



Phytochemical and Antibacterial Activity of *Mentha Arvensis* L

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ABSTRACT

The Phytochemical studies were carried out on the aqueous, chloroform and ethanol extract of the powdered specimen using standard procedures. In this investigation terpenoids, steroids, tannins, saponin, and cardiac glycosides showed positive results in the extracts whereas flavonoids and phlobatannins showed negative results in the aqueous extract. Soxhlet apparatus were used for extracting the antibacterial active compounds from the powdered leaves of *Mentha arvensis*. The discs were prepared and immersed in various solvents. Obtained pathogenic bacteria pure cultures were inoculated nutrient broth respectively. The antibacterial activities against the *Bacillus subtilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Staphylococcus pyogenes*. Ethanol extract was found to be most effective followed by other water and chloroform extracts. *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were more sensitive for ethanol extract. Aqueous extracts showed low inhibition against the tested organisms when compared to other test plant extracts. The present study provides an important basis for the use of chloroform and ethanol extracts from the leaves of *Mentha arvensis* L. for the treatment of skin diseases. The crude extract as well as the isolated compounds found to be active in this study which would be useful for the development of new antibacterial drugs.

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Introduction

In ayurveda and other systems of medicine different parts of the same plant in different seasons and for different therapeutics are used. The specific mention of the part used in each plant species like root, root bark, stem bark; phloem etc. is inevitable (Modak, 1993). These medicinal plants are most valuable natural resources. It is necessary to identify those medicinal plants. Rapid urbanization is resulting in the loss of many important medicinal plants. Scientific documentation of medicinal plants has proved a helpful resource for ayurvedic health care system. Only a quarter of the world population knows the helpfulness of different Indian medicinal plants. With the help of modern scientific knowledge and research we can develop a healthcare system without side effects (Gupta et al., 2005). With the rising prevalence of microorganism showing resistance to antibiotics, there is an urgency to develop new antimicrobial compounds. Since antiquity, plants have been used to treat common infectious diseases.

The healing potential of many plants have been utilized by Indian traditional medicines like siddha, ayurvedha and unani. Being nontoxic and easily affordable, there has been resurgence in the consumption and demand for medicinal plants (Jayashree and Maneemegalai, 2008). Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Diallo et al., 1999). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds (Edeoga et al., 2005). Therapeutically interesting and important drugs can be developed from plant sources which are used in traditional system of medicines. Indian traditional system of medicine is

based on empirical knowledge of the observations and the experience over millennia and more than 5000 plants are used by different ethnic communities in India. The present communication constitutes at a review on the medicinal properties and pharmacological actions of *Mentha arvensis* wall used in Indian traditional medicine. This plant is known contain various active principles of therapeutic value and to possess biological activity against number of diseases. *Mentha arvensis* L, a common plant in Asia, has been widely used in and ayurvedic medicine as avaripanchaga choornam and traditional medicine as a care for rheumatism conjunctivitis and diabetes. This plant materials used for "Fever, diabetes, constipation and urinary" disease. The pharmacological industries have produced a number of new antibiotics in the last three decades; resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are used as therapeutic agents.

The use of plant extracts and photochemical, both with known antimicrobial properties can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. The objective of this research was to evaluate the potential of plant extracts and phytochemicals on standard microorganism strains. Analysis of phytochemical compounds, preparation of plant extracts using different solvent, preparation of different extract disc and assay of antibacterial activity

Materials and methods

Collection and identification of plant material

This study was carried out on phytochemical screening and antibacterial activity of *Mentha arvensis* L. against some clinical pathogens. Plants were selected for this study based on their medicinal use. Fresh *Mentha arvensis* plant leaves were

collected from the cultivated area in Sirupuliur, Thiruvaiyaru Tk, Thanjavur Dt, tamil nadu, India, The study plant were identified with help of available indian literature and identified were verified with the help of Rapinent herbarium St' Josephs College, Tiruchirappalli, Tamil nadu, India.

Preparation of plant powder

Grinding of the selected plant materials

The leaves were air-dried. After drying at 37°C for 7 days the plant material was ground in a grinding machine (Thomas Wiley laboratory mill, model # 4, screen size-1mm) made for the laboratory. Exposure to direct sunlight was avoided to prevent the loss of active components.

Phytochemical screening

Chemical tests were carried out on the aqueous, chloroform and ethanol extracts and on the powdered specimens were using standard procedures to identify the constituents were described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Test for tannins

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for phlobatannins

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatanins.

Test for saponins

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for flavonoids

Three methods were used to determine the presence of flavonoids in the plant sample (Sofowara, 1993; Harborne, 1973). 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H₂SO₄. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Test for sterioids

Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H₂SO₄. The colour changed from violet to blue or green in some samples indicating the presence of sterioids.

