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Research article

Comparative assessment of phytochemical content and antioxidant activity of *mentha arvensis* procured from Summit and Rivulet

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ABSTRACT

Plants and herbs have been used as sources of safest compounds in the management of diseases as well as in the restoration and fortification of body structures in ancient medical systems such as Ayurvedic, Unani, and traditional Chinese medicine. The major motive for incorporating herbs in healthcare is to develop a healthy relationship with the body's chemistry while eschewing off-target and unwanted side effects produced by synthetic medications. A surge in the human population, an insufficient supply of drugs, a non-manageable cost of treatments, and a surge in antimicrobial resistance to presently used drugs have forced the pharmacognostic researchers to trace out novel plant-based bioactive compounds to be used as therapeutic agents against innumerous kinds of diseases and disorders. In this study, we include two different samples of *Mentha arvensis* also known as Pudina or wild mint procured from two different locations in Uttar Pradesh region of India. One sample was taken near the edges of flowing stream in Meerut (Sample 1) and the other sample was taken from hilltop near Dehradun (Sample 2). The major objectives of this study were to analyse the effect of continuous water exposure as cold stress on the phytochemical properties, comparative antioxidant potential as well as antibacterial action of the *Mentha arvensis* leaves procured from Summit and Rivulet. The dried and powered leaves were extracted via four solvent systems of varied polarity viz, Methanol [M], Chloroform [C], Acetone [A], and Water [AQ]. Comparative phytochemical analysis including total phenolic content, total flavonoid content, and antioxidant potential along with antibacterial activity of both samples was evaluated from all extracts. Our results revealed that continuous exposure of water as in Sample 1 has a prominent impact on the phytochemical properties, antioxidant potential and anti-bacterial effect of the *Mentha arvensis* leaves. GC-MS studies of *Menta arvensis* essential oil revealed the presence of various bioactive compounds most of which are secondary metabolites, and we attribute antioxidant and anti-bacterial activity of *Menta arvensis* leaves to these compounds.

Keywords: *Mentha arvensis*; Phytochemicals; Secondary metabolites; Leaf extract; Antioxidants; Antibacterial activity.

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INTRODUCTION

The existence of life is not feasible without plants. In fact, all life forms on this planet are directly or indirectly dependent on plants for carrying on their life processes. Nature has endowed mankind with comprehensive management of health care through herbs, spices, and medicinal plants. Plants are considered the foundation of medicine and are attributed to spiritual and almost supernatural healing abilities. Herbal medicine has a long history in human history, dating back to the dawn of recorded human civilization. Numerous shreds of evidence are available that advocate the use of plant-based chemicals in the treatment of diseases [1]. There is an invigorated curiosity among the researchers and practitioners in herbal products. Phytochemical research provides a one-of-a-kind opportunity to develop new medications to treat illnesses and disorders that have taken a toll on human health. Ethnobotany and ethnopharmacology have emerged as key sources of knowledge in the hunt for novel drugs. Novel phenolic and polyphenolic compounds that show potential as therapeutic agents for a range of illnesses are the focus of current phytochemical research [2]. It is important for scientific journals to enable health care professionals to work diligently to explain the key active ingredients derived from medicinal plants. Furthermore, to define their role in the treatment of current diseases, as well as how they

can be used to create or synthesize more successful drugs. Well-known drugs of plant origin are atropine, aspirin, artemisinin, colchicine, quinine, quinidine, reserpine, digoxin, tubocurarine, ephedrine, physostigmine, pilocarpine, morphine, Taxol, vincristine, and vinblastine. Medicinal plants such as Aloe, St. John’s Wort, Ginkgo Garlic Ispaghula, Ginseng, Tulsi, Neem, Turmeric and Ginger are a few instances of herbs or plants which are achieving popularity amongst modern practitioners and this trend is expected to increase [3]. Mint (Mentha species) belonging to the Lamiaceae family have also been revealed to have various health benefits, including, anti-inflammatory, anti-diabetic, anti-obesity, anti-hypertensive, antibacterial, cardioprotective effects etc. due to its antioxidant potential, presence of essential oils, minerals, vitamins, and a variety of other compounds. One of Mentha species namely Pudina (*Mentha arvensis* L.) is a potent aromatic and rejuvenating medicinal herb that grows up to 60 cm tall, with a cylindrical stem, and simple, opposite type leaves which are 2-5.2 cm long [4].

Pudina has been revealed to possess innumerous types of benign compounds that may be utilized in the management of different diseases and disorders. The leaves act as thermogenic, acrid, dentifrice, stimulant, anodyne, deodorant, vulnerary, anti-helminthic, sudorific, antispasmodic, contraceptive, febrifuge, as well as have been shown to possess other medicinal properties. The phytochemical analysis has revealed that the leaves are loaded with phenolic and polyphenolic compounds that include flavonoids, saponins, alkaloids, tannins, terpenoids and cardiac glycosides as well as essential aromatic oils [5,6]. The leaves' oil content produces 40-50 percent menthol, an antibacterial, stimulant, carminative, diuretic, and astringent used to treat skin infections and hair problems. This essential oil of menthol is also employed in the pharmaceutical, perfumery, and food sectors. Leaves are also flavonoid rich especially in quercetin which imparts the free radical quenching properties [7]. Such a benign property makes this herb a potent oxidative stress reducer which develops due to free radical accumulation and deemed to be the major cause of fatal diseases. Terpenes have also been reported to be possessed in different proportions in *Mentha arvensis*. [8]. It also has vitamin K, thymol, eugenol, methoxide and isorhoifolin among other compounds. *Mentha arvensis* have been traditionally employed in the management of hypertension and ischemia in the past. The juice of the leaves is currently being used in the management of diarrhoea, dysentery, gastrointestinal issues, asthma, jaundice, and allergies. Recent research is revealing the great importance of its leaves in the treatment of liver and spleen problems, dyspepsia, rheumatic aches, arthritis, and swollen joints. Mint also aids digestion and metabolism, particularly of lipids, and has been widely recommended for the treatment of obesity in recent years [9, 10]. In this paper we addressed the alterations in the qualitative phytochemical properties, antioxidant properties, and anti-bacterial activities in the two samples of *Mentha arvensis* leaves, one of which was collected from the hilltop and another from the edges of flowing stream. The main motive of this study was to explore the impact of continuous water exposure on the phytochemical properties, antioxidant potential as well as antibacterial action of *Mentha arvensis* leaves.

