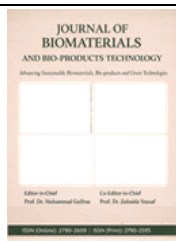
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Research Article

Biochemical Analysis of Leaves of Mentha Species and *In Vitro* Determination of Bioactivities

Summyia Khalid and Anam Abdul Rehman

1. Punch University, Rawalakot, Azad Jammu and Kashmir, Pakistan..
summiyakhalid_31@yahoo.com
2. Mirpur University of Science and Technology (MUST), Mirpur, Azad Jammu and Kashmir, Pakistan. anamabdulrehmanmust786@gmail.com

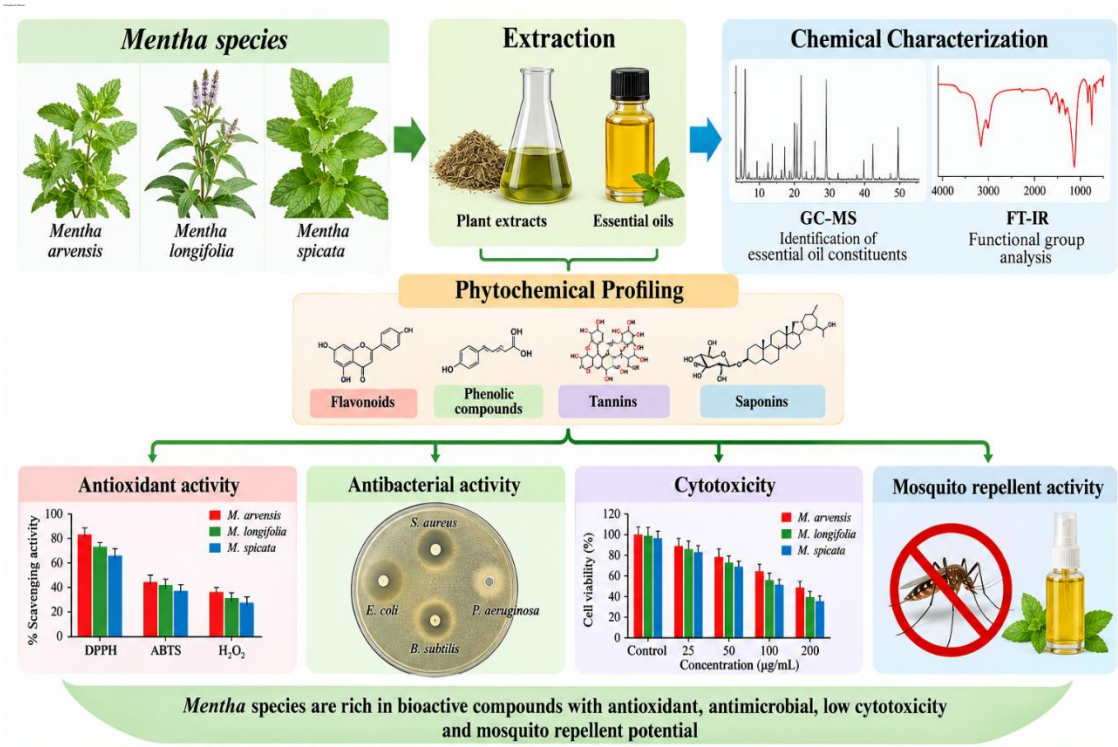
Corresponding author email: summiyakhalid_31@yahoo.com

Abstract

This study investigated the phytochemical composition, essential oil yield, chemical constituents, and in vitro biological activities of three *Mentha* species: *Mentha arvensis*, *M. longifolia*, and *M. spicata*. Plant extracts were screened for flavonoids, total phenolics, tannins, and saponins. Essential oils were extracted by hydrodistillation, and their chemical composition and functional groups were examined using gas chromatography–mass spectrometry (GC–MS) and Fourier-transform infrared (FTIR) spectroscopy, respectively. Antioxidant activity was evaluated using DPPH, ABTS, and hydrogen peroxide scavenging assays, while antibacterial activity was assessed against selected Gram-positive and Gram-negative bacteria. Cytotoxicity was evaluated using the brine shrimp lethality assay. Among the three species, *M. spicata* exhibited the highest flavonoid (15.05 mg/g) and total phenolic (27.64 mg/g) contents, followed by *M. longifolia* and *M. arvensis*. Essential oil yields were highest in *M. arvensis* (1.70%), followed by *M. longifolia* (1.47%) and *M. spicata* (1.20%). GC–MS analysis identified carvone (84.3%) as the predominant constituent of *M. arvensis* oil, piperitone oxide (54.2%) in *M. longifolia*, and 2-hydroxy-3-(3-methyl-2-butenyl)-3-cyclopenten-1-one (47.1%) in *M. spicata*. Among the antioxidant assays, *M. spicata* showed the highest DPPH and ABTS radical-scavenging activities at 50 µg/mL, with inhibition values of 80.1% and 88.1%, respectively. The essential oils also exhibited varying degrees of antibacterial activity against the tested microorganisms. Brine shrimp assay results indicated concentration-dependent cytotoxic effects. Overall, the findings demonstrate interspecific variation in phytochemical composition, essential oil yield, and biological activities, highlighting the potential of *Mentha* species as sources of bioactive compounds for further investigation and potential pharmaceutical applications.

