

Antioxidant and Anti-Mutagenic Potential of Mint (*Mentha Arvensis*) and its Chemical Characterization by HPLC

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ABSTRACT

Background: *Mentha arvensis* has been used for medicinal purposes by Asian countries for ages. It is used to cure various ailments such as diarrhea, asthma, piles, cancer, dementia, hyperglycemia, and aging.

Aim: This study aims to evaluate the antioxidant and anti-mutagenic (geno-protective) properties of four different solvent extracts (methanol, ethanol, acetone, and distilled water).

Methodology: The antioxidant properties were determined by DPPH and reducing power assays, while the DNA damaging assay was used to evaluate the anti-mutagenic activity.

Results: Among the four extracts, the methanol extract of *Mentha arvensis* showed 30.48 ± 0.95 mg QE/gm of flavonoid, and the aqueous extract showed the highest total phenolic content (142.2 ± 0.83 mg QE/gm) and total alkaloids content (78.2 ± 0.21 mg TE/gm) compared to the other three solvent extracts. The ethanol extract of *Mentha arvensis* exhibited the maximum value for total tannin content (17.41 ± 0.38 mg ETA/g CE) and reducing power (41.17 ± 0.87 mg ETA/gm). It also exhibited significant geno-protective activity.

Conclusion: These results indicate that among the four solvent extracts, the ethanolic extract of *Mentha arvensis* has the highest value for phenolic content. Moreover, all four solvents, except distilled water, showed valuable anti-mutagenic behavior. The HPLC results corroborate our findings, suggesting that these plants have great potential for use as antioxidant and anti-mutagenic agents.

Keywords: *Mentha arvensis*, Antioxidant agents, Anti-mutagenic agents, Geno-protective activity.

INTRODUCTION

In recent years, issues related to oxidative stress have become an increasingly important topic in clinical and medicinal research. Studies have demonstrated that diseases such as cancer, diabetes, obesity, hypertension, apoptosis, neurodegenerative disorders, heart failure, and aging are all caused by oxidative stress^{1,2}. As a result, there has been a significant shift towards the use of organic and herbal medicines. These treatments are affordable, easily accessible, and generally have fewer adverse effects than modern medicines. Consequently, many contemporary medical professionals are turning to natural herbs as an ideal treatment option^{3,4}.

Free radicals are produced by metabolic processes. They can damage almost all biomolecules present in the body, including nucleic acids, proteins, lipids, and DNA, which may lead to very serious diseases. Mostly, free radicals are present in reactive oxygen species (ROS) and can have adverse effects on the circulatory system, either by oxidative degradation of lipids or by causing vessels to become so narrow that they may cause myocardial infarction^{5,6}. Singlet oxygen does not act as a free radical nor follow a free radical pathway. When nascent oxygen reacts with the assistance of double bonds, it forms an endoperoxide. This endoperoxide then reduces into an alkoxyl radical, and a complex chain reaction starts. These free radicals can destroy normal body cells or cause mutations in the cells⁷.

The balance between oxidant and antioxidant activity is maintained by our body. When there is an imbalance, the body's defense system is unable to prevent disease-causing agents, and as a result, the whole body mechanism is disturbed. To overcome this imbalance, we have to use some antioxidant and anti-mutagenic agents. Some plants and herbs have good antioxidant and anti-mutagenic activity. These plant extracts are absorbed on the surface of pathogenic bacteria and dissolve their cell walls, resulting in bacterial cell death⁸. They play an important role in reducing cell mutation by preventing DNA damage and work well against carcinogenic diseases⁹.

Mentha arvensis belongs to the Lamiaceae family, which is distributed all over the world and has 25-30 species. Besides being used as an edible and flavoring agent, it is used in cosmetics and has many applications in the pharmaceutical and clinical industry¹⁰. Oil extracted from mint species has a strong ability against reactive oxygen species and microbes, hence preventing *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Salmonella enteritidis*^{11,12}. It inhibits arachidonic acid breakdown and prevents platelet aggregation caused by the metabolism of arachidonic acid, and can enhance glutathione peroxidase, which prevents heart diseases¹³. It is used against some gram-negative bacteria, including *E. coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*¹⁴. It has beneficial effects against hypertension and in patients with heart disease. It is presumed that it acts on cyclooxygenase and lipoxygenase enzymes, which are the basic factors for heart diseases. Both of these enzymes are targets for drugs used in cardiovascular and inflammatory diseases¹³.