MATERIAL AND METHODS

Assemblage of Plant material

Two samples of *Menth arvensis* leaves were procured from their natural habitat, from two different locations in Uttar Pradesh region of India. One sample was collected near the edges of flowing stream in Meerut (Sample 1) and the other sample was taken from hilltop near Dehradun (Sample 2). A taxonomist from Dehradun's Forest Research Institute (FRI) recognised the samples.

Chemicals

The reagents used for the study were acetone, methanol, chloroform, Mayer’s Reagent, Ammonia Solution, ferric chloride, concentrated H_2SO_4, quercetin, ascorbic acid, gallic acid, lead acetate, 2, 2-diphenyl-1-picryl-hydrazyl (DPPH), sodium carbonate, potassium acetate, Folin-Ciocalteau’s phenol reagent, aluminum chloride, hydrogen peroxide, Mueller-hinton agar (MHA), nutrient broth, peptone water, procured from Hi-media, Merck, and sigma. All reagents incorporated in this study were of analytical grade.

Preparation of plant extract

After collecting the leaves, they were rinsed thoroughly with tap water followed by sterilized distilled water. Afterwards, the leaves were dried in shade at room temperature for 20-25 days, then homogenized into a fine coarse powder with the aid of an electric blender, and lastly kept in airtight containers until needed. Individually, the fine leaf powder was extracted using the maceration process and Soxhlet extraction with several organic solvents of varying polarity like Methanol [M], Acetone [A], Chloroform [C], and Water [AQ]. In the conical flasks, dry powder (10 g) was poured in 100 mL of each solvent, plugged with cotton wool, and shaken at 120 rpm for 38 hours on a rotary shaker. After 38 hours, the extract was filtered with sterilized Whatman Filter Paper Grade No 1. The supernatant was collected while the solvent was evaporated. To calculate the extractive yield, the produced greenish gummy exudates residues were weighed using a balance. The crude extract was kept in sealed Eppendorf tubes at 4°C and thereafter undertaken for phytochemical analysis as well as assessed for potential antioxidant and antibacterial properties [11].

Phytochemical preliminary screening

Traditional procedures reported by Trease and Evans in 2002 were used to test the extracts for phytochemicals [12]. Assays for presence of phenols, flavonoids, alkaloids, saponins, Glycosides, Terpenoids, Anthraquinones, and Tannins were carried out.

Assessment of total phenol content

The procedure of determining the quantity of phenolic content in samples is termed as TPC activity. Plant-derived phenolic compounds have redox characteristics, which allow them to act as antioxidants [13, 16]. As reported by McDonald et al [13], total phenolic content was determined using Folin-reagent Ciocalteu's test. 1 mL extract and 0.1 mL Folin-Ciocalteau's reagent (0.5 N) were mixed together and incubated at 25 ° C for 15-20 minutes. After that, 3 mL saturated sodium carbonate solution was added and incubated at room temperature for another 30 minutes. Finally, the absorbance was taken at 760 nm. As a standard, Gallic acid was employed for which a standard curve was developed beforehand. Gallic acid equivalent (mg g^{-1} of extracted component) was used to express the total phenol levels.

Assessment of total flavonoid content

Aluminum chloride colorimetric assay was used to assess the total flavonoid content [14]. The reaction mixture was incubated at room temperature for half an hour, with 1.0 ml of sample (1 mg/ml), 1.0 ml methanol, 0.5 ml of (1.2 percent) aluminium chloride, and 0.5 ml of (120 mM) potassium acetate in a final volume of 3 ml. At 415 nm, the absorbance of all the samples was checked with the aid of photo-spectrometer. As a standard, quercetin solutions in methanol ranging from 50 to 300 µg/ml were used to create the calibration curve. The content of flavonoids is measured in Quercetin equivalents (mg g^{-1} of extracted compound).

Antioxidant assay

The free radical scavenging activity of *Mentha arvensis* leaf extracts was assessed using the 2, 2- Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay [10, 14]. In methanol oxidized DPPH produces a rich violet color. An antioxidant molecule transfers an electron to DPPH, resulting in its reduction and a shift in hue from deep violet to yellow. All extracts were measured for hydrogen donating or radical scavenging ability [15]. Extracts were diluted to obtain concentrations of 0.1, 0.3, 0.6, 0.8 and 1.0 mg/ml. 3 ml of ethanolic DPPH solution was combined with diluted solutions of extracts (1 ml each) (DPPH, 0.004 %). After half an hour of incubation at room temperature, the absorbance at 517nm was measured using a UV-Visible Spectrophotometer to assess the reduction of the DPPH free radical. As a control, absorption of a blank sample with the same amount of ethanol and DPPH solution was made and analyzed. As a positive control, ascorbic acid was employed whose absorbance was detected at different concentrations (0.1-1.0mg/ml). The experiment was carried out three times in all. Equation (1) was involved to derive the percentage inhibition of all samples. The data were reported as mean values with a standard deviation of three (n = three).

