

Original Research Article

Determination of the Nutritive Value of Ceylon Almond (*Terminaliacatappa*) and Butterfly Pea (*Clitoriaternatea*) Seeds from Sabaragamuwa Region of Sri Lanka

ABSTRACT

Aims: The population of the world is increasing at an accelerated rate while food security is moving towards a negative direction. Therefore, Identification and utilization of underutilized food ~~resources re-sources~~ are important to attain sustainability. ~~thus, it inadvertently contributes to improve health and culinary diversity.~~ The present study ~~conducted focused~~ on seeds of two underutilized plants namely; Ceylon almond (*Terminaliacatappa*) and Butterfly pea (*Clitoriaternatea*) which naturally growing in wet zone of Sri Lanka. The seeds of these two plants were collected and evaluated for potential application as a food/feed source.

Methodology: The fat content of the seeds was evaluated through Soxhlet method followed by FAME determination through GC method. The moisture and protein content ~~was~~ determined by AOAC 925.09 and AOAC 991.20, respectively while mineral analysis carried out using ICP-MS.

Results: The study revealed that seeds of *Terminaliacatappa* contained ~~around~~ 18.18% of fat and major fatty acid was oleic (50.87%); a mono-unsaturated fatty acid. The crude protein content of defatted seeds of *T. catappa* was ~~around~~ 35.11% and the major mineral was potassium according to the ICP-MS analysis. *Clitoriaternatea* seed oil contained the least amount of fat (10.8%) and the major fatty acid was palmitic acid; a ~~fully~~ saturated fatty acid. While its crude protein content ~~of it~~ was 50.16%, ~~and~~ major minerals were potassium, magnesium, and calcium.

Conclusion: *C. ternatea* is an excellent source of protein; ~~it~~ contained a relatively low percentage of fat against *T. catappa*. However, both seeds are good sources of minerals.

Keywords Ceylon almond, Butterfly pea, crude protein, fatty acids, minerals

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1. INTRODUCTION

The relationship between plants and humans has always been a challenge, and plants with one or more parts that can be used as a food source for humans are called food plants. It is estimated that around 27,000 plants worldwide have the potential to be used as a food source, although the exact number of plants used is still unknown [1]. Most underutilized plants are considered nutrient-dense and can be grown with few limited resources. Since most of these species are used by local communities, it may and help to meet the nutritional needs of rural ethnic social groups, and thus that help to treat micronutrient deficiencies [2]. Furthermore, relying on a few major crops such as wheat, rice, and maize to fulfill the energy requirement will pose a risk in long-term survival. Since the climatic change negatively effecting on crop yield and put stress on food system security system, thus, undervalued or underutilized crops may add sustainability to the system. However, all underutilized crops may not be able to transform into commercially important crops and a significant level of studies may require in order to accomplish this endeavor [3].

As demand for food escalates, current food supplies may not be sufficient to meet the demand in most countries. In addition, food products may require additional processing steps, which further add the cost to their value, so some food sources may not be available to the downtrodden community. Hence, underutilized crops have the potential to play key roles with respect to food security, while aiding the poor in upkeep their nutritional need and income, reducing dependence on major crops, improving food quality, and preserving cultural and dietary diversity [4].

Clitoria ternatea commonly known as butterfly pea is a perennial climbing herb belonging to the family Fabaceae. The Asian region was identified as the origin of *C. ternatea* and later it is distributed to Africa, America, and the Pacific regions. It is a legume with characteristic of dark blue single flowers, but variations of mauve and white flowers are also available. The pods of *C. ternatea* are flat and elongated, 6 to 12 cm long, with up to 10 seeds per pod. The seeds are brown-black in colour, 4.5 – 7 mm long, and 3 – 4mm wide in size. The extracts and parts of leaves, seeds, fruits, stem, and root are widely used in traditional medicines to cure many illnesses and diseases. In addition, plant extracts showed many pharmacological effects such as antioxidant, antimicrobial, anti-inflammatory, anti-cancer, and anti-diabetic properties. The previous studies of phytochemical analysis of the *C. ternatea* roots and flowers evidenced the presence of alkaloids, tannins, saponins, phenols, flavonoids, and anthocyanins [5, 6].

