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Determine the Efficiency of Two Different Methods Used for the Extraction of Polyphenols from *Moringa Oleifera* Leaves

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ABSTRACT

The plant *Moringa oleifera* is a rich source of bioactive chemicals and antioxidants. The present study was focused to determine the efficiency of two different method used for the extraction of polyphenols present in the leaves of *moringa oleifera.* The attempt was made to check the efficiency of both methods used for the extraction process such as soxhlet extraction method and maceration and determined the accuracy of both methods by HP-TLC, FTIR and UV-Visible spectroscopy. *Moringa oleifera* possesses relatively higher content of polyphenols. Epidemiology research repeatedly shows that eating foods high in phytochemicals, such as fruits, vegetables, and herbs, reduces the prevalence of disease in people. The presence of polyphenolic chemicals is primarily responsible for this advantageous effect. Because of their strong ability to entrap the free radicals linked to many diseases, polyphenols have attracted a lot of attention.

KEYWORDS: *Moringa oleifera, HP-TLC, FTIR, UV-Visible spectroscopy*

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I. INTRODUCTION

The fast-growing, drought-resistant *Moringa oleifera* tree is a member of the Moringaceae family and is indigenous to the Indian subcontinent. Ideal soil temperature for germination of moringa seeds ranges from 20 to 26°C and adapted to a wide range of soil types, but grows well in muddy soil. *Moringa oleifera*, widely distributed in the southern states of India, Africa, South America, the Philippines, and other Asian regions/countries. Whole leaves (leaflets, stalks, and stems), immature, green fruits or seed pods, fragrant flowers, young seeds, and roots are all edible portions of the plant. It is widely grown for its young seed pods and leaves which can be utilize as vegetables, traditional herbal medicine, and water purifier, culinary. Being a large source of B vitamins, vitamin C, pro-vitamin A in the form of beta-carotene, vitamin K, manganese, and protein, the leaves constitute the plant's most nutrient-dense component. According to epidemiological research, M. oleifera leaves contain anti-tumour, anti-inflammatory, anti-ulcer, good vitamin content, anti-atherosclerotic, immunological boosting, sperm boosting, and anti-convulsion properties. The nutrients in moringa leaves can be preserved by drying and powdering them for long-term use and storage. Due to the abundance of bioactive chemicals with significant antioxidant and other pharmacological characteristics in plants, they have recently been widely employed for nutritional and medicinal purposes. These anti-oxidant substances are powerful in the fight against oxidative stress and the diseases it causes. *M. oleifera* is known as a "Miracle tree" because of the highly nutritious and important plant components for pharmacological purposes. The Malayalam word "muringo" from southern India is where the genus Moringa gets its name. Moringa, drumstick tree, horseradish tree, and ben oil tree or Benz olive tree, and Mother's Best Friend are some of the common names. It also improves health and has more therapeutic value due to the presence of bioactive components. Because of the seed pods' resemblance to a thin, bent drumstick, moringa is also known as a "drumstick tree." Drumstick seeds contain oil, that's why the tree is also called as Ben oil tree or

Benz-olive. For the treatment of almost 300 different ailments, traditional Indian Ayurveda recommends using all plant parts, including leaves, flowers, roots, bark, and seeds. Some unique characteristics of various plant parts have been found, for example, the root bark of *M. oleifera* has antiviral, anti-inflammatory, and analgesic qualities, while the flowers of the plant contain tonic, stimulant, and diuretic capabilities. The root possesses the Anti-inflammatory, Anti-viral and Analgesic properties. Other significant pharmacological properties of the plant were antioxidant, anticancer, hepatoprotective, cardiovascular, anti-allergic, antiulcer, analgesic, anticonvulsant, anthelmintic, and wound healing. These days, treatments include plant extracts that can be added to human diet. The leaves, seeds, and pods of *M. oleifera* have significant antioxidant properties. The plant, especially the leaves, gains significant antioxidant properties from the presence of secondary metabolites including phenol and flavonoids. Plant foods like fruits, vegetables, herbs, spices, tea, dark chocolate, and wine all include naturally occurring substances referred to as polyphenols. They can function as antioxidants, preventing dangerous free radicals from harming your cells and raising your risk of diseases including cancer, diabetes, and heart disease.

