Determination of Nutritional Values And Antioxidant Activity Of Seed Of *Terminalia catappa* L.

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Abstract

In this research, the seed of *Terminalia catappa* L. was collected from Mandalay University campus on December, 2017 to determine nutritional values and antioxidant activity from it. Firstly, the phytochemical screening of the seed of *Terminalia catappa* L. was carried out. The mineral content of the sample was determined by using Energy Dispersive X-Ray Fluorescence (EDXRF) spectroscopy method. The examination of moisture and ash contents was tested using oven and muffle furnance. Then, the oil content in the sample was determined by Soxhlet extraction method. The determination of chemical constituents in oil of the seed of *Terminalia catappa*L was performed by gas chromatography. The crude fibre content in the sample was tasted using strong acid and strong alkali. The determination of water soluble carbohydrate in the sample was performed by phenol-sulphuric acid method. The protein content in the sample was examined using Kjeldahl's Analyzer. Finally, the antioxidant activity of the ethanol extract of seed of *Terminalia catappa* L. was investigated by DPPH Radical Scavenging Assay.

Keywords: nutritional values, phytochemical, EDXRF, moisture, antioxidant

Introduction

The nutritional value of food refers to its capacity to nourish the body with the substances needed to live and grow. The body relies on food for fuel and to obtain the chemical compounds it needs to function. The seven major types of nutrients are carbohydrates, fats, proteins, water, fiber, vitamins and minerals. The first five nutrients are considered macronutrients, which are the nutrients the body requires in relatively large quantities. The last two nutrients vitamins and minerals are considered micronutrients, which

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the body only needs in relatively small amount. The body primarily uses carbohydrates and fats as fuel to supply the body with the energy, or calories, it needs for activity (Nutrient data library/ London, 2005).

Antioxidants are naturally occurring plant substances that protect the body from damage caused by harmful molecules called free radicals. Antioxidants help to prevent oxidation which can cause damage to cells. They improve immune function and perhaps lower the risk for infection, cardiovascular disease, and cancer. Antioxidants exist as vitamins, minerals and other compounds in foods. A diet containing plenty of fruits and vegetables, whole grains and nuts can supply all the antioxidants our body needs. Diets rich in antioxidants can be very beneficial. **(**https:// und.edu>-files>docs>fact-sheets/antioxidants.pdf)

Terminalia catappa L. commonly called Tropical Almond is a large deciduous stately tree that thrives mainly as an ornamental tree in many tropical cities in the world. It has a large nutty fruits that taste very much like commercially Tropical Almonds. A fibrous shell surrounds the nut. The nut is edible. *Terminalia catappa* L. have been used as traditional medicines in both East and West African countries to treat infectious diseases. The leaves, bark and fruits are useful in the treatment of dysentery, rheumatism, cough and asthma. (Christian.A, et al., 2006). In this research, nutritional composition of seed of Tropical Almond was measured and the antioxidant activity of this seed was tested.

Botanical Description

Figure 1. Plant and Fruits of *Terminalia catappa* L.

Family name : Combretaceae

Scientific name : *Terminalia catappa* Linn

English name : Tropical Almond

Myanmar name : Badan

Material and Methods

Sampling

The fruits of Tropical Almond were collected from Mandalay University campus. They were pounded with stone to obtain its seed. They were dried at room temperature. They were powdered and obtained the sample.