Ceylon almond (*Terminalia catappa*), locally known as Kottammba, is a monoecious plant native to Southeast Asia and is mainly used for ornamental and shade purposes. In addition, its root, bark, leaf, and fruit extracts have been shown to possess many functional properties, including antibacterial, anti-inflammatory, anticancer, antioxidant, and antidiabetic. The Ceylon almond trees are grown to a height of 35m with an upright symmetrical crown. The leaves are broad, leathery surface, dark green in colour, and are used for treating leprosy, headaches, hepatitis, cancer, anemia, liver-associated diseases, and dermatitis. The fruit is a green drupe that turned into yellow and then red when fully rippled and contains a single seed [7, 8, 9]. According to a study in Nigeria, the seed is on average 2.53cm long and 0.74cm thick and white kernel covered with a thin brown testa. It has been reported that one *T. catappa* tree can produce about 5 kg of kernels per year, but most of the time the fruit is discarded due to ignorance, which will be added to the food waste of a country. However, it is used as a food source in raw or baked form and added as an ingredient in baked goods such as cakes and biscuits [9, 10].

Therefore, current research focuses on determining the nutrient content of the seeds of these two selected plants that widely grow in the wet zone of Sri Lanka. The objective of this study is to compare the nutritional properties of the seeds with a view to evaluate and their importance in the food and feed industry as potential food sources.

2. MATERIALS AND METHODS

2.1 Sample collection and preparation

The Seeds of *Terminaliacatappa*, and *Clitoriaternate* plants are collected from Sabaragamuwa province in Sri Lanka (6.7396° N, 80.3659° E). The seeds of both types were collected from the same plant after they were attained to full maturity.

Thereafter, the pericarp of the collected seeds was removed and they were ground using an electric grinder. The ground seed powder was stored in airtight containers.

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2.2 Determination of Moisture content of seeds (AOAC 925.09)

Using an analytical balance (RADWAG, AS 220 R2), 5.0 g of the powdered seed sample was weighed and dried in a hot air oven at 103 ± 2 °C for 4 hours until weight difference between two subsequent measurements getting 1 mg. The final moisture content of the samples was calculated by wet basis.

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2.3 Determination of Crude crude protein content of seed meals (AOAC 991.20)

The protein content of the seed powder samples was analyzed according to the method specified by AOAC 991.20 using the Kjeldhal method. The calculated N amount was transformed into Protein content by using 6.25 factor.

For the analysis, defatted and dried seed powder samples were used in the current study.

2.4 Determination of Oil-oil content and fatty acid profile

The fat extraction of the seed samples was conducted using the soxhlet extraction method using pet ether as the solvent as per the method described by the AOAC 920.39C and the fat content was calculated using the given formula: ~~given below~~.

$$\text{Crude Fat/Oil \% (m/m)} = (M1-M0)/W \times 100$$

M0 = Empty flask weight (g)

M1 = Flask + Oil weight (g)

W = Moisture free sample weight (g)

Fatty acid methyl esters (FAME) were prepared according to the method specified in the ISO 12966-2:2017 standard. 200 mg of oil sample was boiled with methanolic KOH in a flask until the disappearance of the fat droplets and followed by boiling with Boron trifluoride for 3min. The heating source was removed and 3 mL of Isooctane solution was added. Before the content gets cooled, 20 mL of saturated NaCl was added to the flask and shaken vigorously for 15 sec. Thereafter, the mixture was allowed to cool and the upper organic layer was transferred to a tube with anhydrous Sodium sulfate. Finally, the organic layer was filtered into a GC vial.

FAME analysis was conducted using GC (used FID as detector) and Table 1 represents the temperature programming of the GC for the FAME analysis. The relative percentages of FAMEs were calculated by area normalization and peaks identified by using Supelco 37-component FAME mix.