Test for terpenoids (Salkowski test)

Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for cardiac glycosides (Keller-Killani test)

Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic

of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Assay of antibacterial activity

Extraction

A soxhlet apparatus were used for the extracting antimicrobial active compounds from the plant leaves. 20g of the plant powder sample were ground and soaked with 100ml water, chloroform and ethanol (separately) in a 250ml conical flask. The flask was covered with cotton wool and aluminium foil to the present the solvent from escaping. The flask was placed in a shaker for 24hrs. The plant extracts were collected and stored in a vial for further studies.

Preparation of disc

The 6 mm (diameter) discs were prepared from Whatman NO: 1 filter paper the discs were sterilizing by autoclave at 121°C for 15min. After the sterilization the moisture discs were dried on hot air oven at 50°C. Then various solvents extract discs and control discs were prepared.

Test microorganisms

Authentic pure cultures of human pathogenic bacteria like *Bacillus subtilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Staphylococcus pyogenes* were obtained from MTCC, Chandigarh.

Inoculum preparation

Obtained pathogenic bacteria pure cultures were inoculated nutrient broth respectively. The inoculated broths were incubated at 37°C for 24 hrs. After incubation the strains use further studies.

Antibacterial susceptibility test

Disc diffusion method (Bauer et al., 1966) was adopted for evaluation of antimicrobial activity of *Mentha arvensis* plant leaves. Muller Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45°C. The cooled media was poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The various solvents extract prepared discs individually were placed on the each petriplates and also placed control and standard (Clindamycin) discs. The plates were incubated at 37°C for 24 hrs. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm. Each extract was tested in triplicate for calculation of mean value and standard deviation.

Results

Phytochemical compounds screening of aqueous extract of *Mentha arvensis* L

The analysis of Tannin compounds were brownish green colour developed to indicate the presence of tannin. Similarly based on the presence or absence of colour change indicate positive and negative results. In this investigation terpenoids, sterioids, tannins, saponin, and cardiac glycosides showed positive results and flavonoids, phlobatannins showed negative results in aqueous extracts. Similarly phytochemical compounds investigated in chloroform and ethanolic extracts. The investigated all phytochemicals present in aqueous, chloroform and ethanolic extracts. The results were presented table - 1. Among the three extracts terpenoids, sterioids, tannins, saponin and cardiac glycosides showed the positive results, aqueous extract showed negative results in flavonoids and phlobatannins tests.

Assay of antibacterial activity

In this investigation aqueous, chloroform and ethanol extracts from the leaf of *Mentha arvensis* L. exhibit

antimicrobial activity against *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Staphylococcus pyogenes*. Antibacterial potential of leaf extract were assessed in terms of zone of inhibition of microbial growth. The effect of different extracts of *Mentha arvensis* test plant on bacteria was shown in table – 2. The results clearly showed that plant extracts were specific in action against the growth of bacteria. Ethanol extract was most effective followed by other water and chloroform extracts. *Pseudomonas aeruginosa* was more sensitive for ethanol extract of leaves of the tested plants. Aqueous extracts were low inhibition against the tested organism compared to other test plant extracts. The *Pseudomonas aeruginosa* (12 ± 0.47 ; 15 ± 0.64 mm in diameter) exhibit relatively higher zone of inhibition followed by chloroform, ethanol extracts and then compared then other test organisms. The results showed inhibition diameters ranging from 7 mm to 15 mm. The test organisms were resistant to the aqueous plant extracts except *Bacillus subtilis* (6 ± 0.32 mm in diameter) and *Staphylococcus pyogenes* (7 ± 0.28 mm in diameter).

Table 1. Qualitative screening and phytochemical tests of *Mentha arvensis*

S.No	Phytochemicals	<i>Mentha arvensis</i> L		
		Aqueous	Chloroform	Ethanol
1.	Terpenoids	Positive	Positive	Positive
2.	Steroids	Positive	Positive	Positive
3.	Flavonoids	Negative	Positive	Positive
4.	Tannins	Positive	Positive	Positive
5.	Phlobatannins	Negative	Positive	Positive
6.	Saponins	Positive	Positive	Positive
7.	Cardiac glycosides	Positive	Positive	Positive

Table 2. Assay of antibacterial activity

S.No.	Name of the organisms	Zone of inhibition (mm in diameter) (M $\pm$ SD)				
		Stand Cli*	Con.	Aqueous extract	Chloroform extract	Ethanol extract
1.	<i>Bacillus subtilis</i>	-	-	6 ± 0.32	-	7 ± 0.54
2.	<i>Klebsiella pneumoniae</i>	11	-	-	8 ± 0.29	14 ± 0.66
3.	<i>Pseudomonas aeruginosa</i>	10	-	-	12 ± 0.47	15 ± 0.64
4.	<i>Serratia marcescens</i>	13	-	-	7 ± 0.64	9 ± 0.48
5.	<i>Staphylococcus pyogenes</i>	13	-	7 ± 0.54	-	11 ± 0.40

Con. – control

Cli* - Clindamycin (disc 20mg) Ref. Hi Media Stranded value

The inhibitory effect of *Mentha arvensis* extract showed that water, chloroform and ethanol were 12 ± 0.47 mm; 15 ± 0.64 mm for *Pseudomonas aeruginosa*, 8 ± 0.29 mm; 14 ± 0.66 mm for *Klebsiella pneumoniae*, 7 ± 0.54 mm; 11 ± 0.40 mm for *Staphylococcus pyogenes*, 7 ± 0.64 mm; 9 ± 0.52 mm for *Serratia marcescens*, 6 ± 0.32 mm; 7 ± 0.28 mm in diameter *Bacillus subtilis*, respectively.

