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CHARACTERIZATION OF *Moringa oleifera* (PERIYAKULAM -1), OIL ANALYSIS AND RADICAL SCAVENGING ACTIVITY

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ABSTRACT

A series of laboratory experiments were conducted to evaluate the oil properties of a commercially modified and common edible seed species, *Moringa oleifera* (PKM-1), representing family *Moringaceae*. The oil properties of the seed were analyzed, as the wild variety is known to have distinct medicinal properties but suffers from thelow yield of oil. The experiments were conducted from oil obtained from seeds with pods removed and seeds with pods. The (PKM -1) seed variety gave an oil yield of (45.36% ± 2.05), while the acid value was found to be (178.3% ± 2.8). Deviation in the polarity of the pure seed oil and the seed with pod oil were observed, Laevorotatory and Dextrorotatory respectively. A high value of radical scavenging activity was found with oil extracted with the seed pods (58.330% ± 0.05). The commercial variety of *Moringa oleifera* (Periyakulam - 1) has been proved to be a better alternative to the wild variety with better and sustainable growth.

KEYWORDS: *Moringa oleifera* (PKM-1), Seed Oil Analysis, Radical Scavenging

The commonly called "drumstick tree", *Moringa oleifera* is the most extensively grown species from the genus *Moringa*, which is the only genus in the family Moringaceae. *Moringa*is derived from a Tamil word Murungai. It is a fast-growing tropical tree. It can reach a height of 10-12 m.The flowering begins within the first six months after planting, although the plant reproduces by both sexual and asexual reproduction. The fruit is found in hanging manner,a three-sided brown capsule of 20-45 cm size which holds dark brown globular seeds within a diameter of 1 cm.It has feathery foliage of tripinnate leaves.

PKM-1 or Periyakulam-1 is the superior variety which was made from pure breading so that high productivity plants can be obtained. The pure breed seeds have all the beneficial traits as in the wild variety but with a better production quantity, providing quality fruits as well as oil production.

Moringa oleifera has a long history in the Indian culture, the plant has been a part of most of the ancient literature, and has been known for the myriad of properties that are beneficial for curing many ailments (Brahmachari, 2001). India has been known to use many other forms of medicines for treatment of diseases, i.e., Yoga, unani, Ayurveda, Siddha, Homeopathy, about 70% Indians still use non-allopathic medicines (Vaidya and Devasahayam, 2007). In the African culture, the tree is called "The Miracle Tree" as every part of the plant bears some special medicinal property.

The bark, sap, roots, leaves, seeds and flowers are used in traditional medicines.As a part of the Indian system of herbal medicines (Ayurveda) this tree has a lot of uses for different diseases (Rai, 2005). Traditional uses have found out that all the parts of the plant can be used for some ailment, the plant issued as a cardiac circulatory tonic and antiseptic (Nadkarni, 2009). Research has examined how it might affect the blood lipid profiles.Extracts from the leaves contain low contents of polyphenols which are under basic research for their potential properties. A considerable preliminary research on the biological properties of *Moringa* components has helped in treating various diseases in human.

MATERIALS AND METHODS

Collection of Plant Materials

The seeds of *Moringa oleifera* PKM-1 were purchased from New Rama Seed Corporation (New Delhi). The reagents used were ananalytical grade, obtained from Sigma-Aldrich (USA) and MERCK specialties Private Limited (USA). The facilities of Lucknow Biotech Park were used for performing all the experimental protocols.

Preparation of Seeds

The seeds were air dried and the seeds were obtained by removing them from the pods. The seeds were weighed as per the volume of the Soxhlet extraction unit.

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The seeds were crushed into a fine powder using a clean mortar pestle.

Oil Extraction from *M. oleifera* seeds

Two types of oil extractions were done, one with the seeds only and other with the seeds and the pods.The method for the extraction of *Moringa oleifera* seed oil was followed as described by (Anwar and Bhanger, 2003). *Moringa oleifera* (PKM-1) seeds weighing 500 g, were crushed with a mortar pestle. Oil was extracted from the grounded seeds using n-hexane for 16 h by soxhlet extraction method. The solvent of extraction (n-hexane) was evaporated using a rotary evaporator for 30 minutes at 4^0C.

The extracted oil was degummed by heating the oil in a water bath at 75^0C and adding 20% W/W boiling water. The mixture was stirred for 10 minutes, cooled and centrifuged for 10 minutes at 3500 rpm. The supernatant was collected and stored.

