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A Comparative study on conventional and non-conventional extraction methodologies for extraction yield, quality and antibacterial properties of moringa (*Moringa oleifera* Lam.)

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Abstract

The effect of different extraction methods using ethanol as a green solvent, such as soaking/maceration, Soxhlet, Ultrasound-Assisted Extraction (UAE), and Microwave-Assisted Extraction (MAE), on the percentage of extraction yield, total phenol content, total flavonoid content, antioxidant activity, and antibacterial effect, was investigated. Unknown phytochemicals present in the moringa leaf extract from different methods were identified using Gas chromatography-Mass spectrometry (GC-MS), and identified compounds present in the moringa leaf extract ranged from twenty-eight compounds to thirty-four compounds from different extraction methods. On comparing the conventional and non-conventional methods of extraction, the quantity and quality of extracted moringa biomolecules were found to be high in terms of extraction yield (21.79 ± 0.10%), TPC (144.52 ± 3.44 QE mg/g), TFC (22.93 ± 0.28 GA mg/g) and antioxidant activity (84.15 ± 1.14%) in UAE, followed by MAE in less time than conventional methods. Moringa leaf extract from UAE showed a maximum zone of inhibition (56.25 ± 0.35 mm) against *E. coli* at higher concentrations. Based on the research results, UAE and MAE could be novel extraction methods for various industrial sectors with a wide range of nutrients and bioactivity. These extracted compounds might be further purified, characterized and fortified with other food products to mitigate malnutrition.

Key words: *Moringa oleifera*, ultrasound-assisted extraction (UAE), ethanolic extract, extraction technologies, phytochemical screening, antibacterial activity

Introduction

One of the world's most well-known and valuable trees is *Moringa oleifera*, more often known as moringa. Moringa is a fast-growing tree found throughout the tropics for the human diet, livestock feed, medicine, water purification, *etc.* (Palada and Chang 2003). Moringa is popularly known as the drumstick tree and by other 210 different names (Amjad *et al.,* 2015). Virtually every part of the moringa tree (pods, leaves, flowers, bark, root and pisin) is edible. Various studies regarding moringa revealed that leaves have remarkable nutritional values such as vitamins, minerals, amino acids and other beneficial biomolecules. Nutrients available in moringa leaves play a major role in fighting against malnutrition among pregnant women, nursing mothers and infants (Anwar *et al.,* 2007). Based on genetic makeup, habitat, and cultivation practices, there may be vast variations in the nutrient content of *M. oleifera* leaves. (Brisibe *et al.,* 2009). Several research studies confirmed that the bioactive compounds from moringa leaves contain a wide range of biomolecules with significant biological actions like antioxidant, antimicrobial, anti-inflammatory, etc., that could be tapped by various food and plant drug-based industries (Oyeyinka and Oyeyinka 2018, Verma *et al.,* 2009).

To study plant-derived biomolecules, various extraction methods, solvent concentration, extraction time, temperature, extraction instrument and plant materials are carefully considered for better recovery (Vongsak *et al.,* 2013; Castro-López *et al.,* 2017). Different heterogeneous groups of biomolecules interact with other substances in the plant matrix, making it challenging to extract various compounds with different polarities. Accordingly, the recovery percentage and quality of biomolecules from plant samples may vary with different extraction methods (Rodríguez-Pérez *et al.,* 2015). Distinct extraction methods are used to overcome these difficulties in isolating different metabolites from the plant sample. Adequate extraction conditions provide good recovery of desired compounds with pertinent biological properties (Garcia-Salas *et al.,* 2010; Fattore *et al.,* 2016; Wang *et al.,* 2018).