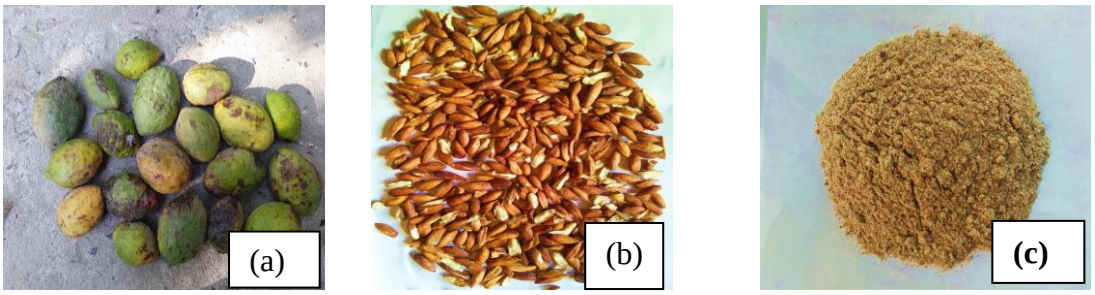

Figure 2. (a) Fruits, (b)Seeds and (c) Powder of Tropical Almond Seeds

Preliminary Phytochemical Test

Phytochemical tests were done on the various extracts of seeds of Tropical Almond according to procedures. (Harbone, J. B, 1973, Sofowora A, 1993)

Determination of Moisture Content

The air dried seeds of Tropical Almond 10g was accurately weighed and then dried in an oven for about 2 hr at 101˚C. It was then removed from the oven and cooled in a desiccator at room temperature and weighed. The procedure was repeated until the constant weight was obtained. (AOAC, 2000)

Determination of Mineral Contents

Mineral contents of the seeds of Tropical Almond were measured at Department of Chemistry, Monywa University by applying EDXRF (Energy Dispersive X-Ray Fluorescence Spectroscopy) method.

Determination of Oil Content

Tropical Almond seeds powder (30g) accurately weighed were introduced into a thimble and a piece of cotton wool was placed the open end of the thimble. The thimble containing sample was then placed in a soxhlet apparatus. Then the apparatus was fixed with round- bottomed flask (500ml) containing petroleum ether (b.p 40˚ - 60˚ C) (350ml). The extraction flask was heated on the water bath for 8 hours at the boiling point of petroleum ether. After the extraction was completed, most of the ether extract was distilled off. The content in the flask were carefully transferred to a weighed specimen tube. The remaining ether in the specimen tube was vapourized until constant weight was obtained. (AOAC, 2000)

Determination of Ash Content

The defatted sample 10(g) was weight and placed in a preheated cooled and weighed the crucible. The crucible was heated carefully in the furnace at 550℃ for 2 hours burned off without flaming or until all the carbon was eliminated. When the materials are converted to white ash powder, the crucible was cooled at room temperature in a desiccator and weighed again. To obtain a constant weight, the heating, cooling and weighing were repeated. (AOAC, 2000)

Determination of Nitrogen and Protein Content

About 5g of defatted sample was weighed and placed in the Kjeldahl's digesting flask. About 5g 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 5ml of distilled water was carefully added with frequent shaking. (AOAC, 2000)

(b) Distillation

The Kjeldahl's distillating apparatus was setup, taking care that the tip of the condenser extended below the surface of the standard sulphuric acid solution 50ml in the receiver. The digested solution was poured into the flask together with 100ml of 40% sodium hydroxide to make mixture strongly alkaline. The evolved ammonia was distilled off.

(c) Titration

The distillate was titrated with standard sodium hydroxide solution using methyl orange as an indicator. A blank determination was carried out without sample using all the reagents as in the case of sample. The nitrogen content of sample can be calculated by using following formula.

$$\text{Nitrogen}(\%) = \frac{(V_2 - V_1) \times N_A \times 0.01401 \times 100}{W}$$

Where, V_2 = the volume of acid used in the test (in millimeter)

V_1 = the volume of acid used in the blank (in millimeter)

N_A = the concentration of acid used (in Normality)

W = the weight of sample (in gram)

Protein (%) = Nitrogen content × 6.25

Where, 6.25 = a factor of protein – nitrogen conversion

Determination of Crude Fibre Contents

About 2g of defatted sample was placed into a 500ml flask and then 200ml of 1.25% sulphuric acid solution was added. The flask was connected with reflux condenser and digested for about 30 minutes. The flask was rotated every few minutes in order to mix the contents and to remove particles from the side of flask. After 30 minutes the boiling solution with insoluble materials was filtered. The insoluble residue was washed with the hot water in order to free from acid. Then the residue was washed down into the flask with 200ml of 1.25% sodium hydroxide solution and boiled for 30 minutes. After boiling, the residue was filtered again and washed with 15ml of 95% ethanol. After washing the residue was introduced into a crucible and it was heated in an oven at 100° C until the constant weighed was obtained. Finally, the substance in the crucible was incinerated in a muffle furnace dull red until the all carbonaceous matter had been removed. The contents with the crucible were cooled and weighed.

