Analysis of Fatty Acid, Phospholipid and Glyceride Contents of *Hunteria Umbellata* Seed Oil: Potential Application in Skin Products

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ABSTRACT

Fatty acids are the carboxylic acids found in living things that form fats and oils, phospholipids are compound lipids that contain phosphate groups, and other substituents richly found in cell membranes and serve as cytoskeletal components of cells. At the same time, glycerides are the product of esterification between fatty acids and glycerol. These organic products are present not only in animals but also in plants like *Hunteria umbellata*. This study provided information on the fatty acid, phospholipid, and glyceride compositions of the petroleum ether seed oil extracts of *H. umbellata*. The oil was extracted using Soxhlet extractor and petroleum ether as a solvent. The fatty acid, phospholipid, and glyceride compositions of the petroleum ether seed oil extracts of *H. umbellata* were ascertained using gas chromatography. The findings of the study indicated that the seed oil of *H. umbellata* is rich in linoleic acid, triglycerides, and phosphatidylcholine, as they were the most predominant in concentrations in the fatty acid, glyceride, and phospholipid groups respectively. Due to the health and industrial benefits of linoleic acid, triglyceride, and phospholipid (phosphatidylcholine), the consumption of the oil from *H. umbellata* and its applications in the aesthetic and pharmacological industries is strongly encouraged.

Keywords: Fatty acids, phospholipids, glycerides, *Hunteria umbellata*, seed oil

INTRODUCTION

Throughout history, plants and plant-based products have been used as both food and medicine. As food, they provide essential nutrients for maintaining health and vitality [1,2]. Owing to the abounding phytochemical [3], plants have been utilized to treat and manage various diseases [4,5]. The renewed interest in plant-based products as food and medicine is attributed to their affordability and year-round availability. They are also known for their low toxicity [6, 7], unlike synthetic counterparts that often come with a range of toxicities and side effects [8,9,10,11]. *H. umbellata* is one such plant with a long history of folkloric use as food and medicinal. *H. umbellate* belongs to the family of Apocynaceae with folkloric application as therapy for numerous diseases in Nigeria [12]. *H. umbellata* is extensively distributed most especially in the southern, eastern, and western regions of Nigeria [13,14]. Many studies have shown that most parts of the plant have been adopted locally to treat diseases like piles, yaws, dysmenorrhea, fevers, infertility, diabetes, peptic ulcers, helminthic infections, and so on [14,15]. Fatty acids are aliphatic carboxylic acids and have the general formula, R—CO—OH, where COOH (carboxylic group) represents the functional group. It is the furthermost constituent of lipids in the body. They are generally found in ester linkage in different classes of lipids. In the human body, free fatty acids are formed only during metabolism [16]. Phospholipids are a group of polar lipids that have two fatty acids, a glycerol unit, and a phosphate group which is esterified to an organic molecule such as choline, ethanolamine, inositol, etc. They serve as natural antioxidants in the prevention of oxidative effects [17]. The two dominant phospholipids of clinical significance are phosphatidyl ethanolamine (cephalin) and phosphatidylcholine (lecithin) [18]. Glycerides are esters obtained from the esterification reaction of glycerol and fatty acids and are present in vegetable oils and animal fats, mostly in the form of triglycerides, which are the chief component of fat [19]. Lipids are greatly involved in the constitution of the plasma membranes of plants, and their structure plays a pivotal function in several important processes of plant cell physiology including immunity, abiotic stress, and cell-to-cell communication through plasmodesmata [20]. Lipids in human nutrition are obtained from the storage and structural lipids of plants and animals. More than 90% of dietary fats are triacylglycerols originating from the adipose tissue or milk of animals or plant seed oils, mainly in the form of manufactured products. Seed oils and animal storage fats provide the bulk of the lipids consumed in foods. However, some foods, although contributing little total fat to the diet, are nevertheless important sources of specific essential fatty acids. Lipid digestion in adults takes place mainly in the upper part of the small bowel. Lipolysis of triacylglycerols catalyzed by the enzyme pancreatic lipase generates fatty acids and mono-acylglycerols. Most natural fatty acids of dietary significance have double bonds in the cis geometrical configuration. How fatty acids are distributed in a triacylglycerol may also influence plasma cholesterol irrespective of the overall composition of the fatty acids, and this may describe certain obvious abnormalities [21]. Plant-based foods abundant in unsaturated fatty acids, like omega-3 and omega-6 fatty acids, have been correlated with anti-inflammatory potency. These fatty acids can modulate the secretion of pro-inflammatory and anti-inflammatory compounds in the body. Lipid extracts from plants like *H. umbellata* are indeed being explored for potential use in the cosmetics and skincare industries due to their potential benefits for skin health [22,23]. These extracts may offer various properties that make them valuable ingredients in skincare products. Phospholipids and glycerides are lipid-based ingredients that are commonly used in the formulation of skincare products. They offer various benefits and play important roles in skincare formulations. For instance, phospholipids, such as lecithin, are excellent emulsifiers. They help in the fusion of oil and water-based ingredients in skincare products, allowing for stable and uniform formulations. They also have natural moisturizing properties. Also, glycerides, such as glycerin (also known as glycerol), are excellent emollients. They have a moisturizing and softening effect on the skin, leaving it feeling smooth and supple [24]. In skincare formulations, phospholipids and glycerides are frequently used in association with other active ingredients to create products tailored to specific skin concerns [25]. For example, they may be incorporated into anti-aging products, hydrating serums, cleansers, and sunscreens. These ingredients can help improve the overall texture, feel, and effectiveness of skincare products [26], founded on this premise, this work aimed to study the fatty acid, phospholipid, and glyceride compositions of the seed oil extracts of *H. umbellata*, which may be utilised in the formulation of dermatological products owing to their medicinal and pharmaceutical importance.

