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RESEARCH ARTICLE

Oleochemistry

Physiochemical characterization of critically endangered *Coscinium fenestratum* (Gaertn.) Colebr. seed fat for potential niche applications in cosmetics and nutritional supplements

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Abstract: *Coscinium fenestratum* (Sinhala *Veniwalgatta*), a medicinal plant native to Sri Lanka and India, is widely utilized in traditional medical practices such as Ayurveda, Unani and Siddha. The global cosmetic and nutritional industries are increasingly shifting towards plant-based feedstock, leading to the exploration of novel sources of fats and oils. However, the seed and fat of *Coscinium fenestratum* remain underutilized with no prior studies conducted on them. Therefore, this study aims to characterize the seed oil of *C. fenestratum* by determining its fatty acid (FA) composition as their methyl esters, chemical constituents in unsaponifiable matter, and other physiochemical properties. The oil was extracted using the Soxhlet extraction method. The ash and moisture contents of the seeds, acid value (AV), iodine value (IV), smoke point and thermal stability of the oil were determined. Fatty acid methyl esters and unsaponifiable matter were analyzed using gas chromatography-mass spectrometry. Results showed an oil yield of 46.94 ± 0.01% with a moisture content of 4.10 ± 0.03%, ash content of 2.30 ± 0.28%, AV of 2.34 ± 0.10 mg KOH/g, IV of 56.33 ± 0.32 g I_2/100 g, smoke point of 202.9 ± 3.9 °C, decomposition temperature of 416.88 ± 1.74 °C, and unsaponifiable matter yield of 0.57 ± 0.01%. The dominant FAs, stearic (C18:0) and oleic (C18:1), contributed to 50.54 ± 0.88% and 39.21 ± 0.86% of the composition, respectively. The main constituents of the unsaponifiable matter were stigmasterol (1.91 mg/g), gamma-sitosterol (1.29 mg/g), campesterol (0.40 mg/g), fucosterol (0.37 mg/g), and squalene (0.13 mg/g). In conclusion, the findings of this study suggest that *C. fenestratum* seed fat has promising potential to be valorized in both cosmetic and nutritional supplement industries.

Keywords: *Coscinium fenestratum*, cosmetics and nutritional supplements, fatty acids, seed fat, unsaponifiable matter.

INTRODUCTION

The global beauty market has consistently grown at an approximate annual rate of 4.5% over the last two decades. Manufacturers are strategically incorporating plant-based feedstocks such as fats and oils into cosmetic formulations in alignment with consumer demand for natural and sustainable products and their health and dermatological properties (Łopaciuk & Łoboda, 2013). Fish oils have long dominated the nutritional supplement industry due to the high content of essential fatty acids (FAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are renowned for their protective properties against various diseases, particularly cardiovascular conditions (Lenihan-Geels *et al.*, 2013). However, concerns about the declining population of fish due to overfishing and ecological impact have promoted the replacement of fish oils with novel plant oils. This has created a new global trend to search for underexplored and underutilized plant resources to incorporate into the cosmetic and nutritional industries (Nasopoulou & Zabetakis, 2012).

Lipids are the main constituent in plant oils, present as triglycerides and play vital roles in various biological

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functions. The human body can synthesize saturated and monounsaturated FAs but cannot produce polyunsaturated fatty acids (PUFAs). Assessing the saturated/unsaturated FA ratio in seed oils provides insights into their potential as essential FAs and suitability for incorporation into food ingredients (Yao & Xu, 2021). The FA composition of plant oils is highly variable and influences their suitability for different applications. Long-chain saturated fatty acids (SFAs) such as myristic (C14:0), palmitic (C16:0), and stearic (C18:0) acids offer skin softening, smoothing, and protective properties. Plant oils with higher SFA content are favourable for cosmetics but may not suitable as nutritional supplements because of their adverse health effects (Johnson, 1978; Rabasco Alvarez & González Rodríguez, 2000). Understanding the saturated/unsaturated FA ratio is crucial for the suitability of plant oils in various applications. A higher proportion of unsaturated fatty acids (UFAs) can lead to deterioration of oil quality due to natural oxidation reactions associated with them (Kostik *et al.*, 2013).

Unsaponifiable matter in fats and oils is another class of chemical constituents that are found in minor levels compared to FAs. Despite their low levels, they contribute significantly to cosmetic and health benefits. Unsaponifiable matter in plant oils mainly contains, but is not limited to, substances such as phytosterols, carotenoids, terpenes, triterpene alcohols, tocopherols and fatty alcohols (Fontanel, 2013). These constituents, whether working synergistically or individually, offer health benefits such as antioxidant, anticancer, anti-inflammatory and dermatological properties and they are also proven to contribute to the prevention of cardiovascular diseases (Caligiani *et al*., 2010). Among the constituents in unsaponifiable matter, squalene is the most commercially explored and utilized compound due to its various cosmetic and health benefits. Despite the diverse bioactivities of squalene, its potential applications in cosmetics are limited due to the lack of natural resources other than fish oils (Kim & Karadeniz, 2012). Therefore, most squalene and its derivatives, which are currently used in cosmetic and nutritional supplement industries, originate from crude oil via synthetic routes. Furthermore, the inclusion of phytosterols in dietary supplements will offer significant health benefits such as direct inhibition of cholesterol absorption, etc. (Ogbe *et al*., 2015). However, some studies have shown that elevated concentrations of certain phytosterols in plasma can lead to risk factors (Glueck *et al.,*1991).

