**QUANTITATIVE AND QUALITATIVE PHYTOCHEMICAL ANALYSIS OF ETHANOIC LEAF EXTRACT OF *MENTHA PIPERITA***

**Abstract**

Mentha piperita (peppermint) is a widely used medicinal plant known for its therapeutic properties, yet its neurobiological and toxicological safety profile remains under-explored in animal models. This study investigates the phytochemical constituents, elemental composition, and acute toxicity (LD₅₀) of ethanolic leaf extract of Mentha piperita, with implications for its neuropharmacological safety and efficacy.

Fresh Mentha piperita leaves were collected, authenticated, and subjected to ethanolic extraction. Qualitative and quantitative phytochemical analyses were performed using Gas Chromatography (GC). Elemental analysis was conducted using Flame Atomic Absorption Spectrophotometry (FAAS). Acute toxicity was assessed using a modified Lorke’s method on Wistar rats, with extract doses ranging from 10 to 5000 mg/kg body weight administered orally.

The phytochemical analysis revealed the presence of bioactive compounds including ammodendrine (22.07 µg/ml), cyanogenic glycosides (20.19 µg/ml), proanthocyanidins (10.48 µg/ml), naringenin (7.43 µg/ml), tannins (12.00 µg/ml), and alkaloids such as spermatine (15.97 ng/ml). Elemental analysis indicated high concentrations of chloride (87.40 mg/L), alongside sodium (33.65 mg/L), potassium (29.74 mg/L), calcium (25.89 mg/L), and phosphate (21.08 mg/L). No mortality or observable signs of toxicity were recorded at doses up to 5000 mg/kg, indicating an LD₅₀ > 5000 mg/kg.

The ethanolic extract of Mentha piperita contains significant levels of phytochemicals and essential elements with no observable acute toxicity in Wistar rats up to 5000mg/kg, suggesting its safety for potential pharmacological and nutraceutical applications. These findings support its traditional use and provide a basis for further neurobiological and therapeutic investigations.

**Keywords:** Mentha piperita, phytochemicals, acute toxicity, LD₅₀, elemental analysis, Wistar rats, neuropharmacology

**1.0 Introduction**

Nutrition is fundamental to optimal brain function, as specific nutrients supply essential chemical compounds critical for brain development and activity. A deficiency in these nutrients can result in cognitive impairments (Ali et al., 2024; Ebuehi, 2012). Nutrition also influences cellular processes and organismal development. Certain phytochemicals derived from plants act as primary precursors to neurotransmitters vital for the central nervous system, facilitating chemical signaling along neural pathways. Neurotransmitters such as serotonin, epinephrine, acetylcholine, and dopamine can be synthesized from nutritional amino acids (Lee, 2015; Ebuehi, 2012).

Herbs are widely recognized and utilized in traditional medicine across the globe for their therapeutic potential. Many of these plants demonstrate pharmacological properties and are promising candidates for the treatment of central nervous system disorders, including anxiety and cognitive dysfunction (Ibrahim et al., 2025; Carlini, 2003; Faustino et al., 2010).

**1.1 Mentha Piperita**

Peppermint (Mentha piperita L.), an aromatic perennial herb of the Lamiaceae (Labiatae) family, is a natural hybrid of spearmint (Mentha spicata L.) and water mint (Mentha aquatica L.) (Spirling & Daniels, 2001). Though native to the Mediterranean, peppermint is now cultivated globally for its applications in flavoring, fragrance, and pharmaceuticals (Shakib et al., 2023). In Nigeria, it is commonly known as "Mint leaf" and is particularly prevalent in the northern region (e.g., Jos). Mentha piperita, a hybrid of Mentha spicata and Mentha aquatica, is known by various names across cultures, including Menthe poivrée (French), Po Ho (Chinese), Pudina (Hindi), Ewe minti (Yoruba), Ahu ogwu (Igbo), and Na’abba (Hausa) (Almatroodi et al., 2021).

