CHEMICAL ANALYSIS AND BIOACTIVITIES OF ESSENTIAL OILS OF SELECTED *MENTHA SPECIES* FROM LOWER HIMALAYA REGION OF PAKISTAN

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ABSTRACT

Plants secondary metabolites are responsible for various biological activities. Essential oils of plants are also secondary metabolites that have curative and pharmaceutical effects such as antibacterial, antifungal, antiviral and insect repellent. The *mentha* species are potential candidates for development of drugs due to their efficient antimicrobial, antioxidant and radical-scavenging activities and reliable cytotoxicity

In the current study *Mentha species* (*Mentha arvensis, M. longifolia* and *M. spicata)* were assessed for their phytochemicals. The essential oils contents by using gas chromatography mass spectroscopy (GC-MS) and FT-IR . The antioxidant, antibacterial, cytotoxic and mosquito repellent activities of plant extracts were also determined It was observed that various mentha extracts are good source of flavonoids, phenolic, tannins and saponins . All mentha specie contained higher quantity of essential oils. The higher antioxidant activity of DPPH assay was found ( ) as compared to ABTS and H2O2. Where as higher zone of inhibition ( ) was obtained for S aures as compared to other microorganism . Where as plant extracts have shown lower cytotoxicity . It is expected that due to out come of this study raw materials of mentha spp will get industrial application and might be used for preparation of drugs required for human health

Keywords; Phytochemicals , Chemical analysis, Bioactivities**,**

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INTRODUCTION

Herbal treatments are used by over 80% of rural communities worldwide to cure a variety of human problems. Medicinal plants play a crucial part in the production of allopathic pharmaceuticals. Many contemporary medications, including aspirin, digoxin, morphine, codeine, vinblastine, cocaine, emetine, ephedrine, vinocristine, and pilocarpine, have been extracted from plants (Abbasi et al., 2015). The pharmaceutical industry's interest in medicinal plants has once again resurfaced after the latter half of the 20th century saw more advancements in synthetic chemistry over natural goods. They now view the sources of herbal plants as chemical scaffolds to produce pharmaceuticals. Approximately 70% of our medications are synthetic; natural chemicals derived from medicinal plants may be a safer alternative (Adedapo *et al*., 2009).

According to WHO estimates, the global market for pharmaceuticals derived from plants might be worth around 200 million Indian rupees. Of the 2,50,000 plant species that are classified as higher plants on land, over 80,000 are essential to medical treatment. The medications were extracted from complete plants or from specific plant parts including seeds, stems, leaves, flowers, roots, bark, etc. Certain products, including gum, latex, and resins, were generated from excretory materials. Numerous medications produced from plants have been included into allopathic treatment. These components have a significant role in the contemporary pharmacopoeia (Ashafa et al., 2010). Additionally, several plants possessed the vital chemical intermediates (solasodine, diosgenin, and betaionine) needed to make current medications (Agor and Ngogan, 2005).

The pharmaceuticals used in modern medicine are either directly or indirectly derived from

natural sources, which are also very significant in the drug discovery process because of their enormous diversity, which makes it possible to identify lead molecules that are highly interesting for the creation of novel therapeutic agents for pathological processes. Approximately 80% of people on the planet are thought to rely mostly on plants for medical treatment (Beauchamp and Fridovic, 1971; Cefarelli et al., 2006). Plant tissue culture and plant molecular biology are two methods used in the cultivation, conservation, and preservation of herbal medicinal species that are greatly aided by modern science and technology. Standardization and other relevant issues must be addressed, as well as the separation of active ingredients and their development into novel therapies Dillard and German, 200). According to Dufour et al. (2000), the plants in the Phyllanthus genus (Euphorbiaceae) are extensively spread in most tropical and subtropical nations, and they have been used for a long time in traditional medicine to treat intestinal infections, diabetes, kidney and urinary bladder disorders, and hepatitis B.

Over the last thirty years, botanical researchers have discovered that some plant elements have been utilized for both illness prevention and treatment (Farnsworth and Morris, 1976). According to Feng et al. (2011), plants contain chemicals that may be used to cure illnesses and find novel medications.

Most medical plants also have the ability to treat bacterial infections. These plants are reasonably priced, readily available in the area, and gaining popularity every day.The body becomes incompatible with metabolic syndrome. The metabolic syndrome was treated using medicinal plants that had a significant excess of phytochemicals. Purified from an alcoholic extract of clausena anisata stem bark, the carbazole alkaloid "clausenol" has been shown to exhibit antibacterial properties. The Cassia alata leaves' alcoholic and acetonic extract has demonstrated

a strong antibacterial effect in vitro (Foster and Duke, 2000). Many plants have demonstrated cytotoxic activity against a single cell line, SF-268 or MCF-7, respectively, at GI50 values ≤10 µg/ml. These plants include Thevetia ahouai, Physalis viscosa, Piper jacquemontianum and Piper barbatum, Senna occidentalis, Tovomita longifolia and Lippia cardiostegia, and Blepharocalyx salicifolius. Worldwide, cancer is a major cause of death. The MTT assay demonstrated significant anti-proliferative activity of methanolic extracts from the bark of Zanthoxylum xanthoxyloides and the roots of Croton membranaceus against three human cancer cell lines, including DLD-1, MCF-7, and M14. These extracts are used in cancer treatment.In vitro, four chemicals that were extracted from the fruit rind of Terminalia belerica and refined into pure forms—termilignan, thannilignan, 7-hydroxy 3, 4 (Methylenedioxy) flavone, and anolignan B—exhibited anti-HIV 1, anti-malarial, and anti-fungal action (Graf et al., 2010). Through both in vitro and in vivo testing, the antimalarial properties of petroleum and ethanolic extracts from species such as Japonica, Artemisia maritimia, and Artemisia nilegarica were evaluated. Using the Rane test in Balb/c, the activities were concluded in vivo and it was seen that all composites increased the mice's endurance time.Numerous chemical components are present in plants. According to Gulfraz et al. (2008), these chemical components are divided into two categories of metabolites: main and secondary metabolites.

