CHEMICAL COMPOSITION AND BIOACTIVITIES ASSESSMENT OF VARIOUS MENTHE SPECIES

Saqia Yousaf 1 , Aafia Islam 2 and Anam Abdul Rehman 3
1. Women University Bagh AJK
2. UIBB-Arid University Agriculture
3. MUST University Mirpur AJK

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 . Results from this work should lead to industrial uses for mentha spp. raw materials and, maybe, their incorporation into pharmaceuticals that are essential to human health.

Keywords; Phytochemicals , Chemical analysis, Bioactiv

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. The plant kingdom has been the source of many modern pharmaceuticals, such as aspirin, digoxin, codeine, vinblastine, cocaine, emetine, ephedrine, vinocristine, and pilocarpine (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).

Modern medicine relies on drugs that have their origins in natural sources, which play a crucial role in the development of new drugs. 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), in traditional medicine, members of the Phyllanthus genus (Euphorbiaceae) have long been used to cure a variety of conditions, including hepatitis B, diabetes, kidney and urinary bladder problems, and intestinal infections. This genus is widely distributed across tropical and subtropical regions of the world.

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. The emollient, operient, demulcent, antidiabetic, and carminative properties of Mentha arvensis and Mentha piperita (podina) are well-known, while the epileptic, colic, and uterine disorder-related uses of Oreganum vulgare (Ban ajwain) are well-known as well. Mentha has several culinary uses, including as a condiment,

Longifolia has several medicinal uses, including those of a carminative, stimulant, headache, digestive, blood purifying, and anti-emetic. When used topically, salvia nubicola alleviates

respiratory issues in cattle, including asthma (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

Mentha arvensis, Mentha longifolia, and Mentha spicata aerial parts were freshly procured from District Abbottabad. We based our sample collection on the local population's ethnobotanical use of menthe species in traditional medicines. The little plastic bags that were used to gather the samples were appropriately labeled with the name, location, and sampling area of the facility. The samples were identified by a trained taxonomist at the Department of Environmental Sciences at the COMSATS Institute of Information Technology in Abbottabad, and voucher specimens (No. 132) were saved for further reference. The biochemistry lab of the Department of Chemistry at the COMSATS Institute of Information Technology in Abbottabad received around 3 kg of plant parts that had been gathered by hand and packed in plastic bags. 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). The gallic acid standard solution was prepared in a conical flask by dissolving 2.5 mg of gallic acid in 25 mL of methanol. The final concentration was 100 ppm. 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

Analysis of the extracted oil's chemical composition was carried out using Gas Chromatography-Mass Spectrometry (GC-MS). The GC-MS apparatus from Hawlett Packard was used to analyze the samples. The Hawlett Packard GC 6890N, equipped with a DB-5 column (30 m length, 0.25 mm internal diameter, and 0.25 µm stationary phase film thickness), was coupled with an Agilent Technologies Inc. USA Mass Spectrometer (HP 5973 MS). The injector was started up at 235ϤC. Starting at 40 ϤC, the GC oven was programmed to maintain that temperature for two minutes. Further heating to 450 ¦C occurred at a rate of four degrees Celsius, and the temperature remained there for eight minutes. A continuous flow of ultrapure helium gas at a rate of 1 mL/min was used as the mobile phase. As per UPadhyay (2015), a diluted essential oil solution measuring 1 µL was administered when the apparatus was operating in split-less mode.

An FT-IR instrument (Model 1:1 FS 25, Bruchure, Germany) was used to record the FT-IR spectra of defatted samples. The instrument was operated and data was processed using PC-based software. A thin film was produced by applying pressure, and a little amount of powdered material was converted into pellets using KBr, in order to conduct FT-IR analysis. While collecting the infrared transmittance data, the wave number range was set between 4000 cm-1 and 500 cm-1. The KBr pellets were used as a control in each

Three times, the sample was analyzed. The spectra were compared to a standard in order to identify the functional groups.

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. The ABTS radical cation was prepared by combining 5 mL of a 7 mM aqueous ABTS solution with about 88µL of 140 mM potassium persulphate (K2S2O8) until the final solution concentration reached 2.45 mM. For a period of sixteen hours, the solution was not exposed to light. After the incubation period, the radical cation was further diluted with ethanol until the initial absorbance value of the solution was reduced to 0.7. To make the plant extracts for the research, 25µL of ethanol was then used. 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

We found a process that scavenges H2O2. Incubation was carried out for 10 minutes after a 4 mL extract solution was mixed with 0.6 mL of a solution of about 4 mM H2O2 (prepared in PBS). A spectrophotometer was used to test the solution's absorbance at 230 nm in contrast to a control solution. H2O2 percentage as a measure of free radical scavenging potency. Reduced H2O2 levels were indicative of more effective free radical scavenging (Ullah and Khan, 2008).

