# Protein Analysis and Nutritional Evaluation of *Payena paralleloneura* Kurz. (Kan-zaw)

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**Abstract**

The Kan-zaw plant is famous in Southern Myanmar, the seeds are used for curing cancer diseases by local people. The oil is the main component present in the seed of this plant. In the present research, the results of mean values showed that the protein contents of the Kan-zaw seeds ranged from defatted, decoated samples 8, 9, 10 (0.220±.114, 0.389±0.141 and 0.653±0.291) respectively and non defatted, decoated samples 11, 12, 13 (0.401±0.228, 0.547±0.256 and 0.905±0.483) respectively. The nutritional values of Kan-zaw seeds revealed the presence of crude protein, carbohydrate, crude fat, total sugar, starch contents were higher in seeds. Physicochemical properties of the Kan-zaw seeds were examined; petroleum ether (60-90°C) extract of seed oils was also investigated for the saponification, iodine value and estimation of free fatty acids.

***Key Words*: Kan-zaw seeds: Protein Analysis, Nutritional Evaluation, Physicochemical properties**

## Introduction

Kan-zaw plant *Payena paralleloneura* Kurz. belong to the family Sapotaceae is naturally abundantly found in only Tanintharyi Region, not widely distributed in other Regions and States of Myanmar and other countries. The plants have the potential value in natural anticancer medicines. Its medicinal value is due to the presence of oils in the seeds. Kan-zaw seeds have traditionally been used as a source of oil. Local people used its fixed oil expressed from the seed as a major remedy traditional medicine for the treatment of breast, ovarian and various cancer, anti-peptic ulcer, paralysis, bronchitis, rash, chest pain, injury, sores and various other ailments.

The role of proteins in human nutrition is substantial. According to modern nutrition recommendations, human beings should rely mostly on vegetable and legume proteins to meet the protein requirement in their diet. In addition to their nutritional value, proteins provide great potential as functional food ingredients enhancing the useful properties when incorporated into food commodities. In order to utilize a by-product as a protein source, it should contain high protein content and protein value (quality) based on well-balanced essential amino acids (Oreopoulou and Tzia, 2007).

The nutritional composition application of Kan-zaw oil by-products with year by year demands has been changed. The effects of nutritional composition from Kan-zaw by products on human performance and product quality in medicine processing are studied. The special interest indicates that the Kan-zaw nutritional value may affect human health.

Saponification value is the number of milligrams of KOH required to neutralise the fatty acids resulting from complete hydrolysis of 1g of fat or oil. This value is useful for studying the chain length of the fatty acids. The shorter the average chain length of the acids, the higher is the saponification value. Iodine value

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(halogen) of a fat or oil is the number of grams of iodine absorbed by 100g of fat or oil. It is always constant for a particular fat or oil. This value may be regarded as a measure of its degree of unsaturation and gives an idea of its drying character. This value thus helps in finding out the purity of oil. The fatty acids are a large group of chemicals that can be used to produce a wide range of socially and economically important products (Atasie, *et. al.*, 2009).

In the present study for the first time, an attempt has been investigated to assess protein analysis, the nutritional evaluation and physicochemical properties benefits of Kan-zaw seeds. The aims and objectives are to develop Myanmar traditional medicine plants based on scientific validation of its therapeutic effects and to promote an intensive application of it in Myanmar traditional medicine. To assess protein analysis of Kan-zaw seeds, to investigate the nutritional evaluation of Kan-zaw seeds and to determine the physicochemical properties benefits of Kan-zaw seeds.

## Materials and Methods

### Protein analysis of Kan-zaw seeds

### Defatting procedures

For hexane or petroleum ether defatting, 1g of Kan-zaw powdered was incubated with 5ml hexane or petroleum ether at (35-60°C) for 15min with gentle agitation. Solutions were centrifuged at 5000 rpm for 10 min at 4°C in a Sorvall RC3B swinging bucket rotor. The supernatant was disposed of and 5ml of hexane or petroleum ether was added to the pellet. The above procedures were repeated until supernatants looked clear, about three times. After the final centrifugation, the supernatant was removed and samples were tapped out into large weigh boats to sit in a chemical fume hood overnight to ensure all hexane or petroleum ether was evaporated from the samples. Kan-zaw protein was extracted from these powders the following morning.

