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Antioxidant Activity, Flavonoid and Phenolic Content of *Mentha piperita* Collected from Rupandehi, Nepal

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Abstract

Medicinal herbs from Himalaya's country like Nepal are considered to have higher bioactive medicinal ingredients as compared to similar herbs from other regions. Preliminary phytochemical screening of Mentha piperita revealed the presence of alkaloids, terpenoids, flavonoids, phenols, tannins, saponins, glycosides, steroids, proteins, amino acids, and polyphenols, indicating rich bioactive composition. Quantitative analyses showed total phenolic content of 370.9 ± 6.45 mg GAE/ 100g dry weight and total flavonoid content of 345.0 ± 3.95 mg QE/ 100g dry weight, determined using Folin–Ciocalteu and aluminum chloride colorimetric assays, respectively. Antioxidant activity, assessed via the DPPH radical scavenging method, exhibited a concentration-dependent response, with % inhibition ranging from 22.40 ± 0.61% at 31.25 µg/mL to 84.89 ± 1.41% at 500 µg/mL. The IC_{50} value was 156.06 ± 2.12 µg/mL with reference to standard ascorbic acid with an R^2 of 0.854, indicating a strong correlation between concentration and activity. These findings confirm Mentha piperita as a potent source of natural antioxidants, attributable to its high phenolic and flavonoid contents. The results support its traditional medicinal use and suggest potential applications in pharmaceutical, nutraceutical, and food preservation sectors.

***Keywords*:** *Mentha piperita*, Phytochemical screening, Antioxidant activity, Total phenolic content, Total flavonoid content

Introduction

The demand of natural substances with antioxidant properties has been increasing in medicine and food industries due to their preventive action on human health caused by free radicals and reactive oxygen. Medicinal herbs from Himalaya's country like Nepal are considered to contain higher bioactive medicinal ingredients due to its geographical condition, climatic action, biodiversity, and ancient ethnomedicinal knowledge as compared to herbs from other regions (Phuyal et al., 2019). Plant-based bioactive molecules help achieve Sustainable Developmental Goal SDG-3 by providing safe, affordable, and effective healthcare solutions, reducing disease burden, and promoting

preventive health, all while being sustainable and accessible worldwide (Howden-chapman & Chisholm, 2018). Although, the synthesized medicines are very effective in treating disease, their chemical constituents can produce free radicals, which in turn may cause oxidative stress leading to long term heath issues (Chaudhary et al., 2023). On the other hand, plant based medicines not only cures the disease their secondary metabolites like alkaloids, flavonoids, phenolic, terpenoids, etc are compatible with human body and making them effective against human diseases (Hussain et al., 2012).
Phytochemical screening, preliminary a qualitative analysis, is carried out to detect the presence of bioactive compounds that are present in herbs/plants. The screening test provides the researchers a pathway to investigate the important aspects of the herbs in many medicinal, industrial, others sectors. In medicine, they are being used as an antioxidant, anti-inflammatory and anticancer (Tang et al., 2024). In industrial sector, their flavors and fragrances are used to increase the taste and aroma of food beverages and sometimes they are also used as preservatives. Likewise, they play a crucial role as direct medicines in drug discovery field, and biodiversity (Alqethami & Aldhebiani, 2021).

Culinary herbs have not only become popular in modern kitchens but have also been valued since ancient times for their ability to enhance and compliment flavors of wide variety of foods and medicines (Kaur et al., 2025). The studies showed that the culinary herbs like Mentha piperita, Origanum vulgaris, Rosmarinus officinalis, are rich in antioxidant capacity, oxygen radical absorbance capacity (ORAC) (Ulewicz-Magulska & Wesolowski, 2019) and total phenolic contents (Zheng & Wang, 2001). Mentha piperita, commonly called Peppermint (Pudina) is a common herbs belonging Lamiaceae family has more than 600 varieties (Khanal, 2019). The herb is commonly used for flavors and particularly used in gastrointestinal disorder treatment (Grigoleit & Grigoleit, 2005). Besides flavors, its essential oil which mainly contains methanol helps to reduce pain by muscle relaxing, and producing cooling sensation along with its antimicrobial activity for treating bronchitis and other infections (Goldstein 2014).