Plant extracts, including seed oils, are increasingly used in cosmetics due to their biodegradability and lower toxicity compared to synthetic or semi synthetic substances. Currently, various seed oils such as almond oil, apricot kernel oil, castor oil, sunflower oil, jojoba oil, and plant fats such as shea-butter, kokum butter and mango kernel butter are extensively used in the cosmetic industry. Soybean and rapeseed oils, rich in PUFAs, are considered promising alternatives to fish oils (Nasopoulou & Zabetakis, 2012). Plant oils such as olive, flax seed, moringa seed, grape seed and perilla seed are currently used in dietary supplements as alternatives to fish oils due to their FAs and unsaponifiable matter composition for their health-enhancing properties (Caligiani *et al*., 2010). The chemical composition of unsaponifiable fractions highlights the superiority of olive oil, jojoba oil, avocado oil, shea butter and kokum butter in commercial applications.

The global cosmetic and nutritional supplement industry is increasingly turning to plant seed oils as renewable feedstock causing a substantial need to explore novel sources of fats and oils. The increasing global population and the escalating demand for plant oils in both the food and industrial sectors pose significant challenges. The main challenge is the extremely high cost of some seed oils due to their rarity and short supply. This opens new avenues to explore alternative sources of fats and oils, focusing on non-conventional and underutilized seeds to address the shortage of currently used plant oils.

Sri Lanka is a tropical country with very high biodiversity and has a wide variety of oil-rich seeds that are widespread across all geographic regions of the country. However, many of these oil seeds are poorly characterized or not chemically characterized at all. *Coscinium fenestratum* (Gaertn.) Colebr. is among the unexplored oil seed species in Sri Lanka. It is a critically endangered plant belonging to the family Menispermaceae due to its over-exploitation for its proven medicinal properties of root, bark and stem. It is native to Sri Lanka and the Western Ghats of India, known locally as "*Veniwalgatta*". The stem of *C. fenestratum* is extensively used in various ayurvedic, siddha and indigenous medicine formulations (Kothalawala *et al*., 2020). Surprisingly, there is no report of the utilization of seeds in these medicinal practices. Therefore, the chemical composition of the seeds of *C. fenestratum* remains unexplored even though the stem has been extensively studied for its medicinal properties and chemical composition (Rai *et al.*, 2013). A wide array of commercial cosmetics and nutritional supplements such as soap, bath oils, face wash, body lotions and fairness creams and medical concoctions that include *C. fenestratum* stem, root or bark extracts have emerged over the last few decades (Warakagoda & Subasinghe,

2014). However, seeds or seed oils have been ignored in these formulations due to the lack of knowledge of their chemical composition.

Therefore, the selection of *C. fenestratum* seeds in this study is driven by their medicinal value compared to other parts of the plant as the well as the lack of prior investigative data for seeds. This study focused on determining the physiochemical properties, FA composition and unsaponifiable matter composition of *C. fenestratum* seeds with the ultimate goal of identifying potential applications in the cosmetic and nutritional supplement industries.

MATERIALS AND METHODS

Extraction of *C. fenestratum* seed oil and determination of oil content

Seeds were collected from the Yagirala forest reserve (6.365506, 80.172835) in the Kalutara District in September based on random sampling. A quantity of 20-30 seeds was obtained from well-ripened, dried fruits of *C. fenestratum* and only high-quality, pest and fungi-free seeds selected for further analysis. After removing the seed coat, kernels were ground and sieved to obtain a fine powder. This powder was oven-dried at 60 °C for 2-3 hours. Approximately 25 g of ground seed kernels were used for oil extraction. Soxhlet extraction was conducted using 150.0 cm^3 of HPLC-grade hexane for six hours. Anhydrous sodium sulphate was added after refluxing to remove traces of moisture, and the solvent was then removed using a rotary evaporator. The crude oil percentage (w/w) was calculated based on the dried ground seed kernel powder (Keneni *et al*., 2021). Seed oil extraction was duplicated as necessary to ensure accuracy.

Determination of moisture content in seed kernel

Moisture content was determined by the modified AOAC 934.06 method. The ground seed kernel sample was dried at 103 ± 2 °C for 2 h in an oven and repeated till a constant weight was achieved. The moisture content was calculated as a percentage of the initial seed sample weight (Zang *et al.*, 2017). Moisture content was determined using the following formula (1):

$$\text{Percentage of Moisture Content (\%)} = \frac{x - y}{x} \times 100$$

x- Weight of the sample used for moisture determination
y- Weight of dried sample of constant weight

Determination of ash content in seed kernel

The modified AOAC 942.05 method was followed using a muffle furnace. A known weight of the dried ground seed kernel was ignited at 550 °C for 5 h. The weight of the resulting ash was measured at room temperature and ash content was calculated as a percentage of the initial seed sample (Yadav *et al.*, 2022) using the following formula (2):

$$\text{Ash Content (\%)} = \frac{W3 - W1}{W2 - W1} \times 100$$

W1 - Weight of the empty crucible
W2 - Weight of the crucible and the sample before ashing
W3 - Weight of the crucible and ash