### Protein extraction

In this investigation, two different extraction methods were used to extract the proteins from Kan-zaw seeds. In methods A, the seeds were frozen in liquid nitrogen and ground into a fine powder using a mortar and a pestle. The first method (called Method A) was performed according to (Mujoo *et al.*, 2003). In this case, 1g of the Kan-zaw powdered was defatted three times with hexane (Baker, Phillipsburg, USA, www.mallbaker.com). Then, the proteins were extracted with 25mL of a solution containing 0.03mol $L^{-1}$ Tris–HCl (Merck, Darmstadt, Germany, www.merck.de) pH 8.0 and 0.01mol $L^{-1}$ β - mercaptoethanol (J. T. Baker) for 1 h, with vortexing every 10 min. Samples were then centrifuged at room temperature for 20 min at 11000 g in a model Bio-Spin-R ultracentrifuge (BioAgency, Sao Paulo, Brazil, www.bioagency.com.br) and the supernatant containing the Kan-zaw proteins was collected. The second method (called Method B) was adapted from the protocol described by (Bellato *et al.*, 2004). In this case, 1 g of the Kan-zaw powdered was defatted three times with petroleum ether, b.p. 60-90°C (J. T. Baker) for 15 min each. Then, the proteins were extracted with 10 ml of a solution containing 50 mmol $L^{-1}$ Tris–HCl pH 8.8, 1.5 mmol $L^{-1}$ KCl (Merck), 10 mmol $L^{-1}$ dithiothreitol (DTT) (Pierce, Rockford, USA, www.piercenet.com), 1.0 mmol $L^{-1}$ phenylmethanesulfonyl fluoride (PMSF) (Sigma, St. Louis, USA, www. sigmaaldrich.com) and 0.1% (m/v)

sodium dodecyl sulfate (SDS) (Synth, Diadema, Brazil, www.synth.com.br). The samples were mixed for 10 min in an ice bath and insoluble materials were removed by centrifugation at 4°C for 5min at 5000 g.

**Separation of proteins by SDS-PAGE**

The samples obtained using both protein extraction methods were submitted to SDS-PAGE separation in order to establish the extraction efficiencies. The separation was carried out with a vertical slab gel apparatus using a 185x135x1mm gel plate. The SDS-PAGE was done using a separation gel composed of 12.5% (m/v) acrylamide at pH 8.8 and 3.5% (m/v) stacking gel at pH 6.8, prepared according to (Laemmli, 1970). The samples were diluted in a solution containing 0.05mol $L^{-1}$ Tris–HCl (pH 6.8), 13.6% (m/v) glycerol (J.T. Baker), 2.7% (m/v) SDS and 5.4% (v/v) β-mercaptoethanol. Then, the diluted samples and the protein marker (MBI Fermentas, Hanover, USA, www.fermentas.com) were heated at 100°C for 5min. For the electrophoretic separation, 20 µL of the diluted samples were applied in different lanes of the gel. The same volume of protein marker was applied in a separate lane of the gel, in order to allow the estimation of the molar masses of the separated proteins. The protein marker contains the proteins β-galactosidade (116.0 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), lactate dehydrogenase (35.0 kDa), restriction endonuclease Bsp981 (25.0 kDa), β-lactoglobulin (18.4 kDa) and lysozyme (14.4 kDa). Electrophoresis was performed at 200V and 30mA for 9 h. As soon as the electrophoretic run was finished, the gel was stained with 1% (m/v) Coomassie brilliant blue (CBB) G-250 for 1 h. The excess of CBB G-250 was removed using a destaining solution, made of deionized water, methanol (J.T. Baker) and acetic acid (J.T. Baker) in a 6:3:1 (v/v) proportion, respectively. The gel was scanned and its image was analyzed by GelPro Analyzer version 3.1 software (Media Cybernetics, Maryland, USA, www.mediacy.com) for estimating protein molar masses.

**Determination of total protein content**

Total protein content in all samples was determined according to the Bradford method, employing bovine serum albumin (Sigma) as a standard, in order to estimate the protein content after each extraction.

**Aim:**

To estimate the amount of protein in the given sample by Bradford Assay (1976).

**Principle**

The protein in solution can be measured quantitatively by different methods. The methods described by Bradford uses a different concept-the protein‘s capacity to bind to a dye, quantitatively. The assay is based on the ability of proteins to bind to coomassie brilliant blue and form a complex whose extinction coefficient is much greater than that of free dye.

