Determinations of Nutritional Compositions and Antioxidant Activity in the Leaves of *Morinda citrifolia* Linn

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Abstract

The leaves of *Morinda citrifolia* Linn. collected from Mandalay University Campus, Mahaaungmyay Township, Mandalay Region were selected for reliminary phytochemical screening, elemental analysis, determinations of nutritional compositions and antioxidant activity. The phytochemical compositions of the sample was investigated using test tube method. The mineral elements present in the sample were determined using Energy Dispersive X-ray Fluorescence (EDXRF spectrometer. The presence of proteins in the sample was investigated by qualitative tests. The nutritional values of the sample were also determined. The antioxidant activity of the sample was tested by DPPH (1,1 diphenyl-2-picryl-hydrazyl) assay using ascorbic acid as standard.

Keywords: *Morinda citrifolia* Linn., EDXRF, antioxidant activity, nutritional compositions

Introduction

Morinda citrifolia Linn. (Myanmar name- Ye-yo) is one of the species of Rubiaceae. Its native range extends through Southeast Asia and Australasia, and the species is now cultivated throughout the tropics. (ab Nelson, 2006). It is one of the medicinal plants discovered by the ancestors of Polynesians and has been used as traditional folk medicinal plant for over 2000 years in Polynesia (Dixon *et. al.*,1999). Its fruit extract is used to treat a wide range of maladies (Ross, 2002).

The size of fruit is about 12 cm due to coalescence of the interior ovaries of many closely packed flowers; it has a foul taste and a soapy smell when it is ripe. The fruit of this plant has been used for food, drink, medicine, colorful dye, cosmetics purpose and as a result it is highly demanded for medicinal purposes especially for such diseases as diabetes, high blood pressure, AIDS, arthritis, cancer, gastric ulcers, sprains, mental depression, poor digestion, atherosclerosis, blood vessels problem etc. Its root, leaves, stem, bark, flowers and fruits are recorded as herbal remedies for different diseases (Wang, 2002 and Wamg, 2001). Noni juice extract is obtained from Noni fruit which is the most effective product that has helped people relieve from the suffering of about 22 conditions, such as arthritis, heart diseases, diabetes, headache and muscle pain, high blood pressure, cancer, etc (Peter, 2007).

Morinda citrifolia is a perennial bush and it is possible to find fruits at different stages of maturity in the same plant at the same time. The species is generally found from sea level to 400m altitude, although it adapts better to coastal regions. About 160 phytochemical compounds have been already identified in the noni plant, and the major micronutrients are phenolic compounds, organic acids and alkaloids (Wang, 2001 and West, 2006).

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Botanical Description

Family name - Rubiaceae
Botanical name - *Morinda citrifolia* Linn.
Myanmar name - Ye-yo
English name - Indian Mulberry, noni
Parts used - leaves

Figure (1) The Leaves and Fruits of Ye-yo

Materials and Methods

Materials

All the chemicals and reagents used were purchased from Able chemical shop, Mandalay. The leaves of Ye-yo (*Morinda citrifolia* Linn) were collected from Mandalay University Campus, Maha Aung Myay Township, Mandalay Region. EDXRF Spectrometer (EDXRF-700) and UV-Visible spectrometer (Shimadsu, Japan) were used for the elemental analysis and for the determination of antioxidant activity.

Methods

Preliminary Phytochemical Tests

The phytochemical constituents of the sample were tested using test tube method at the Department of Chemistry, University of Mandalay for the determination of phytochemical compounds (Harbone, 1973).

Elemental Analysis

The mineral elements present in the leaves of Ye-yo were investigated using EDXRF spectral data at Department of Chemistry, University of Monywa.

Determination of Nutritional Compositions

Preparation of Protein Solution for Qualitative Tests

The air dried powder sample (10 g) was pleaced in a conical flask and distilled water (100 ml) was added in this conical flask. Then the mixture was heated in a water-bath for about 30 minutes. It was cooled and filtered. The filtrate was used for qualitative tests of proteins from the leaf of Ye-yo (AOAC, 2000).

