Phytoconstituents, Nutritional Values and Elemental Analysis of Seeds of *Terminalia Catappa* L. (Almond)

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Abstract

This research deals with phytochemicals, nutritional values and elemental content of seeds of almond (*Terminalia catappa* L.). The preliminary phytochemicals investigation revealed the presence of many valuable phytochemicals such as alkaloids, α-amino acids, carbohydrates, glycosides, flavonoids, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins and terpenoids. Nutritional properties including moisture, ash, fibers, fats, proteins and carbohydrate content in the seeds were also estimated by standard methods. The seeds sample was found to contain 4.16 % of moisture, 3.99 % ash, 25.18 % of protein, 3.46 % of dietary fibers, 50.14 % of crude fats, 13.07 % of carbohydrates, and 604.26kcal /100 g of energy value based on dried sample. Mineral elemental compositions were determined by Energy Dispersive X-ray Fluorescence Spectroscopy (ED XRF) method. It was found to have the highest content of K and P where as minor components of S, Ca, Fe, Zn, Cu and Mn according to ED XRF analysis.

Keywords: *Terminalia catappa*, phytochemicals, nutritional values, elemental contents

Introduction

Terminalia catappa L. (Almond) is a well-known and one of the most plentiful plants in all over Myanmar. Due to the shade and seed of the plant, it is grown in many places in the whole country. In addition to the shade of the tree, many almond have been planted on the campus of West Yangon University to provide shade and eye-catching. Besides, the seeds of the fruit of the almond, their sweet taste attract everyone who has ever tasted it. Therefore, this research was conducted to scientifically study whether it is suitable for those who consume almond seeds.According to the literature survey, almond is a large tree that grows mainly in the tropical regions of Asia, Africa and Australia (Pankaj and Robert, 2008).This is also identified by common English names such as; Country-almond, Indian-almond, Sea-almond, Tropical almond (USDA, 2016). *Terminalia catappa* L. (Tropical almond) belonging to the family Combretaceae. It is planted extensively in the tropics for shade and ornamental purposes, especially in parks, along avenues, as well as home gardens (Mbah *et al*., 2013). The fruit of the tree is a drape 5-7 cm long and 3-5.5 cm broad, green at first, then yellow and red when ripe. The fruit contains a single seed, with sweet edible fibrous pulp which is eaten by children as forage snacks and there has been no report of associated toxicity with its consumption (Mbah *et al*., 2013; Arumugam *et al*., 2015). The aim of the present study is to screen the preliminary phytoconstituents, nutritional values and elemental analysis of seeds of almond.

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Botanical Aspects of *Terminalia catappa* L.

Botanical name - *TERMINALIA CATAPPA* L.
Myanmar name - Banda
English name - Tropical almond
Family - Combretaceae
Genus - *Terminalia*
Species - *catappa*
Part used - Seeds

Plant

Seeds

Figure 1. Plant and seeds of *T. catappa* (Almond)

Materials and Methods

Collection and Preparation of Sample

Terminalia catappa (Almond) seeds were collected from West Yangon University Campus, Yangon Region and identified at the Department of Botany, West Yangon University. After collection, the seeds of *T. catappa* were cleaned thoroughly with distilled water to remove any type of contamination. Then, the collected plant materials were shade dried to retain its vital phytoconstituents and subjected to size reduction. The powder of the samples were separately stored in air tight bottles and kept in a cool, dark and dry place until analyses were commenced.

Preliminary Phytochemical Tests of *Terminalia catappa* Seeds

The preliminary phytochemical such as alkaloids, α-amino acids, carbohydrates, cyanogenic glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids**,** tannins, terpenoids of seeds of *T. catappa* were carried out with standard phytochemical methods.