Experiment: Chemical Reagents: Four different chemicals, namely methanol (90%), ethanol (90%), acetone (90%), and distilled water, were used as solvents for the extraction of the sample plant. Each sample plant was extracted separately using all of these solvents.

Preparation of Extracts from Sample Plants: The leaves of the collected sample plant were washed with fresh water and dried in the shade for three weeks to prevent protein or DNA denaturation due to heat exposure. The dried plant leaves were pulverized into a fine powder using a pestle and mortar. The plant extract was collected using the cold extraction method, with four samples of 20-grams of plant powder each extracted separately in glass conical flasks solvent using different solvents (methanol-90%, ethanol-90%, acetone-90%, and distilled water). The mixture of plant powder and solvents was kept for five days at room temperature and then filtered using Whatman filter paper to remove debris. The extract was then evaporated in open air and further by using the water bath method.

Determination of Total Phenolic Content: The Folin-Ciocalteu assay was used to measure the total phenolic content in the sample plant extracts, including methanolic, ethanolic, acetone, and distilled water extracts. In this assay, the FC reagent oxidizes phenolic compounds to phenolate while the FC reagent itself is reduced (gains ions from phenol) to produce a blue molybdenum-tungsten complex. Phenols are rapidly oxidized in alkaline conditions to give appreciable concentrations of phenolate ions. However, the acidic FC reagent and the blue complex formed are unstable in alkaline conditions. Therefore, a moderate pH of around 9-10 was used to achieve reasonably rapid production. An excess amount of FC reagent was used so that it could survive the alkaline conditions long enough to react with all the phenolate. Gallic acid was used as the standard¹⁵.

Determination of Saponins: For determination of total saponin content in the sample plant extract, a method described by Hiai et al., (1976) was followed and taken as the standard for calibration curve¹⁶. The results were expressed as mg of Aescin Equivalents (AE) per gram (mg of AE per g) and compared with the standard.

Determination of Alkaloids: Total alkaloid compounds were extracted according to Sabri et al. (1973)¹⁷. The extract was dissolved in 1.5 ml of chloroform, followed by the addition of 20 ml of 0.02N H₂SO₄. The resulting solution was warmed to vaporize the chloroform, cooled and finally titrated with 0.02N NaOH using methyl red as an indicator. The amount of alkaloid was calculated per ml of H₂SO₄.

Determination of Flavonoids: Aluminum trichloride (AlCl₃) colorimetric method was modified from the procedure reported by Woisky and Salatino (1998)¹⁸. A calibration curve was prepared using quercetin. Quercetin (10 mg) was dissolved in 80 % ethanol to make a stock solution and further dilutions of 25, 50 and 100 µg/ml were made. For the analysis, 0.5 ml of the diluted quercetin solution was mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of potassium acetate, and 2.8 ml of distilled water. The mixture was kept for 30 minutes at room temperature, and the absorbance was measured using a spectrophotometer.

Determination of Tannins: Tannins have the capacity to precipitate proteins while binding with phenolic acids. Therefore, the Hemoglobin precipitation method was used. For this purpose, a volume of each plant extract was diluted to give a concentration of total polyphenols approximately 450 micrograms/milliliter and mixed with an equal volume of haemolysed sheep blood. After 10 minutes, this solution was centrifuged for 20 minutes and the absorbance of the supernatants was measured at 550 nm. Tannins were quantified as tannic acid equivalents per gram of extract (mg TAE/g of CE)¹⁹.

DPPH Scavenging Assay: The 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity of the sample plant extracts was evaluated. Each plant extract (0.1 ml) was added to 3.9 ml of a 0.06 mM methanolic solution of DPPH. The solution was placed in the dark for almost 60 minutes, and the absorbance was measured at 517 nm²⁰. The Trolox calibration curve was plotted as a function of the percentage of DPPH radical scavenging activity. The final results were expressed as millimoles of Trolox equivalents (TE) per gram.

Reducing Power Assay: The reducing power activity was determined using the method adopted from Oktay et al. (2003). In this method, 1.5 ml of plant extract was mixed with phosphate buffer (2.5 ml, 0.2 mole per liter, pH 6.6) and K₃Fe(CN)₆ (2 ml, 1%)²¹. The mixture was kept at 50°C for 20 minutes, and then 2 ml of 10% trichloroacetic acid (TCA) was added. The mixture was centrifuged at 10,000g for 10 minutes. To the upper layer of the centrifuged solution (2 ml), deionized water (2.5 ml) and FeCl₃ (0.5 ml, 0.1%) were added. The absorbance was measured at 700 nm. An increase in absorbance of the mixture indicated an increased reducing power. The measurement was taken with a standard curve of ascorbic acid (AA) solution. The final results were expressed in terms of milligrams of quercetin equivalents (QE) per gram.

