SJIF Impact Factor (2026): 8.863| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 11 | Issue: 4 | April 2026 - Peer Reviewed Journal

REVIEW ON PHYTOCHEMICAL SCREENING OF MENTHA PIPERITA

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ABSTRACT
The objective of this study was to assess the phytochemical potential of Mentha piperita L. leaf extracts in hexane, acetone, chloroform, ethanol, and water. Mentha piperita L. leaves were used in the experiment.gathered and verified from the Nanded (MS) region of India. Alkaloids, flavonoids, tannins, phenols, saponins, steroids, terpenoids, proteins, and carbohydrates were all found in Mentha piperita L. leaves. When all of the tested phytochemicals were present, acetone and aqueous extracts were shown to be more powerful. Plant extracts in acetone and water have the highest levels of phenols and flavonoids, respectively. Additionally, all of the extracts demonstrated strong antibiotic activity against bacteria, particularly the acetone and chloroform extracts, but very little activity against fungus. These phytochemical substances, sometimes referred to as plant secondary metabolites, are said to have a variety of biological and therapeutic properties. As a result, it is anticipated that this species will have numerous therapeutic applications and can be further researched for the manufacture of pharmaceuticals.

KEYWORDS: *Phytochemical, Antimicrobial, Mint, Extracts.*

INTRODUCTION

One of the most often used families of medicinal plants is the Labiatae family, which serves as both a general source of tastes and a solidified sourceof concentrates that have strong antibacterial and cell-reinforcing qualities. Originally from Europe and the Middle East, the plant is currently widely distributed around the world. It is occasionally found in the wild alongside its parent species. People have long used peppermint for aromatherapy and medicinal purposes. Mentha piperita L., a member of the Labiatae family, is a significant medicinal plant (Kirethekar, 1985). It is a perennial immigrant herbaceous plant with a four-sided stem, oppositely stalked, toothed leaves, and a maximum height of forty inches. The flowers exhibit irregularities in form with a reddish or pink hue (Clark and Menory, 1980).

The therapeutic qualities of the essential oils found in Mentha species have been extensively documented in the literature (Gulluce et al., 2007; Rasooli, 2008). Research has demonstrated the antioxidant, insecticidal, antiviral, antifungal, and antibacterial qualities of essential oils (Burt, 2004; Kordaly et al., 2005). The primary tool used to treat various microbial illnesses is antibiotics. Finding novel antimicrobial medicines is crucial given the evidence of drug-resistant bacteria spreading quickly throughout the world (Basheer and Abdullah, 2013). Serious infections brought on by certain aerobic Gram-negative bacteria are frequently treated with a wide range of medicines (Tumah, 2005).

The widespread use of antibiotics both inside and outside medicine is playing a significant role in the emergence of bacterial resistance (Goossens et al., 2005). Although, there were low levels of preexistent antibiotic resistant microorganisms before the widespread use of antibiotics; evolutionary pressure from their use has played a role in increase in multidrug resistance varieties and the spread of resistance between microbial species (Hawkey and Jones, 2009). In this concern, the present study was aimed to evaluate the phytochemical analysis and antimicrobial potential of Mentha piperita L. leaves extracts.

MATERIALS AND METHODS

Collection of Plant Material

The fresh leaves of Mentha piperita L. was collected from the various localities of Nanded, Maharashtra, India; during August 2020. Leaves were splashed with distilled water to remove soil particles and dirt. The leaves were crushed and dried in a shaded area at room temperature for a period of a week. Then the dried leaves were grinded with an ordinary grinder and then sieved through the server.

Preparation of Plant Extracts

The extracts of selected plant material in different solvents such as aqueous, ethanol, chloroform, hexane and acetone were prepared. The 30 gm of dried powder was extracted with 300 ml solvent using Soxhlet apparatus for 24 hrs. The aqueous, ethanol, chloroform, hexane and acetone extracts were lyophilized and stored in 4O C

Preliminary Qualitative screening of plant extracts

Standard protocols were used for the phytochemical analysis. Phytochemical screening for the presence of major types of compounds in the extract was done by Harborne (1973) with some modifications.

