



Determination of Bioactive Compounds Through Gas Chromatography-Mass Spectrometry in *Moringa Oleifera* Leaves

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Abstract: Medicinal plants have long been recognised for their therapeutic and nutritional importance. Among them, *Moringa oleifera* Lam., known as the “Miracle Tree,” is valued for its diverse pharmacological activities and rich phytochemical profile. The present study aimed to identify and characterise bioactive compounds in petroleum ether extract of *M. oleifera* leaves using Gas Chromatography-Mass Spectrometry (GC-MS) and to evaluate their potential pharmacological relevance. Fresh leaves of *M. oleifera* were collected from Chaksu, Rajasthan, authenticated, shade-dried, and powdered. Petroleum ether extraction was performed by maceration, and the extract was subjected to GC-MS analysis. Compounds were identified by comparing retention times and mass spectra with the NIST library database. The GC-MS profiling indicated that several bioactive compounds, such as derivatives of stearic acid esters, carvacrol, boron-containing heterocycles and bisphenol A are present. These compounds displayed pharmacological properties which included antimicrobial, antioxidant, hypocholesterolemic, anticancer and cardioprotective properties. It is important to note that the discovery of bisphenol A raises the issue of environmental pollution and the importance of adopting rigorous quality assurance in the study of medicinal plants. The findings affirm that the leaves of *M. oleifera* are an excellent source of bioactive compounds with potential use in the medical field. Although the availability of such compounds as carvacrol and stearic acid esters justifies the historical applications of the plant, the occurrence of toxicants such as bisphenol A underscores the need to subject phytochemicals and safety to stringent screening and evaluation.

Keywords: Gas chromatography-mass spectroscopy, drumstick, bioactive compound, *Moringa oleifera*, Moringaceae.

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1. Introduction

For thousands of years, medicinal plants have served as an integral part of human health care, both as nutritional supplements and as conventional therapeutic agents for the treatment of diverse diseases across the globe (1). Their historical application has led to the present-day scientific exploration, with several plant-produced compounds being the foundation of pharmaceutical drugs. One of the most widely studied plants of this group is *Moringa oleifera* Lam. (family Moringaceae) owing to its wide array of pharmacological effects and nutritious properties (2). It is often known as the Miracle Tree and is a fast-growing perennial that performs well in both tropical and subtropical areas of Africa, America, and Asia and has been used as a source of food and medicine (3,4).

The *moringa oleifera* has become known internationally, since practically everything in it has proven itself to be therapeutically significant: leaves, seeds, pods, flowers, bark, and roots. It has traditionally been applied in the treatment of illnesses such as malnutrition, inflammation, infectious diseases and metabolic illnesses (5). Its general bioactivities are cardioprotective, anti-

atherosclerotic, antiviral, anti-inflammatory, hepatoprotective, antioxidant, antibacterial, antimicrobial, antifungal, antitumor, immunomodulatory, cholesterol-lowering, antipyretic, antiepileptic, antiviral, anti-inflammatory, antiulcer, wound healing, antispasmodic, diuretic, antihypertensive, and probably one of the most unique characteristics of *M. oleifera* is its high nutritional content. The leaves are also outstanding because they are rich in essential macronutrients and micronutrients, such as high-quality proteins, vitamins (A, B, C, and E), minerals (calcium, potassium, iron, and magnesium), and a number of bioactive compounds. Some of the most important phytoconstituents reported are β -carotene, niazimicin, -sitosterol, glucomoringin, niazinin, kaempferol, quercetin and chlorogenic acid that have a part to play in its multifaceted biological actions (8,10). These compounds are not only antioxidant and anti-inflammatory, but also possess potential in disease prevention, especially chronic diseases like diabetes, hypertension and cancer.

