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GCMS AND ELEMENTAL ANALYSIS OF *MADHUCA LONGIFOLIA* VAR. *LATIFOLIA* SEEDS

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ABSTRACT: *Madhuca longifolia* var. *latifolia* belonging to the family Sapotaceae which was also known as Mahua and Butter tree. The present study was designed to identify the fatty acids present in oil and to analyze the elements present within the seed and seed cake. After extraction of oil, it was found that the seeds were high in oil content within the kernel. The oil percentage found in seed was highest recorded as 57.59%. In GCMS analysis of oil, the oleic acid which is monounsaturated fatty acid (45.11%) was recorded highest in amount compared to others. Elements within the seed and seed cake were analyzed using Energy Dispersive X-ray Spectroscopy. Concentrations of seven elements such as Calcium (35.53%), Carbon (33.56%), Oxygen (22.46%), Nitrogen (5.20%), Phosphorus (2.18%), Magnesium (0.23%) and Sodium (0.04%) had been determined in seed cake by Energy Dispersive X-ray Spectroscopy. The present study provides information about the availability of some essential minerals and fatty acids, which can be used as nutraceuticals and it may also help in developing the biofuel for industrial purposes.

INTRODUCTION: *Madhuca longifolia* (Koen.) Mac Bride. var. *latifolia* (Roxb.) Chev. belongs to the family Sapotaceae. The name *Madhuca* came from Sanskrit language, *Madhu* means Honey because of its flower which is sweet. *M. longifolia* is one of the multi-use forest tree species present in most of the part of South Asia provides the solution for the timber, fuel, fodder, food and other nontimber forest products (NTFP's) to local people. It is a large deciduous tree growing mostly under dry and hot climatic conditions and found up to an elevation of 1200 meter³. The harvesting time of *M. longifolia* seeds is at the peak of period rainfall (June-July)⁴. The seed oil content is varied from 33 to 61% weight of the kernel⁵. Oil is pale yellow and used as cooking and lamp oil by most of the tribes in Western Ghats of Maharashtra.

Almost all parts of this tree show the medicinal properties⁶. Seed cake (SC) which remains after oil extraction is mostly used as organic manure and to feed cattle⁷. Tribal people of the study areas use this multifarious plant for their livelihood. Global demands of bio-fuel are increasing day by day because of depletion in fossil fuels. Consequently, world biodiesel production had increased from 200 million gallons to 1 billion gallons during 2001-2005¹.

India has the vast potential of more than 0.6 million tons of tree borne oil (TBO). But near about only 80,000 tons of oil is being extracted². It is, therefore, necessary to exploit domestic resources to maximize production and ensure the oil security for the country. The present study was carried out to determine the fatty acids as well as elements of *M. longifolia* seeds. Seeds are the rich source of minerals and can be used as nutraceuticals presenting the oil is used for cooking purposes⁷.

MATERIALS AND METHODS:

Plant Material Collection: The seeds of *M. longifolia* were collected from Pune district in June

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2016. Primarily the precise location (longitude and latitude) and diameter at breast height (DBH) of the plant were recorded. Then approximately 2 kg of seeds were collected in polybags. The collected seeds were brought in the laboratory and kept it in the refrigerator (-20 °C) for further analysis.

Oil Extraction Using Soxhlet: The fine oven dried kernel powder of seeds was made and used for extraction of oil. The dried kernel powder packed in thimble and oil was extracted in petroleum ether (Thomas Baker, 60-80 °C) for 6 h using Soxhlet apparatus⁸. After the extraction solvent was removed in a rotary vacuum evaporator and the yield of oil was calculated from the dry weight of the seeds. The oil was stored in a glass vial and kept at 4 °C for further use. Each experiment was performed in triplicates.

Energy Dispersive X-ray Spectroscopy (EDS) Analysis: The seed and SC were dried in an oven to reduce the moisture. The dried seed and SC were crushed in a blender, and a fine powder was prepared. The powder was passed through sieve 120 µm and used for analysis. The samples were analyzed using SEM-EDS and observed by Bruker XFlash 6130 at the Central Instrumentation Facility of Savitribai Phule Pune University, Pune⁹. Each experiment was performed in triplicates and mean was calculated.

