ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 12, Iss 4, 2023

Nutritional & Physico-Chemical investigation of *Gmelina Arborea (Roxb.)* seeds

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ABSTRACT

Gmelina arborea seed was investigated. The oil was obtained by solvent extraction using petroleum ether. The percentage oil yield was 52.56%. Proximate analyses showed that the seed contained 4.15% ash, 5.09% Nitrogen,31.81%crude protein, and 5.71%moisture. The specific gravity and refractive index were found to be 0.89 and 1.441, respectively. Characterization of the oil showed that the oil had an iodine value of 60.28g/100gm, saponification value of 84.15mgKOH/g, acid value of 2.917mg/g, 1.45%mg/g free fatty acid, and percentage of unsaponifiable matter 1.54. The iodine and saponification values showed that the oil has a low degree of unsaturation, thus non-drying oil, and has high molecular, making the oil suitable for human consumption. Minerals were also determined by Atomic Absorption Spectroscopic method. Fatty acids of oil are analyzed using gas chromatography to detect the potential source of oil. Seventeen fatty acids were found in the seed oil; the most abundant were Oleic Acid Methyl Ester(C18:1n9c) and Linoleic Acid Methyl Ester(C18:2n6c). Linoleic Acid Methyl Ester (an omega-6 fatty acid) and α-Linolenic acid Methyl Ester (an omega-3 fatty acid) are essential for human health. Amino acid results showed the protein contained and quantities of the most crucial amino acids. Eighteen types of amino acids were found in the seed, and the sources are rich in Glutamate, Aspartate, Serine, Glycine, Alanine, Tyrosine, and Valine. The other amino acid is also found in reasonable quantities. The study aimed to evaluate the nutritional level of seeds in livestock feed.

Keywords – Physico-Chemical, Amino acids, Fatty acids, Seeds, GC, HPLC.

INTRODUCTION

Seeds are an essential source of nutrients and can use for high-quality dietary needs. One of the least expensive ways to get a protein-rich diet is by encouraging the consumption of edible seeds that are rich in them [1] [2]. Proteins play a vital role in human nutrition. Humans' amino acid contents, proportions, and digestibility characterize a protein's biological value [3]. Also, dietetic fats provide essential fatty acids and fat-soluble vitamins. Unsaturated fatty acids, specifically linoleic and linolenic, are the essential fatty acids required in the diet. These essential fatty acids have several bodily functions, such as the immune system and cell membranes. Other food sources of essential fatty acids are fish, oils, kernels, and nuts [4]. *Gmelina arborea* is a large deciduous tree with a straight trunk belonging to the family Verbenaceae. Its bark is smooth and scaly, pale brown. This medium size tree grows up to 30-40 m tall. The flower is white and yellow, and the seed and there are one or two filled seeds per fruit, with sizes varying from 6 to 9mm, hard [5]. The fruit is a drupe, 1.8-2.5cm long, obovoid, seated on the enlarged calyx, gloss and yellow when ripe; exocarp succulent and

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Research paper UGC CARE Listed (Group -I) Journal Volume 12, Iss 4, 2023

aromatic; endocarp bony and usually 2-celled—seeds -3, lenticular, ex-albuminous. Mature fruits are produced one week after the flowering peak, and fruiting may be spread over two months [6]. The seeds are distorted oval, 0.5 cm, and light yellow colored. The bark, leaves, and roots contain traces of alkaloids and are used medicinally in the plant's native range. For example, fruit and bark have medicinal properties against bilious fever [7].

This study investigated fatty acids and amino acid composition in Gmelina Arborea seeds. In addition, the study targeted obtaining all-inclusive components of seeds, which may be of medicinal and nutritional interest.

MATERIALS AND METHODS

Plant Material

The seeds sample were collected from the forest area of Chhattisgarh. Dirt and other foreign materials were cleaned up, and the husk shell of the seed was removed manually. The seeds were subsequently dried and grounded in a mixer grinder for analysis.

Oil Extraction

Extraction of oil was from the seeds of Gmelina arborea using a Soxhlet extractor with petroleum ether as the solvent. 239 grams of the pulverized seed was put in the Soxhlet extractor with petroleum ether for about 10 hours (the boiling point was between 60-80° c). Excess solvent was removed through a rotary evaporation procedure. The oil yield was calculated and stored in a refrigerator for further analysis [8] [9].