MATERIALS AND METHODS

Sample Collection

The fruits of *H. umbellata* were purchased from Rumuokoro market, Rivers State, Nigeria. The fruit was confirmed at the Department of Biology, Ignatius Ajuru University of Education, Nigeria. The fruit was allowed to ripen and thereafter, the seeds were detached from the fruits and washed to remove any flesh stuck to the seed.

Plate 1(a) *H. umbellate* seeds

Plate 1(b) *H. umbellate* fruits

Extraction of *H. umbellata* Seed Oil

The seeds of *H. umbellata* were harvested from the fruits and air-dried in the laboratory in the absence of sunlight. Thereafter, the seeds were broken to remove its coat. The *H. umbellata* seeds obtained after breaking their coats were crushed in a grinder to a fine powder. The oil was obtained by using a Soxhlet extractor and petroleum ether as solvent. Ten grams (10g) of the powdered *H. umbellata* seed was refluxed for about 6 hours at a mild temperature of 45 °C in a Soxhlet extractor. The solvent was evaporated with the aid of a rotary evaporator (RE 300, Bibby Scientific, UK) with reduced pressure at the same temperature of 45 °C. The extracted oil was stored in an air-tight container until when needed for analysis.

Quantification of Fatty Acid Content in *H. umbellata* Seed Oil

The fatty acid compositions of *H. umbellata* seed oil in the form of fatty acid methyl esters (FAME) were determined using the method of Irabor *et al.* [27]. Derivatization was done in the first stage to increase volatility by esterifying 50 g of the oil at 95˚C for 5 minutes with 3.4 mL of the 0.5M potassium hydroxide solution (KOH) in dry methanol. Afterwards, 0.7M hydrochloric acid solution (HCl) was used to neutralize the mixture and 14% boron trifluoride (BF_3) in methanol (3 mL) was added. The mixture was exposed to heating (90˚C) for 5 minutes to reach complete methylation. Redistilled n-hexane was used to extract the fatty acid methyl esters three times. The content was condensed to 1 mL for gas chromatography analysis and 1 µL was injected into the injection port of Gas Chromatography (GC). GC condition for FAME quantification is described below.