Peppermint is typically used in the form of leaves, leaf extracts, and stems, though it is primarily cultivated for its essential oil, extracted through the distillation of freshly ground leaves (Mairapetyan et al., 2016; Uribe et al., 2016). Menthol, a key component of peppermint oil, is responsible for its spasmolytic effects (Peat et al., 2016), and has been shown to enhance bile flow and promote belching (Sabir et al., 2023). The plant is also rich in polyphenols, which confer strong antioxidant properties (Dorman et al., 2003; Mairapetyan et al., 2016; Lv et al., 2012). Additionally, peppermint oil and extracts demonstrate antimicrobial and antifungal activity (Shaikh et al., 2014; Sun et al., 2014; Bansod & Rai, 2008), and exhibit antispasmodic, anti-inflammatory, anti-angiogenic, anticancer, and irritable bowel syndrome (IBS) alleviating effects (Hassan & Tawfeeq, 2023; El Omari et al., 2024). Loh et al. (2023) found that the aroma of peppermint, combined with cinnamon, improved driver alertness, reduced frustration, and decreased perceived time pressure. The peppermint scent also reduced anxiety and fatigue. Similarly, nasal inhalation of peppermint oil was shown to mitigate mental fatigue in mice (Pramanik et al., 2023).

Mentha spp., including Mentha piperita, are widely known for their aromatic and medicinal properties. These herbs are cultivated extensively due to their antimicrobial and antioxidant features (Kadam et al., 2011; Nayak et al., 2011). The plant has creeping branches and oval, serrated leaves. The Mentha genus comprises 25–30 species within the Lamiaceae family (Pramanik et al., 2023). Though originally Mediterranean, peppermint is now grown globally for its culinary and therapeutic uses (Iscan et al., 2002). It thrives in moist, humid environments but can also tolerate full sunlight (ALrashidi et al., 2023). Peppermint plants, which grow up to 120 cm tall, are considered invasive due to their rapid spread via stolons—underground stems that cannot be stored long-term due to their high moisture content (Ahmad et al., 2023).

Culinarily, mint leaves are used fresh or dried in various dishes, including gravies, chutneys, salads, soups, teas, and beverages. They are valued for their refreshing flavor and medicinal qualities, including antibacterial, stimulative, diaphoretic, stomachic, and antispasmodic properties. Traditionally, they have been used to treat colds, fever, flu, anorexia, nausea, rheumatism, digestive disorders, and respiratory conditions (Ogbuokiri et al., 2024; Ahmed et al., 2023; Amin et al., 2023).

Peppermint features smooth, dark green leaves, square stems, and clusters of pink to lavender flowers. Due to its propagation via stolons, it can cover large areas efficiently. Natural hybridization with wild Mentha species has produced a wide variety of mint cultivars. Two key commercial varieties are black peppermint—featuring purplish stems (also known as English peppermint or Mitcham mint)—and white peppermint, which is less productive but prized for its superior oil quality.

The leaves have a strong, sweet aroma and spicy flavor with a refreshing aftertaste. Peppermint is rich in minerals such as sodium, magnesium, potassium, calcium, chromium, iron, cobalt, copper, zinc, and selenium (Hassan and Tawfeeq, 2023). It contains 0.5–4% essential oil, primarily composed of menthol (25–78%), menthone (14–36%), isomenthone (1.5–10%), menthyl acetate (2.8–10%), and cineole (3.5–14%) (Ali et al., 2024; Ibrahim et al., 2025; Loh et al., 2023; Sabir et al., 2023). Menthol, also known as peppermint camphor, is widely used pharmaceutically as a soothing agent. Essential oil extraction typically involves hydrodistillation from either fresh or dried leaves.

Peppermint remains a key component of traditional medicine, especially for gastrointestinal and nervous system conditions. Its therapeutic effects include antitumor, antimicrobial, chemopreventive, renal, antiallergenic, and digestive properties, making it effective against ailments such as cramping, nausea, and diarrhea (Shakib et al., 2023; Sabir et al., 2023). The essential oil contains menthol, menthone, and minor constituents like pulegone, menthofuran, and limonene, with its composition influenced by plant maturity, growing region, and processing conditions (Amin et al., 2023; El Omari et al., 2024).

Peppermint (*Mentha piperita*) thrives best in moist, shaded environments and propagates via underground rhizomes. Cultivation typically involves transplanting young shoots from mature plants at approximately 0.5 meters (1.5 feet) apart. Under conditions of consistent moisture, peppermint rapidly spreads across the ground through runners. Due to its invasive nature, home gardeners often prefer container cultivation to restrict overgrowth. Optimal growth is achieved with ample water supply (avoiding water-logging) and exposure to partial sunlight or shade (Liu, 2023).

