ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 19, Issue 2, 2026

Online - 2455-3891
Print - 0974-2441

Research Article

ISOLATION, IDENTIFICATION, AND CHARACTERIZATION OF NATURAL CHEMICAL COMPOUNDS AND GC-MS ANALYSIS OF METHANOL EXTRACT OF STEM BARK OF *MORINGA OLEIFERA* WITH ANTI-HYPERGLYCEMIA AND ANTI-MICROBIAL ACTIVITIES

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Received: 29 September 2025, Revised and Accepted: 13 December 2025

ABSTRACT

Objectives: The study aimed to isolate and identify novel and known phytochemical compounds from the methanol extract of *Moringa oleifera* stem bark, to carry out comprehensive phytochemical profiling using gas chromatography-mass spectrometry (GC-MS), and to evaluate the α-amylase and α-glucosidase inhibitory activities of the isolated compounds for potential anti-diabetic effects, along with assessing their antibacterial and antifungal activities.

Methods: The methanol extract of *M. oleifera* stem bark was prepared and subjected to various chromatographic techniques for compound isolation. The structures of the isolated compounds were confirmed through spectral analysis, including proton nuclear magnetic resonance and carbon-13 nuclear magnetic resonance, infrared, and mass spectrometry spectroscopy. GC-MS analysis was conducted to identify the phytochemical constituents based on peak area, retention time, molecular weight, and molecular formula. The isolated compounds were tested for inhibitory effects on α-amylase and α-glucosidase enzymes, and their antibacterial and antifungal activities were evaluated using standard *in vitro* assays.

Results: Two novel compounds, 4-hydroxy-7-methylmellein and 8-methylluteolin, along with four known compounds, benzyl laurate, 4-hydroxymellein, kaempferol, and quercetin, were isolated from the methanol extract. GC-MS analysis revealed the presence of eighty-five compounds in the extract in which 07 compounds identified as common laboratory contaminants or plasticizers, 70 compounds are identified as bioactive phytochemicals, and eight compounds are repeated at different R. Time. Total relative abundance by common laboratory contaminants or plasticizers is 22.61%. Alcohol, alkane, ester, fatty acid, phenolic, sterol, and miscellaneous bioactive phytochemicals are also identified with their total relative abundance, that is, 22.35%, 2.05%, 5.36%, 15.19%, 2.06%, 14.7%, and 15.7% respectively. Sterol, that is, dl-α-Tocopherol and γ-Sitosterol, and alcohols such as isocitronellol, cis-sinapyl alcohol, n-tetracosanol-1, and humulane-1,6-dien-3-ol are identified. The isolated six compounds exhibited inhibitory activity against α-amylase and α-glucosidase enzymes, indicating potential anti-hyperglycemia properties. In addition, antibacterial and antifungal assays demonstrated significant antimicrobial potential for certain isolated compounds.

Conclusion: The methanol extract of *M. oleifera* stem bark contains diverse bioactive phytochemicals with notable pharmacological activities, including enzyme inhibition relevant to hyperglycemia management and antimicrobial properties. The discovery of two novel compounds expands the phytochemical knowledge of *M. oleifera* and supports its traditional medicinal applications.

Keywords: Anti-hyperglycemia, Anti-microbial, Gas chromatography-mass spectrometry, *Moringa oleifera,* Phytochemicals.

 DOI: http://dx.doi.org/10.22159/ajpcr.2026v19i2.57037. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Moringa oleifera is widely cultivated in India and also known as horse-radish tree/drumstick tree/miracle tree/mother's best friend tree [1,2]. This plant is widely used as a nutritional herb and has a high concentration of Vitamin A, Vitamin C, iron, copper, zinc, magnesium, manganese, potassium, and calcium. The plant contains all the essential amino acids [3-5]. Various pharmacological activities like anti-diabetic, anti-asthmatic, anti-inflammatory, anti-fertility, anti-microbial, anti-cancer, anti-oxidant, anti-ulcer, anti-allergic, anti-pyretic, hepatoprotective, cardiovascular, wound healing, analgesic, central nervous system activity, and HIV-1 reverse transcriptase inhibitor are reported by various researchers [3-5]. Many types of natural compounds class such as alkaloids, protein, quinine, saponins, flavonoids, tannin, steroids, glycosides, and fixed oil and fats are present in different parts of this plant [3-5].

Conventionally, the leaves, fruits, flowers, and immature pods of this tree are edible and are used as a highly nutritive vegetable in many countries, particularly in India, Pakistan, Philippines, Hawaii, and some African nations [6-8]. Phytochemicals such as vanillin, omega fatty acids, carotenoids, ascorbates, tocopherols, beta-sitosterol, moringine, kaempferol, and quercetin have been reported in its flowers, roots, fruits, and seeds [9]. Among glucosinolates, 4-O-(a-L-rhamnopyranosyloxy)-benzylglucosinolate (glucomoringin) is the most predominant in the stem, leaves, flowers, pods, and seeds of *M. oleifera* [10]. Although in the roots, benzyl glucosinolate (glucotropaeolin) is the most prominent. The highest content of glucosinolate is found in the leaves and seeds [11]. Every part of this plant contains versatile valuable medicinal properties [5].

METHODS

General experimental procedures

TLC run on Al-Kieselgel 60F_{254} (E.Merck) sheet. Silica gel (E.Merck, 60-120 mesh, 550gm) used for column (1.5 m × 4.0 cm) chromatography. The infrared (IR) spectra were recorded on Fourier Transform IR SHIMADZU 8400S spectrometer with Nujol. The proton nuclear magnetic resonance (^{1}HNMR) and carbon-13 nuclear magnetic resonance (^{13}CNMR)spectra were recorded in Deuterated Chloroform ($CDCl_3$) at 400 MHz and 100 MHz on a Brucker NMR instrument, respectively, using TMS as internal standard. FAB mass spectra were recorded on JEOL SX 102/DA-6000 mass spectrometer using Argon/Xenon as FAB gas. Shimadzu GCMS-QP2020 used for GCMS analysis,

Specifications: EI Source; Mass range *m/z* 1.5–1090; Mode: Scan, SIM, Fast Automated Scan/SIM Type; High-speed scan rate: 10,000 μ/s; Equipped with NIST Library.

Collection of plant material

Stem bark of *M. oleifera* in quantity 2.87 kg, collected from the local area of Jaipur, Rajasthan, India, and a sample is submitted to the herbarium, Department of Botany, University of Rajasthan. Shade-dried plant material (1.96 kg) was coarsely grind to powder.

Extraction and isolation of the constituents

The plant material was extracted with methanol for 12 h × 6 days. Obtained extract was concentrated under reduced pressure to give crude extract. The methanolic extract was dissolved in the minimum amount of methanol and adsorbed on silica gel to form slurry. The dried slurry was subjected to column chromatography over silica gel [12]. The column was eluted with different solvents of increasing polarity, where a total of six compounds with two novel compounds, were isolated, purified, and characterized.

Anti-hyperglycemia activity

Inhibition of alpha-amylase enzyme

A starch solution (0.1% w/v) was obtained by stirring 0.1 g of potato starch in 100 mL of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha-amylase in 100 mL of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartarate solution and 3,5-dinitrosalicylic acid solution 96 mM. Both control and compound were added with starch solution and left to react with alpha-amylase solution under alkaline conditions at 25°C. The reaction was measured over 3 min. The generation of maltose was quantified by the reduction of 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid. This reaction is detectable at 540 nm [13].

Inhibition of alpha-glucosidase enzyme

The inhibitory activity was determined by incubating a solution of starch substrate (2% w/v maltose or sucrose) 1 mL with 0.2 M tris buffer, pH 8.0, and various concentration of compounds for 5 min at 37°C. The reaction was initiated by adding 1 mL of alpha-glucosidase enzyme (1U/mL) to it, followed by incubation for 40 min at 35°C. Then the reaction was terminated by the addition of 2 mL of 6N hydrogen chloride. Then the intensity of the color was measured at 540 nm [13].

