

Investigation on Antioxidant, Antimicrobial, Glucose Lowering Activities and Acute Toxicity Study of Leaves of *Morinda citrifolia* Linn

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Abstract

In this research work, the leaves of *Morinda citrifolia* Linn, were collected from campus of University of Mandalay, for chemical analysis. The phytochemical constituents of sample were determined by phytochemical screening methods. The elemental content in sample was measured by Energy Dispersive X-ray Fluorescence Spectrophotometer (EDXRF) method. The antioxidant activity of ethanol extract of sample was determined by using 1-1-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay. The antimicrobial activities of three extracts such as n-hexane, ethyl acetate and ethanol of sample were tested by Agar-well diffusion method on six tested organisms. The acute toxicity of ethanol extract of sample was determined by Teng Wash Sam method. Moreover, the glucose lowering activity of ethanol extract of sample was examined by iodometric titration method.

Keywords: *Morinda citrifolia*, antimicrobial, antioxidant, iodometric, toxicity

INTRODUCTION

Morinda citrifolia is known as India noni or Indian mulberry. It is grown in tropical region of the world. It is one of the most important traditional medicinal plants which has been used for over 2000 years, because of medicinal and nutritional values. It is most plant used in the herbal remediation. It has traditionally used for cold flu, diabetes, arthritis, high blood, muscle ached, pains, headache, heart diseases, hypertension, as well as mental depression. It is proved to be best remedial on antibacterial, anticancer, anti-inflammatory, antioxidant, diuretic and cardio-toner etc. (Chunhieng, et al, 2003).

Morinda citrifolia Linn leaves have been consumed as a vegetable by many cultural groups. This reason it is included in the World Health Organization's and Food and Agriculture Organization's Food composition. *Morinda citrifolia* Linn about 160 phytochemical compounds in the plant have already been phenolic compounds, organic acids, and alkaloids. Phenolic compound are example of herbal compounds with widely reported antioxidant and anti-inflammatory properties. (West BJ, et al, 2007).

Therefore, the phytochemical constituents, elemental compositions, antimicrobial activity, antioxidant activity, acute toxicity and the glucose lowering activity of the leaves of *Morinda citrifolia* Linn were studied in my research work.

Botanical Description

Family name: Rubiaceae

Scientific name: *Morinda citrifolia* Linn

Myanmar name: Ye-yo



Figure (1) The Plant of *Morinda citrifolia* Linn

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MATERIAL AND METHODS

Sampling

The leaves of *Morinda citrifolia* Linn were collected from campus of University of Mandalay. The sample was cut into small pieces and dried in air for about three weeks. It was stored in a well- stopper bottle and used throughout the experiment.

Preliminary Phytochemical Test

Preliminary phytochemical test of sample was carried out by usual method. The results were shown in Table (1).

Elemental Composition

Elemental contents of sample were measured at Department of Chemistry, Monywa University by using Energy Dispersive X-ray Fluorescence Spectrophotometer (EDXRF). The results were shown in Table (2).

Antioxidant Activity

The antioxidant activity of ethanol extract of the sample was done by using DPPH Radical Scavenging Assay. The result was shown in Table (4).

Antimicrobial Activities

Antimicrobial activities of the crude extract of the sample were tested in various solvent systems by using Agar well diffusion method on six selected organisms in CRDT (Central Research Development and Technology), Insein, and Yangon as shown in Figure (2). The results were shown in Table (5).

Acute Toxicity

The acute toxicity of ethanol extract of sample was determined by Teng Wah Sem method. The result was shown in Table (6).

Glucose Lowering Activity

Glucose lowering activity of ethanol extract of the sample was done by using iodometric titration method. The results were shown in Table (6).

RESULTS AND DISCUSSION

The results of phytochemical constituent of the sample were shown in Table (1).

Table (1) Results of Preliminary Phytochemical Test of Sample

No.	Constituents	Reagent used	Observation	Result
1.	Alkaloid	(1)Wagner's reagent (2)Dragendorff's reagent	Reddish brown ppt Orange ppt	+ +
2.	Tannin	10%FeCl ₃ ,dilH ₂ SO ₄	No red color solution	-
3.	Saponin	Distilled water	Froth like comb	+
4.	Flavonoid	conc:HCl,Mgturning,Δ	No pink color solution	-
5.	Terpene	Aceticanhydride,CHCl ₃ , conc: H ₂ SO ₄	No red color solution	-
6.	Glycosides	10% lead acetate	White ppt	+
7.	Steroids	Acetic anhydride, conc:H ₂ SO ₄	Green color solution	+

8.	Phenolic	10 % FeCl ₃	Greenish blue color solution	+
9.	Polyphenol	10 % FeCl ₃ , 1 % K ₃ [Fe(CN) ₆]	Greenish blue color solution	+
10.	Reducing sugar	Benedict solution	No Brick-red ppt	-
11.	Lipophilic	0.5M KOH,NaOH	Deep color solution	+

(+) = presence of constituent (-) = absence of constituent

According to this table, *morinda citrifolia* leaves extract consist of alkaloid, saponin, glycosides, steroid, phenolic, polyphenol and lipophilic respectively.