Determination of acid value

The Acid Value (AV) was determined using a modified A.O.C.S Official method, Cd 3a-63, 2006. A weight of 0.2 g of *C. fenestratum* seed fat was dissolved in a freshly prepared, pre-neutralized methanol-diethyl ether 1:1 (v:v) ratio. This was neutralized with a 0.05 M KOH solution using phenolphthalein as the indicator (Abdullahi *et al*., 2021). The AV was calculated using the following formula (3).

$$\text{Acid Value} = \frac{\text{Volume of KOH solution} \times 56.1 \times \text{KOH concentration}}{\text{Weight of Oil}}$$

Determination of iodine value

The modified AOAC 920.159 method was followed using Wij's solution to determine the Iodine Value (IV) of the seed fat. The sample was titrated against 0.1 M $Na_2S_2O_3$ solution, and the IV was calculated using the following formula (4) (Pardeshi, 2020):

$$\text{Iodine Value} = \frac{(V_{blank} - V_{sample}) \times Na_2S_2O_3 \text{ concentration} \times 12.69}{\text{Weight of Oil}}$$

Determination of smoke point

The smoke point was determined by heating 0.5 mL of the seed fat on a hot plate and noting the temperature at which continuous smoke started to appear. The temperature was measured using an IR thermometer (Falade *et al*., 2008).

Determination of fatty acid composition by gas chromatography – mass spectrometry (GC-MS).

To identify and quantify the FAs present in the extraction, the seed fat was converted to Fatty Acid Methyl Esters (FAME). Approximately 0.05 g of oil was mixed with 5.00 cm^3 of iso-octane in a stoppered test tube. To this solution, 200 µL of 2 M methanolic KOH was added, stoppered, and vigorously shaken for 30 seconds until cloudiness appeared. Finally, approximately 1 g of sodium dihydrogen orthophosphate was added and shaken until the cloudiness disappeared. The upper layer of the freshly prepared sample was subjected to GC-MS analysis after drying with anhydrous sodium sulphate and filtration. From the filtered sample, 1.0 µL was injected at a split ratio of 1:20 to an Agilent GC model-7890A and MS model-5975C with a non-polar ultra-inert capillary column containing 5% phenyl methyl siloxane (19091S-433UI HP-5MS with an internal diameter of 250 µm, column length of 30.0 m, and film thickness of 0.25 µm). The temperature program included an initial temperature of 100.0 °C held for 3.0 minutes, increased to 240.0 °C at a rate of 3.0 °C/min and then held for 7.0 minutes. Samples were prepared in four replicates and the average was considered for quantification and comparison.

Compound identification was performed by comparing the total ionic current spectrum of each peak of *C. fenestratum* seed fat extract with that from the NIST mass spectral library and the retention time window of each matching FAMEs of the mass spectrum of the Supelco 37 component FAME reference mix standard (Sigma Aldrich). The quantification was carried out using the internal standard method by considering the response factors with heptadecanoic acid methyl ester (Sigma Aldrich) as the internal standard.

Extraction of unsaponifiable matter

A modified AOAC 933.08 procedure was used to extract unsaponifiable matter from *C. fenestratum* seed fat. Around 5 g of fat was refluxed with 50.0 cm^3 of 1 M methanolic KOH. The unsaponifiable matter was extracted using liquid-liquid extraction with HPLC-grade hexane. The solvent was removed by rotary evaporation and acetone was added to eliminate volatile components. The weight of the obtained unsaponifiable matter was measured to calculate the yield (w/w) as a percentage of the initial fat weight (Giacometti, 2001; Janporn *et al*., 2015).

Identification and quantification of unsaponifiable matter using gas chromatography-mass spectrometry (GC-MS)

The white unsaponifiable matter was dissolved in HPLC-grade hexane, dried with anhydrous sodium sulphate, and analysed using GC-MS. An Agilent GC model-7890A and MS model-5975C were used with the same column as for FAME analysis.

The temperature program started at 70.0 °C was kept for 1.5 minutes, then increased to 280.0 °C at a rate of 10.0 °C/min and held for 38.0 minutes at 280.0 °C. Compound identification followed the same method for FAME identification and quantification was done using an external calibration method with reference standards from Sigma Aldrich.

Thermal analysis

Thermal analysis was conducted using the Discovery SDT 650 model, equipped with simultaneous Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) capabilities (Trios software). The analysis involved heating the *C. fenestratum* seed fat sample from room temperature to 600.0 °C at a rate of 10.0 °C/min under a nitrogen atmosphere. The resulting mass changes were plotted using Origin software to generate TGA and Differential Thermogravimetry (DTG) curves (Solís-Fuentes *et al*., 2010).

Determination of thermal stability of peanut oils as the reference

The seed kernels of fully ripened peanuts, procured from the local market, were extracted by the Soxhlet method outlined in seed oil extraction section (Keneni *et al*., 2021). The thermal stability of the extracted peanut oil was then analyzed according to the procedure described in above (Solís-Fuentes *et al*., 2010).