Anti-mutagenic Activity: Preparation of Phosphate (PO₄)

Buffer 50 mM: A phosphate (PO₄) buffer was prepared by dissolving 0.18 g of NaH₂PO₄ and 0.55 g of Na₂HPO₄ in 100 mL of distilled water.

Preparation of DNA solution: (Calf thymus) CT-DNA at a concentration of 0.5 µg/µL was taken and diluted two-fold to a concentration of 0.5 µg/3 µL with 50 mM phosphate buffer at pH 7.4. The reaction was carried out in micro Eppendorf tubes.

Preparation of 30% H₂O₂: A 30% H₂O₂ solution was prepared from a 36% H₂O₂ solution.

Reaction Mixture preparation: Approximately 3 µL of diluted ctDNA was added to the micro Eppendorf tube, followed by the addition of 20 µL of the selected synthetic compounds' stock solution to the final reaction mixture. Subsequently, 3 µL of TAE buffer and 4 µL of 30% H₂O₂ were added in succession.

Control 1 preparation: 10 µL of diluted ctDNA and 12 µL of phosphate buffer were added to a micro Eppendorf tube.

Control 2 preparation: 10 µL of diluted ctDNA, 12 µL of H₂O₂, and FeSO₄ were added to a micro Eppendorf tube.

Procedure: 1X Tris-Acetate-EDTE (TAE) buffer was prepared by dissolving 10 ml of 50 X buffer in 490 ml of distilled water. Then, a 1% solution of agarose gel in 1X TAE buffer was prepared by dissolving 1 g of agarose in 100 ml of 1X TAE buffer. The mixture was heated in an oven for 1 minute to form a homogeneous mixture. It was then slightly cooled to a bearable temperature, and 20 µl of staining ethidium bromide dye was added. The mixture was shaken and poured into the gel tray of a gel electrophoresis system and allowed to solidify for 30 minutes. After solidification, 1X TAE (gel running buffer) was poured to a level that both electrodes were dipped in buffer. After incubation, 3 µl of loading dye (Bromophenol blue) was added to each reaction mixture, and these samples were loaded into the wells made with the help of a comb on a 1% agarose gel containing TAE buffer and ethidium bromide. Each reaction mixture with a column was run horizontally in TAE buffer at 100 volts for 45 minutes in a gel electrophoresis system. The gel was photographed under UV light using a gel document system (SynGene, England). For each run, a molecular marker, a negative control, and a positive control were loaded, as well as various antioxidant treatments.

HPLC methods: Various antioxidant and anti-mutagenic compounds present in the plant extracts were determined using the High-Performance Liquid Chromatography (HPLC) technique. The procedure settings used were as described by previous studies.^{22,23}

For the HPLC analysis, a Shim-pack column (C-18) with a length of 100 mm was used. Two mobile phases, A and B, were used with gradient elution. Gradient A contained water and ascorbic acid in a ratio of 94:6 and had a pH of 2.27, while gradient B contained 100% acetonitrile. The solvent gradient was run at 15% B from 0-15 minutes, 45% B from 15-45 minutes, and 100% B from 30-45 minutes. The flow rate of the column was set to 1 mL/min. A UV-visible detector with a range of 280 nm was used for detection, and the range of the bipolar solvent was set to 1250 mV with 10 samples per second. The base area of the curve represented the value of different compounds.

RESULTS AND DISCUSSION

Qualitative analysis of sample plant extracts from the two species revealed the presence of different antioxidant and anti-mutagenic compounds such as flavonoids, saponins, tannins, alkaloids, phenolic compounds, and terpenoids. In *Mentha arvensis* (Table 1), the acetone leaf extract showed the highest positive response compared to other solvents such as ethanol, methanol, and distilled water.

Qualitative Analysis of *Mentha arvensis*

DPPH free radical scavenging percentage of *Mentha arvensis*:

The antioxidant potential of mint has been evaluated using different assays, such as DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay, reducing power assay, and total antioxidant capacity assay. The DPPH assay is based on the principle that

antioxidants can donate hydrogen atoms to the free radical DPPH, which results in the reduction of its absorbance. The antioxidant activity of *Mentha arvensis* was determined by a free radical scavenging assay. The results indicate that the sample plant exhibits dose-dependent antioxidant activity. The IC50 value was used as a parameter to interpret the results from the DPPH method. A lower IC50 value indicates higher free radical scavenging activity of *Mentha arvensis* extracts in different solvents is as follows: ethanol > distilled water > acetone > methanol. The results of the antioxidant activity percentage are illustrated in Table 2.

