

INVESTIGATION ON THE NUTRITIONAL VALUES AND ANTIOXIDANT ACTIVITY OF LADY FINGER

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ABSTRACT

The fruit of *Hibiscus esculentus* Linn. (Lady finger) collected from Sintkaing Township, Mandalay Region was selected for the chemical investigations. Firstly, the phytochemical investigation of the sample was tested to study the phytochemical constituents. It consists of alkaloid, flavonoid, steroid, terpene, polyphenol, glycoside, phenolic, tannin, saponin, carbohydrate and lipophenol. The nutritional values of the sample were tested by using various methods. The sample consists of ash (4.45%), moisture (16.1%), oil (0.53 %), nitrogen (0.6445%), protein (4.0281 %), carbohydrate (5.8 %), fiber (9.5 %). Then, the mineral contents of the sample were determined by using EDXRF method. The amount of potassium is the highest percent (2.409 %) in this sample. The vitamin C content of the sample is 20.8 mg/ 100 g by using iodometric titration method. Furthermore, the antimicrobial activities of three solvents extracts of sample were determined by agar-well diffusion method on six selected organisms namely *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans*, *E. coli*. Ethyl acetate extract gives rise to high activities on all tested organisms. Finally, the antioxidant activity of ethanol extract of the sample was determined by using DPPH Radical Scavenging Assay. The IC₅₀ values of ethanol extract of the sample was found to be 66.48 mg/ml.

Keywords : Lady finger, Nutritional values, Mineral contents, antioxidant activity

1. INTRODUCTION

In nature, many plants and plants seed provided source of medicine at the earlier times. Plants have proven to be the most useful in curing diseases and provide on important source of pharms and medicine. The medicinal importance of these plants lies in some chemical substances that produce a distinct physiological action on the body of human. Many plants are cheaper and more simply to get to most people especially in the developing countries and these plants have lower incidence of side effect after use. Due to this reason they are used worldwide. In the current study, we have chosen one plant such as *Hibiscus esculentus* Linn. (Lady Finger). These fruits are rich in vitamins, calcium, potassium and other mineral matters. Its mature fruit and stems contain crude fibre, which is used in the paper industry. Lady finger is a very useful vegetable and inexpensive medicine, as it is available practically round the year in India. It is a very useful to ladies close to menopause. Lady finger has a higher concentration of antioxidant compounds than other high-antioxidant vegetables as well as many high-antioxidant fruits.(website-1)

Botanical Description (website-2)

Family name	: Malvaceae
Botanical name	: <i>Hibiscus esculentus</i> Linn.
Myanmar name	: Yon-padi
English name	: Okra, Lady finger
Part of use	: Fruit



Figure 1 The Plant and Fruits of Lady Finger (*Hibiscus esculentus* Linn.)

2. MATERIAL AND METHODS

2.1 Sample Collection

The fruit of Lady Finger sample were collected from Sintkaing Township, Mandalay Region. The sample was then dried in shade, cut into pieces and grounded by electric grinder.



Figure 2 The Pieces and Powder of Fruits of Lady Finger

2.2 Phytochemical Investigation of Lady Finger (Aparana Buzarbarua)

2.2.1 Preliminary Phytochemical Analysis

Phytochemical tests were carried out to detect the presence or absence of organic constituents in the fruit of Lady Finger.

2.3 Determination of Moisture Content of the Fruits of Lady Finger Procedure

The moisture content of Lady finger sample was determined by oven-drying method. The sample (1 g) was placed in pre-weighed porcelain crucible. Then it was kept in an oven at 101°C for 30 min. It was cooled in desiccator and weighed again. The process of heating, cooling and weighing was repeated until a constant weight was achieved.

$$\text{Moisture content (\%)} = \frac{\text{loss in weight (g)}}{\text{weight of sample (g)}} \times 100 \quad (1)$$

2.4 Determination of Ash Content of the Fruits of Lady Finger

The air dried sample (2 g) was weighed and placed in a preheated, cooled and weighed the 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.

$$\text{Ash \%} = \frac{\text{Residue wt (g)}}{\text{Sample wt (g)}} \times 100 \quad (2)$$

2.5 Determination of Mineral Content from the Fruits of Lady Finger

Mineral contents of the fruits of *Hibiscus esculentus* Linn (Lady finger) were measured at the Department of Physics, University of Mandalay, by applying EDXRF (Energy Dispersive X-Ray Fluorescence Spectroscopy) method.