Keywords: *Mentha arvensis*; *Mentha longifolia*; *Mentha spicata*; essential oils; phytochemical profiling; GC–MS; FT-IR spectroscopy; antioxidant activity; antibacterial activity; cytotoxicity; mosquito-repellent activity

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Keywords: *Mentha arvensis*; *Mentha longifolia*; *Mentha spicata*; essential oils; GC–MS; FT-IR; phytochemicals; antioxidant activity; antibacterial activity; cytotoxicity; mosquito repellent.

1. Introduction

Medicinal plants have played an important role in traditional healthcare and the development of modern pharmaceuticals. Numerous drugs, including aspirin, digoxin, codeine, vinblastine, ephedrine, and pilocarpine, have originated from plant-derived compounds (Abbasi et al., 2015). Plants remain valuable sources of bioactive molecules that can serve as lead compounds for developing therapeutic agents. Their chemical diversity has attracted considerable interest in pharmaceutical research, particularly in the identification and characterization of natural products with potential medicinal applications (Ashafa et al., 2010; Newman and Cragg, 2012). Furthermore, advances in plant biotechnology, tissue culture, and molecular biology have improved opportunities for the cultivation, conservation, and investigation of medicinal plant resources.

The biological properties of medicinal plants are largely associated with their secondary metabolites, which contribute to plant defense and interactions with the environment. Unlike primary metabolites, such as carbohydrates, proteins, lipids, and nucleic acids, secondary metabolites are not directly involved in basic growth and development but often exhibit important biological activities (Gulfraz et al., 2008; Koehn and Carter, 2005). Major classes include alkaloids, terpenoids, phenolic compounds, flavonoids, tannins, and saponins. These compounds have been investigated for their antioxidant, antimicrobial, and other pharmacological properties. For example, plant-derived alkaloids and phenolic compounds have contributed to pharmaceutical research, while terpenoids are widely used in medicinal, food, and fragrance applications (Marles and Farnsworth, 1995; Newman and Cragg, 2012).

Medicinal plants have also attracted attention as potential sources of antibacterial agents. Previous studies have reported antimicrobial activity in extracts from several plant species, demonstrating the importance of investigating natural products against pathogenic microorganisms (Foster and Duke, 2000). In addition, some plant extracts have shown cytotoxic or antiproliferative effects in experimental models, supporting further investigation of their bioactive constituents (Graf et al., 2010). However, biological activity can vary considerably depending on plant species, chemical composition, extraction procedures, and experimental conditions.

Therefore, systematic phytochemical characterization and in vitro bioactivity assessment are important steps in evaluating the potential applications of medicinal plants.

Essential oils are complex mixtures of volatile compounds, frequently rich in terpenoids and other aromatic constituents. They have been investigated for their antibacterial, antifungal, antiviral, and insect-repellent properties. Their chemical composition can be characterized using gas chromatography–mass spectrometry (GC–MS), while Fourier-transform infrared (FTIR) spectroscopy can provide information about the functional groups present in plant-derived samples. Combining these analytical techniques with phytochemical screening and biological assays enables a more comprehensive assessment of medicinal plants and their potential sources of bioactive compounds.

The genus *Mentha*, belonging to the family Lamiaceae, includes aromatic plants widely used in traditional medicine, food preparation, and commercial products. Species such as *Mentha arvensis*, *M. longifolia*, and *M. spicata* are of particular interest because of their essential oils and diverse phytochemical constituents. Their reported traditional uses include supporting digestion and relieving certain common complaints. Nevertheless, the chemical composition and biological activities of *Mentha* species may differ according to species identity, environmental conditions, geographical origin, and extraction methods. Such variation highlights the importance of comparative investigations of different species collected from defined locations.

Pakistan possesses considerable medicinal plant diversity, particularly in its northern regions, where numerous aromatic and medicinal plants are used in traditional healthcare. Members of the Lamiaceae are important components of this diversity, and their phytochemical constituents offer opportunities for scientific investigation and potential commercial utilization. Characterizing locally collected *Mentha* species may contribute to knowledge of their chemical composition and help identify promising candidates for further pharmacological and industrial research.

Accordingly, the present study investigated *M. arvensis*, *M. longifolia*, and *M. spicata* collected from Abbottabad, Pakistan. The objectives were to assess their major phytochemical constituents, determine essential oil yield, characterize essential oil composition using GC–MS, and examine functional groups using FTIR spectroscopy. The study also evaluated antioxidant activity through DPPH, ABTS, and hydrogen peroxide scavenging assays, antibacterial activity against selected Gram-positive and Gram-negative bacteria, and cytotoxicity using the brine shrimp lethality assay. These comparative analyses aim to establish a clearer understanding of interspecific variation in phytochemical composition and in vitro biological activities and to identify potential directions for further research on *Mentha*-derived bioactive compounds.