The aerial parts of the plant (the leaves and flowering tops) are harvested at the onset of flowering, a period correlated with peak essential oil concentration. While wild varieties are less desirable for this purpose, cultivated strains are specifically selected for their superior oil yield and composition. After harvesting, plant materials may be slightly wilted before distillation or processed immediately (Liu, 2023).

Recent studies suggest that peppermint and its constituents may modulate BDNF expression, although this area remains underexplored. Nonetheless, evidence from neuropharmacological research indicates that various phytochemicals—such as those in *Mentha piperita*—could exert neuroprotective effects by enhancing BDNF signaling pathways (Golden et al., 2010).

**2.0 Methodology**

**Study Design and Study area**

The leaves of *Mentha piperita* were obtained from Jos, Plateau State, Nigeria. The hand-picked leaves were washed, refresh leaves sample of *Mentha piperita* were sent to the Botany department of Nnamdi Azikiwe University Awka, for identification, and authentication by a taxonomist. It was identified and authenticated at the Department of Botany at Nnamdi Azikiwe University, Awka with Specimen herbarium number NAUTH: 22OB.

Phytochemical analysis of *m. piperitha* was carried in the Department of medical biochemistry, Nnamdi Azikiwe University, Nnewi Campus.

**2.1 Qualitative Phytochemical Analysis:**
The phytochemical composition of *Mentha piperita* was analysed using Gas Chromatography (GC) as described by Kumar & Sharma (2022).

The sample is carried by an inert gas into a separation column, where various components are separated. A flame ionization detector then measures the quantity of each component.

One gram of sample was weighed and transferred into a test tube. Ethanol (15 ml) and potassium hydroxide (10 ml) were added. The mixture was warmed in a water bath at 100°C for 60 minutes. The solution was transferred to a separating funnel and washed successively with methanol (20 ml), cool water (10 ml), hot water (10 ml), and hexane (3 ml). It was further washed three times with 10 ml of 10% ethanol-aqueous solution, dried using anhydrous sodium sulfate, and the solvent evaporated. The residue was dissolved in 1000 µl of pyridine, and 200 µl was injected into a Buck M910 gas chromatograph (equipped with FID). Injector temperature: 280°C; splitless injection: 2 µl; helium flow rate: 40 ml/min; oven temperature: ramped to 330°C at 3°C/min; detector temperature: 320°C. Concentrations were expressed in mg/g.

**2.2 Elemental Analysis of *Mentha piperita*:**
Elemental analysis was performed using the method described by APHA (1998) with a Flame Atomic Absorption Spectrophotometer (FAAS, Model 400, India).

The sample is atomized in a flame. A light beam of a specific wavelength passes through the flame. The amount of light absorbed by each atomized element is measured and is proportional to its concentration.

Two grams of the dried sample were heated in a furnace at 550°C for 2 hours, digested with 20 ml of concentrated H₂SO₄, and filtered. The digest was analyzed in an air-acetylene flame to determine metal concentrations.

**2.3 Lethal Dose (LD₅₀) Determination:**
The median lethal dose (LD₅₀) of *Mentha piperita* ethanolic leaf extract was determined using the method by Enegide et al. (2013) at the Department of Human Biochemistry Laboratory, NAU, Nnewi Campus. Fifteen rats were grouped and administered increasing doses:

* Stage 1 (4 rats): 10, 100, 300, 600 mg/kg
* Stage 2 (3 rats): 1000, 1500, 2000 mg/kg
* Stage 3 (3 rats): 3000, 4000, 5000 mg/kg

No mortality was observed at any stage. A confirmatory test was performed using 5000 mg/kg extract on two additional rats.

**LD₅₀ Calculation:**
LD₅₀ = (M₀ + M₁) / 2
Where:

* M₀ = Highest dose with no mortality = 5000 mg/kg
* M₁ = Lowest dose with mortality = N/A (none observed)
Thus, LD₅₀ > 5000 mg/kg (indicating low acute toxicity)

A total of 12 apparently healthy adult Albino Wistar rats (150–200 g) were used for LD₅₀ determination.

**3.0 Results**

### **3.1 Acute Toxicity (LD₅₀) of Ethanolic Leaf Extract of** Mentha piperita

The acute toxicity (LD₅₀) study of the ethanolic leaf extract of Mentha piperita as shown in table 1 below was conducted at dosage levels ranging from 10 to 5000 mg/kg body weight. No signs of mortality or observable toxicity were recorded at the highest administered dose of 5000 mg/kg body weight. This indicates that the LD₅₀ of the extract is greater than 5000 mg/kg, classifying the extract as practically non-toxic. These results suggest that the extract is safe for use in both human and animal models at reasonable therapeutic doses.