$$\% \text{ inhibition} = \frac{\text{(Control absorbance – sample absorbance)}}{\text{Control absorbance}} \times 100 \quad \text{equation (1)}$$

Antibacterial assay

The antibacterial potential of *Mentha arvensis* leaf extracts of both samples were tested against Gram-positive bacteria *Bacillus cereus* 10451 (BC), and Gram-negative bacteria *Escherichia coli* GIM1.708 (EC), *Escherichia coli* DH5-Alpha (ECα), *Salmonella enteritidis*10982 (SE). Disc diffusion assay was incorporated to assess the antibacterial potential of different solvent extracts of both the samples of *Mentha arvensis*. One ml of fresh bacterial culture was aseptically swab cultured on Mular -Hinton agar along with introduction of antibiotic discs as positive control, DMSO at a concentration of 15% as a negative control and samples. Sterile discs were aseptically dipped in the different extracts and placed on the agar plates. Antibiotics chloramphenicol and tetracycline were used as positive control. The MHA plates were incubated for 24 hours at 37^0 C and thereafter looked for the zones of inhibition [17].

GC-MS analysis of *Mentha arvensis* essential oil

The leaves were pulverized in an electric grinder and utilised in the hydro distillation technique to obtain essential oil. The essential oil was extracted using a Clevenger-type device using hydro distillation. *Mentha arvensis* leaves (80 g) were placed in a 1 l round bottomed flask with 500 ml of water. The ingredients were properly mixed, and the flask was left at room temperature overnight. The flask was put on the heating mantle and equipped with Clevenger-type gear. For 10 hours, the contents were refluxed and i n a conical flask, the essential oil (top layer) containing minimal water was collected separately. The same approach was used to treat 20 batches of essential oil in order to obtain high yields. Diethyl ether was used to partition the essential oil three times (100, 50 and 50 ml) [19]. The oil-containing diethyl layer (upper layer) was passed through anhydrous sodium sulphate to eliminate any residues of water. Diethyl ether was removed from the oil, which was then kept at 4 °C in dark glass vials for GC-MS examination.

RESULTS

Extractive yield result

The recovery of antioxidant phytochemicals requires the extraction. The solvents utilized and the chemical property of materials is the critical parameters under the same time and temperature conditions [18]. There are numerous findings in the

literature that show that extraction yield varies depending on the solvent and type of plant material to be extracted [19].

Table 1: Extractive yield of two samples of *Mentha arvensis*

Yield w/w (%)				
Mentha arvensis	Methanol [M]	Chloroform [C]	Acetone [A]	water [AQ].
Sample 1	9.61	1.98	4.10	3.26
Sample 2	9.52	2.10	3.99	3.22

Table 1 shows the varying levels of extractive yield of both samples in four distinct solvents: acetone, methanol, chloroform, and water. Among the different solvents, the extractive yield varied between two samples of *Mentha arvensis* (Sample 1 retrieved from the borders of a flowing stream and Sample 2 gathered from a hilltop). Methanolic extracts in both samples showed the highest extractive yield than other extracts. Lowest extract yield was seen in chloroform. Overall extractive yield was slightly higher in case of Sample 1.

Qualitative phytochemical screening result

All the extracts were undertaken for qualitative phytochemical analysis using different tests mentioned above. The results of the phytochemical analysis of secondary metabolites of these two samples showed the presence of flavonoids, phenols, alkaloids, terpenoids, glycosides, and tannins. Saponins and anthraquinones were absent in all extracts of both samples (Table 2 and 3). All tests for secondary metabolites showed a qualitative elevation in case of extracts of sample 1.

Table 2: Phytochemical screening of *Mentha arvensis* (Sample 1)

Phytochemical constituents	[Distilled water] Extract	[Acetone] Extract	[Chloroform] Extract	[Methanol] Extract
Flavonoid	+	++	-	++
Alkaloid	++	++	++	++
Phenols	+	-	-	+
Saponin	-	-	-	-
Glycoside	-	+	-	+
Terpenoid	+	+	+	++
Anthraquinone	-	-	-	-
Tannin	++	+	++	++

Table 3: Phytochemical screening of *Mentha arvensis* (Sample 2)

Phytochemical constituents	[Distilled water] Extract	[Acetone] Extract	[Chloroform] Extract	[Methanol] Extract
Flavonoid	+	+	-	+
Alkaloid	+	+	++	+
Phenols	+	-	-	-
Saponin	-	-	-	-
Glycoside	-	+	-	+
Terpenoid	-	+	-	-
Anthraquinone	-	-	-	-
Tannin	+	+	++	+

+ -Present, + + Present in abundance, - Absent

Total phenolic content result

The phenolic content of all 8 leaf extracts was analyzed by incorporating the Folin–Ciocalteu's reagent as a baseline. All the results were obtained from a calibration curve (y = 0.0022x + 0.13, R^2 = 0.9981) of gallic acid (0–1000 µg/mL) (Figure 1) and expressed in gallic acid equivalents (GAE) per gram dry extract weight. The total phenol content in both samples ranged from 27.88 to 49.13 (Table 4 & 5). Each value is the average of three measurements ± (standard deviation).