RESULTS AND DISCUSSION

Figure 1 shows the seed morphology and seed fat of *C. fenestratum.* The seed kernel of *C. fenestratum* has a distinct yellow color and the seed kernel is solid white at room temperature (Figure 1). Determination of the percentage of crude oil content is significant to evaluate the economic feasibility and commercial potential of *C. fenestratum* seeds as an oil-bearing source. Compared to shea butter (47.5% yield) extracted from shea nuts,

which is extensively used in the food, pharmaceutical, and cosmetic industries, *C. fenestratum* seeds had a calculated oil yield of 46.94 ± 0.01% (Table 1) through solvent extraction (Abdul-mumeen *et al.*, 2019). This suggests that *C. fenestratum* seeds can be considered as high-oil-yielding seeds and could be a potentially advantageous and cost-effective resource for large-scale industrial applications.

To assess the quality of the extracted oils, we determined the moisture content of the seed kernel. While previous studies reported a moisture content of 28% for fresh seeds of *C. fenestratum*, it is important to note that the seeds collected for our study were well-ripened, dried and fallen from the plant (Ramasubbu *et al*., 2012). Consequently, the moisture content determined in this study (Table 1) was significantly lower at 4.10 ± 0.03%.

Figure 1: Seeds (A), kernels (B) and seed fat of *C. fenestratum* (C)

Table 1: Physiochemical properties of *C. fenestratum* seed kernel.

Physiochemical property	Average ± SD
Yield of crude Oil (%)	46.94 ± 0.01
Moisture content (%)	4.10 ± 0.03
Ash content (%)	2.30 ± 0.28

Table 2: Physiochemical properties of the seed fat of *C. fenestratum* seed.

Physiochemical property	Average ± SD
Acid value (mg KOH/g)	2.34 ± 0.10
Iodine value (g I_2/100g)	56.33 ± 0.32
Yield of unsaponifiable matter (%)	0.57 ± 0.01
Smoke point (°C)	202.9 ± 3.9
Decomposition temperature (°C)	416.88 ± 1.74

The low moisture content in the kernel is a positive indicator, suggesting extended storage stability and a lower risk of spoilage, which is consistent with previous research findings (Onyeike & Acheru, 2002).

The ash content of *C. fenestratum* seed kernel was determined to be 2.30 ± 0.28% (Table 1), which is close to the value obtained for groundnut seed kernel in a previous study and notably higher than the values for coconut (0.43%), dika nut (0.63%), melon (1.50%), and palm kernel (1.50%) (Onyeike & Acheru, 2002). This finding provides us an initial insight into the mineral content and nutritional value of *C. fenestratum*, since the ash content indicates the total minerals present in the seed kernel. Essentially, it represents the inorganic residue remaining after the removal of water and organic matter. This suggests a significant mineral richness in *C. fenestratum* seed kernels, suggesting potential nutritional benefits and making it a promising candidate for dietary inclusion (Zang *et al.*, 2017). However, further analysis of metal ion content is required for a comprehensive assessment of the benefits associated with mineral content. This study is currently underway in our group.

The AV of the *C. fenestratum* seed fat was 2.34 ± 0.10 mg KOH/g (Table 2). This value reflects the free fatty acid content of the oil and serves as an important indicator of the rancidity of fats and oils. A significantly low value suggests its suitability for potential edible purposes of the oil. The AV of *C. fenestratum* seed fat was lower compared to the values for sesame, soybean, sunflower and rapeseed oils. This positions the oil as a stable and durable alternative for various applications with further treatments. The IV of *C. fenestratum* seed fat was 56.33 ± 0.32 g I_2/100g (Table 2), which corresponds to the degree of unsaturation of the FA profile. Oils with higher IVs typically contain more UFAs and are more susceptible to oxidative rancidity. In contrast, the relatively low IV of *C. fenestratum* seed fat indicates a higher proportion of SFAs, suggesting greater resistance to oxidative rancidity and enhanced oxidative stability (Kyari, 2008).

Having a low IV of 56.33 ± 0.32 g I_2/100g (Table 2) indicates a high amount of SFAs. A previous study suggested that oils with higher IVs are susceptible to photocatalytic reactions (Kyari, 2008).

The smoke point is an important parameter when assessing the stability and suitability of fats and oils for high-temperature applications. It is determined by the threshold temperature at which a continuous wisp of smoke is produced upon heating. The higher the smoke point the higher the thermal stability of the oil.

According to Canadian government specifications, oils that are subjected to high temperature applications, especially frying, require a smoke point exceeding 200 °C (Przybylski *et al.,* 2020). The value in this study for *C. fenestratum* seed fat is 202.9 ± 3.9 °C (Table 2). Therefore, it can be considered as a moderately thermally stable oil.

Analysis of fatty the acid profile of *C. fenestratum* seed fat

The percentage composition of each identified FA is given in Table 3, and the total ionic current spectrum of the FAMEs of *C. fenestratum* seed fat is shown in Figure 2.

