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ORIGINAL ARTICLE

Fatty Acids and Heavy Metals analysis of *Terminalia catappa* Seeds Oil from Arid Zone of Rajasthan

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ABSTRACT
Terminalia catappa is a huge tropical tree that belongs to the Combretaceae family have an edible nut which is cultivate and grown in various climate conditions. Terminalia catappa seeds were gathered from western Rajasthan and oil percentage is found 51.31%. The physicochemical properties of Terminalia catappa seed oil featured as clear light yellowish colour with a pleasant smell. The iodine value of 119.63 g I_2/100g, moisture content 1.2%, protein content 18.5%,acid value 1.36 mg KOH/g andsaponification value of 173.82 mg KOH/g. On GC-FID analysis, stearic acid (12.04%), palmitic acid (25.74%), linoleic acid (33.58%) and oleic acid (18.23%) were found to be present in fatty acid methyl ester ofTerminalia catappa seed oil. Heavy metals such as Cd, Cr, Zn, Cu, Fe, Ni, and Pb are present in digested seed oil of Terminalia catappa were determined using the atomic absorption spectroscopy (AAS) technique.
***Keywords**: Terminalia catappa, Fatty acids, Heavy metals, Seed oil, AAS.*

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INTRODUCTION

Terminalia catappa is a huge tropical tree that belongs to the *Combretaceae* (Leadwood) family. This tree has grown across the tropics and is even seen in coastal areas. The tree is one of the most abundant plants in the tropics both wild and roadside tree. It is also known as tropical almond, fake kamani, Indian almond, sea almond, beach almond, wild almond and malabar almond [1]. When the green*Terminalia catappa* fruit ripens, it becomes yellow. The endocarp of the fruit includes an edible, oily seed with an almond flavour[2]. In India, the edible seeds like tropical almond have been served as snacks [3]. Furthermore, in some countries, the roasted nuts are sprinkled over oatmeal or curd for breakfast, and the nuts are occasionally mixed with soft cheese to produce a delicious bread spread.

Terminalia catappa is an edible nut which is cultivate and grown in various climate conditions and globally popular for nutritional, sensory and health benefits. Edible nuts are high in proteins and lipids and they also contain significant amount of minerals and vitamins. Nut seeds are a rich source of fibre and eaten as a snack or utilised as an ingredient in a lot of foods, including peanut brittle and peanut butter. Nuts have been used as man's nourishment in various regions of the world from the ancient period [4]. Their importance in human nutrition comes from their high nutritional value. They contain a considerable amount of protein and essential minerals [5]. Nut proteins are a suitable alternative for animal feedstock and a good source of lipids and edible oils due to their high quantity [6].

Tropical almond oil is a type of essential oil that is extracted for use in food flavourings and cosmetics [7]. Flavonols, terpenoids, phenolic, proanthocyanidins, and triterpenoids compounds are among the antioxidants found in it. Tropical almond extract has the potential to be used as a food preservation ingredient as well as a nutraceutical supplement by slowing down the oxidative reactions in the food [8]. Due to existence of almond lipid-association, its consumption has been reduced risk of colon cancer and also beneficial for brain and nerve system [9]. It is supposed to promote lifespan and a high intelligence level [10].

Release of heavy metals such as Cu, Fe, Ni, Pb, Zn, Cd, Mn etc. into the atmosphere has been increasing exponentially and have negative impact on health of those who consume these polluted meals.Heavy metal levels in soil, agriculture and environmental sampling are a significant indicator of pollution and are receiving worldwide attention due to their harmful effects on human even at trace quantities [11-12]. Urban wastes, battery industries waste, fertilizers, industrial emissions, pesticides, metal manufacturing and mining are all variables that contribute to heavy metal contamination in agricultural soils [13]. Heavy metals easily penetrate into the food chain when agricultural soils are polluted with them. Several aspects have been shown to influence the behaviour of heavy metals in agricultural land, their availability to crops, and their transmission from agricultural crops to humans [14-15].

MATERIAL AND METHODS

Sample Preparation

Terminalia catappa seeds were collected from the industrial areas of Rajasthan on a daily basis from completely mature fruits that had fallen to the ground. Fresh nuts were obtained after the non-edible part of the seed was removed by hand and the seed shell was removed with the help of a mortal-pestle then the seeds of *Terminalia catappa* were sun-dried to remove all moisture. The dried seeds were milled with a mortal-pestle into powder, which was stored in an airtight box until extraction was required.

Extraction of *Terminalia catappa* Seed Oil

Terminalia catappa oil was extracted using a Soxhlet extractor apparatus. In the soxhlet extractor, 100 g of milled seeds of *Terminalia catappa* were placed in a thimble. After that, petroleum ether (40-60 °C) solvent was added to the extractor. The extraction procedure was completed in nearly 8 hours at a temperature of 60 °C. A rotating evaporator was used to extract the solvent under a vacuum. A magnetic stirrer was used to evaporate the remaining solvent.Seed oil was stored at 4 °C in a refrigerator for further analysis [16].