Although many studies have been carried out on Mentha piperita found in different geographical locations of the Himalayas and hilly regions of Nepal, most of them have been limited to qualitative screening only (Khanal, 2019; Sharma & Gautam, 2022). Furthermore, a research gap on result obtained TPC and TFC analysis of Himalaya herbs to that of results obtained from other geographical locations. The present study aims to address literature gap by screening out the potential phytochemical ingredients as well as quantifying the antioxidant activity, total phenolic content (TPC) and total flavonoid content (TFC) in the prepared extract from leaves extract of Mentha piperita.

Materials and Methods

Study area and Sample Collection

Fresh Peppermint herbs (*Mentha piperita*) were collected from local markets and villages of Rupandehi district, Nepal. The fresh leaves were collected in the month of June with an average daily temperature of 25°C, with an average humidity of approximately 80%.

Preparation of Extract

Leaves of *Mentha piperita* were washed with distilled water and dried under shade until

Figure 1 *Mentha piperita* and study area

the constant weight was obtained. The shaded dried leaves (230 g) were grinded into powder forms and packed into clean air tight plastic bag. For Analysis, 100 gram of dry extract was used. The powder was dissolved in 500 mL ethanol (Qualigen, 99% pure) solvent under magnetic shakers for 24 hours. The mixture was centrifuged for 30 minutes maintaining 500 rotations per minutes (rpm). The filtrate was collected and evaporated in water bath keeping 65 ºC – 70 ºC. The concentrated filtrate was collected in clean beaker of known weight, covered with aluminum foil. The complete evaporation of solvent was carried out by perforating foil with small holes and obtained constant weight. For TPC and TFC measurement, 100 gram of dry extract leaves were used. The percentage yield of the extract was calculated (Qanash et al., 2023) and samples were stored in refrigerator and used for phytochemical screening and quantitative analysis.

Total Phenolic Content

The total phenolic content of *Mentha piperita* extract was determined using Folin-Ciocalteu, Thermo Fisher (Product Code: Q35953), reagent following a slightly modified method of Ainsworth et. Al. (Ainsworth & Gillespie, 2007). Gallic acid monohydrate, 99% extra pure (Mol. wt. 188.14 $gmol^{-1}$) purchased from Loba Chemie, India was used as reference standard for quantitative estimation of TPC. A volume of 500 µL aliquot of 31.25, 62.5, 125, 250, 500 µg/mL gallic acid solution were mixed with 2 mL of Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water), and neutralized with 4 mL of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 minutes with intermittent shaking for color development and absorbance of solution was measured at 760 nm using UV-vis Spectrophotometer. The same procedure was repeated for the methanol extracts of the *Mentha piperita*. TPC result was calculated and results were expressed in mg gallic acid

equivalent per 100 g of dry extract (mg GAE/ 100 g) using standard calibration curve equation obtained using different concentration of gallic acid. The calibration curve for Gallic acid is given in Figure 2. All measurements were performed in triplicate for each analysis.

Total Flavonoid Content

The quantitative estimation of total flavonoid content of prepared Mentha extract was determined by aluminum chloride colorimetric method described by Kim et al. (Kim et al., 2003). Quercetin, 95% extra pure, of Mol. wt. 302.24 $gmol^{-1}$ purchased from Loba Chemie, India (product code 05526) was used as standard materials for TFC determination and results were expressed in mg quercetin equivalent per 100 g of dry extract (mg QE/ 100 g). Standard stock solution of quercetin of varying concentration ranging from 500 ppm to 31.25 ppm were prepared in ethanol. A 100 µL of each of the quercetin aliquot was mixed with 500 µL (0.5 mL) of distilled water, and 100 µL of 5% $NaNO_2$ (5% w/v) and allowed to stand for 6 minutes. A 300 µL $AlCl_3$ (10%, w/v) was added in the mixture and stand for 6 minutes followed by addition of 2 mL (1M) of NaOH solution. The final volume was made to 10 mL by adding distilled water and incubated for 25 ^oC for 15 minutes, and absorbance at 510 nm was measured and result was calculated using standard calibration curve equation obtained using different concentration of quercetin.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Assay