Table 1: Qualitative Phytochemical Analysis of *Mentha arvensis*

S.No	Phytochemical/ solvent	Ethanol	Methanol	Acetone	Distill Water
1	Tanin	+	+	+	+
2	Flavonoid	-	++	+	+
3	Terpanoids	+	+	+	+
4	Alkaloids	+	+	+	++
5	Phenols	+	+	+	++

Note: ++Present in higher amount, - Not detectable, +Present in moderate amount

Table 2: DPPH (µg/ml) free radical scavenging percentage of *Mentha arvensis*

Sample Plant	Ethanol	Methanol	Acetone	Distill water
<i>Mentha Arvensis</i>	92.62%	70.6%	73.77%	89.34%

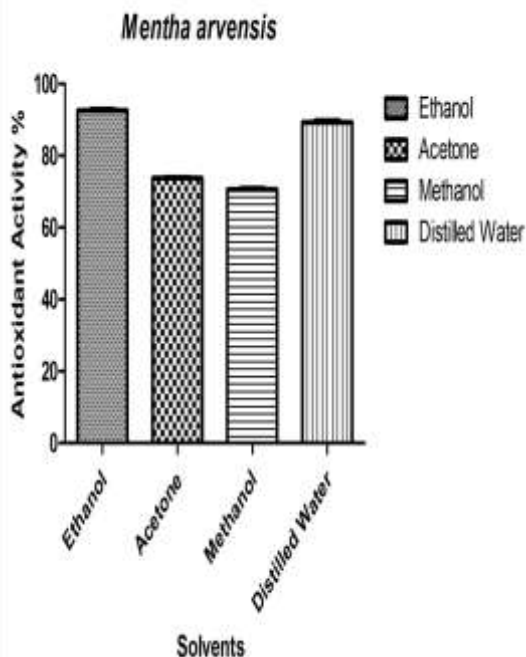


Figure 1: Graphical representation of percentage antioxidant activity of *Mentha arvensis*

Table 4: Total flavonoid content in *Mentha arvensis*

Mentha arvensis	Solvents			
	Methanol	Ethanol	Acetone	Distilled Water
Total reducing power value (mg QE/ gm)	34.48±0.95	41.17±0.87	27.44±0.81	37.12±0.90
Total Flavonoid Content (mg QE/ gm)	30.48±0.95	7.40±0.67	25.11±0.60	27.4±0.63
Total Phenolic Content (mg GE/ gm)	85.11±0.69	111.22±0.80	92.81±0.71	142.2±0.83
Total Alkaloid Content (mg of TE per g)	51.4±0.42	69.34±0.29	42.22±0.30	78.20±.21
Total Tannin content (mg ETA/g CE)	9.31±0.31	17.41±0.38	7.21±0.42	12.6±0.51

In *Mentha arvensis*, the ethanol extract showed the highest free radical scavenging activity of 92.62% (41 µg/ml), while the methanol extract showed a lower value of 70.6% (31.25 µg/ml). The aqueous extract of *Mentha arvensis* showed 89.34% (39.55 µg/ml) while acetone extract showed 73.77% (32.66 µg/ml) DPPH free radical scavenging activity as shown in figure 1. The antioxidant effect of the sample plant extracts varies based on the quality of polyphenols. The mechanism of this reaction between the antioxidant and DPPH is dependent on the structural conformation of the antioxidants (Santos-Sánchez et al., 2019)²⁴. Some polyphenols react rapidly with DPPH, which reduces the number of DPPH, equivalent to that of OH- groups of these antioxidants.

Reducing Power Analysis: The increase in antioxidant activity was observed as the concentration of the analyzed samples increased, as noted through spectrophotometric absorbance measurements. In the case of *Mentha arvensis*, the ethanol extract had a reducing power of 41.17±0.87 (mg QE/gm). Our findings showed that the reducing power of extracts, various organic fractions, and essential oils were in agreement with the higher values of TPC, TFC, and other phytochemicals analyzed during qualitative analysis. There were significant differences (P ≤ 0.05) observed in the reducing power analysis at various concentrations (Table 3).

Estimation of Total Flavonoids Content: The research revealed that the total flavonoid content of *Mentha arvensis* is highest in methanol, with a concentration of 30.48±0.95 mg QE/g. This value is consistent with the flavonoid content estimated by previous researchers^{25,26}. Therefore, *Mentha arvensis* can be utilized as an effective antioxidant and anti-mutagenic agent (Table 3).