Anti-microbial activity

Anti-bacterial activity

Test bacteria, namely, Gram +ve, that is, *Bacillus subtilis*, *Staphylococcus aureus,* and Gram –ve, that is, *Escherichia coli*, were screened by agar-well diffusion method [14] using gentamycin as a standard.

Anti-fungal activity

Agar-well diffusion method [14] was used to screen the test fungi, namely, *Aspergillus flavus, Candida albicans,* and *Trichophyton rubrum* with ketoconozoleas the reference marker.

RESULTS

Experimental data of isolated compounds

Isolation of benzyl laurate

This colorless compound is isolated from fraction no MS-9-16 when column eluted with Hexane + EtOAc in 90: 10 ratio. A total 2000 mL of the solvent system is passed through the column, and each fraction is collected in 250 mL portion. IR (nujol, cm^{-1}): 3075 (Ar -C-H Stretching), 2870, 2925 (Aliphatic C-H Stretching), 1745 (-C=O, Stretching), 1560 (Ar C=C Stretching), 1215, 1185 (Ester C-O, Stretching), 810, 780 (Aromatic C-H Bending).^{1}HNMR (400 MHz, $CDCl_3$, δppm): 0.99 (3H, t, J = 0.4, 0.8 Hz, -CH_3), 1.33-1.37 (16H, m, at C-6 to C-13), 1.69 (2H, m, at C-5), 2.33 (2H, t, at C-4), 5.34 (2H, s, at C-1), 7.22-7.25 (5H, m, C-2' to C-6'). ^{13}CNMR (100 MHz, $CDCl_3$, δppm): C-1 at 66.13, C-3 at 173.10, C-4 at 34.03, C-5 at 25.33, C-6 at 28.93, C-7 to C-10 at 28.96, C-11 at 29.06, C-12 at 31.65, C-13 at 22.94, C-14 at 14.02, C-1' at 137.09, C-2' to C-6' at 128.32. EI-MS (m/z): 290 [M^+], 207, 173, 121, 91. Molecular Formula: $C_{19}H_{30}O_2$.

Isolation of 4-hydroxy-7-methylmellein

This brown color compound is isolated from fraction no MS-22-27 when column eluted with CH_2Cl_2 + MeOH in 95:5 ratio. A total of 1500 mL of the solvent system is passed through the column, and each fraction is collected in a 250 mL portion. Melting point of this compound is determined as 137–139°C. IR (KBr, cm^{-1}): 3565, 3110, 3035, 2980, 2925, 1735, 1645, 1560, 1415, 1350, 1150, 1085.^{1}HNMR (400 MHz, $CDCl_3$, δppm): 5.12 (1H, m, at C-3), 1.57 (3H, d, J = 1.2 Hz, CH_3 at C-3), 5.39 (1H, d, J = 1.6 Hz, at C-4), 6.85 (1H, d, J = 0.8 Hz, at C-5), 7.14 (1H, d, J = 0.8 Hz, at C-6), 2.29 (3H, s, -CH_3 at C-7), 4.86 (1H, s, -OH at C-4), 10.35 (1H, s, -OH at C-8).^{13}CNMR (100 MHz, $CDCl_3$, δppm): C-1 at 171.21, C-3 at 75.90, C-4 at 72.85, C-5 at 116.39, C-6 at 136.16, C-7 at 125.92, C-8 at 158.37, C-9 at 110.40, C-10 at 143.08, 3 -CH_3 at 16.52, 7 -CH_3 at 15.70. MS: 208 [M+H]+, 190, 162, 149, 121, 105, 91, 77. Molecular Formula: $C_{11}H_{12}O_4$.

Isolation of 4-hydroxymellein

This white color amorphous compound is isolated from fraction no MS-31-36 when column eluted with CH_2Cl_2 + MeOH in 95:5 ratio. A total of 1500 mL of the solvent system is passed through the column, and each fraction is collected in a 250 mL portion. Melting point of this compound is determined as 131–133°C. IR (KBr, cm^{-1}): 3615, 3140, 3075, 2990, 2910, 1710, 1625, 1595, 1470, 1365, 1330, 1220, 1165, 1110, 1045, 985, 950, 880, 830. ^{1}HNMR (400 MHz, $CDCl_3$, δppm): 5.15 (1H, m, at C-3), 1.55 (3H, d, J = 0.8 Hz, CH_3 at C-3), 5.37 (1H, d, J = 0.8 Hz, at C-4), 6.91 (1H, d, J = 1.2 Hz, at C-5), 7.29 (1H, m, at C-6), 6.80 (1H, d, J = 1.3 Hz, at C-7), 4.83 (1H, s, -OH at C-4), 10.20 (1H, s, -OH at C-8). ^{13}CNMR (100 MHz, $CDCl_3$, δppm): C-1 at 170.44, C-3 at 75.96, C-4 at 72.92, C-5 at 116.92, C-6 at 135.14, C-7 at 114.45, C-8 at 159.30, C-9 at 110.75. C-10 at 146.37, -CH_3 at 16.52. MS: 194 [M+H]+, 165, 150, 137, 122, 121, 104, 93, 77, 65, 43. Molecular Formula: $C_{10}H_{10}O_4$.

Isolation of kaempferol

This pale-yellow color amorphous compound was isolated from fraction no MS-41-48when column eluted with $CHCl_3$ + MeOH in 90: 10 ratio. A total of 2000 mL of the solvent system is passed through the column, and each fraction is collected in 250 mL portion. Melting point of this compound was determined as 276–278°C. IR (KBr, cm^{-1}): 3490 (O-H, stretching), 3045 (Ar =C-H, stretching), 1675 (-C=O, stretching), 1565, 1535 (Ar =C-C=, stretching), 1210 (Phenolic C-O, stretching), 1035 (Ether C-O-C, stretching), 825, 785 (Ar –C-H Bending). ^{1}HNMR (400 MHz, $CDCl_3$, δppm): 6.18 (1H, s, at C-6), 6.21 (1H, s, at C-8), 7.41 (2H, d, J = 1.2 Hz, at C-2' and C-6'), 6.89, (2H, d, J = 1.6 Hz, at C-3' and C-5') 10.34 (1H, s, -OH at C-3), 12.69 (1H, s, -OH at C-5), 8.82 (1H, s, -OH at C-7), 8.31 (1H, s, -OH at C-4').^{13}CNMR (100 MHz, $CDCl_3$, δppm): C-2 at 145.88, C-3 at 136.35, C-4 at 179.20, C-5 at 159.80, C-6 at 96.90, C-7 at 163.90, C-8 at 94.10, C-9 at, 157.50, C-10 at 104.25, C-1' at 121.95, C-2' and C-6' at 130.95, C-3' and C-5' at 115.95, C-4' at 159.80. EI-MS (m/z): 286 [M^+], 165, 153, 151, 137, 121, 107 etc. Molecular Formula: $C_{15}H_{10}O_6$.

Isolation of quercetin

This yellow color amorphous compound is isolated from fraction no MS-52-57when column eluted with $CHCl_3$ + MeOH in 85:15 ratio. A total of 1500 mL of the solvent system is passed through the column, and each fraction is collected in 250 mL portion. Melting point of this compound was determined as 318–320°C. IR (KBr, cm^{-1}): 3455 (O-H, stretching), 3025 (Ar =C-H, stretching), 1685 (-C=O, stretching), 1590, 1510 (Ar =C-C=, stretching), 1220 (Phenolic C-O, stretching), 1045 (Ether C-O-C, stretching), 915, 830, 795 (Ar –C-H Bending). ^{1}HNMR (400 MHz, $CDCl_3$, δppm): 6.01 (1H, s, at C-6), 6.16 (1H, s, at C-8), 6.95 (1H, s, at C-2'), 6.71, (1H, s, at C-5') 6.93 (1H, s, at C-6'), 10.75 (1H, s, -OH at C-3), 12.65 (1H, s, -OH at C-5), 8.93 (1H, s, -OH at C-7), 8.32 (1H, s, -OH at C-3'), 8.37 (1H, s, -OH at C-4').^{13}CNMR (100 MHz, $CDCl_3$, δppm): C-2 at 146.83, C-3 at

137.09, C-4 at 175.24, C-5 at 159.95, C-6 at 99.62, C-7 at 164.13, C-8 at 94.42, C-9 at, 157.59, C-10 at 104.28, C-1' at 121.81, C-2' at 115.98, C-3' at 145.29, C-4' at 148.35, C-5' at 116.29, C-6' at 121.63. EI-MS (m/z): 302 [M+], 179, 153, 165, 137, 107, 91, 77. Molecular Formula: $C_{15}H_{10}O_7$.