Fatty Acid Profiling using Gas Chromatography-Mass Spectroscopy (GCMS): Fatty acid methyl esters (FAME) was prepared from oil using boron trifluoride acid catalysis method¹⁰. Prepared FAME was identified on Gas Chromatography (GC) model-7890 A, equipped with Mass Spectrometer (MS) model-5975. For carrier gas Helium was used with a flow rate of 3 ml/min. A capillary column HP-5ms, 0.32 mm internal diameter, 30 m length and 0.25 µm film thickness (Agilent Technologies) was used for the

identification of FAME present in the oil. The 1 µl sample was injected at a temperature of 270 °C. A temperature program of 100 °C (5 min), 7 °C/min to 225 °C (5 min), 5 °C/min to 300 (2 min) was used for separation of FAME in GCMS.

The identification of compounds was performed by comparing their mass spectra with NIST library. The relative percentage of fatty acids was calculated from the total area by using the software of the instrument.

RESULTS AND DISCUSSION: The seeds were collected from the healthy plant (Lat. 18°51'38.9"N, Long. 73°45'22.0"E) from Pune district of Maharashtra, India having DBH 47.79 cm. The plant sample was identified and deposited in Western Regional Center of Botanical Survey of India, Pune (Accession no. 136273). Seed cake and extracted oil are used for further analysis with the following results.

Oil Analysis: The oil was extracted using petroleum ether in Soxhlet apparatus. The oil obtained after extraction was pale yellow and having a characteristic odor. The average oil content in the seed was 57.59 %, which was higher as compared to previous reports^{11, 12}. The high oil content in *M. longifolia* seeds is an important parameter for the economic view and industrial applications^{13, 15}.

GCMS Analysis: A total of four major fatty acids have been identified from the chromatogram of the GCMS **Table 1**. The present study showed the high concentration of unsaturated fatty acids (55.21%) and a comparatively low amount of saturated fatty acids (44.77%). The major fatty acid present in *M. longifolia* oil is oleic acid which is 45.11%. The result from the present investigation corroborated well with the previously reported values¹⁴⁻¹⁶.

TABLE 1: FATTY ACID COMPOSITION OF *M. LONGIFOLIA* OIL

S. no.	Fatty acid	Systemic name	Formula	RT	Area %
1	Palmitic acid	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	18.436	23.45
2	Linoleic acid	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	20.703	10.10
3	Oleic acid	13-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	20.828	45.11
4	Stearic acid	Octadecanoic acid, methyl ester	C ₁₉ H ₃₈ O ₂	21.146	21.32

The high oleic acid content is may be responsible for reducing the serum blood cholesterol and blood pressure¹⁵. The presence of high amounts of oleic

acid is unique for seed oils of plant origin. The oleic and linoleic acid ratio are an important criterion to check the seed kernel quality¹⁷. Many

authors have reported the successful production of biodiesel from *M. longifolia* oil¹⁸⁻²². The *M. longifolia* oil has an estimated oil production potential of 0.18 million metric tons per year in India²³. The seed oil is edible, and it is mostly used by tribal's of India. The oil is also used for manufacturing soap, lubricant grease, and fatty alcohols. Thus the seed oil is valuable in meeting demands of food, feed, and fuel to industrial uses.

Elemental Analysis: The results of the elemental composition of *M. longifolia* seed and SC are

shown in **Table 2**. The SEM-EDS spectra of the *M. longifolia* seed and SC were showed in **Fig. 1** and **2** respectively. The EDS analysis revealed that *M. longifolia* seeds and SC contain Carbon (C), Calcium (Ca), Oxygen (O), Nitrogen (N), Magnesium (Mg), Phosphorus (P), Sodium (Na). After the extraction of oil, the elements in SC concentration was varied. In all these elements, C and Ca presented as high concentration while P and N presented as the moderate amount. But Mg and Na presented only in trace quantities.