The new techniques of analysis, including Gas Chromatography-Mass Spectrometry (GC-MS), High-Performance Liquid Chromatography (HPLC), and Liquid Chromatography-Mass Spectrometry (LC-MS), have allowed the identification and quantification of bioactive metabolites in *M. oleifera* to be performed with more precision. To justify this, *M. oleifera* petroleum ether extract (PEE) of the leaf has been used to confirm the presence of potent bioactive compounds with therapeutic values. The research is crucial in the design of pharmacognostic standards, as well as the establishment of the chemical diversity of the plant (11). Notably, novel studies have found synergistic action of multiple or more than one phytochemical in *M. oleifera*, with the therapeutic activity being possibly attained not by the individual molecules, but rather by complicated interactions between the phytochemicals within its phytochemical matrix. Moreover, its potential to prevent instances of malnutrition and overt deficiency of micronutrients in resource-deprived scenarios is also part of the global obsession with *M. oleifera*. It is also being used in an increasing number of functional foods, dietary supplements, and fortified products due to the fact that its leaves and seeds have a high level of protein (12,13). There is also interest in its use in nanomedicine and drug delivery systems where bio-actives of *M. oleifera* are being encapsulated to improve stability, bioavailability and specific therapeutic effect.

Despite substantial research, gaps remain in translating its traditional uses into clinically validated therapies. Variability in cultivation conditions, extraction methods, and phytochemical content poses challenges for standardisation. Therefore, novel investigations focusing on metabolomic profiling, molecular docking, and in vivo pharmacological studies are required to establish mechanistic insights and optimise its therapeutic potential. In light of these perspectives, the present research aims to explore the phytoconstituents and bioactivities of *M. oleifera* in greater depth, with particular emphasis on identifying novel therapeutic compounds, validating their pharmacological effects, and evaluating their potential in the development of nutraceuticals and modern medicines.

2. Materials and Methods

2.1 Plant Collection and Authentication

Fresh leaves of *Moringa oleifera* Lam. were collected from the local areas of Chaksu, Rajasthan, India, during January 2023, when the plant was in its active growth phase. The collected material was initially cleaned to remove dust, debris, and contaminants, followed by thorough washing with distilled water. The plant specimen was authenticated by a botanist at the Department of Botany, [University/Institute Name], and a voucher specimen (No. XXXX) was deposited in the institutional herbarium for future reference.

2.2 Preparation of Plant Material and Extract

The leaves were stripped manually, shade-dried at room temperature ($25 \pm 2^\circ\text{C}$) for 10–12 days to prevent the degradation of thermolabile phytoconstituents and subsequently pulverised into a coarse

homogeneous powder using a mechanical grinder. The powdered material was stored in an airtight, moisture-free container to avoid contamination and deterioration. A total of 50 g of dried leaf powder was subjected to maceration using petroleum ether solvent (analytical grade, HiMedia Laboratories, Mumbai, India). The mixture was kept under constant agitation on a rotary shaker at 150 rpm for 32 hours at room temperature to ensure efficient extraction. After maceration, the mixture was filtered initially through muslin cloth, followed by Whatman No.1 filter paper. The filtrate was then concentrated by slow evaporation of the solvent under reduced pressure and stored in an airtight, amber-coloured glass container at 4°C until further analysis (8).

2.3 Solvent and Sample Preparation

Petroleum ether (boiling point range 60–80°C) of analytical reagent (AR) grade was procured from a reliable firm and used without further purification. For GC-MS analysis, the petroleum ether extract (PEE) was reconstituted in the same solvent to prepare a stock solution at a final concentration of 1 mg/mL. The prepared solution was filtered through a 0.22 µm syringe filter to remove any particulate matter and ensure compatibility with the analytical instrumentation.

2.4 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis of *M. oleifera* petroleum ether extract was performed using a Thermo Scientific TSQ 8000 Evo triple quadrupole mass spectrometer coupled with a TRACE 1300 gas chromatograph. Separation was achieved on a DB-5MS fused silica capillary column (30 m × 0.25 mm, i.e., 0.25 µm film thickness). The oven temperature program was set as follows: initial temperature 100°C (held for 4 min), ramped to 340°C at a constant rate of 5°C/min, and held at 240°C for 10 min to ensure complete elution of bioactive compounds. The injector temperature was maintained at 250°C, with helium gas employed as the carrier at a constant flow rate of 1 mL/min. A sample volume of 1 µL was injected in split mode (50:1). The mass spectrometer was operated in electron impact (EI) ionisation mode at 70 eV, with a source temperature of 200°C and scan range of 40–650 m/z.