Physico-Chemical Analysis

Characteristics of seed oil

The extracted golden yellow oil obtained a 52.56% yield. The oil was analyzed for iodine value, saponification value, acid value, free fatty acid, and unsaponifiable matter by the standard method described in AOAC [10]. With the increase in unsaturation and depending on the chain length of Fatty Acid, the refractive index increases. The refractive index of the oil slightly above room temperature was determined with an Abbe refractometer [11]. Specific gravity is determined to check the density of the oil. The specific gravity bottle is used to determine the density of the oil at room temperature [12]

Proximate analysis of seeds

The proximate analysis provides a top-level, broad classification of the food, seeds, etc. It also provides contents of materials that burn in a gaseous state (volatile matter), in a solid form (fixed carbon), and inorganic waste material percentage. It can provide biomass characteristics [13]. The proximate composition of the seed was determined by the methods mentioned in AOAC & International Scientific on the lines recommended in Wende Analysis [10] [14]. The sample was investigated for the presence of dry matter, organic matter, crude protein, calorific value, and mineral matter (total ash).

Ultimate analysis of seeds

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Ultimate analysis determines the seed's component elements like Carbon, Hydrogen, Nitrogen, Sulphur, etc. It helps select the quantity of air required for combustion and analyze other gases. Ultimate analysis was performed on the seeds of *Gmelina arborea* using the CHN/O analyzer [15]. The study was done both on the original and defatted seeds.

Analysis of metals

Finding inorganic elements is mandatory to ensure the safety and quality of traditional or herbal medicine. For instance, Zinc and Iron are essential metals for the body at certain concentration levels but can be dangerous if concentration levels are high. Microwave digestion and Atomic absorption spectrometry methodology are utilized for checking the element traces and concentration. Approximately 1.0 grams of samples ground in a mortar pestle were taken, and using microwave digestion and atomic absorption spectrometry, the content of copper, iron, and zinc was determined [16]

DETERMINATION OF AMINO ACIDS

Crude protein determination

Nitrogen content was determined by the micro-Kjeldahl method, and the percentage of nitrogen was converted to crude protein by multiplying by 6.25 [17] [18] [19].

Sample analysis

Amino acid content was estimated by high-performance liquid chromatography described in the book Chromatography in Food Science and Technology [20]. The seeds were hydrolyzed using a standard process for 22 h at 110 °C. The mixture was filtered and evaporated to dryness under a vacuum. The hydrolysates were reconstituted with a mobile phase and filtered more with a 0.50-µm pore-size membrane. The analysis was carried out on an Agilent 1260 Infinity HPLC system, attached with a µ-degasser (G1379B), 1260 binary pump (G1312B), 1260 standard autosampler (G1329B), 1260 thermostatic column compartment (G1316A), 1260 diode array and multiple wavelength detector (G1315C), and a Zorbax Eclipse-AAA column (250 mm x 4.6 mm, L x ID) particle size 5µm). The hydrolyzed samples were automatically derivatized with OPA (o-phthalaldehyde for primary amino acids) and FMOC (9-fluorenylmethyl chloroformate for secondary amino acids) by programming the autosampler. After derivatization, 0.5µl of each sample was injected into a Zorbax Eclipse-AAA column at 55 °C, with detection at λ1 = 338 nm and λ2 = 262 nm.

The separation was performed at a flow rate of 0.7ml/min. Each sample is expressed as mg/100g protein.

DETERMINATION OF FATTY ACIDS COMPOSITION [19] [21]

Preparation of methyl esters of fatty acids

Fatty acids analysis was done using methods described in AOAC (2001. 996.06) [22] and the journal of chromatography [23]. The isolated fat was trans-esterified using 0.5 M methanolic KOH to form fatty acid methyl esters (FAME). Fatty acids were determined by a Gas Chromatograph (7890B of Agilent Technologies) equipped with a flame ionization detector and Agilent - DB-FFAP column (nitroterephthalic-acid-modified polyethylene glycol (PEG) of high polarity for the analysis of volatile fatty acids). The column temperature was maintained at an initial temperature of 100°C for 5 min and raised to 240°C at the rate of 4°C/min. The

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carrier gas was Nitrogen at a column flow rate of 1.0. The detector temperature was maintained at 280°C. Individual trans-fatty acids standards, Supelco trans-9-Eliadic methyl ester, 10 mg/ml in heptane, trans-9, 12-Octadecadienoic (linoleliadic) methyl ester, and trans-11-Vaccenic methyl ester, were used.