Plants use primary metabolites in their fundamental metabolism. Chlorophyll, proteins, lipids, carbohydrates, and nucleic acids are a few examples of primary metabolites. The stem, leaves, or roots of plants, which humans eat on a regular basis, are where they store the surplus primary metabolites (Hussain et al., 2014).

Chemical components that do not undergo primary metabolic metabolism are referred to as secondary metabolites. They were previously considered end products or excretory products. The

medical community is now aware of their significance. These components are now recognized as being significant from a medical standpoint. Plant defense is significantly aided by secondary metabolites. They deter herbivores from approaching the plant and shield it from insects (Koehn and Carter, 2005).

Alkaloids, terpenoids, phenolics, and other important classes might be used to categorize plant secondary metabolites.Alkaloids are heterocyclic substances that include nitrogen. They have a bitter flavor, are alkali-like chemicals, include nitrogen, and are basic in nature. These substances are heterocyclics and have pharmacological activity. Alkaloids are further classified into distinct structures based on the number of nitrogen atoms contained in the molecule, such as Ergotamine, which has penta nitrogen, and Atropine, which contains a single nitrogen atom.Alkaloids, like nicotine, are crystalline substances that include oxygen. Alkaloids are normally colorless, although there are rare instances where they are; one such example is serpentine, which has a brownish red tint. Alkaloids are classified into three types and are soluble in ether, chloroform, and other non-polar solvents (Krymow, 2002).

In addition to being utilized in food, terpenoids have therapeutic uses. Foods and beverages are flavored with terpenoids. In addition, they are employed as flavorings in cigarettes and toothpaste (Males and Farnsworth, 1995). There are reports on terpenoids' antimicrobial properties and ability to heal wounds. The resins that are extracted from the plants include these kinds of terpenoids. The aromatic ring-containing hydroxyl group that makes up phenolics. One or more hydroxyl groups might be present.Plants include polymeric phenolic chemicals that are useful for medicine, including tannins, lignin polyphenolics, and melanin. Since sugar moieties make up the majority of phenolic compounds, they are soluble in water (Gulfraz et al., 2008). Flavonoids and polyphenolic substances are a family of secondary metabolites that are frequently

found in the diet of humans. Flavonoids also include saponins and tannins. Drug development and medicinal plants are closely related fields. The history of using medicinal plants to cure illnesses in humans is extensive. The cornerstone of drug development was laid by the isolation of active molecules from medicinal plants. Pharmacist Serturner extracted morphine from opium in 1817. Between 1820 and 1850, atropine, codeine, and caffeine were isolated. Antipyrin, the first synthetic medicine discovered by a German scientist (Newman and Cragg, 2012).

Pakistan is endowed with an abundance of naturally occurring medicinal plant resources. There are twenty-five pharmaceutical businesses in Pakistan that commercially produce tibbi medications. According to data from Pakistan's Export Promotion Bureau, the country earned 6 million US dollars from the export of 8500 tons of medical plant materials in 1999, but it spent 31 million dollars importing raw materials for herbal products from other nations (Newman *et al*., 2000; Rios, 2010). There are over 50,000 herbalists operating in Pakistan. Only 400–600 of the 2000 species of Pakistani plants are known to exist, despite their classification under the Unani system. Pakistan's pharmaceutical industry produces pharmaceuticals from medicinal plants, and local healers employ these plants to treat human diseases (Packer et al., 2004). Seventy species of medicinal plants are comparable (Pandey, 2006). There are 91 species in the family Lamiacea, including 6 Mentha, and a wide range of lamiaceae flora in Pakistan. The country's northern region is a hotspot for floral variety and is home to a large number of significant indigenous medicinal plants that the local populace frequently uses. In traditional medicine, the locals utilize a variety of Lamiaceae plant species. Mentha arvensis and Mentha piperita, also referred to as podina, are used as emollients, operients, demulcents, antidiabetic agents, and carminatives, while Oreganum vulgare, also known as Ban ajwain, is used to treat epilepsy, colic, and uterine disorders. In addition to being used as a condiment, Mentha

longifolia is also used as a carminative, stimulant, headache, stomach, digestion, blood purification, and anti-emetic. Salvia nubicola is used to cure cattle's asthma and other lung conditions (Phillipson, 1994). The current study was conducted with the following goals and objectives in mind, given the ineffectiveness of medicinal herbs, particularly menthe longifolia. Extracting essential oils from plants, analyzing them chemically, and measuring their components using mass spectrometry and gas chromatography Examining antimicrobial

MATERIAL AND METHODS

Collection of samples

District Abbottabad provided fresh aerial portions of Mentha arvensis, Mentha longifolia, and Mentha spicata. The ethnobotanical usage of menthe species in local folk remedies by the local population served as the foundation for sample collection. The plant sample collecting area, name, and location were properly labeled on the thin plastic bags used to collect the samples. The Department of Environmental Sciences at the COMSATS Institute of Information Technology, Abbottabad, employed a skilled taxonomist to identify the samples, and voucher specimens (No. 132) were deposited for future use. Packed in plastic bags, around 3 kg of manually harvested plant components were transported to the Department of Chemistry's Biochemistry Laboratory at the COMSATS Institute of Information Technology in Abbottabad. In addition to extracting essential oils, the aerial sections of the mentha species were used to measure additional phytochemicals and evaluate their bioactivities.


Preparation of samples

To get rid of undesirable components, including dust, plant materials were cleaned with distilled water. Samples were then sun- and oven-dried for one night at a reduced temperature, after which they were shade-dried. After being dried and processed using an electric grinder and an 80 grit screen, the samples were kept at 4 °C until needed again.