The antioxidant activity of the ethanolic extract of *Mentha piperita* was evaluated using the DPPH radical scavenging assay. A 0.1 mM DPPH solution was prepared in ice-cold ethanol solvent. A series of stock solution with varying concentration ranging from 31.25 µg/mL to 500 µg/mL (1 µg/mL=1ppm) were prepared. 1 mL of DPPH solution was then mixed with 3 mL of each stock solution. Ascorbic acid was used as antioxidant. The mixture was shaken thoroughly and incubated in the dark at room temperature for 30 minutes to prevent photo degradation of DPPH. A UV-visible spectrophotometer, (ACZET 0210702206), was used to measure the absorbance at 517 nm. The antioxidant capacity was calculated by the following formula:

$$\text{Antioxidant activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

The concentration of Mentha extract needed to inhibit 50% of the DPPH free radical (IC_{50}) was calculated by from inhibition curve obtained by plotting concentration vs absorbance curve, as described by Al-Rajhi et. al.(Al-Rajhi et al., 2023).

$$\text{Radical scavenging capacity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

Results and Discussion

Preliminary Phytochemical Screening

The preliminary phytochemical screening of the ethanoic extract of *Mentha piperita*, obtained with a percentage yield of 10.6% (w/w), revealed the presence of various bioactive compounds. The analysis indicated strong presence (+++) of terpenoids,

flavonoids, polyphenols, and phenolic compounds, while coumarins, glycosides, tannins, and steroids were moderately present (++). Alkaloids, reducing sugars, saponins, proteins, and amino acids were detected in weak amounts (+), whereas quinones were absent. These results suggest that the extract is rich in antioxidant and pharmacologically important constituents, which could contribute to its potential therapeutic applications.

Table 1: Preliminary Phytochemical Screening Test

S.N.	Test	Result
	Alkaloids	+
	Terpenoids	+++
	Coumarins	++
	Flavonoids	+++
	Quninones	−
	Glycosides	++
	Polyphenols	+++
	Reducing Sugar	+
	Saponins	+
	Tannins	++
	Steroids	++
	Protein	+
	Amino Acid	+
	Phenolic Compounds	+++

Where, +++: Stronghly Present, ++ : Moderately Present, + : Weakly Present , − : Absent

Total Phenolic Content

The total phenolic content (TPC) of the methanolic extract of *Mentha piperita* leaves was assessed using the Folin–Ciocalteu colorimetric assay, employing gallic acid as the calibration standard. A standard curve for gallic acid is shown in Figure 2, and is used to determine the total phenolic content of the extract. The bar graph with standard error (Figure 3) illustrates that phenolic content was highest at the lowest concentration (31.25 ppm) and progressively decreased with increasing concentration. The extract's TPC was measured to be 370.9 ± 6.45 mg gallic acid equivalent per 100 gram of dry weight (mg GAE/g). The obtained result was quite higher than the reported result 226 mg/GAE per 100 gram dry extract (Zheng & Wang, 2001), 3.57 mg/GAE per 100 gram dry extract content in 20 mg/mL alcoholic solution (Farnad et al., 2014) suggested that confirming that *Mentha piperita* contained abundant phenolic compounds, recognized for their strong antioxidant activity and potential therapeutic benefits.

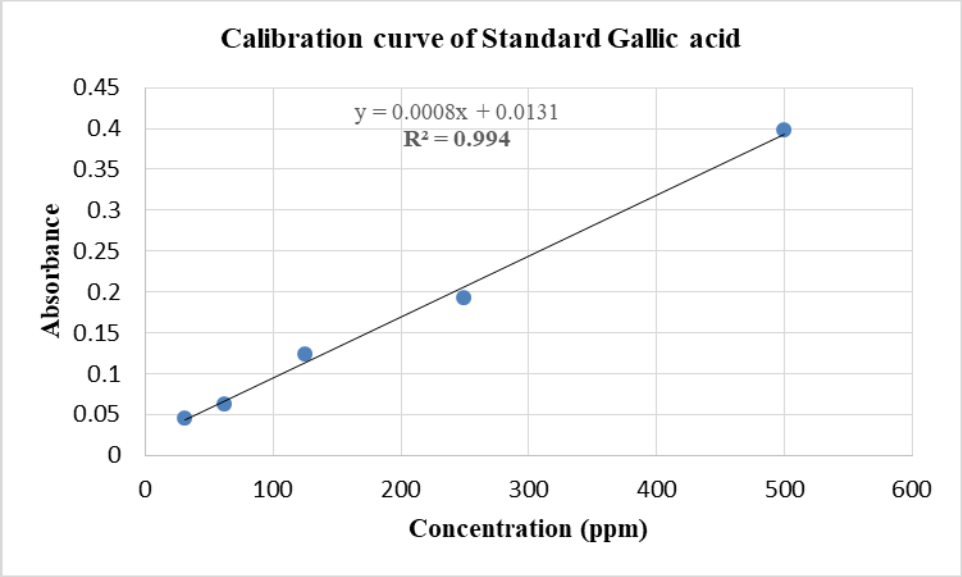

Figure 2 Calibration of standard gallic acid

Table 2: Total Phenolic Content of *Mentha piperita*

Concentration(ppm)	Absorbance	Total Phenolic Content (TPC) mgGAE/ 100 g of dry weight
500	2.154	279.0
250	1.107	273.0
125	0.586	276.0
62.5	0.361	297.9
31.25	0.183	370.9