The toxicity test for brine shrimp

An artificial seawater combination made with a commercial salt blend and double distilled water was used to hatch brine shrimp eggs in a shallow rectangular plate that measured 22 × 30 cm. Before adding the 50 mg eggs to one of the two unequal sections, a plastic divider with 2 mm holes was clamped into the dish to create a divider. The bigger compartment was then darkened, while the smaller compartment was kept lit. The phototropic napulii were collected by pipetting them from the lit side, which was separated from their shells by the divider, after 48 hours. Each sample vial was filled with 5 mL of fake seawater and twenty shrimp were put to it using a pipette. With a lit backdrop, you can count the napulii in the pipette stem. With each vial, we added a drop of dry yeast suspension—a mixture of 3 mg in 6 mL of artificial seawater—as feeding. A light source was used to keep the vials illuminated. Using three different magnifying glasses, we counted the survivors and calculated the percentage of mortality at each dosage and control after 24 hours. The statistics were adjusted using Abbott's technique in every instance where control deaths occurred.:

% 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). In terms of flavonoid content in plant extracts, the findings showed that M. arvensis (12.21 mg/g), M. longifolia (14.17 mg/g), and M. spicata (avital parts) had the greatest levels (15.05 mg/g). For the three species of Malassezia, the corresponding percentages of total phenolics in milligrams per gram of sample extract were 20.32%, 25.19%, and 26.637, respectively. The saponin content of M. arvensis was 0.04 mg/g, whereas that of M. longifolia was 0.01 mg/g, 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)

Chemistry of mentha essential oils using gas chromatography mass spectrometry

Tables 3 and 4 show the findings of the GC-MS analysis of the mentha oils, which were used to determine their chemical components. We found seven major components in the essential oil of M. arvensis, and they make up 99.1 percent of the oil. Eucalyptol(5.3%) and carvone (84.3%) were the most prevalent chemicals. The essential oil of Melaleuca longifolia consisted of six main components, accounting for 98.2% of the total oil content. Pilegone(4%), borneol(4.6%), menthol(3.3%), menthone(9.7%), 2-Hydroxy-3-(3-methyl-2-butenyl)-3-cyclopenten-1-one(8.4%), and piperitone oxide (54.2%) were the main components. There are four main components that make up 96.5% of the essential oil of Melissa spicata. Eucalyptol (12.0%), α-pinene (9.7%), borneol (9.5%), and 2-Hydroxy-3-(3-methyl-2- butenyl)-3-cyclopenten-1-one (47.1%) were the main components.

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

The compounds that were found were listed according to the elution sequence that was used on the DB-5 GC column. In M. arvensis, the primary chemical is carvone, in M. longifolia it is piperitone oxide, and in M. spicata it is 2-Hydroxy-3-(3-methyl-2-butenyl)-3- cyclopenten-1-one, with relative percentages of 84.3%, 54.2%, and 47.1%. Some compounds were also found to be abundant in plants. To demonstrate, eucalyptol concentrations ranged from the lowest (0.4%) in M. longifolia to the greatest (5.3%) in M. arvensis. The concentration was highest in M. spicata (12%). In a similar vein, M. longifolia and M. spicata both had 0.7% germacrene-D, whereas M. arvensis only had 2.5%. As for borneol, M. spicata contained 9.5% and M. longifolia 4.6%. Caryophyllene was present in all of the plants, however the concentrations ranged from 0.6% to 1.7%.

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

At a concentration of 300 µg/mL, the extracts of M. arvensis, M. longifolia, and M. spicata considerably reduced H2O2 by 34.9%, 29.66%, and 37.9% correspondingly, as shown in Table 7. As shown in table 9, the extracts of M. arvensis, M. longifolia, and M. spicata demonstrated a dose-dependent increase in H2O2 scavenging activity.

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

Antibacterial activity against Gram+ bacteria, such as Bacillus cereus (KX262674) and Staphylococcus aureus (KX262674), and Gram-negative bacteria, such as Escherichia coli (ATCC 10536), Salmonella typhi (ATCC 6539), and Pseudomonas aeruginosa (ATCC 9027), was assessed in the essential oils of Meravicinia arvensis, Meravicinia longifolia, and Meravicinia spicata. At different concentrations, the results show that essential oils significantly inhibit the growth of bacteria. The antibacterial activity of M. arvensis was shown to be higher at dosages of 100 µg/mL and 200 µg/mL compared to streptomycine, with inhibition zones of 9.87, 11.31, (9.36, 11.43), and (6.78, 10.87) mm for S. aureus, B. cereus, and S. typhi, respectively. Similarly, at doses of 100µg/mL and 200µg/mL, respectively, M. longifolia exhibited significant antibacterial activity against B. cereus, with inhibition zones of 8.06 mm and 8.25 mm. After being subjected to doses of 100 µg/mL and 200 µg/mL, 8.18 mm and 9.34 mm, respectively, of M. spicata essential oil showed very potent antibacterial action 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

An experiment was conducted to determine the cytotoxicity of extracts from three different plants: Moringa arvensis, Moringa longifolia, and Moringa spicata. The results showed that the cytotoxicity of the extracts rose as the concentration of the plant extracts increased, but overall, the extracts shown reduced 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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