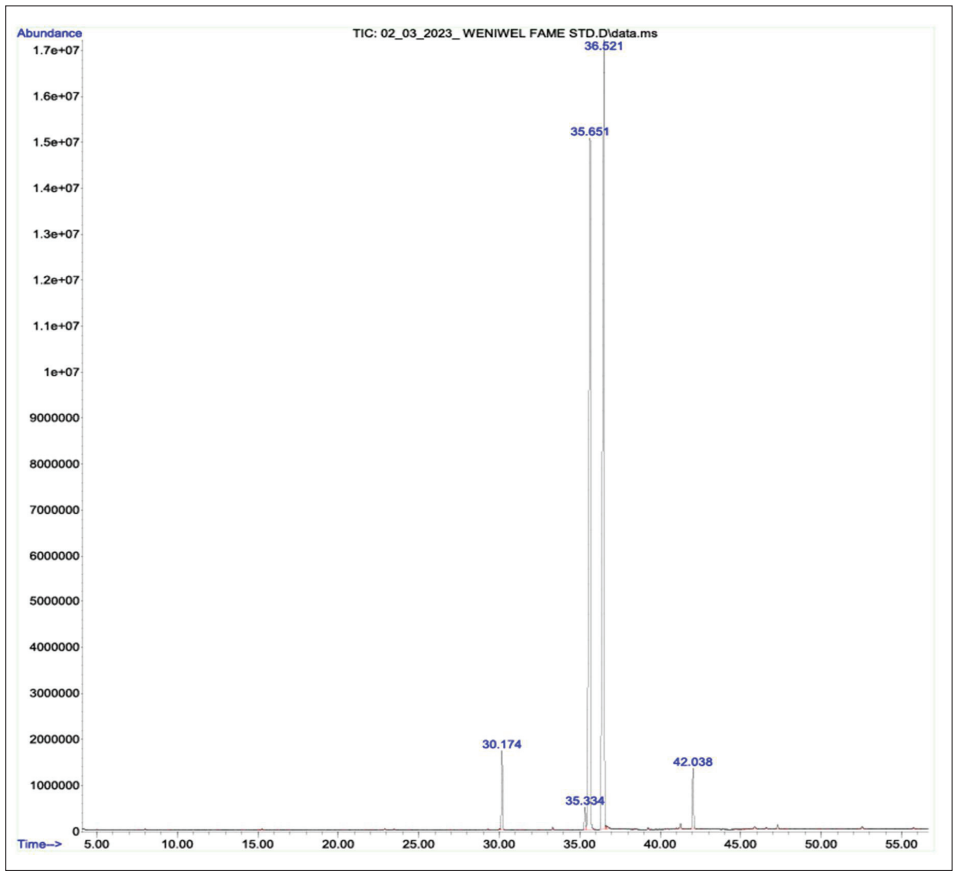

Figure 2: Total ion chromatogram of the FAME present in *C. fenestratum* seed fat obtained from the GC-MS analysis in this study. The retention times of relevant FA are; 30.174 min (C16:0), 35.334 min (C18:2), 35.651 min (C18:1), 36.521 min (C18:0), 42.038 min (C20:0)

The gas chromatography-mass spectrometry (GC-MS) method was used to identify and quantify the FAs as their methyl esters of *C. fenestratum* seed fat. Typically, when the level of SFAs significantly exceeds that of UFAs, most plant triglycerides remain solid at room temperature. However, exceptions exist, as seen in palm oil and coconut oil.

According to the FA profile in Table 3, the total composition of FAs is 97.01 ± 1.25%. The most abundant are the SFAs with a total percentage of 56.65 ± 0.91%. The specific amounts of SFAs are palmitic (C16:0) 3.14 ± 0.18%, stearic (C18:0) 50.54 ± 0.88% and arachidic (C20:0) 2.62 ± 0.13%. It is also noteworthy to observe that very long-chain fatty acids including C20 to C24 are present in minor levels (3.24 ± 0.14 %) These very long-chain fatty acids are associated with various cosmetic properties. The total percentage of UFAs is 40.36 ± 0.86%. The most abundant UFAs are the oleic acid (C18:1) 39.21 ± 0.86% with linoleic (C18:2) 0.85 ± 0.07% and gondoic (C20:1) 0.30 ± 0.02% acids present at minor levels. The calculated SFA/UFA ratio is 1.40, which supports the appearance of a solid physical state.

Table 3: Fatty acid composition of *C. fenestratum* seed fat (n=4)

Fatty Acid		%
Saturated Fatty Acids		
C14:0	Myristic acid	0.03 ± 0.01
C16:0	Palmitic acid	3.14 ± 0.18
C18:0	Stearic acid	50.54 ± 0.88
C20:0	Arachidic acid	2.62 ± 0.13
C22:0	Behenic acid	0.20 ± 0.04
C24:0	Lignoceric acid	0.12 ± 0.04
Unsaturated Fatty Acids		
C18:1	Oleic acid	39.21 ± 0.86
C18:2	Linoleic acid	0.85 ± 0.07
C20:1	Gondoic acid	0.30 ± 0.02
Total FAs		97.01 ± 1.25
Total Unsaponifiable Matter		0.57 ± 0.01
Unidentified FAs		2.42 ± 1.25

SFAs such as stearic and palmitic and UFAs such as oleic acids are noted for their ability to enhance skin permeation, while linoleic acid is recognized for providing moisturizing and skin healing effects (Vermaak *et al*., 2011). Having a higher SFA composition offers advantages in resisting oxidative rancidity (Jayadas & Nair, 2006). Another notable observation in the FA composition is the simplicity of the composition. Only two FAs, steric and oleic, contribute to 89.75 ± 1.23% of the total FA content. This simple composition makes the *C. fenestratum* seed fat a potential candidate for a wide variety of cosmetic and dietary supplement applications without concerns for other FAs that have potential health risks such as cholesterol elevation effects. This also stamps *C. fenestratum* seed fat as a stearic acid and oleic acid-rich clean plant source for other industrial applications in the lubrication industry.

