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Extraction, Chemical Characterization, and Functional Modification of Moringa oleifera Seed Oil for Health Applications

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Abstract

Essential oils (EOs) are valuable plant-derived extracts with diverse applications in medicine, cosmetics, food preservation, and industry due to their antioxidant and antimicrobial properties. Among these, *Moringa oleifera*, widely known as the "tree of life," is gaining recognition for its nutritional richness and therapeutic potential. This study aimed to extract, modify, and characterize *Moringa oleifera* seed oil to evaluate its physicochemical and chemical properties for potential applications. The seed oil was extracted using the Soxhlet apparatus, yielding a pale-yellow oil with a peanut-like odor. Preliminary analysis revealed a high proportion of monounsaturated fatty acids, particularly oleic acid, which contribute to its oxidative stability. The crude oil was assessed for pH, specific gravity, viscosity, saponification value, acid value, and iodine value. To enhance its functional properties, the oil was chemically modified through iodine treatment, with the goal of improving iodine content and altering fatty acid composition. Advanced analyses using Gas Chromatography–Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography (HPLC) were performed to determine the fatty acid profile and chemical constituents of both crude and modified oil. Results indicated that iodine modification caused significant differences in viscosity, iodine value, and acid value, suggesting structural changes that may influence stability and bioactivity. The characterization confirmed the presence of valuable bioactive compounds, reinforcing its potential as a multifunctional bioresource. In conclusion, *Moringa oleifera* seed oil demonstrates promising physicochemical and chemical properties, both in crude and modified forms, making it suitable for applications in pharmaceuticals, nutraceuticals, cosmetics, food processing, and biodiesel production. This study provides scientific evidence to support the industrial and therapeutic relevance of *Moringa* seed oil as a sustainable natural resource.

Introduction

Now days, Aromatic plants are in high demand due to their medicinal, culinary and anthropogenic properties. Consumers are getting awareness about the benefits of medicinal and aromatic plants so they are taking interest in these plants. They are more concerned about their health and nutrition. Aromatic plants essential oils and beneficial for health and nutritional values.

Given the complexity of essential oils' composition, it is worthwhile to enhance the chemistry and other characteristics of their constituent parts in order to create new and advantageous uses in the fields of environment, food, medicine, and agriculture. Essential oils are substitutes for chemical industrial components that do not have the same side effects.

Plant extracts are employed today for their antimicrobial properties in a variety of industries, including the culinary, medicinal, cosmetic, and agricultural sectors. Essential oils are multicomponent, complex compounds that are derived from various plant components. They are significant commerce goods that are made all over the world. Their wide range of uses is contributing to the fast expansion of aromatherapy. Essential oils have anti-inflammatory, anti-microbial properties.

These oils can be inhaled, distributed by air but usually applied to skin. EOs has a good safety side view. EOs may have severe or mild toxicity effects. Adverse effects include abortions or abnormalities in pregnancy, retardation of infant growth, hepatotoxicity. But these effects are rare to see. A variety of medicinal, cosmetic and nutritional benefits are observed. (de Groot, A. C., & Schmidt, E. (2016). These are used in food for flavoring and pleasant odor. Not only aromatic compounds are responsible for color, different pigments also contribute in varying color. Moreover, they are also used as additives due to their antioxidant and antimicrobial behavior and as active compounds in packaging material providing the hydrophobicity to the material. (Phakawat Tongnuanchan and Soottawat Benjakul 2014). This oil has been used in cosmetics, medicines since ancient times. It is difficult to know when it was first used but carbon of cave painting showed its use since 18000 B.C.E. In sixteenth century, essential oil was recognized by Paracelsus von Hohenheim on the base of its deduction from drug Quinta essential in Switzerland. It can be defined as the volatile products derived from the vegetables either by steam distillation or dry distillation. (Dhifi, Bellili, Jazi, Bahloul and Mnif 2016).