2. MATERIALS AND METHODS

2.1. Collection of samples

Fresh aerial parts of *Mentha arvensis*, *M. longifolia*, and *M. spicata* were collected from Abbottabad District, Pakistan, based on their traditional medicinal uses. Samples were placed in labeled plastic bags containing collection and location details. Plant identification was performed by a trained taxonomist at the Department of Environmental Sciences, COMSATS Institute of Information Technology, Abbottabad. Voucher specimens (No. 132) were preserved for future reference. Approximately 3 kg of plant material was collected for phytochemical analysis, essential oil extraction, and biological activity assessment.

2.2. Preparation of samples

Plant materials were washed with distilled water to remove dust and other impurities. The samples were dried using the reported sun-drying, low-temperature oven-drying, and shade-drying procedures. Dried materials were ground using an electric grinder, passed through an 80-mesh screen, and stored at 4 °C until analysis.

2.3. Analysis of plant extracts for phytochemicals

2.3.1. Determination of flavonoids

Total flavonoid content (TFC) was determined spectrophotometrically following a modified method of Skehan et al. (1990). A quercetin

stock solution (100 µg/mL) was prepared by dissolving 2.5 mg of quercetin in 25 mL of methanol. Standard solutions of 10, 20, 40, 60, 80, and 100 µg/mL were used to construct the calibration curve. For each sample, 0.5 mL of extract, 0.5 mL of distilled water, and 0.5 mL of $AlCl_3 \cdot 6H_2O$ solution were mixed and incubated at room temperature for 5 min. The reported NaOH addition was then performed, and absorbance was measured at 510 nm. TFC was calculated from the quercetin calibration curve and expressed as quercetin equivalents (QE) per gram of sample. Analyses were conducted in triplicate.

2.3.2. Estimation of total phenolic content

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method (Phillipson, 1994). Gallic acid (2.5 mg) was dissolved in 25 mL of methanol to prepare a 100 µg/mL stock solution. Standards of 10–100 µg/mL were used to establish the calibration curve. The sample solution was mixed with distilled water and 10% Folin–Ciocalteu reagent, followed by incubation for 5 min at room temperature. Sodium carbonate solution was added, and the mixture was incubated in darkness for 90 min. Absorbance was measured at 760 nm against a blank. TPC was calculated using the gallic acid calibration curve and expressed as gallic acid equivalents (GAE). Measurements were performed in triplicate.

2.3.3. Determination of saponins

Saponin content was determined using a solvent-extraction procedure based on Skehan et al. (1990), Schwartmann et al. (2002), and Sharma et al. (1975). Each 20 g plant sample was extracted with 200 mL of 20% ethanol at approximately 55 °C for 4 h with continuous stirring. The residue was re-extracted with a further 200 mL of 20% ethanol. Combined extracts were concentrated to approximately 40 mL at 90 °C. The concentrate was partitioned with 20 mL diethyl ether, and the aqueous layer was retained. The aqueous fraction was extracted with 60 mL n-butanol, and the combined n-butanol fractions were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solvent was evaporated, and the residue was oven-dried to constant weight for saponin determination.

2.3.4. Quantification of tannins

Tannins were extracted according to Ullah and Khan (2008). Dried plant powder (100 g) was refluxed with 400 mL of 70% acetone for 12 h at 60 °C, protected from light. The extract was filtered and centrifuged at 3,500 rpm for 15 min. The solvent was removed using a rotary evaporator below 60 °C. For quantification, 800 µL of extract was mixed with 800 µL of Folin–Ciocalteu reagent and allowed to stand for 5 min. After dilution with 5 mL distilled water, absorbance was measured at 725 nm. Tannin content was calculated using a tannic acid calibration curve and expressed as tannic acid equivalents per gram of extract.

2.4. Extraction of essential oils

Essential oils were extracted from fresh aerial plant parts by hydrodistillation. The weighed, chopped plant material was placed in a distillation apparatus containing approximately 2,000 mL of distilled water. The mixture was boiled for 3 h using a heating mantle, and the condensate was collected in a separating funnel. The separated oil layer was recovered for further purification.

2.4.1. Liquid–liquid extraction

The distillate was extracted with 70 mL of HPLC-grade n-hexane, and the extraction was repeated three times. The organic fractions were combined and treated with anhydrous magnesium sulfate to remove residual water. Hexane was evaporated using a Büchi R100 rotary evaporator at 25 °C under reduced pressure, initially at 200 mbar and subsequently at 100 or 80 mbar as required. The recovered oil was weighed, and yield was calculated as:

$$Essential\ oil\ Yield\ \% = \frac{Mass\ of\ essential\ oil\ (g)}{Mass\ of\ fresh\ Plant\ material} \times 100$$

2.5. Chemical analysis of essential oils by GC–MS

Essential oil composition was analyzed using a Hewlett-Packard GC 6890N coupled with an HP

5973 mass spectrometer. Separation was performed on a DB-5 column (30 m × 0.25 mm internal diameter; 0.25 µm film thickness). The injector temperature was 235 °C, and helium was used as the carrier gas at 1 mL/min. A 1 µL aliquot of diluted essential oil was injected in splitless mode. The oven program began at 40 °C and was held for 2 min before temperature ramping. Compound identities were assigned using the reported instrumental and library-based analysis procedures.