II. MATERIALS AND METHODS

The present study entitled "Determine the efficiency of two different methods used for the extraction of polyphenols from ***moringa oleifera*** leaves" was carried out in the department of food technology, Parul institute of applied sciences, Parul University, Vadodara. The materials utilised are listed in this part, along with a description of the processing methods.

2.1. MATERIALS AND METHODS

2.1.1. Raw materials used in study

Moringa oleifera (Variety: Moringa) leaves were handpicked in the month of November 2022 in the outskirts of Vadodara district. These leaves were ground using a mixer grinder (Ultra, India).

2.1.2. Chemicals and Glassware's

Glassware and chemicals (70% methanol, petroleum ether) for this process were available in the department of Food Analysis and Food Processing lab, Department of Food Technology, Parul Institute of Applied Sciences, Parul University, Vadodara.

2.1.3. Processing Equipment

Equipment required for the preparation were: Weighing balance, Tray dryer, Soxhlet apparatus, grinder, HPTLC (High-performance Thin-layer chromatography), FTIR Spectroscopy and other utensils were obtained from Food Processing Lab, Department of Food Technology, Parul Institute of Applied Sciences, Parul University, Vadodara.

2.2. METHODS

2.2.1. Sample collection and Pre-treatment

The leaves of *M. oleifera* were collected from Vadodara district and washed with distilled water to remove dust and foreign particles. After cleaning, moringa leaves were allowed to dry up to 2 to 3 days under sun. The leave dried were ground using a mixer grinder (Ultra, India) for further extraction process.

2.2.2. Preparation of extract

2.2.2.1. Soxhlet Extraction Method

The most widely used method for extraction process was soxhlet extraction method. The solvent used for the extraction was 70% methanol. The powdered sample (20 gm) was placed in the thimble. A 100 ml of solvent was placed in the round bottom flask. The round bottom flask was in the contact with heating apparatus. In the condenser, the generated vapour was condensed. Now, the condensed solvent was dropped on the sample, filling up to the level of the siphon tube. Once it reaches the level of siphon tube, it was emptied into the round bottom flask as an extract and heated again. This cycle was continued until the extraction was completed. After completion of the extraction process, all the extract was stored at 4°C until further use.

2.2.2.2. Maceration

The leaves of *M. oleifera* were collected and washed with distilled water to remove dust and foreign particles. After cleaning, moringa leaves were allowed to dry up to 2 to 3 days under sun. The leave dried were ground using a mixer grinder (Ultra, India) now, dried powdered of moringa leaves were stored with 70% methanol for 3 days in refrigerator at 4-5ºC. After 3 days solution was filtered through Muslin cloth and centrifuge at 3500 rpm for 10 minutes. Then supernatant were collected and evaporated in Rota vapor. After evaporation of compound in rota vapour, essential compound stored in Eppendorf tube for further analysis.

2.2.3. PROXIMATE ANALYSIS

The following tests were included in the approximate analysis: moisture content, crude fat, crude protein, crude fibre, and ash content.

2.2.3.1. Ash content

Ash content was determined using (AOAC 1990) procedure. 5g of sample was taken into pre-weighed container. The ash is the inorganic residue left after heating at 600ºC. For determining ash, the powdered was incinerated so as to burn out all organic matter. The percent ash was calculated by knowing the difference between the initial and final weight.

% Ash = Weight before heating – Weight after heating/ weight of sample × 100

2.2.3.2. Moisture content

Moisture content was estimated by drying the empty petri plate. 5g of sample was taken and placed in petri plate. Then plate was placed in oven at 105^0C for 4hrs. Sample was taken from oven after 4 hrs and placed in desiccator till attainment of constant weight. The total loss in weight was calculated as moisture content.

Moisture % = Initial weight (W1) - final weight (W2) / Initial weight (W1) × 100

2.2.3.3. Protein content

Protein content was determined by Micro-Kjeldhal method.

Crude Protein % = (Sample titre – Blank titre) × 0.0014 × 6.25 / Sample weight × 100

2.2.3.4. Crude Fat

The fat analysis of *Moringa oleifera* leaves was done using Soxhlet Extraction method.

Fat = (W2 – W1)/ W × 100

2.2.3.5. Determination of Carbohydrates

The amount of carbohydrates was determined by subtracting the total amount of moisture, fat, protein, total Ash and crude fibre.