To date, conventional and non-conventional method extraction is the most vital classification for the better isolation of phytochemicals. Both extraction methods have pros and cons in recovering bioactive compounds. The plant matrix is homogenized and soaked in solvent in the conventional extraction before bioactive chemicals are extracted from the plant matrices with or without heat treatment. The conventional extraction method will be simple, but the drawbacks are the poor efficiency and yield of bioactive compounds (Barba *et al.,* 2017). On the other hand, non-conventional extraction methods yield superior

extraction efficiency in terms of cost, yield, extraction time and/or selectivity due to the exploitation of energy on the plant matrix to improve extraction yield and efficiency (Kovačević *et al.,* 2018, Barba *et al.,* 2017, Putnik *et al.,* 2018). The non-conventional method of extraction is currently being widely adopted to recover a wide variety of biomolecules. These methods include electrical pulse technologies, microwave-assisted extraction, supercritical fluid extraction, Ultrasound-Assisted Extraction (UAE), and Microwave-Assisted Extraction (MAE). (Roselló-Soto *et al.,* 2015).

To assess the relative efficacy of traditional and unconventional extraction techniques, an experimental study was performed. This study aimed to examine the extraction efficiency, characterize the obtained biomolecules, and explore the impact of moringa leaf compounds. The objectives were to determine the extraction yield percentage, assess extract quality using phytochemical screening, analyze bioactive components through GC-MS profiling, and investigate their antioxidative and antibacterial properties *in vitro*.

Materials and methods

In this study, four different extraction methods, *viz.* maceration (traditional soaking), Soxhlet, Ultrasound-Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE) have been carried out with standardized hydroethanolic solvent (Vongsak *et al.,* 2013).

Plant material: Fresh leaves of PKM-1 Moringa were harvested from the organic field located at the western block in Horticultural College and Research Institute (HC&RI), Periyakulam, Tamil Nadu, India. Harvested leaves are cleaned, washed and dried under a solar cabinet dryer at 45 ºC for 4 hours. Dried moringa leaves are milled using commercial grade pulverized by passing through 200 µm mesh and stored in an airtight container under dark, refrigerated conditions at 4ºC.

Extraction procedure

Maceration method: Powdered moringa leaves were weighed (50g) and 500 mL of 70% ethanol is added into a conical flask. The mixture is kept in a dark, dry place with occasional shaking for 24 hours. After 24 hours, extracts were centrifuged, and the supernatant was collected. The filtrate was concentrated *in vacua*. The extract obtained after concentration was freeze-dried and stored in a refrigerator for further analysis.

Soxhlet method: 50g of powdered sample was taken in the porous thimble and placed in the extraction chamber. 500 mL of 70% ethanol was taken in the boiling flask and boiled at 40ºC for 16 hours or 8 siphons. After 16 hours of extraction, the extract is concentrated under a vacuum rotary evaporator. Obtained concentrated extracts were freeze-dried and stored at -4ºC.

Ultrasound-assisted extraction (UAE) method: Powered moringa leaves of 50g leaves were taken in the 500 mL beaker containing 70% ethanol, and extraction was performed using an Ultrasound Probe Sonicator VCX 1500 equipped with a 1 inch diameter probe (20 kHz, 15 amps, 1500 W) manufactured by Sonics & Materials Inc. USA. The probe was placed inside the mixture and the thermostat to maintain the extraction temperature of 30ºC with pulse mode, 5s on and off. Extraction was carried out for 30 min with sonication energy of 70% amplitude by slight modifications in the optimized extraction proposed by Lin *et al.* (2021). The entire sample mixture was extracted under ice-cold conditions using an ice bath to avoid the degradation of the biomolecules in the sample due to an increase in temperature during sonication. Once the sonic extraction was completed, the extracts were centrifuged, and the supernatant was concentrated using a rotary evaporator. Concentrated extracts were lyophilized and stored at - 4 °C until further analysis.

Microwave-assisted extraction (MAE) method: Extraction of biomolecules from moringa leaves was achieved by placing the 3g sample with 30 mL of 70% ethanol in each of 12 microwave cells of Milestone Ethos™ X. Extraction was conducted with 600 W power for 10 min with slight modifications in the extraction protocol proposed by Rocchetti *et al.* (2018) based on the equipment. The mixtures were instantaneously cooled to room temperature following the extraction cycle, and the resulting marc was then extracted three more times. Then, the pooled extracts were concentrated *in vacua*. Concentrated extracts were freeze-dried, and the dried extracts were stored under dry, cold conditions at - 4 °C until further analysis.