Physicochemical Properties of Oil

The physicochemical parameters of *Terminalia catappa* seed oil was determined using AOCS methods for acid value, iodine value, saponification value, unsaponification value, peroxide value, free fatty acid, and specific gravity [17, 18].

Preparation of FAME and Analysis

Fatty acid analysis was done in triplicate and consisted of two steps: FAME preparation and Chromatographic analysis. For FAME preparation, first of all, the seed oil sample was heated with methanolic NaOH for esterification. To retrieve the methyl ester in the form of an organic phase, 5 ml of n-hexane was added. A separating funnel was used to separate the aqueous and organic layers after adding a saturated NaCl solution to the mixture. The upper layer of n-hexane was pipetted out and transferred to a 5ml glass bottle. This sample was stored in the refrigerator until the **Gas chromatography-mass spectrometry (**GC-MS) analysis was completed.

Preparation of Metal Standard

Standards for metals (like Zn, Fe, Ni, Cd, Pb) are prepared by dissolving one gram of metal in a minimal amount of aqua regia (HCl and HNO_3 in 1:3) and then making it up with double distilled water in a 1 litre volumetric flask. This is the stock of 1000 µg/L. After that, we prepare the working standard by suitable dilution of the stock solution.

Digestion of Seed Oil

1 g of seed oil is mixed with 5 mL of concentrated HNO_3, and the mixture is slowly heated for nearly 45 minutes on a heating plate. After cooling the solution, add 2.5 mL of conc. $HClO_4$ and then continuously heat until white fumes appear. After cooling, 10 ml of double distilled water was added. After that, the solution was filtered and used for analysis.

RESULTS AND DISCUSSION

Physicochemical Properties

Phytochemical properties such as peroxide value, moisture content, protein content, specific gravity, acid value, saponification value, unsaponification value and iodine value were determined by AOCS methods and shown in table 1.

Table 1: Phytochemical properties of *Terminalia catappa* seed oil

S. No.	Characteristics	Value
1	Oil Content (%)	51.31
2	Specific Gravity	0.89
3	Moisture Content (%)	1.20
5	Saponification Value (mg KOH/g)	173.82
6	Unsaponifiable Matter (%)	1.91
7	peroxide value (Meq KOH/g)	3.98
8	Acid Value (mg KOH/g)	1.36
9	Iodine Value (g I_2/100g)	119.63
10	Protein Content(%)	18.50

*Terminalia catappa*oil yield determined by soxhlet extraction which is high in quantity and the yield is 51.31%. The moisture content of ***Terminalia catappa* is very low at 1.2%, so it has a high**dry matter content. Due to this, it reduces oxidation-reduction reactions, microbial activities, and algae-fungi growth. This seed also increases their shelf life and can be stored for a long time. They will be an appropriate source of protein and amino acids for humans due to their high protein content of 18.50%.

Fatty Acid Analysis

Gas chromatography-mass spectrometry (GC-MS) technique was used to analysis Fatty acid methyl ester of *Terminalia catappa*oil. The amount of linoleic acid is highest 33.58% in unsaturated fatty acidsand palmitic acid is highest 25.74% in saturated fatty acids. Fatty acids are shown in table 2 and figure 1.

Table 2: Fatty acid composition of *Terminalia catappa* seed oil

S.No.	Fatty acid %	Lipid Number	Composition
1	Linoleic acid	C 18:2	33.58
2	Palmitic acid	C 16:0	25.74
3	Oleic acid	C 18:1	18.23
4	Stearic acid	C 18:0	12.04
5	Margaric acid	C 17:0	4.12
6	Palmitoleic acid	C 16:1	1.88
7	Arachidic acid	C 20:0	0.83