Test for alkaloids

Dried powder sample 2 g was boiled with 1 % hydrochloric acid for about 10 minutes and filtered after cooling. The filtrate was divided into two portions and tested with Dragendorff's reagent and Mayer's reagent, the following observations were found to be the presence of alkaloids. The first portion of the filtrate was treated with a few drops of Dragendroff's reagent, orange solution was formed. Finally, a few drops of Mayer's reagent were added to the second portion of the filtrate, white precipitate was formed .

Test for α - amino acids

About 2 g of dried powder sample was boiled with distilled water 25 mL for about 10 minutes and then filtered. An aliquot portion of filtrate was transferred to a filter paper with the help of micropipette and allowed to dry. Then the filter paper was sprayed with ninhydrin reagent and dried at 110° C in an oven for about 5 minutes. The presence of α- amino acid gives a purple spot appeared on the filter paper .

Test for carbohydrates

The powdered sample 3 g was boiled with 20 mL of distilled water for about 30 minutes and then filtered. The filtrate taken in a test tube was added with a few

drops of 10 % α- naphthol and the mixture was shaken. The test tube was then inclined at an angle 45° and about 1 mL of concentrated sulphuric acid was slowly added along the inner side of the test tube. Observation was made to see if red ring was formed at the interface of two liquids .

Test for cyanogenic glycosides

5 mL of water extract was placed into the test tube and about 5 drops of concentrated sulphuric acid were added and sodium picrate paper was trapped in the neck of the test tube by means of a loosely closed cock. The resultant mixture was heated by using a spirit burner. Observation was made to see if the paper turned brick red.

Test for flavonoids

Hydrochloric acid 5-10 drops was added to 1mL of alcoholic extract and then a small piece of magnesium ribbon was added. The appearance of reddish pink indicates the presence of flavonoids.

Test for glycosides

About 3g of powdered sample materials were boiled with distilled water 25 mL for about 10 minutes and filtered after cooling. The filtrate was tested with 10 % lead acetate solution. Observation was made to see if the solution turned to white precipitate.

Test for organic acids

5 mL of water extract of the selected medicinal plant was taken and treated with a few drops of bromocresol green indicator. Observation was made to see if blue colouration appeared .

Test for phenolic compounds

Sample 3 g was boiled with distilled water 30 mL for about 10 minutes and then filtered. The filtrate was treated three drops of freshly prepared (1:1) mixture containing 1% potassium ferricyanide and 1% ferric chloride solution. The solution gives deep blue color indicates the presence of phenolic compounds .

Test for reducing sugars

Sample 2 g was boiled with distilled water 25 mL for about 10 minutes and then filtered after cooling. The filtrate was tested with Benedict's solution. Brick-red precipitates indicate the presence of reducing sugars .

Test for saponins

Sample 1 g was put into a test tube followed by the addition of distilled water and the mixture was vigorously shaken for a few minutes. Formation of froth indicates the presence of saponins.

Test for starch

Sample 0.5 g was extracted with distilled water 5 mL and treated with a few drops of freshly prepared iodine solution. Deep blue colouration appeared .

Test for steroids

Petroleum ether extract of the powder was treated with a few drop of acetic anhydride. Concentrated sulphuric acid was added carefully down the inside wall of the test tube. Green colour indicates the presence of steroids.

Test for tannins

About 5 g of dried powder was boiled with distilled water and then filtered. The filtrate was treated with ferrous sulphate solution. Deep blue colour indicates the presence of tannin.

Test for terpenoids

3 g of dried powdered sample was extracted with chloroform 20 mL for about 30 minutes and filtered. The solution was added with acetic anhydride 2 mL,

followed by concentrated sulphuric acid 1 mL. Formation of red or pink colour indicates the presence of terpenoids.

Determination of Nutritional Values of *Terminalia catappa* Seeds

Nutritional values of almond seeds were determined at Department of Chemistry, West Yangon University.