Sample fatty acid composition was compared with standard fatty acid composition, and percentages were calculated by standardization of peak areas.

RESULTS

The results obtained from oil extraction, Physico-chemical analysis of the seed, proximate analysis, and ultimate analysis & analysis of metals are given below from Table 1-4, respectively.

Table 1. **Physico-Chemical Characteristicseristics of *Gmelina Arborea* seeds sample**

PARAMETER	VALUE
Colour	Golden Yellow
OIL (%)	52.56
REFRACTIVE INDEX	1.441
SPECIFIC GRAVITY	0.894
SAPONIFICATION VALUE	84.15mg KOH/gm
IODINE VALUE	60.28gm/100gm
ACID VALUE	2.917 mg/g
UNSAPONIFIABLE MATTER	1.54
FREE FATTY ACID %	1.45 mg/g

Table 2. **Proximate analysis of *Gmelina Arborea* seeds sample**

PARAMETER	VALUE
ASH%	4.34
MOISTURE%	4.15
CRUDE PROTIEN%	31.8125
CALORIFIC VALUE	7192.01
FIXED CARBON	4.11
VOLATILE MATTER	87.39

Table 3. **Ultimate analysis of *Gmelina Arborea* seeds sample**

TYPE	C%	H%	N%	S%
Original	62.31	9.544	5.09	0.3
Defatted	42.06	6.588	10.12	0.6

Table 4. **Analysis of metals in *Gmelina Arborea* seeds sample**

METALS	Fe(ppm)	Zn(ppm)	Cu(ppm)	Mn(ppm)

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VALUE	281.3	167.8	59.9	41.5

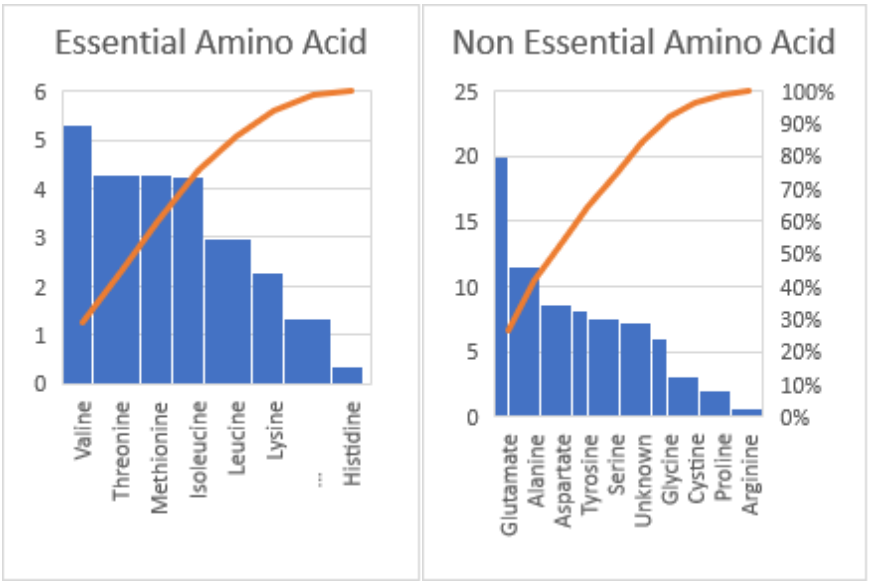
The amino acid profile of the seed oil of *Gmelina arborea* is shown in Table-5 and compositions is depicted by graph in **Graph-1** shows the summary of various essential and non-essential amino acids present in the seed oil.

Table 5. **Amino Acids Profile of *Gmelina Arborea* seeds sample (g/100gm)**

ESSENTIAL	COMPOSITION	NON-ESSENTIAL	COMPOSITION
HISTIDINE	0.34	ASPARTATE	8.59
THREONINE	4.3	GLUTAMATE	19.9
VALINE	5.32	SERINE	7.58
METHIONINE	4.3	GLYCINE	6
PHENYLALANINE	1.32	ARGININE	0.66
ISOLEUCINE	4.26	ALANINE	11.52
LEUCINE	2.96	TYROSINE	8.22
LYSINE	2.28	CYSTINE	3.08
		UNKNOWN	7.3
		PROLINE	2.07

Total EAA %= 25.14(approximate)

Graph 1. Amino Acids Profile of *Gmelina Arborea* seeds sample



The fatty acid profile of the seed oil of *Gmelina arborea* is shown in Table-6, Table-7. Graph 2 summarizes various saturated and unsaturated fatty acids in the seed oil.