Table 1 Temperature programming for FAME analysis

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•	Rate (°C/min)	Temp / °C	Hold time(min)
0	-	110.0	7.00
1	3.00	190.0	2.00
2	0.50	205.0	0.00
3	5.00	230.0	5.00
4	5.00	240.0	5.00

2.5 Determination of Mineral content of defatted seed meal

The mineral content of the defatted seed samples was determined according to the method specified by the AOAC 2011.14. Using the analytical balance (RADWAG, AS 220 R2), 0.5 g of defatted and moisture-free sample was measured into a micro-digester tube. Then 8 mL of Conc. HNO₃ acid solution was added to the tube which was followed by placing the tubes inside the Microwave digestion system for 15 min at 200°C. The mixture was transferred into a 50 mL volumetric flask and top-up using deionized water. Finally, the mixture was filtered using a membrane filter and the filtrate was injected into the ICP OES instrument (Thermo Scientific iCAP 7200).

All tests pertaining to the study were replicated thrice and results are expressed as $X \pm SD$ (Mean \pm SD).

3.0 RESULTS AND DISCUSSION

The physical and chemical composition of *Terminaliacatappa* and *Clitoriaternatea* seeds are given in Table 2.

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Table 2 – Moisture and crude fat content of raw seeds and crude protein content of the defatted seeds of *Terminaliacatappa* and *Clitoriaternatea*.

Seed	Moisture content of raw seed % (on wet basis)	Crude Fat %	Crude protein of defatted seed % (on dry basis)
Butterfly pea	18.08 ± 0.04	10.8 ± 0.71	50.16 ± 0.05
Ceylon almond	30.00 ± 0.12	18.18 ± 0.06	35.11 ± 0.64

3.1 Moisture content of seeds

The initial moisture content of seeds is important for preservation and food processing. In general, for safe storage moisture content of cereals, legumes, and oilseeds must be reduced below 14%, 12%, and 8%, respectively. The resulting moisture content of the *T. catappa* seeds pertaining to the current study was high (18-30%). Therefore, these seeds were not suitable for keeping long periods of storage under normal ambient conditions unless a proper drying method is adopted. The current result was similar to the study conducted in Nigeria, where the moisture content of the seed was 30.47% [16]. However, seeds in the Congo region were evidenced to be far low in moisture (4.13%) [11]. In the case of Butterfly pea seed, which moisture content was lower than that of the Ceylon almond; but this value is still considerably high and thus susceptible to microbial infestations during post-harvest storage. However, according to the study done by Joshi et al (1981), the resulted observed low moisture content in seed moisture content was low in value (1.75%) and had a significantly high shelf life [14]. The moisture content of the seeds can vary with the type of grain, chemical composition, moisture level at harvesting, method of harvesting, and relative humidity, which justify the variation of in moisture content of the current-present study as well as the result of the previous experiments [17].

3.2 Crude protein content of defatted seed meals

Crude protein is an important nutrient but all proteins found in foods may not be used as food proteins. Food proteins are classified as easily digestible, non-toxic, nutritionally adequate, functionally usable in food products, available in abundance, and agriculturally sustainable [18]. According to this study, the The crude protein content of the defatted meal of Ceylon almond seed was 35.11 ± 0.64, but Benin et al (2016) [19] reported more crude protein (55.3%) than that of our study The previous studies reported the crude protein range of 33.69% to 21.98% in seeds was in the range of 33.69% – 21.98% [10, 20]. However, the values of raw seeds are expected to be lower than those of the defatted seeds due to the fact that the fat component has been removed through defatting the process of this study.

The defatted meal of butterfly pea seed contained a higher proportion of protein than that of Ceylon almond seeds. According to Turnos (2021), [15] raw seeds of butterfly pea contained 40.59% of crude protein, and also, a

study done by Joshi and Shrivastava (1981), [14] expressed-obtained the value was of 38.4% based on their study in India. The protein content is concentrated more in butterfly pea seeds and followed by flowers and leaves [21]. Hence the leaves of the *C. ternatea* are used as a forage legume due to their high palatability and protein content which was around 21.5%. Therefore, mixing the defatted seed meals with leaves may improve the protein fraction of the forages [22]. The legumes are generally considered lacking in Sulphur-containing amino acids and the presence of anti-nutritional compounds. Such compounds include trypsin inhibitors, which would decrease the bioavailability of the proteins [23]. Therefore, further studies are required with local plant varieties.