Determination of moisture content

The moisture contents of the *T. catappa* seeds were determined by oven drying method. The moisture content of sample is the weight lost due to the evaporation of water at the drying temperature. Sample 5.00 g was placed in the moisture basin which had previously been dried and cooled in air-desiccators and accurately weighed. The basin with the sample was placed in an oven and dried at 105 °C for 30 minutes. It was then removed from the oven and cooled followed by in air-tight desiccators to room temperature and weighed. The procedure was repeated until a constant weight was obtained. The moisture content can be calculated by the following formula.

$$\text{Moisture (\%)} = \frac{\text{Loss in weight(g)}}{\text{Weight of sample(g)}} \times 100$$

The experiments were repeated three times and the average content is presented in Table 2.

Determination of ash content

The ash contents in the dried powder samples were determined by the method given in "The Chemical Analysis of Foods" (Joslyn, 1970). Carefully weighed dried powdered 10 g was placed in a preheated, cool and weighed porcelain crucible. The crucible was heated carefully on a hot plate until the organic matter burned off without the flame. The practically decomposed sample was then incinerated in a muffle furnace at 600 °C (873 K) until the resultant ash was uniform in colour (i.e. white or gray). The crucible containing ash was then cooled to room temperature in a desiccator and weighed. Heating, cooling and weighing were repeated until a constant weight was obtained. The percent content of ash in the sample could be calculated by the following equation.

$$\text{Percent of ash} = \frac{\text{weight of ash}(g)}{\text{weight of dried powder (g)}} \text{x} 100$$

Ash content in the dried powdered sample was determined by this method for three more times and average ash content was shown in Table 2.

Determination of fibre content

The crude fibre contents in the dried powder samples were determined by the method given in "The Chemical Analysis of Foods" (Joslyn, 1970). 2 g of sample was placed in a 500 mL conical flask. The 200 mL of dilute sulphuric acid (1.25 %) was added and heated the mixture to the boiling point (continued boiling for half an hour). The level was maintained constant by adding water periodically to make good the loss by evaporation. The sample solution was filtered through a fine piece of muslin cloth and washed three times with boiling water.

The sample on the cloth carefully transferred into the same flask with 200 mL of hot sodium hydroxide solution, 1.25 %. The sample was boiled gently to half an hour maintaining the level constantly by adding water at intervals to make good the loss by evaporation. The sample was filtered through a piece of fine muslin and washed with boiling water till the washing were neutral. Finally it washed with 5 mL of alcohol and transferred to a clean and dried crucible dried at 100° C to obtain constant weight and then weighed.

Incinerated completely and determined the weight of ash. The difference in weight of the fibre before and after incineration gave the weight of fibre. The average fibre content is presented in Table 2.

$$\text{Fibre (\%)} = \frac{\text{Weight of fibre}}{\text{Weight of original sample}} \text{x} 100$$

Determination of fat content

The fat contents were determined by the Soxhlet extraction method (Peason, 1970 & 1976). The dried powder sample 10 g was weighed, place in a cloth bag and the bag was then placed in a Soxhlet extractor. Petroleum ether was poured into the extractor until some of it overflowed into the flask and some pieces of pumice stones were added. The extraction was allowed to continue for four hours at the boiling point of petroleum ether. After the completion of extraction, most of the petroleum ether in the extract was distilled off. Then the content in the flask were carefully combined and transferred to a weighed beaker. The remaining ether in the beaker was vapourized at (100 °C, 373 K) until a constant weight was obtained. The difference in weight of before and after refluxing with petroleum ether was the fat extracted from the sample. The fat content can be calculated using the following equation. The data are shown in Table 2.

$$\text{\% of fat content} = \frac{\text{Weight of extracted fat (g)}}{\text{Weight of powdered sample (g)}} \text{x } 100$$

Determination of protein content

The crude protein contents of the samples were determined by Macro-kjeldahl Method (Steyermart, 1961; Willainn, 1984). Accurately weight amount of powdered sample 1 g was placed in a clean kjeldahl flask. Anhydrous sodium sulphate 3.84 g, anhydrous copper(II) sulphate 0.14 g and concentrated sulphuric acid 10 mL were added into the flask. A small funnel was placed at the opening of the digestion flask and the content was digested by heating the flask in an inclined position. The mixture was heated gently for about 2 hours until the initial frothing ceased. Heating was continued vigorously for about 12 hours (intermittent heating) until the solution become clear. The flask was gently shaken from time to time during the digestion process.