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Table 6. Saturated fatty Acids Profile of *Gmelina arborea* seeds oil

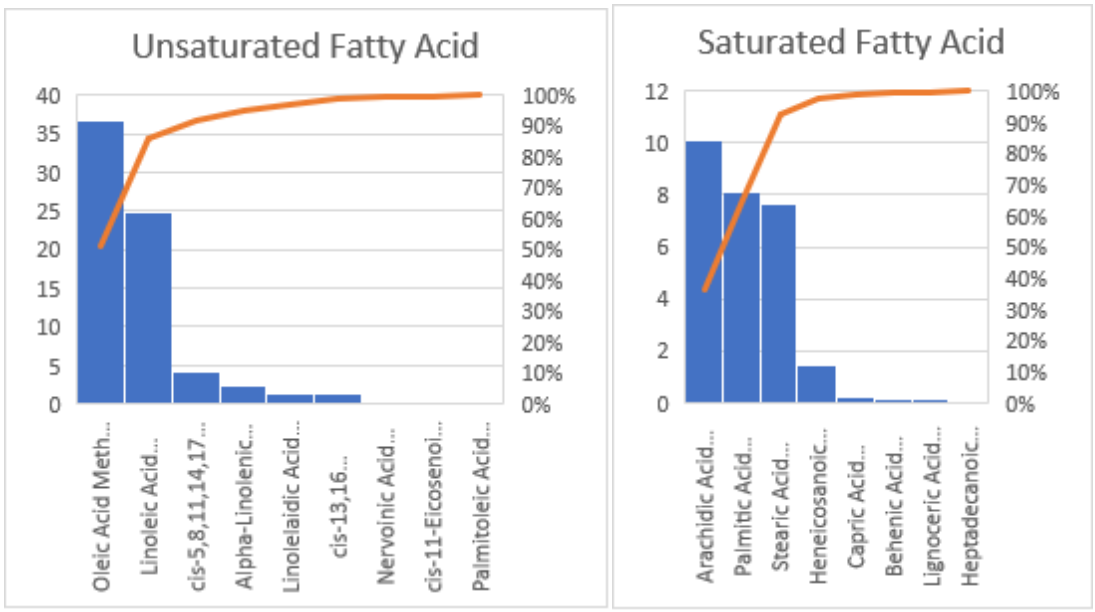
SATURATED	COMPOSITION
CAPRIC ACID (C10:0)	0.25
PALMITIC ACID (C16:0)	8.08
HEPTADECANOIC ACID (C17:1)	0.08
STEARIC ACID (C18:0)	7.68
ARACHIDIC ACID (C20:0)	10.12
HENEICOSANOIC ACID (C21:0)	1.44
BEHENIC ACID (C22:0)	0.17
LIGNOCERIC ACID (C24:0)	0.11

Table 7. Unsaturated fatty Acids Profile of *Gmelina arborea* seeds oil

UNSATURATED	COMPOSITION
PALMITOLEIC ACID (C16:1)	0.23
OLEIC ACID (C18:1n9c)	36.8
LINOLEIC ACID (C18:2n6c)	24.95
LINOLEADIC ACID (C18:2n6t)	1.41
ALPHA-LINOLENIC ACID (C18:3n3)	2.46
CIS-11-EICOSENOIC ACID (C20:1n9)	0.31
CIS-5,8,11,14,17-EICOSAPENTAENOIC ACID (C20:5)	4.2
CIS-13,16-DOCOSADIENOIC ACID (C22:2)	1.27
NERVOINIC ACID (C24:1n9)	0.45

Total unsaturated fatty acid %= 72.08(approximate)