It is recorded that the first essential oil was used in Chinese and Indian medicines in 2000 to 3000 B.C (Baser & Buchbauer 2010). Chinese and Indian ointments include the substances like cinnamon and sandalwood as they are effective in healing. Firstly the essential oils used between 500 to 400 B.C on the substances like thyme, marjoram peppermint and cumin (Pauli & Schilche 2009). In ancient Egypt civilization aromatic oils were used as early as 4500 in medicine, cosmetic, and in embalming for mummification of dead bodies. Bottles of essential oils were also found in graves of dead. They still had the healing power. In ancient Asia and other ancient civilizations essential oils were used as a fragrance in perfumes, religious ceremonies and as anti-microbial agents. Aromatic oils and perfumes were also used for the liturgical and analeptic purposes (José-Luis Ríos 2016). About 118 references is present in the Bible for the use of essential oil. Romans were very much fond of fragrances. They used to scent their hair, clothes and every part of their body. Mostly their bodies and outfits, even walls were scented with perfumes. Costly and cheap fragrances were derived from essential oils.

Arab: Fragrance science reached the highest point of alloy in the Arab world after the fall of Roman Empire. Later, in Middle Ages art of fragrance Crusaders brought back knowledge of fragrance to Europe. Alchemists used this knowledge to seek the means of formulating the elixir and priories were used to produce different products and medicines from essential oils (Sonwa 2000).

Modern time: In 18th and 19th centuries chemists identify many substances like caffeine, quinine, morphine and atropine which shoes important biological effects. (Tisserand and Balacs 1995). In 19th century, a large number of essences were prepared scientifically. Chamberland published his research on antiseptic properties of EOs. After few decades, lavender oil was prepared and consider as natural healer when French chemist Rene-Maurice burned his hand. He unintentionally put on his hand in lavender oil and his hand healed without any infection. Later on, many researches were done on lavender and also introduced in many hospitals of France. No fatal effects were observed.

Essential oils are the mixture of different components. More than 400 components are identified. Chemical structure of Essential oils possesses two distinct groups terpenes and terpenoids and another group of aliphatic and aromatic compounds. Terpenes are the chemical compound formed by 5 carbon isoprene units monoterpene contain 10 carbon atoms and produced from 2 isoprene units. Monoterpenes is the major constituent of the EOs that show antimicrobial activity. (Freire's, Denny, Benso, Alencar and Rosalen 2015). Usually, three forms of monoterpenes occur acyclic, monocyclic and bicyclic. Sesquiterpenes contribute 25% of Terpenes fraction. It is built up from 3 isoprene units and contains 15 carbon atoms. It is classified into acyclic, monocyclic, bicyclic and tricyclic forms. Terpenoid is the chemically modified form of the terpenes. i.e. oxidation. (Dhifi, Bellili, jazi, Bahloul and Mnif 2016). In some cases, these components may be hydrocarbons while in other oxygenated compounds (Bakkali et al., 2008) Aromatic compounds like aldehyde (citronellal, methoxy derivatives are present (Sell, 2010; Chamorro et al., 2012)

Nonterpenic compounds are also present in the essential oil; that are bio generated by phenylpropanoids, i.e. eugenol, safrole and cinnamaldehyde. Both terpenoids and phenylpropanoids are generated by two different pathways due to their different primary metabolic precursors. Composition mainly depends on the part of plant used to extract the oil (leaf, flower, fruits, roots, stem, seed). For example, green parts and roots of Achillea ptarmica possess very small amount of essential oil while flower contain high level of EOs (Kuropka, Neugebauer and Glombitza 1991).

Today, plant extracts are used in many fields especially cosmetics, medical, agriculture and food industries for antimicrobial activities. Many researchers declared that essential oils can be used in cooking as they provide a good taste to food and can be effective in aromatherapy (El Shafie and Camele:2017). The isolation is done by steam distillation, expression cold pressing, solvent extraction and simultaneous distillation and extraction method. These techniques are time consuming and are not cost affective. Other disadvantages, such as loss of some febrile components, a little extraction, presence of toxic solvent in extract and denaturation of some compounds by heating also seen and a large amount of sample is also required. (Mohammad hosseinia, Sarkerb, Akbarzadeh 2017). So new more efficient extraction techniques are available like super critical fluid extraction, pressurized liquid extraction, membrane assisted solvent extraction and many others. These methods have the advantage like lower oxidation of the compounds. Fractionations cleanup is required to remove the high molecular weight lipids that are also extracted along with essential oil. (Pavela 2015). Modern techniques are environmental friendly, secure and safe.

