Antioxidant Assessment of *Mentha spicata* Leaf Extract And GC-MS Profiling of Metabolite

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Abstract

Mentha spicata is a member of Lamiaceae family and containing diverse array of secondary metabolites, having significant role in medicinal contexts. The importance of the secondary metabolites of the M. spicata is widely acknowledged; however, some metabolites need further assessment to determine their potential applications in medicine. In the present study, the metabolites are extracted from the M. spicata using ethanol and are analyzed through Gas Chromatography-Mass Spectrometry (GC-MS). The list of metabolites such as Undecane, Hexadecanoic acid methyl ester, 12,15-Octadecadienoic acid methyl ester, 6-Octadecenoic acid methyl ester (Z), 6-Octadecenoic acid methyl ester, Cyclohexane, methyl ester, Phenol, 4,4'-(1-methylethylidene) bis, Sulfurous acid cyclohexylmethyl dodecyl ester, Octasiloxane -hexadecamethyl are identified in the extract. The antioxidant efficacy of the extract is evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assays. Results show that at a concentration of 1 mg/mL, scavenging activity of the extract is 20 ± 2%, which is enhanced in a dose dependent manner by increasing the extract concentration. Concentrations 2 mg/mL and 6 mg/mL result the activities up to 22.4 ± 2% to 28.6 ± 2.5. GC-MS analysis facilitates identification of metabolites, improving the knowledge about their nature and antioxidant properties and DPPH assay highlights to scavenge free radicals. Both GC-MS and DPPH assay highlight the role of the M. spicata in reducing oxidative stress and its potential applications in pharmaceuticals.

Keywords: *Mentha spicata*; Bioactive Metabolites; GC-MS; DPPH; Antioxidant.

Introduction

Mentha spicata, an aromatic herb, has a great medicinal application, belongs to the Lamiaceae family, and has drawn great attention of researchers due to its antitumor, antimicrobial, and properties (D'Agostino, et al., 2024). It is distinguished by its spear and dark green leaves (Saba et al., 2024). One major economic role of *M. spicata* is the extracts and essential oils production. The commercial advantages of *M. spicata* has also drawn much attention due to its wide use in traditional medicine. Oxidation in a living system is extremely lethal because reactive oxygen species (ROS) produce in the reaction are extremely reactive that react with any biological molecule and trigger the chemical reaction,

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resulting cell damage and hence cause of cancer (Abbas et al., 2024). The antioxidants properties of the natural metabolites have drawn magnanimous attention globally. These antioxidants are important because of their free radical-scavenging activities and have great values for health benefits and medicine (Chen et al., 2023).

A number of plant genera, including the genus of *Mentha*, contain high level of antioxidants such as phenolic compounds, ascorbic acid, and carotenoids. These antioxidants scavenge the molecules thus causing oxidation with the help of their free radical scavenging properties. A very common example is Phenolic metabolites, which behave as free radical scavengers and inhibit lipid peroxidation (Yazar et al., 2011). *M. spicata* is known to contain a diverse array of secondary metabolites, which has important medicinal applications (Bezerra et al., 2023; Belakhdar et al, 2015). While the importance of these secondary metabolites is well-acknowledged, further assessment is needed to determine their potential in various medicinal contexts (Fatiha et al., 2015). *M. spicata* contains rich array of phytometabolites in order to establish medicinal values (Saba et al., 2024).

These phytochemicals have been profiled using gas chromatography-mass spectrometry (GC-MS). The GC-MS is a technique that is commonly used to identify the metabolites present in the and characterize specific substance on the pooled extracted portion (Al-Owaisi et al., 2014). GC-MS breaks down the metabolites through mass to charge ration (*m/z*) to identify the metabolites through comparing its mass spectra with established libraries. The characterization of the metabolites of *M. spicata* extract led the researcher to understand its medicinal values. The analysis of extracts through GC-MS identifies the antioxidant molecules that help protect body cells from damage caused by harmful free radicals. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is frequently applied to test the antioxidant potential of the extract (Bouyahya et al., 2024).