This procedure, such as heating, cooling and weighing were made until a constant weight was obtained. The loss in weight during the incineration was referred to as crude fibre. (AOAC, 1990)

Determination of Water-Soluble Carbohydrate

The water soluble carbohydrate content was also determined by phenol-sulphuric acid colourimetic method in terms of glucose. (James N. B., 2009).

Preparation of Sample Solution : The defatted sample powder 0.1g was dissolved in (100ml) of hot water and shaken for ten minutes. (1ml) of this solution was then dilute to (10ml) with water and this solution was taken as the sample extract.

Preparation of Standard Sugar Solution: Glucose 100mg (0.1g) was exactly weighed and dissolved in (100ml) of distilled water. 1,2,4,6,8 and 10ml of these solution were drawn out and put in each (100ml) volumetric flask and diluted to the mark with distilled water. These solutions contained 10, 20, 40, 60, 80 and 100μg of glucose per ml respectively.

Procedure

The sample solution (1ml) and six standard sugar solutions containing 10, 20, 40, 60, 80 and 100 μg of glucose per ml were put in each test tube. 1ml of 5% phenol solution was also added to each test tube and mixed. A blank also prepared with 1ml of distilled water instead of sugar solution. 5ml of 96% sulphuric acid was again added to each tube so that the stream 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 colour was stable for several hours. Absorbance was 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.

Determination of Antioxidant Activity of Seed of Tropical Almond

The antioxidant activity of ethanol extract of seed of Tropical Almond was determined by DPPH (1, 1-Diphenyl-2-picryl-hydrazyl) Radical Scavenging Assay in Department of Chemistry, University of Mandalay. (Manzocco *et al*., 1998)

Determination of Chemical Compositions of Tropical Almond Seed Oil

(a)Esterification of Tropical Almond Seed Oil: Esterification was carried out by refluxing the mixture of methanol and Tropical Almond seed oil in the ratio of 2:1 in the water bath. Concentrated sulphuric acid was used as the catalyst and added drop by drop (1 ml) into the reacting mixture. After esterifying the oil for one hour, the flask was cooled to room temperature. Cooled reaction mixture was poured into a separating funnel containing the NaCl solution and n-hexane. The flask was sealed and shaken vigorously for 2 minutes to make sure the 2 layers are mixed very well. The flask was vented occasionally to release the pressure. The layers were allowed to settle for 5 minute. The hexane layer (the upper layer) was decanted into the beaker containing 2 grams of sodium sulfate. 25 mL of hexane was added to the salt/methanol solution and any remaining fatty acid methyl esters were extracted from the salt water and methanol. The second n-hexane extract was combined with the first extract. (Hammond, Earl G., 2005)

(b) Chemical Compositions in Ester of Oil of Seed of Tropical Almond : Chemical compositions in ester of oil of Tropical Almond seed was measured by gas chromatography and mass spectrometry (GCMS) at the Department of Chemistry, University Research Center (URC), University of Mandalay (David S.O., et al, 2011).

Results and Discussion

Results of phytochemical tests in seed of Tropical Almond

From the experimental results, the seed of Tropical Almond consists of alkaloids, flavonoid, polyphenol, terpene, glycoside, phenolic compound, reducing sugar, sponin, tannin, lipophenol compounds respectively. However, steroid compound was not detected.

Results of Elemental Composition of Seed of Tropical Almond

Relative abundance (%) of elemental composition in the sample was shown in Table 1.

