



Antibacterial Activity And Phytochemical Analysis Of Peppermint Leaves (Mentha Piperita)

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Abstract

The hybrid mint, *Entha piperita*, is a mix between spearmint and water mint. It is a perennial herbaceous rhizomatous plant. *Mentha piperita* leaves were gathered in Bhaktapur, Nepal. Methanol was used as a solvent in the Soxhlet extraction procedure to create the peppermint leaf extract. The antibacterial properties and phytochemicals found in the plant sample's extracted leaves were examined. Methanol was employed as the extraction solvent. Peppermint's methanolic extraction revealed the presence of alkaloids, flavonoids, carbohydrates, glycosides, phenols, and tannins. Nevertheless, there was no antimicrobial activity in the extracted material.

Keywords: Soxhlet extraction, Phytochemicals, Antibacterial activity

Introduction

The study of botany and application of plants for therapeutic purposes is known as herbalism. For a large portion of human history, medicinal remedies have been derived from plants. Potential medications can be found in abundance in the kingdom of plants. A variety of flora have served as inspiration in the creation of new medications. The first documented record of using medicinal herbs to make medications was discovered on a block of Sumerian clay from Nagpur that is thought to be around 5000 years old. It included twelve recipes for medication preparation using more than 250 different plants, including alkaloid species like poppies. The ancient scholars only believed that herbs are only solutions to cure a number of health related problems and diseases. They conducted thorough study about the same, experimented to arrive at accurate conclusions about the efficacy of different herbs that have medicinal value. Most of the drugs, thus formulated, are free of side effects or reactions. This is the reason why herbal treatment is growing in

popularity across the globe. These herbs that have medicinal quality provide rational means for the treatment of many internal diseases, which are otherwise considered difficult to cure. Medicinal plants such as Aloe, Tulsi, Neem, Peppermint, Turmeric and Ginger cure several common ailments. These are considered as home remedies in many parts of the country. It is known fact that lots of consumers are using Basil (Tulsi) for making medicines, black tea, in pooja and other activities in their day to day life. Apart from the medicinal uses, herbs are also used in natural dye, pest control, food, perfume, tea and so on. In many countries different kinds of medicinal plants/ herbs are used to keep ants, flies, mice and flee away from homes and offices. Nowadays medicinal herbs are important sources for pharmaceutical manufacture. All plants produce chemical compounds known as phytochemicals which give them an evolutionary advantage. Phytochemicals are the chemical compounds that occur naturally in plants are responsible for colour and organoleptic properties, such as the deep purple of blueberries and smell of garlic. These phytochemicals have potential for use as drugs, and the content and known pharmacological activity of these substances in medicinal plants is the scientific basis for their use in modern medicine. There are several phytochemicals found in several medicinal plants. Some of them are: tannins, terpenes, glycosides, polyphenols, alkaloids, flavonoids etc. Many inside problems that are otherwise thought to be difficult to cure can be rationally treated with quality. Many common illnesses can be cured by medicinal plants like ginger, peppermint, tulsi, neem, aloe, and turmeric.

In many regions of the nation, these are regarded as home treatments. It is well known that many customers use basil, also known as tulsi, in their daily lives are utilized in cuisine, tea, perfume, natural dyes, pest control, and other products. Various types of medicinal plants and herbs are used in various nations to keep ants, flies, mice, and other pests away from homes and workplaces. These days, medicinal herbs play a significant role in the production of pharmaceuticals.

Plant profile

- **Peppermint Plant**
- **Synonym:** brandy mint, balmint, curled mint, lamb mint
- **Biological source:** It is fresh and dried leaves of mentha perita
- **Family:** lamiaceae

There type

1. Chocolate mint
2. Spearmint
3. Apple mint
4. EAUDE cologne mint
5. Curly mint
6. Double mint
7. Ginger mint
8. Mentha spicata
9. Grapefruit mint
10. Calamintha
11. Lavender mint
12. Magehrebi mint tea
13. Pennyroyal
14. Round leaved mint
15. Banana mint
16. Buddleia mint
17. Cat mint
18. Corn mint
19. Mintha alaica
20. Mentha aquatica
21. Mentha langifolia

Part of plant

1.leaves 2.stems 3.flowers 4.calyx 5.corolla 6.stamens 7.style

Chemical formulation of mint

- Chemical formula : $C_{10}H_{20}O$
- Molar mass : $156.269 \text{ g mol}^{-1}$
- Appearance : white or colourless crystalline solid
- Odour : mint licorice