**A. Equipments**

Test tubes, Graduated cylinder, Weight Balance, UV spectrophotometer

**B. Reagents**

Dissolve 100mg of Coomassie-Brilliant blue G-250 in 50 ml of 95% Ethanol. Add 100 ml of 85% phosphoric acid and makeup to 600 ml with distilled water. Filter the solution and add 100 ml of glycerol, then makeup to 1000ml. The solution can be used after 24 hrs. BSA.

**C. Procedure**

Prepare various concentration of standard protein solutions from the stock solution (say 0.02, 0.04, 0.06, 0.08 and 0.1 ml ) into series of test tubes and make up the volume to 1 ml. Pipette out 0.02, 0.04, 0.06 ml of the sample in three other test tubes and make up the volume to 1ml respectively. A tube with 1 ml of water serves as blank. Add 5.0 ml of coomassie brilliant blue to each tube and mix by vortex or inversion. Wait for 10-30 minutes and read each of the standards and each of the samples at 595nm. Plot the absorbance of the standards versus their concentration. Plot graph of optical density versus concentration. From graph find amount of protein in an unknown sample.

**Nutritional evaluation of Kan-zaw seeds**

The Kan-zaw seeds of *Payena paralleloneura* Kurz. were calculated for its nutritive values were analyzed for moisture, ash, crude protein, crude fiber, crude fat, carbohydrate, energy value, starch and total sugar according to the Association of Official Analytical Chemists (AOAC, 2000) methods (925.40, 942.05, 920.152, 978.10 respectively) and (Horwitz, 1980).

**Determination of Physico-chemical properties of Kan-zaw oils**

Saponification value, iodine value and free fatty acid value were determined by (B.P., 1998; Buzarbarua, 2002-2003) procedures. Unless otherwise, the data shown represent the average of three replicate analyses with the standard deviation from the mean for each value.

**Determination of Saponification Value**

**(i) Materials**

Alcoholic potash : Dissolved 40g of KOH in one litre of distilled alcohol keeping the temperature below 15.5°C; the alkali will dissolve giving a clear solution; Phenolphthalein indicator:1% in 95% alcohol; HCl:0.5N, accurately standardized; Water or air condenser

**(ii) Procedure**

The fat was extracted from seeds. Oil 1g was weighed into a 250ml conical flask and added about 25 ml alcoholic potash (approx.) and 0.5N hydrochloric acid from a burette. A blank was prepared also by taking only 25 ml alcoholic potash. Connected an air condenser to the flasks and boiled for about 1 hour. The flasks were cooled and titrated with 0.5N HCl using phenolphthalein indicator. The end point was indicated by the disappearance of the pink colour. The saponification value is calculated by the following equation:-

$$\text{Saponification value} = \frac{56.1 \times (B - S) \times N}{W}$$

B = Titration volume of blank
S = Titration volume of sample
N = Normality of standard acid
W= Weight of sample

## Determination of Iodine Value

**(i) Materials**

Hanus Iodine Solution:13.6g of iodine was weighed and dissolved in 825ml glacial acetic acid by heating and cool. Titrated 25 ml of this solution against 0.1N sodium thiosulphate. Another portion of 200-ml of glacial acid was measured and add 3ml of bromine to it. Ten ml of 15% potassium iodide solution was added 5ml of this solution add and titrated against 0.1N sodium thiosulphate. Calculated the value of bromine solution, to double halogen content of the remaining 800ml of the above iodine solution as follows: X=B/C, where X=ml of bromine solution required to double the halogen content; B=800 x thiosulphate equivalent of 1 ml of iodine solution and C= thiosulphate equivalent of 1 ml of bromine solution; 15% Potassium Iodide Solution; 0.1% Sodium Thiosulphate Solution; 1% Starch indicator

**(ii) Procedure**

0.5 or 0.25 g of oil was weighed into an iodine flask and dissolved in 10 ml of chloroform. 25 ml of Hanus iodine solution was added using a pipette, draining it in a defined time. Mixed well and allowed to stand in the dark for exactly 30min with occasional shaking. 10 ml of 15% KI was added shaken thoroughly and added 100 ml of freshly boiled and cooled water, washing down any free iodine on the stopper. 0.1N sodium thiosulphate was titrated until yellow solution turns almost colourless. A few drops of starch solution was added as indicator and titrated until the blue colour disappears completely. Towards the end of titration, stoppered the flask and shook vigorously so that any iodine remaining in solution in $CHCL_3$ is taken up by potassium iodide solution. Ran a blank without the sample. The iodine values were calculated by the following equation:-

$$\text{Iodine value} = \frac{(a-b) \times 0.0127 \times 100}{\text{Weight of sample}}$$

a = titration of blank

b = titration of sample

## Determination of Free Fatty Acid Value

**(i) Reagents**

25l ether, 25ml 95% alcohol; 1 ml of 1% Phenolphthalein in 95% ethanol; 0.1N KOH (VS)