After the digested liquid become clear, it was cooled to below 289.2 K and transferred into the distilling flask 250 mL from the macro-kjeldahl apparatus. The digestion flask was rinsed with small portion of distilled water 4 x 25 mL.

In the receiving flask, boric acid 50 mL (2 % w/v) and 0.2 mL of methyl red indicator was added and the flask was rotated to mix the contents thoroughly. The distillation unit was then set up. Some pieces of pumice stones were placed in the distilling flask containing the digested liquid to prevent bumping and sodium hydroxide solution (50 % w/v) at least 75 mL were added to the solution by the use of the dropping funnel. The flask and its content were heated when ammonia diffused into the acid solution.

The colour of boric acid solution gradually changed from pink to yellow. The distillation was continued until 200 mL of distillate were collected in the receiving flask. The stopper of the flask was opened and the condenser and the delivery tube were washed down with a little distilled water. The distillate in the flask was titrated with approximately 0.05 M standard sulphuric acid solution. A blank determination was carried out in the same manner but distilled water was used in place of the same solution. The protein content in the sample was calculated by using the following relation.

$$\text{Protein (\%)} = \frac{(V_2 - V_1) \times 0.01401 \times M \times 100}{W} \times 6.25$$

Where, V_1 = volume in mL of standard acid for blank titration

V_2 = volume in mL of standard acid for sample solution

M = molarity of standard acid solution in mol dm^{-3}

W = weight in grams of the sample used for the digestion procedure and the results are recorded in Table 2.

Determination of carbohydrate content

Total carbohydrate content was determined by subtraction method. The total carbohydrate content of samples can be obtained as the difference between 100 and the sum of the percentages of ash, fats, fibres, moisture and proteins by the following equation.

Carbohydrate (%)=100 - % (ash+ fats + fibres + moisture + proteins)

Determination of energy values

The energy value of samples was calculated by the following equation.

Energy values (kcal/100 g) = (4 × proteins %) + (4 × carbohydrates %) + (9 × fats %)

Determination of Mineral Elements of *Terminalia catappa* Seeds

Some elements (nutrient elements as well as toxic elements) in *T. catappa* seeds were determined by using Energy Dispersive X-rays Fluorescence (ED XRF) spectroscopic method at Monywa University, Monywa. Dried powder sample 2.5g was fabricated into the pallet for ED XRF spectrometry. The sample was placed in the sample chamber of EDX-700 spectrometer that can measure the sixteen samples at a time. The chamber was pumped up to vacuum. The vacuum pressure was about 38 Pa and the detector temperature is about 170 °C. Therefore, liquid nitrogen needs to be added at the time of analysis. Rhodium target was used in EDX-700 spectrometer. Each sample was run for a counting time of about 100 seconds and the spectrum obtained was stored and analyzed in PC based multichannel analyzer using EDX-700 software. The resultant EDXRF spectrum was illustrated in Figure 2 and relative abundances and normalized percent of some elements in the sample were tabulated in Table 3.

Results and Discussion

According to the preliminary phytochemical analysis of *T. catappa* seeds showed the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins and terpenoids. However, the toxic phytochemical, cyanogenic glycosides were not found in this sample. The main constituents such phenolic compounds, flavonoids, terpenoids and steroids, present in seeds of *T. catappa* may contribute to bioactivities such as antimicrobial, antioxidant and anticancer properties. Test reagents, observations and inferences for the analyses are summarized in Table 1.