Chemical constituent and there uses

1. Menthol : The main component of peppermint oil menthol is a calcium channel blocker that can help with spasms.
2. Menthone : The second most common component of peppermint perfume and cosmetic.
3. Menthyl acetate : A carboxyl ester found in peppermint oil.
4. Cineol : Respiratory tract issues, muscle pain, headaches.
5. Limonene : Pesticides, cleaning industrial.
6. Pulegone : Insecticide
7. Caryophyllene and Pinene : Gastroprotective, antibacterial, antioxidant.
8. Terpenoids : Anticancer, antiviral, antidepressant.
9. Flavonoid : flavours

Classification

1. DOMAIN: Eukarya
2. KINGDOM: Plantae
3. SUBKINGDOM: Tracheobionta
4. SUPERDIVISION: Spermatophyta
5. PHYLUM: Angiospermophyta
6. CLASS: Magnoliopsida (Dicotyledon)
7. ORDER: Lamiales
8. FAMILY: Lamiaceae (Mint Family)
9. GENUS: Mentha
10. SPECIES: *Mentha x piperita*
11. COMMON NAME: Peppermint



Fig.1. *Mentha piperita* plant

Peppermint typically occurs in moist habitats, including stream sides and drainage ditches. Being a hybrid, it is usually sterile, producing no seeds and reproducing only vegetatively, spreading by its runners. It can grow almost anywhere. Peppermint generally grows best in shaded locations, and expands by underground

rhizomes. Young shoots are taken from old stocks and dibbled into the ground about 1.5 feet apart. They grow quickly and cover the ground with runners if it is permanently moist. For the home gardener, it is often grown in containers to restrict rapid spreading. It grows best with a good supply of water, without being water-logged, and planted in areas with part-sun to shade. In Nepal, peppermint is locally known as "pudina". It is widely distributed from Himalayan region to Terai region of Nepal. People cultivate peppermint as a source of diet here. used for curing several diseases since a long time. It has been used in home remedy and also as a precursor of drugs.

Peppermint Extract and Oil

The leaves of the peppermint plant (*Mentha piperita*) are used to make peppermint extract, a natural remedy. Mentha (23.4%) and menthol (40.7%) are the extract's primary ingredients. Menthyl acetate, 1,8-cineole, limonene, beta pinene, and betacaryophyllene are other constituents. Among other things, it contains flavonoids, polyphenols, glycosides, and terpenoids. In essence, the liquid (extract) is made by utilizing alcohol to extract the oil from the plant's blooming tops and dried or fresh leaves.

Uses of Oil

Peppermint oil is frequently used as fragrance soap and cosmetics as well as a flavorful in foods. Peppermint oil also is used for a variety of health conditions and can be taken orally in dietary supplements or topically as a skin cream. According to Clinical data alleviate that peppermint oil likely can help with symptoms of irritable bowel syndrome symptoms. It may also help indigestion and prevent spasms in the GI tract caused by endoscopy or barium enema. Some studies show that used topically it may help sooth tension headaches and cracked nipples from breastfeeding but more research is needed to confirm these studies. In most adults, the small doses of peppermint oil contained in dietary supplements and skin preparations appear to be safe. Pregnant and breastfeeding women, however, should avoid such products because little is known about their safety during pregnancy and lactation. Possible side effects of peppermint oil include.

Phytochemical Screening

It describes the process of removing examining, and identifying the compounds in plants that have therapeutic use. Nonnutritive substance called phytochemical are made by plants and contain components that protect against disease. There are already about 900 phytochemical known to be present in food and many more are still to be found. Flavonoids, alkaloids, carotenoids, tannins, and phenolic compounds that can be extracted from plants. The phytochemical category contains substances that are known to be important nutrients. These substance are found naturally in plants and are necessary for normal physiological processes meaning that humans must consume them through their diet.

Antibacterial Activity

In the current state of pharmaceutical and medical development, microorganisms alter their genetic makeup and metabolism to become resistant to medications used to treat common infection diseases.

These drug resistant candidates provide a significant challenge to the pharmaceutical and healthcare industries since they are more pathogenic and have a high death rate.

Researchers are anticipating the creation of new and alternative medications to combat microbial antibiotic resistance.

Natural resources like animal, plants, and algae.

Mentha piperita (peppermint): A phytochemical and Antibacterial analysis offers a variety of natural therapeutic ingredients to treat a range of infectious disorders.

Since ancient times, plants have been used as a source of medicine.