The results of elemental contents of the leaves of *morinda citrifolia* were shown in Table (2).

Table (2) Results of Elemental Contents of Sample

Elements	Relative Abundance (%)
Chlorine	1.434
Calcium	1.058
Potassium	0.557
Manganese	0.481
Silicon	0.244
Sulphur	0.223
Phosphorus	0.103
Iron	0.016
Strontium	0.003
Magnesium	0.003
Copper	0.002
Tin	0.001
Zinc	0.001

According to this table, chlorine is the highest amount in the sample. Chlorine helps to relief from stroke, blood pressure, heart and kidney disorder and stress.

The results of antioxidant activity using DPPH assay in standard ascorbic acid was shown in Table (3).

Table(3) Results of % Inhibition of Various Concentration of Standard Ascorbic Acid

Concentration (µg/mL)	Mean Absorbance	Mean % inhibition	IC ₅₀ (µg/mL)
50	0.561	89.936	
25	0.400	64.103	
12.5	0.336	53.846	11.98
6.25	0.283	45.353	
3.125	0.227	36.378	

IC₅₀ value was calculated by using linear regressive equation.

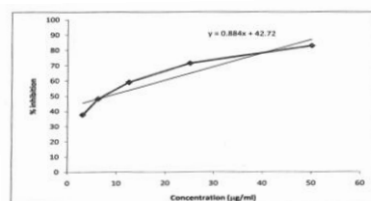


Figure (2) Plot of % Inhibition Vs Concentration of Standard Ascorbic Acid

The results of antioxidant activity using DPPH assay in ethanol extract of sample was shown in Table (4).

Table (4) %inhibition of Various Concentration of Sample

Concentration(µg/mL)	MeanAbsorbance	Mean%inhibition	IC ₅₀ (µg/mL)
100	0.134	26.070	
50	0.098	19.066	
25	0.092	17.899	388.569
12.5	0.087	16.926	
6.25	0.097	18.872	

IC₅₀ value was calculated by using linear regressive equation.

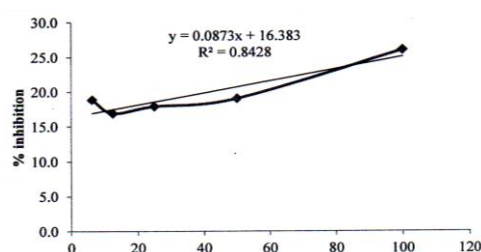


Figure (3) Plot of % Inhibition Vs Concentration of the Sample

According to Table (3) and (4), the IC₅₀ value of the standard ascorbic acid was 11.98 µg/mL and that of ethanol extract of leaves of *morinda citrifolia* L. was 388.569 µg/mL. The IC₅₀ value of sample was found to be higher than that of standard ascorbic acid. Therefore, the sample extract has lower antioxidant activity than standard ascorbic acid.

The results of antimicrobial activities of the various extracts of sample were shown in Table (5).

Table (5) Results of Antimicrobial Activities of the Sample

Samples	Solvent	Inhibition Zone					
		I	II	III	IV	V	VI
<i>Morinda</i>	n-	12mm	13 mm	13 mm	13 mm	13 mm	12mm
<i>citrifolia</i>	hexane	(+)	(+)	(+)	(+)	(+)	(+)
Linn	EtOAc	14mm	13 mm	12 mm	15 mm	14 mm	15mm
		(+)	(+)	(+)	(++)	(+)	(++)
	EtOH	15mm	15 mm	14 mm	17 mm	14 mm	15mm
		(++)	(++)	(+)	(++)	(+)	(++)

Agar well – 10 mm

Organisms

10 mm ~ 14 mm (+)

I= *Bacillus subtilis*

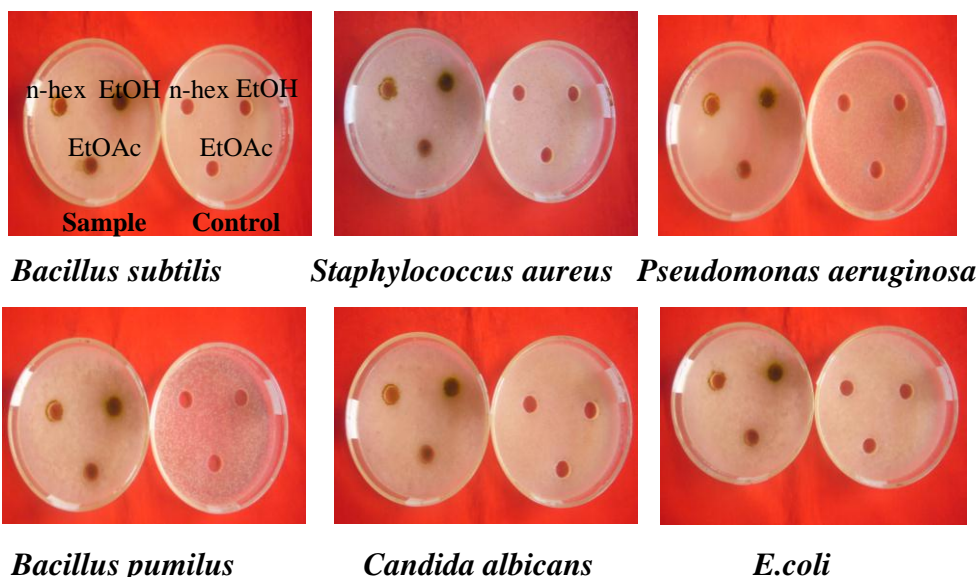
15 mm ~ 19 mm (++)

