CHARACTERIZATION OF SEED OILS OF THREE FABACEAE SPECIES

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As a tropical country, Sri Lanka has wide varieties of oil-bearing seeds which often end up as waste without proper utilization. Plant seed oils have emerged as a promising avenue for the use in cosmetics and dietary supplements due to their triglycerides with a useful fatty acid composition and other non-polar constituents. However, most of these oils are not characterized by chemical composition. This study aims to characterize the seed oils of *Pongamia pinnata L., Clitoria ternatea L.* and *Adenanthera pavonina L*. by determining Fatty Acids (FA) composition as their methyl esters, nonpolar constituents in unsaponifiable matter, and other physical properties. The oil was extracted using the soxhlet extraction method. Ash content of seeds, Acid Value (AV), Iodine value (IV), and Smoke Point of oils were also determined. Prepared Fatty Acid Methyl Esters (FAME) and unsaponifiable matter were analyzed using GC-MS. Thermogravimetric Analysis (TGA) was performed to assess the thermal stability. The results indicated that *P. pinnata*, *A. pavonina* and *C. ternatea* show oil yields (w/w) of 28.32%, 28.25%, and 12.90%, respectively. Smoke point values ranged from 151.6 ± 12.1 °C to 213.5 ± 14.3 °C. *P. pinnata* showed a higher AV of 67.73 ± 1.36 mgKOH/g, while the other oils showed 10.17 ± 0.93 mgKOH/g and 8.46 ± 1.13 mgKOH/g. IVs ranged from 10.54 ± 2.65 to 13.97 ± 1.46 gI2/100g and ash content values varied from 2.61 ± 0.16% to 4.58 ± 0.32%. A higher yield of unsaponifiable matter of all three of these oils is found which ranged from 2.05 ± 0.01 % to 5.02 ± 0.01 %. Long-chain fatty acids, such as Oleic, Linoleic, Stearic, and Palmitic acids, were the most abundant FA constituents in all the oils. *C. ternatea* contained γ-sitosterol, stigmasterol, campesterol and taraxasterol as phytosterols that show antioxidant, and anti-inflammatory effects on human. *P. pinnata* contained a higher amount of squalene which is a good emollient in the unsaponifiable fraction. In conclusion, the results suggest that all three oils show a high potential to be used in both dietary supplements and cosmetics.

Keywords: Family Fabaceae, unsaponifiable matter, fatty acids, cosmetics, dietary supplements

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INTRODUCTION

Globally there's an emerging demand for novel sources of fats and oils in the cosmetic and nutritional industries. This trend is mainly driven by consumers' preference for eco-friendly and less-toxic products that have minimal side effects. As a result, these industries are increasingly shifting towards renewable plant-based sources. The plant seed oils offer an exciting avenue towards cosmetic and dietary supplements due to their low toxicity and fewer side effects.[1] As a tropical country, Sri Lanka is home to a vast array of oil-bearing seeds, some of which have been traditionally used in Ayurvedic and other traditional medicinal practices. However, there is currently a lack of organized data on the chemical composition and physical characteristics of these oils, which limits their further utilization. This study aims to analyze and characterize the seed oils of *Pongamia pinnata L., Clitoria ternatea L.* and *Adenanthera pavonina L.* of the family Fabaceae (or Leguminosea). The Fabaceae family is globally the 3rd largest family of flowering plants comprising 19,400 species classified in approximately 730 genera that are widely distributed in tropical, subtropical and temperate regions. This family possesses extremely diverse characteristics that have enabled humans for a long period to use these plants for synthesizing natural products for medicinal purposes, including the treatment of numerous diseases. The legumes of this family are a unique and significant feature due to their nitrogen fixation ability.[2]*P. pinnata* is distributed all over the world but has been extensively used in Ayurvedic and Siddha medicine in India. Therefore, numerous studies have been conducted on the fatty acid composition of *P. pinnata* seed oil in India. The seed oil exhibits an average oil yield of approximately 28-34% with a high proportion of Polyunsaturated Fatty Acids (PUFA). A study done on this oil has demonstrated that the oil has potential as a sustainable source of biodiesel. Due to the presence of various chemical constituents, *P. pinnata* is recognized as an essential medicinal plant with a diverse range of pharmacological activities.[3,4] *C. ternatea* which is commonly known for its blue flowers, has been used in the Ayurvedic system of medicine for its medicinal properties. Extracts from the roots, seeds, and leaves of *C. ternatea* have been in clinical use for some time. Previous studies done on the fatty acid composition of *C. ternatea* seed oils have found that they are abundant in Oleic (C18:1), Palmitic (C16:0) and Linoleic (C18:2) in decreasing order of abundance.[5] *A. pavonina* is a tropical plant that is traditionally used for medicinal purposes and is widely distributed in various Asian countries. Recent studies have shown that *A. pavonina* is particularly rich in lipids, crude proteins and fatty acids compared to other legume oils. Additionally, *A. pavonina* has a high nutritional value and may have a positive impact on human health, which makes it a promising source of edible oil. Not having sufficient scientific data available on their efficacy, safety, specific benefits and lack of awareness of the potential of these oils are the reasons for the limitation of utilization of these oil sources in the industry at the moment [6,7]