Comparison of FA composition of *C. fenestratum* seed fat with commonly used seed oils for possible use in cosmetics and dietary supplements

This study focuses on identifying the potential of *C. fenestratum* seed fat to be incorporated into cosmetics and nutritional supplements. To achieve this objective the FA composition of *C. fenestratum* seed fat was compared with plant oils and fats that are already established in these industries. The primary objective of this comparison is to assess the possibility of substituting *C. fenestratum* seed fat with expensive fats and oils currently utilized in the industry. Our comparison reveals distinct similarities in the FA profile of *C. fenestratum* with that of shea butter, sal fat, mango kernel fat, and kokum butter (Table 4). All these seed fats are already used in various cosmetic and dietary supplement formulations and are high in oleic and stearic acid, which are in demand in the cosmetic industry.

Table 4: Comparison of FA profile of *C. fenestratum* seed fat with selected commercially available fats (Rabasco Alvarez & González Rodríguez, 2000; Firestone, 2013)

Species	SFA (%)			UFA (%)	
	C16:0	C18:0	C20:0	C18:1	C18:2
	Palmitic	Stearic	Arachidic	Oleic	Linoleic
Shea butter	0.5-8.5	35.1-47.4	0.1-2	43.5-50	4-5
Sal fat	5.3-23	33-57	1-8	31-52	0.3-5
Mango kernel fat	3-18	26-57	1.6-6	38-50	1-13
Kokum butter	1.4-5	49-60.4	-	39-49	1-2
C. fenestratum fat	3.0 -3.3	49.7 -51.4	2.5 – 2.8	38.4– 40.1	0.8 - 0.9

Mango kernel fat, a newly introduced source in the cosmetic industry, has shown promise in producing stable emulsions (Shukla & Kragballe, 1998). Cocoa butter, known for its use in night creams, lip balms, and bath oils, and shea butter, widely employed in skin care applications in massage creams, makeup, and baby-care products, possess unique characteristics due to their high content of unsaponifiable matter. This unsaponifiable

matter is rich in terpenic alcohols and phytosterols, imparting soothing properties and sun protection abilities to cosmetic products. As a result, shea butter is extensively incorporated into various cosmetic products, such as hair care, baby care, sun care, and body lotions, in suitable amounts (Shukla & Kragballe, 1998). The significant presence of oleic acid in *C. fenestratum* seed fat makes it suitable for use as excipients in cosmetic formulations and as penetration enhancers in pharmaceutical formulations. Kokum butter is a highly stable exotic butter, widely recognized for its applications in skin and hair care products, acne treatments, skin tonics, and as an enhancer for cocoa butter in the global market (Shukla & Kragballe, 1998). While sal butter is locally employed in cooking, soap production, and the formulation of skin and hair care products, it is often combined with mango butter to enhance emollient and oxidative properties (Shukla & Kragballe, 1998). Furthermore, some studies have shown that UFAs exhibit an effect on reducing blood levels of cholesterol, LDL and HDL, while all SFAs, except stearic acid (C18:0), are believed to have an opposing effect on this physiological aspect (Williams, 2000). Based on this FA profile comparison, *C. fenestratum* seed fat has a promising potential to substitute or to be used as a mixture with all the above seed fats.