2.6. FTIR analysis

FTIR spectra were recorded using an FS 25 instrument (Bruker, Germany, as reported). Samples were analyzed as thin films or KBr pellets, with KBr serving as the control. Spectra were recorded over 4,000–500 cm^{-1}. Each sample was analyzed three times, and characteristic absorption bands were used to identify functional groups.

2.7. Determination of bioactivities

2.7.1. DPPH scavenging assay

DPPH radical-scavenging activity was assessed using a modified method of Graf et al. (2010). A 100 µL aliquot of extract was mixed with 4 mL of 0.1 mM DPPH solution, vortexed, and incubated at room temperature for 30 min in darkness. Absorbance was measured at 517 nm. Scavenging activity was calculated as:

$$Inhibition\ \% = \frac{Ab - As}{Ab} \times 100$$

Where (Ab) is the absorbance of the blank or control and (As) is the absorbance of the sample reaction.

2.7.2. ABTS scavenging assay

The ABTS radical solution was prepared by mixing 5 mL of 7 mM ABTS with approximately 88 µL of 140 mM potassium persulfate. The mixture was incubated in darkness for 16 h and diluted with ethanol to an initial absorbance of approximately 0.7. Extract (10 µL) was added to the reaction mixture, and absorbance was measured at 730 nm. Percentage scavenging was calculated using the absorbance-based inhibition equation described above.

2.7.3. Hydrogen peroxide (H_2O_2) scavenging assay

Hydrogen peroxide-scavenging activity was assessed by mixing 4 mL of extract with 0.6 mL of approximately 4 mM H_2O_2 prepared in phosphate-buffered saline. After 10 min of incubation, absorbance was measured at 230 nm against a control. Percentage scavenging was calculated using the absorbance-based inhibition equation.

2.7.4. Brine shrimp lethality assay

Brine shrimp eggs were hatched in artificial seawater for 48 h under light and dark conditions. Nauplii were transferred to sample vials containing 5 mL of artificial seawater, with 20 nauplii per vial and a drop of yeast suspension (3 mg in 6 mL seawater) as feed. Samples were tested at the reported concentrations, and survivors and dead nauplii were counted after 24 h. Mortality was calculated as:

$$Mortlaity\ \% = \frac{Mortality\ of\ dead\ nauplii}{Total\ nauplii\ exposed} \times 100$$

Where control mortality occurred, Abbott's correction was applied:

$$Mortlaity\ \% = \frac{Pt - Pc}{100 - Pc} \times 100$$

Where (Pt) and (Pc) are the percentage mortalities in the treatment and control groups, respectively.

2.8. Determination of antibacterial activity

Bacterial suspensions were prepared from 24-h cultures and adjusted to an optical density of 0.5 at 600 nm, following the reported McFarland-based procedure. Antibacterial activity of the three essential oils was evaluated by agar well diffusion (Upadhyay, 2015). Nutrient agar was prepared using 13 g nutrient broth and 14 g agar per litre of distilled water, sterilized as reported, and poured into plates. Bacterial suspensions (200 µL) were spread over the agar surface, and 6 mm wells were prepared. Essential oil solution (30 µL) was added to each well. Streptomycin (1 mg/mL) served as the positive control, while hexane served as the negative control. Plates

were incubated at 37 °C for 24 h, and inhibition zones were measured in millimetres. Essential oil solutions at concentrations of 1–5% were prepared in absolute ethanol.

2.9. Statistical analysis

Experiments were conducted using the reported replicate numbers. Results were expressed as mean ± standard deviation where applicable. One-way analysis of variance (ANOVA) was used to evaluate differences among treatments.

3. Results and Discussion

Phytochemical composition, essential-oil yield and composition, antioxidant activity, antibacterial activity, and brine-shrimp lethality were evaluated in three *Mentha* species: *M. arvensis, M. longifolia,* and *M. spicata*. Results are presented alongside their interpretation to avoid repeating the same findings in separate Results and Discussion sections.

3.1. Phytochemical composition

All three extracts contained measurable flavonoids, total phenolics, saponins, and tannins (Table 1). M. spicata had the highest reported values for flavonoids (15.05 mg/g), total phenolics (27.637 mg/g), saponins (0.134 mg/g), and tannins (3.51 mg/g). M. longifolia showed intermediate flavonoid and phenolic contents (14.17 and 25.149 mg/g, respectively), while M. arvensis had the lowest values for these measures. The higher phenolic and flavonoid contents in M. spicata may be relevant to its activity in the in-vitro antioxidant assays, although the present data do not establish a direct causal relationship. Phenolics and flavonoids comprise chemically diverse groups, and their biological effects depend on composition as well as total concentration.