Figure 1: Standard curve of Gallic acid (765 nm)

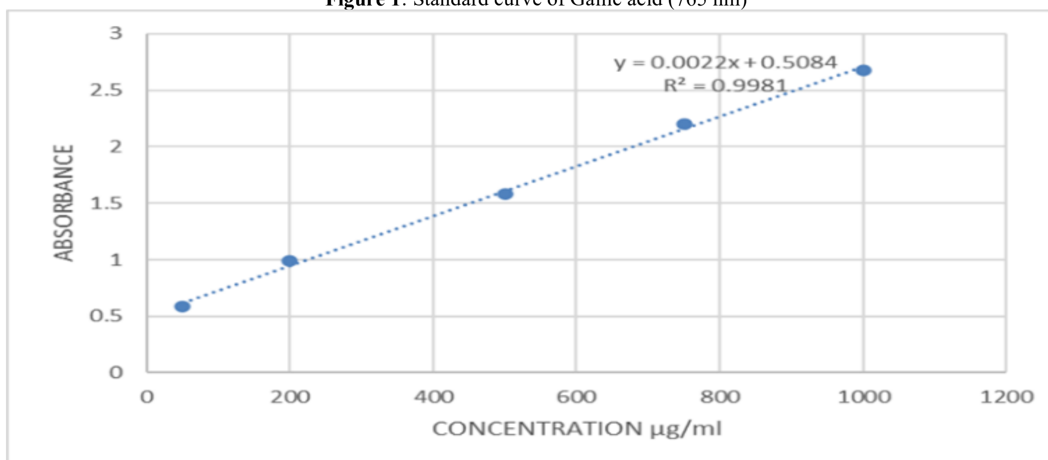

Table 4: Total phenolic contents in the extracts (Sample 1) expressed in terms of gallic acid equivalent (mg of GA/g of extract).

Sample 1 extracts	Total phenolic content mg of GA/g of extract (Mean ± S.E.)
Water	41.78 ± 0.396
Acetone	38.98 ± 0.533
Chloroform	27.87 ±0.673
Methanol	49.13 ± 0.861

Table 5: Total phenolic contents in the extracts (Sample 2) expressed in terms of gallic acid equivalent (mg of GA/g of extract).

Sample 2 extracts	Total phenolic content mg of GA/g of extract (Mean ± S.E.)
Water	39.64 ± 0.292
Acetone	35.05 ± 0.843
Chloroform	29.53 ± 0.764
Methanol	46.20 ± 0.391

The concentration of phenolic compounds was higher in methanol extracts and lower in chloroform extracts in both samples. But in almost all the four extracts of sample 1 total phenol content (mg of GA/g) was slightly higher than sample 2 except for chloroform extract.

Total flavonoid content result

The flavonoid concentration was expressed in terms of Quercetin equivalents (mg of Q/ g of extracted compound) for which the standard curve equation was (y = 0.002x - 0.0076, R^2 = 0.9988) (Figure 2). The concentration of flavonoids in both samples

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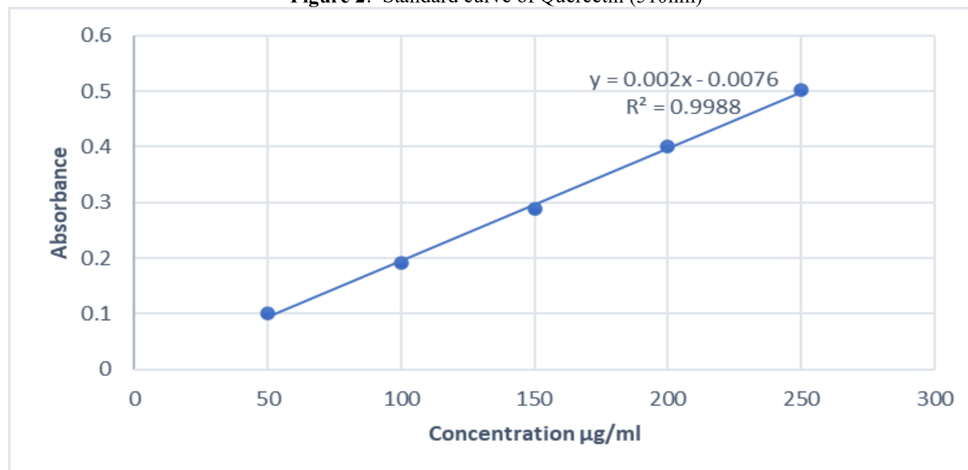
ranged from 10.56 to 56.13 (Table 6 & 7). Each value is the average of three measurements ± (standard deviation). The flavonoid content was higher in methanol extracts and lower in acetone & chloroform extracts in both samples.

Figure 2: Standard curve of Quercetin (510nm)



This result revealed that total flavonoid content is overall higher in the extracts of Sample 1.

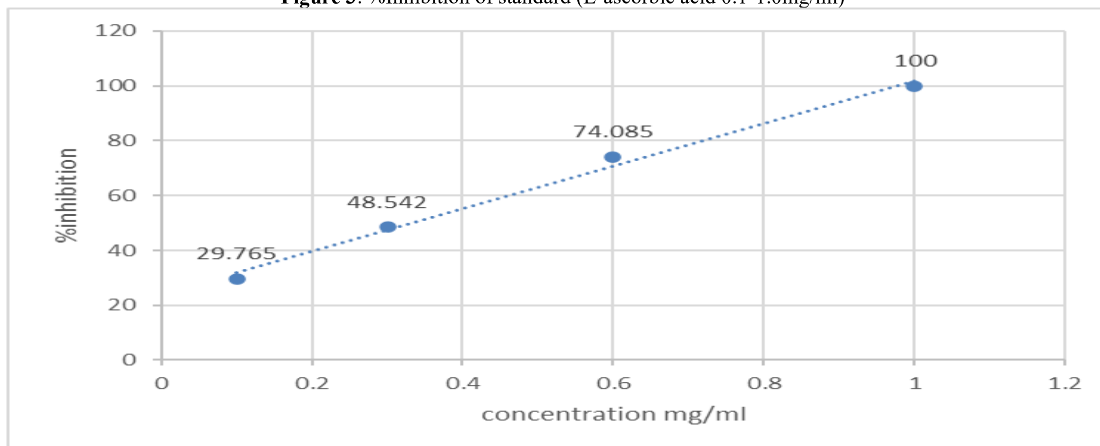
Table 6: Total Flavonoid contents in the extracts expressed in terms of Rutin equivalent (mg of RU/g of extract).

