

COMPARATIVE PHYTOCHEMICAL SCREENING OF MENTHA PIPERITA L. (MINT) LEAVES EXTRACTED WITH DIFFERENT SOLVENTS

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Copyright @Author**Corresponding Author: *****Tahira Bibi****Abstract**

Peppermint (*Mentha piperita* L.), a member of the Lamiaceae family, is a widely cultivated aromatic herb valued for its medicinal and commercial applications. This study investigated the phytochemical constituents of *M. piperita* leaves using different solvent extraction methods. Leaves were collected from Hazargangi, Quetta, dried, powdered, and subjected to extraction with chloroform, methanol, ethanol, acetone, and water; methanolic extracts were prepared using Soxhlet extraction. Phytochemical screening was performed through standard qualitative assays for major secondary metabolites. Results confirmed the presence of carbohydrates, alkaloids, flavonoids, saponins, terpenoids, tannins, glycosides, and steroids. The richest phytochemical profiles were obtained from methanol, ethanol, and chloroform extracts, highlighting the influence of solvent polarity on metabolite recovery. These findings demonstrate that *M. piperita* leaves contain diverse bioactive compounds that may underpin their therapeutic potential. These results support the traditional uses of peppermint and suggest its value as a source of natural health-promoting agents for future nutraceutical and pharmaceutical applications.

INTRODUCTION

Medicinal plants are important sources of secondary metabolites (phytochemicals) that contribute to human health by exhibiting antioxidant, antimicrobial, anti-inflammatory, and other bioactive properties. These compounds include alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, phenolics, and glycosides (Javaid et al., 2021; Polyphenol Profile et al., 2020). For plants used in traditional medicine, knowing which phytochemicals are present and how extraction method and solvent polarity affect their recovery is critical for both

scientific validation and potential pharmaceutical applications. *Mentha piperita* L. (peppermint), of the family Lamiaceae, is cultivated globally for its aromatic leaves, essential oils, and bioactive compounds. It has been used traditionally for treating digestive disorders, respiratory ailments, headaches, and microbial infections (Ganesan & Rahman, 2020; "Review on pharmacological characteristics of *Mentha piperita*," 2023). Scientific investigations confirm that peppermint leaves and their extracts are rich in phenolic acids (e.g., rosmarinic acid, caffeic acid),

flavonoids, volatile oils (menthol, menthone, eucalyptol), and other compounds (Polyphenol Profile et al., 2020; Phytochemical & nutraceutical attributes of *Mentha* spp., 2021; Ethnomedicinal phytochemical and pharmacological updates..., 2020). These constituents are believed to underlie peppermint's antioxidant, antimicrobial, anti-inflammatory, antiviral, larvicidal, anticancer, anti-diabetic, and radioprotective properties (Ganesan & Rahman, 2020; *Mentha* spp. review, 2021).

Extraction technique and solvent selection strongly influence what phytochemicals are obtained. Solvent polarity determines solubility of compounds in general, polar solvents like methanol, ethanol, and water extract polar compounds (phenolics, flavonoids, glycosides, tannins), while non-polar or less polar solvents (chloroform, hexane, acetone) extract more lipophilic substances (terpenoids, essential oils, steroids) (Javaid et al., 2021; Polyphenol Profile et al., 2020). Studies comparing different extracts of *Mentha × piperita* populations show that methanolic and aqueous extracts typically have higher total phenolic and flavonoid contents, whereas essential oils or nonpolar fractions show higher amounts of volatile compounds like menthol, menthone, and related terpenes (Polyphenol Profile et al., 2020; Ganesan & Rahman, 2020). This impacts not only the qualitative presence/absence of phytochemical classes but also their quantity and biological activity. Phytochemical screening (qualitative tests) remains a useful first step to determine presence of classes of compounds. It is often followed by quantitative analysis (total phenolic content, total flavonoid content) or profiling (HPLC, GC-MS) for detailed characterization (Polyphenol Profile et al., 2020; Jamshed & Jabeen, 2022). For example, in an in vitro and in vivo study, *M. piperita* crude extract showed presence of phenols, alkaloids, carbohydrates, flavonoids, glycosides, saponins, tannins, and terpenes, and demonstrated significant antioxidant activity (Jamshed & Jabeen, 2022). Another study, "Phytochemical and nutraceutical attributes of *Mentha* spp.," reviews traditional uses and bioactivities, emphasizing that presence of secondary metabolites correlates with observed therapeutic effects in *Mentha* species (Javaid et al., 2021). Despite many studies, gaps exist. Some studies focus only on essential oil components or volatile compounds, rather than the broad