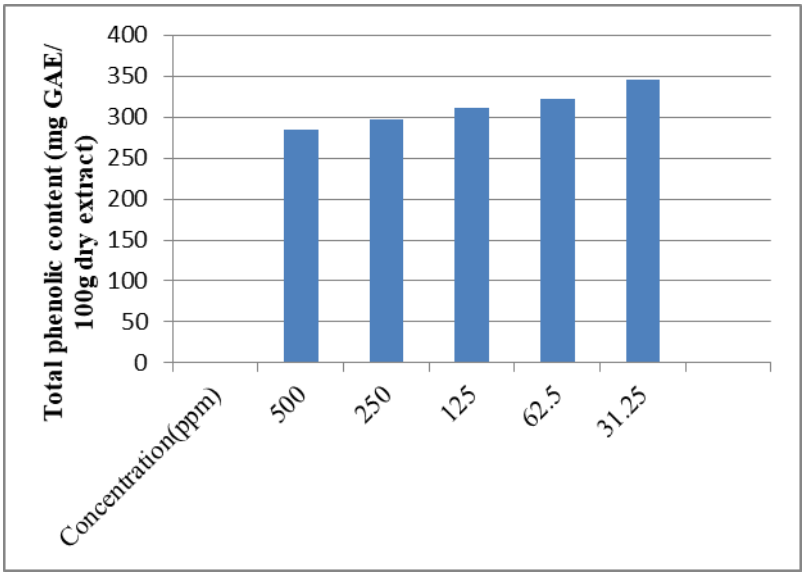

Figure 3 Bar graph of standard error of phenolic content

Total Flavonoid Content

The total flavonoid content (TFC) of the Ethanolic leaf extract of *Mentha piperita* was measured using the aluminum chloride colorimetric technique, employing quercetin as the reference standard. A calibration curve for standard quercetin (Figure 4) was generated from absorbance readings taken at 415 nm for various concentrations. The concentration-wise TFC values are provided in Table 6, showing the highest flavonoid level (345 mg QE/g) at the lowest concentration (31.5 ppm) and the lowest value (284 mg QE/g) at the highest concentration (500 ppm). The extract's overall TFC was found to be 345 ± 3.95 mg quercetin equivalent per 100 gram dry extract (mg QE/ 100 g dry extract), highlighted the abundance of flavonoids in the leaves. The relationship between flavonoid content and concentration is illustrated in the bar graph with standard error (Figure 6), underscoring the potential of *Mentha piperita* as a rich source of pharmacologically important flavonoids. However, TPC and TFC values, which is based on absorbance reading, have indicated that both values are higher at lower concentration i.e. 31.25 ppm. This discrepancy can be explained as higher concentration, saturation of solution could exceed the absorbance range as compared to that at lower concentration (Prior et al., 2005).

Table 3: Total Flavonoids content of *Mentha piperita*

Concentration(ppm)	Absorbance (Y)	Total Flavonoids Content (TFC) mg QE/g
500	0.365	284
250	0.192	297
125	0.104	331
62.5	0.089	332
31.25	0.045	345