Sample 1 extracts	Total Flavonoid content mg of Q/g of extract (Mean ± S.E.)
Water	19.73 ± 0.396
Acetone	16.98 ± 0.531
Chloroform	11.87 ± 0.820
Methanol	56.13 ± 0.451

Antioxidant activity result

The DPPH free radical scavenging activity of Methanolic [M] extract, Chloroform [C] extract, Acetone [A] extract, and water [AQ] extract of sample 1 and sample 2 were detected and juxtaposed with ascorbic acid as standard (Figure 3).

Figure 3: %Inhibition of standard (L-ascorbic acid 0.1-1.0mg/ml)



The percentage inhibition (% inhibition) of all 8 samples at various concentration (0.1- 1.0 mg/ml) as well as standard ascorbic acid (0.1-1.0 mg/ml) was calculated and plotted in graphs using Microsoft Excel 2018. Our result revealed that % inhibition of methanol extracts in both samples is higher among all while juxtaposing with standard L-ascorbic acid (Figure 4 & 5). Sample 1 extracts showed more antioxidant potential than the extracts of Sample 2.

Figure 4: Graphical representation of free radical scavenging activity of extracts compared to standard L-ascorbic acid at concentration of 1mg/ml.

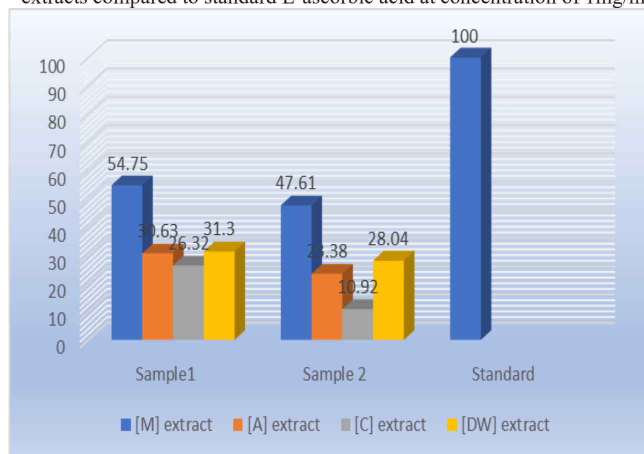


Table 7: Total Flavonoid contents in the extracts expressed in terms of Rutin equivalent (mg of RU/g of extract)

Sample 2 extracts	Total Flavonoid content mg of Q/g of extract
Water	17.77 ± 0.211
Acetone	12.05 ± 0.365
Chloroform	10.53 ± 0.892
Methanol	51.20 ± 0.529

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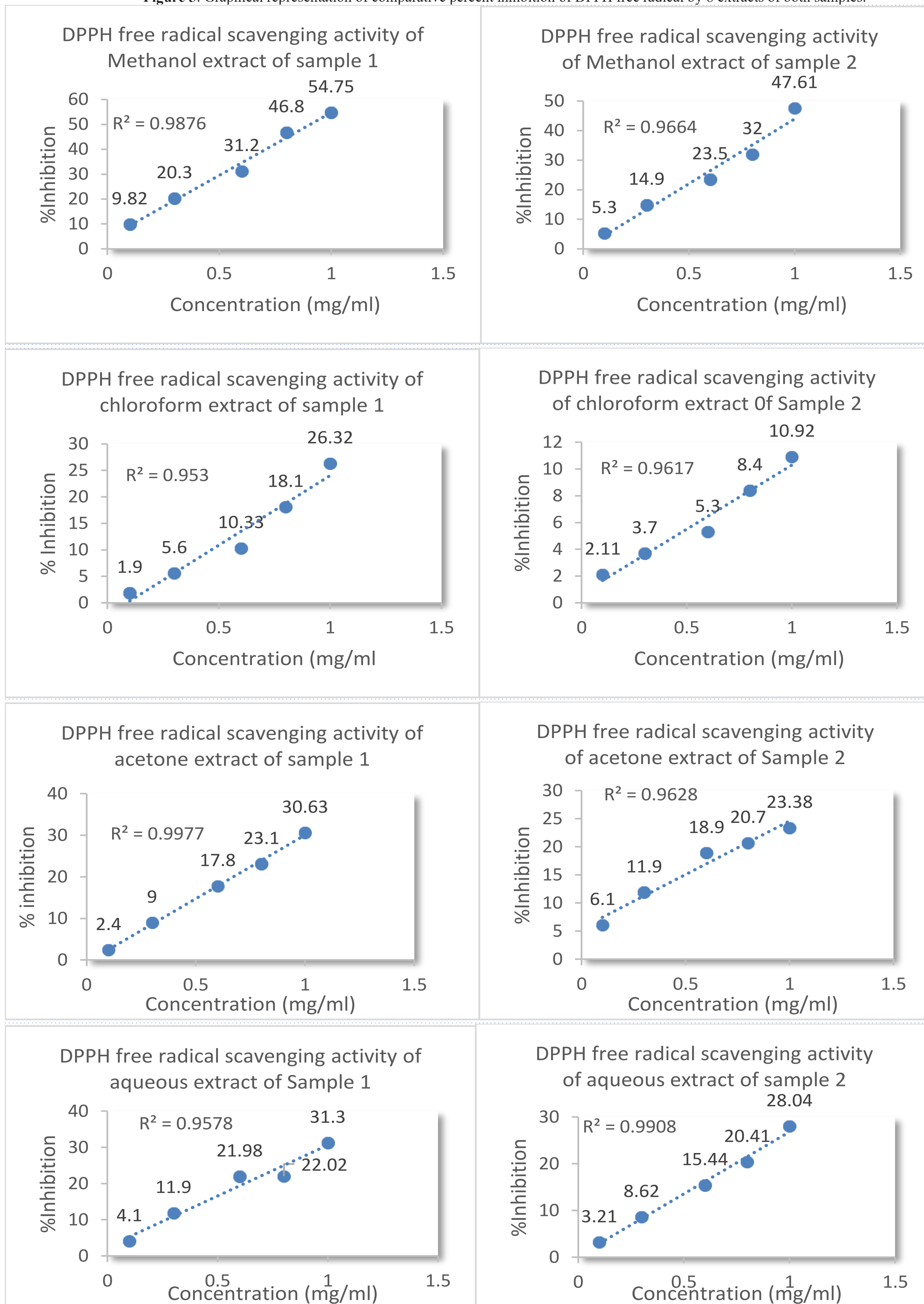
Figure 5: Graphical representation of comparative percent inhibition of DPPH free radical by 8 extracts of both samples.