nonvolatile secondary metabolites. Others do not compare a wide range of solvents or do not use standardized extraction methods (same durations, same parts of plant, drying conditions etc.), making comparisons difficult (Polyphenol Profile et al., 2020; Ganesan & Rahman, 2020). Also, geographical and harvesting differences (soil, climate, time of harvest) can influence phytochemical content but are sometimes underreported. This study aims to contribute to closing these gaps by performing a qualitative phytochemical screening of peppermint leaves using multiple solvents of differing polarity (chloroform, methanol, ethanol, acetone, water), including use of Soxhlet extraction for the methanolic fraction. The objective is to compare the presence or absence of key classes of phytochemicals (alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, steroids, etc.) across these solvent extracts, to inform both traditional uses and potential pharmacological or nutraceutical applications.

2. Materials and Methods:

2.1. Study site:

Mentha piperita (peppermint) leaves were collected from the Hazargangi region of Quetta, an area known for its abundant growth of peppermint and its long history of traditional use by local communities. The plants were found thriving in shaded, moist environments with an adequate water supply. Harvesting is typically carried out twice a year: the first in June, after approximately 100–120 days of growth, and the second in October, after about 80–90 days, under bright sunny conditions.

2.2. Collection of plant material:

Leaves of *Mentha piperita* L. (peppermint) were collected from the Hazargangi region of Quetta, Pakistan, an area well known for the natural abundance of peppermint and its long-standing use in local traditional practices. The plants were found growing in shaded, moist habitats with a reliable water supply, which provided favorable conditions for vigorous growth. Harvesting was performed twice during the growing season: the first collection took place in June, after approximately 100–120 days of growth, while the second was carried out in October, following 80–90 days of growth. Both harvests were conducted on clear, sunny days to ensure high-quality

leaves. The collected specimens were authenticated by a qualified taxonomist, and a voucher specimen was prepared and deposited in the herbarium of Govt. Girls Post Graduate Collage Quetta Cantt for future reference. After collection, the leaves were thoroughly washed with distilled water to remove surface impurities, air-dried in the shade at room temperature to preserve heat-sensitive phytochemicals, and finely powdered using a mechanical grinder. The powdered material was stored in airtight containers until extraction and phytochemical screening.

2.3. Extraction Protocol:

2.3.1. Aqueous Extraction

For aqueous extraction, 5 g of dried leaf powder was transferred into a conical flask containing 150 mL of distilled water at room temperature. The mixture was heated in a water bath at 90 °C for 1 hour. After heating, the flask was removed and allowed to cool at room temperature. The extract was then filtered using Whatman No. 1 filter paper, and the filtrate was stored at 4 °C for subsequent analysis.



Figure1: Sequential steps for collection, authentication, and extraction of *Mentha piperita*.

2.3.2. Solvent Extraction by Soxhlet Method

Methanolic extracts of *Mentha piperita* were prepared using a Soxhlet apparatus. For this procedure, 5 g of powdered plant material was placed in the extraction chamber, and 150 mL of methanol was added as the solvent. The Soxhlet extraction was carried out at 60–80 °C until the siphoning solvent became colorless, indicating complete extraction. The obtained extract was then concentrated by evaporating the solvent in a hot air oven. The dried extract was collected in airtight bottles and stored at 4 °C until used for phytochemical screening.