The yield of oil depends on the method of extraction. For example, yield of Satureja hortensis is affected by the method of extraction. Different distillation methods are used for oil extraction. The oil yield is represented in Table1. The oils were dried with anhydrous sodium sulfate (F. Sefidkon et al. 2006).

Table 1: Oil yield of Satureja hortensis

Distillation method	Means of yield (g)
Hydro distillation	0.93 a
Water and steam distillation	0.69 a
Steam distillation	0.27 b

Plants like moringa can be eaten. In Pakistan, it is one of the most underutilized resources. In addition to minerals, it is the primary source of many vitamins. Primarily, these vitamins are A, B, E, and K. The minerals copper, calcium, iron, magnesium and potassium are found in Moringa. Furthermore, it contains around forty naturally occurring antioxidants**.** The well-known species of the Moringaceae genus is Moringa oleifera. The ancient Greeks, Romans, and Egyptians extracted oil from seeds and used it to make treatments and perfumes. The oil was used as lubricant for machines and for making perfumes. About 35% of the Moringa seeds grown in Kohat Forest are yielded as oil.

In seeds, there are 31.4% protein, 18.4% carbs, 7.3% fiber, and 6.2% ash. Along with legumes that have lower levels of amino acids, Moringa seeds also contain significant concentrations of digestible cysteine and methionine. These seeds do not exhibit urease activity because they lack trypsin inhibitor. That demonstrates how highly digestible it is. The high concentration of behenic acid in this oil is why it is named ben oil. This oil has a high concentration of monounsaturated fatty acids—nearly 76.73%. There are 21.18% saturated fatty acids in MO seed oil. 76.73% of monounsaturated fatty acids are present in high concentrations. Oleic, palmitoleic, and gadoleic acids are examples of these monounsaturated fatty acids. MO oil contains a negligible quantity (1.18%) of polyunsaturated fatty acids. Additionally present are 0.46% and 0.76% of linoleic and linolenic acid. (Ogunsina, Indira, Bhatnagar, Radha, Debnath, Krishna 2014).

A component of 92% of total sterol is composed of stigma and camp sterols, also known as β-sterol. Trace levels of other sterol components are found. Although sterol components are reduced throughout the degumming process, the extraction techniques have little effect on the composition of sterol. Plant variety and cultivation-related agroclimatic conditions both influence the composition of sterol found in oil (Farooq and Umer 2007). Because it has so many significant health applications, cholesterol is significant. It reduces cholesterol because it plays a part in the metabolism of cholesterol. Additionally, B-sterol has anti-diabetic properties. Tocopherol is another important component of Moringa seed oil.

The oil from Moringa seeds reduces inflammation. Consequently, it's utilized in the formulation of skin cream with anti-inflammatory properties. This cream reveals the anti-inflammatory properties of Moringa seed oil by both in vivo and ex vivo inflammatory activities (Fernandes, Sousa, Oliveira, Morais, Richter, Munoz2015)**.** Another treatment for the hypertension that causes high blood pressure is Moringa seed oil. If hypertension is not treated, it may develop into cardinal disorder, which can be fatal (Okorei, et.al, 2019). M. oleifera seeds, either fresh or crushed, are used in folk medicine to treat stomach pain, ulcers, joint pain, blindness, and stomach discomfort. For the purpose of antibacterial movement against many microscopic organisms and parasite species, this kind of seed removal is beneficial (Leone et.al, 2016). Numerous cancer cell lines are cytotoxically affected by M. Oleifera oil. Asthma and rheumatoid arthritis are two conditions where a chronic immunemediated inflammatory response can be reduced by Moringa extract (Elsayed, Sharaf-Eldin, Wadaan 2015).

This study is designed to extract, chemically characterize, and functionally modify Moringa oleifera seed oil in order to explore its potential health applications. With its richness in monounsaturated fatty acids, sterols, tocopherols, and bioactive compounds, M. oleifera seed oil holds significant promise as a natural resource for food, nutraceutical, cosmetic, and pharmaceutical industries. Through the use of advanced analytical techniques, the study seeks to determine the physicochemical and compositional properties of the crude oil and to evaluate the impact of iodine modification on its stability and bioactivity. By providing scientific evidence on both crude and modified forms, this work aims to highlight the industrial and therapeutic relevance of Moringa oleifera seed oil as a multifunctional bioresource.

