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Determination of the Nutritive Value of Ceylon Almond (*Terminalia catappa*) and Butterfly Pea (*Clitoria ternatea*) Seeds from Sabaragamuwa Region of Sri Lanka

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The Global population is increasing at an accelerated rate while food security is moving in towards a negative direction. Therefore, identification and utilization of underutilized food resources are important to attain sustainability. The present study focused on seeds of two underutilized plants namely; Ceylon almond (*Terminalia catappa*) and Butterfly pea (*Clitoria ternatea*) as a source of edible oil production and to evaluate nutritional factors of the seed meal for potential application as a food/feed source.

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Methodology: The moisture content of the seeds was determined according to the method of AOAC 925.09. The fat content of the seeds was evaluated through the Soxhlet method followed by FAME determination through GC method. The crude protein content of the defatted seed meal was analyzed according to the method of AOAC 991.20 and AOAC 2011.14 procedures was followed to determine the mineral content of the seed meal using ICP-OES.
Results: The study revealed that both of the seeds contained high levels of moisture content and *Terminalia catappa* seed exhibited the highest moisture content (30.0%). The seeds of *Terminalia catappa* contained 18.08% of fat and major fatty acid was palmitic. The crude protein content of defatted seeds of *T. catappa* was 35.11% and it had a high level of potassium (K) according to the ICP-OES analysis. *Clitoria ternatea* seed oil contained the least amount of fat (10.8%) and the major fatty acid was oleic (50.87%); an unsaturated fatty acid. The seed meal of it contained 50.16% of crude protein and major minerals were potassium, magnesium, and calcium.
Conclusion: The *C. ternatea* seed meal is an excellent source of protein. However, seeds contain relatively low level of fat compared to *T. catappa* seeds. Both seed meals are good source of minerals.

Keywords: Ceylon almond; Butterfly pea; crude protein; fatty acids; minerals.

1. INTRODUCTION

The relationship between plants and humans has always been a challenge; plants with one or more parts can be used as a food source for humans are called food plants. It is estimated that approximately 27,000 plants worldwide have the potential to be used as a food, although the exact number of plants used is still unknown [1]. Most underutilized plants are nutrient-dense and can be grown with limited resources. Since most of these species are used by local communities, and help meet the nutritional needs of ethnic social groups, and thus 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 for long-term survival. Because, climatic change negatively affects for the crop yield and put stress on food security, thus undervalued or underutilized crops may add sustainability to the system; however, not all underutilized crops may be able to transform into commercially important crops, and a significant number of studies may require in order accomplishing 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 cost to the final product, 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 Fabaceae family. The Asian region was identified as the origin of *C. ternatea* and later it was distributed to Africa, America, and the Pacific regions. It is a legume characterized by single dark blue flowers, but variations in 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 were brown-black in colour, 4.5 – 7 mm long, and 3 – 4 mm wide. The extracts and parts of the leaves, seeds, fruits, stems, and roots are widely used in traditional medicine to treat many illnesses and diseases. In addition, plant extracts have showen many pharmacological effects, including 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*) trees were 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 activities. 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 ripped 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 approximately 5 kg of kernels per year, but most of the time the fruit is discarded because of ignorance. However, some instances, its used as a raw or baked food source and added as an ingredient in baked goods such as cakes and biscuits [9, 10].

This study focused in determining the nutrient content of the seeds of these two selected plants that grow widely in the wet zone of Sri Lanka with objective of comparing the nutritional properties and their importance in the food and feed industries as potential food sources in the future.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Seeds of *Terminalia catappa,* and *Clitoria ternatea* plants were collected from Sabaragamuwa province in Sri Lanka (6.7396^0 N, 80.3659^0 E). Seeds of the two plant species were collected after they reached full maturity. Thereafter, the pericarps of the collected seeds were removed and ground using an electric grinder. Ground seed powder was stored in an airtight container for the subsequent use of the study.

2.2 Determination of Moisture Content of Seeds (AOAC 925.09)

Using an analytical balance (RADWAG, AS 220 R2), powdered seed samples were weighed and dried in a hot air oven at 103 ± 2 ^{o}C for 4 hours until the weight difference between the two subsequent measurements was getting 1 mg. The final moisture contents of the samples were calculated on a wet basis.

2.3 Determination of Crude Protein Content of Seed Meals (AOAC 991.20)

The protein content of the seed powder samples was analyzed according to the method described by AOAC 991.20 using the Kjeldhal method. The calculated amount of N was transformed into protein content by using the factor of 6.25.
For the analysis, defatted and dried seed powder samples were used in the current study.