2.2.4. STRUCTURAL PROPERTIES

2.2.4.1. Fourier Transform Infrared Spectroscopy (FTIR)

The sample analysis was conducted using the FTIR spectrometer (ALPHA Bruker, Germany) at room temperature. The ATR (attenuated total reflection) plate was cleaned with isopropyl alcohol, and scanning was done across a 4000-400 cm-1 scale.

2.2.4.2. High-Performance Thin-Layer Chromatography (HPTLC)

In this article, HPTLC methods was suitable for the analysis of polyphenols from *moringa oleifera* leaves extract were presented, using HPTLC (LINOMAT 5, Switzerland), MilliporeSigma Silica gel 60 TLC plates, analytical standards and extract reference materials.

RECOMMENDED CAMAG DEVICES:

Automatic TLC Sampler, Automatic Developing Chamber, TLC Visualizer 2, Chromatogram Immersion Device 3, TLC Plate Heater 3, and vision CATS.

Sample: Prepared extract in 70% methanol and petroleum ether

Stationary phase: HPTLC Si 60 10 x 10 cm

Mobile phase: 70% Methanol, Petroleum Ether

2.2.4.3. UV-Visible spectroscopy:

Moringa oleifera leaves extract was analysed spectrophotometrically using UV-Visible spectrophotometer. The extract was diluted to 1:10 with methanol and same solvent was taken as blank. The extract was scanned in the wavelength ranging from 300-700 nm and characteristic peak was detected.

III. RESULT AND DISCUSSION

The result obtained during investigation of "Determine the efficiency of two different methods used for the extraction of polyphenols from ***moringa oleifera*** leaves" is discussed here.

3.1. PROXIMATE ANALYSIS

Sample	% moisture content	%Ash content	% crude Fat	% crude protein	% crude Fibre
Leaf extract	7.9	9.0	32.0	22.0	14.0

Table 1: Proximate percentage content of *Moringa oleifera* leaves extract

3.2. Fourier Transform Infrared spectroscopy (FTIR)

For maceration:

The functional group present in the *moringa oleifera* leaves were detected by the infrared material analysis. The FTIR concludes the molecular vibrational measurement of polyphenols. It shows major peaks near 1378 and 2924 cm^{-1}. The wide band at 1378 cm^{-1} was commonly found due to stretch vibration of O-H bonding and it corresponded to phenols. The peak at 2924 cm^{-1} found due to the stretch vibration of C-H bonding and it corresponds to alkane.