Analysis of Extracted Oil

The Total Percentage Yield of Oil

The total percentage of oil extracted from the seeds used were calculated by gravimetric method. The extracted oil was degummed before calculating the total yield and the percentage of total oil yield was calculated using the formula, given below. The yield was obtained on the basis of W/W and V/W.

$$\% \text{ yield of oil (W/W)} = \frac{\text{(weight of oil) x100}}{\text{Weight of sample}}$$

$$\% \text{ yield of oil (V/W)} = \frac{\text{(Volume of oil) x 100}}{\text{Weight of sample}}$$

As the extraction process was divided into two types i.e., only seeds and seeds with pods, the yield for two samples were calculated.

To Determine the Moisture Content of the Oil Sample

The moisture content was determined by thegravimetric method. The samples were heated at 50^0C in a pre-weighed beaker and were regularly check till the time the weight became constant. The moisture content was calculated as percentage moisture.

To Determine the Specific Gravity of Oil

The specific gravity of the extracted oil sample was determined by agravimetric method using a pycnometer. The specific gravity both the samples was calculated as the ratio of the weight of 1ml of oil to that of 1ml of distilled water.

To Determine the Optical Rotation of Oil

The optical rotation of *Moringa oleifera* (PKM-1) oil samples was calculated using a polarimeter (Rudolph digipole). Hexane was set as the blank as the oil was extracted using n-hexane as the organic solvent.

To Determine the Acid Value of the Oil Sample

The acid value of the oil sample was determined using Potassium hydroxide (KOH). The oil sample was added with 95% ethanol and few drops of Phenolphthalein and titrated against 0.1N KOH. The acid value was calculated according to the formula given below.

$$\text{Acid Value} = \frac{\text{56.1 x titre value x N}}{\text{The weight of oil sample}}$$

N = normality of KOH

To Determine the Free Radical Scavenging Activity

The free radical scavenging potential of *Moringa* seeds was determined against 1,1-diphenyl-2-picrylhydrazyl (DPPH), by the method described by(Adesegun et al., 2008). Various concentrations of the oil samples (Seed and Seed with pods) were prepared in methanol (25, 50, 75, and 100µg/mL).

RESULTS AND DISCUSSION

Oil Analysis

The oil analysis of the superior variety of Moringa, i.e., *Moringa oleifera* (PKM-1) has a significantly high oil yield as compared to the wild type variety, other experimentally tested aspects in this study have been compared with previously done studies on different varieties and regions of cultivation of *Moringa oleifera.*

The values calculated by this study are provided with mean and SD with samples taken in triplicates. Table 1 highlights the major oil properties analyzed with the comparative data of previous literature.

Table 1: highlights the three basic oil properties of *Moringa oleifera* (PKM-1) as compared to previous studies on the wild varieties of *Moringa* [a]

Oil properties	Oil Source	*M. oleifera* (PKM-1)	*M. oleifera* (Sindh Pakistan)	*M. oleifera* Var. Mbololo (Kenya)	*M. oleifera* Var. Wild (Malawi)
Oil Yield (%)	Seeds (W/W)	45.36 ± 2.05	40.39	35.7	35.3
	(V/W)	36.43 ± 1.51	Present Study	Present Study	Present Study
	Seeds & Pods				
	(W/W)	37.34 ± 1.36	Present Study	Present Study	Present Study
	(V/W)	31.43 ± 1.01	Present Study	Present Study	Present Study
Moisture Content (%)	Seeds	8.37 ± 0.51	5.70	Present Study	Present Study
	Seeds & Pods	2.05 ± 0.38	Present Study	Present Study	Present Study
Specific Gravity (mg/ml)	Seeds	0.858 ± 0.04	0.9032	0.8809	0.8882
	Seeds & Pods	0.905 ± 0.03	Present Study	Present Study	Present Study
Acid Value (mg of KOH/ g of oil)	Seeds	178.3 ± 2.8	186.67	178.11	184.16
	Seeds & Pods	198.1 ± 1.6	Present Study	Present Study	Present Study

[a] All the values were taken with five simultaneous readings represented as mean ± SD

The extended time of extraction of oil using n-hexane gave an oil yield of 45.36% (W/W). This was the highest oil yield obtained from any other variety of *Moringa* seeds. A significantly close value of extraction was found in the *Moringa oleifera* of Sindh which gave a yield of 40.39%.

The oil yield of *Moringa* varieties of Kenya (35.7%) and wild variety Malawi (35.3%) were mentioned to have a low oil yield (Tsaknis et al., 1999)(Tsaknis et al., 1998). As stated in theprevious literature by (Lalas and Tsaknis, 2002) the oil yield for *Moringa oleifera* (Periyakulam 1) was reported to be (38.30%) from India.The moisture content of the PKM-1 variety was found to be higher than the wild-type, 8.37% as compared to 5.70% of the wild-type(Anwar and Bhanger, 2003).