2.6 Determination of Oil Content of the Fruits of Lady Finger

20 g of sample powder was introduced into a thimble and a piece of cotton wool was placed the open end of the thimble. The thimble was then placed in a Soxhlet apparatus. Then the apparatus was fixed with round-bottomed flask (1000 ml) containing petroleum ether (b.p 60-80°C) (250 ml). The extraction flask was heated on a water bath for 10 to 12 hours. After extraction, the thimble containing the meal cake was placed in an oven until no odour of ether remains.

$$\text{Oil \%} = \frac{\text{weight of oil (g)}}{\text{weight of sample (g)}} \times 100 \quad (3)$$

2.7 Determination of Nitrogen and Protein Contents by using Kjeldahl's Method

About 5 g of sample and analar sodium sulphate (ca. 5 g), anhydrous copper sulphate (ca. 0.25 g) and sulphuric acid (98 %, 12.5 ml) were added placed in the Kjeldahl's digesting flask. The flask was heated till the mixture become colourless. It was allowed to cool and 10 ml of distilled water was carefully added with frequent shaking. The digested solution was poured into the flask together with 40 % sodium hydroxide (100 ml) to make mixture strongly alkaline. The evolved ammonia was distilled off and the distillate was titrated with standard sodium hydroxide solution, using phenolphthalein as an indicator. A blank determination was carried out exactly as above, but instead of sample, 20 ml of distilled water was used.

2.8 Determination of Water-Soluble Carbohydrate of the Fruits of Lady Finger

1 ml of sample solution and six standard sugar solutions containing 10, 20, 60, 80 and 100 µg 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 also prepared with 1 ml of distilled water instead of sugar solution. 5 ml of 96 % sulphuric acid was again added to each tube. After ten minutes, the tubes were reshaken and placed in water bath at 25°C-30°C for twenty minutes. The yellow orange colour 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 µg per ml. Using this standard curve, the concentration of glucose in the sample was calculated.

2.9 Determination of Crude Fibre Contents of the Fruits of Lady Finger

About 2 g of the defatted sample was placed into a 500 ml flask and then 200 ml of 1.25 % sulphuric acid solution was added. The flask was connected with reflux condenser and digested for about 30 minutes. After 30 minutes the boiling solution with insoluble materials was filtered. Then the residue was washed down into the flask with 200 ml of 1.25 % sodium hydroxide solution and boiled for 30 minutes. After boiling, the residue was filtered again and washed with 10 ml of 90 % ethanol. The residue was heated in an oven at 100°C until the constant weighed was obtained.

$$\text{Fibre \%} = \frac{\text{weight of fibre}}{\text{weight of sample}} \times 100 \quad (4)$$

2.10 Determination of Vitamin C Content of the Fruits of Lady Finger

2.10.1 Determination of Ascorbic Acid Content

25 ml of fresh juice sample was taken into a 250 ml conical flask. 10 drops of 1 % starch indicator solution were added and then titrated with standard iodine solution until blue-black colour appeared. Then the amounts of ascorbic acid contents were calculated.

2.11 Antimicrobial Activities of Fruits of Lady Finger

The antimicrobial activities of various extracts of Lady finger sample were done by Agar-well diffusion method on six selected organisms in Central Research and Development Centre (CRDC), Insein, Yangon.

2.12 Determination of Antioxidant Activity of Fruits of Lady Finger (Nonald R. Buhler)

In this experiment, 1-1 diphenyl -2- picrylhydrazyl (DPPH) powder was used as stable free radical. Ascorbic acid was used as standard antioxidant and ethanol (analar grade) was used as solvent. The absorbance was determined at 517 nm wavelength.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Test of Fruits of Lady Finger

Fruits of Lady Finger were tested by phytochemical screening and the results were shown in Table (1).