Qualitative Tests of Protein

Biuret Test

Equal amount of 10% sodium hydroxide solution was added to the 3 ml of protein solution of leaf and mixed well. Then, copper (II) sulphate solution was added drop by drop. Appearance of orange color shows the presence of albumin in the test sample.

Millon's Test

About 5 ml of protein solution of leaf was mixed with a few drop of Millon's reagent. The mixture was heated up to the boiling point and white precipitate was formed. And then it turned brick red indicating the presence of albumin in the test sample.

Xanthoproteic Test

2 ml of protein solution was mixed with 0.5 ml of concentrated nitric acid in the test tube and boiled. When the mixture was cooled down under tap water, yellow color was appeared. Concentrated ammonium hydroxide was added to make the alkaline solution. Yellow color was observed and then it changed to orange. Appearance of orange color denotes the prescence of amino acids like tyrosine, tryptophan and phenylalanine. Therefore protein may be present in the test sample.

Formaldehyde Test

About 2 ml of protein solution was mixed with dilute formaldehyde solution. A few drops of concentrated sulphuric acid was slowly added to the mixture. A purple violet ring developed in the surface of contact shows that proteins containing tryptophan was confirmed in the test sample.

Determination of Nitrogen and Protein Content Using Kjeldahl's Method

(a) Digestion

About 1g of sample (finely ground) was weighed and placed in the Kjeldahl's digesting flask. About 5 g of annular sodium sulphate, 0.25 g of anhydrous copper (II) sulphate and 12.5 ml of 98% sulphuric acid were added into it in such a way as to wash down any solid adhering to the neck. The flask was shaken until the contents were thoroughly mixed and it was heated till the mixture became colourless.

The digestion was continued for half an hour to make sure that all the nitrogen in the sample was converted to ammonium sulphate. It was allowed to cool and 10 ml of distilled water was carefully added with frequent shaking.

(b) Distillation

The Kjeldahl's distillation apparatus was set up, taking care that the tip of the condenser is extended below the surface of the standard sulphuric acid solution 25 ml in the receiver. The digested solution was poured into the flask together with 100 ml of 40 % sodium hydroxide to make mixture strongly alkaline. The evolved ammonia was distilled off.

Determination of Water-Soluble Carbohydrate

The water soluble carbohydrate was determined by phenol-sulphuric acid colorimetric method in terms of glucose (AOAC, 2000).

Preparation of Standard Sugar Solution

Stock solution – Glucose 100 mg was dissolved in 100 ml of distilled water. Working standard solution – 10 ml of stock solution was diluted to 100 ml with distilled water.

To prepare the standard curve, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard glucose solutions were transferred into a series of test tubes. Then the volume of each test tube was made up to 1 ml with distilled water and 1 ml of distilled water was set-up for a blank solution.

Procedure

1 ml of sample solution, five standard sugar solutions containing 0.2, 0.4, 0.6, 0.8 and 1ml of glucose per ml were put in each test tube. 1 ml of 5 % phenol solution was also added to each test tube and mixed. A blank solution was also prepared with 1 ml of distilled water instead of sugar solution. 5 ml of 98 % sulphuric acid was again added to each test tube so that the steam hit the liquid surface directly to produce good mixing. Each test tube was agitated during the addition of acid. After ten minutes, the tubes were reshaken and placed in water bath

at 25°-30°C for twenty minutes. The yellow orange color was stable for several hours. Absorbances were measured at 490 nm using UV-visible spectrophotometer.

A standard curve was plotted by the absorbance of the standard solution against the concentration in mg per ml. Using this standard curve, the concentration of glucose in the sample was calculated.