Analysis of plant extracts for phytochemicals

Determination of flavonoids

After minor adjustments, the spectrophotometric approach (Skehan et al., 1990) was used to estimate the total flavonoid content (TFC), and the analysis was done in triplicate. 2.5 mg of quercetin were added to 25 mL of methanol to create a 100 ppm standard solution in a conical flask. with a standard curve created with six distinct doses of (Sigma-Aldrich) (10, 20, 40, 60, 80, and 100 ug/mL), the TFC of the sample was ascertained. Each Falcon tube received 0.5 mL of the sample solution, 0.5 mL of distilled water, and 0.5 mL of AlCl3.6H2O solution. Following that, the falcon tubes were incubated at room temperature for five minutes. Following the incubation period, 2 milliliters of a 1 milliliter NaOH solution were added and thoroughly mixed. At 510 nm, the absorbance was determined using a spectrophotometer. The amount of flavonoids

present in the sample was represented as µg of quercetin equivalent (QE) per g. Each sample was run through three replicates, and the average result was expressed as QEg/100g.

Estimation of Total Phenolic Content

The Folin-Ciocalteu technique was often used to assess the total phenol concentration (TPC) in plant extracts (Phillipson, 1994). In a conical flask, a 100 ppm standard solution of gallic acid was made by dissolving 2.5 mg of gallic acid in 25 mL of methanol. Using a standard curve created with varying doses of gallic acid (10, 20, 40, 60, 80, and 100 µg/mL) from Sigma-Aldrich, the TPC of the sample was ascertained. Folin-Ciocalteu's phenol reagent (10%) was combined with 0.5 mL of sample solution, 0.5 mL of distilled water, and 0.5 mL of the mixture. Following a 5-minute room temperature incubation period, 2 milliliters of sodium carbonate solution were added and well mixed. The combination was then allowed to sit at room temperature in the dark for ninety minutes. Using a UV-visible spectrophotometer, the absorbance of the solution was measured at 760 nm in comparison to the blank following incubation. Gulfraz et al. (2008) said that the final results were represented as standard Gallic acid equivalent and that each sample was taken three times.

Determination of Saponins

Using the procedure outlined by (Skehan et al., 1990), the saponin content of three plant samples was measured. A 20% ethanol solution of 200 mL was used to disseminate 20g of each plant

sample. About 55 °C was continuously stirred while the suspension was cooked over a hot water bath for four hours. Following filtration of the mixture, 200 mL more of 20% ethanol was used to extract the residue once again. Using a water bath heated to about 90 °C, the mixed extracts were reduced to 40 mL. 20 milliliters of diethyl ether were added to the concentration in a 250 milliliter separatory funnel, and the mixture was violently agitated. And the ethyl ether was thrown away, leaving behind the aqueous layer. N-butanol (60 mL) was added and the purification procedure was repeated. Ten milliliters of 5% aqueous sodium chloride were used to wash the mixed n-butanol extracts twice. We cooked the leftover solution in a water bath. Following evaporation, the samples were dried in an oven to a consistent weight, and the amount of saponin was determined (Schwartmann *et al*., 2002; Sharma *et al*., 1975).

Quantification of tannins

According to the procedure described by Ullah and Khan (2008), tannin extraction was carried out. In an Erlenmeyer flask, 400 mL of 70% acetone was added to a mass of 100g of dried plant powder. Aluminum foil was placed over the flask to protect it from light. After refluxing this mixture for 12 hours at 60 °C. The material was then centrifuged for 15 minutes at 3500 rpm after being filtered using Whatman filter paper No. 41. Aluminum foil was used to protect the flask from light exposure while the solvent was extracted using a rotary evaporator operating at a temperature below 60 °C. The Folin-Ciocalteu technique was used to quantify the total tannins. For the experiment, 800 µL of the sample and the same volume of Folin-Ciocalteu reagent were combined in a test tube, shaken, and allowed to sit for five minutes. The total tannin content of the solution was measured in a UV-Visible spectrophotometer at 725 nm after it had been diluted with 5 mL of distilled water. The resulting absorbance readings were represented as milligram

standard equivalent per gram of plant extract and compared to standard curves for total tannins made with tannic acid.

Extraction of Essential oils

One method of purification or isolation for temperature-sensitive materials, such as naturally occurring aromatic compounds, is steam distillation. The distillation equipment is filled with steam or water to reduce the compounds' boiling points. The fundamental idea behind steam distillation is that it may extract a chemical or mixture of compounds at a temperature far lower than the boiling point of any individual component. Lower boiling point chemicals found in essential oils volatilize at temperatures of around 100 °C when exposed to steam or boiling water. Fresh aerial portions of the plant were used to extract the essential oil. Using a knife, the chosen plants' aerial portions were divided into smaller pieces. The smaller plant fragments may produce more biomass that can be efficiently and readily harvested. An electronic digital balance was used to weigh the plant's aerial parts after they had been chopped into tiny bits. After the plant material was weighed, it was charged into a stain-free distillation equipment tank. The vase was filled with around 2000 mL of distilled water and set on a heating mantle. After that, a condenser that was chilled by cold tap water was attached to the vessel. After starting the water flow via a condenser, the mixture was brought to a boil for three hours. After going through the water condenser, the distillate containing volatile chemicals was gathered in a separating funnel. The presence of essential oil was evident from the oil layer at the top of the water in the separating flask. Following the separation of essential oil floating on distillate, oil was extracted from it using liquid-liquid extraction using the distillate that remained (Upadhyay, 2015).