In the present study, the metabolites of *M. spicata* are extracted using ethanol and analyzed through GC-MS. The analysis identified several metabolites, including Undecane, Hexadecanoic acid methyl ester, 12,15-Octadecadienoic acid methyl ester, 6-Octade cenoic acid methyl ester (Z), 6-Octadecenoic acid methyl ester, Cyclohexane, methyl ester, Phenol, 4,4'-(1-methylethylidene) bis, Sulfurous acid cyclohexylmethyl dodecyl ester, and Octasiloxane -hexadecamethyl (Brighenti et al., 2014). The antioxidant efficacy of the extract is evaluated using DPPH scavenging assays, which show that the extract exhibit a dose-dependent free radical scavenging activity, reaching up to 28.6 ± 2.5% at a concentration of 6 mg/mL (Figat et al., 2020; Brighenti et al., 2014; Souza et al., 2016; Navarro-Hoyos et al., 2018).

Maerials and Methods

Sample Collection

M. spicata sample is collected from a local market transported to the laboratory, where the leaves of s *M. spicata* are dried in shed at 40ºC. Dried leaves sample weighed 100 g is crushed in to a fine powder and is soaked subsequently in 1000 ml of 75% ethanol. The mixture is kept on stirrer for 72 h at 25°C. The extract is then filtered through Whatman filter paper and then subjected to dehydration using rotary evaporator at 45°C in a vacuum to remove the ethanol. The resulting dehydrated residue is used for GC-MS and the DPPH assay.

Gas Chromatography-mass Spectrometry Analysis

The dried residues are added with 100% ethanol and then concentrated using a rotary evaporator. The sample is prepared for GC-MS (GC-MS, Agilent, USA) analysis. Precisely 1 µL sample is injected in to GC-MS at a flow rate of 0.5 mL/min. The inlet temperature is kept at 250°C, while the oven temperature is initially set at 110°C, which is gradually raised to 240°C after in a period of 4 h and is lasted for 5 min. The transfer line temperature of the mass spectrometer and the of the filament temperature are at 200°C and 180°C, respectively. The compounds are initially identified and quantified by using the electron impact ionization method at 70 eV with the help of the total ion count (TIC). The data included in the GC-MS database are used as referenced in order to get the names, molecular weights.

Antioxidant Assay (Free radical scavenging)

In order to evaluate the antioxidant potential of the extract, the stable radical DPPH scavenging assays is carried out. Different concentration of the ethanolic extract are prepared (1, 2, 3, 4, 5, 6 and 7 mg/ml). for the test, 2 ml of the DPPH solution is added in to 1 ml off each extract concentration and vitamin C is used as positive control. The blank is prepared by adding 1 ml of ethanol into 2 ml of the DPPH. All the solutions e.g., test, positive control and blank are incubated for 45 min at 25°C in the dark. The absorbance is measure through spectrophotometer at 517 nm and the readings are put in the formula to get the results.

$[(Ab - Aa)/Ab] \times 100$ = percentage of scavenging activity.

Where, Ab = the absorbance of the control (without antioxidant)

Aa = absorbance of the antioxidant sample.

Statistical Analysis

The statistical analysis is carried through one way ANOVA using MS Excell (version 2019) and the values are represented as mean ± standard deviation.

Results

Gas chromatography mass spectroscopy analysis of the M. spicata extract

M. spicata is known for rich secondary metabolite profile, and hence provided multiple health benefits. In the present analysis, GC-MS method is used to analyze the useful metabolites in the extract of *M. spicata*. These metabolites are necessary for many body functions, including the enhancement of the immune system and resistance against pathogens. The extract is injected into the GC-MS for the identification of the bioactive metabolites. The GC using helium gas as carrier separated the metabolites based on the retention time, area, height of the peaks. Results of CG analysis are shown in the form of GC chromatogram (Figure 1).

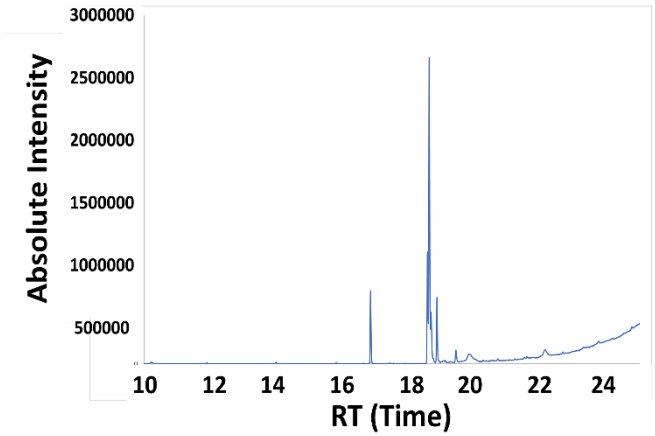

Figure 1: GC spectrum of the metabolites in the extract of Mentha plants. RT shows specific time for each compound.