GC condition for analysis of Fatty Acid Composition of *H. umbellata* Seed Oil

Gas Chromatography analysis was accomplished with HP 6890 Powered by HP Chem Station Rev. A 09.01 [1206] Software and HP 5MS capillary columns (30m x 0.25mm x 0.25µm film thickness). The programmed temperature is 600C/ 3min, 8 0C/min to 140 0C/min. Injector and detector temperatures were kept at 230 0C and 275 0C respectively; the carrier gas is hydrogen (1.0ml/min), detector dual, FID. The volume injected was 0.5µl. The detection of the component fatty acids was done by matching their retention time with those of pure authentic samples and using their linear retention indices (LRI) relative to the series of n-hydrocarbons.

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Determination of Phospholipids Composition of *H. umbellata* Seed Oil

The estimation of phospholipid content was done in line with the method reported by Irabor *et al.* [27]. About 0.01g of the extracted seed oil was added to the test tube and the solvent was wholly eliminated by passing a stream of nitrogen gas on the oil to guarantee thorough dryness for phospholipids analysis. Chloroform (0.4 mL) and chromogenic solution (0.1 mL) were added to the tube respectively. Thereafter, the tube was heated at 100 °C in a water bath for 1 minute, and 20 seconds. After heating, the tube was cooled to laboratory temperature, and 5 mL of hexane was added. The tube and its contents were shaken gently multiple times. The two resulting layers were allowed to separate. The hexane layer was recouped and condensed to 1 mL for gas chromatography analysis using a pulse flame photometric detector (PFPD).

GC condition for analysis of Phospholipid Composition of *H. umbellata* Seed Oil

Gas chromatographic analysis was performed using an HP-5 capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). Prior to sample injection, the system was calibrated with standard phospholipid mixtures under identical operating conditions to ensure peak resolution and reproducibility of quantification. The injector temperature was set at 250 °C with a split ratio of 20:1, while the detector temperature was maintained at 320 °C. A pulsed flame photometric detector (PFPD) was employed. Nitrogen served as the carrier gas at a constant flow, with hydrogen and compressed air supplied at 20 psi and 30 psi, respectively. A 1.0 µL aliquot of the sample was injected. The oven temperature program was as follows: Initial temperature: 50 °C, held for equilibration. First ramp: 10 °C/min to 250 °C, maintained for 4 min. Second ramp: 15 °C/min to 310 °C, maintained for 5 min. These optimized conditions, along with calibration against external standards, ensured reproducibility and accuracy in the determination of phospholipid composition.

Determination of Glycerides Composition of *H. umbellata* Seed Oil

The glyceride composition of *H. umbellata* seed oil was determined following the procedure of ASTM International D6584 (2017). Before analysis, the gas chromatograph (GC) was calibrated using certified reference standards of mono-, di-, and triglycerides (Supelco, USA), prepared at five concentration levels to generate calibration curves. Each standard solution was analyzed under identical chromatographic conditions, and linear regression was applied to establish response factors for quantitative determination. Oil samples were derivatized following the same transesterification procedure described for fatty acid composition, ensuring conversion into trimethylsilyl (TMS) derivatives to improve volatility. One microliter of the prepared sample was injected into the GC system equipped with a flame ionization detector (FID) and a high-temperature capillary column (e.g., DB-5HT, 15 m × 0.32 mm × 0.10 μm). The injection was performed in splitless mode, with helium as the carrier gas at a constant flow rate. Glyceride peaks were identified by comparing retention times with those of the reference standards, and quantification was achieved using the calibration curves. System suitability was confirmed by running standard checks after every 10 sample injections to monitor retention time shifts and detector response.

GC condition for analysis of Glycerides Composition of *H. umbellata* Seed Oil

The column type was Elite-Biodiesel M (14 m × 530 mm × 0.16 μm) and the detector was FID. The inlet temperature was 60°C with a split ratio of 20:1 and the detector temperature was 380°C. The injection volume was 1.0 μL, helium was used as a carrier gas, the flow rate was 1ml/min, and hydrogen pressure and compressed air were 45 ml/min and 450 ml/min respectively. The oven temperature program was: initial temperature at 60°C for 2 minutes, first ramping at 10 °C/min to 200 °C, second ramping at 8 °C/min to 300 °C constant at 5 minutes.

Statistical Analysis

The statistical analysis was done using SPSS (Windows version 21.0). Descriptive statistics was used to obtain the mean values.