According to the current study, if the seeds are used in extracting oil, the byproduct seed meal can be used in the food or feed industry as a source of protein.

3.3 Oil content and fatty acid profile

The fatty acid profile of *Terminaliacatappa* and *Clitoriaternateaseeds* was analyzed and results were given in Table 3.

Table 3 – Major fatty acids present in the *Terminaliacatappa* and *Clitoriaternateaseed* samples.

Fatty acids	Butterfly pea	Ceylon almond
C 12	0.16 ± 0.03	0.17 ± 0.02
C 14	0.23 ± 0.05	0.20 ± 0.01
C 16	14.36 ± 0.27	37.05 ± 0.08
C 18	8.44 ± 0.24	5.53 ± 0.18
C 20	2.17 ± 0.18	0.65 ± 0.03
C 22	2.63 ± 0.05	0.18 ± 0.02
C 18:1	50.87 ± 0.32	26.58 ± 0.29
C 20:1	0.40 ± 0.07	0.06 ± 0.01
C 18:2	17.11 ± 0.47	28.26 ± 0.36
C 18:3	0.80 ± 0.18	0.10 ± 0.02

Values illustrate as percentage of total fatty acids, n=3; (C 12 = lauric, C 14 = myristic, C 16 = palmitic, C 18 = stearic, C 18:1 oleic, C 18:2 = linoleic, C 20 = arachidic, C 20:1 = eicosanoic acid, C 20:2 = alpha Linolenic acid, C 22 = behenic acid).

The ~~According to the~~ results of Table 2, ~~showed~~ the oil content of the Ceylon Almond seed is lower than that of the previously conducted studies in Congo, Thailand, and Cote d'Ivoire regions. The ~~results of those studies showed the~~ oil content was higher than 50%, which was relatively two times higher than the current study [11, 12, 13]. According to this study, butterfly pea contained low amount of oil and it may not be suitable as a source for commercial-scale oil extraction. The results ~~are in agreement with -also shows a similarity with~~ Joshi et al., (1981), ~~who also conducted a study using butterfly pea seeds in India~~ [14]. Another study ~~based-conducted~~ in Thailand ~~also~~ demonstrated a slight increase in crude oil which was ~~around~~ 12.26% [15]. The crude oil content, get varied with climatic factors (rainfall and temperature), ripening stage of seeds, and harvesting time [10, 13]. The seed samples collected for this study were also from mature seeds and those seeds may have been influenced by climatic factors in variation of the crude oil percentage.

In Ceylon almond seeds, the major fatty acid was palmitic acid followed by linoleic acid, which is a polyunsaturated FA. According to previous studies, the palmitic acid content of these seeds ~~in the~~ ranging from 30.1% - 40.0%, and values obtained from the present study was aligned with the aforementioned range [10]. However, studies were done by Weerawatanakorn et al., (2015) [12] and Santos et al., (2022) [21] using *T. catappa* seeds (same type strain but different terminology) found that the major fatty acid of these seeds was oleic. Except for lauric acid, other medium-chain FAs were not detected during the analysis, and the majority of the fatty acids were belonging to the long-chain FA group. Regarding the saturated fatty acid level in Ceylon almond which was ~~around~~ 43.78% and major acids were palmitic and stearic, the rest contribute marginally (Table 3). However, the lowest saturated fatty acids levels were detected in the study of Weerawatanakorn et al., (2015) [12] in Thailand and the highest value was reported by Menkitie et al., (2015) [24] (43.89%) ~~which is based on a~~ Nigerian study. Since the major unsaturated fatty acids, are oleic and linoleic acids and those are the omega 9, and omega 6 categories, respectively. The total unsaturation of the fatty acids of Ceylon almond seed oil was ~~around~~ 55% and those include essential fatty acids for human nutrition too. According to the literature, the unsaturated fatty acid levels were found to be in the range of 65.9% to 54.23% and the current study revealed it was closer to the lower margin of this range [12, 19].