Subsequently the GC separation of metabolites present in the ethanolic extract of *M. spicata*. The MS part ionize the metabolites through mass to charge ration (*m/z*) to identify the separated metabolites through comparing its mass spectra with established libraries. By matching the mass spectra with the existing libraries, these metabolites are characterized and documented. The main metabolites identified through the analysis are as follows: *Undecane, Hexadecanoic acid methyl ester, 12,15-*

Octadecadienoic acid methyl ester, 6-Octadecenoic acid methyl ester (Z), 6-Octadecenoic acid methyl ester, Cyclohexane, methyl ester, Phenol, 4,4'-(1-methylethylidene) bis, Sulfurous acid cyclohexylmethyl dodecyl ester, Octasiloxane -hexadecamethyl. These metabolites appear to have significant role in shaping the unique chemical profile of the *M. spicata* extract (Table 1).

Table 1: GC-MS profile of the metabolites in the extract of M. spicata.

Peak #	Ret. Time	Area	Height	A/H	Name
1	6.232	235833	56079	4.21	Undecane
2	16.853	1146651	622031	1.84	Hexadecanoic acid, methyl ester
3	18.575	1809625	958219	1.89	12,15-Octadecadienoic acid, methyl ester
4	18.627	5131843	3E+06	1.96	6-Octadecenoic acid, methyl ester, (Z)-
5	18.683	928724	438325	2.12	6-Octadecenoic acid, methyl ester, (Z)-
6	18.75	90871	42510	2.14	Cyclohexane, (1,2,2-trimethylbutyl)-
7	18.86	1079535	559387	1.93	Methyl stearate
8	19.435	246014	102543	2.4	Phenol, 4,4'-(1-methylethylidene)bis-
9	19.827	359054	44559	8.06	Sulfurous acid, cyclohexylmethyl dodecyl ester
10	24.765	40735	17525	2.32	Octasiloxane, hexadecamethyl-

Antioxidant Activities of the Extracts of M. spicata

The antioxidant potential of *M. spicata* extracts is evaluated through DPPH scavenging assays. Results show that the antioxidant scavenging activity is gradually increasing as the concentration of the extract is increased. The extract shows antioxidant scavenging efficiency of 20 ± 2% at the concentration 1 mg/ml, which is raised to 22.4 ± 2% when the concentration is increased to 2 mg/ml. Finally, the activities are 27.6 ± 2.5%, and 29.2 ± 1.5 % when the concentration is reached to 6 mg/ml and 7 mg/ml respectively, showing significant improvement in the activities as compared to the activities at lower concentrations. This development focuses the potent antioxidative capabilities of extract, which significantly enhanced the activities with an increase in the dosage (Figure 2).

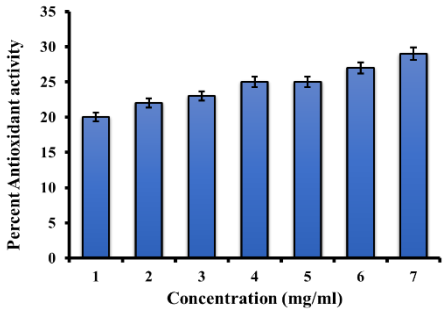

Figure 2: the antioxidants in the extract of Mentha.