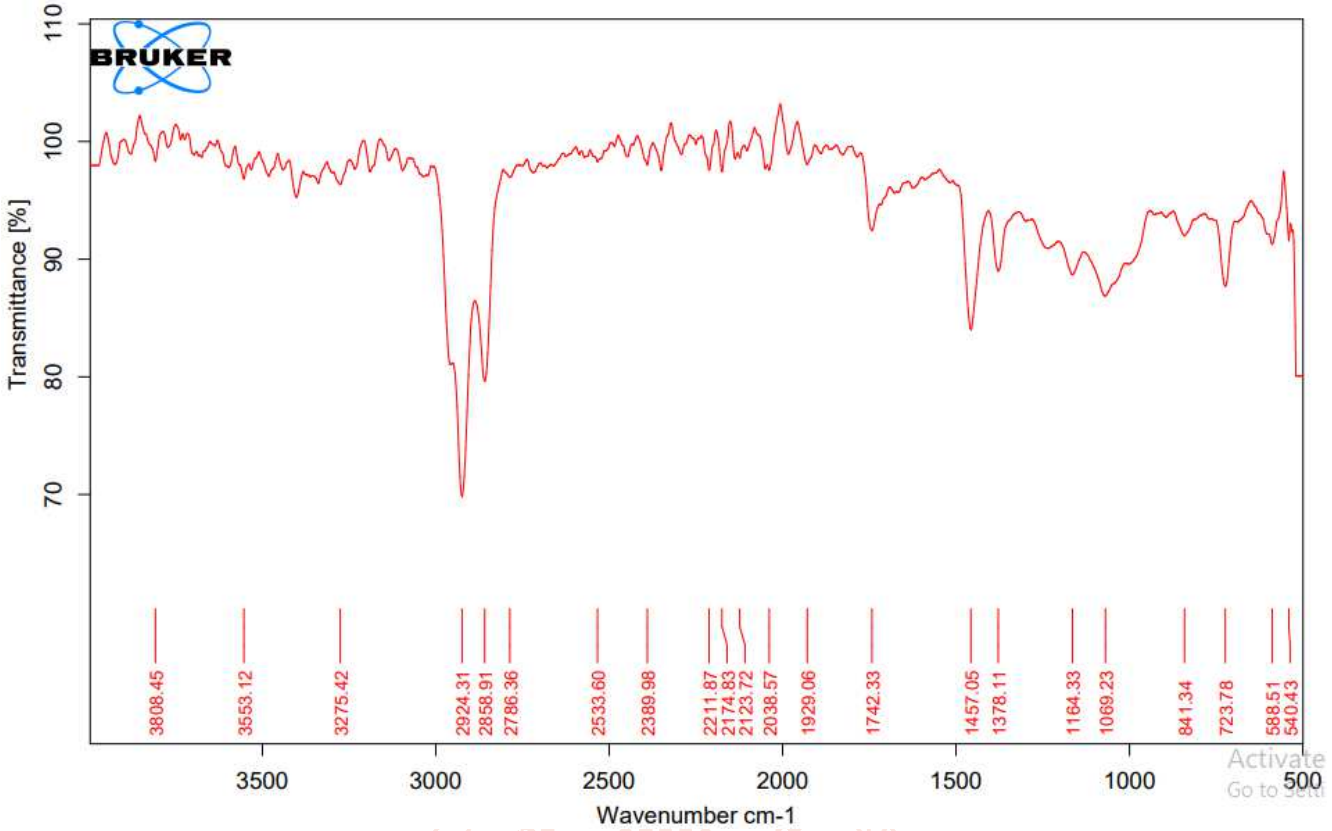

Figure 1: FTIR spectrum of *Moringa oleifera* leaves (Extracted from Maceration).

For Soxhlet extraction method:

The functional group present in the *moringa oleifera* leaves were detected by the infrared material analysis. For soxhlet extraction method, FTIR shows different major peaks near 1017, 1651and 3357 cm^{-1}. The wide band at 1017 cm^{-1} which is in the range below 1050 cm^{-1} was commonly found due to stretch vibration of O-H bonding and it corresponds to Primary Alcohol. The peak at 1651 cm^{-1} found due to the stretch vibration of C=C bonding and it corresponds to alkane. The another peak at 3357 cm^{-1} was observed which is in the range between 3200-3400 cm^{-1} was found due to the stretch vibration of O-H bonding and corresponds to Alcohol and Phenols.