The butterfly pea seed oil contains more unsaturated fatty acids, and oleic acid ~~was~~ the major fatty acid. The total unsaturated fatty acids (oleic, linoleic, alpha-linolenic, and eicosanoic acids) content of the ~~present study was around~~ 69.18%. The results pertaining to the oleic and linoleic acid fractions of this study are similar to Joshi et al., (1981). However, the values highly deviate from the study of Knothe et al., (2016), [29] that state linolenic acid (38.9%) was the major fatty acid in *C. ternatea* seeds. In addition, the same study disclosed a high percentage of unsaturated fatty acids too which was ~~around~~ 80.3%.

Similar to the Ceylon almond, only lauric acid was present as medium chain FA and palmitic acid was the major saturated FA which was ~~around~~ 28% out of the total saturated FA in the oil sample. Based on the studies of coconut kernels, the fatty acid composition of the kernels gets varied as a result of the extraction method, variety, and area of cultivation, which explains the variation of the Fatty acid profile of the seed samples of the present study with previous experiments. [25].

3.4 Mineral content of defatted seed meal

Minerals are a dietary component required by both animals and plants. ~~They are required at different levels and deficiencies will cause certain diseases and adverse effects [26]. Primarily minerals are categorized into two groups such as macro and microelements. In the current study sodium (Na), potassium (K), magnesium (Mg), and calcium (Ca) levels were analyzed as macro-minerals while micro or trace minerals as iron (Fe), copper (Cu), and zinc (Zn).~~

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The result ~~of mineral analysis are~~ given in Table 4. ~~The results shows that illustrated that~~ *T. catappa* seed contains a relatively high amount of minerals. When compared ~~with ison of the present and~~ previous studies, it was observed that the results are varying with a high degree of variability. ~~It was also observed that~~ The trace minerals were represented in the meal of Ceylon almond (defatted) seed, which was below 100 ppm and the level of major minerals, was higher than 2000 ppm. According to WHO, ~~while~~ Ca daily requirement for both adult males and females are ~~1000mg, Mg requirement, 260mg, and 220mg respectively.~~ According to this study, while the Butterfly pea contains around 25 mg of Mg and 10 mg of Ca per 100g of seed, the Ceylon almond is around 21 mg and 26 mg per 100g respectively. Therefore, the seeds of these underutilized plants can be recommended as a good source of minerals.

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However, previous ~~studies done by~~ researchers ~~disclosed reported~~ that *T. catappa* contained anti-nutrient constituents such as oxalate (prevent absorption of Ca, Zn, and Mg by the formation of insoluble compounds), phytic acid (Ca and Fe absorption prevented by the formation of insoluble phytates), and tannins (binding with proteins) thereby bioavailability of the minerals can be challenged [20, 27]. In addition, such anti-nutrients are present in different concentrations depending on the region where the plant is growing; therefore, further studies are required [28].

The butterfly pea seed also contains a high amount of minerals except copper and calcium; however, the rest of the minerals were higher than those in the defatted Ceylon almond seeds.

4. CONCLUSION

Ceylon almond ~~seeds~~ contains a higher percentage of oil than ~~that of~~ butterfly pea seeds. However, both seeds may not be suitable for commercial scale oil extraction by pressing because they contained less percentage of oil. Hence, solvent extraction is the best option. The moisture content of both raw-seed samples was high and recommended to carry out a proper drying method until achieve a safe moisture content in order to maintain the quality as well as shelf life of the seeds. The defatted butterfly pea seeds had a high percentage of protein than that of Ceylon almond and both ~~samples seeds~~ can be recommended as a viable protein source based on the quantity. ~~Although F~~ further studies are required to evaluate the biological value of the protein fraction and its palatability with other food ~~or feed~~ sources. It is concluded that Ceylon almond seed and butterfly pea seeds in Sri Lanka have more unsaturated fatty acids namely palmitic and oleic acids than saturated fatty acids. In addition, both seeds contain essential fatty acids for human nutrition. The mineral analysis revealed that both seeds contained Ca, Mg, and K while Cu was present in the lowest level.

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