**(ii) Procedure**

Five g of the substance into a 250 ml flask, and were added 50 ml of equal volumes of ethanol and ether solvent which has been neutralized with 0.1 N potassium hydroxide after the addition of 1 ml of phenolphthalein. Heated gently on a water-bath, if necessary, until the substance has completely dissolved, cooled; titrated with 0.1N KOH. Note the number of ml required. Shake constantly until the appearance of a pink colour which persists for 15 seconds is obtained. Note the number of ml required (a).

**(iii) Calculation**

$$\text{Free fatty acid value} = \frac{a \times 0.00561 \times 1000}{\text{Weight (g) of substance}}$$

## Results and Discussion

### Protein analysis of Kan-zaw seeds

### Defatting and nitrogen liquid does not affect the protein profile of Kan-zaw extracts

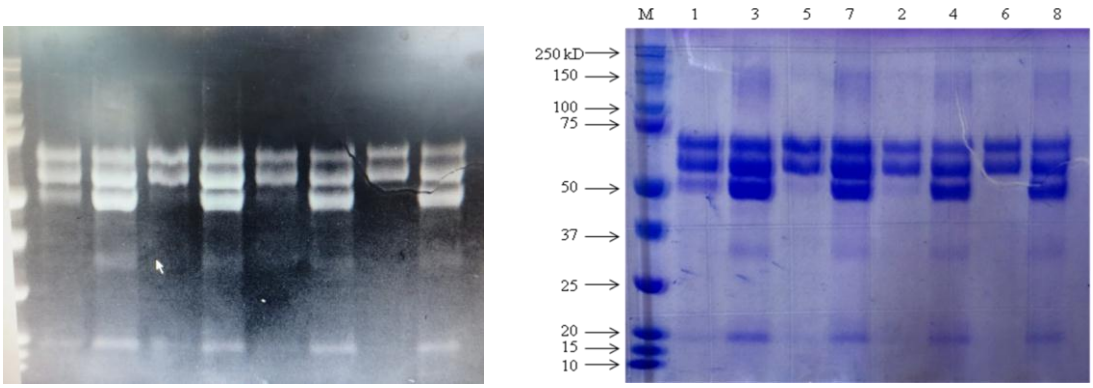
To determine if defatted, coated and non defatted, coated changed the protein profile of Kan-zaw extract, a 12 or 14% SDS-PAGE gel was run under reducing conditions. Kan-zaw powdered were either defatted (df), nitrogen liquid using or non defatted (ndf), no nitrogen liquid using then extracted. The resulting df, nitrogen liquid using and ndf, no nitrogen liquid using extracts were then either centrifuged at 10,000xg in a microcentrifuge for 1 min, 4°C. There was no visible qualitative change in the protein profile of hexane or petroleum ether defatted extracts (lanes: 1, 3, 5 and 7) and non defatted extracts (lanes: 2, 4, 6 and 8). Centrifugation of the df, decoated and ndf, decoated samples had an more apparent impact on protein yield lanes 3, 7, 4 and 8 than lanes 1, 5, 2 and 6.

### The procedure of the SDS-PAGE protein profile of Kan-zaw extracts

Evaluation of protein extraction methods and separation by SDS-PAGE. The initial evaluation of Kan-zaw seeds protein extraction methods was made by comparison of the total protein concentrations determined by the Bradford method. For Method A, and Method B: a protein concentration were found the same (mg of protein per g of sample). Although using nitrogen treatments affect the protein profiles of the Kan-zaw samples, the defatted, decoated and non defatted, decoated samples significantly affected the extractable Kan-zaw protein profile. In SDS-PAGE analysis of the various Kan-zaw extracts it can be seen that df, coated and ndf, coated samples are two bands at the molecular weights where known major Lanes: 1, 5, 2 and 6 (60-75 kDa) and df, decoated and ndf, decoated samples are three bands Lanes: 3, 7, 4,and 8 (50-75 kDa) have been shown in Fig. 1. To confirm the difference in protein profiles between decoated and coated samples, a higher percentage (14%) gel was run and protein samples were significant can be seen in extracts.