Numerous expert's studies have demonstrated that plants are one of the main suppliers.

2. Literature Review

1. *Mentha piperita* commonly known as peppermint, is a hybrid mint resulting from the crossbreeding of watermint and spearmint.
2. It is regarded as a significant medicinal plant and contains various phytochemical compounds. Numerous studies have been conducted on peppermint, including one by Diane and Jefferey (2006) which focused on the bioactivity and health benefits of peppermint tea.
3. Their findings indicated that menthol and menthone are the primary volatile compounds in its essential oil.
4. Peppermint exhibits considerable antimicrobial and antiviral properties strong antioxidant and antitumor effect, as well as some potential for alleviating allergies.
5. Pramila et al. (2012) conducted a study on the phytochemical composition and antimicrobial properties of peppermint leaf extract via methanol.
6. They evaluated the extract derived from the locally found *mentha piperita* for its phytochemical constituents, antioxidant capacity, and antimicrobial effect.
7. In a separate review, sachan et al. (2013) analyzed *mentha piperita* and found that its primary compounds are menthol (30-55%) and menthone (14-32%).
8. They also provided extensive information regarding the medicinal application of peppermint oil, noting its effectiveness for various internal conditions such as irritable bowel syndrome, digestive issues like flatulence and gastritis, as well as external ailments like muscle pain and headaches.
9. Additionally loolaie et al. (2017) published a review titled "peppermint and its functionality" which included molecular docking studies indicating that cineol and menthyl acetate from peppermint interact with the active site of the aryl amine n-acetyltransferase enzyme, suggesting inhibitory effects.
10. Their quantum studies indicated that menthol ($E_{gap}=16.9\text{eV}$) is unstable.
11. This evidence highlights peppermint as a promising area for further research, particularly regarding its potential in disease prevention.
12. According to the German commission E monographs peppermint oil and peppermint leaves have been used internally as an antispasmodic for the upper gastrointestinal tract and bile duct as well as to address conditons such as irritable bowel syndrome, respiratory tract catarrh, and oral mucosa inflammation.
13. Externally, peppermint oil is applied for muscle and nerve pain.
14. The commission E also suggest that peppermint oil may possess carminative, cholagogue, antibacterial, and secretolytic properties, and it has a cooling effect.
15. Enteric-coated peppermint oil capsules (Colpermin) are utilized as an orally administered antispasmodic for colonoscopy preparation.
16. Brian et al. (2001) from New York State Integrated Pest Management investigated peppermint's health benefits and environmental impacts, noting that a small portion of the population may be allergic to peppermint and its oil and derivatives.
17. Most chronic toxicity tests of peppermint, its oil and water extracts yilded negative result, although some of peppermint's chemical isolates showed ambiguous or mildly positive outcome in a few instances.
18. Zuhair 2016 published the article "phytochemical Screening and Evalution of Antioxidants and Antibacterial Activities of Five Medical Plants" in the International journal of pharmacognosy and phytochemical Research, which highlighted the presence of alkaloids, flavonoids, triterpenoids, tannins, and carbohydrates in the extracts of five plants, including *mentha piperita*, while saponins and steroids were found only in *syzygium aromaticum* and *zingiber purpurea* extracts, absent in peppermint. Additionally, Ebenezer et al. 2011 published a study in the Asian Pacific Journal of Tropical Biomedicine comparing the effectiveness of peppermint oil with standard antibiotic against specific bacterial pathogens.
19. Dr Aruna 2018 wrote in the Himalayan Times about the nutritional benefits of moringa and mint. Consequently the phytochemical and antibacterial properties of peppermint leaf extracts were analyse in the laboratory.

3. Materials and Method

Chemicals:

- Hydrochloric acid
- Sodium hydroxide
- Aluminum chloride
- Dimethyl sulphoxide (DMSO)
- Neomycin

Equipments:

- Soxhlet
- Weighing machine
- Heating mantle
- Incubator
- Oven

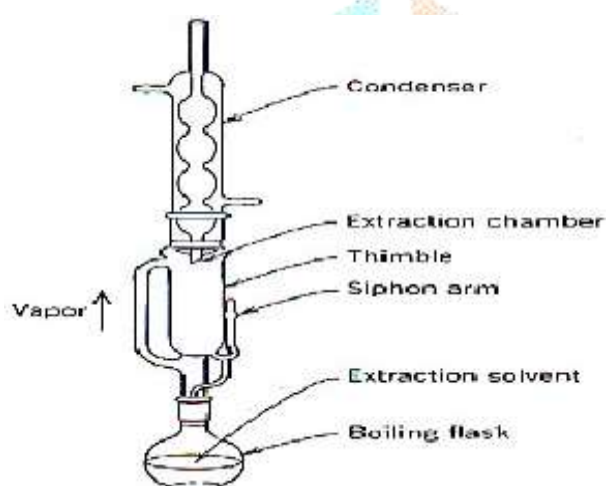


Fig.2. Soxhlet apparatus

Methods Sample collection and drying

In September 2018, fresh green leaves of *Mentha piperita* were gathered from Bhaktapur. The leaves were washed with water to eliminate any epiphytic organisms present on their surface and then shaded to dry for two weeks.

Once dried, the leaves were ground by hand into a powder and stored at a low temperature.

Extraction of plant materials

Ninety grams of dried leaves were ground into a powder. The powdered leaves of *Mentha piperita* weighing 50 grams, were then extracted using the Soxhlet extraction method with methanol the solvent in a volume of 250 ml. the apparatus was continuously heated with a heating mantle. The sample was immersed directly in boiling solvent. After undergoing several cycle over a period of 6-7 hours, the solvent. Used for Soxhlet extraction was collected and concentrated using a rotary evaporator under reduce pressure, ensuring that the temperature remained below the boiling point of methanol. The resulting extract were stored in vile tubes at very low temperature.

Phytochemical analysis

This method involves the selective and successive extraction of the plant phytochemical. The analysis of the presence of main group of natural constituents present in the plant extract was done using different specific reagents.

Chemical tests were done to identify bioactive compounds of pharmacological importance through standard methods.

Phytochemicals such as tannins, alkaloids, saponins, flavonoids, terpenoids, phenols etc. were qualitatively determined as following:

- Screening of phenol and tannins:

Two drop of crude extract were combined with 2ml of a 2% FeCl₃ solution. A black colour signifies the presence of phenols and tannins.

- Screening of glycosides (Keller Kilianin Test): 2ml of extract was mixed with 2 ml of glacial acetic acid which was followed by the a few drops of ferric chloride solution and 1ml of concentrated sulphuric acid. A brown ring at interface confirms the presence of glycosides.

- Screening of Flavonoids (Shinoda's test): The extract was blended with 2-3 pieces magnesium ribbon fragments and 4 drops of conc. HCl. The Red change indicates the presence of flavonoids in the extract.

- Screening of carbohydrates: 1ml of each extract was added to 1ml of Benedict's reagent and heated on a boiling water bath for 2 minutes. A green solution appeared confirming the presence of reducing sugar.

- Screening of alkaloids: Crude extract was mixed with 2ml of 2% HCl. Then, heated on a boiling water bath, cooled, filtered and divided into two tubes.

- Mayer's Reagent: In the first tube a few drop of Mayer's reagent was added. The Appearance of yellow precipitation or turbidity indicates the presence of alkaloids.

- Dragendroff's Reagent: To the solution of second tube, 3 drops of Dragendroff's reagent were added and the Presence of white precipitation indicated alkaloids.

Antibacterial activity

The antibacterial activity was investigated using the agar well diffusion technique. The effectiveness of the antibacterial agent was assessed by measuring the zone of inhibition. (ZOI).

Preparation of stock or working solution

A working solution of 50mg/ml was prepared by aseptically placing 0.01g of dry crude into a sterile vial with 1ml of DMSO solvent. The extract was then dissolved dissolved in the DMSO. After preparing the stock solution, the test tubes were capped, sealed, and stored in the refrigerator for future use.

Test Organisms

The microbial strains were collected and analyzed at NIST college in Khusibu. The strains studied included rod shaped gram-negative bacteria such as *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Escherichia coli*, as well as aspherical gram-positive bacterium, *Staphylococcus aureus*.

Antibacterial assay of plant extract

The plant extract was dissolved in DMSO at a concentration of 50 mg/ml. A bacterial culture was inoculated onto muller Holton agar (MHA) plates using swabs and allowed to diffuse for 15 minutes.

The prepared sterile MHA plates were dried at the appropriate temperature to eliminate moisture.

Each plate for the assay was labelled with the corresponding bacteria name and rotated 60° after each swab.

The inoculated plates were allowed to dry for a few minutes at room temperature with the lids on. Different extracts, along with 50% DMSO as a negative control (NC) and 1mg/ml.

Neomycin as a positive control (PC) were then added to the plates.