Method for extraction of oil

Sample preparation

A mortar and pestle were used to crush the Moringa oleifera seed kernels. Soxhlet apparatus was used to defatted weighed seed powder. Using the solvents under reflux, the oil was extracted. For an hour, the procedure persisted. To get the most oil yield possible, the extracted oil was heated to remove any remaining water. The method of extracting oil involves several steps. The following are the measures to take place. The seed coating covers the kernels. The process of grinding breaks down the seed covering and separates the kernels from the husk. The seed kernels were dried in the shade to prevent the release of crucial information. Using a pestle or mortar, dried seeds are ground into a fine paste. Water is utilized as a solvent when crushed seeds are put into the Soxhlet equipment. It is extracted using oil. To get rid of the extra moisture, extracted oil is cooked. Unadulterated oil is enclosed in a sealed container.

Soxhlet extraction operator

Ten grams of seed powder were added to a flask with a round bottom. Water, the solvent, was put into this flask. For one hour, the Soxhlet was heated to 60 degrees Celsius. As it heated, the sample released odors. The condenser, which was implanted vertically, allowed these vapors to ascend. After the condensation, fumes descend into the mixture. Using a fractional distillation assembly, oil was extracted after refluxing for one hour. After being boiled to eliminate extra moisture, the extracted oil was sealed in an airtight container.

Modifications made in extracted oil

20ml oil was heated at 35^{o}C. Conc. 15 ml. Iodine that was already on hand was added while swirling constantly. Warm distilled water was used to wash the product. The water was evacuated after two hours. The improved fatty acid values were established.

Result

The Soxhlet process was used to isolate the oil from Moringa oleifera seed. MO seed oil had a scent similar to peanuts and was pale yellow in color. We calculated different parameters like acidity, viscosity, saponification, specific gravity, and iodine values. Various analytical parameters were measured for unrefined oil: specific gravity, viscosity, refractive index, saponification value, acidic value, iodine value, 7.97, 0.092, 42.2, 1.450, 190.50, 3.9, and 74.80, respectively. Distinct outcomes were acquired via various assessments.

Table 2: Physiochemical characteristics of oil

Characteristics	Unrefined	Refined
pH	7.970	8.020
Specific gravity	0.091	0.092
Viscosity	42.200	9.120
Refractive index	1.450	1.462
Saponification value	190.520	180.60
Acidic value	3.900	2.822
Iodine value	74.800	63.040

Table 3 Percentage [Determination] of extracted oil

Determination	Value (g)
Weight of empty flask (M_1)	105.21
Weight of thimble (W_1)	2.90
Weight of sample + thimble (W_2)	32.22
Weight of sample ($W_2 - W_1$)	29.20
Weight of empty flask + oil (M_2)	158.50
Weight of oil ($M_2 - M_1$)	53.39
Second weight of sample	36.20
Third weight of sample	35.12
Fourth weight of sample	38.50

The percentage of extracted oil = 36.77%, percentage Moisture content = 4.00%

Table 4: HPLC analysis of MO seed oil

Detector A 250nm

Peak value	Retention time	Peak Area	Peak area	Theoretical Plates/ meter (USP)	Tailing factor value
1	3.014	19823	2122	15578	1.278
2	4.147	10100	673	11978	1.093
3	5.523	15361	729	10822	1.034
Total		45284	3524		

Table 5: Fatty acid composition

Fatty acid	Value
$C_{8:0}$	Not detected
$C_{14:0}$	Not detected
$C_{16:0}$	6 – 7.0
$C_{16:1}$	0.8 – 1.20
$C_{18:0}$	4.67 – 7
$C_{18:1}$	74.0 – 78.0
$C_{18:2}$	0.65 – 2.0
$C_{18:3}$	Not detected
$C_{20:0}$	3.0 – 4.2
$C_{22:1}$	1.20 – 0.6
$C_{22:0}$	4.17 – 6.00
$C_{22:1}$	Not detected
$C_{26:0}$	Not detected

The purpose of refining oil was to alter unrefined oil. After comparing the outcomes, it was found that refined oil is higher-quality oil with enhanced characteristics. The calculated values were in good agreement with the literature prior to change. Following the change, the oil's saponification readings were in good accord, but its iodine value was lower and its free fatty acid value higher.