2.5 Identification of Phytoconstituents

The identification of bioactive components in the extract was based on the comparison of their retention time (RT), relative peak area (% area), and corresponding mass spectra. The obtained spectra were interpreted by matching with the National Institute of Standards and Technology (NIST) library database, which contains over 62,000 reference compounds. Only compounds with ≥90% similarity index were considered as positively identified. Quantification of each compound was expressed as a relative percentage of the total chromatogram area.

2.6 Statistical and Data Analysis

All GC-MS analyses were performed in triplicate to ensure reproducibility. The mean values of retention time and relative abundance (% peak area) were calculated. Identified compounds were further classified into their respective chemical groups (alkaloids, terpenoids, flavonoids, fatty acids, sterols, etc.) based on structural similarities. Preliminary biological significance of the identified compounds was assessed through a literature review, emphasising their reported pharmacological activities.

3. Results and Discussion

The petroleum ether extract of *Moringa oleifera* leaves, prepared through the maceration technique, revealed a diverse range of phytochemical constituents when subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The chromatographic separation and identification provided clear evidence of four major bioactive compounds, namely stearic acid, 3-(octadecyloxy) propyl ester, 1,8-Dioxa-5-thiaoctane,8-(9-borabicyclo[3.3.1]non-9-yl)-3-(9-borabicyclo[3.3.1]non-9-yloxy)-1-phenyl-, carvacrol, and bisphenol A. Each of these compounds displayed unique retention times,

peak areas, molecular formulas, and molecular weights, all of which were precisely documented and presented in both tabular and graphical form, thereby ensuring the reliability of the analytical process. The findings of this analysis provide a strong foundation for understanding the chemical nature of *Moringa oleifera* leaves and also highlight their potential contribution to pharmacological activities. GC-MS analysis of plant extracts remains one of the most reliable approaches for detecting, isolating, and quantifying phytoconstituents, and in this case, it has revealed the complexity and therapeutic promise of *Moringa oleifera*.

The role of bioactive compounds of medicinal plants like *Moringa oleifera* cannot be underrated. Phytochemicals are also applicable in therapeutics due to their structural diversities, which gives them a wide spectrum of pharmacological effects. Traditionally, many modern medicines and scientific investigations are based on plants that have had a historical foundation and are still discovered for their chemical structures and medical possibilities. The identification of phytochemicals by high-end methods such as GC-MS is not only used in describing a plant metabolite; rather, it is used as a guideline when designing a pharmaceutical (14,15). This data is useful in the examination of biological activity, toxicity, and therapeutic index of phytoconstituents, hence helps design safer and effective medicines. In addition to this, the findings confirm the effective utilisation of conventional systems of medicine, whereby *Moringa oleifera* has been established as a plant that possesses numerous health benefits. The plant has had a long history of traditional medical use by traditional healers as a treatment against a host of diseases, and the growing scientific interest in the phytochemicals of this plant has helped close the ethnomedicine-to-evidence-based medicine gap (16).

One of the compounds that is interesting, but controversial, is bisphenol A. Neither a naturally occurring phytochemical nor a naturally occurring phyto-toxicant, its presence gives reason to suspect that the environment has been affected and actively expresses concern about potential contamination. One of the most common industrial chemicals is bisphenol A, which is commonly used in plastics and resins as well as food packaging materials. It is known to be an endocrine disruptor that is low in estrogenic activity and disrupts hormonal signalling in animals and humans (17,18). The fact that it is available in plant extracts signifies the likelihood of environmental leaching or accumulation and consequent exposure via dietary intake. Although bisphenol A is not a desirable compound in medicinal plants, the discovery attracts focus towards proper quality control, sourcing of plants and the impact of environmental pollutants on medicinal flora. The fact that this compound was included in the GC-MS spectrum also underscores the importance of conducting intense toxicological analysis before using crude plant extracts in the clinical setting.