Liquid-Liquid extraction

A separation technique called liquid-liquid extraction, also known as solvent extraction, is predicated on the disparate distribution of the components that need to be separated between two liquid phases. Essential oils may be readily extracted using organic solvents such as hexane, chloroform, ethyl acetate, methanol, or acetone since they are a combination of organic components. Hexane, ethyl acetate, chloroform, and other water immiscible solvents are examples of the solvents that can only be utilized in liquid-liquid extraction from a water substrate. This procedure involved adding 70 mL of HPLC-grade n-hexane to the distillate that was gathered in the separating funnel. As soon as the hexane was added, the separating funnel's stopper was opened to lessen the pressure that the hydration of the hexane caused. To dissolve the most essential oil possible in the hexane, the funnel's stopper was closed and the resultant mixture was gently shook for a while. To alleviate part of the pressure, the separating funnel's stopper was released. At least three iterations of the shaking procedure were carried out, and the separating funnel was suspended from a stand. The water that was separated in a different flask developed a transparent layer of hexane-containing essential oil atop it after a few minutes. By mixing 70 mL of hexane into the leftover distillates, the same process was carried out three times. By mixing a tiny quantity of anhydrous magnesium sulfate with hexane extract, some water traces that were still present were eliminated. magnesium-containing hexane extract(Valko *et al*.,2006).

Using a Buchi R100 rotary evaporator set at 25°C and low pressure, the extra hexane was turned into vapor. By dividing the mass of the extracted oil by the mass of the plant used to extract the oil, the yield (%) of the hexane-free essential oil was calculated by weighing on an analytical

scale. A glass adapter was used to connect a round-bottom flask carrying hexane extract to a rotary evaporator. After hexane evaporated, it was collected in a flask that was attached to the rotary evaporator's condenser. At 25°C, the rotary evaporator's initial vacuum setting was 200 mbar. Following a 3-minute duration, the pressure was lowered to either 100 or 80 mbar once the flask's evaporation stabilized. The round bottom flask was subjected to this method until all of the hexane had evaporated. The mass of the pure essential oil was weighed, and the mass of fresh plant material needed to extract the oil was divided to find the essential oil's yield (%).

Chemical analysis of the essential oil by GC-MS

Using Gas Chromatography-Mass Spectrometry (GC-MS), the chemical makeup of the extracted oil was examined. Sample analysis was performed using the Hawlett Packard GC-MS equipment. A HP 5973 Mass Spectrometer (MS; Agilent Technologies Inc. USA) was attached to the Hawlett Packard GC 6890N, which had a DB-5 column (30 m length, 0.25 mm internal diameter, and 0.25 µm stationary phase film thickness). At 235tsC, the injector was run. The temperature of the GC oven was set to start at 40 ¹C and stay there for two minutes. After that, it was raised to 450 ¦C at a pace of four degrees Celsius and it stayed there for eight minutes. As the mobile phase, a steady flow of 1 mL/min of ultrapure helium gas was employed. In split-less mode, a 1 µL amount of the diluted essential oil solution was injected (UPadhyay, 2015).

Using PC-based software to handle instrument operation and data processing, the FT-IR spectra of defatted samples were recorded in an FT-IR instrument (Model 1:1 FS 25, Bruchure, Germany). For FT-IR analysis, a thin film was created by exerting pressure and a tiny quantity of powdered material was formed into pellets using KBr. A wave number range of 4000 cm-1 to 500 cm-1 was used to acquire the IR transmittance data. Using KBr pellets as a blank, each

sample was examined three times. To determine the functional groups, the spectra were matched to a reference.

Determination of bioactivities

The procedure previously published by several authors was followed in evaluating the antioxidant activity of plant extracts. Therefore, the following bioassays were employed to determine the antioxidant content.

With a few adjustments, DPPH scavenging activity was carried out using the Graf et al., 2010 approach. The foundation of this technique was the antioxidant's capacity to scavenge the 1, 1-diphenyl-2-picryl hydrazyl (DPPH) action radical. After adding 4 mL of DPPH solution (0.1 mM) to 100 µL of the sample solution in a falcon tube, the mixture was violently vortexed. After that, the mixture was allowed to sit at room temperature for 30 minutes while covered with aluminum foil to prevent heat exposure. Using a UV-Vis spectrophotometer, the solution's absorbance at 517 nm was measured. Inhibition percentage

$$\% \text{ Inhibition} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

ABTS scavenging bioassay

It was done using the ABTS radical scavenging bioassay. To create the ABTS radical cation, 5 mL of an aqueous ABTS solution with a concentration of 7 mM was mixed with about 88µL of 140 mM potassium persulphate (K2S2O8) until the final concentration of the solution was 2.45 mM. The solution was left in the dark for sixteen hours. Following incubation, the radical cation was further diluted in ethanol in order to bring the solution's original absorbance value down to 0.7. Next, 25µL of ethanol was used to produce the plant extracts for the study. At 730 nm, the

final percentage decrease in absorbance was determined after adding 10µL of the sample solution to the reaction mixture. It was anticipated that the end absorbance would be 20–80% lower than the reaction mixture's starting absorbance (Wall *et al*., 1966).

Scavenging of H_2O_2

H2O2 scavenging was identified. After adding around 4 mM H2O2 0.6 mL solution (made in PBS) to a 4 mL extract solution, it was incubated for 10 minutes. Using a spectrophotometer, the absorbance of the solution was measured at 230 nm in comparison to a blank solution. Potency of free radical scavenging as measured by %age H2O2. Stronger free radical scavenging activity was indicated by lower H2O2 (Ullah and Khan,2008).