The Acid value or saponification value was similar as compared to previous works. The acid value in the *Moringa* variety at Sindh was (186.67 mg/ml) (Anwar and Bhanger, 2003), while in the Mbololo and Malawi it was found to be (178.11mg/ml) and (184.16 mg/ml) respectively (Tsaknis et al., 1999) (Tsaknis et al., 1998). A significant increase in the acid value was observed in the oil extracted with the pods. The increase in the acid value because of the combine oil extraction of the seeds and the pods suggests that more fatty acids are released by this type of oil extraction strategy.

Optical Rotation

The optical rotation of both the oil samples is presented in Table 2.

Table 2: The optical rotation activity of oil samples calculated with Hexane as blank [a]

Blank (n-Hexane)	*Moringa* Seed oil extract	*Moringa* Oil extracted with pods
0.674 ± 0.004	- 0.077 ± 0.008	+ 0.014 ± 0.022

[a] All the values were taken with five simultaneous readings represented as mean ± SD

The Polarimeter data was calculated at 31^0C at a wavelength of 589nm and 50mm path length. The optical rotation of both the extracted oils gave completely different polarity on analysis. The oil sample which was extracted with the pods displayed a Dextrorotatory (+) polarity, while the extracted oil without the pods displayed a Laevorotatory (-) polarity.

Radical Scavenging Activity

On determining the radical scavenging activity of *Moringaoleifera* seed oil samples it was observed that the sample with the extraction of oil with the pods provided a

higher level of radical scavenging activity (58.330%) in comparison to the seed oil (38.637%).

As the concentration of each diluted oil sample was increased the radical scavenging property increased and was found highest at 100µg/ml. Table 3 depicts the data for the radical scavenging property of both the oil samples.

Table 3: Radical scavenging activity of *Moringa* oil samples using DPPH (I%) assay with BHT as standard [a]

Samples	Concentration (µg/ml)	% Radical Scavenging
Seed oil	25	16.316 ± 0.04
	50	22.005 ± 0.01
	75	38.036 ± 0.02
	100	38.637 ± 0.09
Seed oil and pods	25	26.328 ± 0.01
	50	38.249 ± 0.01
	75	45.009 ± 0.06
	100	58.330 ± 0.05

[a] all the values were taken with five simultaneous readings represented as mean ± SD

The current study as well as previous studies on the different varieties of *Moringa* that are being cultivated in different geographical locations of the world, we observed that the oil yield, acid value,and other such basic oil characteristics differ greatly based on the environment in which these plants are grown.

The high yielding variety of *Moringaoleifera* (Periyakulam 1) has become a more economical variant of *Moringa*variety as found in our study. Our study investigated the basic oil characteristics optimizing the yield parameters to get a better extraction, as in our study we found out the oil yield to be (45.36%), which was considerably higher than those of the previous researchers. We also took a variant in the extraction process to investigate the extraction efficiency of seed oil extracted with pods. The seed oil when extracted with pods gave some distinct results in the parameters of moisture content, acid value, optical rotation and radical scavenging potential.

The moisture content in Periyakulam 1 was found out to be higher than other varieties of Mbololo and Malawi, while the acid value came out to be similar to the previous study. The low titer at which this oil provided its acid value shows that the oil is not prone to fast rancidity(Li et al., 2007).As the *Moringa* oil has a low acid titer, it is an acceptable oil for consumption and is edible. The fact that *Moringa*seed oil has a high oleic acid with its highly unsaturated in nature with peroxide potential, this oil can be suitable for use in food as well as biofuel industries, varnishes,and agrochemicals (Manzoor et al., 2007).

(Nepolean et al., 2009) studied the various phytochemicals present in *Moringaoleifera*, flowers, leaves and seeds. The major compound found by him in the seeds were Roridin E, Veridiflorol, and 9 – Octadecenoic acid. According to(Gupta, 2008 and Mehrotra, 2004), the seeds of Moringa were found to contain Moringyne, 7-(α-L-rhamnosyloxy) benzyl isothiocyanate,and many other amino acids.

This study has highlighted the important aspects of the pure breed Periyakulam 1, this commercially important variety of *Moringaoleifera* has the potential to become a sustainable asset to society. The high oil yield and good radical scavenging activity can have multiple applications from industries to home remedies.

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