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Antibacterial activity result

After 24 hours of incubation period, zones of inhibition developed around the positive control discs and the samples showing potent antibacterial action. These zones were measured to assess antimicrobial activity of *Mentha arvensis* leaf extracts (Figure 6).

Table 8: Antimicrobial activity

Zone of inhibition mm				
Test strain	**Methanol extract**	**Water extract**	**Chloroform extract**	**Acetone extract**
Bacillus cereus	S1 [11.2 ± 0.8] S2 [10.9 ± 0.9]	S1 [9.2 ± 1.1] S2 [9.0 ± 0.7]	S1 [N] S2 [7.5 ± 0.8]	S1 [N] S2 [N]
Escherichia coli GIM1	S1 [12.1 ± 1.2] S2 [11.9 ±O.9]	S1 [9.2 ± 0.8] S2 [9.4 ± 1.1]	S1 [N] S2 [N]	S1 [8.1± 1.3] S2 [N]
Escherichia coli DH5-Alpha	S1 [N] S2 [N]	S1 [8.3 ± 1.2] S2 [7.9± 0.7]	S1 [N] S2 [N]	S1 [8.6 ± 0.9] S2 [N]
Salmonella enteritidis	S1 [10.3±1.2] S2 [1O.0±1.1]	S1 [N] S2 [N]	S1 [N] S2 [N]	S1 [9.1 ± 1.3] S2 [9.0 ± 1.2]
Values in triplicate determination (n=3) ± standard deviations, N- no zone of inhibition, S- Sample 1, S2- Sample 2				

Methanolic and aqueous extracts of sample 1 showed highest antibacterial activity against selected strains. Table 8 shows how the results were interpreted.

GC-MS analysis of essential oil derived from leaves

The essential oil of Mentha leaves was separated using gas chromatography-mass spectrometry (GC-MS) (QP2012 Plus, Shimadzu, Japan) and a Rtx-5 MS capillary column (0.30 m film thickness). The injector was kept at 265 °C and operated in split injection mode for 2 minutes with the split valve closed. Helium gas was employed as the carrier gas at a constant pressure of 69 kPa. The column oven was originally kept at 65 °C for 5 minutes before being elevated to 210 °C at 3 °C/min and subsequently to 280 °C at 10 °C/min. The interface temperature was 270 °C, and the ionization mechanism was electron impact (70 eV). During the scan, the mass selective detector was turned on. The relative size of each peak measured by the flame ionization detector was used to quantify the proportion of components in the essential oil (Figure 7). Peak identification was accomplished by comparing the mass spectra to data from the WILEY8 and NIST08 libraries. Table 9 lists the constituents of fractioned Mentha oil.

Figure 6: A typical gas chromatogram of the chemical ingredients of Mentha leaf essential oil

