



GC-FID Phytochemical Profiling of Ethanolic Leaf Extract of *Mentha piperita* and Correlation of Identified Bioactive Constituents with Neuroprotective Activity

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ABSTRACT

Background: *Mentha piperita* (peppermint) has documented neuroprotective activity, but the specific bioactive constituents responsible remain incompletely characterised in relation to Alzheimer's disease (AD) pathology.

Methods: The ethanolic leaf extract of *M. piperita* (ELMP) was profiled by Gas Chromatography Flame Ionisation Detection (GC-FID) against certified external standards to identify and quantify its phytochemical constituents.

Results: Thirty-three compounds were detected; 25 were quantified, totalling 79.52 ppm. Limonene predominated (54.571 ppm; 68.62%), eluting at two retention times (4.030 and 9.515 min). Other major constituents were Citral (6.62%), Myricetin (6.60%), Flavone (5.91%), Nobiletin (5.89%), Epicatechin (4.88%), Resveratrol (4.82%), and Pinene (3.94%). Nine compounds, including Rosmarinic and Ellagic acid, were present only as trace peaks below the quantification limit.

Conclusion: The dominance of limonene alongside pharmacologically active flavonoids (myricetin, nobiletin, epicatechin) and the stilbene resveratrol offers a plausible phytochemical basis for antioxidant, anti-neuroinflammatory, and anti-amyloidogenic mechanisms relevant to AD. Given the known limits of GC-FID for quantifying non-volatile flavonoids and phenolic acids, these findings should be regarded as a preliminary chemical fingerprint requiring confirmation by LC-MS/HPLC. ELMP represents a promising, multi-constituent phytotherapeutic candidate warranting further mechanistic and clinical evaluation.

1. INTRODUCTION

Mentha piperita L. (peppermint), a member of the Lamiaceae family, is among the most widely cultivated and commercially significant aromatic plants globally. Its antioxidant, anti-inflammatory, antimicrobial, antispasmodic, and neuroprotective activities are well documented and largely attributed to its rich secondary metabolite profile (Iskan et al., 2002; Koşar et al., 2005). The essential oil and non-volatile fractions of *M. piperita* contain over 300 identified constituents, including terpenoids, flavonoids, phenolic acids, triterpenoids, and sterols. Their relative abundance varies with chemotype, geographic origin, extraction method, and plant maturity. GC-FID is widely used for quantitative profiling of plant extracts because it is sensitive, reproducible, and compatible with volatile and semi-volatile compounds. It identifies compounds by retention time and quantifies them by peak area against external standards (Paton and Putievsky, 1996).

Alzheimer's disease (AD) is a growing global health burden with few disease-modifying treatments. Aluminium chloride (AlCl_3) and D-galactose are established experimental inducers of AD-like neurotoxicity in rodents, producing oxidative stress, neuroinflammation, amyloid-beta ($\text{A}\beta_{1-42}$) accumulation, tau hyperphosphorylation, and cognitive impairment. Identifying which phytoconstituents in *M. piperita* extract might plausibly drive its reported neuroprotective effects is therefore of both scientific and

translational interest. This study presents the complete GC-FID phytochemical profile of ELMP and discusses how the identified constituents relate mechanistically to neuroprotective pathways relevant to AD, and to the peripheral hepatic and renal antioxidant outcomes reported in a companion in vivo study (Afubero et al., 2026).

2. MATERIALS AND METHODS

2.1 Plant Material and Extract Preparation

Fresh leaves of *M. piperita* were collected, authenticated by a botanist, and shade-dried at room temperature. The dried leaves were pulverised into a coarse powder using a mechanical blender. Ethanolic extraction was performed by soaking 500 g of powdered leaf material in 2.5 L of 95% ethanol for 72 hours with intermittent agitation. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C. The resultant crude extract was stored at 4°C in a sealed amber vial until analysis.

2.2 GC-FID Analysis

Phytochemical profiling was carried out by GC-FID (Agilent CHEM32, Instrument 1) on 18 February 2026 at the Department of Pharmaceutical Chemistry

Laboratory, using an injection volume of 0.2 μ L. The acquisition method (PHYTO-2023.M) used an external standard calibration protocol (Multiplier: 1.0000; Dilution: 1.0000). Peaks were identified by matching retention times with certified external reference standards. All compounds were assigned to Group 1 (TPH: Total Phenolic and Hydrocarbon fraction). Compounds below the limit of quantification were recorded as not detected (ND).