Table 1.Phytochemical Test Results of *T. catappa* seeds

Sr. No.	Tests	Extracts	Test Reagent	Observation	Inferences
1	Alkaloids	1% HCl	(i) Dragendorff's reagent (ii) Mayer's reagent	Orange ppt White ppt	+ +
2	α-amino acids	H_2O	Ninhydrin reagent	Purple colour	+
3	Carbohydrates	H_2O	10% ∝-naphthol, conc:H_2SO_4	Red ring	+
4	Cyanogenic glycosides	H_2O	Sodium picrate	No brick red colour	–
5	Flavonoids	EtOH	Mg turnings, conc : H_2SO_4	Pink colour	+
6	Glycosides	H_2O	10% lead acetate	White ppt	+
7	Organic acids	H_2O	Bromocresol green	Blue colour	+
8	Phenolic compounds	H_2O	10% $FeCl_3$	Deep blue	+
9	Reducing sugars	H_2O	Benedict's solution	Brick-red ppt	+
10	Saponins	H_2O	Distilled water	Frothing	+
11	Starch	H_2O	I_2 solution	Deep blue	+
12	Steroids	PE	Acetic anhydride, conc:H_2SO_4	Green colour	+
13	Tannins	H_2O	$FeSO_4$	Deep blue	+
14	Terpenoids	$CHCl_3$	Acetic anhydride, conc:H_2SO_4	Pink colour	+

(+) present (-) absent (ppt) precipitate

Nutritional Values of *Terminalia catappa* Seeds

In this research, some nutritional values of *T. catappa* seeds were determined by standard procedures and the results are shown in Table 2.

Table 2**.** Some Nutritional Contents of *T. catappa* seeds

Sample	Almond seeds (%)
Moisture	4.16
Ash	3.99
Fibre	3.46
Protein	25.18
Fat	50.14
Carbohydrate	13.07
Energy value (kcal/100g)	604.26

Elemental Analysis of *Terminalia catappa* Seeds

Elemental compositions in almond seeds sample was determined by ED XRF spectrometer. The relative compositions of the elements predominantly found in the sample are presented in Table 3 and Figure 2.

Table 3. Some Mineral Contents of *T. catappa* seeds

No.	Name of Element	Symbol	Content (%)
1.	Potassium	K	0.576
2.	Phosphorus	P	0.557
3.	Sulphur	S	0.179
4.	Calcium	Ca	0.171
5.	Iron	Fe	0.006
6.	Zinc	Zn	0.004
7.	Copper	Cu	0.003
8.	Manganese	Mn	0.002
9.	Nickel	Ni	0.001