Table 1. Elemental Composition of Seed of Tropical Almond

No	Elements	Symbols	Relative Abundance (%)
1	Potassium	K	0.561
2	Phosphorus	P	0.554
3	Calcium	Ca	0.213
4	Sulfur	S	0.209
5	Iron	Fe	0.008
6	Zinc	Zn	0.004
7	Copper	Cu	0.003
8	Manganese	Mn	0.002

According to Table 1, the seed of Tropical Almond contains essential element for human body such as potassium, phosphorus and calcium, sulfur, iron, zinc, copper, and manganese.

Results of Nutritional Values of Seed of Tropical Almond

The results of nutritional values of seed of Tropical Almond were shown in Table 2.

Table2. The results of nutritional values of seed of Tropical Almond

No	Nutritional Values	Content (%)
1	Moisture	2.95 ± 0.05
2	Ash	4.95 ± 0.05
3	Oil	51.58 ± 0.02
4	Crude Fibre	6.84 ± 0.16
5	Protein	26.06 ± 0.06
6	Soluble Carbohydrate	7.27 ± 0.01

Results of Antioxidant Activity of Seed of Tropical Almond

Table 3. % Inhibition and IC_{50} Value of Standard Ascorbic Acid

Standard Concentration µg/mL	DPPH Concentration		% inhibition	IC_{50} µg/mL
	Initial Absorbance	Final Absorbance		
50	0.5140	0.0511	90.0584	15.528
25	0.5140	0.1250	75.6809	
12.5	0.5140	0.2240	56.4202	
6.25	0.5140	0.3260	36.5759	
3.125	0.5140	0.4210	18.0934	

Table 4. % Inhibition and IC_{50} value of Seed of Tropical Almond

Standard Concentration µg/mL	DPPH Concentration		% inhibition	IC_{50} µg/mL
	Initial Absorbance	Final Absorbance		
100	0.624	0.282	54.808	84.956
50	0.624	0.386	38.141	
25	0.624	0.444	28.846	
12.5	0.624	0.483	22.596	
6.25	0.624	0.506	18.910	

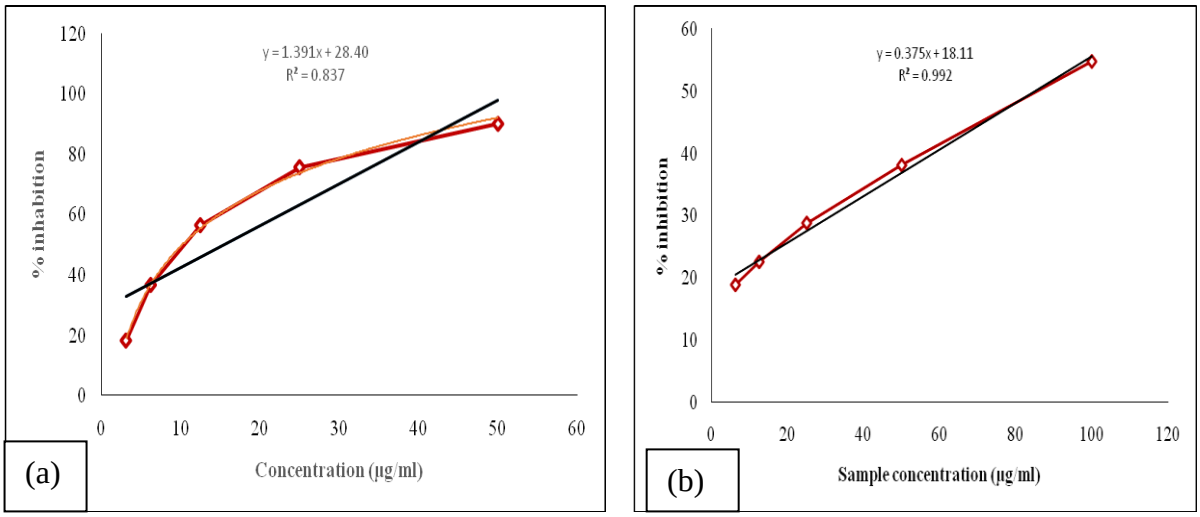

Figure 3. % Inhibition of Different Concentration of (a) Standard Ascorbic Acid (b) Sample

IC_{50} value (50 % inhibition concentration) was calculated by using linear regressive equation.. IC_{50} value of standard ascorbic acid was found to be 15.528 µg/mL. IC_{50} value of seed of Tropical Almond was 84.956 µg/mL. The seed of Tropical Almond has lesser antioxidant activity than the standard ascorbic acid.