2.4. Types of Solvents and Extraction Methods

In this study, five different solvents were employed for the extraction of phytochemicals from *Mentha piperita* leaves: methanol, ethanol, chloroform, acetone, and distilled water. Each solvent was selected to evaluate its efficiency in extracting a broad range of secondary metabolites for phytochemical analysis.

2.4.1. Methanolic Extract

A total of 25 g of dried leaf powder was mixed with 100 mL of methanol and allowed to stand for one hour with occasional shaking. The mixture was

filtered, and the filtrate was collected and used for phytochemical screening.

2.4.2. Ethanolic Extract

For ethanolic extraction, 25 g of dried plant powder was dissolved in 100 mL of ethanol and left undisturbed for one hour. The solution was filtered, and the resulting extract was preserved for phytochemical tests.

2.4.3. Acetone Extract

In this method, 25 g of powdered leaves was suspended in 100 mL of acetone. After standing for

one hour, the mixture was filtered, and the filtrate was collected for further analysis.

2.4.4. Chloroform Extract

Chloroform extraction was performed by dissolving 25 g of dried powder in 100 mL of chloroform. The mixture was kept for one hour and then filtered. The filtrate was collected and used for phytochemical estimation.

2.4.5. Aqueous Extract

For aqueous extraction, 25 g of dried leaf powder was dissolved in 100 mL of distilled water and allowed to stand for one hour. The mixture was filtered, and the clear extract was used for phytochemical analysis.



Figure2: Solvent-based extraction of *Mentha piperita* leaves for phytochemical analysis.

2.5. Phytochemical screening test:

2.5.1. Molisch's test:

Two milliliters of the plant extract were transferred into a clean test tube. A few drops of alcoholic α -naphthol solution were added, followed by carefully layering 0.2 mL of concentrated sulfuric acid along the side of the test tube. The formation of a purple or violet ring at the interface confirmed the presence of carbohydrates in the sample.

2.5.2. Benedict's Test

An aliquot of the plant extract was placed in a test tube, followed by the addition of a few drops of Benedict's reagent, which contains an alkaline cupric citrate complex. After shaking, the tube was heated in a water bath. The formation of a reddish-brown precipitate indicated the presence of reducing sugars.

2.5.3. Fehling's Test

Equal amounts of Fehling's solution A (copper sulfate solution) and Fehling's solution B (potassium sodium tartrate in sodium hydroxide)

were mixed in a test tube. The plant extract was then added, and the mixture was heated in a water bath. The appearance of a brick-red precipitate of cuprous oxide confirmed the presence of reducing sugars.

2.6. Test for alkaloids:

2.6.1. Wagner's Test

A small volume of the plant extract was treated with Wagner's reagent, which is a solution of iodine in potassium iodide. The formation of a reddish-brown precipitate was taken as evidence of alkaloids.

2.6.2. Hager's Test

The extract was mixed with Hager's reagent, a saturated solution of picric acid. A yellow precipitate was considered a positive indication of alkaloids.

2.6.3. Mayer's test

Few milliliters of the extract, two drops of Mayer's reagent (potassium mercuric iodide solution) were added. The appearance of a creamy-white precipitate confirmed the presence of alkaloids.