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• **Alkaloids:** 1.36gm of mercuric chloride was dissolved in 60ml distilled water and 5gm of potassium iodide and diluted to 100ml with distilled water. To 1.0ml of an acidic aqueous solution of samples, few drops of reagent were added. Formation of white or pale precipitate showed the presence of alkaloids.
• **Flavonoids:** In a test tube containing 0.5ml of various extracts of the samples, 5-10 drops of dilute HCl and a small piece of Zn or Mg were added and then the solution was boiled for a few minutes. In the presence of flavonoids, the reddish-pink or dirty brown colour was produced.
• **Phenols:** To 1.0ml of alcoholic solution of samples, 2.0 ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added and the formation of blue or green colour indicates the presence of phenols.
• **Saponins:** In a test tube containing 5ml of various extract of the sample, a few drops of sodium bicarbonate was added. The mixture was shaken vigorously for 3mins. A honeycomb like froth was formed and it showed the presence of saponins.
• **Steroids:** To 2.0ml of various extracts of samples, 1.0 ml of concentrated H2SO4 was added carefully along the sides of the test tube. Formation of red colour chloroform layer indicates the presence of steroids.
• **Tannins:** In a test tube containing about 5.0 ml of a various extract, a few drops of 1% solution of lead acetate was added. A yellow or red colour precipitate was formed, indicating the presence of tannins.
• **Terpenoids:** 0.5 ml of extract was mixed with 2 ml of chloroform in a test tube. 3 ml of concentrated sulfuric acid was carefully added to the mixture to form a layer. A reddish-brown colouration was formed for the presence of terpenoids.
• **Carbohydrate:** In a test tube containing 2.0 ml of plant sample, 2 drops of freshly prepared 20% alcoholic solution of a naphthol was added and mixed. To this solution 2.0 ml of concentrated sulphuric acid was added so as to form a layer below the mixture, the formation of the red-violet ring at the junction of the solution and its disappearance on the addition of an excess of alkali solution indicates the presence of carbohydrates.
• **Protein:** 1 part of mercury was digested with 2 parts of HNO3 and the resulting solution was diluted with 2 volumes of water. To a small quantity of decoction, 5- 6 drops of Million's reagent was added. A precipitate which turned red on heating was formed and it indicates the presence of proteins.

Quantitative estimation of plant extracts:
• **Estimation of total phenolic content:** Antioxidant compounds generally contain phenolic group(s) and hence, the amounts of total phenolic compounds in the extracts of the flowers, leaves and seeds were estimated by using Folin–Ciocalteu reagent with Gallic acid as standard. The total phenolic content was estimated according to the method of Singh et al. (2011). The aliquot of the extract was taken and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) was added sequentially to the test tube. Soon after overtaxing the reaction mixture, the tubes were placed in the dark for 40 min. and the absorbance was recorded at 725 nm using UV-Vis Spectrophotometer against the reagent blank. A standard curve was prepared using Gallic acid. The linearity obtained was in the range of 1-10 µg/ml. Using the standard curve, the total phenolic content was calculated and expressed as Gallic acid equivalent in mg/g of extract.

• **Estimation of total flavonoid content:** The antioxidant activity of medicinal plants could be attributed to its flavonoid content. Flavonoids act as scavengers of various oxidizing species i.e. superoxide anion, hydroxyl radical or per-oxy-radicals, they also act as quenchers of singlet oxygen (Ratty and Das, 1988). The Aluminium chloride colorimetric method was used to determine the total flavonoids content in plant extract using Quercetin as standard. The total flavonoids content was estimated according to the method of Chang et al. (2002).

Briefly, 0.5 ml solution of extract in methanol was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water, and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with the help of a UV-Visible spectrophotometer. The calibration curve was prepared by preparing Quercetin solutions at concentrations 10 to 100 µg/ml in methanol.

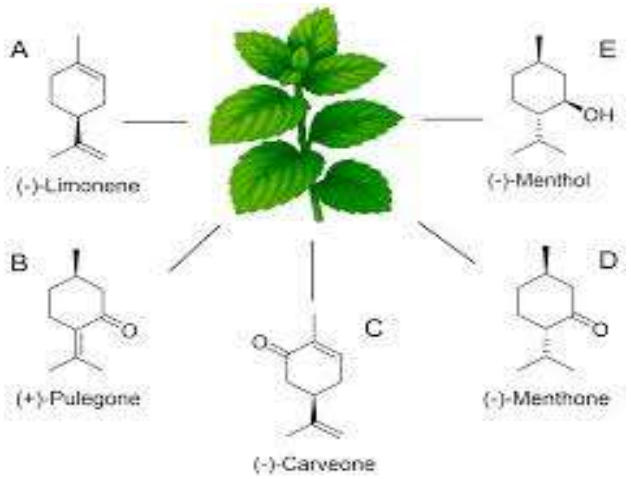
Antimicrobial properties
The antimicrobial activity of the plant extracts was evaluated using Agar Well Diffusion Method of Firdaus et al., (2011) with minor modifications. 0.1 ml of diluted inoculum (105 CFU/ml) of the microbial strains was swabbed on the Nutrient agar plates. Wells of 5 mm diameter were punched into the agar plates with the help of sterilized cork borer (5 mm). Using a micropipette, 100 µl of the plant extracts were added to the wells made in the plate. The plates were incubated aerobically in an upright position at 37±2 °C for 24-48 h. Antimicrobial activity was evaluated by measuring the zone of inhibition (mm) against the Clostridium perfringens, Bacillus cereus, Staphylococcus aureus, Aspergillus niger and Rhizopus stolonifer strains.
Result