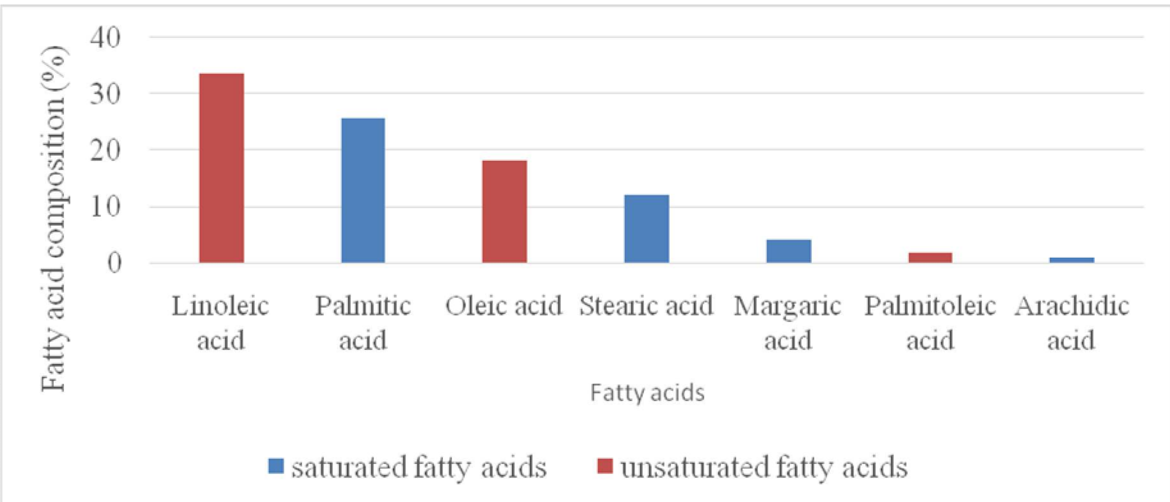

Figure 1: Fatty acid composition of *Terminalia catappa* seed oil

Heavy Metal Analysis

Atomic absorption spectroscopy (AAS) with Air Acetylene mixture flame was used to investigate heavy metals in *Terminalia catappa*.Heavy metals concentration found in seed oil is in the range of 0.001 to 2.447. *Terminalia catappa* seeds are used as a rich source of Zn and Fe for human and animals. Heavy metals concentrations found in *Terminalia catappa* seed oil samples shows in Table 3.

Table 3: Heavy metal concentration of *Terminalia catappa*

S.No	Metals	Wavelength(nm)	Type of flame	Concentration (ppm)
1	Cr	359.7	Air Acetylene mixture	0.237
2	Co	241.4	Air Acetylene mixture	0.001
3	Ni	232.0	Air Acetylene mixture	0.075
4	Cu	324.8	Air Acetylene mixture	0.237
5	Zn	213.9	Air Acetylene mixture	2.447
6	Cd	228.8	Air Acetylene mixture	0.007
7	Pb	283.8	Air Acetylene mixture	0.290
8	Fe	243.3	Air Acetylene mixture	1.600

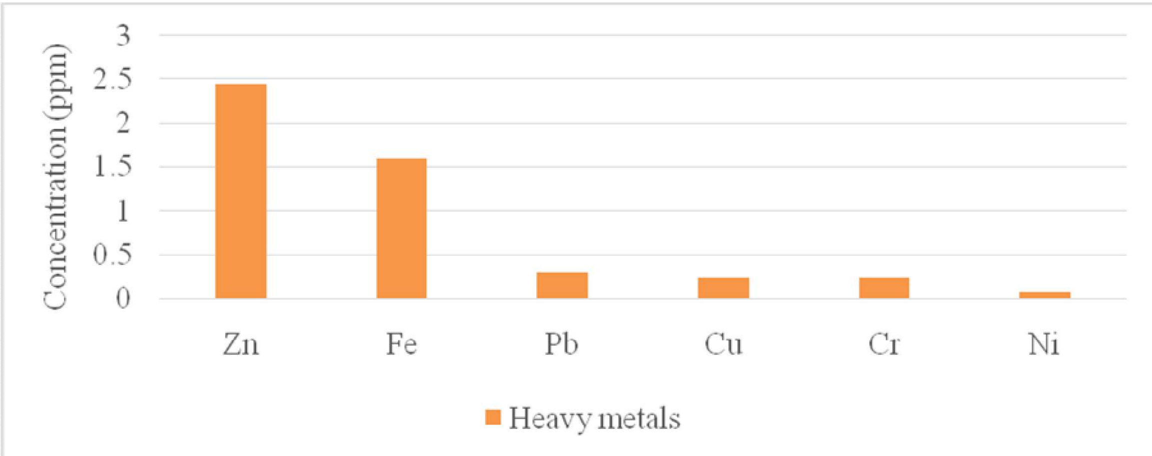

Figure 2: Plot of heavy metal concentration in seed oil of*Terminalia catappa*

CONCLUSION

On the basis of results, we conclude that *Terminalia catappa* seed oil is a good source of linoleic acid (ω-6) and oleic acid (ω-9). Linoleic acid is good for human health because it protects the body from vascular and heart diseases. Oleic acid is an important fatty acid because it decreases bad cholesterol and increases good cholesterol. It also helps to prevent heart disease by reducing plaque build-up in the arteries. In *Terminalia catappa* seed oil palmitic acid also present which is used in cosmetic products, detergents and soap making. According to heavy metal analysis, these metals are within the permissible limits and also contains a high amount of protein content, so it can be used as a dietary supplement.

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