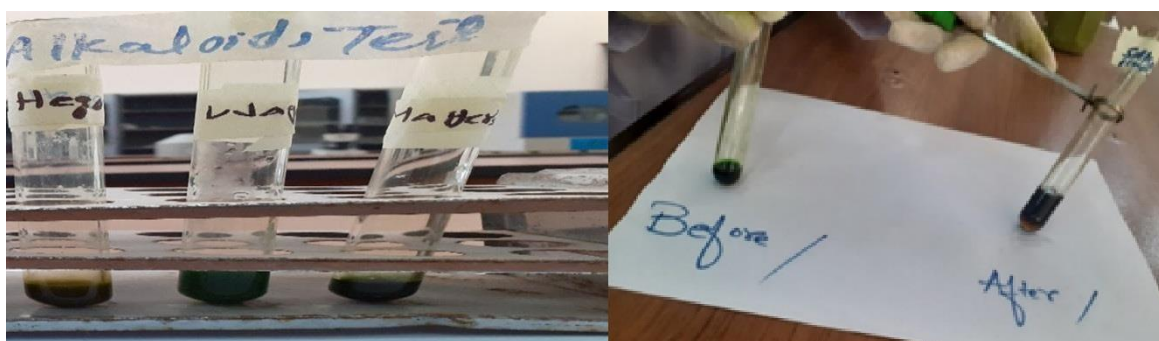


Figure 3: Phytochemical Profiling of Leaf Extracts

2.7. Test for flavonoids:

2.7.1. Shinoda test:

A small quantity of the plant extract was mixed with a few fragments of magnesium ribbon, followed by the dropwise addition of concentrated hydrochloric acid. After several minutes, the development of greenish-blue, pink, scarlet, or crimson coloration indicated the presence of flavonoids.

2.7.2. Alkaline reagent test:

The aqueous extract of the plant was treated with a few drops of sodium hydroxide solution,

producing a yellow coloration. The subsequent addition of dilute acetic acid caused the yellow color to disappear, confirming the presence of flavonoids.

2.7.3. Test for Saponins:

Two grams of the plant extract were placed in a test tube and mixed with 10 mL of distilled water. The solution was shaken vigorously for a few minutes. The formation of a stable, honeycomb-like froth indicated the presence of saponins in the sample.



Figure 4: Screening for Flavonoid Compounds

2.8. Test for terpenoids:

2.8.1. Salkowski's test:

0.5 mL of the plant extract, 2 mL of chloroform was added, followed by the careful addition of 3 mL of concentrated sulfuric acid along the side of the test tube. The development of a reddish-brown layer confirmed the presence of terpenoids.

2.8.2. Test for tannins:

About 0.5 g of dried plant material was boiled in 20 mL of distilled water, and the mixture was filtered. A few drops of ferric chloride solution (FeCl_3) were added to the filtrate and shaken well. The formation of a bluish-black or greenish-black coloration indicated the presence of tannins.

2.8.3. Test for glycosides:

Take a test tube add 0.2 gram of plant sample extract and mixed in 1ml of glacial acetic acid and also add 1 drop of ferric chloride in solution. This was then under layered with 1 ml of conc. sulfuric acid. A brown colored ring is formed which showed that glycoside is present in the sample.

2.8.4. Test for steroids:

Take a test tube add 2.0 ml of plant sample extract, and also add 1.0 ml of concentrated H_2SO_4 along the side of test tube. The formation of red color chloroform layer shows the presence of steroids test.



Figure 5: Assay for Terpenoid Identification

3. Results:

The qualitative analysis of the major bioactive constituents of *Mentha piperita*, a medicinally significant plant, was performed through phytochemical screening. Different solvents, including methanol, ethanol, acetone, chloroform, and aqueous medium, were employed for extraction. For each solvent, 25 g of dried leaf powder was macerated in 100 mL of the respective solvent, and the resulting extracts were subjected to qualitative phytochemical evaluation.

3.1. Methanolic extract:

Qualitative phytochemical analysis of *Mentha piperita* leaves using methanol as a solvent revealed varied

results across different tests. Among the carbohydrate assays, only Fehling's test gave a positive result, whereas Molisch's and Benedict's tests were negative. Similarly, steroids were not detected in the methanolic extract. On the other hand, methanolic extracts showed strong positivity for alkaloids, terpenoids, saponins, tannins, and glycosides. These findings are consistent with previous reports by Valsala et al. (2021) and Patel et al. (2021), who also noted high concentrations of these phytochemicals in methanolic extracts of *Mentha piperita*. Comparable results were documented by Khanal et al. (2019), particularly regarding the presence of tannins and terpenoids in peppermint leaf extracts.

Table 1: Detection of pharmacologically active compounds in methanolic extract of *Mentha piperita* leaves.