### Proteins contents of Kan-zaw seeds

Results shown represent for the average of three replicate analyses with the standard deviation from the mean values for protein contents ranged from defatted, decoated samples 8, 9, 10 (0.220±.114, 0.389±0.141 and 0.653±0.291) mg/ml respectively. Means values composition of samples 10 were higher as compared to sample 8 and 9. Protein concentration of non defatted, decoated samples 11, 12, 13 determined as (0.401±0.228, 0.547±0.256 and 0.905±0.483) mg/ml respectively. Among these results non defatted, decoated sample 13 protein contents were higher as compared to sample 11 and 12. Abbas *et al*., (2015) reported that protein contents of two peanut varieties were determined as 26.17±0.56 and 27.42±0.61 in Golden and Bari 2011, respectively. The results for protein content also fall within the limits to the findings of Aurelia, *et al.*, (2009) found the protein content ranging from 29.5% to 30.1%. The protein content of pearl millet is comparable to wheat (11.6 vs 11.8 g/100 g), is higher than rice (6.8 g/100 g), sorghum (10.4 g/100 g) and maize (4.7 g/100 g) as per the Nutritive value of Indian foods NIN, (2003). Verma and Patel (2013) evaluated the proteins 9.8g. The results are shown in Fig.2 and 3.




Before stained Stained with 1% Coomassie brilliant blue

Fig. 1 SDS-PAGE electrophoresis of Kan-zaw seed Samples. Lanes: M-protein marker, 1, 5-defatted, coated: 3, 7-defatted, decoated; 2, 6-non defatted, coated; 4, 8-non defatted, decoated

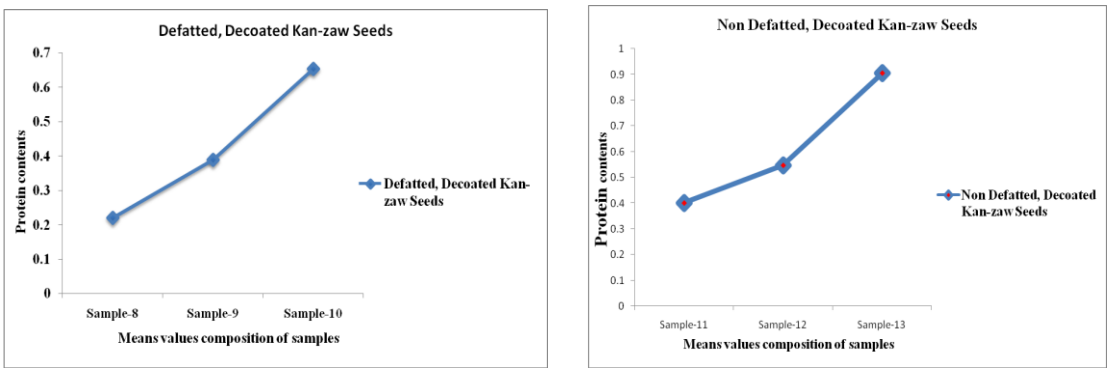



Fig. 2 Histogram showing protein contents of Defatted, Decoated and Non Defatted, Decoated Kan-zaw seeds

**Nutritional evaluation of Kan-zaw seeds**

The nutritional evaluation revealed the Kan-zaw seeds moisture was found to be 7.09%. The results as shown in Table. 1. Abbas *et al*., (2015) two peanut varieties (Golden and Bari 2011) showed moisture content of 5.28±0.32 in Golden and 5.41±0.23 in Bari 2011. Atasie *et al.*, (2009) interpreted that peanut contains about 5-7% moisture on average basis.

Results showed that Kan-zaw seeds contained 1.75% ash. Abbas et al.(2015) who showed that mean values (2.06±0.18) for ash content of Golden were higher as compared to Bari 2011 (1.84±0.18). According to the findings of Sai-Ut *et al*., (2009), found that the ash content ranged from 2.2 to 3.8%. The results are also in agreement with the findings of Grosso *et al.*, (2000) reported the ash content for peanut ranging from 2.3 to 2.6%.

Kan-zaw seeds showed crude protein content of 7.51%. Abbas *et al*., (2015) the value for crude protein revealed in isolates of Bari 2011 were 81.62±1.41 and in Golden variety, it was found to be 74.84±1.38. Sharma, *et al*., (2017) reported that the content of proteins 3.7% in Kodo millet, 14.4% in Wheat, 7.5 % in Rice and 11.5% in Barley.