The other chemical found in the petroleum ether extract is Stearic acid, 3-(octadecyloxy) propyl ester, which is a fatty acid ester with many industrial and pharmacological applications. It has been noted that fatty acid esters, particularly those of stearic acid, can exhibit hypocholesterolemic effects, which lower serum cholesterol levels and, thus, give cardioprotective effects. Besides its pharmacological uses, this compound finds its application in perfume, flavouring, and lubrication, thus justifying why it is a highly versatile material. Its hypocholesterolemic effect is also something worth mentioning, medically, since the prevalence of heart diseases increases worldwide (19). As long as additional research is conducted *in vivo*, the substance has the possibility of being incorporated into the development of plant-based supplements or therapeutic treatments to control lipid metabolism.

It is also worth mentioning that 1,8-Dioxo-5-thiaoctane,8-(9-borabicyclo[3.3.1]non-9-yl)-3-(9-borabicyclo[3.3.1]non-9-yloxy)-1-phenyl-, a boron heterocyclic compound has been observed. Boron-based compounds, which are a relatively neglected area in phytochemical research, are gaining scientific interest due to their unique chemical reactivity as well as the potential pharmacological applications. This is a reducing agent and could contribute to the antioxidant action

of the *Moringa oleifera* leaves (20). Antioxidants play a critical role in overcoming the free radicals, thereby preventing oxidative stress that is implicated in the pathogenesis of many chronic diseases like cancer, diabetes, cardiovascular diseases, and neurodegenerative diseases. These compounds imply that the *Moringa oleifera* has therapeutic potential as a natural source of antioxidants, and also, it is agreeable with the historical use of the compound in the prevention and cure of degenerative illnesses.

The next notable compound that is detected in GC-MS analysis is carvacrol, which is an extremely studied and reported compound that has been proven to have a wide variety of biological applications. Carvacrol is a phenol of monoterpenoids, and it is mostly commonly applied because of its antimicrobial properties. It has been said to exhibit an effect with bacteria strains such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Besides its antibacterial properties, carvacrol has been proven to be antifungal, antiparasitic, antioxidant and anticancer. It is among others, antitubercular, which can be of serious interest in the emergence of the global burden of tuberculosis and the emerging phenomenon of drug resistance (21). It is also highly effective in reducing the level of oxidative stress and cell damage, and that is why its antioxidant effect adds extra therapeutic potential. In addition, carvacrol's anticancer properties have been examined in various experimental models, whereby it was stated that it causes apoptosis and inhibits tumour progression. The existence of this substance in *Moringa oleifera* supports the scientific validation of the importance of the historical use of the plant in medicine and indicates the potential pharmaceutical application of this substance.

The overall findings of this study are untimely yet” promising. They highlight the significance of *Moringa oleifera* as the source of different bioactive compounds with potential therapeutic value. The compounds emphasised in the analysis provide data about the pharmacological basis of the medicinal purposes of the plant and precondition the subsequent scientific research (22). Despite the presence of bioactive molecules in the crude extract, further processes are needed to isolate, purify and identify the compounds in isolation to determine their respective biological effects. The outcome of this type of research is that standardised extracts or pure phytoconstituents with proven therapeutic effects may be developed.

The second important point that emerges as a result of this work is that the toxicity and safety profile of the identified compounds needs to be evaluated (23). In illustration, though carvacrol is generally perceived to be safe in specific doses, bisphenol A is perceived to be of concern since it is an endocrine-disrupting chemical. This resistance brings out the dual aspect of the researchers to isolate useful compounds and simultaneously avoid or remove harmful compounds. These bioactive molecules still need clinical trials and animal research to establish the efficacy, safety, dosage and pharmacokinetics of these bioactive molecules before they can translate into clinical practice (24).