**Table 1: Result for acute toxicity (LD50) of** **ethanolic leaf extract of *Mentha piperita***

|  |  |  |  |
| --- | --- | --- | --- |
| **PHASE** | **DOSE** | **DEATH** | **OBSERVATION** |
|  1 | 10mg/kg100mg/kg1000mg/kg | 0/30/30/3 | The rats remained normalThe rats remained normal The rats remained normal  |
| 2 | 1200mg/kg1600mg/kg2900mg/kg5000mg/kg | 0/10/10/10/1 | The rats remained normal The rats remained normal The rats remained normal No death occurred |

Table 1 result revealed that the acute toxicity (LD50) of ethanolic leaf extract of *Mentha piperita* is above 5000mg/kg, which is safe for human and animals’ consumption.

### **3.2 Elemental Composition of** Mentha piperita

Table 2 displays the elemental composition of the ethanolic leaf extract of Mentha piperita. The analysis identified the presence of essential electrolytes, including sodium (Na⁺), potassium (K⁺), chloride (Cl⁻), phosphate (PO₄³⁻), and calcium (Ca²⁺). Among these, chloride was recorded at the highest concentration (87.40 mg/L). The presence of these elements in moderate to high concentrations indicates that the extract may contribute to electrolyte balance and physiological homeostasis.

**Table 2: Result for elemental analysis of ethanolic leaf extract of *Mentha piperita***

|  |  |
| --- | --- |
| **Element** | **Concentration (ppm)** |
| Sodium | 7.898 |
| Potassium | 5.787 |
| Chloride  | 87.40 |
| Phosphate  | 8.467 |
| Calcium | 8.367 |
| Zinc | 1.898 |
| Copper | 0.787 |
| Magnesium | 7.677 |
| Manganese | 0.378 |
| Iron  | 0.895 |

**Note:** Elements identified were of triplicate values

### **3.3: Phytochemical Analysis of Ethanolic Leaf Extract of** Mentha piperita

Table 3 presents the results of the qualitative and quantitative phytochemical analysis of the ethanolic leaf extract of Mentha piperita. The qualitative analysis showed the presence of all tested phytochemicals except ribalinidine. The analysis revealed the presence of several bioactive compounds. Notably, ammodendrine (22.07 µg/ml), cyanogenic glycoside (20.19 µg/ml), and proanthocyanidin (10.48 µg/ml) were detected, indicating a significant presence of flavonoids. Additional flavonoid constituents include Mentha piperita-specific flavonoids (3.33 µg/ml) and naringenin (7.43 µg/ml), along with tannins (12 µg/ml).

The extract also contained alkaloids such as spermatine (15.97 ng/ml), as well as phytic acid (8.81 µg/ml). Other compounds including oxalates, aphyllidine, ephedrine, and saponins were also detected in moderate quantities, suggesting a rich phytochemical profile with potential medicinal properties.

**Table 3: Result for Phytochemical analysis of ethanolic leaf extract of *Mentha piperita***

|  |  |  |
| --- | --- | --- |
| **Identification** | **Concentration (ug/ml)** |  **Qualitative** |
| Kaempferol | 3.33 | + |
| Steroid | 8.29 | + |
| Proanthocyanidin | 10.49 | ++ |
| Anthocyanin | 2.07 | + |
| Narigenin | 7.43 | + |
| Dihydrocytisine | 5.91 | + |
| Cyanogenic glycosides | 20.19 | +++ |
| Ammodendrine | 22.07 | +++ |
| Tannin | 12 | ++ |
| Flavonones | 8.74 | + |
| Cardiac glycoside | 4.01 | + |
| Flavones | 5.72 | + |
| Ribalinidine | 0.00 | - |
| Phytic acid | 8.81 | + |
| Spartein | 15.97 | ++ |
| Oxalate | 1.31 | + |
| Aphyllidine | 4.24 | + |
| Epihedrine | 3.94 | + |
| Sapogenin | 11 | + |

**4.0 Discussion of the Findings**

**4.1 Median lethal dose** (LD50) **of Ethanolic Leaf Extract of *Mentha Piperita***

The LD50 of ethanoic leaf extract of Mentha *piperita* (table 1) suggests that the extract of *Mentha piperita* is not toxic to the experimental animals up to the dose of 5000 mg/kg/bw. This implies that the leaf extract of this plant may have low toxicity profile and therefore presents reduced health risk.