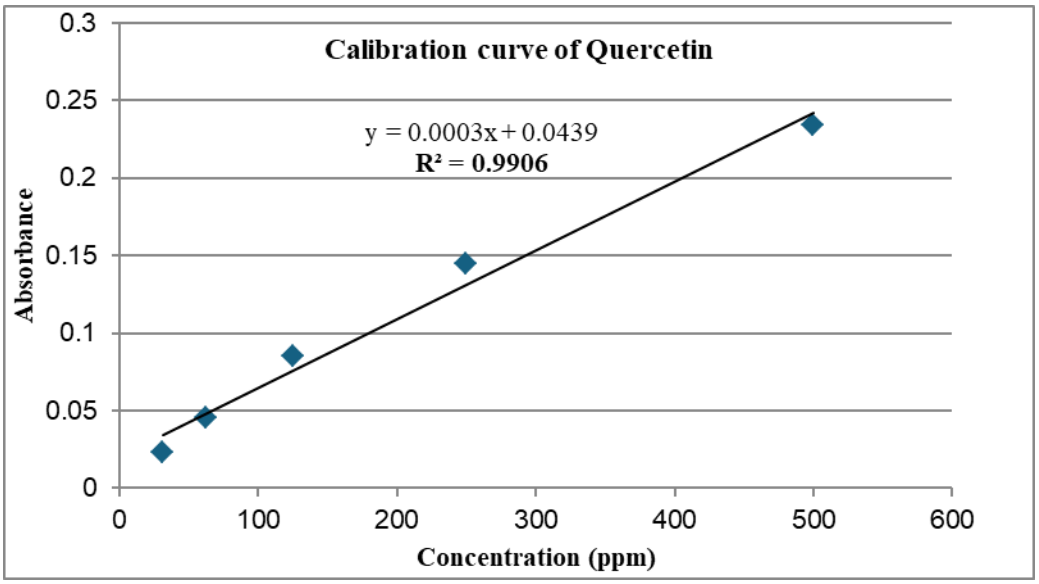

Figure 4 Calibration curve of standard quercetin.

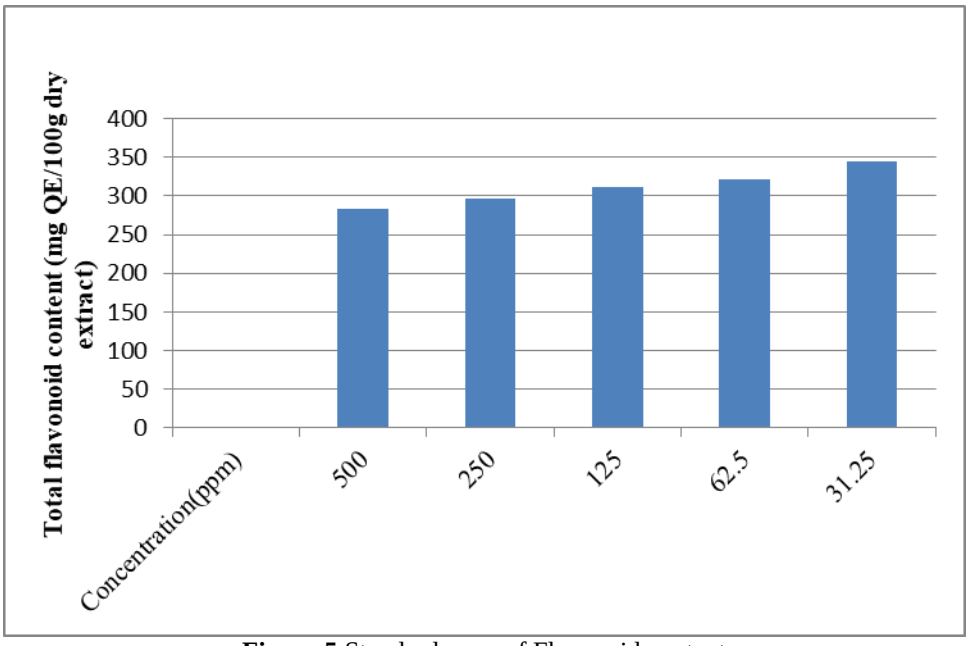

Figure 5 Standard error of Flavonoid content

Antioxidant activity

The antioxidant activity of the methanolic extract of *Mentha piperita* was evaluated using the DPPH radical scavenging assay, revealed a clear dose-dependent trend. As given in Table 4 and Figure 6, the percentage inhibition increased steadily from 22.40% at the lowest concentration (31.25 µg/mL) to 84.89% at the highest concentration tested (500 µg/mL). The DPPH radical scavenging activity (% inhibition) for *Mentha piperita* extract was found to 84.89 ± 1.41% with IC_{50} = 156.06 ± 2.12 µg/mL (Table 4), as compared to that standard ascorbic acid, which was found to be 92.30 ± 0.26% with IC_{50} = 160 ± 0.01 µg/mL (Farnad et. al 2014). These findings suggest that the plant is a valuable natural source of bioactive compounds capable of neutralizing free radicals, supporting its possible use in pharmaceutical formulations or as a natural food preservative to protect against oxidative stress and related damage.