Phytochemical screening of moringa extract: Phytochemical analysis of moringa leaf extract was performed for the qualitative detection of alkaloids, flavonoids, steroids, reducing sugar, tannins and saponins

Alkaloids: In Moringa leaf extract, a few drops of Wagner's reagent (dilute Iodine solution) were added. The development of reddish-brown colouration confirmed the presence of alkaloids.

Flavonoids: 3 mL of moringa leaf extract was added with 10 mL of distilled water, and the solution was vortexed with a few drops of magnesium. The presence of flavonoid was confirmed with the observance of a yellowish colour with the addition of concentrated hydrochloric acid.

Saponins: To a 3 mL of moringa leaf extract, 2 mL of distilled water was added to dilute and the extract was warmed and vortexed. The presence of saponins was confirmed upon the formation of froth.

Steroids: A few drops of concentrated sulphuric acid were added to the moringa leaf extract in a test tube containing 2 mL of chloroform. The presence of steroids was confirmed with a blowfish green colour at the interface.

Tannins: A few drops of moringa leaf extract were taken in the test tube and boiled gently for 2 min, and to the cooled extract, 3 drops of ferric chloride were added. Blue-green precipitate confirms the presence of tannins in the extract.

Reducing sugars: 0.5 mL of plant extracts were warmed in a water bath with 1 mL of water, 5-8 drops of Fehling's solution, and 1 mL of Fehling's solution. The presence of reducing sugar was confirmed by forming a red (brick) precipitate.

Determination of extraction yield: The extraction yield was assessed based on the weight of freeze-dried extract after the complete removal of the solvent and the mass of the leaf sample used for extraction. The ethanolic extract was concentrated under

vacuum by a rotary evaporator at 40 °C and then lyophilized. Dried extracts were weighed, and the results were expressed in percentages (Cho *et al.,* 2020).

Determination of total phenol content in extracts: Total phenolic content in the moringa leaf extract was determined spectrophotometrically by the modified Folin-Ciocalteu method (Rakesh *et al.,* 2021; Chavan *et al.,* 2013). Dried extracts were reconstituted in double-distilled water (1mg/mL). To 100 µL of reconstituted extract, 400 µL of double-distilled was added to dilute the extract, and 150 µL of 1:1 (v/v) diluted FC reagent was added and vortexed. To the mixture, 20% of 500 µL Sodium carbonate (Na_2CO_3) was added and incubated for 1 hour under dark conditions to develop a greenish-blue colour. The absorbance of the extract was read at 650 nm using a microplate reader (BIO-RAD). For blank, 500 µL of ethanol with all reagents were added except plant extract. Gallic acid was taken as a standard to estimate the phenolic content of the extract.

Total flavonoid content: Total flavonoid content in the moringa leaf extract was determined by the aluminium chloride method with slight modification proposed by Daghaghele *et al.* (2021; Rakesh *et al.* (2021). To the 100 µL reconstituted extract 400 µL double distilled water is added to dilute the extract concentration and vortexed. To the sample solution, 100 µL of 10% aluminium chloride solution was added. Later, 100 µL of 1 M sodium acetate was mixed well with the extract and incubated for 45 min at room temperature in dark conditions. The absorbance of the developed golden yellow colour was read at 415 nm using a microplate reader (Bio-Rad). Ethanol 500 µL with all reagents without moringa extract was considered blank. The total flavonoid content in the extract was calculated using quercetin as standard.

Free radical scavenging activity by DPPH Assay: The antioxidant activity of the leaf extract was estimated using DPPH (2,2'-Diphenyl-1-Picryl-Hydrazyl Method with slight modifications (Aryal *et al.,* 2019, Rakesh *et al.,* 2021). The experiment was conducted under dark conditions as the DPPH chemical is light-sensitive. Dried moringa leaf extract was redissolved in 50% methanol. The sample extract of 50 µL was taken and made up to 600 µL by adding 50 % methanol. To the mixture, 200 µL of 0.004 % DPPH solution (4 mg DPPH dissolved in 100 mL of methanol) was added, vortexed and incubated under dark conditions for 30 min. The change in colour of the sample solution and its absorbance was read at 517 nm using a UV-VIS spectrometer (MODEL). As a blank, 600 µL methanol with 200 µL DPPH without adding plant extract is considered. Ascorbic acid was taken as the standard. The percentage of free radical scavenging activity was calculated using the below-mentioned formula:

$$\text{Radical scavenging activity (\%)} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100$$