 **4.2 Elemental/Mineral analysis of the Leaf Extract of *Mentha Piperita***

The elemental analysis done on the leaves of Mentha *piperita* (table 2) using atomic absorptive spectrophotometer (AAS) indicated the presence of Iron, Sodium, Potassium, Chloride, Phosphate, Calcium, Zinc, Copper, Magnesium and Manganese present in this leaf extract is suggestive that *Mentha piperita* leaf may be good sources of some of these trace elements mentioned above. The plant leaves may be useful in managing electrolyte imbalance in the body. Elemental analyses are essentially used to find out the usefulness of medicinal plants in managing electrolyte imbalance. Trace elements are involved in antioxidant actions in the body. The presence of iron in *Mentha piperita* ethanolic leaf-extract also suggest that the leaf-extract may be good in the treatment and management of iron deficiency anaemia. Iron is involved in blood formation in the body. However, the above assertions are collaborated by the report of Abdur *et al.*, (2023) on phytochemical and elemental Analysis of Methanol leaf- extract of Peppermint tea; except that phosphate (PO42-) indicated in this work were absent in the above-mentioned work reported by Abdur *et al.*, (2023). Phosphates and calcium are involved in bone formation in the body. The presence of these elements is suggesting that ethanolic leaf *Mentha piperita* may be a good supplement for teething and bone formation in the body especially in newborn still undergoing teething.

**4.3: Result of qualitative and quantitative phytochemical analysis**

The qualitative phytochemical analysis of *Mentha piperita* ethanolic-leaf extract shown in table 3 revealed the presence of kaempfereal, steroid, proanllocyanin, anthocyanin, narigenin, dihydrolytisine, cynogenic, glycosides and ammodendrine. Other nutrients present were tannin, flavonone, ribalinidine, phytate, spartein, oxalate, aphyllidine, epihedrine and sapogenin. This result suggests that the leaf extract of *Mentha piperita* may be a rich source of the above phytochemicals and therefore may have potentials in treating many diseases. The above findings are in agreement with the study done by Nataliia *et al* (2023) which reported that *Mentha piperita* essential oil and extracts contained many phytochemicals which made it rich source of phytochemicals. However, the above findings partially agree and disagree with the findings of (Patil, 2016), this disagreement may be as a result of the type of method used, as (Nataliia *et al.*,2023) did not use gas chromatograph technique as was used in this work.

Result from the quantitative phytochemical analysis of *M. piperita* partly agreed with the works of Mainasara et al (2018), which reported the presence of flavonoids, saponins, alkaloids, tannins, steroid, glycosides and volatile oils. Our study however, did not reveal the presence of votile oil and this could be as the result of methodology employed here and/or geographical location. Gas chromatographic machine helps to bring out more phytochemicals in plants. The presence of phytochemicals in this plant leaf-extract as found in this work such as Kaempferal, antholyanin, flavones, tannins and narigenin, is suggesting that the plant may have strong antioxidant potentials against some free radicals (Dike *et al*.,2018b) reported the antioxidant activities of tannin, Kaempferal, anthocyanein and flavonoids. Antioxidants prevent the antioxidative effects of reactive species released by free radicals on biological molecules in the body.

## **4.4 Conclusion**

The **acute toxicity (LD₅₀) analysis** indicates a high margin of safety, with no signs of mortality or observable toxicity even at doses as high as 5000 mg/kg body weight. This study demonstrates that Mentha piperita ethanolic leaf extract contains pharmacologically important phytochemicals, including ammodendrine, cyanogenic glycosides, proanthocyanidins, and naringenin. These compounds are known for their antioxidant, anti-inflammatory, and neuroprotective effects, which support the potential therapeutic applications of the plant in neuropharmacology and metabolic health. The **elemental analysis** revealed that Mentha piperita contains significant amounts of essential electrolytes such as sodium, chloride, potassium, and calcium. Together, these findings provide a scientific basis for the traditional use of peppermint in herbal medicine and support its continued exploration for pharmacological applications, especially in supporting neurobiological health.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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