Isolation of 8-methylluteolin

This yellow color amorphous compound was isolated from fraction no MS-58-61 obtained when the column eluted with $CHCl_3$ + MeOH in 75:25 ratio. A total of 1000 mL of the solvent system is passed through the column, and each fraction is collected in 250 mL portion. Melting point of this compound was determined as 331–333°C. IR (KBr, cm^{-1}): 3390 (O-H, stretching), 3065 (Ar =C-H, stretching), 1715 (-C=O, stretching), 1545, 1505 (Ar =C-C=, stretching), 1240 (Phenolic C-O, stretching), 1070 (Ether C-O-C, stretching), 845, 730 (Ar –C-H Bending). ^{1}HNMR (400 MHz, $CDCl_3$, δppm): 6.61 (1H, s, at C-3), 5.97 (1H, s, at C-6), 2.36 (3H, s, at C-8), 6.90 (1H, s, at C-2'), 7.10 (1H, s, at C-5'), 6.79 (1H, s, at C-6'), 13.65 (1H, s, -OH at C-5), 9.80 (1H, s, -OH at C-7), 8.97 (1H, s, -OH at C-3'), 9.42 (1H, s, -OH at C-4'). ^{13}CNMR (100 MHz, $CDCl_3$, δppm): C-2 at 164.05, C-3 at 105.44, C-4 at 182.86, C-5 at 158.11, C-6 at 98.16, C-7 at 161.83, C-8 at 102.54, C-9 at, 157.71, C-10 at 103.81, C-1' at 122.59, C-2' at 114.13, C-3' at 146.43, C-4' at 150.03, C-5' at 116.31, C-6' at 119.39. EI-MS (m/z): 300 [M+], 285, 165, 153, 151, 137, 107, etc. Molecular Formula: $C_{16}H_{12}O_6$.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC–MS analysis of the methanol extract of Moringa oleifera stem bark led to the identification of 85 compounds belonging to various chemical classes with sterols, fatty acids, alcohols and phenolic constituents as major components.

Anti-hyperglycemia activity

The isolated compounds exhibited significant concentration dependent inhibition of α-amylase and α-glucosidase enzymes. Flavonoids such as quercetin, kaempferol and 8-methylluteolin showed comparatively stronger inhibitory activity, suggesting their potential role in the management of post-prandial hyperglycemia.

Anti-microbial activity

The isolated compounds exhibited strong antibacterial and antifungal activities against selected gram-positive, gram-negative bacteria and pathogenic fungi. Flavonoid rich compounds, particularly quercetin and 8-methylluteolin, showed comparatively higher inhibition zones, indicating broad spectrum antimicrobial potential. The results suggest that hydroxylated phenolic structures play a key role in the observed antimicrobial efficacy.

DISCUSSION

Characterization of isolated compounds

Characterization of benzyl laurate

Molecular formula $C_{19}H_{30}O_2$ was calculated on the basis of the mass spectrum data as observed with a molecular ion peak at m/z 290, and other prominent peaks were observed at m/z 207, 173, 121, etc. The IR spectrum (nujol, cm^{-1})of this compound showed significant absorptions at 3075 cm^{-1} was assigned to aromatic C–H stretching, while strong bands at 2925 $cm^{-1}$and 2870 cm^{-1}corresponded to aliphatic C–H stretching vibrations, indicating a long hydrocarbon chain. A sharp absorption at 1745 $cm^{-1}$was characteristic of ester carbonyl (C=O) stretching. The aromatic ring was further supported by C=C stretching at 1560 cm^{-1} and C–H bending bands at 810 and 780 cm^{-1}. Additionally, strong peaks at 1215 and 1185 cm^{-1} confirmed ester C–O stretching vibrations.

^{1}HNMR spectrum (400 MHz, $CDCl_3$, δppm) displayed a triplet at δ 0.99 ppm integrating for three protons, characteristic of a terminal methyl group (-CH_3) at the end of the aliphatic chain. A multiplet at δ 1.69 ppm for two protons corresponded to the methylene group at C-5, while C-4 methylene, appeared as a triplet at δ 2.33 ppm. A singlet at δ 5.34 ppm integrating for two protons was assigned to the benzylic methylene (-CH_2-Ph) attached to the ester oxygen. The aromatic region showed a multiplet at δ 7.22–7.25 ppm integrating for five protons. A multiplet in the region δ 1.33–1.37 ppm integrating for remaining.^{13}CNMR spectrum (100 MHz, $CDCl_3$, δppm) displayed a distinct signal at δ 173.10 ppm corresponding to the ester carbonyl group. Thebenzylic methylene carbon (C-1)appeared at δ 66.13 ppm, consistent with a –CH_2- group attached to an oxygen atom. Remaining ^{13}CNMR peaks were observed for C-4 at 34.03, C-5 at 25.33, C-6 at 28.93, C-7 to C-10 at 28.96, C-11 at 29.06, C-12 at 31.65, C-13 at 22.94, C-14 at 14.02, C-1' at 137.09, and C-2' to C-6' at 128.32. On the basis of various findings and literature, Shonle and Row [15], this compound (Fig. 1) was identified as Benzyl laurate, and the molecular formula was calculated as $C_{19}H_{30}O_2$.

Characterization of 4-hydroxy-7-methylmellein

The mass spectrum showed a molecular ion peak at m/z 208 [M+], and other significant peaks were observed at m/z 193, 180, 165, 137, 123, 95 & 85, and calculated the molecular formula as $C_{11}H_{12}O_4$. This type of pattern suggested to be isocoumarin. The IR spectrum (KBr, cm^{-1}) exhibited a broad absorption at 3565 cm^{-1}, indicative of –O-H stretching, suggesting the presence of a hydroxyl group. Aromatic C-H stretching vibrations were observed at 3110 cm^{-1} and 3035 cm^{-1}, while aliphatic C–H stretches appeared at 2980 cm^{-1} and 2925 cm^{-1}. A strong and sharp absorption at 1735 cm^{-1} corresponded to C=O stretching, characteristic of a lactone or ketone group, and a band at 1645 cm^{-1} was assigned to aromatic C=C stretching, supporting the presence of an aromatic system.

The ^{1}HNMR spectrum (400 MHz, $CDCl_3$, δppm)displayed a multiplet at δ 5.12 ppm (1H) corresponding to the one proton at C-3, while a doublet at δ 1.57 ppm (3H, J = 1.2 Hz) was assigned to the methyl group at the C-3 position. A doublet at δ 5.39 ppm (1H, J = 1.6 Hz) was attributed to the proton at C-4. The presence of hydroxyl protons was confirmed by a singlet at δ 4.86 ppm, corresponding to the -OH group at C-4, and a downfield singlet at δ 10.35 ppm, characteristic of a phenolic -OH group at C-8, which is hydrogen-bonded. The aromatic region displayed two doublets at δ 6.85 ppm and δ 7.14 ppm (each 1H, J = 0.8 Hz) corresponding to the protons at C-5 and C-6 position. A singlet at δ 2.29 ppm (3H) was assigned to the –CH_3 group at C-7 position. On the analysis of ^{13}CNMR spectrum (100 MHz, $CDCl_3$, δppm) signal observed at δ 171.21 ppm was assigned to the lactone carbonyl carbon (C-1). Carbons at C-3 and C-4 appeared at δ 75.90 ppm and δ 72.85 ppm, respectively. Remaining ^{13}CNMR peaks were observed forC-5 at 116.39, C-6 at 136.16, C-7 at 125.92, C-8 at 158.37, C-9 at 110.40, C-10 at 143.08, 3 -CH_3 at 16.52, and 7-CH_3 at 15.70. On the basis of various findings, Reveglia *et al*. [16], this is a novel compound (Fig. 1) and identified as 4-hydroxy-7-methylmellein. Molecular formula was calculated as $C_{11}H_{12}O_4$.