Estimation of Total Phenolic Content: Results showed that in aqueous extract of *Mentha arvensis* has 142.2±0.83 (mg GE/ gm) phenolic content. The values resembles with total phenolic content estimated by previous studies using phytochemical screening of leaves.^{27,28} This indicates that *Mentha arvensis* has more potential for antioxidant activity since the total phenolic content in *Mentha arvensis* is comparatively high. Other three solvent extract of sample plant also show a considerable amount of phenolic content (Table 3).

Total Alkaloid Content (mg of TE per g): The aqueous extract of *Mentha arvensis* was found to contain a significant amount of alkaloids, with a total alkaloid content of 78.2021 mg of TE per gram as shown in table 3. This value is consistent with previous findings in the literature, indicating the potential value of *Mentha arvensis* as a source of alkaloids.^{29,30}

Tannins Contents: Tannin content of *Mentha arvensis* is highest in ethanol extract as 17.41±0.38mg ETA/g CE. This value resembles with that obtained by a research conducted by Malik et al. (2012)^{31,32}. Leaves of *Mentha arvensis* has valuable amount of tannin, so this plant can also be used to add fragrance in drinks. The ethanol extract of *Mentha arvensis* has the highest tannin content, with a value of 17.41 ± 0.38 mg ETA/g CE, which is consistent with previous research^{33,34,35}. The leaves of *Mentha arvensis* contain a significant amount of tannins, which makes it a potential candidate for use as a flavoring agent in beverages (Table 3).

Anti-mutagenic Activity Analysis: Genoprotective Activity:

Samples of plant extracts in all four buffers, i.e., ethanol (90%), methanol (90%), acetone (90%), and distilled water, were loaded onto an agarose gel along with positive and negative controls. This depicts the genoprotective ability of the sample plant in specific buffers. Results indicate that *Mentha arvensis* has a relatively greater potential to protect DNA against oxidative damage. Ethanol, methanol, and acetone extracts were found to be effective for the genoprotective activity of *Mentha arvensis*, while the genoprotective activity was not found in distilled water as shown in the figure 2 (Table 4).

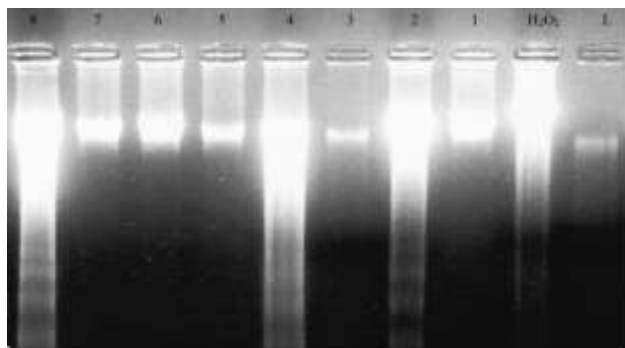


Figure 2: Bands obtained in DNA damaging assay

Result interpretation:

L: Ladder containing only DNA, where the band appears because it is protected.

H₂O₂: Contains DNA and oxidative agent, resulting in DNA damage and a smear formation.

1 Sample of *Mentha arvensis* in "acetone solvent" containing DNA and oxidative agent, as well as the sample. The appearance of a band indicates that the sample protects the DNA from oxidative damage.

2 Sample of *Mentha arvensis* in "methanol solvent" where a band is formed, indicating that the sample protects the DNA from oxidative damage.

3 Sample of *Mentha arvensis* in "ethanol solvent" where a band is formed, indicating that the sample protects the DNA from oxidative damage.

4 Sample of *Mentha arvensis* in "distilled water solvent" results in a smear formation, indicating that the sample does not protect the DNA from oxidative damage.

Table 4: Summary of genoprotective activity of *Mentha arvensis*

Sample Plant	Ethanol	Methanol	Acetone	Distill Water
<i>Mentha Arvensis</i>	+	+	+	-