Table 1 Result of Phytochemical Constituents of Fruits of Lady Finger

No.	Constituents	Extract	Reagents used	Observation	Result
1.	Alkaloid	1 % HCl	(i) Dragendroff's reagent (ii) Wagner's reagent	(i) Orange color solution (ii) Reddish brown color solution	+ +
2.	Flavonoid	95 % ethanol	Conc: HCl, Mg	Pink colour solution	+
3.	Steroid	95 % ethanol	Acetic anhydride, conc: H ₂ SO ₄ , CHCl ₃	Green colour solution	+
4.	Terpene	95 % ethanol	CHCl ₃ , conc: H ₂ SO ₄	Pink colour ppt	+
5.	Polyphenol	95 % ethanol	1 % FeCl ₃ + 1 % K ₃ [Fe(CN) ₆]	Greenish blue colour solution	+
6.	Glycoside	water	10 % lead acetate	White ppt	+
7.	Phenolic compound	water	10 % FeCl ₃	Greenish blue colour solution	+
8.	Reducing sugar	water	Benedict's solution	no brick-red ppt	-
9.	Saponin	water	shake	Froth	+
10.	Lipophenol	water	NaOH, 0.5 M KOH	Deep colour solution	+
11.	Tannin	water	10 % FeCl ₃ , dil H ₂ SO ₄	Yellowish brown ppt	+
12.	Carbohydrate	ethanol	Fehling's (A) + (B)	Yellow ppt	+

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

According to this table, the fruits of Lady Finger contain alkaloid, flavonoid, steroid, terpene, polyphenol, glycoside, phenolic compound, saponin, lipophenol, tannin and carbohydrate.

3.2 Determination of Moisture Content of Lady Finger

The results of Moisture Content of fruits of Lady Finger were shown in Table (2).

Table 2 Results of Moisture Content of Lady Finger

No. of experiment	Wt. of sample (g)	Wt. of loss (g)	Moisture (%)
1.	1.00	0.14	14
2.	1.00	0.16	16
3.	1.00	0.16	16

3.3 Determination of Ash Content of Lady Finger

The results of Moisture Content of fruits of Lady Finger were shown in Table (3).

Table 3 Results of Ash Content of Lady Finger

No. of experiment	Wt. of sample (g)	Wt. of loss (g)	Ash (%)
1.	2.00	0.89	89
2.	2.00	0.87	87
3.	2.00	0.89	89

3.4 Elemental Composition of Fruits of Lady Finger by EDXRF Spectroscopy

The mineral content for fruits of Lady Finger were determined by using EDXRF method at Department of Physics, Mandalay University. This results shown in Table (4).

Table 4 The Results of Elemental Composition in Lady Finger by EDXRF

No.	Elements	Symbols	Results (Mass %)
1.	Potassium	K	2.409
2.	Calcium	Ca	1.058
3.	Phosphorus	P	0.556
4.	Iron	Fe	0.027
5.	Zinc	Zn	0.008
6.	Manganese	Mn	0.004
7.	Copper	Cu	0.001

From these data, some of elements (K, Ca, P, Fe, Zn, Mn, Cu, Se) were found in the fruits of Lady Finger. Among them, potassium is the highest amount in the sample.

3.5 Determination of Oil Content of the Fruits of Lady Finger

Table 5 Results of Oil Content of Lady Finger

No.	Wt. of sample (g)	Wt. of oil (g)	Oil content (%)
1.	20.0000	0.1060	0.53
2.	20.0000	0.1080	0.54
3.	20.0000	0.1060	0.53

3.6 Determination of Nitrogen Content and Protein Content of Fruits of Lady Finger

Table -6 Results of Nitrogen and Protein Content of Lady Finger

No.	Nitrogen content (%)	Protein content (%)
1.	0.6444	4.0277
2.	0.6444	4.0277
3.	0.6445	4.0281

3.7 Determination of Water-Soluble Carbohydrate of the Fruits of Lady Finger

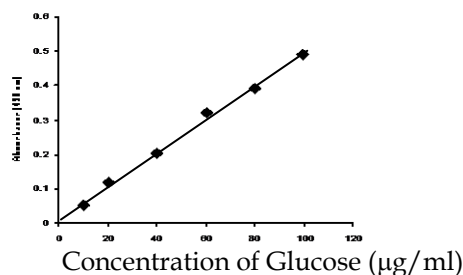


Figure 3 Standard Calibration Curve for Water Soluble Carbohydrate

Table 7 Results of Soluble Carbohydrate Content of Fruits of Lady Finger

Experiment	Soluble carbohydrate content (%)
1.	5.9
2.	5.8
3.	5.8

3.8 Determination of Crude Fibre Content of the Fruits of Lady Finger**Table 8 Results of Crude Fibre Contents of Lady Finger**