Characterization of 4-hydroxymellein

The mass spectrum showed a molecular ion peak at m/z 194 [M+], and other significant peaks were observed at m/z179, 166, 151, 123, and 95, and calculated the molecular formula as $C_{10}H_{10}O_4$. This type of pattern suggested being isocoumarin. The IR spectrum (KBr, cm^{-1}) exhibited a broad absorption at 3615 cm^{-1}, characteristic of -O-H stretching, indicating the presence of hydroxyl group. Aromatic C-H stretching vibrations appeared at 3140 cm^{-1} and 3075 cm^{-1}, while aliphatic C-H stretches were observed at 2990 cm^{-1} and 2910 cm^{-1}. A prominent absorption at 1710 cm^{-1} was assigned to the lactone carbonyl (C=O) stretching. The presence of an aromatic ring was supported by C=C stretching at 1625 cm^{-1} and 1595 cm^{-1}, along with other aromatic-related vibrations at 1470 cm^{-1} and 1365 cm^{-1}.

A multiplet in ^{1}HNMR spectrum (400 MHz, $CDCl_3$, δppm), at δ 5.15 ppm (1H) was assigned to the proton at C-3 and this was supported by a doublet at δ 1.55 ppm (3H, J = 0.8 Hz) corresponding to the methyl group attached to C-3 position. A doublet at δ 5.37 ppm (1H, J = 0.8 Hz) was attributed to the proton at C-4, which is connected to a hydroxyl group, evidenced by a singlet at δ 4.83 ppm for the -OH proton at C-4 position. Signals were observed at δ 6.91 ppm (1H, d, J = 1.2 Hz) for C-5, δ 7.29 ppm (1H, m) for C-6, and δ 6.80 ppm (1H, d, J = 1.3 Hz) for C-7, indicating the presence of one protons at each position. A singlet at δ

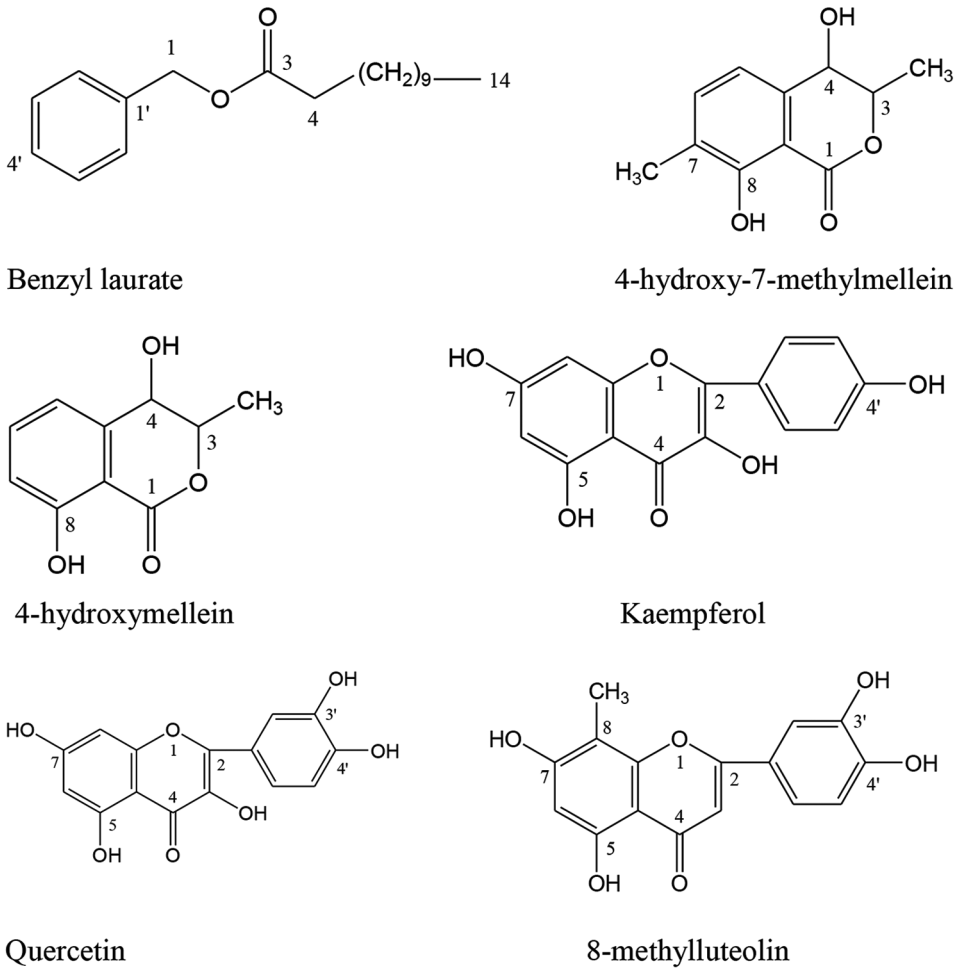

Fig. 1: Isolated compounds from methanol extract of stem bark of *Moringa oleifera*

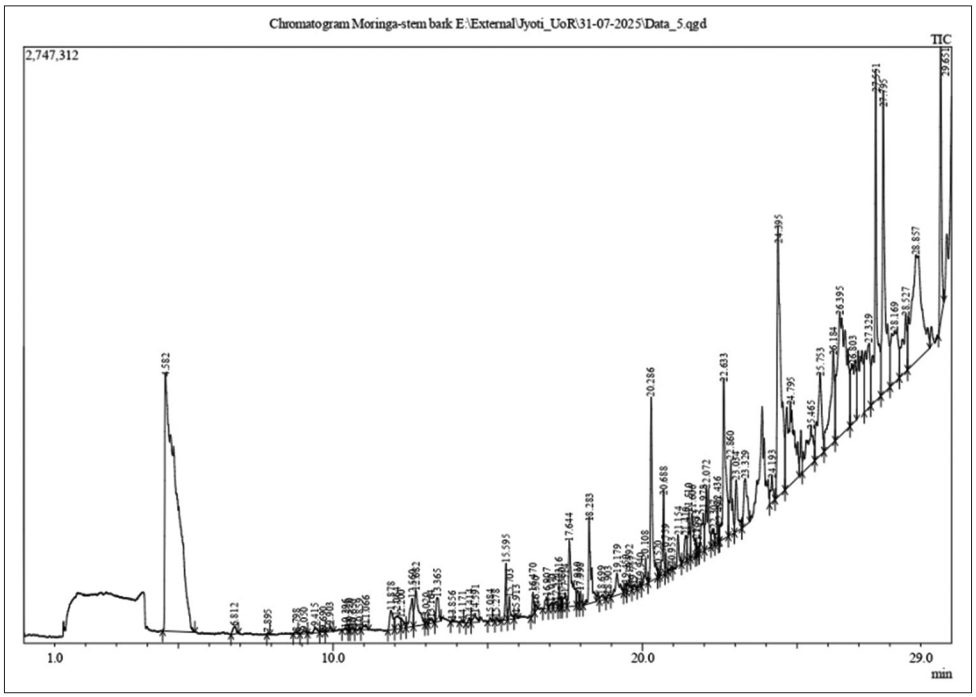

Fig. 2: Gas chromatography-mass spectrometry chromatogram of methanol extract of stem bark of *Moringa oleifera*

10.20 ppm corresponded to the phenolic -OH proton at C-8 position, which appeared downfield due to intra-molecular hydrogen bonding. On the analysis of ^{13}CNMR spectrum (100 MHz, $CDCl_3$, δppm) signal observed at δ 170.44 ppm, corresponding to the lactone carbonyl carbon (C=O) while methine carbons, C-3 and C-4 appeared at δ 75.96 ppm and δ 72.92 ppm, respectively. Remaining ^{13}CNMR peaks were observed forC-5 at 116.92, C-6 at 135.14, C-7 at 114.45, C-8 at 159.30, and C-9 at 110.75. C-10 at 146.37, -CH3 at 16.52. On the basis of various findings Reveglia *et al*. [16], this compound (Fig. 1) was identified as 4-hydroxymelleinand molecular formula was calculated as $C_{10}H_{10}O_4$.