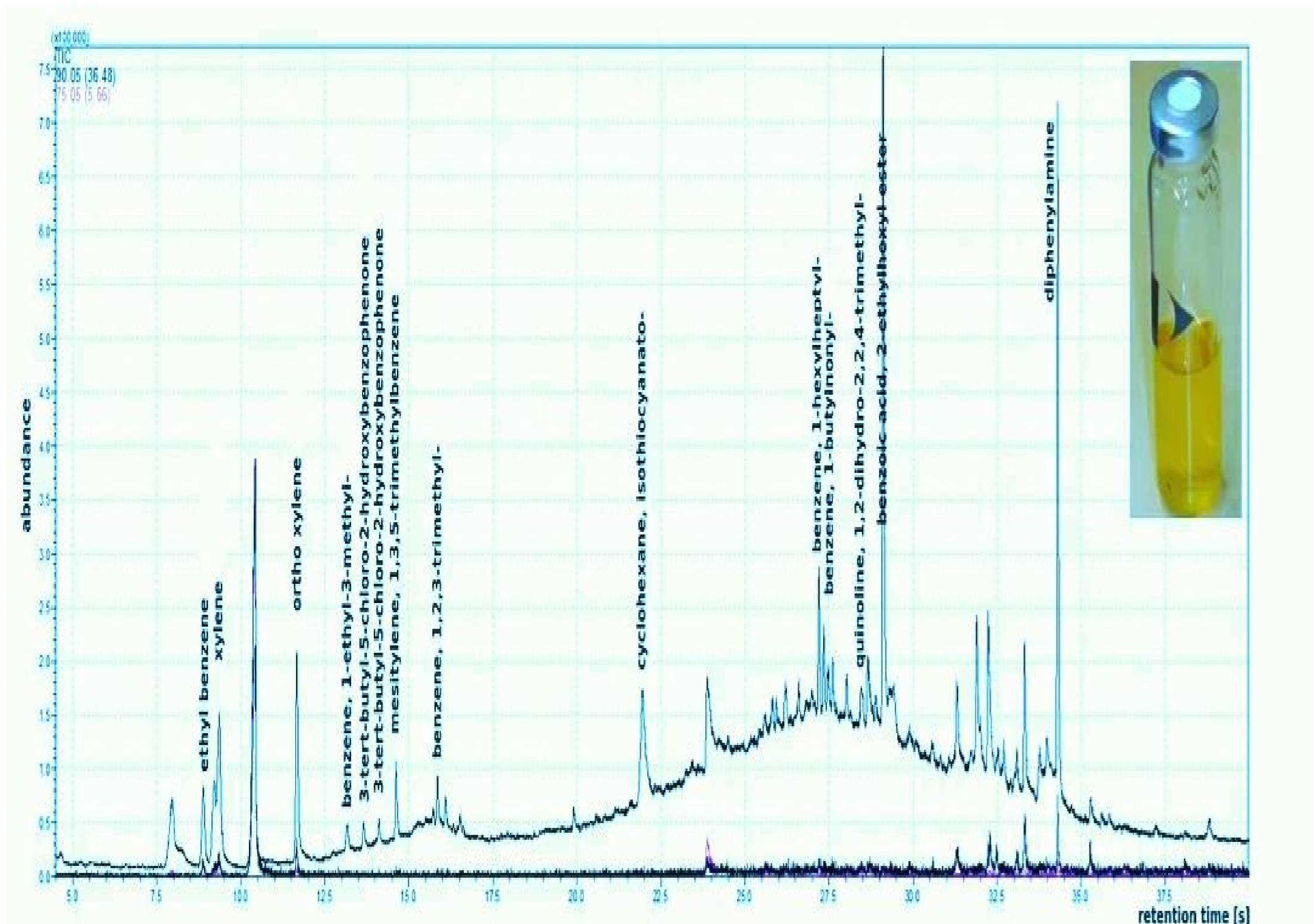

Figure 7: Antibacterial effects of *Mentha arvensis* methanol extracts of sample A and B.

