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RESEARCH ARTICLE

Relationship between phyto-chemicals in *Mentha piperita* and their Antibacterial Activity

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ABSTRACT

A plant with as diverse a role as Peppermint leaves is a versatile resource for all forms of life. The plant extracts have active compounds in the form of alkaloids, glycosides, lactones and steroids. All these active compounds have immunomodulatory and physiological roles of different types, thereby demonstrating the diverse versatility of the plant. Studies need to be conducted with aspects of how the active compounds actually interact with the living systems and affects the structure-function relationships. Aqueous extract of Peppermint leaves were extracted by aqueous method. In the qualitative phytochemical testing presence of various secondary metabolites were found in aqueous extract of Peppermint leaves were Alkaloid, Flavonoids, Tannins and Saponin. In the quantitative analysis carbohydrate was found in Peppermint leaves sugar conc. is found (470 µg/ml). Antimicrobial activity was also quite good, in Peppermint leaves respectively against *Escherichia coli* and *Staphylococcus aureus*. The study demonstrates that the *Mentha piperita* contains the presence of different bioactive compounds having the potential as herbal drug.

1) INTRODUCTION

Peppermint is a hybrid mint, a cross between watermint and spearmint. Indigenous to Europe and the Middle East, the plant is now widely spread and cultivated in many regions of the world. It is occasionally found in the wild with its parent species.

Although the genus *Mentha* comprises more than 25 species, the most common one used is peppermint. While Western peppermint is derived from *Mentha piperita*, Chinese peppermint, or "Bohe" is derived from the fresh leaves of *Mentha haplocalyx*. *Mentha piperita* and *Mentha haplocalyx* are both recognized as plant sources of menthol and menthone and are among the oldest herbs used for both culinary and medicinal products.

Peppermint was first described in 1753 by Carl Linnaeus from specimens that had been collected in England; he treated it as a species, but it is now universally agreed to be a hybrid. It is an herbaceous rhizomatous perennial plant that grows to be 30–90 cm tall, with smooth stems, square in cross section. The rhizomes are wide-spreading, fleshy, and bear fibrous roots. The leaves can be 4–9 cm long and 1.5–4 cm broad. They are dark green with reddish veins, and they have an acute apex and coarsely toothed margins. The leaves and stems are usually slightly fuzzy. The flowers are purple, 6–8 mm long, with a four-lobed corolla about 5 mm diameter; they are produced in whorls (verticillasters) around the stem, forming

thick, blunt spikes. Flowering season lasts from mid- to late summer. The chromosome number is variable, with 2n counts of 66, 72, 84, and 120 recorded. Peppermint is a fast-growing plant; once it sprouts, it spreads very quickly [1].

India is world's largest producer and exporter of mint oil. Mint oil and its constituents and derivatives are used in food, pharmaceutical and perfumery and flavouring industry. Its main constituent, menthol, is used in the manufacture of lozenges, toothpastes, pain balms, cold balms, Pudín Hara, etc. The basic raw material for mint oil is leaves of a plant *Mentha arvensis*. The oil is used for treating certain stomach disorders like indigestion, gas problem, acidity, etc. It is the main ingredient of ayurvedic medicines like Daburs 'Pudín Hara'. The oil is a natural source of menthol, which is the main ingredient of cough drops and ointments like Vicks Vaporub, etc.

In addition, the leaves and the flowers of *M. piperita* have medicinal properties. An alternative approach to overcome antibiotic resistance might be using natural products and phytochemicals. It has also been shown that some plants extracts efficiently inhibit the biofilm formation of *C. albicans*. *Mentha piperita* L., a medicinally important plant belongs to the Family Lamiaceae [2] and commonly known as

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peppermint is a hybrid of *M. spicata* L. (spearmint) and menthe aquatic. It was cultivated by the ancient Egyptians and documented the Icelandic pharmacopoeia of the thirteenth century. It is widely grown in temperate areas of the world, particularly in Europe, North America and North Africa but now a days cultivated throughout all regions of the world