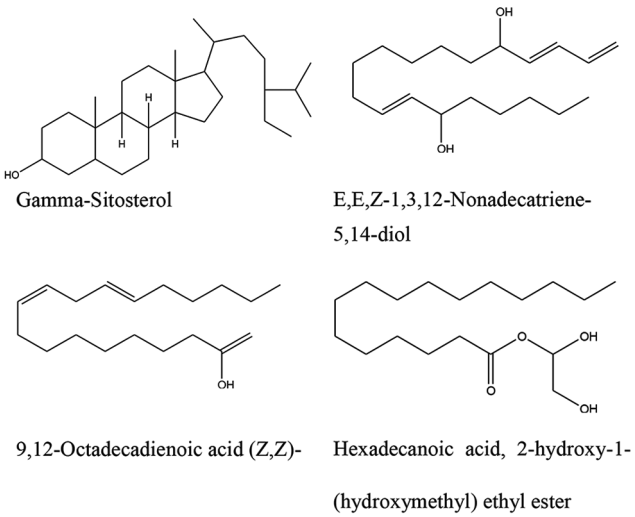

Fig. 3: Identified compounds in major amount from methanol extract of the stem bark of *Moringa oleifera* using gas chromatography-mass spectrometry

Characterization of kaempferol

The EI-MS spectrum shows a molecular ion peak at m/z 286 [M^+], consistent with the molecular formula $C_{15}H_{10}O_6$. Other fragmentation peaks at m/z 165, 153, 151, 137, 121, and 107 are characteristic of a flavonoid skeleton. The IR spectrum (KBr, cm^{-1}) shows a broad absorption at 3490 cm^{-1}, corresponding to -O-H stretching. Aromatic C-H stretching appears at 3045 cm^{-1} and a strong band at 1675 cm^{-1} represents carbonyl (C=O) group. Aromatic skeletal vibrations were evident at 1565and 1535 cm^{-1}. C-O stretching at 1210 cm^{-1} and the ether C-O-C stretch at 1035 cm^{-1}further support the presence of hydroxylated and oxygenated aromatic systems. The C-H bending vibrations of the aromatic ring were observed at 825 and 785 cm^{-1}.

In the ^{1}HNMR spectrum (400 MHz, $CDCl_3$, δppm) a singlet at δ 6.18 ppm (1H) and another singlet at δ 6.21 ppm (1H) were observed to the protons attached at position C-6 and C-8, respectively. The B-ring shows two doublets at δ 7.41 ppm (2H, J = 1.2 Hz) and δ 6.89 ppm (2H, J = 1.6 Hz) corresponding to the protons attached at C-2', C-6', and C-3', C-5' positions, indicating a para-substituted phenyl ring. The spectrum also reveals another four singlets due to hydroxyl protons, that is, 10.34 (1H, s, -OH at C-3), 12.69 (1H, s, -OH at C-5), 8.82 (1H, s, -OH at C-7), 8.31 (1H, s, -OH at C-4').

In the ^{13}CNMR spectrum (100 MHz, $CDCl_3$, δppm), carbonyl carbon (C-4) resonates at δ 179.20 ppm, while C-2 and C-3 carbons appear at δ 145.88 and 136.35 ppm, respectively, supporting the double bond and hydroxyl substitution at C-3. The A-ring carbons are observed at δ 159.80 (C-5), 96.90 (C-6), 163.90 (C-7), 94.10 (C-8), 157.50 (C-9), and 104.25 (C-10), showing the presence of hydroxyl groups at C-5 and C-7. In the B-ring, the quaternary C-1' appears at δ 121.95 ppm, while the C-2' and C-6' (δ 130.95) and C-3' and C-5' (δ 115.95) reflect a symmetrical para-substituted phenyl ring. The C-4', bearing a hydroxyl group, resonates at δ 159.80 ppm, indicating strong deshielding due to phenolic substitution. On the basis of various findings, Sermkaew

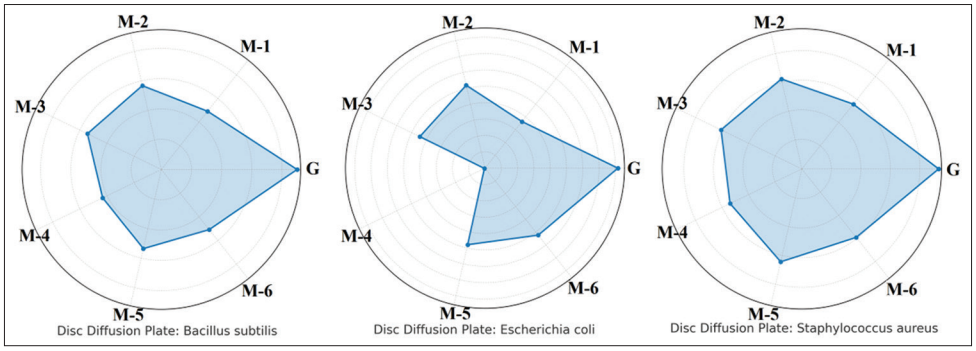

Fig. 4: Antibacterial activity against *Bacillus subtilis*, *Escherichia coli,* and *Staphylococcus aureus*. Wells: 1. Gentamycin (Std), 2. M-1, 3. M-2, 4. M-3, 5. M-4, 6. M-5, 7. M-6

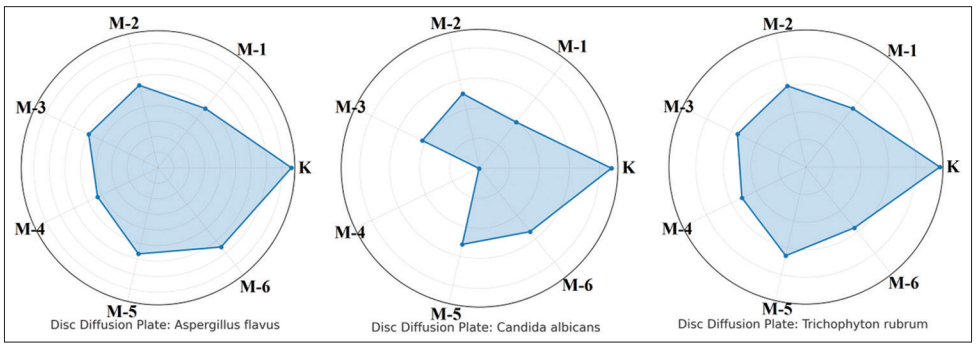

Fig. 5: Anti-fungal activity against Aspergillus flavus, Candida albicans, and Trichophyton rubrum. Wells: 1. Ketoconazole (Std), 2. M-1, 3. M-2, 4. M-3, 5. M-4, 6. M-5, 7. M-6

Table 1: Category/class of identified compounds using gas chromatography-mass spectrometry from methanol extract of stem bark of *Moringa oleifera*