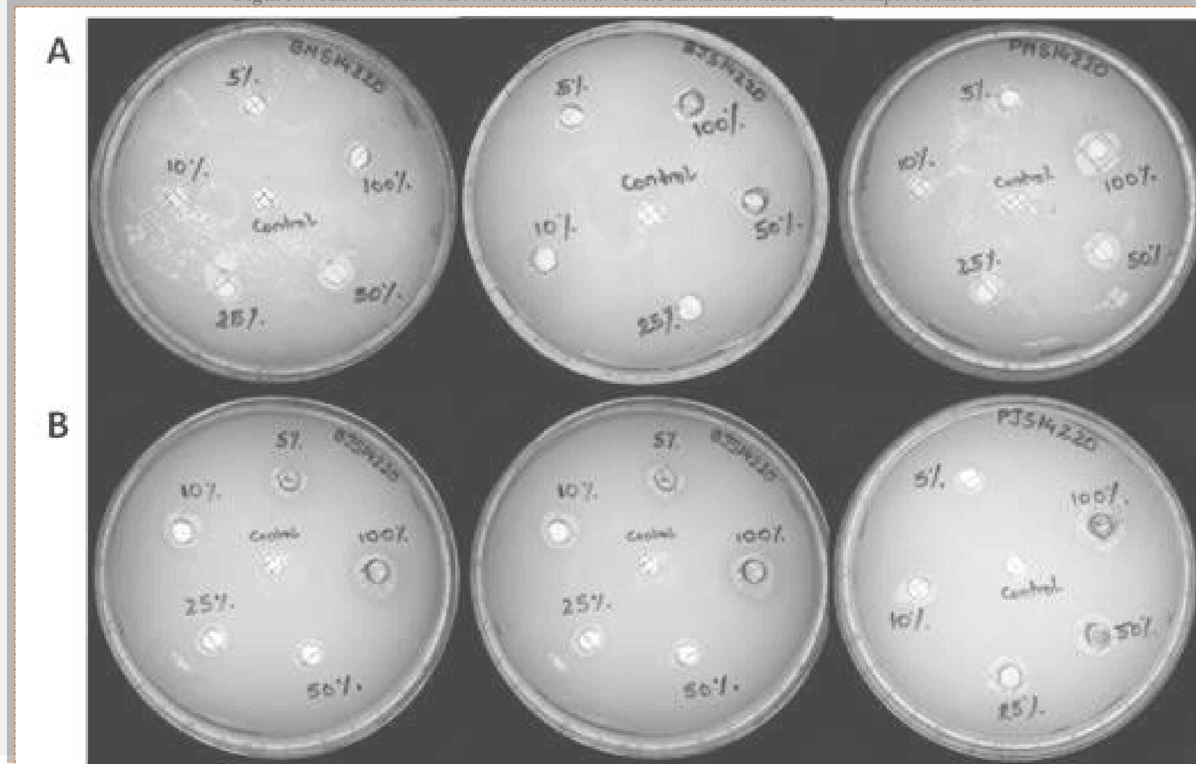

Table 9: GC–MS revealed bioactive compounds of Mentha leaf essential oil

Name	Retention time	Percent area
Eucalyptol	5.103	2.35
2-Propen-1-one	5.735	1.50
β-Pinene	6.846	3.74
Eugenol	7.957	5.54
Carvomenthone	9.957	4.21
3-Hexen-1-ol	11.635	0.13
α-terpeniol	12.624	0.64
1-Phenyloxirane	13.986	0.86
Linalool oxide	13.542	1.58
Limonene	15.423	1.56
Mentha-2,8-dien-1-ol	16.123	0.46
Isopulegol	18.534	0.87
Piperitone	19.967	5.68
Menthone	20.534	3.57
Cardinene	21.756	9.56
2-Furanmethanol	23.234	1.66
Cyclopentanone	24.465	2.57
Hexanoic acid	25.454	1.35
Pulegone	25.968	0.68
Isopulegol acetate	26.231	2.79
Caryophyllene	27.345	2.57
Cyclopentanone	29.009	4.65
Neomenthyl acetate	25.053	6.53
Isomenthyl acetate	25.531	0.95
Isomenthone	26.286	0.09
Isoeugenol	27.005	3.85
β-Bourbonene	30.463	0.64
Methyl acetate	31.456	0.95
Thujone	31.986	2.84
Menthol	32.087	41.63
Dipentene,	34.987	0.63
Phellandrene	35.761	1.53
Carvacrol	38.643	2.96
Aromadendrene	39.009	15.98

DISCUSSION

The leaves of *Mentha arvensis*, popularly known as Pudina or wild mint, were picked in October 2021. The current study uses two different *Mentha arvensis* samples collected from two different places in India's Uttar Pradesh region. Sample 1 was taken near the edge of a flowing stream in Meerut, and Sample 2 was taken from a hilltop near Dehradun (Sample 2). Alterations in phenotype among organisms are directly influenced by gene expression as well as environmental parameters, resulting in inter-specific variance [19]. Variations in agro-climatic circumstance have been shown to possess significant impact on antioxidant potential and phytochemical diversity of herbs and plants. Continuous biotic or abiotic stressors, such as pathogenic microbe invasion, high temperature, humidity shift, snowfall, prolonged water exposure, and cold stress conditions, cause plants to produce more phytochemicals as a self-defense strategy [21]. According to Kumar et al., 2016 [22], samples from colder regions and semi-arid regions have higher antioxidant activity, which

supports the theory that plants create more phytochemicals when they are stressed. Plants under stress produced more flavonoids, anthocyanins, saponins and mucilaginous compounds, according to research [23]. Low temperature is related to enhanced unsaturated fatty acid levels, which leads to the fast development of antioxidants as a self-defense strategy to combat environmental stresses [24]. Our current study is also in harmony with these studies and demonstrated that all the parameters including, total flavonoid content, total phenol content, antioxidant action as well as antibacterial activity was higher in *Mentha arvensis* leaves grown on the edges of a flowing stream [Sample 1]. Also, all tests for secondary metabolites showed a qualitative elevation in case of extracts of sample 1. Solvent type according to its polarity and extraction procedure also affects extraction yield and phytochemical concentration. [18, 19]. One study had shown that methanolic extract resulted in the highest extraction yield and a more complex phenolic content. [25]. The present work also revealed that methanolic and water extracts demonstrated best extraction yield, high secondary metabolite extraction, high flavonoid content, high antioxidant potential and potent anti-bacterial action in the sample that was under cold stress due to water exposure. As a matter of fact, it is important to quantify the effect of temperature variation, continuous water exposure, cold stress, soil moisture, precipitation levels, and fertility on medicinal herbs by growing the plants in these conditions and the analyzing how these geo-climatic factors impact the phenology, nutrient value, antioxidant potential, and secondary metabolite levels. Our findings show that continuous water exposures as a stress have a significant impact on the phytoconstituents and antioxidant potential of *Mentha arvensis* leaves. Finally, we performed GC-MS analysis of Menta oil which revealed a number of bioactive compounds. We attribute the important bioactive functions of *Mentha arvensis* leaves to these bioactive compounds. Various studies have reported the same compounds in the Mentha oil and in this way our current study is in harmony with such studies [26, 27, 28].

CONCLUSION

Leaves of *Mentha Arvensis* are promising source of benign phytochemicals as well as have potent antioxidant and antibacterial activity. The current study showed that Phyto-constituents and antioxidant activity in distinct *Mentha arvensis* samples are affected by a variety of regional and environmental conditions. According to current study continuous water exposure has significant effect on total flavonoid and total phenolic content along with antioxidant potential of *Mentha arvensis* leaves procured from two different locations. Screening the antioxidant potential of *Mentha arvens* is herbs in relation to their activity in various geographical regions might aid in the selection of locations for their mass production to improve its pharmacological and marketing value. The antioxidant and antibacterial qualities of *Mentha arvensis* could be investigated for uses in the food, medical, and cosmetic industries. GC-MS analysis of Mentha oil revealed the presence of benign bioactive compounds that have innumerous therapeutic activities. Thus, these leaves could be used as a source in the formulation of novel and more potent antioxidant medications that could be used in the management of various diseases that have taken a toll on human health. This work is an attempt to incite young researches to perform their research in this field of Pharmacognosy.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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