Table 1. Quantitative phytochemical composition of the three Mentha extracts (mean ± SD, n = 3).

Species	Flavonoids (mg/g)	Total phenolics (mg/g)	Saponins (mg/g)	Tannins (mg/g)
M. arvensis	12.21 ± 0.07	20.32 ± 0.09	0.040 ± 1.3	2.31 ± 1.5
M. longifolia	14.17 ± 0.07	25.149 ± 0.06	0.013 ± 1.2	3.14 ± 0.9
M. spicata	15.05 ± 0.14	27.637 ± 0.06	0.134 ± 1.2	3.51 ± 0.8

3.2. Essential-oil yield and GC–MS composition

Hydrodistillation yielded 1.70% essential oil from *M. arvensis,* 1.47% from *M. longifolia*, and 1.20% from *M. spicata* (Table 2). Thus, **M. arvensis** had the highest reported yield and M. spicata the lowest. Yield differences may reflect species-level variation and the condition of the plant material, but the present comparison alone cannot identify the factors responsible.

Table 2. Essential-oil yield from fresh aerial material (mean ± SD, n = 3).

Species	Family	Essential-oil yield (%)
M. arvensis	Lamiaceae	1.70 ± 0.11
M. longifolia	Lamiaceae	1.47 ± 0.31
M. spicata	Lamiaceae	1.20 ± 0.03

GC–MS profiling indicated different dominant constituents among the species (Table 3). Carvone was reported as the principal constituent of M. arvensis (84.3%), piperitone oxide as the principal constituent of *M. longifolia* (54.2%), and 2-hydroxy-3-(3-methyl-2-butenyl)-3-cyclopenten-1-one as the major reported constituent of *M. spicata* (47.1%). Other compounds reported at appreciable proportions included 1,8-cineole (5.3% in *M. arvensis* and 12.0% in *M. spicata*), limonene (3.2% in *M. arvensis*), menthone (9.7% in *M. longifolia*), borneol (4.6% and 9.5% in *M. longifolia* and *M. spicata*, respectively), and α-

pinene (9.7% in *M. spicata*). These profiles suggest marked differences in the relative composition of the oils. Because the source compound tables are misaligned and contain inconsistent totals/nomenclature, these summary values should be checked against the original GC–MS output and compound-identification report before publication.

Table 3. Summary of the major constituents reported in the essential oils by GC–MS.

Species	Reported dominant constituent	Reported relative abundance	Other reported constituents
M. arvensis	Carvone	84.3%	1,8-Cineole (5.3%); limonene (3.2%)
M. longifolia	Piperitone oxide	54.2%	Menthone (9.7%); borneol (4.6%); pulegone (4.0%); 2-hydroxy-3-(3-methyl-2-butenyl)-3-cyclopenten-1-one (8.4%)
M. spicata	2-Hydroxy-3-(3-methyl-2-butenyl)-3-cyclopenten-1-one	47.1%	1,8-Cineole (12.0%); α-pinene (9.7%); borneol (9.5%)

The complete compound-by-compound GC–MS table should be retained as supplementary material after its retention indices, retention times, compound names, and total identified percentages have been checked.

3.3. FTIR profile

The FTIR profile of the Mentha extracts showed bands assigned in the original analysis to O–H, C–H, C=O, C–O, and other vibrations (Table 4). The band at 3337.45 cm^{-1} was assigned to O–H stretching, consistent with hydroxyl-containing constituents, while bands at 2924.51 and 1710.21 cm^{-1} were assigned to C–H and C=O vibrations, respectively. These assignments provide a broad functional-group profile, rather than definitive identification of individual compounds. Assignments to nitro groups and alkyl halides should be independently checked against the original spectrum and peak positions.

Table 4. Reported FTIR bands and tentative assignments for Mentha extracts.

Wavenumber (cm^{-1})	Reported bond/vibration	Reported functional-group assignment
3337.45	O–H, hydrogen-bonded	Alcohols/phenols
2924.51	C–H stretching	Alkanes
1710.21	C=O stretching	Carboxylic acids/carbonyls
1514.05	CO asymmetric stretch (as reported)	Nitro compounds (as reported)
1451.07	C–C stretching (as reported)	Aromatic structures (as reported)
1030.75	C–O	Alcohols, esters, ethers
817.84	C–Cl stretching (as reported)	Alkyl halides (as reported)
725.27	C–Cl stretching (as reported)	Alkyl halides (as reported)
632.43	C–Br stretching (as reported)	Alkyl halides (as reported)

3.4. Antioxidant activity

Antioxidant activity was assessed using DPPH, ABTS, and hydrogen-peroxide (H_2O_2) scavenging assays. The values from all three assays are consolidated in Table 5 to reduce

duplication. In the DPPH and ABTS datasets, *M. spicata* generally showed the highest reported scavenging values at the same nominal concentration, followed by *M. longifolia* or *M. arvensis* depending on concentration. At 50 µg/mL, for example, reported DPPH values were 80.1% for M. spicata, 70.33% for *M. longifolia*, and 60.7% for *M. arvensis;* corresponding ABTS values were 88.1%, 78.73%, and 67.7%. The H_2O_2 values also varied among species.