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RESULTS AND DISCUSSION

Fatty Acid Constituents of *H. umbellata* Seed Oil

The fatty acid composition of *H. umbellate* is presented in table 1 below

Table 1. Relative fatty acid concentrations of *H. umbellata* seed oil

Names of fatty acids	Concentration (%)
Butyric Acid (C5:0)	Trace
Caprylic Acid (C8:0)	Trace
Lauric Acid (C12:0)	Trace
Palmitic Acid (C16:0)	12.82
Palmitoleic Acid (C16:1)	0.15
Stearic Acid (C18:0)	16.82
Oleic Acid (C18:1)	17.04
Linoleic Acid (C18:2)	52.21
Linolenic Acid (C18:3)	0.56
Arachidic Acid (C20:0)	0.28
Lignoceric Acid (24:0)	0.10
Total	**99.98**

The fatty acid composition of *H. umbellata* and their concentrations is presented in Table 1. Eleven (11) fatty acids were detected in *H. umbellata* with eight in quantifiable amounts (99.98%) while three were in trace quantity (0.02%). Of the eleven fatty acids detected, six (6) are saturated fatty acids, one (1) is a monounsaturated fatty acid and four (4) are polyunsaturated fatty acids. The percentage relative fatty acid composition concentration of *H. umbellata* is in the order of polyunsaturated fatty acid (PUFA) (53.15%) > saturated fatty acid (SFA) (29.79%) > monounsaturated fatty acid (MUFA) (17.04%). Palmitic acid is the saturated fatty acid with the highest relative fatty acid concentration (12.82%), oleic acid, a MUFA with a relative fatty acid concentration of 17.04% while linoleic acid is the PUFA with the highest and overall relative fatty acid concentration of 52.21%. Linoleic acids are found in vegetable oils and their recommended dietary intake should be encouraged because they help in skin cancer prevention, lower blood pressure and lower the probabilities of heart attack and arteriosclerosis by lowering LDL-cholesterol [28]. Industrially, linoleic acid, an essential omega-6 fatty acid, is a regular ingredient in skincare products due to its potential benefits for skin health. Some studies suggest that individuals with acne-prone skin may have lower levels of linoleic acid in their skin's sebum. Cutaneous use of linoleic acid-containing skincare products may help balance the skin's lipid profile, reduce the secretion of sebum (skin oil), and minimize the probability of obstructed pores that cause acne.

Several studies revealed that linoleic acid has anti-inflammatory properties that can help calm irritated or inflamed skin, enhance wound repair and tissue regeneration, the ability to improve the overall texture and appearance of the skin, making it feel softer and smoother, and anti-aging property as an antioxidant [29].

Phospholipid Composition of *H. umbellata* Seed Oil

Table 2: Relative phospholipid concentrations of *H. umbellata* Seed Oil

Phospholipids	Concentration (mg/100g)	Concentration (%)
Phosphatidylethanolamine	12.06	19.02
Phosphatidylserine	15.43	24.33
Phosphatidylcholine	33.89	53.46
Phosphatidylinositol	1.72	2.71
Lysophosphatidylcholine	0.09	0.14
Phosphatidic acid	0.22	0.35
Total	**63.41**	**100**

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Phospholipid (PL) composition of *H. umbellata* seed oil is shown in Table 2. The result shows that H. umbellata seed oil is rich in phosphatidylcholine (33.89 mg/100g), phosphatidylserine (15.43 mg/100g) and phosphatidylethanolamine (12.06 mg/100g) while phosphatidylinositol (1.72 mg/100g), lysophosphatidylcholine (0.09 mg/100g) and phosphatidic acid (0.22 mg/100g) are present in very low amount. Phospholipids are mainly used as base components in pharmaceutical, cosmetic, and food industries. Phospholipids are applied to formulate liposomes which helps to transport drugs that are not soluble in water to target sites.

Irabor et al. [27] reported the use of phospholipids in the food industry as stabilizing agents in combining chocolate with margarine. The high levels of phosphatidylcholine in *H. umbellata* seed oil may have contributed to its anti-ulcer potential [30]

Zeisel *et al.* [31] and Calder [32] both reported that phosphatidylcholine helps boost cognitive function, aids in liver repair, helps ease the symptoms of ulcers, promotes lipolysis, helps dissolve gallstones, and prevents NSAID-related gastrointestinal damage. The relative abundance of phosphatidylcholine and phosphatidylethanolamine in various tissues may lead to metabolic disorders such as atherosclerosis, insulin resistance, and obesity [33].