Brine Shrimp toxicity assay

Brine shrimp eggs were hatched in a shallow rectangular dish (22 × 30 cm) filled with artificial sea water which was prepared with a commercial salt mixture and double distilled water. A plastic divider with 2 mm holes was clamped in the dish to make two unequal compartments, the eggs (50 mg) were sprinkled into the larger compartment which was darkened while the smaller compartment was illuminated. After 48 hrs, the phototropic napulii were collected by pipette from the illuminated side, which was separated by the divider from their shells. Twenty shrimps were transferred to each sample vial using pipette and 5 mL artificial seawater was added. The napulii can be counted in the stem of pipette against a lighted background. A drop of dry yeast suspension (3 mg in 6 mL artificial seawater) was added as food to each vial. The vials were maintained under illumination. Survivors were counted with the aid of 3 magnifying glasss and after 24 hrs percent death at each dose and control were determined. In each case where control deaths occurred, the data were corrected using Abbott’s formula:

% death = [Test – Control] / Control × 100

Determination of Antibacterial activity

The preparation of the organism suspension followed McFarland's guidelines. Bacterial suspension was prepared using a culture that was 24 hours old. Inoculation loops were used to select bacterial strains (colonies) for Eppendorf tubes, which were then incubated for 30 minutes at 37°C. Following the collection of each bacterial colony, it was combined with 3 mL of distilled water and forcefully shaken or vortexed. The optical density was then measured using a spectrophotometer set at 600 nm, and it was determined to be equal to 0.5.

Antibacterial activity of mentha essential oil

Using the agar well diffusion technique, the antibacterial activity of essential oils isolated from M. arvensis, M. longifolia, and M. spicata was evaluated (UPadhyay, 2015). In order to create nutrient agar, precisely weighed 13 grams of nutrient broth and 14 grams of agar technical were added to 1 liter of distilled water in a reagent container. The mixture was then vigorously stirred to ensure it was well combined. After that, the mixture was autoclaved for 60 minutes at 110 °C. After that, 30-35 mL of autoclaved nutritional medium were added to agar plates, which were then covered and sealed with parafilm. Petri plates were placed in an incubator at 37 °C for the whole night after being covered and left for an hour to allow the agar to harden. Each bacterial strain was equally distributed across the surface of petri plates using a glass spreader containing 200 µL. Next, using a cork borer with a 6 mm diameter in each plate, four equal and spaced

wells were created. For every test petri plate, two duplicates were created and 30µL of the essential oil test solution was added to each well. The positive control for all bacterial strains was chosen to be streptomycin (1 mg/ml), whereas hexane was utilized as the negative control. The plates were in an incubator set at 37°C for 24 hours after 30 minutes of incubation. A scale was used at four separate locations around the inhibition zone circle to measure the individual millimeters of each well's zone of inhibition in a petri plate (Wall et al., 1966).

Various concentrations ranging from 1 to 5% were used for the biological activity testing. Absolute ethanol was used to create the essential oil solutions for the biological activities. For every 50 milligrams of essential oil (50 mg/mL), about 1 mL of ethanol was added in order to create the 5% solution. For the 1% solution in ethanol (10 mg/mL), the identical process was carried out.

Statistical analysis

The bioassay findings are displayed as the mean of four to five data replicates. One-way ANOVA (analysis of variance) was used to examine the collected data in order to determine the statistical difference between various treatments.

RESULTS

Analysis of Phytochemicals

The proportion of flavonoids, phenolics, saponins, and tanins in extracts is shown by quantitative analysis (Table 1). As per the results, the aerial portions of M. spicata had the highest concentration of flavonoids (15.05 mg/g), followed by M. longifolia with 14.17 mg/g and M. arvensis with 12.21 mg/g in plant extracts. For M. arvensis, M. longifoilia, and M. spicata, the

corresponding percentage mg per gram of sample extract of total phenolics was 20.32, 25.149, and 27.637. While M. arvensis and M. longifolia had 0.04 and 0.01 mg/g of saponins, respectively, the plant extract of M. spicata had the highest concentration of saponins at 0.134 mg/g. Likewise, the tannin output from M. spicata (3.51 mg/g) and M. longifolia extract (3.14 mg/g) was noteworthy.

Table 1. Quantitative analysis of phytochemicals

Plant name	Flavonoids (mg/g)	Total phenolics (mg/g)	Saponins (mg/g)	Tannin (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

Mean ± Standard Deviation (n=3)

Assessment of yield of essential oils

By hydro distilling fresh aerial portions of Mentha arvensis, Mentha longifolia, and Mentha spicata, essential oils were extracted. Both M. longifolia and M. arvensis fresh aerial portions produced significant amounts of essential oil—1.70% and 1.47%, respectively. Although it wasn't as much as other mentha species, M. spicata also shown a decent output (Table 2).

Table 2. Percentage yield of essential oils

Latin name	Family	%age yield of essential oils

Mentha arvensis	Lamiaceae	1.70±0.11
Mentha longifolia	Lamiaceae	1.47±0.31
Mentha spicata	Lamiaceae	1.20±0.03

Mean ± Standard Deviation (n=3)

Chemical analysis of essential oils of *mentha* with GC-MS

The chemical constituents of *mentha* oils were obtained by analysis of oil by GC-MS and results are presented in Tables 3 and 4. In the essential oil of *M. arvensis*, seven main compounds were identified representing (99.1%) of the essential oil. The most abundant compounds were carvone (84.3%) and eucalyptol(5.3%). *M. longifolia* essential oil contained six major compounds representing 98.2% of the oil. Major compounds were piperitone oxide (54.2%), menthone (9.7%), 2-Hydroxy-3-(3-methyl-2-butenyl)-3-cyclopenten-1-one (8.4%), borneol (4.6%), pulegone (4%) and menthol (3.3%). In *M. spicata* essential oil, four major compounds were identified constituting 96.5% of the oil. The major compounds were 2-Hydroxy-3-(3-methyl-2-butenyl)-3-cyclopenten-1-one (47.1%), eucalyptol (12.0%), α-pinene (9.7%) and borneol (9.5%).