However, the reported percentages decline as concentration increases in all three assay tables. This trend conflicts with the original narrative describing concentration-dependent increases in scavenging. Before drawing a firm conclusion, verify the column headings, calculation formula, blank/control definitions, and concentration order against the laboratory records. Until verified, the data should be described as reported rather than interpreted as a conventional dose-response pattern.

Table 5. Combined antioxidant-assay results (mean ± SD as reported; n = 3).

Assay	Concentration (µg/mL)	M. arvensis (%)	M. longifolia (%)	M. spicata (%)	Ascorbic acid (%)
DPPH	50	60.7 ± 1.07	70.33 ± 0.4	80.1 ± 0.8	49.29 ± 0.5
DPPH	100	55.45 ± 1.9	63.77 ± 0.6	73.24 ± 0.62	41.34 ± 1.3
DPPH	150	48.9 ± 0.13	51.81 ± 0.5	61.33 ± 0.3	33.41 ± 1.7
DPPH	200	33.62 ± 0.36	36.18 ± 1.0	49.67 ± 0.03	28.67 ± 1.03
DPPH	250	21.8 ± 0.33	25.42 ± 1.01	33.33 ± 0.2	21.8 ± 1.01
DPPH	300	19.9 ± 0.33	14.33 ± 2.1	21.9 ± 0.7	13.55 ± 1.23
ABTS	50	67.7 ± 1.23	78.73 ± 0.5	88.1 ± 0.9	67.29 ± 0.9
ABTS	100	59.42 ± 1.8	65.45 ± 0.9	78.44 ± 0.7	56.76 ± 1.09
ABTS	150	51.89 ± 0.45	58.21 ± 0.7	63.81 ± 0.8	49.87 ± 1.8
ABTS	200	44.32 ± 0.23	47.81 ± 1.1	55.54 ± 0.01	40.54 ± 1.08
ABTS	250	38.61 ± 0.10	36.72 ± 1.01	49.01 ± 0.3	34.6 ± 1.1
ABTS	300	33.62 ± 0.33	29.88 ± 2.1	35.62 ± 0.7	22.55 ± 1.23
H_2O_2	50	92.7 ± 1.7	96.46 ± 2.3	87.1 ± 0.6	64.29 ± 0.9
H_2O_2	100	90.45 ± 0.9	91.06 ± 0.9	79.05 ± 0.88	52.07 ± 1.2
H_2O_2	150	76.9 ± 0.13	83.33 ± 0.77	78.98 ± 0.1	33.41 ± 1.44
H_2O_2	200	69.77 ± 0.56	67.11 ± 5.1	67.01 ± 0.05	31.05 ± 1.08
H_2O_2	250	51.8 ± 0.45	52.01 ± 1.13	49.34 ± 0.7	24.8 ± 1.1
H_2O_2	300	34.9 ± 0.34	29.66 ± 3.22	37.9 ± 0.1	20.45 ± 1.01

3.5. Antibacterial activity

The essential oils were tested against five bacterial species. In the screening results (Table 6), the oils produced smaller inhibition zones than streptomycin, while n-hexane produced no inhibition. *M. spicata* showed the largest reported zone against *S. aureus* in the screening table (4.75 mm), whereas *M. longifolia* produced a 3.62-mm zone against B. cereus. *M. arvensis* showed zones of 4.50 mm against S. aureus and 4.43 mm against B. cereus. These observations suggest variable activity across the tested oil–bacterium combinations, but do not support the original claim that the oils outperformed streptomycin.

A second table reports results at two oil concentrations, but its headings do not clearly map each value to a concentration and the positive-control entries are incomplete. Those results should not be combined numerically with the screening data until the concentration labels and control values are confirmed. For publication, combine both datasets into one table with separate columns for each concentration only after the original laboratory sheet confirms the mapping.

3.6. Brine-shrimp lethality

The brine-shrimp assay showed increasing numbers of dead nauplii at higher nominal concentrations for the reported species-level data, although the table is not consistently formatted and some totals differ between rows (Table 7). For *M. arvensis*, the reported dead counts rose from 2 at 10 µg/mL to 9 at 600 µg/mL; for M. longifolia, counts rose from 1 to

9 over the same range. *M. spicata* showed 0, 2, 5, and 9 deaths at 10, 100, 300, and 600 µg/mL, respectively. These counts suggest concentration-related lethality, but mortality percentages and LC_{50} values should not be reported until the total number of nauplii per treatment and control mortality are confirmed.