Table 4: Antioxidant Activity of *Mentha piperita*

Concentration (µg/mL)	% Inhibition	Standard Deviation
31.25	22.40	0.61
62.50	38.89	1.35
125	56.03	0.34
250	70.87	2.11
500	84.89	1.41

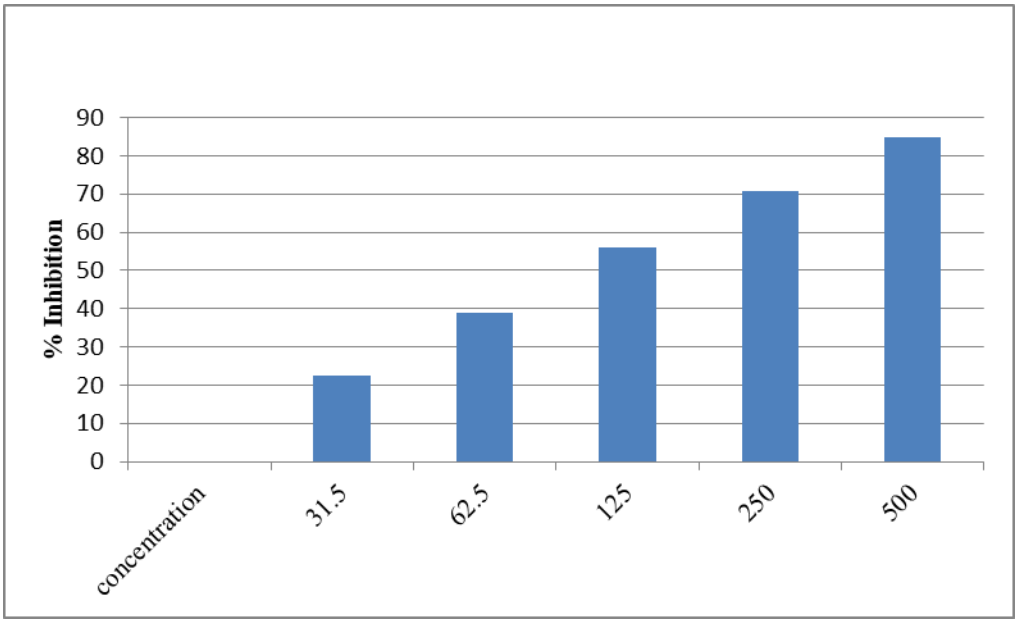

Figure 6 Antioxidant activity of *Mentha piperita*

Conclusion and Recommendations

The present study shows the *Mentha piperita* found in local markets and villages of Rupandehi district is a potent radical scavenger. Phytochemical screening of ethanolic extract revealed presence of secondary metabolites; alkaloids, terpenoids, flavonoids, glycosides, polyphenols, saponins, tannins, and steroids. The metabolites like terpenoids, flavonoids, and phenolic compounds are found in high concentration. The total phenolic content (TPC) and total flavonoid content (TFC) are found to be 370.9 ± 6.45 mg GAE/100 g dry extract and 345 ± 3.95 mg QE/100 g dry extract, respectively, revealed higher amount of phenolic and flavonoid compounds as compared to other similar studies.

Antioxidant activity assessed by the DPPH assay showed increasing inhibition from 22.40% at 31.25 µg/mL to 84.89 ± 1.41 % at 500 µg/mL, with an IC_{50} of 156.06 ± 2.12 µg/mL, indicating a dose-dependent response and strong free radical scavenging activity.

Thus, *Mentha piperita* demonstrates significant antioxidant properties with enrich phenolic and flavonoid compound making it a valuable natural product bioactive materials in chemistry and biochemistry.

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References

Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nature Protocols*, *2*(4), 875–877. https://doi.org/10.1038/nprot.2007.102

Al-Rajhi, A. M. H., Qanash, H., Bazaid, A. S., Binsaleh, N. K., & Abdelghany, T. M. (2023).