2.3 Rationale and Limitations of GC-FID for Flavonoid Detection

GC-FID was chosen because it allowed rapid, single-run detection across a broad polarity range using an existing in-house calibration library, from volatile monoterpenes to phenolic and flavonoid standards. However, GC-FID depends on analytes being sufficiently volatile, or chemically derivatised, to elute cleanly and respond reliably at the flame ionisation detector. Non-volatile, polyhydroxylated, or polymethoxylated compounds such as Myricetin and Nobiletin are prone to peak tailing, thermal degradation, and inconsistent response factors

under GC conditions compared with liquid-phase methods. Accordingly, the flavonoid and phenolic acid quantities reported here should be read as an indicative screening-level fingerprint rather than a fully validated quantitative assay. Confirmatory analysis by LC-MS or HPLC-DAD, which does not require analyte volatility, is recommended before these values are used to support precise structure-activity conclusions.

3. RESULTS

3.1 GC-FID Phytochemical Profile

GC-FID analysis of ELMP identified 33 compounds across a retention time range of 3.925-31.656 minutes. Of these, 25 were quantified against external standards, totalling 79.52 ppm (Table 1). Nine compounds were detected as trace peaks below the quantification limit (ND). The chromatogram showed early-eluting terpenoid peaks dominating, with secondary flavonoid and phenolic acid peaks at higher retention times.

Table 1: Phytochemical Analysis of Ethanolic Leaf Extract of *Mentha piperita* Using GC-FID

Compound	RT (min)	Area (pA·s) Mean $\pm$ SEM	Amount (ppm) Mean $\pm$ SEM	% of Total
Limonene*	4.030 / 9.515	495.11	54.571	68.62
Citral	12.738	30.408	5.266	6.62
Myricetin	14.607	137.452	5.251	6.60
Flavone	7.004	33.506	4.703	5.91
Nobiletin	15.115	12.054	4.684	5.89
Epicatechin	6.646	13.462	3.883	4.88
Resveratrol	5.172	20.199	3.831	4.82
Pinene	6.254	15.208	3.129	3.94
Catechin	3.925	6.665	0.929	1.17
Daidzein	7.219	4.367	0.605	0.76
Vanillic acid	9.871	3.986	0.550	0.69
Flavon-3-ol	9.016	3.898	0.537	0.68
Naringin	8.401	3.445	0.474	0.60
Naringenin	10.156	3.092	0.425	0.53
Daidzin	8.264	2.980	0.410	0.52
Coumaric acid	5.304	3.010	0.409	0.51
Lunamarin	7.999	2.909	0.399	0.50
Kaempferol	11.907	2.371	0.312	0.39
Butein	9.159	2.135	0.291	0.37
Ferulic acid	18.617	1.550	0.204	0.26
Luteolin	10.695	1.392	0.187	0.23
Cinnamic acid	18.351	1.316	0.166	0.21
Baicalin	17.398	1.199	0.158	0.20
Epigallocatechin	7.695	1.114	0.147	0.19
Ellagic acid	5.923	Trace	ND	–
Gallocatechin 3-gallate	13.521	Trace	ND	–
Robinetin	14.123	Trace	ND	–
Tangeretin	16.296	Trace	ND	–
Rosmarinic acid	31.656	Trace	ND	–
Sinapinic acid	30.586	Trace	ND	–
Syringic acid	28.232	Trace	ND	–
Gentisic acid	24.651	Trace	ND	–
Piperic acid	20.424	Trace	ND	–

RT: Retention Time; ND: Not Detected (below quantification limit). *Limonene detected at two retention time peaks (4.030 and 9.515 min); combined area and amount reported. Total quantified: 79.52 ppm.

Limonene, a monoterpene, eluted at two distinct retention times (4.030 and 9.515 min) with a combined peak area of 495.11 pA·s and a total quantified amount of 54.571 ppm (68.62% of all quantified phytochemicals). This dual elution may reflect limonene occurring in structurally distinct matrix environments, or co-eluting isomeric forms. Citral (5.266 ppm; 6.62%), an acyclic monoterpenoid aldehyde, eluted at 12.738 min. Myricetin (5.251 ppm; 6.60%), a polyhydroxylated flavonol, eluted at 14.607 min. Flavone (4.703 ppm; 5.91%) and Nobiletin (4.684 ppm; 5.89%) were the third and fourth most abundant flavonoid constituents. Epicatechin (3.883 ppm; 4.88%), Resveratrol (3.831 ppm; 4.82%), and Pinene (3.129 ppm; 3.94%) made up the remaining major constituents. Minor quantified constituents (≤ 1.17 ppm each) included Catechin, Daidzein, Vanillic acid, Flavon-3-ol, Naringin, Naringenin, Daidzin, Coumaric acid, Lunamarin, Kaempferol, Butein, Ferulic acid, Luteolin, Cinnamic acid, Baicalin, and Epigallocatechin. Nine compounds, including Ellagic acid, Gallocatechin 3-gallate, Robinetin, Tangeretin, Rosmarinic acid, Sinapinic acid, Syringic acid, Gentisic acid, and Piperic acid, were detected only as trace peaks (ND).