It is a medicinal plant that has received more attention from both food and pharmaceutical industries because of its health benefits for human society. Herein, the chemical structure of peppermint compounds evaluated using theoretical studies. Indeed, the health benefits of peppermint were reviewed. Molecular docking showed that among peppermint compounds, cineol and menthyl acetate apparently bound to the active site of arylamine N-acetyltransferase enzyme. This type of interaction indicates the inhibitory effects of these compounds against this enzyme. The plant is a good target for research and further studies should be focus on evaluating of peppermint in prevention of human diseases [3] the species is a natural interspecific hybrid. *M. piperita* yield peppermint oil (principal component is menthol. waxy white crystalline monoterpene substance, solid at room temperature, produced and accumulated specifically in the peltate glandular trichomes of the aerial parts) of commerce. Although peppermint oil is obtained from three species namely, *M. arvensis* L. var. *piperascens malinvaud*, *M. piperita* L. var. *piperita* and *M. spicata*, oil quality of *M. piperita*) and efficacy are reported to be the best. India is the dominant source of mint oil and menthol in the world market; however, despite the quantity of peppermint being at par with world standards much headway in export trade could not be made due to fierce competition from USA [4]. Various plant extracts have great potential against infectious agents and can be used for therapeutic purposes [5,6]. This study was carried out to evaluate the antimicrobial activities of peppermint (*Mentha piperita*) extracts against 10 multidrug resistant pathogenic bacteria clinical isolates. The antibacterial activities of ethanol, methanol, ethyl acetate and chloroform peppermint extracts were assessed using the standard minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) methods. Overall, the ethyl extract of peppermint had strong growth inhibitory effects on the tested pathogens, followed by the chloroform, ethanol and methanol extracts [1]. The present investigation aims to assess the phytochemical content, antioxidative activity and antimicrobial activity of the methanolic leaf extract of locally available *Mentha piperita*, the mint plant. The methanolic leaf extract of mint leaves was analyzed qualitatively for the phytochemical contents as previously described. The antimicrobial susceptibility test, including minimum inhibitory concentration and minimum bactericidal concentration (MIC and MBC) were determined. The functional chemical groups were determined by Fourier transform infrared spectroscopy (FTIR). The methanolic extract was found to contain tannins and flavanoids, with considerable free radical scavenging activity.

2) METHOD AND MATERIAL

Sample collection

Leaves of *M. piperita* were collected from local market of Barabanki. The samples were stored at room temperature (37° C) until further use. Drying of Leaves of *M. piperita* was done at room temperature for 3-4 days.

Maceration extraction

The plant part was cleaned, shade dried and powdered mechanically and stored in airtight containers. The extraction was carried out by maceration. About 10gm of powder was extracted with 100ml saline water (any polar solvent). The extract was concentrated to dry under controlled temperature 40-50°C. The extract was preserved in refrigerator.

Phytochemical test

I. Saponin: 1ml sample was dissolved in 5ml distilled water. It was shaken well, froth formation took place. Stability of froth confirms the presence of saponin in sample.

II. Tannin: 1ml sample was dissolved in 1ml 5% FeCl₃ Appearance of dark blue, black or dark green confirms presence of tannin in sample.

III. Flavonoid: 1ml sample was dissolved in 2ml 1% NaOH Presence of yellow colour indicates the presence of flavonoid in sample.

IV. Protein: 1ml of 1% CuSo₄ and 1ml of 1% NaOH was dissolved in 2ml sample. Appearance of purple color confirms the presence of protein in sample.

V. Alkaloid: 1ml iodine was dissolved in 1ml sample. Appearance of reddish-brown precipitate confirms the presence of alkaloid in sample.

Quantitative Test

1. Salicylic acid DNS method for carbohydrate estimation

Take 10 clean, dry test tubes, pipette and standard sugar solution in the range of 0 to 3ml in different test tubes and make up the volume of all test tubes to 3ml with distilled water concentrations ranging from 0 to 750mg. Add 1ml DNS reagent to all the test tubes and mix plug the test tube with cotton or marble and keep the test tube in a boiling water bath for 5 minute. Take the tubes and cool to room temperature. Read extinction at 540nm against the blank. Please note that all the tubes must be cooled to room temperature before reading, since the absorbance is sensitive to temperature. Prepare standard curves of the sugars provided and use them to estimate the concentration of the unknowns provided.

2. Thin Layer Chromatography (TLC)

Silica gel was mixed with water in ratio of 1:2. the glass slide or TLC plate was coated with suspension (thickness of silica gel in glass slide should be 0.1 to 0.25 mm for analytical purpose and 0.5 to 2mm for preparative purpose). The plate was air dried. The sample was loaded in on silica glass plate. Organic solvent (mobile phase) was prepared. Isopropanol and acetone (2:1) were used as a running solvent. After that silica plate was dipped in organic solvent and waited for some time.

Visualisation Technique

Solid iodine was placed in a closed container and vaporized through gentle heating. After the tank atmosphere was filled with iodine, the developed iodine TLC plate in the tank attain a brown colour.

R_f (Retention Factor) value = Distance travelled by solute from line of origin/ Distance travelled by solvent from the line of origin

3. Antibacterial Activity by Agar Well Diffusion Method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similarly, to the procedure used in disk diffusion method, the agar plate surface is inoculated by spreading a volume of the

microbial inoculum over the entire agar surface. Then, a well with diameter of 6-8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20µl-100µl) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested. NAM media were prepared for three plates of 15 ml. subjected it to autoclave. Immediately after autoclaving, it was poured into a petri plate. Agar media was allowed to cool and solidify at room temperature. The glass spreader and plates were put in UV light. 10µl of sample was put in plates and spread with the spreader evenly on surface of the plate. It was dried for 4-5 minutes. Then the well was punched out of four wells, antibiotic was added in one well and rest three well was added with the extracts. Plate was kept in incubator.