S.NO	Pharmacologically Active compounds	Test Performed	Extracting solvent
			Methanol
1	Carbohydrates	Molisch's test	—
		Benedict's test	—
		Fehling's test	++
2	Alkaloids	Wagner's test	—
		Hager's test	+
		Mayer's test	++
3	Flavonoids	Shinoda test	++
		Alkaline reagent test	++
4	Saponins	Foam test	+++
5	Terpenoids	Salkowski's test	++
6	Tannins		+++
7	Glycosides		+++

3.2. Ethanolic extract:

Phytochemical screening of *Mentha piperita* leaves with ethanol revealed carbohydrates in Molisch's test, while Benedict's and Fehling's tests were negative. Alkaloids were detected by Wagner's and Hager's tests but absent in Mayer's test. Flavonoids, tannins, and steroids were not observed, whereas glycosides showed moderate presence. Notably, saponins and terpenoids were abundant. These compounds are of pharmacological interest due to their roles in respiratory relief, including cough and hay fever.

Table 2: Phytochemical profile of *Mentha piperita* leaves extracted with ethanol.

S.NO	Pharmacologically Active compounds	Test Performed	Extracting solvent
			Ethanol
1	Carbohydrates	Molisch's test	+++
		Benedict's test	—
		Fehling's test	—
2	Alkaloids	Wagner's test	++
		Hager's test	+++
		Mayer's test	—
3	Flavonoids	Shinoda test	—
		Alkaline reagent test	—
4	Saponins	Foam test	+++
5	Terpenoids	Salkowski's test	+++
6	Tannins		-
7	Glycosides		++
8	Steroids		-

3.3. Acetone extract:

The acetone extract of *Mentha piperita* leaves showed moderate levels of alkaloids, flavonoids, terpenoids, and tannins. Carbohydrates and glycosides were absent. In contrast, saponins were strongly present in this mixture.

Table 3: Qualitative analysis of phytoconstituents in acetone extract of *Mentha piperita*.

S.NO	Pharmacologically Active compounds	Test Performed	Extracting solvent
			Acetone
1	Carbohydrates	Molisch's test	—
		Benedict's test	—
		Fehling's test	—
2	Alkaloids	Wagner's test	—
		Hager's test	++
		Mayer's test	++
3	Flavonoids	Shinoda test	++
		Alkaline reagent test	—
4	Saponins	Foam test	+++
5	Terpenoids	Salkowski's test	++
6	Tannins		++
7	Glycosides		—
8	Steroids		—

3.4. Aqueous extract:

In the aqueous extract of *Mentha piperita* leaves, carbohydrates, alkaloids, flavonoids, and tannins were absent. In contrast, saponins, terpenoids and glycosides were strongly present.

Table 4: Screening of bioactive compounds in aqueous extract of *Mentha piperita*.

S.NO	Pharmacologically Active compounds	Test Performed	Extracting solvent
			Aqueous
1	Carbohydrates	Molisch's test	—
		Benedict's test	—
		Fehling's test	—
2	Alkaloids	Wagner's test	—
		Hager's test	+
		Mayer's test	—
3	Flavonoids	Shinoda test	—
		Alkaline reagent test	—
4	Saponins	Foam test	+++
5	Terpenoids	Salkowski's test	+++
6	Tannins		—
7	Glycosides		+++
8	Steroids		—

3.5. Chloroform Extract:

The chloroform extract of *Mentha piperita* leaves revealed varied phytochemical profiles. Carbohydrates, flavonoids, and tannins were absent. Alkaloids were moderately present, while saponins and glycosides showed strong presence. Terpenoids were detected at low levels.

Table 5: Phytochemical screening of *Mentha piperita* leaf extract using chloroform solvent.

S.NO	Pharmacologically Active compounds	Test Performed	Extracting solvent
			Chloroform
1	Carbohydrates	Molisch's test	++
		Benedict's test	—
		Fehling's test	—
2	Alkaloids	Wagner's test	++
		Hager's test	+
		Mayer's test	++
3	Flavonoids	Shinoda test	—
		Alkaline reagent test	—
4	Saponins	Foam test	+++
5	Terpenoids	Salkowski's test	+
6	Tannins		—
7	Glycosides		+++
8	Steroids		—

Table 6: Extraction yield and physical characteristics of *Mentha piperita* leaf extracts obtained with different solvents.