2.4 Determination of 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.

Crude Fat/Oil % (m/m) = (M1-M0)/W× 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. The oil sample (200 mg) was boiled with methanolic KOH, isooctane solution and boron trifluoride use as a catalyst. Before the contents were cooled, saturated NaCl added to the flask and shaken vigorously. Thereafter, cool upper organic layer was transferred to a tube containing 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 were identified by using Supelco 37-component FAME mix.

Table 1. Temperature programming for FAME analysis

Step number	Rate (℃/ min)	Temperature / ℃	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 Meals

The mineral content of the defatted seed samples was determined according to the method specified by the AOAC 2011.14. Defatted and moisture free samples (0.5 g) were measured into a micro-digester tube using an analytical balance (RADWAG, AS 220 R2). Thereafter, 8 mL of Conc. HNO3 solution was added to the tube, and the tubes were placed inside the microwave digestion system for 15 min at 200 ^{o}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 an ICP OES instrument (Thermo Scientific iCAP 7200).

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

3. RESULTS AND DISCUSSION

The physical and chemical compositions of *Terminalia catappa* and *Clitoria ternatea* seeds and seed meals are listen in Table 2.

3.1 Moisture Content of Seeds

The resulting moisture content of the *T. catappa* seeds pertaining to the current study was high. Therefore, these seeds were not suitable for keeping long periods of storage under normal ambient conditions unless a proper drying method is adopted. The initial moisture content of seeds is important for preservation and food processing. In general, for safe storage moisture content of, cereals, legumes, and oilseed must be reduced below 14%, 12%, and 8% respectively. The current result was similar to the study conducted in Nigeria, where the moisture content of the seed was 30.47% [11]. However, seeds in the Congo region were evidenced to be far low in moisture (4.13%) [12].

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. 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 moisture content of the current study as well as the result of the previous experiments [13].

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, abundantly available, and agriculturally sustainable [14]. The crude protein content of the defatted meal of Ceylon almond seed was 35.11 ± 0.64%. Benin et al (2016) [15] reported more crude protein (55.3%) than that in our study and previous studies reported that crude protein content in seeds was in the range of 33.69% to 21.98% [10,16]. However, the values of raw seeds are expected to be lower than those of defatted seeds because the fat component was removed through the defatting process. Previous studies had also showed that fermented *T. catappa* seed meal diet was suitable for poultry diet formulations as a protein source because, it was capable to enhanced growth performance in broiler chicks [17].

The defatted meal of butterfly pea seed contained a higher proportion of protein than that of the Ceylon almond seeds. According to Turnos (2021), [18] butterfly pea raw seeds contained 40.59% of crude protein. The protein content is highest in butterfly pea seeds, followed by flowers and leaves [19]. Hence the leaves of the *C. ternatea* are used as forage legumes because of their high palatability and protein content (approximately 21.5%). Therefore, mixing defatted seed meals with leaves may improve the protein fraction of the forages [20]. The legumes are generally considered to lack Sulphur containing amino acids and anti-nutritional compounds. Such compounds include trypsin inhibitors, which would decrease the bioavailability of proteins [21]. Therefore, further studies on the local plant varieties are required before incorporating to food/feed formulations.

The seeds are used to extract oil, the by-product 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 profiles of *Terminalia catappa* and *Clitoria ternatea* seeds were given in Table 3.

The results given in Table 2, exhibit the oil content of the Ceylon almond seed was lower than that of previously conducted studies in the Congo, Thailand, and Cote d'Ivoire regions. The oil content was higher than 50%, which was two times higher than the current study [12, 22, 23]. According to this study, butterfly pea contains a low amount of oil and it may not be suitable as a source for commercial-scale oil extraction. Another study conducted in Thailand demonstrated a slight increase in crude oil, which was 12.26% [18]. The crude oil content varies with climatic factors (rainfall and temperature), ripening stage of seeds, and harvesting time [10, 23]. The seed samples collected for this study were also mature seeds, and these seeds may have been influenced by climatic factors in variation of the crude oil percentage.

In Ceylon almond seeds, the major fatty acid is palmitic acid, followed by linoleic acid, which is a polyunsaturated FA. According to previous studies, the palmitic acid content of these seeds ranges from 30.1% - 40.0%, and the values obtained in the present study were aligned with the aforementioned range [10]. However, studies were conducted by Weerawatanakorn et al., (2015) [22] and Santos et al., (2022) [19] using *T. catappa* seeds (same type strain but different terminology), and found that the major fatty acid of these seeds was oleic. Except for lauric acid, medium-chain FAs were not detected during the analysis and the majority of the fatty acids belonging to the long-chain FA group. Regarding the saturated fatty acid level in Ceylon almond, which was 43.78% and major acids were palmitic and stearic, the rest contributed marginally (Table 3). However, the lowest saturated fatty acid levels were detected in study by Weerawatanakorn et al., (2015) [22] in Thailand and the highest value was reported by Menkitie et al., (2015) [24] (43.89%) in Nigerian study. The major unsaturated fatty acids, are oleic and linoleic, which belong to the omega 9, and omega 6 categories respectively. The total unsaturation of the fatty acids in Ceylon almond seed oil was 55%, including 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 that it was closer to the lower margin of this range [15, 22].