Graph 2. Fatty Acids Profile of *Gmelina Arborea* seeds oil



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DISCUSSION

The iodine value of the seed is 60.28 mg/100gm, which shows that this is a non-drying oil(<100). Therefore the oil is stable concerning oxidative storage. The oil seed of *Gmelina arborea* has a low acid value of 2.917 mg/g, which indicates that it can be used for edible oil and is suitable for human consumption [24]. The acid value is also an indication of contents of free fatty acids and thus contributes to overall shelf life. The lower free fatty acid value (1.45 mg/g) indicates that the oil can be stored for a longer duration [25]. The saponification value refers to the mass in mg of potassium hydroxide (KOH) required to offset the free fatty acids and saponify the esters contained in a gram of material. The saponification number indicates fatty acid components present in FAMEs of oil & depends on the molecular weight and the percentage concentration of fatty acids. The higher molecular weight (84.15mg KOH/gm) indicates the usage of the oil for human consumption. Contents of Iron (281.3 ppm) and Zinc (167.8) are also present in seed in reasonable quantities. Traces of copper and manganese also were found in the seed. Ash content (4.34%) indicates minerals present in the seed. The lower moisture content (4.15%) means, the longer shelf life of these oil seeds and is also prone to microbial activity.

Quantitative analysis shows the variation in amino acid composition in the seed proteins. The outcomes of amino acid composition are listed in Table 1. Among essential Amino Acids, Valine seems to be in the highest quantity (around 5.32 %), followed by Methionine, Threonine, & Isoleucine (having around 4.3%, 4.3%, and 4.26 %, respectively. Traces of other essential Amino acids were also found, like Leucine, Lysine, Phenylalanine & Histidine. Among non-essential Amino acids, Glutamate is found to be in the highest quantity (around 19.9%). It was followed by Alanine, Aspartate, Tyrosine & Serine (having around 11.52%, 8.59%, 8.22% & 7.58%)

Results of the fatty acids profile of fats of Gmelina Arborea seeds are shown in ***Graph-1***. The yield (oil) obtained from the seed was 52.56%. The estimation of FAME derivatives showed seventeen types of fatty acids. Total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) exhibited significant variation in their contents. Oleic acid methyl ester (36.80%) was the most abounded fatty acid. The other major fatty acid found was Linoleic acid (24.95%), Arachidic methyl ester (10.12%), Palmitic acid methyl ester (8.08%), Stearic acid methyl ester (7.68%) using GC.

CONCLUSION

Results of Physio-Chemical analysis & proximate analysis show that oil from seeds of *Gmelina arobrea* is good, edible, and has a good shelf life. The presence of Zinc and iron indicates that the seed and oil can be a source of minerals to overcome deficiencies. Omega-3 in seeds protects the heart from potentially dangerous abnormal rhythms [26] [27]. Oleic acid, which is monounsaturated fatty acid contributes to lowering bad cholesterol (LDL) without lowering good (HDL) cholesterol [28]. Linoleic acid, called omega-6, was found in the seed (around 24.95) and α-Linolenic Acid Methyl Ester (2.46) in Gmelina Arborea seed oils. Omega-6 fatty acid also contributes to the prevention of cardiovascular diseases [29]. The fatty acids are also used in the cosmetic industry for skin care and body care creams [30].

Methionine, an essential amino acid, a building block of protein containing sulfur, contributes to properly functioning cells' growth and repair of tissues. It also plays a critical role in protein

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 12, Iss 4, 2023

synthesis. Methionine chelates heavy metals, such as lead. Although the body can synthesize some amino acids, Methionine must be obtained from the dietary intake [31]. Glutamate improves immunity and contributes to metabolic activities. Also, it contributes to the brain, lungs & other organ systems benefiting from the presence of Glutamate [32]. Cysteine is a non-essential amino acid that contains sulfur, like Methionine; Cysteine also contributes to protein synthesis and multiple metabolic functions. Unlike Methionine, Cysteine can be synthesized by the human body, but it needs sufficient Methionine [33].

The quality of Oleic & Linoleic (Omega-6) fatty acids in Gmelina Arborea samples is excellent. Also, Methionine, an essential fatty acid, and Glutamate, a non-essential fatty acid, are in suitable proportions in the seed samples. *Gmelina arborea* seed oil seems to be an excellent source of Amino and Fatty acids, which can be a good choice of healthy food and can be a source for application in the pharmaceutical industry. Due to the derivatives of fatty acids, seed usage in the cosmetic industry can also be explored.

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