The objective of this study is to evaluate the oil yield, the chemical composition of Fatty Acids (FA), percentage yield, and chemical composition of unsaponifiable matter and other physical characteristics of *Pongamia pinnata L., Clitoria ternatea L.* and *Adenanthera pavonina L.* to find new potential oil candidates for the use in cosmetic ingredients and dietary supplements. Previous studies have been carried out on these three species focusing primarily on the analysis of Fatty Acid (FA) composition. However, a comprehensive analysis of the unsaponifiable matter present in these oils has not been conducted to date.

Unsaponifiable matters are the substances in fats and oils that can't be saponified by alkali hydroxide. They provide beneficial properties such as antioxidant, anti-aging, and anti-inflammatory effects that can be incorporated into cosmetic and dietary applications.

METHODOLOGY

The oil was extracted from high-quality dried seed kernels via the soxhlet extraction method using hexane as the solvent. To determine the ash content, acid value (AV), and iodine value (IV) of the extracted oils, standard procedures were followed, including AOAC 942.05, A.O.C.S Official method, Cd 3a-63, 2006, and AOAC 920.159. However, minor modifications were made to the availability of resources. The smoke point, which refers to the temperature at which the sample emits a thin, continuous stream of smoke, was determined using an infrared (IR) thermometer.[8] The conditions employed during the analyses were specified as appropriate. A modified AOAC 933.08 method was followed to extract the unsaponifiable matter from the samples.[9] Fatty Acid Methyl Esters (FAME) were derived by trans-esterification of fats and oils with methanol. Both of these were analyzed from GC-MS to identify their chemical composition. To determine the thermal stability Thermal Gravimetric Analysis (TGA) was performed. TGA was performed by increasing the temperature up to 600 °C under a nitrogen atmosphere.

RESULTS AND DISCUSSION

Table 01 – Table of all the test results of three species

Results	**Name of the Seed Oil**		
	P. pinnata	***C. ternatea***	***A. pavonina***
Yield of Crude Oil (%)	28.32 ± 0.01	12.90 ± 0.01	28.25 ± 0.01
Ash Content (%)	2.61 ± 0.16	4.55 ± 0.20	4.58 ± 0.32
Moisture Content (%)	7.04 ± 0.75	4.75 ± 0.52	1.44 ± 0.26
Acid Value (mg KOH/g)	67.73 ± 1.36	10.17 ± 0.93	8.46 ± 1.13
Iodine Value (g I2/ 100g)	11.68 ± 0.74	10.54 ± 2.65	13.97 ± 1.46
Yield of Unsaponifiable Matter (%)	3.22 ± 0.01	2.05 ± 0.01	5.02 ± 0.01
Smoke Point (^{0}C)	151.6 ± 12.1	213.5 ± 14.3	206.7 ± 5.7
Decomposition Temperature (^{0}C)	287.06	416.74	425.14