Table 6. Antibacterial screening of essential oils (inhibition-zone diameters, mm; values as reported).

Sample	P. aeruginosa (mm)	S. aureus (mm)	B. cereus (mm)	E. coli (mm)	S. typhi (mm)
M. arvensis	2.56 ± 0.23	4.50 ± 0.25	4.43 ± 0.23	2.75 ± 0.20	0.87 ± 0.43
M. longifolia	0	3.25 ± 0.16	3.62 ± 0.32	0	3.12 ± 0.43
M. spicata	3.84 ± 0.31	4.75 ± 0.20	3.00 ± 0.23	1.75 ± 0.50	0
Streptomycin	9.82 ± 0.43	11.65 ± 0.51	11.07 ± 0.32	9.90 ± 0.25	10.79 ± 0.63
n-Hexane	0	0	0	0	0

Table 7. Brine-shrimp assay counts transcribed from the supplied results; verify totals and treatment labels against the original data.

Species	**Concentration (µg/mL)**	**Total nauplii (reported)**	**Dead after 24 h (reported)**
M. arvensis	10	20	2
M. arvensis	100	20	5
M. arvensis	300	18	7
M. arvensis	600	19	9
M. longifolia	10	18	1
M. longifolia	100	20	6
M. longifolia	300	20	8
M. longifolia	600	19	9
M. spicata	10	20	0
M. spicata	100	19	2
M. spicata	300	18	5
M. spicata	600	18	9

4. Conclusion

A significant number of phytochemicals, such as flavonoids, phenolics, annins, and saponins, are present in the chosen mentha species. Carvone (84.3%), 1,8-cineol (5.3%), and limonene (3.2%) were the main constituents of *M. arvensis* essential oil, whereas piperitone oxide (54.2%), menthone (9.7%), 2-Hydroxy-3-(3-methyl-2-butenyl)-3-cyclopenten-1-one (8.4%), borneol (4.6%), and menthol (3.3%) were the main constituents of *M. longifolia* essential oil. 2-Hydroxy-3-(3-methyl-2-butenyl)-3-cyclopenten-1-one (47.1%), 1,8-cineol (12%), α-pinene (9.7%), borneol (9.5%), and germacrene-12% (2.5%). The primary antioxidants most likely come from the chemical substances identified by GC-MS. According to the antibacterial investigation, plant essential oils outperformed tested strains when used at greater concentrations. It was discovered that the zone of inhibition closely matched the published data. According to a bioassay measuring the lethality of brine shrimp, these herbs are safe to use as traditional medicine. Mosquito repellent properties may be obtained from the essential oils of *M. arvensis* and *M. longifolia*.

References

Abbasi, A. M., Shah, M. H., Li, T., Fu, X., Guo, X., & Liu, R. H. (2015). Ethnomedicinal values, phenolic contents and antioxidant properties of wild culinary vegetables. *Journal of Ethnopharmacology*, *162*, 333–345. https://doi.org/10.1016/j.jep.2014.12.051

Adedapo, A. A., Jimoh, F. O., Afolayan, A. J., & Masika, P. J. (2009). Antioxidant properties of the methanol extracts of the leaves and stems of *Celtis africana*. *Records of Natural Products*, *3*(1), 23–31.

Agbor, A., & Ngogang, Y. (2005). Toxicity of herbal preparations. *Cameroon Journal of Ethnobotany*, *1*, 23–28.

Ashafa, A. O. T., Sunmonu, T. O., & Afolayan, A. J. (2010). Toxicological evaluation of aqueous leaf and berry extracts of *Phytolacca dioica* L. in male Wistar rats. *Food and Chemical Toxicology*, *48*(7), 1886–1889. https://doi.org/10.1016/j.fct.2010.04.029

Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*, *44*(1), 276–287. https://doi.org/10.1016/0003-2697(71)90370-8

Cefarelli, G., D'Abrosca, B., Fiorentino, A., Izzo, A., Mastellone, C., Pacifico, S., & Piscopo, V. (2006). Free-radical-scavenging and antioxidant activities of secondary metabolites from reddened cv. Annurca apple fruits. *Journal of Agricultural and Food Chemistry*, *54*(3), 803–809. https://doi.org/10.1021/jf052632g

Dillard, C. J., & German, J. B. (2000). Phytochemicals: Nutraceuticals and human health. *Journal of the Science of Food and Agriculture*, *80*(12), 1744–1756. https://doi.org/10.1002/1097-0010(20000915)80:12%3C1744::AID-JSFA725%3E3.0.CO;2-W

Dufour, D. R., Lott, J. A., Nolte, F. S., Gretch, D. R., Koff, R. S., & Seeff, L. B. (2000). Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory tests in screening, diagnosis, and monitoring. *Clinical Chemistry*, *46*(12), 2050–2068. https://doi.org/10.1093/clinchem/46.12.2050

Farnsworth, N. R., & Morris, R. W. (1976). Higher plants—the sleeping giant of drug development. *American Journal of Pharmaceutical Sciences Support and Public Health*, *148*(2), 46–52.