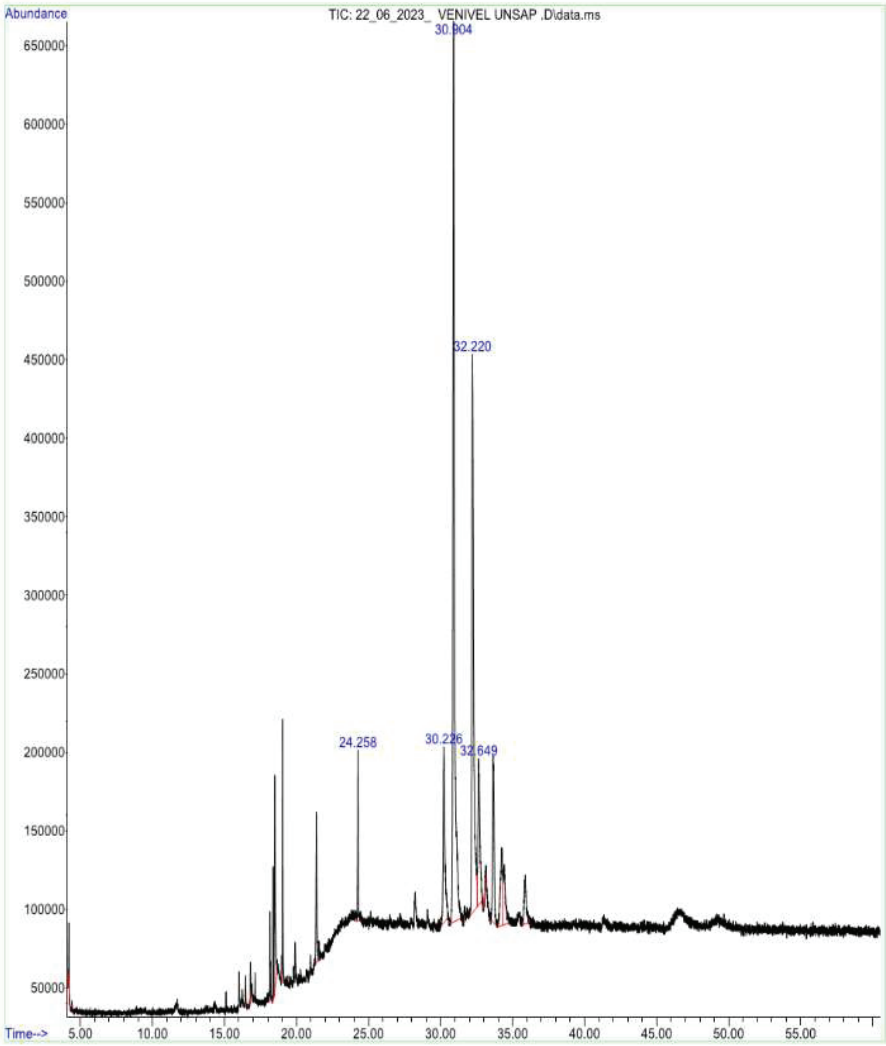

Figure 3: Constituents of unsaponifable matter present in *C. fenestratum* seed fat. They are; retention time (min) = 24.258 (Squalene), 30.226 (Campesterol), 30.904 (Stigmasterol), 32.220 (gamma-Sitosterol) and 32.649 (Fucosterol)

Analysis of unsaponifiable fraction of *C. fenestratum* seed fat

C. fenestratum seed fat has an unsaponifiable matter content of 0.57 ± 0.01% (Table 2) when calculated as a percentage of total lipids. Compared to the unsaponifiable contents of cocoa (0.5%), kokum (0.5%), mango (0.8%), sal (0.9%) and shea butter (6.0%), *C. fenestratum* seed fat showed a close similarity to all except shea butter (Shukla & Kragballe, 1998). Shea butter is a unique cosmetic ingredient due to its very high percentage of unsaponifiable matter making it difficult to find a suitable substitute.

Figure 3 shows the total ionic current spectrum of the unsaponifiable fraction of *C. fenestratum* seed fat and Table 5 shows the weight of each unsaponifable matter constituent identified per 1 g of the *C. fenestratum* seed fat. The most abundant constituent is stigmasterol (1.91 mg/g) followed by gamma-sitosterol (1.29 mg/g) and campesterol (0.40 mg/g). Other minor constituents present are fucosterol (0.37 mg/g) and squalene (0.13 mg/g).

Table 5: The major constituents of the unsaponifiable matter of *C. fenestratum* seed fat.

Compound	Concentration (mg/g)
Stigmasterol	1.91
gamma-Sitosterol	1.29
Campesterol	0.40
Fucosterol	0.37
Squalene	0.13

Phytosterols, which are steroidal compounds similar in structure and function to cholesterol, have demonstrated cholesterol-lowering effects in human and animal plasma mitigating the risk of coronary heart diseases. Besides cardiovascular benefits, phytosterols also possess anti-inflammatory properties, lowering the effects of total cholesterol and low-density lipoprotein levels (Ogbe *et al*., 2015). β-sitosterol, campesterol, and stigmasterol are prevalent phytosterols. Among these, β-sitosterol is noted for its various health benefits. It is reported to have cholesterol-lowering, anti-inflammatory, antibacterial and antifungal properties. Moreover, β-sitosterol may help to inhibit the development of cancer and has antioxidant and UV stabilizing properties. Therefore, the presence of stigmasterol (1.91 mg/g), gamma-sitosterol (1.29 mg/g) and campesterol (0.40 mg/g) in *C. fenestratum* seed fat (Table 5) enhances the potential applicability of this fat in both cosmetics and nutritional supplements (Phương *et al*., 2018).

A relatively low level of squalene (0.13 mg/g) is found in *C. fenestratum* seed fat (Table 5). Squalene is arguably one of the most popular ingredients in cosmetic and dietary supplement formulations. It is mainly sourced from animal fats such as whales, sharks, salmon, etc. Studies have highlighted potential benefits of squalene including, but not limited to, reducing skin damage from UV radiation, lowering LDL and cholesterol levels, and antitumor and anticancer effects against various cancers. Incorporating squalene into the human diet is recommended without adverse health risks (Lozano-Grande *et al*., 2018). Despite the diverse bioactivities of squalene and its potential applications in cosmetics, pharmaceuticals and nutrition, human intake remains low due to limited natural sources and extraction methods.

Fucosterol is also found as a minor constituent in *C. fenestratum* seed fat. A study done on the constituents present in the unsaponifiable matter of 10 Indian seed oils highlighted the presence of fucosterol in minor quantities (Saeed *et al*., 1991). Due to the presence of fucosterol, the incorporation of *C. fenestratum* seed fat could provide therapeutic properties associated with fucosterol, including anti-cancer, anti-diabetic, reduction of oxidative stress and cholesterol-lowering effects (Maeda, 2013).