Discussion

Recently, much attention has been directed toward plant extracts and biologically active compounds isolated from popular plant species. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms (Munoz Mingarro et al., 2003).

In this study phytochemical prospection of *Mentha arvensis*, leaves aqueous extract indicated the presence of different secondary metabolites classes (table – 1). In this investigation terpenoids, steroids, tannins, phlobatannins, saponin, and cardiac glycosides were present. Many of them are known to have

different therapeutic. Tannins possess antibacterial, antiviral, molluscicidal and antitumoral properties (Scalbert, 1991). While steroids, also present in *Mentha arvensis*, is recognized to have anticancer, antiviral and antihemorrhagic properties (Simoes et al., 2002). On the basis of the results obtained in this present investigation, conclude that the ethanol extract of *Mentha arvensis* leaves had significant in vitro antibacterial activity.

The present study *Pseudomonas aeruginosa* showed the most susceptibility to the extract. In contrast, *Klebsiella pneumoniae* was the least susceptible bacterium. This may be due to the fact that *Pseudomonas aeruginosa* has intrinsic resistance from a restrictive outer membrane barrier and transverse loop multidrug resistance pumps (MDRs). The results of present research highlights, the fact that the organic solvent extracts exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted only through the organic solvent medium (Britto, 2001).

In the present work ethanolic and chloroform extracts of *Mentha arvensis*, extract showed higher activity to the test bacteria such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus pyogenes*, *Serratia marcescens* and *Bacillus subtilis*. Aqueous extracts low inhibition against the tested organisms compared to other test plant extracts. The results showed that the inhibition diameters ranging from 7 mm to 15 mm in diameter. The present observation suggests that the organic solvent extraction was suitable to verify the antimicrobial properties of medicinal plants and they supported by many investigators (Mohanasundari et al., 2007). Similarly supported by Singh, (1986) has been studied Ethanol and aqueous extracts of *Acalypha indica*, *Cassia auriculata*, *Eclipta alba*, *Mentha arvensis* and *Phyllanthus niruri* against *Bacillus subtilis* revealed that ethanolic root extract seem to be more active compared to aqueous extract.

The work supported by Ramasubramania raja and Parimala Devi, (2010) have been analyzed on hydro alcoholic dry extracts of *Gymne masylvestre*, *Mentha arvensis*, *Solanum surratense*, for treatment of a dental caries were screened for antimicrobial activity by Agar well diffusion method against *Streptococcus mutans*, *Staphylococcus aureus*, *Streptococcus mitis* and *Candida albicans*. Among them the extracts of *Gymne masylvestre* dry extract showed strong antimicrobial activity against the bacteria and fungi with the zone of inhibition ranges from 16-20mm at 25mg/ml. The other extracts such as *Solanum surratense*, *Mentha arvensis* showed concentration dependent activity against all the tested micro-organisms with the zone of inhibition ranges from 12-24mm at various concentrations which adds more detail for our present work.

Results of present research work were supported by the work done by various researchers. Alcoholic leaf extract was found to have antibacterial effect against the pathogen by Gehlot and Bohra (2000). Several workers have reported that many plants possess antimicrobial properties including the parts which include; flower, bark, stem, leaf, etc. It has been shown that when solvents like ethanol, hexane and methanol were used to extract plants, most of them are able to exhibit inhibitory effect on both gram positive and gram negative bacteria (Bushra Beegum and Ganga Devi, 2003).

Many substances may have antimicrobial activity, but only a few of them will be potential therapeutic agents for the simple reason that mammalian cells are more sensitive to chemical inhibition than microbial cells (Sivakumar and Alagesaboopathi, 2006). Moreover emphasized the need for toxicity testing of drugs derived from medicinal plants because the crude products

obtained from such cheaper sources are often associated with a large number of compounds that have discomforting abilities (Ramdas et al., 2006). Hence the herbal drugs have been subjected to extensive pharmacological, toxicological and clinical tests to confirm the prescribed status. Thus the ethnobotanical approach will be like a search for molecular diversity subjecting a wide variety of new molecules from plant sources and testing them with as many different tests as possible (Muhammad and Muhammad, 2005).

The present study has shown a spectrum of antibacterial activities, which provides a support to some traditional uses of these few medicinal plants. But the effective biomolecules which act as antibacterial have to be identified, isolated and subjected to extensive scientific and pharmacological screening that can be used as source for new drugs. This study also encourages cultivation of the highly valuable plant in large scale to increase the economic status of the cultivation in our country.

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