II = *Staphylococcus aureus*

20 mm above (+++)

III= *Pseudomonas aeruginosa*IV= *Bacillus pumilus*V= *Candida albica*VI= *E. coli*

According to this table, n-hexane extract of sample responds low activities on all tested organisms. EtOAc extract gives medium activities on *Bacillus pumilus*, *E-coil* and low activities on *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albican*. EtOH extract responds medium activities on *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus pumilus*, *E.coil* and low activity of *Pseudomonas aeruginosa*, and *Candida albicans*.

**Figure (4) Antimicrobial Activities of various Extract of Sample**

The total of 20 *Artemia salina* were used to give orally the sample extract. The result of acute toxicity was shown in Table (6).

Table (6) Result of Mortality for Acute Toxicity of Sample

Dosage (ppm)	Log Dosage	Alive	Dead	Accumulated alive	Accumulaed Dead	Ratio Dead: Total	Mortality
4000	3.60	0	20	0	56	56:56	1
2000	3.30	3	17	3	36	36:39	0.92
1000	3	10	10	13	19	19:32	0.59
500	2.69	15	5	28	9	9:37	0.24
250	2.39	16	4	44	4	4:48	0.08
125	2.09	20	0	64	0	0:64	0

Number of brine shrimp larvae = 20

As described in this table, the *Artemia salina* was treated with different dose of ethanol extract of the sample. After 6 hours, acute toxicity on *Artemia salina* was studied. It was found that 20 *Artemia salina* were alive when 125 ppm of ethanol extract of the sample.

The result of percent of decrease in amount of glucose by the ethanol extract of leaves of *Morinda citrifolia* Linn was shown in Table (7).

Table (7) % of Decrease in Amount of Glucose (Ethanol Extract)

No.	Contact time (min)	Initial amount of glucose	Left amount of glucose (m mole)	Decrease (m mole)	%of decrease amount of Glucose
1	15	2.5	2.05	0.45	18
2	30	2.5	2.0875	0.4125	16.5
3	45	2.5	2.1375	0.3625	14.5
4	60	2.5	2.1875	0.3125	12.5
5	75	2.5	2.1875	0.3125	12.5

Among five conditions, conditions 4 are the best of glucose lowering activity of leaves of *Morinda citrifolia* Linn.

CONCLUSION

In this research work, the preliminary phytochemical tests of sample contain alkaloid, flavonoid, glycoside, phenolic compound, polyphenol, saponin, steroid, and lipophenol compound respectively.

The elemental compositions of the leaves of *Morinda citrifolia* Linn were determined by EDXRF method. The results of the element analysis show that the leaves sample consists of Cl (1.433), Ca (1.058), K (0.016), Mn (0.481), Si (0.244), S (0.223), P (0.103), Fe (0.016), Sr (0.003), Mg (0.003), Cu (0.002), Ti (0.02), Zn (0.01) and Br (0.001).

The antioxidant activity of ethanol extract of the sample was studied by DPPH assay. It was found that ethanol extract of sample ($IC_{50} = 388.569$) has lower antioxidant activity than standard ascorbic acid ($IC_{50} = 11.98$).

The antimicrobial activities of various extract of sample were tested by Agar-well diffusion method on six selected organisms. In this study, it can be observed that n-hexane extract gives low activities on all tested organisms. Ethyl acetate extract gives medium activities on *Bacillus pumilus*, *E-coil* and low activities on *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albican*. Ethanol extract responds medium activities on *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus pumilus*, *E-coil* and low activity of *Pseudomonas aeruginosa*, and *Candida albican*.

The acute toxicity of ethanol extract of sample was determined by Teng Wash Sam method. After 6 hours, acute toxicity on *Artemia salina* was studied. It was found that 20 *Artemia salina* were alive when 125 ppm of ethanol extract of the sample. The acute toxicity of ethanol extract of the sample gave the normal condition of their health.

The glucose lowering activity of ethanol extract of sample solution was determined by iodometric titration using sodium hydroxide. From the results, it was observed that the percent of decrease in amount of glucose for leaves sample solution was highest for the contact time, 60 min.

From the chemical analysis of the leaves of *Morinda citrifolia* Linn, the sample contains valuable elemental compositions for human.

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ONLINE MATERIALS

http://en.m.wikipedia.org/wiki/Morinda_citrifolia_Linn.