Solvents	Initial weight of the powder(g)	Weight of the crude extract	Crude extract (%)	Color of the extract
Acetone	25g	4.56	18. 24	Dark brown
Chloroform	25g	3.53	14. 12	Green
Methanol	25g	10.44	41.76	Dark green
Ethanol	25g	8. 56	34.24	Dark brown
Aqueous	25g	8. 37	33.48	Dark brown

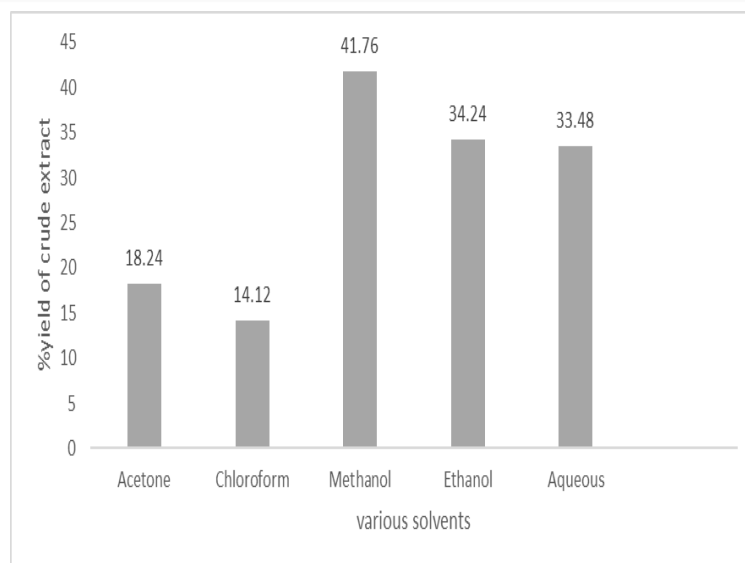


Figure 6: Comparative crude extract yield (%) of *Mentha piperita* leaves across various solvents.

4. Discussion

The phytochemical screening of *Mentha piperita* conducted in this study revealed distinct variations in the presence of bioactive compounds across different solvent extracts. These differences highlight the solvent-dependent solubility of phytoconstituents and their potential pharmacological applications. Among the tested solvents, ethanol and chloroform extracts exhibited a wider range of phytochemicals compared to acetone and aqueous extracts, while methanol was intermediate. This finding aligns with prior studies that emphasized the influence of solvent polarity in extracting plant metabolites of varying chemical nature (Abdullahi et al., 2020; Tiwari et al., 2011). Ethanolic extracts displayed strong presence of terpenoids and saponins, compounds that are well recognized for their antimicrobial, anti-inflammatory, and expectorant activities (Sujana et al., 2013; Mogosanu & Grumezescu, 2014). These results confirm the therapeutic relevance of ethanol as an efficient solvent for isolating terpenoids and saponins from *Mentha* species. Tannins and flavonoids were not detected, which differs from findings in some earlier studies where tannins were consistently reported in ethanolic extracts of mint (Valsala et al., 2021). This variation may be attributed to differences in plant chemotypes, soil conditions, and extraction procedures, factors previously shown to significantly affect phytochemical yields (Brahmi et al., 2017).