The compounds found in *Moringa oleifera*, besides their therapeutic value, also lead to the economic and industrial potential of the plant. In the flavour, perfume and grease industry, and carvacrol as a food preservative and antimicrobial, the applications are not only relevant to human health but also to food and cosmetic usage (25). The multidimensional potential is another value addition to the *Moringa oleifera* plant in terms of medicine and commercial value.

The GC-MS analysis of the petroleum ether extract of *Moringa oleifera* leaves has provided an important first step in unravelling the chemical complexity and therapeutic potential of this plant. The presence of compounds with antibacterial, antioxidant, hypocholesterolemic, and anticancer activities validates the ethnomedicinal uses of *Moringa oleifera* and opens new avenues for pharmacological research. However, given the detection of bisphenol A, stringent measures must be taken to ensure quality control and safety of plant-derived medicines. More extensive *in vivo* studies, toxicological evaluations, and clinical trials are needed to establish a comprehensive pharmacological profile (26,27). This research, therefore, not only contributes to the phytochemical

documentation of *Moringa oleifera* but also lays the groundwork for its future use as a natural, safe, cost-effective, and broad-spectrum therapeutic agent.

4. Conclusion

M. oleifera is a traditional medicinal plant and represents a vital source of bioactive compounds possessing numerous properties that can be used for the prevention of several ailments. Evaluation of phytochemicals requires a large screening process for bioactive compounds. This also helps in determining their mechanism of action. It may lead to the development of a potential drug, its full utilisation by the local community, and the further isolation and characterisation of naturally active compounds for different bioactivities and bio-efficacy.

Declaration

We declare that all authors of this Ms. Have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

Conflict of Interest

The authors announce that they have no conflict of interest.

Authors Contribution

RA and YS conceived the original idea for the article, while HB, VK, and HV carried out the literature search, performed the data analysis, and contributed to the writing of the manuscript. RA and YS further provided critical feedback and valuable guidance, which helped refine and shape the final draft into its present form.

Ethical Approval

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, and no ethical approval was obtained from the committee concerned.

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Table 1. GC-MS profile of PEE of M. oleifera leaf.

Compound name	RT	Area %	Molecular formula	Molecular weight
1,8-Dioxa-5-thiaoctane, 8-(9-borabicyclo [3.3.1] non-9-yl)-3-(9-borabicyclo [3.3.1] non-9-yloxy)-1-phenyl-	26.18	9.09	C ₂₇ H ₄₂ B ₂ O ₃ S	468
Bisphenol A	23.06	15.17	C ₂₇ H ₄₄ O ₂ Si ₂	456
Carvacrol	23.06	15.17	C ₁₆ H ₂₈ OSi	264
Stearic acid, 3-(octadecyloxy)propyl ester	27.44	9.01	C ₃₉ H ₇₈ O ₃	594