S. No.	Category/ class of compounds	Compounds name	Total relative abundance (%)
1.	Common laboratory contaminants or plasticizers	Toluene p-Xylene Chloroacetic acid, 4-methylpentyl ester Heneicosyl heptafluorobutyrate Diethyl Phthalate 1,2-Benzenedicarboxylic acid, diundecyl ester Phthalic acid, 2,7-dimethyloct-7-en-5-yn-4-yli	22.61
2.	Alcohol	Phenol 1-Hexanol, 2-ethyl- Phenol, 2-methoxy- 2-Heptanol, 5-ethyl- Maltol 2-Methoxy-4-vinylphenol Phenol, 2,6-dimethoxy- Isocitronellol Phenol, 2-methoxy-4-(1-propenyl)-, (Z)- 2,4-Di-tert-butylphenol 1-Decanol, 2-hexyl- cis-Sinapyl alcohol 6,10,14-Trimethyl-pentadecan -2-ol 1-Triacontanol n-Tetracosanol-1 Humulane-1,6-dien-3-ol E, E, Z-1,3,12-Nonadecatriene-5,14-diol 1-Heptacosanol	22.35
3.	Alkane	Tetradecane 2-Bromotetradecane Heptadecane Eicosane Nonacosane	2.05
4.	Ester	1-Hexene, 3,5,5-trimethyl- Cyclohexane, [(1-methylpropyl) thio]- Dodecane, 4,6-dimethyl- Benzofuran-2,3-dione, 4,7-dimethyl-2,3-dihyd 2,2,7-Trimethyl-octa-5,6-dien-3-one Benzene, 1-(1,1-dimethylethyl)-4-ethenyl- Dodecane, 4,6-dimethyl- Oxalic acid, dodecyl 3,5-difluorophenyl ester Undec-10-ynoic acid, dodecyl ester (3,7,7-Trimethyl-bicyclo[2.2.1] hept-2-yl)-met 2-Methyltetracosane 4-Tetradecene, 2,3,4-trimethyl- Carbonic acid, decyl 2,2,2-trichloroethyl ester 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-dien Methyl stearate Undec-10-ynoic acid, tetradecyl ester	5.36
5.	Fatty Acid	Octanoic acid, phenyl ester Hexadecanoic acid, methyl ester n-Hexadecanoic acid 9,12-Octadecadienoic acid (Z, Z)- Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	15.19
6.	Phenolic	Benzoic acid, methyl ester Benzoic acid	2.06
7.	Sterol	dl-.alpha.-Tocopherol .gamma.-Sitosterol	14.7
8.	Miscellaneous	2-Cyclopenten-1-one, 2-hydroxy- 1-Tetradecene 1-Dodecene 1,4:3,6-Dianhydro-.alpha.-d-glucopyranose Benzofuran, 2,3-dihydro- 2-Undecanone 1-Tetradecene 17-Pentatriacontene Methoxamine Cetene (E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxypl 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphe 1-Nonadecene Octadecane Neophytadiene Tyramine, N-formyl- 10-Nonadecanone Hexacosane, 1-iodo- Tetrapentacontane, 1,54-dibromo- 1-Hexacosene Oxirane, heptadecyl- 1-Heneicosyl formate	15.7

(Contd...)

Table 1: ***(Continued)***

et al. [17], this compound (Fig. 1) was identified as kaempferol, and molecular formula was calculated as $C_{15}H_{10}O_6$.

Characterization of quercetin

The mass spectrum showed a molecular ion peak at m/z 302 [M^+] along with characteristic fragment ions peaks at m/z 179, 165, and 153. The IR spectrum (KBr, cm^{-1}) showed a broad absorption at 3455 cm^{-1}, indicated the presence of hydroxyl group/s (-O-H), while a strong absorption at 1685 cm^{-1} corresponds to the C=O stretching of a ketone. The aromatic character was confirmed by C-H stretching at 3025 cm^{-1} and C=C stretching bands at 1590 and 1510 cm^{-1}. Peaks at 1220 cm^{-1} (phenolic C–O) and 1045 cm^{-1} (ether C–O–C) supported the presence of flavonoid skeleton features.

The ^{1}HNMR spectrum (400 MHz, $CDCl_3$, δppm) revealed singlets at δ 6.01 and δ 6.16 ppm corresponding to the protons attached at C-6 and C-8 positions on the A-ring of the flavonoid. The B-ring showed three singlets for three different protons at δ 6.95, δ 6.71, and δ 6.93 ppm attributed to C-2', C-5', and C-6' positions. Five downfield singlets at δ 10.75, δ 12.65, δ 8.93, δ 8.32, and δ 8.37 ppm were assigned to hydroxyl protons attached at C-3, C-5, C-7, C-3', and C-4', respectively. In the ^{13}CNMR spectrum (100 MHz, $CDCl_3$, δppm), carbonyl carbon (C=O) appeared at δ 175.24 ppm, while C-2 and C-3 resonated at δ 146.83 and 137.09 ppm**,** respectively. The remaining A-ring carbons were observed

at δ 159.95 (C-5), 99.62 (C-6), 164.13 (C-7), 94.42 (C-8), 157.59 (C-9), and 104.28 (C-10). The B-ring signals observed at δ 121.81 (C-1'), 115.98 (C-2'), 145.29 (C-3'), 148.35 (C-4'), 116.29 (C-5'), and 121.63 (C-6'). On the basis of above data conclusively Sermkaew *et al*. [17] identified the isolated compound as quercetin (Fig. 1), and molecular formula calculated as $C_{15}H_{10}O_7$.

Characterization of 8-methylluteolin

The EI-MS spectrum revealed a molecular ion peak at m/z 300 [M^+] consistent with the other fragmentation ion peaks at m/z 285, 165, 153, 151, 137, and 107. The IR spectrum (KBr, cm^{-1}) showed a broad absorption at 3390 cm^{-1}, indicative of -O–H stretching, confirming the presence of phenolic hydroxyl group/s. The aromatic C–H stretching appeared at 3065 cm^{-1} and a strong absorption at 1715 cm^{-1} was assigned to the carbonyl group (C=O). Aromatic skeletal vibrations were observed at 1545 and 1505 cm^{-1}, supporting the presence of a substituted aromatic system. The phenolic C–O stretching band at 1240 cm^{-1} and ether C–O–C stretching at 1070 cm^{-1} further confirmed the polyhydroxyflavone structure. The bending vibrations of aromatic C–H were observed at 845 and 730 cm^{-1}.

In the ^{1}HNMR spectrum (400 MHz, $CDCl_3$, δppm), a singlet at δ 6.61 ppm (1H) was assigned to the one proton at position C-3, while a singlet at δ 5.97 ppm (1H) corresponded to the proton attached to C-6, and a singlet at δ 2.36 ppm (3H) was assigned to the methyl group at C-8 position. In the B-ring region, three singlets at δ 6.90, 7.10, and 6.79 ppm were attributed to the protons attached to the C-2', C-5', and C-6' positions, respectively. The presence of four hydroxyl groups was confirmed by downfield singlets observed at δ 13.65 ppm (1H, s, OH at C-5), highly deshielded due to intramolecular hydrogen bonding with the carbonyl group, δ 9.80 ppm (OH at C-7), δ 8.97 ppm (OH at C-3'), and δ 9.42 ppm (OH at C-4'). In the ^{13}CNMR (100 MHz, $CDCl_3$, δppm), carbonyl carbon (C=O) appears at δ 182.86 ppm, while C-2 and C-3 carbons resonate at δ 164.05 and 105.44 ppm, respectively. Remaining ^{13}CNMR peaks were observed for C-5 at 158.11, C-6 at 98.16, C-7 at 161.83, C-8 at 102.54, C-9 at 157.71, C-10 at 103.81, C-1' at 122.59, C-2' at 114.13, C-3' at 146.43, C-4' at 150.03, C-5' at 116.31, and C-6' at 119.39. On the basis of the above data, conclusively [18] identified novel compound as 8-methylluteolin (Fig. 1) and the molecular formula was calculated as $C_{16}H_{12}O_6$.

GC-MS analysis

The GC-MS chromatogram (Fig. 2) of the methanol extract shows a total of 85 compounds, present in the extract were determined with the help of NIST library, as shown in Table1 in which 07 compounds identified as common laboratory contaminants or plasticizers, 70 compounds are identified as bioactive phytochemicals, and 08 compounds are repeated at different R. Time. Total relative abundance by common laboratory contaminants or plasticizers is 22.61%. Alcohol, alkane, ester, fatty acid, phenolic, sterol, and miscellaneous bioactive phytochemicals are also identified with their total relative abundance, that is, 22.35%, 2.05%, 5.36%, 15.19%, 2.06%, 14.7%, 15.7%, respectively. Major compounds (Fig. 3), that is, gamma-sitosterol (Area 12.41%), E,E,Z-1,3,12-nonadecatriene-5,14-diol (Area 7.36%), 9,12-octadecadienoic acid (Z,Z)- (Area 6.49%), and hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Area 5.46%), other compounds were found in trace amount.