Pharmacological Evaluation of Acacia nilotica Flower Extract against Helicobacter pylori and Human Hepatocellular Carcinoma In Vitro and In Silico. *Journal of Functional Biomaterials*, *14*(4). https://doi.org/10.3390/jfb14040237

Alqethami, A., & Aldhebiani, A. Y. (2021). Medicinal plants used in Jeddah, Saudi Arabia: Phytochemical screening. *Saudi Journal of Biological Sciences*, *28*(1), 805–812. https://doi.org/10.1016/j.sjbs.2020.11.013

Chaudhary, P., Janmeda, P., Docea, A. O., Yeskaliyeva, B., Abdull Razis, A. F., Modu, B., Calina, D., & Sharifi-Rad, J. (2023). Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. *Frontiers in Chemistry*, *11*(May), 1–24. https://doi.org/10.3389/fchem.2023.1158198

Farnad, N., Heidari, R., & Aslanipour, B. (2014). Phenolic composition and comparison of antioxidant activity of alcoholic extracts of Peppermint (Mentha piperita). *Journal of Food Measurement and Characterization*, *8*(2), 113–121. https://doi.org/10.1007/s11694-014-9171-x

Goldstein, M. C., & Goldstein, M. A. (2014). Peppermint Oil. *Healthy Oils: Fact Versus Fiction*, *1*(1), 164–168. https://doi.org/10.5040/9798400662560.ch35

Grigoleit, H. G., & Grigoleit, P. (2005). Pharmacology and preclinical pharmacokinetics of peppermint oil. *Phytomedicine*, *12*(8), 612–616. https://doi.org/10.1016/j.phymed.2004.10.007

Howden-chapman, P., & Chisholm, E. (2018). Goal 3. Ensure Healthy Lives and Promote Well-Being for All at All Ages. *A New Era in Global Health*, 81–126. https://doi.org/10.1891/9780826190123.0014

Hussain, M. S., Fareed, S., Ansari, S., Rahman, M. A., Ahmad, I. Z., Saeed, M., & Calabrò, S. (2012). Current approaches toward production of secondary plant metabolites. *Rumen Microbiology: From Evolution to Revolution*, *4*(October), 153–159. https://doi.org/10.1007/978-81-322-2401-3_11

Kaur, R., Zhang, S., Berwal, B., Ray, S., Kumar, R., & Varshney, L. R. (2025). From phytochemicals to recipes: health indications and culinary uses of herbs and spices. *Npj Science of Food*, *9*(1), 1–12. https://doi.org/10.1038/s41538-025-00458-z

Khanal, B. (2019). *Phytochemical and Antibacterial Analysis of Mentha piperita (Peppermint)*. *June 2019*, 1–13. https://doi.org/10.13140/RG.2.2.23973.73444

Kim, D. O., Jeong, S. W., & Lee, C. Y. (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry*, *81*(3), 321–326. https://doi.org/10.1016/S0308-8146(02)00423-5

Phuyal, A., Ojha, P. K., Guragain, B., & Chaudhary, N. K. (2019). Phytochemical screening, metal concentration determination, antioxidant activity, and antibacterial evaluation of Drymaria diandra plant. *Beni-Suef University Journal of Basic and Applied Sciences*, *8*(1), 1–9. https://doi.org/10.1186/s43088-019-0020-1

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, *53*(10), 4290–4302. https://doi.org/10.1021/jf0502698

Qanash, H., Bazaid, A. S., Binsaleh, N. K., Alharbi, B., Alshammari, N., & Qahl. (2023). Phytochemical Characterization of Saudi Mint and Its Mediating Effect on the Production of Silver Nanoparticles and Its Antimicrobial and Antioxidant Activities. *Plants*, *12*(11). https://doi.org/10.3390/plants12112177

Sharma, M., & Gautam, D. (2022). Phytoconstituents and Medicinal Value of Mentha piperita. *Modern Phytomorphology*, *15*(March), 156–160. https://www.researchgate.net/publication/358667638

Tang, H. P., Zhu, E. L., Bai, Q. X., Wang, S., Wang, Z. Bin, Wang, M., & Kuang, H. X. (2024). Mentha haplocalyx Briq. (Mint): a comprehensive review on the botany, traditional uses, nutritional value, phytochemistry, health benefits, and applications. *Chinese Medicine (United Kingdom)*, *19*(1), 1–29. https://doi.org/10.1186/s13020-024-01037-2

Ulewicz-Magulska, B., & Wesolowski, M. (2019). Total Phenolic Contents and Antioxidant Potential of Herbs Used for Medical and Culinary Purposes. *Plant Foods for Human Nutrition*, *74*(1), 61–67. https://doi.org/10.1007/s11130-018-0699-5

Zheng, W., & Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry*, *49*(11), 5165–5170. https://doi.org/10.1021/jf010697n