3) RESULTS AND DISCUSSION

The sample was collected from village of Barabanki. The samples were stored at room temperature (37° C) until further use. Drying of Leaves of *M. piperita* was done at room temperature for 3-4 days. All the Phyto-chemicals are presents in *M. piperita* (Table 1).

Table 1. Quantitative phytochemical analysis of crude extract of Peppermint leaves.

Tests	Peppermint leaves
Flavonoid	++
Alkaloids	+++
Saponin	+
Tanin	++

KEY = +++ abundantly present, + fairly present, ++ moderately present, - absent

1. Quantitative Estimation of Carbohydrate

Carbohydrate concentration is found to be maximum in Peppermint sugar conc. is found (470 µg/ml). So, peppermint can prove to be a good source of carbohydrate which is a major source of energy for humans (Table2).

Table 2. DNS assay for carbohydrate conc. estimation

S. No.	Glucose standard	Distill water	Concen.	DNS (ml)	Incubation n(min)	O.D. at 540 nm
Blank	0	3	0	2	Incubation for 5 min at room temp	0
1	0.5	2.5	100	2		0.449
2	1.0	2.0	200	2		0.914
3	1.5	1.5	300	2		1.210
4	2.0	1.0	400	2		1.640
5	2.5	0.5	500	2		1.910
Pepper mint leaves	1.0	2.0	103	2		0.419

2. Thin Layer Chromatography

Thin layer chromatography of the extract of Peppermint leaves was done on silica gel plate for the determination of type of compound present in Peppermint leaves. The compound determination was done by Rf value (Table 3).

Table 3. Rf value of extracted sample of Peppermint leaves.

Sample	Amount of sample loaded	Distance travelled by compound (cm)	Distance travelled by solvent (cm)	Observed Rf value
Peppermint leaves	5µl	1.4	4.5	0.311
	5µl	0.6	4.5	0.133

3. Antibacterial Test Against Pathogens

The antifungal effect of selected medicinal extracts can be applied at petri plat method. The extract being of plats origins has hazardous effects on the seeds as well as on soil. Zone of inhibition was measured in Peppermint leaves it proves that Peppermint leaves is effective on *E. coli*. and *S. aureus* (Table 4, fig1 and fig.2).

Table 4. Minimum inhibition concentration (MIC) *Mentha piperita* against *E. coli* and *S. aureus*.

Concentration	Zone of inhibition	
	<i>E. coli</i>	<i>S. aureus</i>
CONTROL	16 nm	16 nm
50µl	6nm	6 um
75µl	5 nm	4 nm
100µl	4 nm	3 nm



Fig. 1: Antibacterial activity against *E. coli*

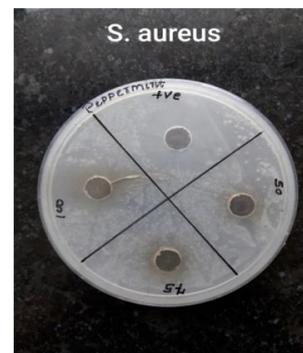


Fig. 2: Antibacterial activity against *S. aureus*

Mentha piperita (Lamiaceae), the peppermint (mint) plant is an aromatic perennial herb cultivated in most part of the world, have traditionally been used in folk medicine. Leaves of mint plant are frequently used in herbal tea and for culinary purpose to add flavour and aroma. The distinctive smell and flavour, a characteristic feature of *Mentha* spp. is due to the naturally occurring cyclic terpene alcohol called menthol. Menthol is prescribed as a medication for gastrointestinal disorders, common cold and musculoskeletal pain [7].

The medicinal parts are the essential oil extracted from the aerial parts of the plants. A mouthwash with peppermint oil included can help with bad breath and gum infections. The use of peppermint oil given orally can cure certain internal ailments such as gallstones or ureteric stones. The doses of them sometimes exceed 45 ml/day in France and Germany. Phenols, phenolic ethers, ketones, and oxides (1,8-cineole) appear to be the major toxic components of these essential oils when used on lice. Aldehydes and sesquiterpenes may also play a role. Obstruction of bile ducts, gall bladder

inflammation, severe liver damage. In case of gallstones, to be used only after the consultant of physician. [7].