The plates were incubated at 37°C for 18-24 hours.

After incubation, the plates were examined for sig of bacterial growth inhibition, indicated by clear zones surrounding the wells. The absence of a zone of inhibition suggested a lack of activity.

The zones of inhibition (ZOI) were measured using a Millimeter ruler and the mean values were recorded.

4. Results and Discussion Phytochemical analysis

The different phytochemical components in the extract were detected through their colour reactions with various reagents.

The primary aim of the phytochemical screening was to recognize the different categories of chemical compounds found in the plant extract.

The finding of the phytochemical screening are outlined below:

Table 1: Phytochemical screening of extract of leaves of *Mentha piperita*

S.N	Phytochemical	Methanol Extract
1.	Tannins	+
2.	Steroids	-
3.	Glycosides	+
4.	Flavonoids	+
5.	Anthraquinone	-
6.	Alkaloids	+
7.	Carbohydrates	+
8.	Phenols	+
9.	Saponins	-

(+) = presence & (-) = absence An initial study indicated that the leaf extract is rich in various bioactive secondary compounds, including phenols. Alkaloids, tannins, glycosides, carbohydrates, and flavonoids. The existence of these compounds in this species suggests it could possess medicinal properties.

Antibacterial Activity

The diameter of the zone of inhibition (ZOI) that the plant extract produced on specific bacteria was evaluated in order to estimate the antibacterial activity of peppermint. *Mentha piperita* leaf methanol extract was investigation. The extract's antibacterial analysis results are shown in the table below. The diameter of the zone of inhibition (ZOI) that the plant extract produced on specific bacteria was evaluated in order to estimate the antibacterial activity of peppermint. *Mentha piperita* leaf methanol extract was investigated. The extract's antibacterial analysis results are shown in the table below.

Table 2: Antibacterial analysis of methanolic extract of leaves of *Mentha piperita*

S.N	Name of the Bacteria	ZOI (mm) of extract
1.	<i>Pseudomonas aeruginosa</i>	0
2.	<i>Salmonella typhi</i>	0
3.	<i>Escherichia coli</i>	0
4.	<i>Staphylococcus aureus</i>	0

Gram-positive and gram-negative bacteria were among the several bacterial strains against which the antibacterial activity of *Mentha piperita* leaf extract in methanol solvent was investigated. No zone of inhibition was found in the results for any of the tested microorganisms.

This demonstrated that none of the examined microorganisms were affected by the methanolic extract of *Mentha piperita* leaves.

Despite being one of the most significant therapeutic plants (based on earlier research) this investigation yielded no fruitful findings. This unfavorable outcome could be caused by a number of factors. One of the main causes can be the location or altitude at which the plant sample was taken.

Plant phytochemical composition might vary depending on location and altitude. Additionally the extract's impurity might have contributed.

5. Conclusion

Phytochemical analysis of peppermint leaf extract revealed the presence of several bioactive compounds, including alkaloids, flavonoids, carbohydrates, glycosides, phenols, and tannins in methanol.

However, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus* were not significantly inhibition by the peppermint leaf extract in methanol.

A phytochemical analysis of peppermint leaf extract revealed the presence of several bioactive compounds, including alkaloids, phenols, and tannins in methanol.

However, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus* were not significantly inhibition by the peppermint leaf extract in methanol.

6. Recommendations

It can be concluded from the study's aforementioned finding that the plant extract include chemical components with pharmacological importance.

The presence of these chemical compounds in a plant suggests that it may produce pharmaceutically significant medication if it is thoroughly screened with additional solvents. Furthermore, peppermint can be considered one of the therapeutic plants with antibacterial properties if it is appropriated processed and tested against a variety of other germs. Additionally, more research on *mentha piperita* may demonstrate that it can serve as the foundation for medications in the future.

8. Appendix

• Test of terpenoids and steroids:

Crude extract was combination with 2 ml chloroform. Then it were evaporated to dryness and 2ml of conc. H₂SO₄ and heated about 2 minutes. The reddish brown coloration or greyish color indicates the presence of terpenoids whereas red colour produced in the lower chloroform indicated the presence of steroids.

• Test for saponins:

Crude extract was added with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam were taken as an indicated for the presence of saponins.

• Test for carbohydrates (Molisch Test):

Crude extract was combination with 2 ml of Molisch's reagent and mixture was shaken properly. Then the 2 ml of conc. H₂SO₄ were poured carefully along the side of test tube. A violet ring at the appearance interphase indicated the presence of carbohydrate.

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