Anti-hyperglycemia activity

The α-amylase and α-glucosidase inhibitory assays were performed to evaluate the anti-hyperglycemic potential of isolated molecules (M-1 to M-6) in comparison with the standard drug Acarbose, which served as the positive control. All values were expressed as mean±standard deviation from three independent replicates. The results demonstrated a clear dose-dependent inhibition of both enzymes across all compounds (Tables 2 and 3). Statistical analysis using one-way ANOVA followed by Tukey's *post hoc* test confirmed significant differences ($p<0.05$) between the tested molecules and the control, validating the reproducibility and reliability of the obtained data.

In the α-amylase inhibition assay (Table 2), Acarbose exhibited the highest inhibitory potential, with 91±1.3% inhibition at 100 μg/mL and an IC_{50} value of 35±1.2 μg/mL. Among the isolated compounds, M-5 (Quercetin) and M-4 (Kaempferol) showed potent inhibition comparable to Acarbose, recording 82±1.3% and 75±1.4% inhibition at the same concentration, with IC_{50} values of 38±1.5 μg/mL and 47±1.9 μg/mL, respectively. M-2 (4-Hydroxy-7-methylmellein) and M-3 (4-Hydroxymellein) showed moderate activity with IC_{50} values of 64±2.1 μg/mL and 70±2.8 μg/mL, while M-1 (Benzyl laurate) exhibited the weakest inhibition (25±0.8% at 100 μg/mL; IC_{50} = 130±4.5 μg/mL). Statistical comparison indicated that the inhibition profiles of M-4

Table 2: The percent inhibition of α-amylase by molecules M-1 to M-6 at varying concentrations

Concentration (μg/mL)	% Inhibition±SD						
	Acarbose	M-1	M-2	M-3	M-4	M-5	M-6
20	42±0.7	5±0.3	18±0.5	15±0.4	30±0.8	38±0.6	25±0.5
40	58±0.9	10±0.4	30±0.7	27±0.6	45±0.9	52±0.8	40±0.7
60	72±1.0	15±0.5	42±0.9	39±0.8	58±1.0	65±1.1	53±1.0
80	84±1.2	20±0.6	51±1.0	47±0.9	69±1.2	76±1.2	63±1.1
100	91±1.3	25±0.8	58±1.2	54±1.1	75±1.4	82±1.3	70±1.2
IC_{50} (μg/mL)	35±1.2	130±4.5	64±2.1	70±2.8	47±1.9	38±1.5	55±2.3

Values represent mean±standard deviation (SD) (n=3). Statistical significance determined by One-way ANOVA followed by Tukey's *post hoc* test ($p<0.05$). Acarbose used as positive control, Molecule M-1: benzyl laurate, Molecule M-2: 4-hydroxy-7-methylmellein, Molecule M-3: 4-hydroxymellein, Molecule M-4: Kaempferol, Molecule M-5: Quercetin, Molecule M-6: 8-methylluteolin

Table 3: The percent inhibition of α-glucosidase by molecules M-1 to M-6 at varying concentrations

Concentration (μg/mL)	% Inhibition±SD						
	Acarbose	M-1	M-2	M-3	M-4	M-5	M-6
20	49±0.8	4±0.3	16±0.4	13±0.4	33±0.8	42±0.7	26±0.6
40	66±1.0	8±0.4	28±0.6	23±0.6	49±0.9	59±0.9	43±0.8
60	80±1.2	12±0.5	39±0.7	34±0.8	63±1.0	71±1.1	56±1.0
80	88±1.3	17±0.6	47±0.9	42±0.9	73±1.1	81±1.2	66±1.1
100	94±1.4	21±0.8	55±1.1	48±1.0	79±1.3	86±1.3	72±1.2
IC_{50} (μg/mL)	25±1.0	140±5.1	68±2.2	76±3.0	42±1.8	32±1.4	52±2.0

Values are mean±standard deviation (SD) (n=3). Statistical differences were analyzed using one-way ANOVA followed by Tukey's *post hoc* test ($p<0.05$). Acarbose used as positive control, Molecule M-1: benzyl laurate, Molecule M-2: 4-hydroxy-7-methylmellein, Molecule M-3: 4-hydroxymellein, Molecule M-4: Kaempferol, Molecule M-5: Quercetin, Molecule M-6: 8-methylluteolin

Table 4: Antibacterial activity of compounds/molecules isolated from methanol extract of *Moringa oleifera* stem bark

S. No.	Test microbes	Inhibition zone (in mm)							F (df=6, 14)	Significant differences (Tukey, p<0.05)
		Standard: Gentamycin	M-1	M-2	M-3	M-4	M-5	M-6		
1	*Bacillus subtilis*	22.5±0.5	12.3±0.6	14.2±0.4	13.6±0.4	10.8±0.7	13.4±05	12.7±0.5	67.4	Gent>M-1, M-2, M-3, M-4, M-5, M-6>M-4 (p<0.05)
2	*Escherichia coli*	20.4±0.4	9.1±0.6	13.0±0.7	11.1±0.6	-	12.0±0.5	13.1±05	89.2	Gent>all compounds (p<0.001); M-2≃M-6>M-1 (p<0.05)
3	*Staphylococcus aureus*	23.2±0.4	14.0±0.6	15.6±0.3	15.2±0.3	13.5±0.4	16.1±0.5	14.8±0.5	71.6	Gent>M-1- M-6; M-5>M-4 (p<0.05)

p<0.001; Inhibition zone (in mm) including the diameter of well (6 mm, mean±standard deviation); Standard: Gentamycin; (-) = No activity; Concentration of tested compounds=20 µg/mL

Table 5: Antifungal activity of compounds/molecules isolated from methanol extract of *Moringa oleifera* stem bark

S. No.	Test microbes	Inhibition zone (in mm)							F (df=6, 14)	Significant differences (Tukey, p<0.05)
		Standard: Ketoconazole	M-1	M-2	M-3	M-4	M-5	M-6		
1	*Aspergillus flavus*	21.5±0.3	12.2±0.5	13.6±0.6	12.4±0.6	10.8±0.7	14.2±0.4	16.3±0.4	74.8	Keto>M-1-M-6; M-6>M-4 (p<0.05)
2	*Candida albicans*	22.0±0.3	9.8±0.6	12.7±0.5	10.6±0.5	–	13.0±0.4	13.5±0.4	92.5	Keto>all (p<0.001); M-6≃M-5>M-1 (p<0.05)
3	*Trichophyton rubrum*	24.3±0.3	13.6±0.5	15.1±0.3	13.8±0.3	12.9±0.4	16.5±0.3	14.1±0.3	78.2	Keto>M-1-M-6; M-5>M-4 (p<0.05)

p<0.001; Inhibition zone (in mm) including the diameter of well (6 mm, mean±standard deviation); Standard: Ketoconazole; (-) = No activity; Concentration of tested compounds=20 µg/mL; F value=One way ANOVA; df=Degree of freedom

and M-5 were not significantly different (p > 0.05) from Acarbose, suggesting that these flavonoids could act through similar enzyme-binding mechanisms.

Similarly, in the α-glucosidase inhibition assay (Table 3), Acarbose again demonstrated the strongest activity, with 94±1.4% inhibition at 100 µg/mL and an IC_{50} of 25±1.0 µg/mL. Among the test compounds, M-5 (Quercetin) recorded the highest inhibition (86±1.3%) and an IC_{50} of 32±1.4 µg/mL, followed by M-4 (Kaempferol) with 79±1.3% inhibition and an IC_{50} of 42±1.8 µg/mL. The remaining molecules (M-2, M-3, and M-6) exhibited moderate activity, while M-1 again showed the lowest inhibitory effect (21±0.8%, IC_{50} = 140±5.1 µg/mL). The One-way ANOVA revealed that M-4 and M-5 exhibited inhibition statistically similar (p>0.05) to Acarbose, indicating their potential as natural enzyme inhibitors.

Overall, the inclusion of Acarbose as a reference standard provided a meaningful benchmark for evaluating the efficacy of isolated compounds. The results clearly indicate that Quercetin (M-5) and Kaempferol (M-4) possess strong α-amylase and α-glucosidase inhibitory activities, nearly comparable to the pharmaceutical standard.