Discussion

Plant-based natural antioxidants are safer and have medicinal benefits, therefore, the importance of their use and is growing interest as compared to the synthetic counterparts (Abbas et al., 2024). Evidences have been reported about the reactivity of the natural antioxidant towards reactive oxygen species and free radicals and hence their application as antioxidants is in high demand (Bouali et la., 2024). *M. spicata*, has a significant value not within the scientific community due its rich polyphenolics and other secondary metabolites e.g., caffeic acid, alkaloids, Flavonoids and many other. In the present study, the extract of *M. spicata* is assessed for the metabolites profiling using GC-MS and antioxidant analysis through DPPH assay. The analysis results metabolites including Undecane, Hexadecanoic acid methyl ester, 12,15-Octadecadienoic acid methyl ester, 6-Octade cenoic acid methyl ester (Z), 6-Octadecenoic acid methyl ester, Cyclohexane, methyl ester, Phenol, 4,4'-(1-methylethylidene) bis, Sulfurous acid cyclohexylmethyl dodecyl ester, and Octasiloxane -hexadecamethyl and DPPH assay shows a significant antioxidant activity. Previous studies support the results of the present study and confirm the presence of various compounds in the ethanolic extract as well as in the essential oils of *M. spicata* (El Omari et al., 2024; Hameed et al., 2015).

GC-MS analysis of the present study reveal the list of metabolites including Undecane, 3,7-dimethyl- Hexadecanoic acid methyl ester, 11,14-Octadecadienoic acid methyl ester, 6-Octadecenoic acid methyl ester, 9-Octadecenoic acid methyl ester, Methyl stearate, Phenol 4,4'-(1-methylethylidene), Naphthalene-1-sulfonic acid 4-methoxy-, (2-adamantan. Boukhebti et al. (2011) has identified several major metabolites in the ethanolic extract and essential oil of *M. spicata* responsible for the antioxidant activities. The DPPH assay is very effective and generally approved technique to evaluate the antioxidant activity of extracts by measuring their capability to scavenge free radicals to disrupt chains of oxidative reactions (Chen et al., 2023; Bouyahya et al., 2024). Cakir et al. (2003) reported in their study that different species of Mentha show 88% to 93% of the antioxidant activities at the concentration of 100 µL/mL. Conversely, one other study provide the data of antioxidant activity, which are as follows: starting from the highest level of DPPH scavenging: *M. piperita*, *M. pulegium, M. rotundifolia, M. spicata,* and *M. longifolia*. In the present study, the extract of *M. spicata*, is evaluated for antioxidant properties using DPPH activity and results show significant antioxidant activity. These results are supported by previous studies that highlight the antioxidant potential of both the aqueous and extracts of *M. spicata* (Saba et al., 2024; Bouyahya et al., 2024).

Phenolics and flavonoid compounds have been known for antioxidant activities and several studies have reported that polyphenolic compounds are mostly responsible for the significant antioxidant activities (Bouali et la., 2024; Rajeswari et al., 2018). The metabolites such as Hexadecanoic acid methyl ester, 12,15-Octadecadienoic acid methyl ester, 6-Octade cenoic acid methyl ester (Z), 6-Octadecenoic acid might be involved in the antioxidant properties (Devi & Muthu, 2014). Results of the present study are supported by the findings of Boukhebti et al. (2015), reported that the profile of the *M. spicata* extract is rich with the metabolites known for the antioxidant activities. The metabolites identified in both the extract and the essential oil of the *M. spicata* are consistent in their antioxidant potentials (D'Agostino et al., 2024). This consistency supports the significance of the bioactive metabolites (El Omari et al., 2024). In addition, previous studies strengthen these results, revealing a consistent pattern concerning the antioxidant efficacy of the extract, which is attributed to phenolic and flavonoid and other metabolites present in extract (D'Agostino et al., 2024; Hameed et al., 2015). However, there could be more secondary metabolites in the extract contributing directly or synergistically in the scavenging activity of the reactive oxygen species (Devi & Muthu, 2014; El Omari et al., 2024). In short, the present study would contribute to the understanding metabolite array in the extract of *M. spicata* and their potential role to be used as antioxidant.

Conclusion

In conclusion, the interaction between conventional knowledge and modern scientific research has emphasized the remarkable potential of *M. spicata*. The unique bioactive properties, of *M. spicata*, particularly its strong antioxidant potential assessed in its ethanolic extracts, ranked the *M. spicata* as a capable natural solution for reducing oxidative stress and related health challenges. More research into its chemical profiling and medicinal applications could pave the way for its broader acceptance in mainstream medicine, which would be contributing to improved health benefits worldwide.

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