Where,

$A_{control}$: Absorbance of the control at 517 nm

A_{Sample}: Absorbance of the plant extract at 517 nm

GC-MC profiling of moringa leaf extract: The profiling of phytochemicals present in *M. oleifera* leaf extract was done using GC-MS Thermo Scientific Trace GC ultra-chromatograph system (Thermo Fischer Scientific, Austria) coupled to Thermo Scientific DSQ II quadruple mass spectrometer. The lyophilized moringa leaf extract was reconstituted into the HPLC grade methanol (99.9% Pure) in the ratio of 1mg in 1 mL of methanol. The mixture is sonicated using a water bath sonicator for 16 mins. The moringa leaf extracts were passed through a syringe filter, and the introduction was fully automated using Triplus RSH headspace autosampler. The methanolic extract was separated using a 5% phenyl methyl silicone fused silica capillary column (TG-SQC, 15 m in length, 0.25 mm I.D., and 0.25 µm film thickness). An electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.99%) was used as carrier gas with a flow rate of 1 mL/min, and the split mode was used with a split flow of 10 mL. The injector temperature was set at 60ºC. The column temperature programs consisted of the following: initial temperature 50 ºC (held 1 min), increased to 150 ºC at a rate of 25 ºC/min. After each injection, the column temperature was increased to 250 ºC and held for 7 min to remove the potentially retained residues in the column. The transfer line and MS source temperatures were 265 and 200 ºC, respectively. Mass spectra were recorded using a voltage of 70 eV. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas, using Xcalibur software (Qual, Quan and library) to view spectra and chromatogram analysis. Based on their retention time, percentage of peak area, pattern of mass spectra, and its comparison were done with the data of the National Institute of Standards and Technology's library of NIST11.LIB, the phytochemicals in moringa extracts, were identified (NIST).

Test organism: Gram-negative *E. coli* bacteria were used in this study to assess the antibacterial effect of moringa leaf extracts. The stock culture of the organism was obtained from the Department of Plant Pathology in Tamil Nadu Agricultural University, Coimbatore, stored on a slanted agar test tube and stored at 4 ºC.

Preparation of stock solution for moringa leaf extracts: The stock solution was prepared by reconstituting 10 g freeze-dried leaf extract in 10 mL sterile distilled water. Freeze-dried leaf extract was vortexed until complete dilution and passed through a bacteriological syringe filter. The prepared stock of different quantities was tested for bioactive compounds with antibacterial effects by Agar Well Diffusion method Valgas *et al.* (2007).

Antibacterial screening: Collected bacterial culture was subcultured in a liquid broth and incubated. After the development of bacterial cultures, Petri plates were prepared by the pour plate technique (Kodaka *et al.,* 2005). After the solidification of nutrient agar along with bacterial culture, sterile cork borer wells were created. Moringa extracts of different levels (40, 60, 80, 100 µL) were added to the created wells and diffused into the nutrient agar medium. Sample-added bacterial plates were prepared in 5 replicates for each level and incubated at 37°C for 24 h. Along with these plates, streptomycin and sterile distilled water were used as positive and negative controls of the inoculated *E. coli*.

Determination of inhibition: Antimicrobial activity was detected by measuring the zone of inhibition by placing ruler or vernier caliper around the inhibition zone appearing after the incubation period around the well diffused with moringa extract. The degree of sensitivity of the organisms to the extracts was determined by

measuring the diameter of visible inhibition zones to the nearest millimetre (Hudzicki, 2009). The observed result of the clear zone in Petri dish was compared to the standard zone of inhibition: <8 mm = no sensitivity; <10 mm= insignificant sensitivity; 10-15mm = moderately sensitive; >16mm = highly sensitive (Mukhtar and Ghori 2012). The procedure was repeated thrice for each batch, and the average result was counted for statistical analysis.