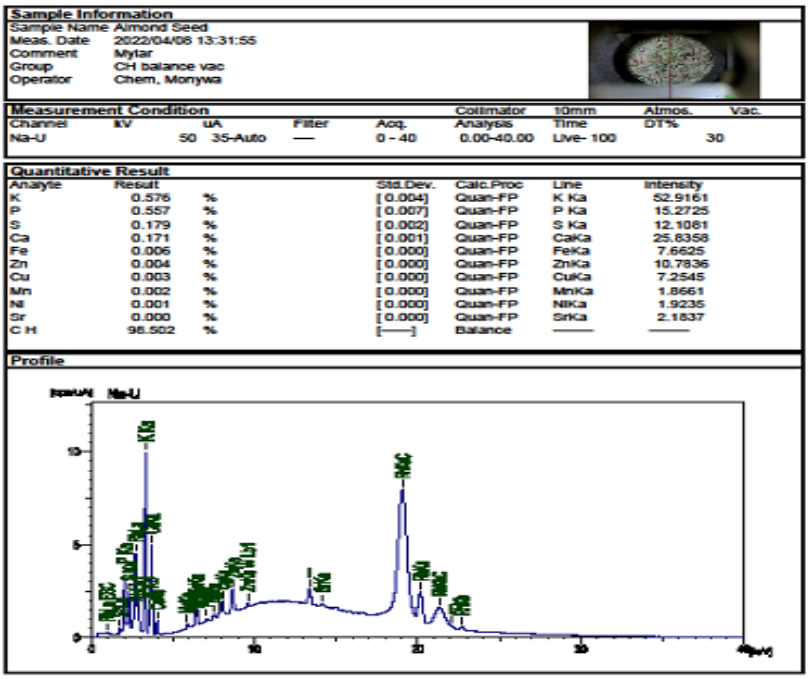

Figure 2. ED XRF spectrum of *T. catappa* seeds

Conclusion

According to the phytochemical investigation of *T. catappa* seeds, various types of bioactive organic constituents were found in the sample, except cyanogenic glycosides. The nutritional values of almond seeds were observed that the almond seeds contain fat 50.14 %, protein 25.18 % and carbohydrate 13.07 %, and have low moisture content. Semi-quantitative elemental analysis of *T. catappa* seeds was performed by ED XRF method. It was found that potassium, phosphorous, sulphur and calcium are major constituents and the other is trace minerals were observed in seeds of *T. catappa*. These elements in this sample play an important role for the nutritional and biological functions for health of consumers. Almonds have calcium and phosphorus, which improve bone health and can protect from fractures. The high content of potassium is effective for the persons with hypertension.The highest content of potassium 0.576 % was observed. Therefore, Plants of banda in West Yangon University campus are not only shading but also lack of toxic phytochemicals contain many nutritional values and valuable mineral elements for consumers.

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References

A.O.C.S. (2017) *Official and Tentative Methods of the American Oil Chemist's Society*. Research Laboratories and Company, Chicago, A.O.C.S. Official Method,20-45.

Arumugam, V. A., Natarajan, D. and P. K. Pannerselvan (2015) "An update Review of *Terminalia catappa*". *J. Pharmacognosy Review*, **9**(18), 93-98.

Harborne, J. B. (1984) *Phytochemical Method, A Guide to Modern Techniques of Plant Analysis*. 2nd Ed., New York, Chapman and Hall, 120-126.

Marini-Bettolo, G. B., Nicolettice, H. M. and M. Patamia (1981) "Plant Screening by Chemical and Chromatographic Procedure under Field Conditions". *J. Chromatog*, **45**, 121 – 123.

Mbah, B. O., Eme, P. E. and C. N. Eze (2013) "Nutrient potential of Almond Seed (*Terminalia catappa*) Sourced From Three States of Eastern Nigeria". *African journal of Agricultural Research*, **8**(7), 629-633.

M-Tin Wa. "Phytochemical Screening, Methods and Procedures". *Journal of Phytochemical Bulletin of Botanical Society of America*, **5**(3), 4-10.

Pakaj O., Robert E. P. (2008) *West India Almond Terminalia catappa L. Combretaceae. Encyclopedia of fruit and Nuts.* Wallingford, United Kingdom. 273-276.

Robinson, T. (1983) *The Organic Constituents of Higher Plants*. 5th Ed, North Amherst, Cordus Press, 285-286.

Shriner, R. L., Fuson, R. C., Curtin, D. Y. and T. C. Morrill (1980) *The Systematic Identification of Organic Compounds, A Laboratory Manual*. New York, John Wiley and Sons, 15-20.

Trease, G.E. and W.C. Evans (1980) *Pharmacognosy*. London, Spottis Woode Ballantyne Ltd,108-529.

USDA (2016) "California Almond Acerage Report, California Department of Food and Agriculture, National Agricultural Statistics Service, Pacific Regional Office". *CDFA Journal*, 1-8.

Venkatalakshmi, P., V. Vadivel and P. Pant and M. M. Padhi.(2014). "A Reviewn on Phytochemistry and Pharmacological Activity of Parts of Mucuna pruriens Used as an Ayurvedic Medicine". *World Journal of Pharmaceutical Research*, **3**(5), 138-158

Willainn, S. (1984) *Official Methods of Analysis of the Association of Official Analytical Chemists*. 14th Ed., 16.