min	180 min	225 min
Sample extract (*Morinda citrifolia*)							
1	1 g/kg	127	158	125	96	74	60
2	1 g/kg	125	147	123	102	78	63
3	1 g/kg	131	152	120	99	82	65
4	1 g/kg	133	155	120	98	83	70
5	1 g/kg	129	149	122	101	85	67
Mean		129	152	122	99	80	65
Positive control (Glibenclamide)							
1	0.5 mg/kg	120	143	112	98	70	56
2	0.5 mg/kg	118	140	115	94	68	56
3	0.5 mg/kg	124	146	113	93	70	54
4	0.5 mg/kg	117	138	114	98	73	55
5	0.5 mg/kg	126	148	110	95	68	52
Mean		121	143	112	95	69	54
Negative control (Water)							
1	0.2 mL/kg	116	158	143	122	110	99
2	0.2 mL/kg	122	160	145	123	113	101
3	0.2 mL/kg	118	158	142	120	108	98
4	0.2 mL/kg	118	159	144	125	112	102
5	0.2 mL/kg	123	160	145	120	106	96
Mean		119	159	144	122	110	99

Table 3. Anti- diabetic activity of ethanol plant extract, positive control (glibenclamide) and negative control (water)

Group	Dose	Blood Glucose Level Mean ± SD (mg/dL) 0 hr	45 min	90 min	135 min	180 min	225 min
I	1 g/kg	129 ±3.16	152 ±4.44	122 ±2.12	99 ±2.40	80 ±4.18	65 ±3.81
II	0.5 mg/kg	121 ±3.87	143 ±4.12	112 ±2.12	95 ±2.40	69 ±2.24	54 ±1,80
III	0.2 mL/kg	119 ±3.00	159 ±1.00	144 ±1.32	122 ±2.12	110 ±4.30	99 ±2.40

SD - Standard Deviation
I - (Ye-yo) *Morida citrifolia* linn. extract
II - Glibenclamide (Standard drug)
III - Diabetic negative control (water only)

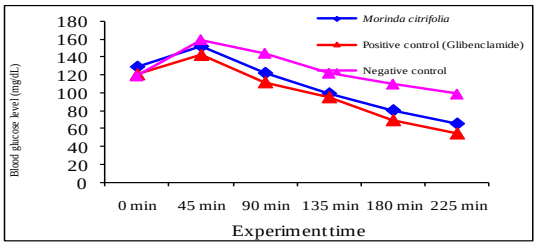

Figure 3. Levels of fasting blood sugar during anti-diabetic activity test with plant extract (*morinda citrifolia* linn.), positive control (glibenclamide) and negative control (water)

3.4. Determination of Antioxidant Activity of the Leaf of *Morinda citrifolia* Linn.

Antioxidant activity of ethanol extract of the leaf of *Morinda citrifolia* Linn. was expressed as percentage of DPPH radical inhibition and IC_{50} values (µg/mL). Free radical scavenging activities values of ascorbic acid and sample extract in percentage range from 47.39% to 57.86% and 30.94% to 75.51% respectively. The results of antioxidant activity using DPPH assay method in sample extract and ascorbic acid used as a positive control are shown in Figure 4 and 5 and Table 4 and 5.

Table 4. Various concentration and % inhibition of standard ascorbic acid

Standard	Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
Ascorbic acid	4	57.86	0.91
	2	51.67	
	1	51.49	
	0.5	49.58	
	0.25	47.39	

Table 5. Various concentration and % inhibition of ethanol extract of leaf of *morida citrifolia* linn.

Extract	Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
	10	75.51	3.26
	5	62.44	
	2.5	49.74	
	1.25	44.41	
	0.625	30.94	

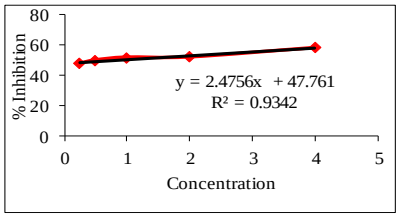

Figure 4. Plot of % inhibition Vs concentration of standard ascorbic acid

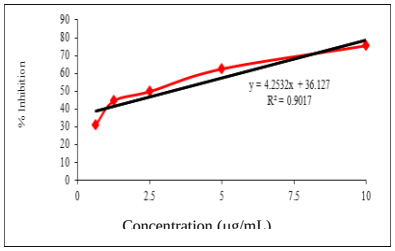

Figure 5. Plot of % inhibition Vs concentration of sample extract

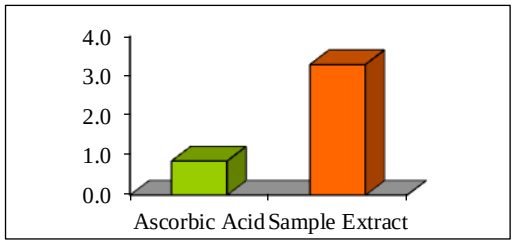

Figure 6. Comparison of IC_{50} values of standard ascorbic acid and ethanol extract of leaf of *morinda citrifolia* linn.

The IC_{50} value is a parameter used to measure antioxidant activity and it is defined as the sample extract concentration required for 50 % scavenging of DPPH radicals under experimental condition employed. The smaller IC_{50} value corresponds to a higher antioxidant activity. According to above IC_{50} values, the ethanol extract of the leaf of *Morinda citrifolia* Linn. was found to exhibit significant antioxidant property which is comparable to standard ascorbic acid. Moreover, in accordance with Fig.4and 5, increase in concentration implies increase in % inhibition of oxidation. From these results, it is also observed that increase in concentration shows to increase in % inhibition, it means that increase the free radical scavenging activity. According to Table 4,5 and Figure 6, the leaf of *Morinda citrifolia* Linn. has lower than antioxidant activity than standard ascorbic acid.

4. Conclusion

According to the preliminary phytochemical screening the leaf of *Morinda citrifolia* Linn. contained many valuable phytochemical constituents which are health benefits to human. Alkaloids, flavonoids, phenolic compounds and polyphenols are effective medicinal properties. Which are useful phytochemicals in drug. The results of antimicrobial activity determination showed the selected plant extracts have high effective activity on all tested organisms except chloroform extract. Ethanol, methanol and ethyl acetate extracts more responded against on tested organisms than other extracts. Chloroform extract responded against low activity on *Bacillus pumilus* and *Candida albicans*. Therefore the selected plant extracts should be consumed and used as antimicrobial drugs. Moreover the leaf of selected plant should be used for treatment of microorganism infections, various ulcers, diarrhea and allergy. The significant findings were that the tested plant extract possessed high remarkable anti-diabetic effects like that of the standard drug, Glibenclamide. High activity was found to possess significant anti-diabetic activity, which supported the traditional application of this plant in treatment of diabetes. IC_{50} value of the ethanol plant extract has higher than standard ascorbic acid. Higher IC_{50} value correspond lower antioxidant activity. But the free radical scavenging activity of selected plant extract has significant activity like ascorbic acid. So, the ethanol extract of the selected plant should be used as antioxidant for maintaining human health, protection of cancer, improve blood circulation, regulate blood pressure, anti-inflammatory and anti-ageing. From the results of experimental data, the quality of pharmaceutical preparations from the leaf of *Morinda citrifolia* Linn. should be done and may be used a variety of medicinal purposes.

Acknowledgments

We would like to thank Dr Khin May Aung, Pro-rector, Panglong University to permit this research paper. We also express our profound gratitude to Dr Nyan Tun, Professor and Head, Department of Chemistry, Panglong University for the provision of the encouragement and suggestions.

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