Statistical analysis: The mean values of extraction yield, total phenol content, total flavonoid content, antioxidant activity and antimicrobial activity of moringa leaf extracts were compared statistically with the aid of IBM SPSS Statistics Version 25.0 to express mean ± standard deviation for each extraction method. The critical difference was worked out for a 5% level of significance (Panse and Sukhatme 1954). Graphical representations of the observed data were executed using GraphPad Prism software (version 9.3.1).

Results and discussion

The present research trial reveaed that the *M. oleifera* leaves on extraction through four different methods such as maceration, Soxhlet, ultrasound-assisted extraction and microwave-assisted extraction with 70% ethanol, showed the presence of alkaloids, flavonoids, Saponins, Steroids, Tannins, Reducing Sugars and secondary metabolites (Table 1).

Table 1. Phytochemical analysis of different extraction methods of *M. oleifera* leaves

Extraction Method	Maceration/ Soaking Method	Soxhlet Method	Ultrasound-Assisted Extraction	Microwave Assisted Extraction
Alkaloid	+	+	+	+
Flavonoid	+	+	+	+
Saponin	+	+	+	+
Steroid	+	+	+	+
Tannin	+	+	+	+
Reducing Sugar	+	+	+	+

+ Presence; - Absence

The hydro-ethanolic extracts from moringa leaves, through different extraction methods, contained many phytochemicals such as alkaloids, flavonoids, glycoside, phenols, saponins, steroids and tannins. This data validated the conclusions of other research where these compounds exhibited antimicrobial activities (Nikkon, 2003). Percentage of extraction yield, total phenol content, total flavonoid content and antioxidant components were extracted in high quantity (21.79 %,14452,22.93, 84.15 and 17.55 %, 136.42,21.35, 81.73) in UAE and MAE, respectively. This might be due to the external energy acting on the moringa leaf matrix. Besides, the quantity of the above-mentioned biomolecules (Table 2) was recorded as low in conventional extraction methods as the solvent alone interferes with the plant matrix and has no energy implemented to increase the recovery of biomolecules. Among these maceration and Soxhlet extraction methods, Soxhlet recorded lower recovery of bioactive compounds that might be due to the prolonged extraction time under heated conditions (45 ºC), which could have degraded the extracted compounds during siphoning for 16 hours, whereas

Table 2. Effect of different extraction methods on extraction yield, TPC, TFC and Anti-oxidant activity

Extraction method	Extraction yield (%)	Total phenol content (qe mg/g)	Total flavonoid content (ga mg/g)	Antioxidant activity (%)
Maceration/ soaking	15.55 ± 0.01	130.16 ± 4.33	18.62 ± 0.13	77.46 ± 1.21
Soxhlet	6.16 ± 0.05	123.17 ± 3.10	17.90 ± 0.08	73.17 ± 2.29
Ultrasound assisted extraction	21.79 ± 0.10	144.52 ± 3.44	22.93 ± 0.28	84.15 ± 1.14
Microwave assisted extraction	17.55 ± 0.27	136.42 ± 2.78	21.35 ± 0.07	81.73 ± 1.27
SE(d)	0.1616	1.7939	0.1580	0.6214
CV (%)	1.50	1.90	1.11	1.11

UAE and MAE extracted more compounds than conventional methods in lesser extraction period. These results were similar to the research findings of Rocchetti *et al.* (2018).

The total phenol and flavonoid content obtained through these extraction methods showed significant differences in the quantity as the ultrasound-assisted and microwave-assisted extraction methods utilize sonication and microwave energy, respectively. This energy produced by the equipment caused a serious impact on the plant matrixCavitation, or the generation of minute vacuum bubbles in the solvent (liquid phase), is caused by probe sonication. These bubbles explode at the plant sample matrix, increasing localized high temperatures (approximately 4500 °C) and pressures (about 50 MPa). These energies result in sonolysis, cell membrane disruption, and the outflow of intracellular substances (Weggler *et al.,* 2020). Similarly, microwaves interact with the plant sample with free water molecules in the gland, vascular system and solvent, resulting in the dramatic expansion of the cells and rupturing of the plant cells (Paré *et al.,* 1994). This might have increased the recovery of biomolecules in higher quantities when compared to other extraction methods. Similar results were found in the research findings of Sin *et al.* (2014), Yuan *et al.* (2018), Rodríguez-Pérez *et al.* (2015) and Yen (2022).