Determination of Fat Content by Soxhlet Method

About 50 g of leaves were placed in a thimble and the bag was then placed in a Soxhlet extractor. Petroleum ether (300 ml) was poured into the extractor until some of it overflowed into the flask. The flask was heated in a water bath until the oil was previously removed from the sample. A duration of about 8 hours was required for complete extraction. After the extraction, the oil dissolved in the solvent was removed by distillation. The last trace of the solvent was then removed by placing the content in an oven at about 105°C until the constant weight was obtained (AOAC, 2000).

Determination of Moisture Content

The moisture content of sample was determined by oven drying method. The moisture content of sample is the lost weight due to the evaporation of water at the drying temperature.

About 5 g of the sample was placed in a weight known crucible which was previously dried and cooled in a desiccator. The crucible containing the sample was placed in an oven and dried for 15 minutes at 105°C. Then, it was removed from the oven and cooled in desiccator and weighed. The procedure was repeated until a constant weight was obtained (AOAC, 2000).

Determination of Ash Content

The air dried sample 5g was weighed and placed in a preheated, cooled and weighed crucible. The crucible was heated carefully in the furnace at 550°C for 2 hours burned off without flaming or until all the carbon was eliminated. When the materials were converted to white ash powder, the crucible was cooled at room temperature in a desiccator and weighed again. To obtain a constant weight the process of heating, cooling and weighing were repeatedly done (AOAC, 2000).

Determination of Antioxidant Activity

The antioxidant activity of the sample was estimated using DPPH (1,1 diphenyl-2-picryl-hydrazyl) free radical scavenging activity at Department of Chemistry, Mandalay University. The antioxidant activity was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in mg/ml) of extract that inhibits the formation of DPPH radicals by 50%.

Results and Discussion

Preliminary Phytochemical Screening of Leaves of Ye-yo

The sample consists of alkaloids, glycosides, lipophenols, phenols, polyphenols, reducing sugars, saponins, steroids, tannins and terpenes respectively according to the results obtained from the preliminary phytochemical tests.

Table (1) Results of Phytochemical Screening of the Leaves of Ye-yo

No.	Test	Reagent	Observation	Results
1.	Alkaloid	Wagner's solution	Red ppt	+
2.	Flavonoid	Conc: HCL, Mg turning	No pink color solution	-
3.	Glycoside	10 % lead acetate	Yellow ppt	+
4.	Lipophenol	0.5 KOH, 4 drops of NaOH	Deep color solution	+

5.	Phenolic	10% $FeCl_3$	Reddish brown color solution	+
6.	Polyphenol	1% $FeCl_3$ and 1% $K_3[Fe(CN)_6]$	Greenish blue color solution	+
7.	Reducing Sugar	Benedict's solution	Yellow ppt	+
8.	Saponin	$NaHCO_3$	Froth	+
9.	Steroid	Acetic anhydride, conc: H_2SO_4	Green color solution	+
10.	Tannin	10% $FeCl_3$, dil H_2SO_4	Yellowish brown ppt	+
11.	Terpene	Acetic anhydride, $CHCl_3$, conc: H_2SO_4	Pink color solution	+

Determination of Elemental Compositions in the Leaves of Ye-yo

According to the EDXRF report, it was found that there are ten mineral elements in the leaves of Ye-yo. Among them, chlorine is the most abundant element followed by calsium, potassium, sulphur and iron. The least elements present in the selected sample are manganese, copper, strontium, zinc and bromine.