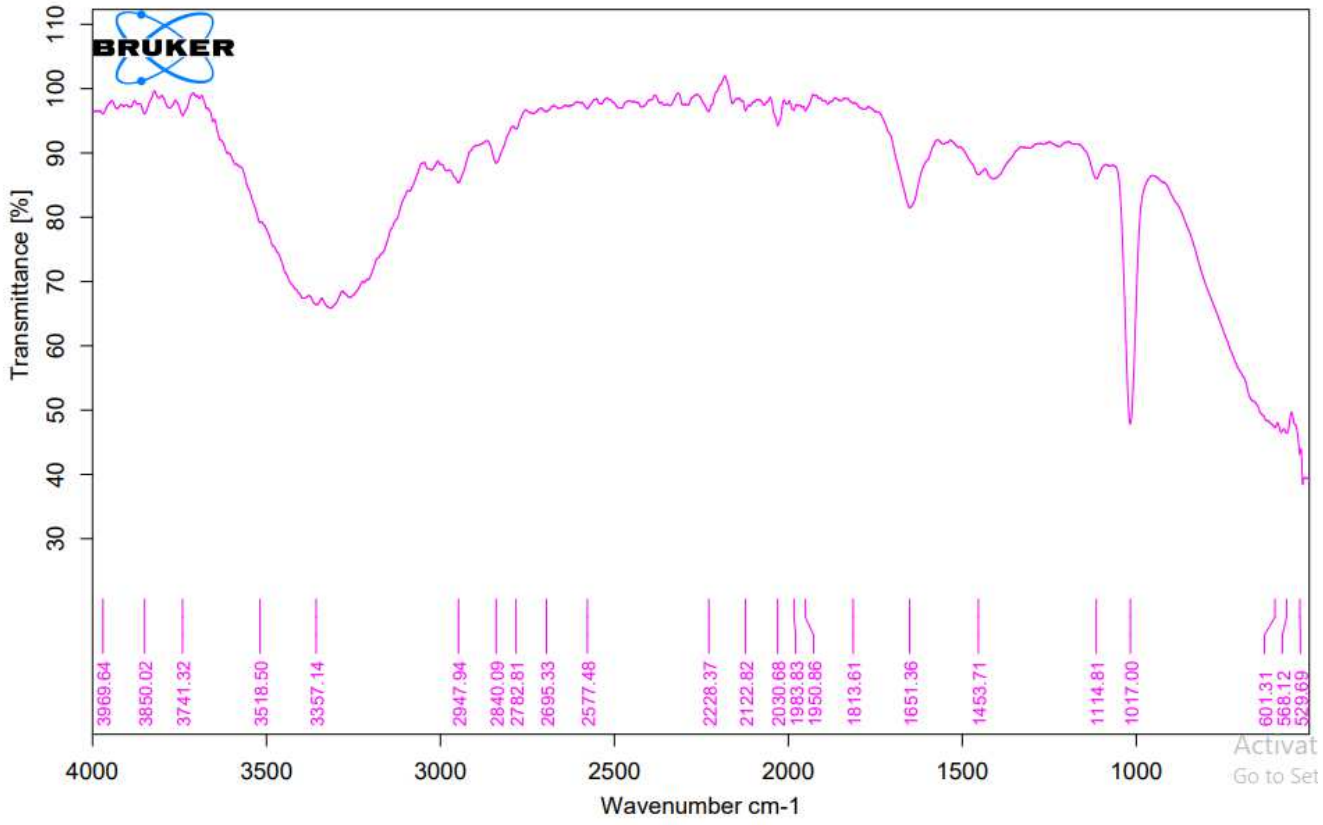

Figure 2: FTIR spectrum of *Moringa oleifera* leaves (Extracted from Soxhlet Extraction Method).

3.3. High-Performance Thin-Layer Chromatography (HPTLC)

The TLC plate works by chromatographic principles: a mobile phase (solvent mixture) will climb up the plate material (stationary phase). Compounds that are less polar will travel farther on the plate due to the less attraction towards stationary phase. While, Compounds that are more polar will travel lower on the plate due to more attraction towards stationary phase. This process is separating the different compounds present in the extract on the basis of their polarity. An important property of any compound is its retention factor (R_f-value). A high R_f -value indicates that the compound has travelled far upto the plate and is less polar, while a lower R_f -value indicates that the compound has not travelled far and is more polar. Since, polyphenols are less polar in nature it travelled far in TLC plate. The calculated Rf values are given in following Table:

Sample	Solvent systems with respective Rf values	
	Maceration	Soxhlet Extraction method
	Methanol (RF value)	Petroleum Ether (RF value)
Moringa oleifera leaves	0.787	0.193

Above table shows RF value for both the methods used for the extraction of polyphenols and determines that due to difference in the concentration methanol travels more in TLC plate than the petroleum ether.

3.4. UV-Visible spectroscopy:

Based on the ability to absorb light in the UV-visible region, the UV-Visible spectrophotometer detects the presence of compounds in *Moringa oliefera* leaves extract (from 300 to 800 nm). The number of peak in the UV-Visible light absorption spectrum will be displayed and it shows the presence of phenols in the *Moringa oleifera* leaves extract. The absorption spectra can give a basic idea about the presence of compounds in the extracts.

Sr. No	Sample	Absorbance peak W/L (nm)
1	Methanol Extract	360, 507, 662
2	Petroleum Ether	410, 500

Table 1.2 Absorbance peaks obtained in UV-Visible range in all the extracts.

According to the result, different absorbance peak at 360, 507 and 662 nm indicates the presence of polyphenolic compounds such as phenols and flavonoids in *Moringa oleifera* leaves extract.

IV. CONCLUSION

From all the performed analysis, it can be concluded that *Moringa oleifera* leaves contains 7.9% moisture, 9.0% total ash, 32.0% crude fat, 22.0% protein and 14.0% crude fibre. It was also concluded that *Moringa oleifera* leaves are rich source of polyphenols. The above results determine the efficiency of two different methods used for the extraction of polyphenols such as soxhlet extraction method and maceration. From the different analysis it is concluded that the maceration was more efficient as it gives more accurate results but it is a time consuming method.

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