No.	Wt. of sample (g)	Wt. of fibre (g)	Crude fibre (%)
1.	2.00	0.21	10.5
2.	2.00	0.19	9.5
3.	2.00	0.19	9.5

3.9 Determination of Vitamin C Content of Fruits of Lady Finger**Table 9 Results of the Titration of 25 ml Fresh Juice of Lady Finger with 0.01M Iodine Solution (Indicator-starch)**

No.	Initial volume (cm ³)	Final volume (cm ³)	Volume used (cm ³)
Rough	0	4	4
1.	4	7.3	3.3
2.	7.3	10.5	3.2
3.	10.5	13.7	3.2

According to the experiment, the amount of ascorbic acid (vitamin C) in Lady finger is (20.8 mg/100 g) of the fresh sample.

3.10 Antimicrobial Activities of Fruit of Lady Finger

The extracts of fruits of Lady finger with various solvents such as n-hexane, ethyl acetate and ethanol were taken and examined the antimicrobial activities.

Table 10 Antimicrobial Activities of Fruits of Lady Finger

Sample	Solvents	Inhibition zone					
		I	II	III	IV	V	VI
Lady's finger	n-hexane	-	-	-	-	-	-
	EtOAc	25 mm (+++)	21 mm (+++)	25 mm (+++)	25 mm (+++)	21 mm (+++)	24 mm (+++)
	EtOH	-	-	-	-	-	-

Agar-well ~ 10 mm			Organisms
10 mm ~ 14 mm	(+)	I	= <i>Bacillus subtilis</i>
15 mm ~ 19 mm	(++)	II	= <i>Staphylococcus aureus</i>
20 mm above	(+++)	III	= <i>Pseudomonas aeruginosa</i>
(+)	= low activity	IV	= <i>Bacillus pumilus</i>
(++)	= medium activity	V	= <i>Candida albicans</i>
(+++)	= high activity	VI	= <i>E. Coli</i>

According to the experimental data, EtOAc extract of sample show high activity on *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans*, *E. coli*. n-hexane and EtOH extract show no activity on six selected organisms.

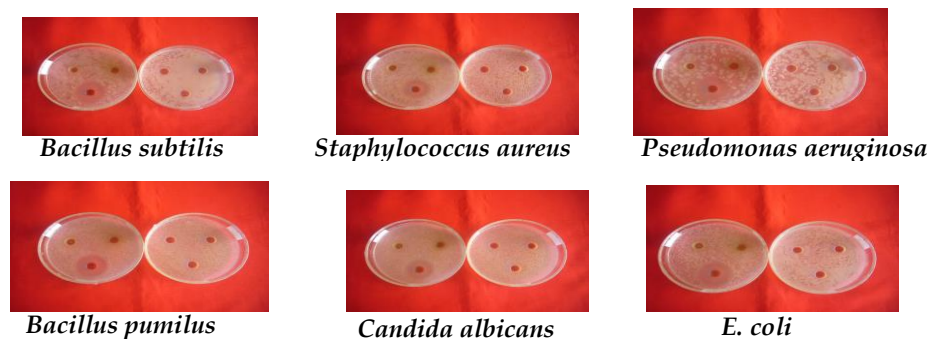


Figure 4 Antimicrobial Activities of Fruit of Lady Finger

3.11 Determination of Antioxidant Activity of the Standard Ascorbic Acid

The results of antioxidant activity using DPPH assay in standard ascorbic acid was shown in Table (11).