Identification of compounds from different extraction methods through GC-MS profiling: The GC-MS analysis of moringa leaf extract from different methods found to have twenty-eight compounds in the maceration method, twenty-two compounds in Soxhlet method, thirty-four compounds in the ultrasound-assisted extraction method, and thirty-one major compounds in microwave-assisted extraction. The mass spectra of the bioactive compounds for the identified compounds were characterized using NIST library database. Among various extraction methods, Thieno[2,3-c] furan-3-carbonitrile,2-amino-4,6-dihydro-4,4,6,6-tetramethyl, Arsenous acid, tris(trimethylsilyl) ester, 2-Methyl-3,5-dinitrobenzyl alcohol, tert-butyldimethylsilyl ether, Ethyl iso-allocholate 5H-Cyclopropa[3,4]benz[1,2-e] azulen-5-one,9,9a-bis(acetyloxy)-,7b- trihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-[1aR-(1aà,1bá,2á,4aá,7aà,7bà,8à,9á,9aà)]-,1,1a,1b,2,4a,7a,7b,8,9,9a-decahydro-2,4a,9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol 9-Desoxy-9x-chloroingol 3,7,8,12-tetraacetate, 9,12,15-Octadecatrienoic acid,2,3-bis[(trimethylsilyl)oxy] propyl ester, (Z, Z, Z)-

Table 3. List of identified compounds from GC-MS with antibacterial activity

Name of the compound	Molecular Weight (g/mol)	Molecular Formula	Retention time (Min)	Uses	Reference
5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,9,9a-bis(acetyloxy)-,7b- trihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-[1aR-(1aà,1bá,2á,4aá,7aà,7bà,8à,9á,9aà)]-,1,1a,1b,2,4a,7a,7b,8,9,9a-decahydro-2,4a	506.5	$C_{26}H_{34}O_{10}$	36.56	Antibacterial and Antioxidant	Majumder *et al.* (2019)
9,12,15-Octadecatrienoic acid,2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-	496.9	$C_{27}H_{52}O_4Si_2$	40.65	Antimicrobial, Anti-Inflammatory, Anticancer, Antimicrobial, Antioxidant and Hypercholesterolemia	Haber *et al.* (2007); Good *et al.* (2009)
Propanoic acid,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	430.6	$C_{27}H_{42}O_4$	35.46	Anti-Microbial and Anti-Tumour	Kadhim *et al.* (2016)
8,14-Seco-3,19-epoxyandrostane-8,14-dione,17-acetoxy-3á-methoxy-4,4-dimethyl-	420.5	$C_{24}H_{36}O_6$	14.49	Antimicrobial	Gupta and Kumar (2017)
1-Monolinoleoylglycerol trimethylsilyl ether	500.9	$C_{27}H_{56}O_4Si_2$	33.97	Property Of Anti-Oxidant and Antimicrobial Activities	Huda *et al.* (2015)
Tris(tert-butyldimethylsilyloxy)arsane	468.7	$C_{18}H_{45}AsO_3Si_3$	10.64	Antioxidant, Antibacteria1 and Antifunga1	Salim (2018)
Silicic acid, diethyl bis(trimethylsilyl) ester	168.30	$C_3H_{12}O_4Si_2$	5.79	Antibacterial	Musini *et al.* (2015)
1,2-Benzisothiazol-3-amine tbdms	264.46	$C_{13}H_{20}N_2SSi$	14.45	Antibacterial	Hameed *et al.* (2018) and Priyanka *et al.* (2015)