The result of Kan-zaw seeds found fiber content 3.98%. Abbas *et al*.(2015) who reported mean values of fiber contents were 2.56±0.01 in Golden while 2.57±0.01 in Bari 2011. Verma and Patel, (2013) found that 9.0 g of crude fiber.

Sharma, *et al*., (2017) showed that the content of crude fibers ranging from 9.7g in Kodo millet, 2.9g in Wheat, 10.2g in Rice and 5.6g in Barley.

Kan-zaw seeds obtained higher fat 33.14%. Abbas *et al*., (2015) results showed that Golden contains 43.87±0.21 fat, while Bari 2011 contains 44.57±0.74 fat. The fat content is comparable to the findings of Atasie *et al*., (2006) reported the fat content ranging from 46-52%. Sharma, *et al*., (2017) showed that the content of crude fat 0.12g in Kodo millet, 2.3g in Wheat, 2.4g in Rice and 2.2g in Barley.

Carbohydrate contents of Kan-zaw seeds were determined as 46.53%. Verma and Patel, (2013) reported carbohydrates content in kodo and ragi millets and it contains 66.6 g. Sharma, *et al*., (2017) showed that the content of carbohydrate 54mg/g in Kodo millet, 50mg/g in Wheat, 51 mg/g in Rice and 52 mg/g in Barley.

Kan-zaw seeds starch was found to be 19.76%. Sharma, *et al*., (2017) showed that the content of starch 41mg/g in Kodo millet, 64mg/g in Wheat, 77.2 mg/g in Rice and 58.5 mg/g in Barley.

**Table. 1 Nutritional evaluation of Kan-zaw seeds**

| Sr.No | Test Parameter | Results (%) |
|---|---|---|
| 1 | Moisture | 7.09 |
| 2 | Ash | 1.75 |
| 3 | Crude Protein | 7.51 |
| 4 | Crude Fiber | 3.98 |
| 5 | Crude Fat | 33.14 |
| 6 | Carbohydrate | 46.53 |
| 7 | Energy Value (Kcal/100g) | 517 |
| 8 | Starch | 19.76 |
| 9 | Total sugar | 21.36 |

[a]Expressed as percentage of Kan-zaw seeds

**Determination of physico-chemical properties of Kan-zaw oils**

Results showed saponification value of Kan-zaw oil were determined as 14.03%. Rhee *et al*., (1971) showed that the content of peanut saponification value 194.0±1.0%. Kan-zaw oil iodine value was found to be 158.75%. Rhee *et al*., (1971) reported that peanut iodine value content 92.4±0.6%. Free fatty acid value contents of Kan-zaw oil were determined as 8.77%. Rhee *et al*., (1971) found to be 1.5±0.2 in peanut. The results are shown in Table. 2.

**Table 2. Determination of physico-chemical properties of Kan-zaw oils**

| Description | Saponification value | Iodine value | Free Fatty Acid value |
|---|---|---|---|
| Golden Yellow colour, Odour aromatic, characteristic Taste slightly bitter | 14.03 | 158.75 | 8.77 |

[a]Expressed as milliequivalent peroxides per g of oil.

## Conclusion

It is concluded from the overall results that defatted, decoated and non defatted, decoated Kan-zaw sample is a vital source of protein. These protein isolates in order to enhance the nutritional value of the commodity. Results for the standard deviation from the mean values for protein contents ranged from non defatted, decoated samples were higher as compared to defatted, decoated samples. Nutritional evaluation of crude protein, carbohydrate, crude fat, total sugar, starch contents were found to be a lot richer in seeds. The Kan-zaw oil is famous in Myanmar. Although the oil is popularly used by local people and have commercial value, the scientifically research of this plant has not been undertaken previously. In addition to the above there are numerous records (locally in Dawei elsewhere in the Myanmar country) of remarkable recovery from several cancers, serious burns, boils, carbuncles, rash, sores and strokes. So it should be very interesting for further research can be continued and conducted.

**Acknowledgments**

I would like to express my profound gratitude to Dr. Mie Mie Soe, Professor and Head, Department of Botany, Lashio University, for their kind permission to read research paper presentation. I would also like to express my heartful gratitude to Wannian Yang, Dr, Professor of Botany, School of Life Science, Central China Normal University, China for providing instrument facilities to carry out analysis and supervision.

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