Table 3. Chemical composition of *M. arvensis* essential oil based on total ion chromatogram of GC-MS

S. No	Compound name	RI	RT	%age
1	α-Pinene	930	10.81	0.1
2	Sabinene	970	12.23	0.1
3	β-Pinene	973	12.34	0.1
4	β-Myrcene	988	12.86	0.1
5	3-Octanol	991	12.99	0.1
6	Limonene	1026	14.25	3.2
7	1,8-Cineol	1028	14.33	5.3

8	β-cis-Ocimene	1035	14.58	0.1
9	Cis-Sabinene hydrate	1064	15.66	0.1
10	Linalool	1096	16.86	0.3
11	Trans-p-mentha-2,8-dienol	1118	17.63	0.1
12	Trans-Limonene oxide	1135	18.27	0.1
13	Borneol	1164	19.33	0.7
14	4-Terpineol	1175	19.73	0.1
15	α-Terpineol	1188	20.21	0.4
16	Dihydrocarveol	1193	20.38	0.1
17	Cis-Dihydrocarvone	1195	20.43	0.4
18	Cis-Carveol	1231	21.69	0.3
19	Carvone	1246	22.22	84.3
20	Isopiperitenone	1269	23.03	0.3
21	Piperitenone	1339	25.39	0.2
22	β-Bourbenone	1386	26.94	0.4
23	Jasmone	1396	27.27	0.5
24	Caryophyllene	1422	28.07	0.6
25	β-Farnesene	1455	29.1	0.1
26	Germacrene-D	1483	29.97	0.7
27	Elixene	1499	30.46	0.1
28	Caryophyllene oxide	1586	33.04	0.1
	Total % Identified compounds			**99.1**
25	Piperitenone	1339	25.39	0.2
26	1-Cyclohexene-1-methanol, 4-(1-methylethenyl)-, acetate	1345	25.57	0.1
27	2-Hydroxy-3-(3-methyl-2-butenyl)-3-cyclopenten-1-one	1365	26.25	8.4
28	β-Bourbenone	1386	26.94	0.1
29	Caryophyllene	1422	28.07	1.7

30	Germacrene-D	1483	29.97	0.7
31	Caryophyllene oxide	1586	33.04	0.3
	Total % Identified compounds			**98.2**

Chemical Constituents of *mentha* oils

As per the elution sequence on the DB-5 GC column, the detected chemicals were enumerated. Present in M. arvensis, M. longifolia, and M. spicata, respectively, are three main compounds: carvone (84.3%), piperitone oxide (54.2%), and 2-Hydroxy-3-(3-methyl-2-butenyl)-3-cyclopenten-1-one (47.1%). A few chemicals were also discovered to be prevalent in plants. To illustrate, the concentration of eucalyptol varied across the species: it was most (12%) in M. spicata, lowest (0.4%) in M. longifolia, and highest (5.3%) in M. arvensis. Similarly, M. longifolia and M. spicata each had a concentration of 0.7%, whereas M. arvensis had a concentration of 2.5% of germacrene-D. M. spicata had 9.5% of borneol, whereas M. longifolia contained 4.6%. All of the plants had caryophyllene, yet the amounts of it varied depending on the plant: 0.6%, 1.7%, and so on.

Analysis of extracts with FT-IR

The Fourier Transform Infrared Spectroscopy (FT-IR) method is a useful tool for screening and scanning samples for a wide range of components. It works by identifying chemical bonds in a molecule to provide a profile of the sample, unique to each one. Finding the functional groups and describing covalent bonds can be done efficiently with FT-IR.

Table 4. FT-IR analysis of methanolic extracts of *mentha* species

Sr. No	Wavelength	Bond	Functional Group

1	3337.45	O-H, H-bond	Alcohol, Phenol
2	2924.51	C-H stretch	Alkanes
3	1710.21	C=O stretch	Carboxylic acids, Carbonyls
4	1514.05	CO asymmetric stretch	Nitro compounds
5	1451.07	C-C stretch	Aromatic
6	1030.75	C-O	Alcohols, Esters, Ethers
7	817.84	C-Cl stretch	Alkyl halides
8	725.27	C-Cl stretch	Alkyl halides
9	632.43	C-Br stretch	Alkyl halides

Antioxidant activities

DPPH and ABTS scavenging potential

The DPPH and ABTS scavenging bioassays were used to assess the antioxidant properties of plant extracts of M. arvensis, M. longifolia, and M. spicata. When using the ABTS scavenging test instead of the DPPH scavenging assay, the plant extracts' ability to scavenge free radicals was significantly increased. (Tables 5 to 6).

Table 5. Scavenging potential of DPPH

	DPPH Scavenging activity			
Plant extracts Conc. µg/mL	***M. arvensis***	***M. longifolia***	***M. spicata***	***Ascorbic acid***
50	60.7±1.07	70.33±0.4	80.1±0.8	49.29±0.5
100	55.45±1.9	63.77±0.6	73.24±0.62	41.34±1.3
150	48.9±0.13	51.81±0.5	61.33±0.3	33.41±1.7
200	33.62±0.36	36.18±1.0	49.67±0.03	28.67±1.03
250	21.8±0.33	25.42±1.01	33.33±0.2	21.8±1.01
300	19.9±0.33	14.33±2.1	21.9±0.7	13.55±1.23

Table 6. Scavenging potential of ABTS

	ABTS Scavenging activity			
Plant extracts Conc. µg/mL	***M. arvensis***	***M. longifolia***	***M. spicata***	***Ascorbic acid***
50	67.7±1.23	78.73±0.5	88.1±0.9	67.29±0.9
100	59.42±1.8	65.45±0.9	78.44±0.7	56.76±1.09
150	51.89±0.45	58.21±0.7	63.81±0.8	49.87±1.8
200	44.32±0.23	47.81±1.1	55.54±0.01	40.54±1.08
250	38.61±0.10	36.72±1.01	49.01±0.3	34.6±1.1
300	33.62±0.33	29.88±2.1	35.62±0.7	22.55±1.23

H_2O_2 Scavenging potential

The extracts of *M. arvensis*, *M. longifolia* and *M. spicata* significantly scavenged H_2O_2 by 34.9%, 29.66% and 37.9% respectively at concentration of 300 µg/mL (Table 7). *M. arvensis*, *M. longifolia* and *M. spicata* extracts exhibited significant scavenging activity for H_2O_2 on dose dependent manner as shown in table 9.