Acetone extracts showed moderate levels of alkaloids, flavonoids, terpenoids, and tannins, with saponins being the most abundant constituent. Similar observations were reported by KS and Shinde (2019), who demonstrated acetone's efficacy in extracting saponins and secondary alkaloids from mint species. Interestingly, carbohydrates and glycosides were not detected in our acetone extracts, a result that contrasts with reports by Muhammad et al. (2019). The absence of these compounds could be linked to the relatively low polarity of acetone, which may limit extraction of glycosidic compounds (Tiwari et al., 2011). The aqueous extracts yielded saponins, terpenoids, and glycosides in high concentrations, while carbohydrates, alkaloids, and flavonoids were absent. These results support the findings of Patil et al. (2016), who reported aqueous extracts of *Mentha piperita* to be particularly rich in saponins and terpenoids. The dominance of saponins in water extracts is consistent with their hydrophilic nature, which enables better solubility in polar solvents. However, the absence of tannins and flavonoids diverges from some reports in other *Mentha* species (Sontakke & Shinde, 2019), again suggesting environmental or varietal influences on phytochemical profiles. Chloroform extracts, by contrast, contained moderate amounts of alkaloids and low levels of terpenoids, with saponins and

glycosides showing strong presence. Carbohydrates, flavonoids, and tannins were absent. This profile is similar to previous work by Muhammad et al. (2019), who also highlighted chloroform as an effective solvent for saponins. The relatively lower presence of terpenoids in chloroform compared to ethanol suggests that ethanol may be the more suitable solvent for isolating volatile terpenoids, which are central to the aroma and pharmacological properties of peppermint essential oils (McKay & Blumberg, 2006). The methanolic extracts showed intermediate phytochemical diversity, consistent with methanol's polarity, which allows extraction of both moderately polar and polar metabolites. Previous studies confirm methanol as one of the most versatile solvents for plant phytochemistry, yielding a wide range of compounds including phenolics, flavonoids, and alkaloids (Khare et al., 2012; Abdullahi et al., 2020). The overlap of methanol results with those obtained from ethanol further supports the complementary use of alcohol-based solvents in medicinal plant research. Overall, the solvent-dependent variability observed in this study underscores the importance of selecting appropriate extraction methods when evaluating medicinal plants for therapeutic purposes. The dominance of saponins and terpenoids across most

extracts indicates that these compounds are core phytoconstituents of *Mentha piperita*, consistent with its traditional uses in treating cough, colds, and digestive ailments (Sujana et al., 2013; McKay & Blumberg, 2006). Moreover, the absence of tannins and flavonoids in some extracts may suggest that the medicinal effects of peppermint are less reliant on phenolic compounds compared to other medicinal plants. These findings carry significant implications for pharmacognosy and natural product research. First, they confirm *Mentha piperita* as a promising source of saponins and terpenoids, both of which have established roles in modern drug development. Second, the solvent-specific differences highlight the need for standardized extraction protocols to ensure reproducibility in phytochemical and pharmacological studies. Future work should integrate quantitative assays, bioactivity testing, and chromatographic profiling (e.g., HPLC, LC-MS) to validate and expand upon these preliminary observations. Additionally, ecological and agronomic variables such as cultivation conditions, soil chemistry, and harvest stage should be considered to better understand the biochemical diversity of *Mentha piperita*.

5. Conclusion

This study demonstrated that the phytochemical composition of *Mentha piperita* leaves varies substantially with the choice of extraction solvent. Ethanol and chloroform extracts showed the widest range of metabolites, with saponins and terpenoids consistently abundant across multiple solvents. Acetone extracts revealed moderate levels of alkaloids, flavonoids, and tannins, while aqueous extracts yielded high levels of saponins and glycosides but lacked carbohydrates, flavonoids, and alkaloids. Methanol extracts provided intermediate results, confirming its efficiency as a general-purpose solvent for phytochemical screening. These findings reinforce the importance of solvent selection in natural product research and confirm *Mentha piperita* as a reliable

source of bioactive compounds, particularly saponins and terpenoids, which are known for their antimicrobial, anti-inflammatory, and therapeutic effects. The absence of certain phenolic compounds such as tannins and flavonoids in some extracts highlights the variability of phytochemical yields due to solvent polarity and plant-specific factors. Overall, the study provides valuable insights into the phytochemical diversity of *Mentha piperita* and its pharmacological potential. Standardization of extraction protocols, coupled with advanced analytical techniques, will be essential in future research to establish consistent profiles and validate the bioactivities of these compounds for therapeutic use.

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