Thermal stability *C. fenestratum* seed fat

Thermogravimetric Analysis (TGA) and Derivative Thermogravimetry (DTG) curves for *C. fenestratum* seed fat and peanut oil, illustrate the mass loss at increasing temperatures in an inert nitrogen atmosphere (Figure 4). The peak in the DTG curve signifies the temperature at which the maximum fat mass loss occurs in a nitrogen atmosphere, serving as an indicator of the thermal stability of fat. A heating rate of 10 °C/min was chosen in this study for its proven effectiveness in providing comprehensive insights into the thermal behaviour of oils, as established in previous studies (Jayadas & Nair, 2006).

The onset temperature in TGA analysis refers to the temperature at which the TGA curve first deviates from the baseline before the occurrence of the thermal event. The onset temperature, determined at a 2% loss of

mass accounts for potential weight loss due to moisture and volatile compounds (Jayadas & Nair, 2006). The comparison in Table 6 reveals that the onset temperature of the thermal decomposition of *C. fenestratum* seed fat is higher than that of sesame and coconut oils but lower than for sunflower oil (Jayadas & Nair, 2006). This analysis provides valuable information about the relative thermal stability of *C. fenestratum* seed fat compared to other commonly used oils. The final decomposition temperature of *C. fenestratum* seed fat obtained from the DTG curve was 416.88 ± 1.74°C (Table 1). This temperature signifies the point at which the fat undergoes complete degradation, and understanding this parameter is pivotal for assessing the thermal stability and potential applications of *C. fenestratum* seed fat. The decomposition temperature is compared with that of peanut oil (422.22 ± 1.73°C), which is considered as one of the most thermally stable oils. The close decomposition temperature to the peanut oil confirms the high thermal stability of *C. fenestratum* seed fat, a crucial property for industrial applications of plant oil.

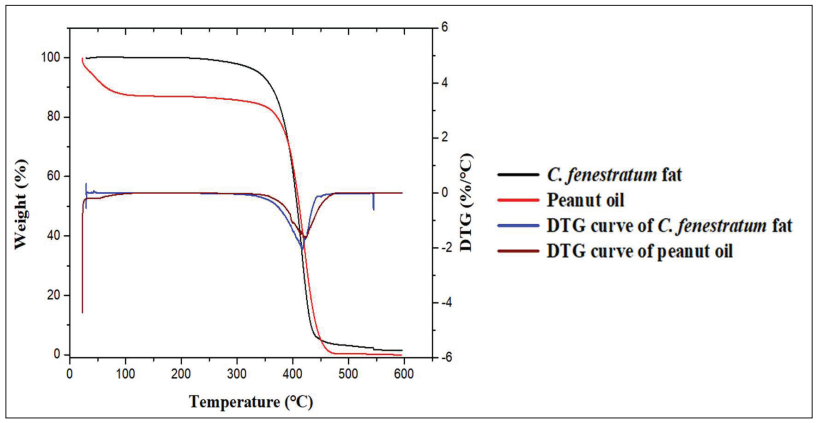

Figure 4: TGA and DTG curves of C. fenestratum seed fat and peanut oil (for comparison) obtained from thermogravimetric analysis

Table 6: Onset temperatures of thermal degradation obtained by *C. fenestratum* seed fat compared with commercially available plant oils. (Jayadas & Nair, 2006)

Name of the oil	Onset temperature (°C)
Sunflower oil	345
Sesame oil	282
Coconut oil	257
Peanut oil	128
C. fenestratum fat	293

CONCLUSION

This study reveals that mature seeds of *C. fenestratum* are a new source of high oil/fat bearing seeds, a property demanded in industrial applications of oil seeds. The ripened seeds yielded substantial oil content of 46.94 ± 0.01%, along with favorable attributes such as low moisture content (4.10 ± 0.03%), AV (2.34 ± 0.10 mg KOH/g), IV (56.33 ± 0.32 g I_2/100g), and a promising FA profile comparable to shea, sal, mango and kokum butter. The most abundant SFA is stearic acid, indicating potential applications in cosmetics for its emollient properties while the most abundant UFA is oleic acid, which is considered as a component of heart-healthy fat. The simple FA profile also signifies its potential use in other industrial applications as well. *C. fenestratum* seed fat also shows thermal stability closer to that of peanut oil. The chemical constituents in unsaponifiable matter indicate enhanced cosmetic properties such as moisturizing, anti-inflammatory and antioxidant effects. From a nutritional standpoint, the presence of phytosterols and squalene indicates potential applications in dietary supplements. While these preliminary findings are promising, further confirmation studies are required to confirm its full potential in cosmetics and dietary supplements. Future investigations could include comprehensive tests such as shelf-life, toxicity assessments, microbial analysis, and phase behaviour studies etc. These additional steps

will provide a thorough understanding of the potential industrial, cosmetic, and nutritional applications of *C. fenestratum* seed fat. However, *C. fenestratum* is considered a rare and critically endangered vine plant that is native to Sri Lanka and small parts of South Asia. Despite its critically endangered status, the plant is extensively used in a large number of commercial herbal medicine products. The plant is almost entirely sourced for commercial products from natural habitats due to the extreme difficulty of cultivation. However, this plant produces large number of seeds during its annual fruiting season, which are currently not used in any commercial applications. Therefore, we believe that the finding of this study would not pose a new threat to the critically endangered *C. fenestratum* plant and would provide more insight to its already known medicinal properties.

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