Table 7 . Scavenging potential of hydrogen peroxide (H_2O_2) by plant extracts

	Percentage of H_2O_2 (%)			
Plant extracts Conc. µg/mL	***M. arvensis***	***M. longifolia***	***M. spicata***	***Ascorbic acid***
50	92.7±1.7	96.46±2.3	87.1±0.6	64.29±0.9
100	90.45±0.9	91.06±0.9	79.05±0.88	52.07±1.2
150	76.9±0.13	83.33±0.77	78.98±0.1	33.41±1.44
200	69.77±0.56	67.11±5.1	67.01±0.05	31.05±1.08
250	51.8±0.45	52.01±1.13	49.34±0.7	24.8±1.1
300	34.9±0.34	29.66±3.22	37.9±0.1	20.45±1.01

Mean±S.D after triplicate analysis

Determination of antibacterial activity

The essential oils of Meravicinia arvensis, Meravicinia longifolia, and Meravicinia spicata were tested for their antibacterial activity against strains of Gram+ bacteria, including Bacillus cereus (KX262674) and Staphylococcus aureus (KX262674); and Gram- bacteria, including Escherichia coli (ATCC 10536), Salmonella typhi (ATCC 6539), and Pseudomonas aeruginosa (ATCC 9027). According to the findings, essential oils have notable antibacterial action at various doses. In comparison to the standard antibiotic streptomycine, M. arvensis had the greatest antibacterial activity against S. aureus, B. cereus, and S. typhi at doses of 100 µg/mL and 200 µg/mL, with inhibition zones of 9.87, 11.31, (9.36, 11.43), and (6.78, 10.87) in mm, respectively. Likewise, M. longifolia had notable antibacterial activity against B. cereus, with inhibition zones of 8.06 mm and 8.25 mm at concentrations of 100µg/mL and 200µg/mL, respectively. Upon exposing 8.18 mm and 9.34 mm to concentrations of 100 µg/mL and 200 µg/mL, the essential oil of M. spicata demonstrated exceptionally strong antibacterial activity against S. aureus.

Table 8. Antibacterial activity of essential oils

Sample	**Zone of inhibition (mm)**

Essential oil	*P. aeruginosa*	*S. aureus*	*B. cereus*	*E. coli*	*S. typhi*
M. arvensis	2.56±0.23	4.5±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±0.23	1.75±0.5	0
Streptomycine	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 11. Antibacterial activity of essential oils at

Sample	**Zone of inhibition (mm)**				
Essential oil	*P. aeruginosa*	*S. aureus*	*B. cereus*	*E. coli*	*S. typhi*
M. arvensis	4.75±0.20	6.25±0.30	6.31±0.47	3.56±0.12	3.37±0.14
M. longifolia	0	5.43±0.23	7.18±0.23	2.18±0.12	5.68±0.12
M. spicata	5±0.88	6.18±0.32	4.62±0.14	2.62±0.25	0
Streptomycine	±	10.18±0.23	13.18±0.37	10.18±0.12	±
n-hexane	0	0	0	0	0

Determination of cytotoxicity

The brine shrimp cytotoxicity of extracts of *M. arvensis, M. longifolia and M. spicata* was carried out which indicates that the cytotoxicity of extracts increased by increasing concentration of plant extracts, however overall extracts has shown less cytotoxicity.

Table 9. Brine shrimp assay of plant extracts

Sample name	**Conc. (µg/mL)**	**Total napuli**	**% of death after 24 hours**	
			Live	**Dead**
M. arvensis	10	20	18	2
	100	20	15	5
	300	18	11	7
	600	19	10	9
M. longifolia	10	18	17	1
	100	20	14	6
	300	20	12	8

	600	19	10	9
	10	20	20	0
M. spicata	100	19	17	2
	300	18	13	5
	600	18	9	9

DISCUSSION

Estimation of phytochemicals

Plants contain phytochemicals that can be utilized as food and medication. Worldwide interest in the characterisation, isolation, and in vitro and in vivo evaluation of the biological activity of phytochemicals that may be useful in treating a variety of human illnesses is expanding. Consequently, a range of chemical compounds derived from the plants under examination exhibit notable anti-proliferative, cytotoxic, antibacterial, and anti-tuberculosis properties. The flavonoid, total phenolic, saponin, and tannin content of the plant extracts was evaluated in this investigation. The research suggests that all three of the plants contain these compounds. A significant quantity of flavonoids, phenolics, and tannins were present in the M. spicata extract (Flowler, 2006; Graf et al., 2010; Koehn and Carter, 2005).The polyphenolic compounds known as flavonoids have fifteen carbons. According to Newan and Cragg (2012), flavonoids are a class of plant metabolites that may have positive impacts on health via cell signaling pathways and antioxidant properties. The majority of secondary metabolites are phenols, which can range in size from basic structures with an aromatic ring to complex ones. Owing to their potent antioxidant qualities, phenolic compounds—which are found in many plants—are an essential component of the human diet. A family of chemical molecules called saponins is present in many different types of plants. Additionally, saponins and glycosides provide a variety of health advantages. Tannins, also known as flavonoids, are astringent polyphenolic biomolecules that

attach to and precipitate different chemical substances, such as alkaloids and amino acids (Rios, 2010, Packer et al., 2004).