9,12,15-Octadecatrienoic acid, 9,12,15-Octadecatrienoic acid,2,3-bis[(trimethylsilyl)oxy] propyl ester, (Z, Z, Z)-, Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate, 9,12,15-Octadecatrienoic acid,2,3-, 9,12,15-Octadecatrienoic acid,2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-, .psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-, Bis [di(trimethylsiloxy) phenylsiloxy] trimethylsiloxyphenylsiloxane 9-Desoxy-9x-chloroingol 3,7,8,12-tetraacetate, Propanoic acid,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-, (22S) -21-Acetoxy-6á,11á-dihydroxy-16à,17à-propylmethylen edioxypregna-1,4-diene-3,20-dione, Olean-12-ene-3,15,16,21,22,28-hexol, (3á,15à,16à,21á,22à)-, (22S)-6à,11á,21-Trihydroxy-16à,17à-propylmethylenedioxypregna-1,4-diene-3,20-dione were the compounds identified under GC-MS with retention time from 1.51 min to 48.78 min. From the various research findings, it has been identified that these compounds were found to have various bioactivity properties like analgesic, anti-anginal, non-opioid, anti-hypertensive, anti-arthritic, dementia treatment, Antimicrobial, anti-inflammatory, anti-cancer, antioxidant and anti-hypercholesterolemia (Majumder *et al.,* 2019; Igwe *et al.,* 2015; Good *et al.,* 2009; Haber *et al.,* 2007).

Among these compounds, major phytochemicals from different extraction methods identified under GC-MS analysis against bacteria with antibacterial activity are listed in Table 3.

Table 4. Antibacterial activity of moringa extracts from different extraction methods against *E. coli*

Zone of Inhibition	Soaking Method	Soxhlet Method	Ultrasound-Assisted Extraction Method	Microwave-Assisted Extraction Method
40 µL	16.75 ± 0.15	16.75 ± 0.05	13.75 ± 0.15	19 ± 0.14
60 µL	21.00 ± 0.08	20.00 ± 0.27	17.00 ± 0.08	20 ± 0.12
80 µL	32.25 ± 0.09	22.75 ± 0.20	36.50 ± 0.19	27 ± 0.14
100 µL	41.25 ± 0.09	24.25 ± 0.095	56.25 ± 0.35	36 ± 0.14
Mean±SD	27.81 ± 11.09	20.93 ± 3.30	30.87 ± 19.67	25.5 ± 25.5
Positive Control	62 ± 0.42			
Negative Control	-			

Antibacterial activity of *M. oleifera* from different methods showed significant differences in the diameter of the inhibition zone compared to the positive control with streptomycin and negative control with sterile water. The clear zone inhibition diameter results showed the antibacterial activity of moringa leaf extracts obtained from different extraction methods. Moringa extracts from various extraction methods showed results of inhibition zone ranging for soaking, Soxhlet, ultrasound-assisted extraction and microwave-assisted extraction methods from 16.75 to 41.25 mm, 16.75 to 24.25 mm, 13.75 to 56.25 mm, 19 to 36 mm, respectively to the *E. coli* gram-negative bacteria (Table 4). Compared to different extraction methods, moringa leaf extracts obtained through Soxhlet methods recorded low inhibition zone (24.25 mm) at a higher concentration level of 100 µL. Whereas moringa extract obtained from UAE recorded a high zone of inhibition (56.25 mm) at the concentration level of 100 µL followed by soaking (41.25 mm) and microwave-assisted (36.00 mm) method of extraction (Fig. 1).