Butterfly pea seed oil contains more unsaturated fatty acids and oleic acid was the major fatty acids. The total unsaturated fatty acids (oleic, linoleic, alpha-linolenic, and eicosanoic acids) content in this study was 69.18%. However, the values highly deviated from the study of Knothe et al., (2016), [25] which state linolenic acid (38.9%) was the major fatty acid in *C. ternatea* seeds. In addition, the same study reported a high percentage of unsaturated fatty acids (approximately 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 28% of the total saturated FA in the oil sample. Based on studies of coconut kernels, the fatty acid composition of the kernels varies 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. [26].

3.4 Mineral Content of Defatted Seed Meals

Minerals are dietary components that are required by both animals and plants. In the current study sodium (Na), potassium (K), magnesium (Mg), and calcium (Ca) levels were analyzed as macro-minerals while micro or trace minerals such as iron (Fe), copper (Cu), and zinc (Zn) were analyzed and results are given in Table 4.

The results given in Table 4 illustrate that *T. catappa* seed contains a relatively high amount of minerals. Comparing the present and previous studies, it was observed that the results are varied with a high degree. It was also observed that trace minerals presented in the meal of Ceylon almond (defatted) seeds, was below 100 ppm, and the level of major minerals was higher than 2000 ppm. The RDA value of Calcium (Ca) and Magnesium (Mg) for adults are 1000 mg (both female and male) and 320 mg (female) 420 mg (male) respectively [27]. According to this study, 100g of defatted Butterfly seed meal can fulfill 10.43% of daily requirement of Ca. Further, 79.6 and 60.69% (male) of Mg requirements for female and male respectively. In case of Ceylon almond, which can fulfil 26.44% of daily requirement of Ca while Mg can contribute 66.69% and 50.81% for female and male respectively. Hence, the results demonstrate that both seed meals are a good source of Calcium and Magnesium.

Table 2. Moisture and crude fat content of raw seeds and crude protein content of the defatted seed meals of *Terminalia catappa* (Ceylon almond) and *Clitoria ternatea* (Butterfly pea)

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

Table 3. Major fatty acids present in the *Terminalia catappa* (Ceylon almond) and *Clitoria ternatea* (Butterfly pea) seed samples

Type of 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 are expressed as a 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 18:3 = alpha Linolenic acid, C 22 = behenic acid).

Table 4. Mineral content of *Terminalia catappa* (Ceylon almond) and *Clitoria ternatea* (Butterfly pea) seed samples

Type of mineral	Butterfly Pea (µg/g)	Ceylon Almond (µg/g)
Fe	74.87 ± 4.63	61.56 ± 3.34
Cu	21.16 ± 1.20	24.80 ± 1.07
Zn	106.62 ± 5.41	61.21 ± 4.23
Na	158.65 ± 2.73	81.93 ± 8.84
K	11815.74 ± 60.40	5237.16 ± 340.41
Mg	2549.10 ± 38.83	2134.13 ± 180.67
Ca	1043.53 ± 25.03	2644.57 ± 34.10

Values are the average of three samples of each meal type and those were analyzed individually (n=3).

Considering the microelements (iron, copper, and zinc), the RDA values of both seed meals demonstrate that they are a good source of iron and zinc. However, both seed meals cannot be recommended as a source of copper, because RDA value for which is 900 mg [27].

However, previous studies conducted by researchers, disclosed 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 (which bind with the proteins) thereby, it can disturb the bioavailability of the minerals [16, 28]. In addition, such anti-nutrients are present in different concentrations depending on the region where the plant is growing; therefore, further studies are recommended [29].

4. CONCLUSION

Ceylon almond contains a higher percentage of oil than butterfly pea seeds. However, both seeds may not be suitable for commercial scale oil extraction by pressing because they contain less percentage of oil. Hence, solvent extraction is the best option. The moisture content of both raw-seed samples was high and recommend further drying method until a safe moisture content in order to maintain the quality as well as the shelf life of the seeds. The defatted butterfly pea seeds contained a higher percentage of protein than that of Ceylon almond and both samples can be recommended as a viable protein source based on the quantity. Ceylon almond seed is an edible, underutilized food source. Therefore, experimenting on its nutrition value determination is highly valuable.

In addition, those meals can be used as alternative protein sources for animal feeds, and recommend to further analysis with relevant to sensory properties along with the, physical and textural properties of seed meal. However, 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 linoleic 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. Overall, the findings of the present study would open-up many useful pathways in the fields of product development pertaining to the animal feed as well as human food.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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