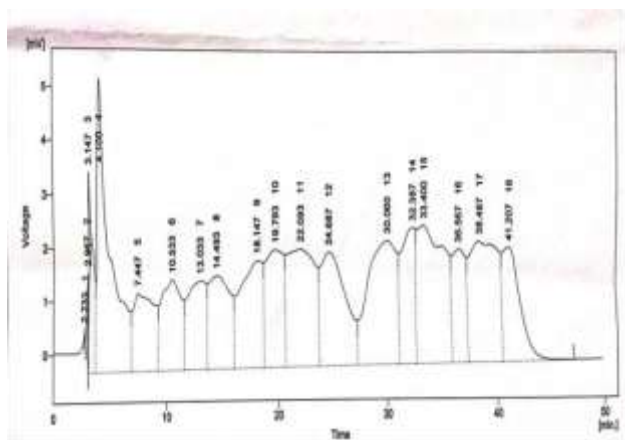


Figure 3: HPLC graph of *Mentha arvensis* in acetone solvent

Chemical Characterization by HPLC: HPLC analysis of the leaf extracts of the sample plant, *Mentha arvensis*, in specific solvents revealed the presence of various antioxidant and anti-mutagenic compounds (Figure 3 and 4). The analysis confirmed the presence of polyphenolic compounds in the plant, which makes it useful against diseases caused by oxidative mutagenic agents (Table 5). The graph below illustrates the concentration of each compound and their respective details.

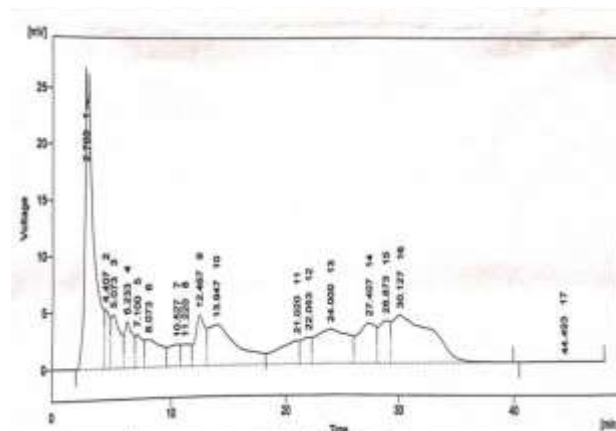


Figure 4: HPLC graph of *Mentha arvensis* in ethanol solvent

Table 5: Chemical compounds in leaf extracts of *Mentha arvensis* analysed by HPLC.

Leaf extract solvent	Retention Time	Chemical compounds	Area percentage (%)	Amount (ppm)
Ethanol	2.760	Quercetin	21.8	72.049
	4.407	Gallic acid	2.4	5.471
	22.053	Ferulic acid	2.3	10.548
	12.467	Caffeic acid	4.2	12.145
Acetone	13.033	Vanilic acid	4.1	11.762
	2.967	Quercetin	0.3	0.7761
	4.100	Gallic acid	9.4	15.5356
	18.147	p-coumeric acid	6.2	3.681
	22.093	Ferulic acid	8.5	28.161
	14.493	Benzoic acid	5.0	24.413
	19.793	M- coumeric acid	5.5	3.0027
	24.687	Cinmic acid	7.6	12.1546

CONCLUSION

The results of the study suggest that *Mentha arvensis* has significant potential as a source of natural antioxidants and anti-mutagenic compounds. The presence of various phytochemicals, including phenols, flavonoids, alkaloids, saponins, and tannins, among others, is evidence of this potential. However, further research is required to fully understand the therapeutic properties of these compounds and their potential applications in treating various diseases.

The DPPH scavenging assay and reducing power assay results confirm the plant's antioxidant potential, with the ethanol extract showing the highest activity. The anti-mutagenic activity of the plant in all solvents except for distilled water further supports the potential of *Mentha arvensis* in protecting DNA from oxidative damage. Moreover, the HPLC analysis of the plant's leaf extracts revealed the presence of various antioxidant and anti-mutagenic compounds, including polyphenols. The concentration of these compounds and their respective details were illustrated in the study. This chemical characterization further confirms the potential of *Mentha arvensis* as a source of natural antioxidants and anti-mutagenic compounds.

Finally, the study highlights the need for further investigation into isolating antioxidant and anti-mutagenic compounds from the stem and roots of the plant. Such research could help develop new

therapeutic treatments for diseases caused by mutations, chromosomal aberrations, or DNA fragmentation. Overall, *Mentha arvensis* has demonstrated significant potential for use in both traditional medicine and modern healthcare as a source of natural antioxidants and anti-mutagenic compounds.