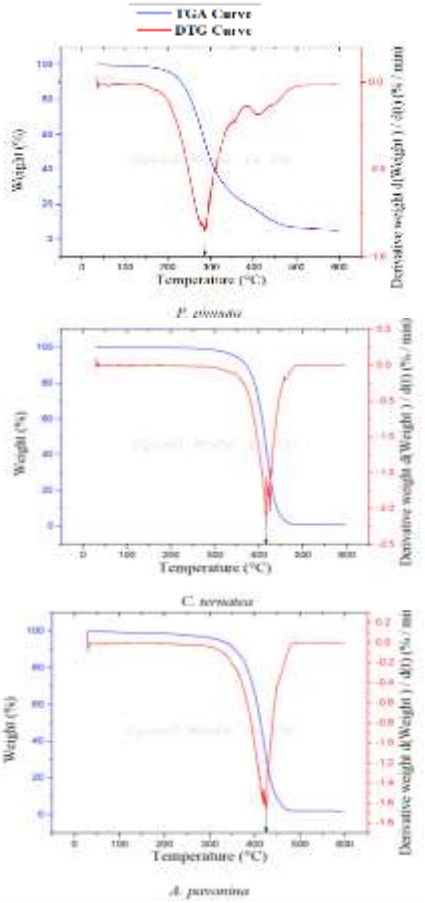

Figure 01 – TGA-DTG Curves

Table 02 – Fatty acid profile of the three seed oils

		P.	***C.***	***A.***

		pinnata	***ternatea***	***pavonina***
Saturated Fatty Acids (%)				
C8:0	Caprylic acid	-	-	0.05
C9:0	Pelargonic acid	-	-	0.07
C12:0	Lauric acid	0.07	-	0.04
C16:0	Palmitic acid	9.21	13.49	10.07
C17:0	Margaric acid	0.08	-	-
C18:0	Stearic acid	4.26	6.39	2.26
C20:0	Arachidic acid	0.98	1.58	1.24
C22:0	Behenic acid	7.78	0.89	2.63
C23:0	Tricosylic acid	-	-	0.36
C24:0	Lignoceric acid	3.13		22.9
Unsaturated Fatty Acids (%)				
C10:1		-	-	0.03
C16:1	Palmitoleic acid	-	-	0.1
C18:1	Oleic acid	26.59	51.48	13.67
C18:2	Linoleic acid	18.54	11.23	33.68
C18:3	α-Linolenic acid	1.17	-	2.57
C20:1	Gondoic acid	1.45	-	3.13
C22:1	Erucic acid	-	-	0.44
C24:1	Nervonic acid	-	-	0.58
Total FAs (%)		73.26	85.06	93.82
Total % of Unsaponifiable Matter		3.22	2.05	5.02
Unidentified FAs (%)		23.52	12.89	1.16
SFA/ UFA Ratio		0.53	0.36	0.73

According to the data in Table 01 oil yields obtained from *P. pinnata and A. pavonina* are particularly advantageous and cost-effective for industrial-scale production. The high ash content indicates a higher mineral content and hence a higher nutritional value. The low moisture content can serve as a rough indication of the shelf-life and quality of the extracted oils. The acid value of an oil is used to determine its potential for rancidity and is measured by its free fatty acid content. *P. pinnata* is a drying oil. Therefore it showed a very high acid value compared to the other two oils. The unsaturated fatty acids in drying oils are prone to reacting with oxygen and water over time, leading to the formation of free fatty acids, which ultimately increases the acid value of the oil. The iodine value is a measure of the unsaturation of fatty acids. The thermal stability of oils obtained from *C. ternatea* and *A. pavonina* was higher than that of coconut oil, with decomposition temperatures exceeding 380 °C. The results in Table 02 show that Oleic and Linoleic were the most abundant Unsaturated Fatty Acids (UFA) in all 3 oils with palmitic, stearic and behenic acids being the most abundant Saturated Fatty Acids (SFA). However, the percentage of UFA was higher than SFA in all 3 oils. Based on the SFA/UFA ratio, all three oils should be in liquid form. However, *P. pinnata* remains in a solid form at room temperature due to the drying oil effect. Drying oils are liquid triglycerides that cross-link and solidify through reaction with atmospheric oxygen. Essential fatty acids such as omega-3 and omega-6 are critical for human biological activities, but cannot be synthesized in the body. α-Linolenic and linoleic acids are the parent FAs that synthesize the rest of the omega 3 and 6 series. *A. pavonina* oil contains a higher percentage of linoleic acid (33.68), making it more applicable for dietary applications. A study done on six seed oils belonging to the Fabaceae family showed linoleic as the most common fatty acid. Long-chain SFA and their fatty alcohol derivatives are

beneficial for obtaining moisturizing properties and also serve as vehicles for other active ingredients present in cosmetic formulations. Palmitic (C16:0) and stearic (C18:0) acids in plant oils provide oiling, softening, smoothing and protective properties that help to keep skin properly moisturized. The presence of omega-3, and omega-6 polyunsaturated fatty acids in cosmetic products is important as they provide anti-allergic properties and healing effects. [10,11]

According to Table 01, all 3 oils exhibited a considerably high yield of unsaponifiable matter, but the highest yield was obtained from *A. pavonina.* Unsaponifiable matter of *C. ternatea* contained 36.50% of taraxasterol, 31.61% of gamma-Sitosterol, 4.56% of campesterol and 1.75% of stigmaterol. These derivatives of phytosterol have blood cholesterol level-lowering properties as well as antioxidant activity and anti-inflammatory effects. Squalene is present in both *P. pinnata* oil (1.86%) and *C. ternatea* oil (0.64%). Scientific research has shown that squalene reduces skin damage caused by UV radiation and has antitumor and anticancer effects and can also prevent cardiovascular diseases. When comparing the unsaponifiable fractions, it can be concluded that *C. ternatea* possesses the highest potential as an ingredient in both cosmetic and food formulations. Figure 02 shows the TGA and DTG curves plotted using OriginPro 9.0 64-bit software for the data obtained from the thermogravimetric analysis (TGA) done separately on the three seed oils. DTG stands for derivative thermogravimetry which is the first derivative of the TGA curve. The decomposition temperatures mentioned in Table 01 were obtained from the DTG curve. *A. pavonina* oil decomposes at a higher temperature than the other two oils, hence the higher thermal stability.

CONCLUSIONS/RECOMMENDATIONS

These three oils demonstrated potential in industrial applicability. *A. pavonina* and *C. ternatea* in particular, have the potential to be used as alternatives to deep-frying oils due to their thermal stability. These oils also have significant potential to replace rare and expensive oils that are currently used in the cosmetic and nutritional industries, thereby providing a more cost-effective and sustainable option. By recognizing the market value of these previously overlooked seeds, we can avoid waste during the harvesting process and promote sustainable resource utilization.

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