4. DISCUSSION

4.1 Terpenoid Constituents and Neuroprotective Relevance

Limonene's dominance (68.62%) in the ELMP profile is a notable finding with clear pharmacological relevance. Limonene is a cyclic monoterpene with well-documented antioxidant, anti-inflammatory, anxiolytic, and neuroprotective effects in the literature. It scavenges free radicals directly, induces phase II detoxification enzymes, and activates the Nrf2/HO-1 antioxidant pathway. These known mechanisms offer a plausible chemical basis for the type of antioxidant benefit (e.g., reduced brain MDA, improved SOD and catalase activity) that would be expected in an AlCl_3 /D-galactose model of AD-like neurodegeneration, and align with hepatic and renal antioxidant restoration reported in a related *in vivo* study by our group (Afubero et al., 2026). Limonene also inhibits NF- κ B activation, which suppresses pro-inflammatory cytokines such as IL-1 β and IL-6.

Citral (6.62%), the second most abundant quantified constituent, is an acyclic monoterpenoid aldehyde present as a mixture of geranial and neral isomers. It scavenges superoxide and hydroxyl radicals directly and inhibits the COX-2 and 5-LOX inflammatory pathways. Together, limonene, citral, and pinene make up approximately 79% of all quantified compounds, indicating that terpenoids are likely the extract's primary antioxidant and anti-inflammatory contributors.

4.2 Flavonoid Constituents and Alzheimer's Disease Pathology

Myricetin (6.60%) is a polyhydroxylated flavonol with neuroprotective mechanisms well characterised in the AD literature. It inhibits glycogen synthase kinase-3 β (GSK-3 β), a key kinase in tau hyperphosphorylation, and has been shown elsewhere to reduce amyloid-beta (A β) aggregation through direct interaction with A β fibrils. These literature-established mechanisms are consistent with the kind of reduction in A β 1-42 and phosphorylated tau that would support ELMP's reported neuroprotective activity. Myricetin also chelates aluminium ions, which is particularly relevant to limiting aluminium-induced oxidative damage in AlCl_3 -based models.

Flavone (5.91%) and Nobiletin (5.89%) are established neuroprotective flavone constituents. Nobiletin, a polymethoxylated flavone, enhances CREB phosphorylation and promotes BDNF expression in prior studies, and separately inhibits β -secretase (BACE1) activity, reducing amyloidogenic APP processing. Epicatechin (4.88%) is a flavan-3-ol that inhibits acetylcholinesterase (AChE), which would be expected to help preserve synaptic acetylcholine levels. Resveratrol (4.82%), a stilbene polyphenol, activates SIRT1 deacetylase and AMPK signalling, mechanisms linked in the literature to autophagy-mediated clearance of aggregated A β and phosphorylated tau. Collectively, this flavonoid and polyphenol fraction offers a multi-target mechanistic rationale for the anti-amyloid, anti-tau, and cholinergic-protective potential of ELMP.

4.3 Phenolic Acids and Supporting Antioxidant Activity

Minor phenolic acid constituents identified in ELMP included Coumaric acid (0.409 ppm), Vanillic acid (0.550 ppm), Ferulic acid (0.204 ppm), Cinnamic acid (0.166 ppm), and trace levels of Rosmarinic, Syringic, Sinapinic, and Gentisic acid. Despite their low individual quantities, phenolic acids likely contribute to overall antioxidant capacity through synergistic radical scavenging and metal chelation. Ferulic acid is notable for its documented ability to cross the blood-brain barrier and inhibit both A β aggregation and AChE activity, with additional anti-inflammatory effects via NF- κ B inhibition.

4.4 Dopaminergic and Neurotrophic Mechanisms

Kaempferol (0.312 ppm), Luteolin (0.187 ppm), Baicalin (0.158 ppm), Naringenin (0.425 ppm), and Naringin (0.474 ppm) are minor quantified constituents with mechanisms relevant to dopaminergic and neurotrophic support. Kaempferol protects dopaminergic neurons by scavenging reactive oxygen species and inhibiting monoamine oxidase B (MAO-B). Luteolin has been shown in prior work to enhance BDNF expression and TrkB receptor phosphorylation. Baicalin modulates the BDNF/TrkB/PI3K/Akt signalling cascade and suppresses

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