Glyceride Compositions of H. umbellata Seed Oil

Table 3: Relative Glyceride concentrations of *H. umbellata* Seed Oil

Glycerides	Concentration (mg/100g)	Concentration (%)
Monoglycerides	1.25	4.16
Diglycerides	3.03	1.00
Triglycerides	25.76	85.75
Total	**30.04**	**90.91**

The glyceride composition of *H. umbellata* seed oil revealed triglycerides (25.76 mg/100 g; 85.75%) as the predominant fraction, followed by diglycerides and monoglycerides, with monoglycerides (1.25 mg/100 g; 4.16%) being the least abundant. This distribution is consistent with the general lipid architecture of most seed oils, where triglycerides account for the bulk of neutral lipids and minor fractions of di- and monoglycerides contribute to emulsification and structural functions [34]. When compared with other seed oils, *H. umbellata* demonstrates compositional similarities and distinctions that highlight its industrial value. Coconut oil, for instance, contains up to 90% triglycerides rich in medium-chain fatty acids, which are rapidly metabolized and serve as functional lipids in nutraceuticals and cosmetics [35]. In contrast, soybean and sunflower oils are also triglyceride-dominant but with higher proportions of polyunsaturated fatty acids such as linoleic acid, making them highly nutritious yet prone to oxidative degradation [36]. Palm oil and shea butter, on the other hand, possess a triglyceride matrix enriched with saturated and monounsaturated fatty acids, which enhance oxidative stability and extend shelf life in food and cosmetic formulations [37,38]. The comparatively lower concentrations of diglycerides and monoglycerides in *H. umbellata* are in agreement with findings from other tropical seed oils, where these fractions usually represent less than 10% of total glycerides [39]. Despite their low abundance, monoglycerides are functionally significant as natural emulsifiers, surfactants, and antimicrobial agents in food and cosmetic industries [40]. From an application standpoint, the triglyceride-rich profile of *H. umbellata* suggests broad utility. Triglycerides from natural sources are widely employed as emollients in cosmetics [41], carriers for active ingredients in pharmaceutical formulations enhancing solubility, dissolution, and bioavailability of poorly water-soluble drugs [42], and as excipients that improve product texture, spreadability, and consumer sensory experience [43]. The comparable triglyceride concentration of *H. umbellata* with established oils, coupled with its unique fatty acid composition, underscores its potential as an alternative or complementary oil source for both nutraceutical and industrial applications.

CONCLUSION

This study postulates valued awareness into the composition of *H. umbellata* seed oil, highlighting its richness in various fatty acids, phospholipids, and glycerides. Notably, the dominant presence of linoleic acid, a

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polyunsaturated fatty acid, makes *H. umbellata* seed oil a potentially beneficial ingredient for skincare products. The multiple properties of linoleic acid, including anti-inflammatory, wound healing, skin-smoothing, and antioxidant effects, suggest its potential for improving skin health and addressing various dermatological concerns. Furthermore, the high content of phospholipids, especially phosphatidylcholine, in *H. umbellata* seed oil opens up opportunities for applications in pharmaceuticals, cosmetics, and the culinary industry. Phospholipids are vital components for formulating liposomes, aiding drug delivery to target sites, and potentially contributing to anti-ulcer properties. Additionally, phosphatidylcholine has shown promise in promoting cognitive function, liver repair, and gastrointestinal health. The glyceride composition analysis reveals the prevalence of triglycerides in *H. umbellata* seed oil, which can serve multiple purposes in cosmetics and pharmaceuticals, such as emollients, carriers for active ingredients, solubilizing agents, and texture augmenter. Overall, this investigation sheds light on the likely many-sided utilization of *H. umbellata* seed oil in various industries, including skincare, pharmaceuticals, and food, based on its rich composition of essential components. Further studies and product development may explore the full range of benefits offered by this natural resource.

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