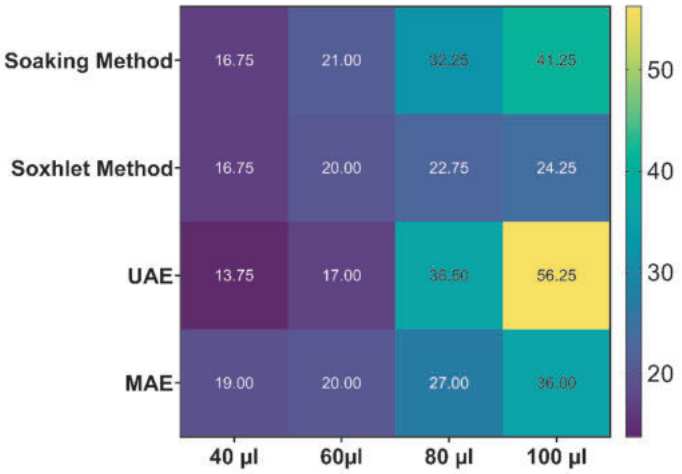

Fig. 1. Heat map representation for the zone of inhibition of different extraction methods

Based on earlier reports, it is clear that moringa leaf extracts have remarkable antibacterial properties, targeting a wide range of microorganisms. As a result, moringa leaf extract is a formidable foe of pathogenic microorganisms, outperforming traditional antibiotics. Notably, the use of novel and non-traditional extraction methods such as ultrasound and microwave-assisted extraction has transformed the process of recovering bioactive compounds. In contrast to the lengthy durations of 24 hours for soaking and 16 hours for Soxhlet methods, these novel techniques yield a variety of phytochemicals with potent antimicrobial activity in just 30 minutes. The efficacy of the obtained phytochemicals, which have antimicrobial properties, is supported by meticulous GC-MS profiling. These ground-breaking findings are consistent with seminal works by Abalaka *et al.* (2012), Dahot (1998), Kumar *et al.* (2012), and Rahman *et al.* (2009), effectively corroborating plant extracts' ability to control pathogenic microorganisms.

An intriguing narrative emerges when the landscape of moringa extracts derived from different extraction methodologies—Soaking, Soxhlet, UAE, and MAE—is delineated. The extracts exhibit promising antibacterial effects against the selected bacterial strain, *E. coli*, demonstrating their potency. Notably, the ultrasonic and microwave-assisted extraction methodologies (UAE and MAE) stand out for their enhanced antibacterial activities.

The current trend toward environmentally friendly practices is exemplified by the use of 70% ethanol, ultrasound-assisted extraction, and microwave-assisted extraction. These cutting-edge techniques outperform the traditional Soaking and Soxhlet methods in a variety of ways. They increase the content of total phenols and total flavonoids while also increasing extraction yield. These innovative approaches significantly increase radical scavenging activity, a key indicator of antioxidant potential.

The mechanisms underlying the success of these novel techniques are complex. Shear forces and cavitation are generated by ultrasonic waves, effectively disrupting the structural integrity of plant cell membranes. According to Lv *et al.* (2011), this transformative interaction increases the contact surface between the solvent and the plant matrix, accelerating the leaching of bioactive compounds. In contrast, microwaves cause rapid internal heating within plant cells. According to Djilani *et al.* (2006), this results in an accelerated release of bioactive phytochemicals in a significantly shorter timeframe than traditional methods.

As a result, unconventional methods such as ultrasound-assisted and microwave-assisted extraction emerge as efficiency and effectiveness leaders. Their ability to rapidly yield abundant phenols, flavonoids, antioxidant compounds, and antibacterial phytochemicals defies the limitations of traditional soaking and Soxhlet extraction techniques, which are characterized by prolonged heat exposure and extraction times. In the ever-changing landscape of botanical extractions, these cutting-edge methods pave the way for expedited yet potent results, setting new industry standards.

The study demonstrated that extracting bioactive compounds from *Moringa oleifera* leaves using advanced methods like ultrasound and microwave-assisted extraction yielded superior results compared to traditional solvent extraction. These modern techniques exhibited higher extraction yield, total phenol and flavonoid content, antioxidant activity, and antibacterial effects. Notably, ultrasound and microwave methods were more efficient and quicker, preserving biomolecule quality. *In vitro* analysis confirmed abundant, high-quality bioactive compound recovery through these energy-intensive methods, benefiting industries seeking premium ingredients. This approach holds the potential for producing top-grade encapsulated plant extracts to enhance nutrition and develop plant-based antimicrobial drugs against human pathogens. The research findings are invaluable for further exploration of these biomolecules' functional and biological properties for fortifying diverse food products.

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