The percentage of extracted oil is displayed in Table 3. The weight of the empty flask was determined for this purpose (M1). Weighing the empty thimble (W_1), we then measured the weight (W_2) after adding the sample, and computed the sample weight (W_2 - W_1). After adding oil, the flask was weighed and the oil's weight was computed.

Table 4 displays the values of several peaks that were acquired using HPLC. A sample volume of 20 ul was injected. Sample was conducted at 250 nm wavelength for 0 to 10 minutes. With retention times of 3.014, 4.147, and 5.523, respectively, three distinct peaks were observed at different intervals of three minutes, four minutes, and five minutes. Pari, Karamac, Kosinska Rybarczyk and Amarowicz (2007) also showed the similar peaks.

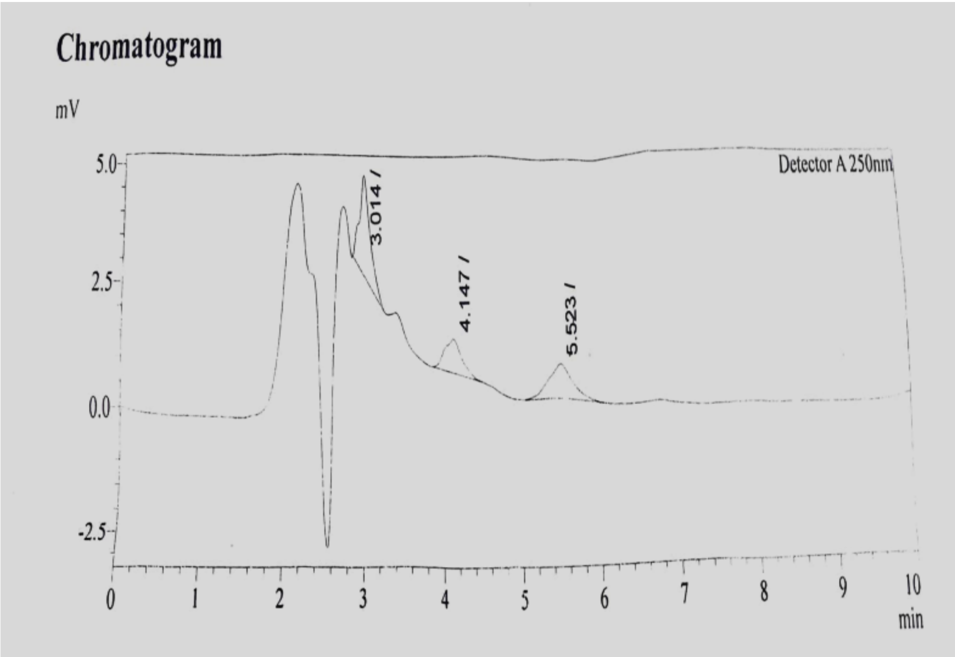

Figure 1: HPLC chromatogram of Moringa oleifera

The various fatty acid compositions found in oil were displayed in Table 4.4. Understanding the behavior of Moringa seed oil as biodiesel oil is helpful when the oil has been modified.

Over 70% of Moringa oleifera's fatty acid content is found to be oleic acid, according to research. Oleic acid is employed in the food sector, water treatment, and pharmaceuticals because of its excellent oxidative stability. Additionally, because of its ability to absorb and hold onto volatile compounds, this oil is utilized in the perfume business to stabilize scents. Eicosanoic acid is C20. It is also a small amount of saturated fatty acid found in nature. Its sealing, lubricating, and adhesive qualities make it useful in several sectors.

Additionally, C18 has been found in Moringa oleifera seed oil. It is referred to as stearic acid and is a saturated fatty acid. Its uses can be seen in the production of detergent, soap, and cosmetics. Soap can be created by saponification of triglycerides, or esters of stearic acid.