Feng, Y., Wang, N., Zhu, M., Feng, Y., Li, H., & Tsao, S. (2011). Recent progress on anticancer candidates in patents of herbal medicinal products. *Recent Patents on Food, Nutrition & Agriculture*, *3*(1), 30–48. https://doi.org/10.2174/2212798411103010030

Foster, S., & Duke, J. A. (2000). *A field guide to medicinal plants and herbs of eastern and central North America* (2nd ed.). Houghton Mifflin Harcourt.

Fowler, M. W. (2006). Plants, medicines and man. *Journal of the Science of Food and Agriculture*, *86*(12), 1797–1804. https://doi.org/10.1002/jsfa.2598

Graf, B. L., Raskin, I., Cefalu, W. T., & Ribnicky, D. M. (2010). Plant-derived therapeutics for the treatment of metabolic syndrome. *Current Opinion in Investigational Drugs*, *11*(10), 1107–1115.

Gulfraz, M., Mehmood, S., Ahmad, A., Fatima, N., Praveen, Z., & Williamson, E. M. (2008). Comparison of the antidiabetic activity of *Berberis lyceum* root extract and berberine in alloxan-induced diabetic rats. *Phytotherapy Research*, *22*(9), 1208–1212. https://doi.org/10.1002/ptr.2438

Hussain, L., Akash, M. S. H., Tahir, M., Rehman, K., & Ahmed, K. Z. (2014). Hepatoprotective effects of methanolic extracts of *Alcea rosea* against acetaminophen-induced hepatotoxicity in mice. *Bangladesh Journal of Pharmacology*, *9*, 322–327.

Koehn, F. E., & Carter, G. T. (2005). The evolving role of natural products in drug discovery. *Nature Reviews Drug Discovery, 4*(3), 206–220. https://doi.org/10.1038/nrd1657

Krymow, V. (2002). *Healing plants of the Bible: History, lore & meditations*. Wild Goose Publications.

Marles, R. J., & Farnsworth, N. R. (1995). Antidiabetic plants and their active constituents. *Phytomedicine, 2*(2), 137–189. https://doi.org/10.1016/S0944-7113(11)80059-0

Newman, D. J., & Cragg, G. M. (2012). Natural products as sources of new drugs over the 30 years from 1981 to 2010. *Journal of Natural Products, 75*(3), 311–335. https://doi.org/10.1021/np200906s

Newman, D. J., Cragg, G. M., & Snader, K. M. (2000). The influence of natural products upon drug discovery. *Natural Product Reports, 17*(3), 215–234. https://doi.org/10.1039/A902202C

Ríos, J.-L. (2010). Effects of triterpenes on the immune system. *Journal of Ethnopharmacology, 128*(1), 1–14. https://doi.org/10.1016/j.jep.2009.12.045

Packer, L., et al. (2004). *Herbal and traditional medicine: Biomolecular and clinical aspects*. CRC Press.

Pandey, B. (2006). *A textbook of botany: Angiosperms, taxonomy, anatomy, embryology (including tissue culture) and economic botany*. S. Chand & Company.

Phillipson, J. D. (1994). Natural products as drugs. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 88*(Suppl. 1), S17–S19. https://doi.org/10.1016/0035-9203(94)90464-2

Schwartsmann, G., Ratain, M. J., Cragg, G. M., Wong, J. E., Saijo, N., Parkinson, D. R., Fujiwara, Y., Pazdur, R., Newman, D. J., Dagher, R., & Di Leone, L. (2002). Anticancer drug discovery and development throughout the world. *Journal of Clinical Oncology, 20*(18 Suppl.), 47S–59S.

Sharma, M., et al. (1975). Observations on oxytoxic activity of a flavonol glycoside isolated from *Curculigo orchioides*. *Journal of Research in Indian Medicine, 10*, 104.

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S., & Boyd, M. R. (1990). New colorimetric cytotoxicity assay for anticancer-drug screening. *Journal of the National Cancer Institute, 82*(13), 1107–1112. https://doi.org/10.1093/jnci/82.13.1107

Ullah, M. F., & Khan, M. W. (2008). Food as medicine: Potential therapeutic tendencies of plant-derived polyphenolic compounds. *Asian Pacific Journal of Cancer Prevention, 9*(2), 187–196.

Upadhyay, R. K. (2015). GC-MS analysis and in vitro antimicrobial susceptibility of *Foeniculum vulgare* seed essential oil. *American Journal of Plant Sciences, 6*(7), 1058–1075. https://doi.org/10.4236/ajps.2015.67110

Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M., & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions, 160*(1), 1–40. https://doi.org/10.1016/j.cbi.2005.12.009

Wall, M. E., Wani, M. C., Cook, C. E., Palmer, K. H., McPhail, A. T., & Sim, G. A. (1966). Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. *Journal of the American Chemical Society, 88*(16), 3888–3890. https://doi.org/10.1021/ja00968a057