Anti-microbial activity

The concentration of 20 µg/mL was selected based on preliminary dose response experiments showing measurable inhibition without compound precipitation or toxicity to microbial cultures. This concentration is within the lower range of clinically relevant antimicrobial concentrations and facilitates comparative evaluation of compound potency relative to standard drugs (Gentamycin and Ketoconazole).

Anti-bacterial activity

The isolated compounds from the methanol extract of *M. oleifera* stem bark (M-1 to M-6) were evaluated for their anti-bacterial activity against a panel of gram-positive and gram-negative bacteria using the agar well diffusion method (Fig. 4). Gentamycin was used as the reference standard. Among the test organisms, *Staphylococcus aureus* (23.2±0.4 mm) and *Bacillus subtilis* (22.5±0.5 mm) were highly sensitive to the standard antibiotic (Table 4). Compounds M-2 (4-hydroxy-7-methylmellein) and M-5 (quercetin) exhibited significant antibacterial activity against *S. aureus* with inhibition zones of 15.6±0.3 mm and 16.1±0.5 mm, respectively (Table 4), suggesting strong potential. M-3 and M-6 also displayed good inhibition against *S. aureus*, indicating that hydroxylated isocoumarin and flavonoid structures contribute to antimicrobial efficacy. *Bacillus subtilis* was moderately inhibited by all test compounds, especially M-2 (14.2±0.4 mm) and M-5 (13.4±0.5 mm), indicating broad-spectrum antibacterial properties. *E. coli,* although Gram-negative, was inhibited by M-2 and M-5, pointing to the role of specific flavonoid or phenolic moieties in breaching bacterial defenses.

Anti-fungal Activity

The antifungal screening revealed notable activity of the isolated compounds against a range of pathogenic fungi (Fig. 5). Ketoconazole was used as the positive control and displayed strong inhibition against *Trichophyton rubrum* (24.3±0.3 mm) and *Candida albicans* (22.0±0.3 mm). Among the isolated compounds, M-5 (Quercetin) and M-6 (8-methylluteolin) demonstrated the highest antifungal activity. M-5 showed inhibition zones of 16.5±0.3 mm against *T. rubrum* and 14.2±0.4 mm against *A. flavus*, while M-6 showed significant zones of 16.3±0.4 mm (*A. flavus*) and 14.1±0.3 mm (*T. rubrum*) (Table 5). These findings suggest that hydroxyl and methoxyl groups on the flavonoid skeleton enhance antifungal potential. M-2 (4-hydroxy-7-methylmellein) and M-3 (4-hydroxymellein) also displayed moderate to good antifungal activity, particularly against *C. albicans* and *A. flavus*.

CONCLUSION

The methanol extract of Moringa oleifera stem bark was found to be a rich source of structurally diverse bioactive phytochemicals, including two novel compounds. The isolated constituents exhibited significant α-amylase and α-glucosidase inhibitory activities along with notable antimicrobial potential. These findings scientifically support the traditional medicinal use of M. oleifera and highlight its promise as a natural source of lead molecules for managing hyperglycemia and microbial infections..

ACKNOWLEDGMENT

Authors wish to thanks Head, Department of Chemistry University of Rajasthan, Jaipur, Rajasthan, India for providing Laboratory facilities.

AUTHORS CONTRIBUTIONS

All authors confirm their significant involvement in the research and accept full responsibility for its content, including experimental, data, and analysis.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

FUNDING

Nil

REFERENCES

1. Abd Rani NZ, Husain K, Kumolosasi E. *Moringa* genus: A review of phytochemistry and pharmacology. Front Pharmacol. 2018;9:108. doi: 10.3389/fphar.2018.00108, PMID 29503616
2. Indriawan D, Najatullah, Nugroho T, Susilaningsih N. The effectiveness of *Moringa oleifera* burn healing: *In vivo* study. J Biomed Transl Res. 2022;6(11):2342-6.
3. Purwoningsih E, Arozal W, Lee HJ, Munim A. Neuroprotective and antioxidant activities of aqueous extract *Moringa oleifera* leaves. Int J App Pharm. 2022;14(5):127-31. doi: 10.22159/ijap.2022.v14s5.26
4. Fitriana M, Mun'im A, Firdayani Adikusuma W. Exploration of the active compounds of *Moringa oleifera* lam as HIV-1 reverse transcriptase inhibitor: A network pharmacology and molecular docking approach. Int J App Pharm. 2024;16(2):237-46. doi: 10.22159/ijap.2024v16i2.49855
5. Alsammarraie HJ, Khan NA, Mahmud R. Formulation, evaluation, and *in vivo* anti-inflammatory and anti-arthritic activities of *Moringa* granules. Int J App Pharm. 2021;13(3):112-20. doi: 10.22159/ijap.2021v13i3.40478
6. Anhwange BA, Ajibola VO, Oniye SJ. Chemical studies of the seeds of *Moringa oleifera* (Lam) and *Detarium microcarpum* (Guill and Sperr). J Biol Sci. 2004;4(6):711-5. doi: 10.3923/jbs.2004.711.715
7. Anwar F, Ashraf M, Bhanger MI. Interprovenance variation in the composition of *Moringa oleifera* oilseeds from Pakistan. J Am Oil Chem Soc. 2005;82(1):45-51. doi: 10.1007/s11746-005-1041-1
8. Oluduro AO. Evaluation of antimicrobial properties and nutritional potentials of *Moringa oleifera* Lam. Leaf in South-Western Nigeria. Malays J Microbiol. 2012;8(2):59-67.
9. Thasmi CN, Hafizuddin H, Husnurrizal H, Dasrul D, Sutriana A, Gani BA, *et al*. *Moringa oleifera* as a potential bioactive agent against Gram-positive and negative bacteria: *In-silico* analysis of 1YN5 and 3RG1 receptor binding. Biodivers J Biol Divers. 2024;25(8):3411-21. doi: 10.13057/biodiv/d250812
10. Amaglo NK, Bennett RN, Lo Curto RB, Rosa EA, Lo Turco V, Giuffrida A, *et al*. Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree *Moringa oleifera* L., grown in Ghana. Food Chem. 2010;122(4):1047-54. doi: 10.1016/j.foodchem.2010.03.073
11. Barbosa M, Freire C, Mota D, Almeida L, Souza R, Pereira M, *et al*. Nutritional quality of *Moringa oleifera* seeds and physicochemical properties of its crude oil. J Bioeng Technol Health. 2020;3(4):341-6.
12. Sharma AK, Sharma TC, Gupta N. Phytochemical study of petroleum ether extract of stem bark of *Bombax* melabaricum. J Adv Sci Res. 2021;12(4 Suppl 1):302-6. doi: 10.55218/jasr.s1202112435
13. Nair SS, Kavrekar V, Mishra A. *In vitro* studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. Eur J Exp Biol. 2013;3(1):128-32.
14. Boyanova L, Gergova G, Nikolov R, Derejian S, Lazarova E, Katsarov N, *et al*. Activity of Bulgarian propolis against 94 *Helicobacter pylori* strains *in vitro* by agar-well diffusion, agar dilution and disc diffusion methods. J Med Microbiol. 2005;54(5):481-3. doi: 10.1099/jmm.0.45880-0, PMID 15824428
15. Shonle HA, Row PQ. New benzyl esters possessing AN anti-spasmodic action. J Am Chem Soc. 1921;43(2):361-5. doi: 10.1021/ja01435a015
16. Reveglia P, Masi M, Evidente A. Melleins-intriguing natural compounds. Biomolecules. 2020;10(5):772. doi: 10.3390/biom10050772, PMID 32429259
17. Sermkaew N, Plyduang T. Self-microemulsifying drug delivery systems of *Moringa oleifera* extract for enhanced dissolution of kaempferol and quercetin. Acta Pharm. 2020;70(1):77-88. doi: 10.2478/acph-2020-0012, PMID 31677372
18. Ravichandran YD, Sulochana N. Isolation and characterization of 8-methylluteolin 5-O-6"-2-methyl propanoyl glucoside and patuletin 3-O-glucuronide from *Tithonia* tagetiflora. Asian J Chem. 2006;18(4):3173-5.