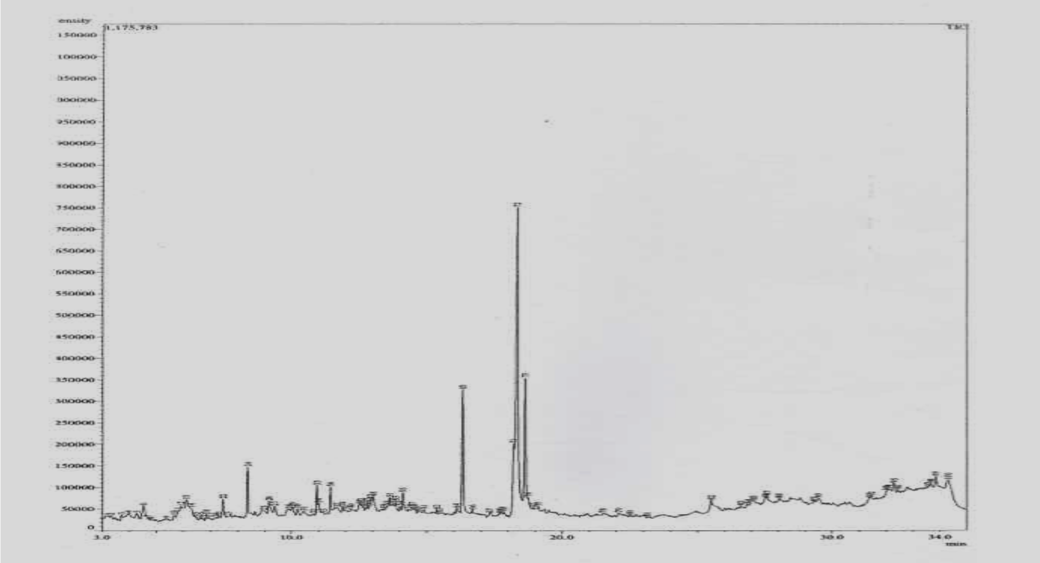

Figure 2: **GC-MS chromatogram of Ethanolic Moringa seed oil**

Discussion

Using a Soxhlet system, Moringa oleifera seed oil was extracted. The oil's moisture content was examined, 4.00% was noted. Analyses were done on physiochemical properties. Its pH value, which ranged from 7.99 to 8.02, indicated the basic character of the oil. There was no discernible difference in the specific gravity value before and after the oil adjustment. There was a noticeable change in the oil's viscosity following the alteration of the seed oil. There was a discernible drop in viscosity, from 42.2 to 9.12. The refractive index values of modified and crude oil did not significantly differ from one another. After adding pure iodine, the result increased to 74.8. The stability of this iodized oil is increased due to the increased unsaturation. The method of extraction has an impact on oil yield. The yield of Soxhlet equipment is negligible. 36.77% was the highest yield that Soxhlet extraction could produce. To achieve a high yield, a different method needs to be applied. Oleic Acid is employed in the food sector, water treatment, and pharmaceuticals because of its excellent oxidative stability. Because of its ability to absorb and hold onto volatile compounds, this oil is also utilized in the perfume business to stabilize scents. In trace amounts, Eicosanoic acid can be found in nature. Its adhesive, lubricating, and sealing properties make it useful in industries. Iodine was added to the oil in order to modify it. It raises the oil's saturation content. The unsaturation content of fats and oils determines their oxidative stability. The more unsaturated the content, the more oxidation-prone it is. This altered oil demonstrated its use as a diesel and edible oil.

Conclusion

Moringa oleifera [oil] was extracted using the Soxhlet Process. For Moringa oleifera seeds, the oil content was found to be 36.77%. Examined were the extracted oil's physiochemical characteristics. The study examined the pH, refractive index and iodine value of the Moringa oleifera seed oil generated, both before and after alteration. The pre-modification values of 7.97 (pH), 0.092 (specific gravity), 42.2 (viscosity), 1.450 (RI) and 74.80 (IV) were compared to the post-mod values of pH (8.02), specific gravity (0.092), viscosity (9.12), refractive index (1.462), saponification value (180.60), acidic value (2.8), and iodine value (63.04). Over 70% of the sample had iodine. The amount of unsaturated content increases with iodine value. The unsaturation content affects the oxidative stability of lipids.

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