Various constituents of peppermint oil as per monographs of International Pharmacopoeia are limonene (1.0-5.0%), cineole (3.5-14.0%), menthone (14.0-32.0%), menthofuran (1.0 - 9.0%), isomenthone (1.5-10.0%), menthyl acetate (2.8-10.0%), isopulegol (max. 0.2%), menthol (30.0- 55.0%), pulegone (max. 4.0%) and carvone (max. 1.0%). The ratio of cineole content to limonene content should be minimum two. Store in well-filled, tightly-closed, light-resistant containers in a cool place [8].

The inhibitory activity of the ethyl acetate extract against all Gram-negative pathogens was higher than that of chloroform methanol *Streptococcus pyogenes* lowest MIC value was seen for tate extract), followed by methicillin-resistant *Staphylococcus epidermidis* (MRSE) and *Enterococcus faecalis* The MBC values of all extracts were higher than the corresponding MIC values for the majority of pathogens [2]. This study highlights the potential antibacterial activity for *M. piperita* extracts, especially the ethyl acetate extract, against MDR *S. pyogenes*, *E. faecalis*, methicillin-resistant *Staphylococcus aureus* (MRSA), MRSE and carbapenem-resist *E. coli*, and *Klebsiella pneumonia* clinical isolates. Further *in vitro* and *in vivo* studies on a large number of clinical isolates of MRSA, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* are necessary to further investigate and standardize the inhibitory effect of peppermint extracts against these emerging pathogens. *Mentha piperita* has numerous pharmacological, cosmetic and alimetal applications due to its ability to produce terpene and terpenoid compounds. This plant produces oils rich in menthol and flavonoids, making it economically very important [9].

Peppermint (*Mentha piperita*) is widely consumed by the population for different purposes, but not for the treatment of dyslipidemias. The objective of the study was to examine the effects of this plant on human biochemical and anthropometric profiles and blood pressure, based on the administration of peppermint juice twice daily for 30 days. Blood samples were collected before and after the treatment in order to determine the glycemic and lipid profiles, and the Body Mass Index (BMI) analysis was performed. The use of peppermint by humans can be considered beneficial in the prevention and treatment of risk factors of chronic degenerative diseases [10].

The plant extract showed antimicrobial activity against clinical isolates of *Escherichia coli*, *Acinetobacter*, *Staphylococcus aureus* and two fungi such as *Candida albicans*, *Candida glabrata* [6]. The FTIR results indicated the molecular configuration of different functional groups in the plant extract. The mint leaf methanolic extract showed considerable antibacterial and antifungal activity against selected bacteria and fungi. Regular intake of mint leaves presumed to ward-off the initial colonization of selected pathogenic microbes [11].

The present study showed the presence of tannins and flavonoids in the methanolic mint leaf extract. A correlative relationship has been reported between the phytochemicals such as tannins and flavonoids and the free radical scavenging activity and antibacterial activity [12] Tannins and flavonoids have therapeutic uses due to their anti-inflammatory, antifungal, antioxidant and healing properties [13].

The present study indicated the antibacterial activity of mint leaves against the selected oral pathogens. The formation of biofilms in the form of plaques that harbours oral bacteria on

teeth, causes tooth decay and has been implicated as a cause of serious infections Frequent and continued intake of mint leaves in daily diet may prove beneficial in keeping the pathogenic microbes below the threshold level. The aqueous extract of *M. piperita* has considerable antibacterial activity against *Helicobacter pylori*, the main etiological agent of chronic gastritis and peptic ulcer disease [14].

Aqueous extract of Peppermint leaves was extracted by aqueous method. In the qualitative phytochemical testing presence of various secondary metabolites were found in aqueous extract of Peppermint leaves were Alkaloid, Flavonoids, Tannins and Saponin. In the quantitative analysis carbohydrate was found in) Peppermint leaves sugar conc is found (470 µg/ml). Antimicrobial activity was also quite good, in Peppermint leaves respectively against *Escherichia coli* and *Staphylococcus aureus*. The study demonstrates that the *Mentha piperita* contains the presence different of bioactive compounds having the potential as herbal drug.

A plant with as diverse a role as Peppermint leaves is a versatile resource for all forms of life. There are reports as already discussed that the plant extracts have active compounds in the form of alkaloids, glycosides, lactones and steroids. All these active compounds have immunomodulatory and physiological roles of different types, thereby demonstrating the diverse versatility of the plant. Studies need to be conducted with aspects how the active compounds interact with the living systems and affects the structure-function relationships.

4) CONCLUSION

Based upon the present study it could be concluded that plant extracts from *Mentha arvensis* and its major constituents menthone (14.0-32.0%), possess Antibacterial activity worth exploiting for bio management of diseases of stored commodities. In pilot experiments it can be concluded that this plant extracts can serve as natural Antibacterial or at least template for the synthesis of noble fungicides. The findings suggest that the *Mentha arvensis* can be exploited as a potent and ecofriendly Antibacterial fumigant against bacterial because of its high yield, strong and durable antibacterial activity.

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