REFERENCES

- Cojocaru KA, Luchian I, Goriuc A, Antoci LM, Ciobanu CG, Popescu R, et al. Mitochondrial Dysfunction, Oxidative Stress, and Therapeutic Strategies in Diabetes, Obesity, and Cardiovascular Disease. *Antioxidants*. 2023;12:658.
- Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, et al. Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. *Front. Physiol.* 2020; 11:694.
- Yu Long, Qiyue Yang, Yan Xiang, Yulu Zhang, Jinyan Wan, Songyu Liu, Nan Li, Wei Peng. Nose to brain drug delivery - A promising strategy for active components from herbal medicine for treating cerebral ischemia reperfusion. *Pharmacological Research*. 2020; 159:104795.
- Hassen G, Belete G, Carrera KG, et al. Clinical Implications of Herbal Supplements in Conventional Medical Practice: A US Perspective. *Cureus* 2022; 14:e26893.
- Shields HJ, Traa A and Van Raamsdonk JM. Beneficial and Detrimental Effects of Reactive Oxygen Species on Lifespan: A Comprehensive Review of Comparative and Experimental Studies. *Front. Cell Dev. Biol.* 2021. 9:628157.
- Jakubczyk K, Dec K, Kalduńska J, Kawczuga D, Kochman J, Janda K. Reactive oxygen species-sources, functions, oxidative damage. *Polski merkuriusz lekarski: organ Polskiego Towarzystwa Lekarskiego*. 2020 Apr 1;48(284):124-7.
- Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria journal of medicine*. 2018;54(4):287-93.
- Hassan AA, Abd-Elaziz GO. Genotoxicity and antimicrobial activity of *Myrtus communis* L., *Ziziphus spina-christi* (L.) Willd and *Cassia angustifolia* Vahl extracts. *Bangladesh Journal of Botany*. 2020 Sep 20;49(3):557-66.
- Engwa GA. Free radicals and the role of plant phytochemicals as antioxidants against oxidative stress-related diseases. *Phytochemicals: Source of Antioxidants and Role in Disease Prevention*. BoD-Books on Demand. 2018 Nov 7;7:49-74.
- Prakash J, Arora NK. Phosphate-solubilizing *Bacillus* sp. enhances growth, phosphorus uptake and oil yield of *Mentha arvensis* L. *3 Biotech*. 2019 Apr;9(4):126.
- Wei H, Kong S, Jayaraman V, Selvaraj D, Soundararajan P, Manivannan A. *Mentha arvensis* and *Mentha piperita*-Vital Herbs with Myriads of Pharmaceutical Benefits. *Horticulturae*. 2023 Feb 8;9(2):224.
- Heydari M, Zanfardino A, Taleei A, Shahnejat Bushehri AA, Hadian J, Maresca V, Sorbo S, Di Napoli M, Varcamonti M, Basile A, Rigano D. Effect of heat stress on yield, monoterpene content and antibacterial activity of essential oils of *Mentha x piperita* var. *Mitcham* and *Mentha arvensis* var. *piperascens*. *Molecules*. 2018 Jul 30;23(8):1903.
- Gul S, Gul H, Nawaz R. Possible mechanism of action of *Mentha arvensis* in cardiovascular diseases. *International Journal of Endorsing Health Science Research*. 2014;2(1).
- Satorov S, Mirzoeva F, Sh S, Vakhidova M, Dushenkov V. Comparative characteristics of antibacterial activity of plants growing in the central part of the Republic of Tajikistan. *Вестник Авиценны*. 2019;21(4):643-54.
- Zhou J, Zhao W, Miao Z, Wang J, Ma Y, Wu H, Sun T, Qian H, Zha Z. Folin-Ciocalteu assay inspired polyoxometalate nanoclusters as a renal clearable agent for non-inflammatory photothermal cancer therapy. *ACS nano*. 2020 Feb 6;14(2):2126-36.
- Hiai S, Oura H, Nakajima T. Color reaction of some saponins and saponins with vanillin and sulfuric acid. *Planta Medica*. 1976 Mar;29(02):116-22.
- Sabri NN, El-Masry S, Khafagy SM. Phytochemical investigation of *Hyoscyamus desertorum*. *Planta medica*. 1973 Feb;23(01):4-9.
- Woisky RG, Salatino A. Analysis of propolis: some parameters and procedures for chemical quality control. *Journal of apicultural research*. 1998 Jan 1;37(2):99-105.
- Bizuayehu D, Atlabachew M, Ali MT. Determination of some selected secondary metabolites and their invitro antioxidant activity in commercially available Ethiopian tea (*Camellia sinensis*). *SpringerPlus*. 2016 Dec;5(1):1-9.