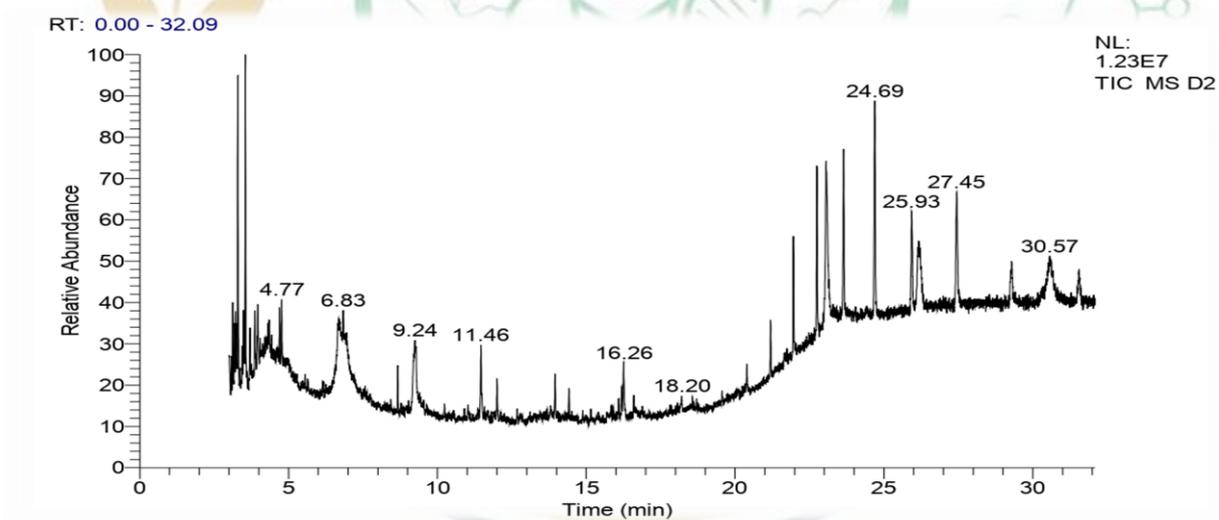


Figure 1. Total ion chromatogram of petroleum ether extract of M. oleifera leaf by GC-MS analysis.

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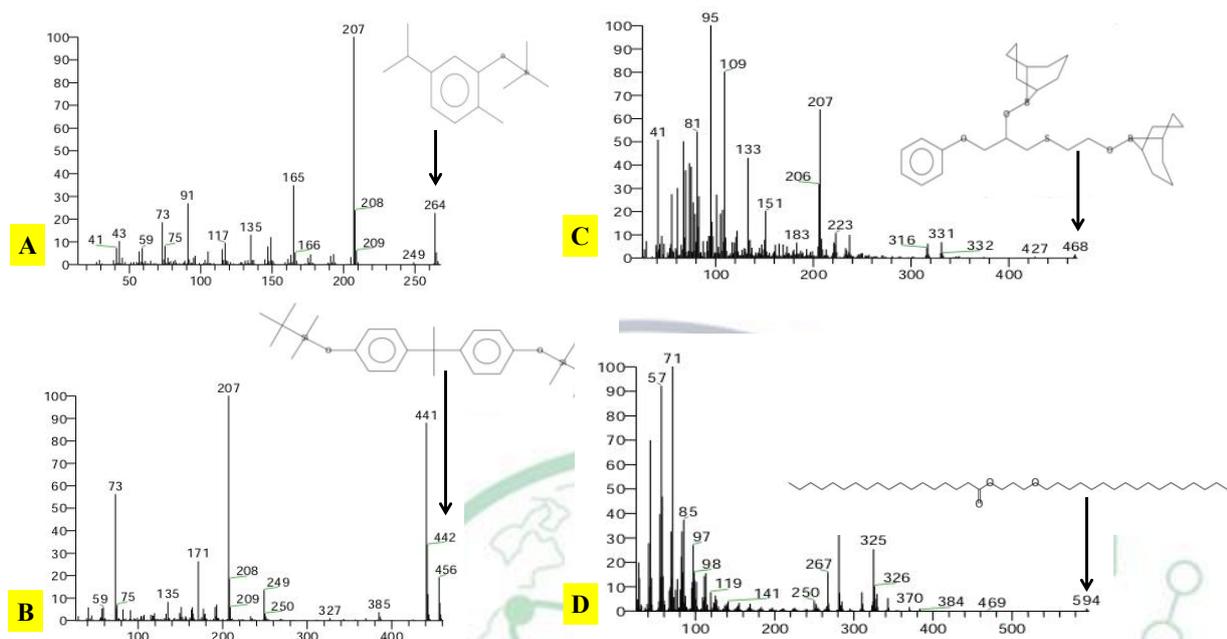


Figure 2. Relative abundance by GC-MS analysis of (A) Carvacrol, (B) Bisphenol A, (C) 1,8-Dioxo-5-thiaoctane, 8-(9-borabicyclo [3.3.1] non-9-yl)-3-(9-borabicyclo [3.3.1] non-9-yloxy)-1-phenyl-, (D) Stearic acid, 3-(octadecyloxy)propyl ester.

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