Table 11 Percent Inhibition of Various Concentration of Standard Ascorbic Acid

Sample Concentration ($\mu\text{g/ml}$)	Mean Absorbance	Mean % inhibition	IC_{50} ($\mu\text{g/ml}$)
50	0.297	68.50	
25	0.350	61.61	
12.5	0.483	48.78	17.99
6.25	0.562	40.41	
3.125	0.608	35.52	

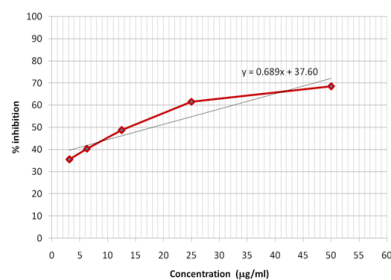


Figure-5 Plot of % Inhibition Vs Concentration of Standard

IC_{50} value was calculated by using linear regressive equation.

3.12 Determination of Antioxidant Activity of Ethanol Extract of Lady Finger by DPPH Radical Scavenging Assay

The results of antioxidant activity using DPPH assay in fruits of Lady finger was shown in Table (12).

Table 12 Percent inhibition of Various Concentration of Sample

Concentration (mg/ml)	Mean Absorbance	Mean % inhibition	IC ₅₀ (mg/ml)
100	0.244	62.34	66.48
75	0.261	59.77	
50	0.356	45.06	
25	0.486	25	

IC₅₀ value was calculated by using linear regressive equation.

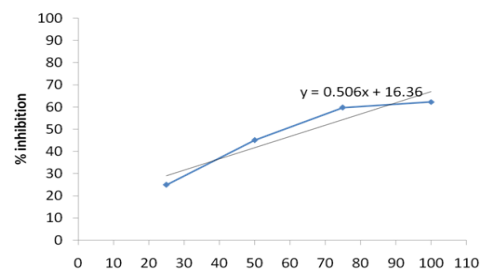


Figure-6 Plot of % Inhibition Vs
Concentration of Fruits of Lady Finger

According to this table, the antioxidant activity of fruits of Lady finger was determined in DPPH free radical scavenging assay. In DPPH screening assay, IC₅₀ value of fruits of Lady finger was found to be 66.48 mg/ml. It was very much higher than that of standard ascorbic acid (IC₅₀ 17.99 µg/ml). So, the sample extract has lower antioxidant activity than standard ascorbic acid.

4. CONCLUSION

The fruits of Lady finger was collected from Sintkaing Township, Mandalay Region. The phytochemical investigation of the fruits of *Hibiscus esculentus* Linn. was done. The fruits of Lady finger consists of alkaloids, flavonoids, steroid, terpene, polyphenol, glycoside, phenolic, tannins, saponin, carbohydrate, lipophenol and the absence of reducing sugar. The elemental contents of sample were determined by EDXRF method. The amount of potassium is the highest percent (2.409 %) in this sample. And other constituents of minerals are calcium, phosphorous, iron, zinc, manganese and copper. The nutritional composition of the fruits of Lady finger such as ash (89 %), moisture (16 %), oil (0.53 %), nitrogen (0.6445 %), protein (4.0281 %), carbohydrate (5.8 %) and fiber (9.5 %). The vitamin C content of the sample is 20.8 mg/100 g. Moreover, antimicrobial activities of the fruit of Lady finger in three solvents were also determined by agar well diffusion method on six selected organisms namely, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans*, *E. coli*. Ethyl acetate extracts gives rise to high activities on all tested organisms. Ethanol and n-hexane extracts showed no activity. The antioxidant activity of the sample was determined at University of Mandalay. Percent inhibition of standard ascorbic acid and IC₅₀ value of ethanolic extract of the sample were determined by using DPPH. The IC₅₀ values of ethanol extract of the sample was found to be 66.48 mg/ml. It was very much higher than that of standard ascorbic acid (IC₅₀ = 17.99 µg/ml). So, the sample extract has lower antioxidant activity than standard ascorbic acid. From the experimental data, the fruit of *Hibiscus esculentus* Linn. contains valuable chemical constituents and nutrients for human.

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REFERENCES

- [1] Dr. nonald R. Buhler, "Antioxidant Activities of Flavonoids" November, 2000.
- [2] Aparana Buzarbarua, Dr, "A Textbook of Practical Plant Chemistry" 1st Edition, 2000

Website

1. <https://en.m.wikipedia.org/wiki/Okra/>
2. <https://plantvillage.psu.edu/topics/.../infos>