- Salarbashi D, Tajik S, Shojaee-Aliabadi S, Ghasemlou M, Moayyed H, Khaksar R, Noghabi MS. Development of new active packaging film made from a soluble soybean polysaccharide incorporated *Zataria multiflora* Boiss and *Mentha pulegium* essential oils. *Food chemistry*. 2014 Mar 1;146:614-22.
- Oktay M, Gülçin İ, Küfrevioğlu Öİ. Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. *LWT-Food Science and Technology*. 2003 Mar 1;36(2):263-71.
- Santos SA, Villaverde JJ, Freire CS, Domingues MR, Neto CP, Silvestre AJ. Phenolic composition and antioxidant activity of *Eucalyptus grandis*, *E. urograndis* (*E. grandis* x *E. urophylla*) and *E. maidenii* bark extracts. *Industrial Crops and Products*. 2012 Sep 1;39:120-7.
- Hasni S, Rigane G, Ghazghazi H, Riguene H, Bouallegue A, Khedher O, Oueslati MA, Ben Salem R. Optimum Conditions and LC-ESI-MS Analysis of Phenolic Rich Extract from *Eucalyptus marginata* L. under Maceration and Ultrasound-Assisted Extraction Methods Using Response Surface Methodology. *Journal of Food Quality*. 2021 Nov 8;2021:1-4.
- Santos-Sánchez NF, Salas-Coronado R, Villanueva-Cañongo C, Hernández-Carlos B. Antioxidant compounds and their antioxidant mechanism. *Antioxidants*. 2019 Mar 22;10:1-29.
- Itam A, Wati MS, Agustin V, Sabri N, Jumanah RA, Efdi M. Comparative study of phytochemical, antioxidant, and cytotoxic activities and phenolic content of *Syzygium aqueum* (Burm. f. *Alston f.*) extracts growing in West Sumatera Indonesia. *The Scientific World Journal*. 2021 Jun 14;2021.
- Akhtar N, Mirza B. Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species. *Arabian journal of chemistry*. 2018 Dec 1;11(8):1223-35.
- Belkacem N, Azzi R, Djaziri R. Phytochemical Screening, Total Phenolics Contents and in Vitro Antioxidant Activity of *Salvia Officinalis*, *Satureja Calamintha*, *Mentha Pulegium* and *Marrubium Vulgare*. *Phytothérapie*. 2022 Feb 1;20(1):23-8.
- Naseer S, Iqbal J, Naseer A, Kanwal S, Hussain I, Tan Y, Aguilar-Marcelino L, Cossio-Bayugar R, Zajac Z, Jordan YA, Mahmood T. Deciphering chemical profiling, pharmacological responses and potential bioactive constituents of *Saussurea lappa* Decne. Extracts through in vitro approaches. *Saudi Journal of Biological Sciences*. 2022 Mar 1;29(3):1355-66.
- Heinrich M, Mah J, Amirka V. Alkaloids used as medicines: Structural phytochemistry meets biodiversity—An update and forward look. *Molecules*. 2021 Mar 25;26(7):1836.
- Rajkumar G, Panambara PA. Comparative Analysis of Qualitative and Quantitative Phytochemical Evaluation of Selected Leaves of Medicinal Plants in Jaffna, Sri Lanka.
- Malik F, Hussain S, Sadiq A, Parveen G, Wajid A, Shafat S, Channa RA, Mahmood R, Riaz H, Ismail M, Raja FY. Phyto-chemical analysis, anti-allergic and anti-inflammatory activity of *Mentha arvensis* in animals. *Afr J Pharm Pharmacol*. 2012 Mar 8;6(9):613-9.
- Akhtar N, Mirza B. Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species. *Arabian journal of chemistry*. 2018 Dec 1;11(8):1223-35.
- Kim SY, Han SD, Kim M, Mony TJ, Lee ES, Kim KM, Choi SH, Hong SH, Choi JW, Park SJ. *Mentha arvensis* essential oil exerts anti-inflammatory in LPS-stimulated inflammatory responses via inhibition of ERK/NF- κ B signaling pathway and anti-atopic dermatitis-like effects in 2, 4-dinitrochlorobenzene-induced BALB/c mice. *Antioxidants*. 2021 Dec;10(12):1941.
- Thakur S, Walia B, Chaudhary G. *Mentha arvensis* (Pudina): A review based upon its medicinal properties. *Research journal of Pharmacognosy and Phytochemistry*. 2021;13(3):143-8.
- Hussain S, Tanvir M, Ahmad M, Munawar KS. Phytochemical Composition of Mint (*Mentha*), its Nutritional and Pharmacological Potential. *Lahore Garrison University Journal of Life Sciences*. 2021 Dec 23;5(04):241-58.