Different secondary metabolites were found in the M. arvensis extract according to phytochemical prospection (Koehn and Carter, 2005). Therefore, a substantial concentration of these crucial phytoconstituents gives the plant strong therapeutic properties including the ability to scavenge free radicals and exhibit antibacterial properties (Marles and Farnsworth, 1995).

Chemical composition of Mentha spp

The area of peaks of the compounds was determined by comparing the GC-MS chromatogram, and other necessary information was collected from the NIST library data source. The chromatogram of M. arvensis's essential oil revealed that carvone (84.3%), eucalyptol (5.3%), and limonene (3.2%) were the main components, with the remaining chemical compounds being present in lesser amounts. According to published reports, a study carried out in Brazil using GC-MS and M. arvensis analysis revealed a notable presence of menthone (69.77%), whereas p-Mentone (12.00%) demonstrated strong antibacterial activity. On the other hand, additional evidence suggests that the essential oil of M. arvensis contains menthol (78.90%) and isomenthone (6.35%) (Hussan *et al*., 2014).

The primary constituents of M. longifolia's essential oil composition are piperitone oxide (54.2%), menthone (9.7%), 2-Hydroxy-3-(3-methyl-2-butenyl)-3-cyclopenten-1-one (8.4%), Borneol (4.6%), Pulegone (4%), 1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-6-one (3.6%), and Caryophyllene (1.7%). Comparably, 2-Hydroxy-3-(3-methyl-2-butenyl)-3-cyclopenten-1-one (47.1%) was the main ingredient in M. spicata's essential oil, according to the GC-MS examination of its chemical composition. The essential oil included 1,8-cineol (12.0%), α-Pinene

(9.7%), Borneol (9.5%), Germacrene-D (2.5%), β-Pinene (1.7%), β-Myrcene (1.6%), Caryophyllene (1.3%), and Linalool (1%) as well. In M. spicata's essential oil, the proportion of 1,8-cineol was determined to be 6.36%, 4.7%, and 14.5%[173], [116], [106]. According to reports, the essential oil in India contains 2.35% caryophyllene [94]. Rios (2010) revealed that the essential oil of the plant also contains α-terpineol.

The farmed mentha species may vary, which might account for the diversity in the chemical composition of essential oils. While the chemistry of mentha oil is complicated and varied, as previously indicated, its composition directly influences the efficacy of biological activities, which have different ingredients according on the growing area. The variance in chemical contents of plants cultivated in different regions of the world may be explained by seasonal fluctuations. The antioxidant-active chemical components found in plants have a major role in the prevention of several degenerative illnesses. Herbs and other human dietary supplements have a greater concentration of chemicals that can neutralize free radicals. The antioxidant capacity of the plant extracts was evaluated using three different techniques: DPPH scavenging, ABTS scavenging, and H2O2 scavenging bioassays. The plants' methanolic extracts demonstrated a notable ability to scavenge free radicals. comparing the outcomes of the DPPH and ABTS bioassays. In comparison to the ABTS bioassay, it was anticipated that the DPPH bioassay would significantly enhance the antioxidant capacity of the three plant extracts. Rios (2010) found that among the three plants, M. longifolia had the highest antioxidant potential, followed by M. arvensis, and that the extract of M. spicata had the lowest antioxidant capacity among the tested extracts.

Similar to this, M. arvensis has demonstrated a significant level of antioxidant potential in the current study. Nonetheless, research carried out in Bangladesh and Malaysia reaffirmed M.

arvensis's strong antioxidant capacity and anti-inflammatory effects. In comparison to the other two plant extracts, M. spicata exhibits lower levels of antioxidant capacity (Packer et al., 2004).

Concentration-dependent plant extracts demonstrated H2O2 scavenging. The entire plant extract of M. spicata was found to have significant total radical scavenging activity (superoxide and hydroxyl radicals), which demonstrated effectively in a dose-dependent manner and suppressed the production of H2O2 (37%) at the dose concentration of 300 µg/mL. This is a feature of chain-breaking antioxidants and has been observed in the oxidation of linoleic acid emulsion with extract (Upadhyay, 2015).

Examples of phenolic components with antioxidant qualities are flavonoids, phenolic acids, and phenolic diterpenes. Extracts' ability to scavenge may be related to their phenolic components, which have the ability to transfer electrons to H2O2 and neutralize it with water. Despite its low reactivity, H2O2 can occasionally be harmful to cells by increasing the levels of hydroxyl radicals within them. Food systems' removal of H2O2 is crucial (Valko et al., 2006).

Tests have been conducted on the antimicrobial activity of M. arvensis, M. longifolia, and M. spicata essential oils in hexane against both Gram +ve and Gram -ve bacterial strains. The chosen herbs have a long history of usage as a treatment for common colds, bronchitis, sinusitis, and TB. The main cause of these actions is the presence of oils such as limonene, carvone, and menthol. The findings show that essential oils have strong antimicrobial properties. Nonetheless, in comparison to other examined bacterial strains, M. arvensis essential oil shown significant activity against S. aureus and B. cereus at concentrations of 100µg/mL and 200µg/mL, as well as better antibacterial activity against S. typhi at 200 µg/mL (Upadhyay, 2015).

According to the antibacterial research, plant essential oils exhibit remarkable antibacterial action against a range of bacterial strains, which are the primary causes of stomach issues, even at greater concentrations. The zone of inhibition was discovered to be greater than the outcomes that other writers have previously reported.

The term "cytotoxicity" describes a substance's capacity to kill live cells in the human body. Healthy living cells can either cause necrosis, or unintentional cell death, or apoptosis, or planned cell death, by means of a cytotoxic substance. The utilization of the brine shrimp lethality bioassay for M. arvensis, M. longifolia, and M. spicata reveals that all plant extracts are extremely low in toxicity and can be employed in traditional medicine (Skehan et al., 1990). This also highlights the significance of mentha extracts for their application in the pharmaceutical industry for the development of drugs.

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.

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