Results of Chemical Compositions of Tropical Almond Seed Oil

The Chemical Compositions in oil of Tropical Almond Seed were measured by GC MS chromatography. The Tropical Almond seed oil could contain palmitic acid, ascorbic acid 2,6-dihecadecanoatenoic acid, linoleic acid, butyl 9,12- octadecadienoic acid, oleic acid, stearic acid, elaidic acid and octadecanoic acid.

Conclusion

In this research, the seed of Tropical Almond was selected for chemical analysis. The phytochemical test indicated that the seed of Tropical Almond contained the valuable phytochemical constituents, such as alkaloids, flavonoid, glycoside, terpene, saponin, reducing sugar, phenolic, polyphenol, tannin, and lipophenol compounds.

The moisture content of sample was found to be 2.95 %. This indicates that the seeds will have good keeping properties. The ash content of Tropical Almond seed was 4.95 %. Ash content signifies the level of mineral present in the sample. Minerals are important in the diet because of their various functions in the body. The seed of Tropical Almond contained higher amount of potassium, phosphorus and calcium than other elements.

The present study shows that the seed of Tropical Almond is a good source of oil. From the results of gas chromatography, the Tropical Almond seed oil could contain palmitic acid, ascorbic acid 2,6-dihecadecanoatenoic acid, linoleic acid, butyl 9,12- octadecadienoic acid, oleic acid, stearic acid, elaidic acid and octadecanoic acid. The protein content of seed was found to be 26.06 %. The results of the analysis also show that the seed consist of some amount of carbohydrate and crude fibre.

The antioxidant activity of the seed of Tropical Almond was measured by DPPH assay method. As a result, the IC_{50} value of seed of Tropical Almond is 84.956 µg/mL which compares with the standard ascorbic acid IC_{50} 15.528 µg/mL. The seed of Tropical Almond has lower antioxidant activity than the standard ascorbic acid but it has significant antioxidant activity.The results of this present study inform that the seeds of Tropical almond have valuable chemical constituents.

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References

AOAC,1990 “Official Methods of Analysis of Association of Official Analytical Chemists”.

AOAC,2000 “Official Methods of Analysis of Association of Official Analytical Chemists”.

Christian, A., and Mark, E. Ukhun., 2006 “Nutritional Potential of the Nut of Tropical Almond (*Terminalia catappa* L.)”, *Pakistan Journal of Nutrition* 5 (4), 334.

David, S.O., Zelda Penton; Fulton G. Kitson (17 May 2011), “Gas Chromatography and Mass Spectrometry: A Practical Guide”, Academic Press. ISBN 978-0-08-092015-3.

Hammond, Earl G. and Wang., 2005, “Method of Converting Free Fatty Acids to Fatty Acid Methyl Esters”, *Iowa State University Patents. 1.*

Harbone, J. B. 1973, “Phytochemical methods: A Guide to Modern Techniques of Plant Analysis”, New York, Chapman and Hall

James N. BeMiller., 2009, “Carbohydrate Analysis”, IN 4790-2009, USA

Manzocco, L., Anese, M., Nicoli, M.C., 1998. Antioxidant properties of tea extracts as affected by processing. Lebens-mittel-Wissen-schaft Und-Technologie 31; (7-8), 694-698.

Nutrient Deta Library / London, UK, Europe 29092005.

Sofowora A., 1993, “Medicinal Plants and Traditional Medicine in Africa”, John Wiley and Sons Limited, 2:96-106.

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https:// und.edu>-files>docs>fact-sheets/antioxidants.pdf