Table (2) Mineral Elements in Leaves of Ye-yo

No.	Symbol	Elements	Relative Abundance (%)
1.	Cl	Chlorine	1.255
2.	Ca	Calcium	0.884
3.	K	Potassium	0.305
4.	S	Sulfur	0.176
5.	Fe	Iron	0.008
6.	Mn	Manganese	0.002
7.	Cu	Copper	0.002
8.	Sr	Strontium	0.002
9.	Zn	Zinc	0.001
10.	Br	Bromine	0.001

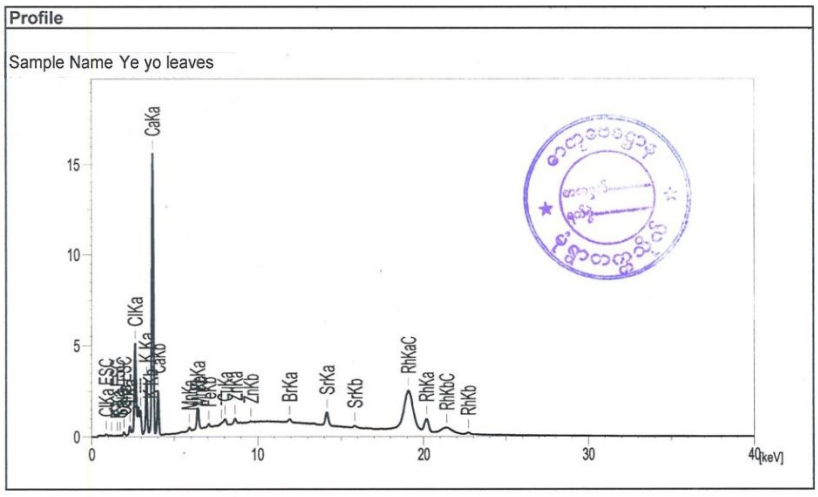

Figure (2) EDXRF Chromatogram of Leaves of Ye-yo

Qualitative Tests of Protein from Leaves of Ye-yo

The results of qualitative tests of protein from the leaves of Ye-yo were shown in Table (1). The tested sample showed positive in protein test except formaldehyde test.

Table (3) Results of Qualitative Tests of Protein

No.	Experiment	Reagent used	Observation	Remark
1.	Biuret test	10 % NaOH, $CuSO_4$	Orange color solution	Presence of albumin
2.	Millon's test	$HgSO_4$ in H_2SO_4	White ppt	Presence of albumin
3.	Xanthoproteic test	Conc:HNO_3, Conc:NH_4OH	Yellow color solution	Presence of tyrosine, tryptophan and phenylalanine
4.	Formaldehyde test	Dil:HCHO solution, Conc: H_2SO_4	No violet ring	Absence of tryptophan

Nutritional Values of Leaves of Ye-yo

The nutritional values such as protein, carbohydrate, fat, moisture and ash contents were determined according to the appropriate reported methods. The results are shown in Table (4), (5), (6) and (7).

Table (4) Results of Nitrogen and Protein Contents in Leaves of Ye-yo

Sample	Nitrogen content (%)	Protein content (%)
Leaf	2.354	14.711

According to this table, the protein contents of the leaves of Ye-yo were found to be 14.711 %. Protein is one of the essential components of human body.

Table (5) Absorbance of Standard Glucose Solutions

No.	Concentration of glucose (mg/mL)	Absorbance at 490 nm
1.	0.2	0.1502
2.	0.4	0.3005
3.	0.6	0.4600
4.	0.8	0.6400
5.	1.0	0.8650

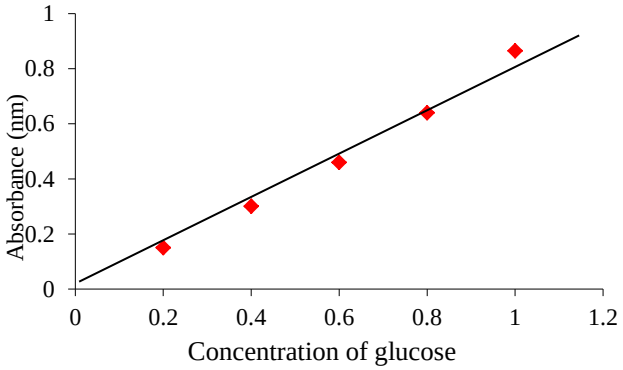

Figure (3) Standard Calibration Curve for Glucose

Table (6) Water Soluble Carbohydrate Contents in Leaves of Ye-yo

Sample	Water Soluble Carbohydrate Content (%)		
Leaf	3.84	3.82	3.84

The carbohydrate content of the leaves was found to be 3.84 % from this table.