Mentha piperita L. leaves were subjected to a preliminary phytochemical investigation using several solvents extracts. Alkaloids, flavonoids, tannins, phenols, saponins, steroids, terpenoids, proteins, and carbohydrates were all present in Mentha piperita L. leaves. Alkaloids were found to be most prevalent in acetone and aqueous extracts, but missing in chloroform extracts. Similarly, it was discovered that the chloroform extracts lacked proteins, terpenoids, and flavonoids. The majority of phytochemicals were detected in ethanol, acetone, and aqueous solvent extracts. (Table 1).

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Several Mentha piperita L. leaf extracts were tested for their antibacterial effectiveness against various microorganisms. Among all the studied extracts, the acetone extract showed the higher zone inhibition. The aqueous and ethanol extracts exhibited the least amount of inhibition.Overall, the extracts demonstrated a strong antimicrobial response against bacteria, but very little against fungus.

CHEMICAL STRUCTURE OF MENTHA PIPERITA



CHEMICAL PROPERTIES OF MENTHA PIPERITA



DISCUSSION

Preliminary qualitative phytochemical analysis of the different successive crude extracts of seed of Mentha piperita L. revealed the presence of alkaloids, flavonoids, proteins,carbohydrates, phenols, saponins, steroids, tannins and terpenoids. These secondary metabolites are reported to have many biological and therapeutic properties, so this species is expected to have many medicinal uses (Kamal et al., 2015). The phytochemical properties and antioxidant potential of Mentha plants make them the preferred candidates for nutritional and pharmaceutical applications. Due to the presence of these compounds, Mentha piperita L. plant show biological activity against various diseases and have been found to be effective in treating various diseases in humans (Lakshmi and Shalini, 2016).

The Singh et al. (2011) method was used to assess the total phenolic content. The acetone extract outperformed the other crude extracts in terms of 7.82 mg/g of phenolic content, 6.82 mg/g of ethanol extracts, 3.40 mg/g of aqueous extracts, and the lowest content of 1.64 mg/g and 0.67 mg/g, respectively, of chloroform and hexane extracts. The Chang et al. (2002) technique was used to estimate the total flavonoid content. Aqueous extracts had the highest total flavonoid content (7.34 mg/g), followed by chloroform (3.00 mg/g), acetone (2.21 mg/g), ethanol (1.75 mg/g), and hexane (0.19 mg/g).

The antibacterial properties of certain Mentha piperita Extracts from L. leaves were tested against various microorganisms. Of all the extracts examined, the acetone Higher zone inhibition against Bacillus cereus was demonstrated by the extract. The ethanol and aqueous extracts demonstrated the least amount of inhibition. Overall, the extracts demonstrated a strong antimicrobial response against bacteria, but very little against fungus. These phyto-constituents, sometimes referred to as plant secondary metabolites, are said to possess a variety of biological and therapeutic qualities. Therefore, it is anticipated that this species will have numerous therapeutic applications and can be investigated further for the manufacturing of pharmaceuticals.

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Conflict of interest
The author declares that there is no conflict of interest

Applications and Uses
Uses in Medicine
Both conventional and contemporary medicine make extensive use of peppermint. It helps relieve digestive issues such as indigestion, irritable bowel syndrome (IBS), and nausea. Menthol has analgesic, antispasmodic, and antimicrobial properties. Peppermint oil is also used for headaches, muscle pain, and respiratory conditions.

Culinary Uses
Peppermint leaves and extracts are used to flavor teas, confectionery, chewing gum, toothpaste, and other food products.

Cosmetic and Industrial Uses
The essential oil is widely used in cosmetics, personal care products, and pharmaceuticals due to its refreshing fragrance and cooling effect.

Economic Importance
Peppermint cultivation is commercially significant due to the high demand for its essential oil in global markets. Countries like the United States and India are major producers.

CONCLUSION
With numerous uses in industry, food, and medicine, Mentha piperita is a multipurpose and commercially significant herb. Its therapeutic effect is attributed to its bioactive components, especially menthol. Due to its effectiveness and natural origin, peppermint continues to be an important plant in both traditional and modern systems of healthcare.

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