TABLE 2: THE PERCENTAGE OF TRACE ELEMENTS IN SEED AND SEED CAKE OF *M. LATIFOLIA*

	Seed			Seed Cake		
	Weight %	Atom Weight %	Sigma Weight %	Weight %	Atom Weight %	Sigma Weight %
C	44.33	65.39	5.81	33.56	50.13	4.44
Ca	40.26	18.13	11.38	35.53	16.08	9.41
O	11.92	13.23	2.17	22.46	25.30	3.40
P	1.54	0.85	0.14	2.18	1.23	0.18
N	1.88	2.37	0.73	5.20	6.67	1.26
Mg	0.15	0.10	0.05	0.23	0.17	0.06
Na	0.05	0.03	0.04	0.04	0.03	0.03

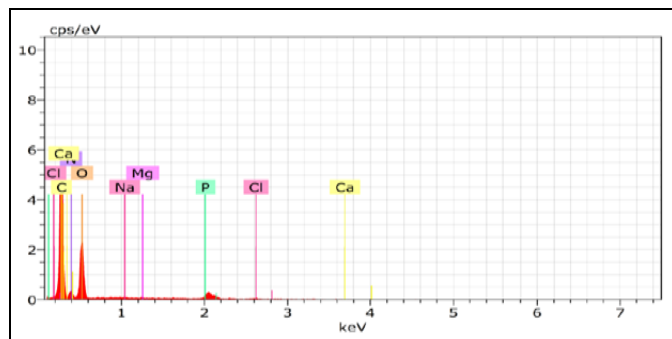


FIG. 1: ENERGY DISPERSIVE X-RAY SPECTROSCOPY SPECTRUM FOR *M. LONGIFOLIA* SEED POWDER

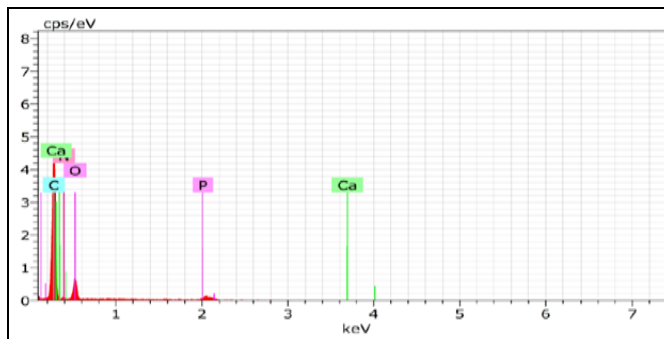


FIG. 2: ENERGY DISPERSIVE X-RAY SPECTROSCOPY SPECTRUM FOR *M. LONGIFOLIA* SEED CAKE POWDER

The concentration of C is reduced in seed cake may be because of low fatty acids concentration present in SC. *M. longifolia* seed cake contains a high amount of saponin, therefore it is used for non feed purposes, *i.e.* biogas, fertilizer, insecticide, pesticide and for mushroom productions^{15, 24-26}.

For the utilization of seed in food and feed purposes, it is essential to detoxify the seed and SC. The seed and SC contain a high amount of Ca. Ca is an essential element in the human body, for teeth and bone development animals need the Ca.

Ca also play an important role in heart rhythm, blood clotting and vitamin synthesis^{27, 28}. The oil extraction waste (SC) with high C content may be used as biofertilizer which is eco-friendly and cheap in comparison to the expensive fertilizers.

CONCLUSION: *M. longifolia* is distributed in most parts of India. The research on elemental analysis is meager, and hence present investigation was undertaken very limitedly. Fatty acid within seed kernel oil is important for effective biofuel production. The present study revealed that *M. longifolia* seeds are rich in oil and minerals. Oleic acid is dominantly present in the oil. The seed and SC are rich in mineral elements *viz.* C, Ca, O, N, Mg, Na, and P which can be used to treating various diseases that are mainly caused due to deficiency of these minerals.

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