Table (7) Results of Fat, Moisture and Ash Contents in Leaves of Ye-yo

Sample	Fat Content (%)	Moisture Content (%)	Ash Content (%)
Leaf	4.2	38.32	1.32

According to this table, fat, moisture and ash contents in leaves of Ye-yo were found as 4.2 %, 38.32% and 1.32 respectively. Fat and oils function as a major store house energy. The moisture contents sometimes reflect the shelf-life of material. The larger the moisture content, the shorter the shelf-life of food sample. The ash of a sample is inorganic residue remaining after organic matter has burnt away.

Determination of Antioxidant Activity of Ethanol Extract of the Sample by DPPH Assay

The antioxidant activity of the ethanol extract of the leaves of Ye-yo was determined in DPPH free radical scavenging assay. From this assay, IC_{50} value of the sample was found to be 132 µg/ml and showed lower activity than standard ascorbic acid compared to its IC_{50} (8.23 µg/ml) value.

Table (8) Radical Scavenging Activity of Various Concentrations of EtOH Extract and IC_{50} Value of Ye-yo

Sample Concentration (µg/ml)	Absorbance	% Inhibition	IC_{50} (µg/ml)
300.00	0.078	86.75	
150.00	0.284	51.78	
75.00	0.334	25.5	132
37.50	0.376	36.13	
18.75	0.387	34.29	

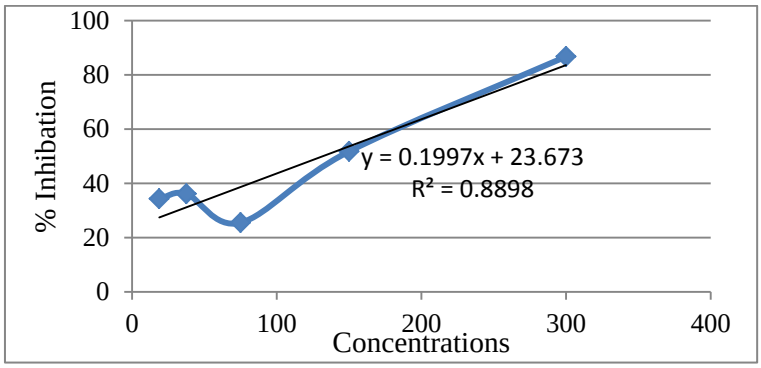

Figure (4) Plot of Concentration Vs % Inhibition of EtOH Extract of Sample

Conclusion

The leaves of *Morinda citrifolia* Linn. were used for chemical investigation. The phytochemical analysis revealed that the sample contains the valuable phytochemical constituents such as alkaloids, glycosides, lipophenols, phenols, polyphenols, reducing sugars, saponins, steroids, tannins and terpenes. Elemental analysis indicated that chlorine is the highest amount in the leaf sample followed by calcium and potassium.

The presence of protein in the sample was investigated by qualitative tests. The protein content in the sample was observed as 14.71% and thus it is observed that it contains the

largest amount of amino acids. The carbohydrate and fat contents in the leaves of Ye-yo were found to be 3.84% and 4.2%. In addition, it was found that the sample contains 38.32% of moisture and 1.32% ash contents respectively. So the leaves of *Morinda citrifolia* contain adequate amounts of basic nutrients to maintain human health and it is valuable to be used as nutrients and traditional medicines.

The antioxidant activity of the sample was determined using DPPH (1,1-diphenyl-2-picryl-hydrazyl). The antioxidant activity of the sample was lower than that of standard ascorbic acid from the comparison of their IC_{50} values (132 and 8.23 µg/ml).

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