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Studies on Antimicrobial Potential and Phytochemical Analysis of Mint Leaves Extracts

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ABSTRACT

The present study was carried out to find out the antimicrobial activity and phytochemical analysis of Mint (*Mentha arvensis*) leaves solvent extracts against selected enteric pathogens. Plants have been an important source of medicine with qualities for thousands of years. In the present study, the antimicrobial efficacy of Mint leaves solvent extracts was examined using Ethanol, Methanol, Diethyl ether and Acetone as solvents and tested against nine enteric pathogens namely *E.coli*, *Salmonella typhi*, *Sal.Para.A*, *Sal. Para. B*, *Shigella sonnei*, *Shigella dysenteriae*, *Enterobactor spp.*, *Citrobactor spp.* and *Klebsiella spp.* Antimicrobial analysis was done by using agar well diffusion method against selected enteric pathogens. The MIC values were determined by both agar and broth dilution method. The functional chemical group was determined by Fourier Transform Infrared Spectroscopy (FTIR). The Ethanol extract of Mint showed highest antimicrobial activity against *Shigella sonnei*, while lowest antimicrobial activity was observed by Ethanol extract of Mint against *Citrobactor spp* and by Acetone extract of Mint against *Shigella dysenteriae*. The phytochemical analysis of Mint leaves solvent extracts showed presence of alkaloid, flavonoid, phenol, tannin, glycosides etc.

Keywords: Antibacterial activity, *Mentha arvensis*, Enteric pathogens, MIC, Phytochemical analysis, FTIR etc.

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INTRODUCTION

Plant material have been used for the treatment of serious diseases throughout the world before the advent of modern clinical drugs ¹.The use of medicinal plants still plays an important role to cover the basic health needs in the developing countries ².Several top selling drugs of modern times such as Quinine, Artemisinin, Shikonin, etc. are obtained from plants¹.Most of the phytochemicals, secondary metabolites of plants, are physiological active². Majority of phytochemicals are known to produce therapeutic activities like antibacterial, antifungal, antioxidant etc. Alkaloids, tannins, flavonoids and phenol compounds are the most important of bioactive constituent of plants. In addition to their use for therapeutic purposes, natural phytochemicals are effective as precursors for the synthesis of novel useful drugs. About 50% of modern drugs are natural products, which play an important role in drug development in pharmaceutical industry.

The exploitation of plants by man for treatment of diseases has been in practice for a very long time. Medicinal plants are of great value to mankind and society. The medicinal value of these plants can be observed from the chemical agents they possess which may alter certain physiological actions in the human body. The most important of these bioactive constituents of plants are alkaloid; tannins, flavonoids and phenolic compounds³.Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to food meant for pregnant and nursing mothers for medicinal purposes⁴.These plants are used as sources of therapeutic agents due to their higher properties. These include among others reduced cost, relative lower incidence of adverse reactions compared to modern synthetic pharmaceuticals ⁵. Knowledge of the chemical constituents of medicinal plants are desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of economic materials such as tannins, oils, gums, precursors for the synthesis of complex chemical substances .Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds etc.⁶

Medicinal and aromatic plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals, perfumery, flavor and cosmetic industries. The plant kingdom has served as an inexhaustible source of useful drugs, foods, additives, flavoring agents, lubricants, coloring agents and gums from time immemorial. The therapeutic power of herbs had been recognized since creation of the universe and botanic medicine is one of the oldest practiced professions by mankind ⁷.Medicinal plants have been found useful as antimaleria, antisickling, anti-helminthic, anti-microbial and anti-hypertensive agent's etc.⁸

Medicinal plants have been used by human being since ages in traditional medicine due to their therapeutic potential and the search on medicinal plants have lead to the discovery of novel drug candidates used against diverse diseases. According to World Health Organization (WHO) in 2008 more than 80% of the world population relies on traditional medicine for their primary healthcare needs ⁹. Abundant medicinal plants have been used in many forms over the years to treat, manage or control mans ailments, therefore any effort to further explore the medicinal or natural products from mans botanical flora towards improving health care delivery deserves attention. Management of plant and human disease is generally achieved by the use of synthetic pesticides and antibiotics ¹⁰; however the incessant and indiscriminate use of these synthetic antibiotics has caused health hazards in animals and humans due to their residual toxicity and many a times management of pathogenic microbes becomes difficult due to the resistance of pathogens.

Mint is aromatic, almost exclusively perennial, rarely annual herb. They have wide spreading rhizomes and erect, branched stems. The leaves are arranged in opposite pairs, from simple oblong to lancetate, often downy and with a serrated margin. Mint was originally used as a medicinal herb to treat stomach ache and chest pains. The leaf, fresh or dried, is the culinary source of mint. Mint leaves are used in teas, beverages, syrups, candies and icecreams. Treatment of diseases with modern medicine is often and generally associated with the development of side effects. Hence the plant products have been increasing worldwide, to lower side effects.

In ayurveda, Mint is considered as appetizer and useful in gastric troubles. In Europe, wild mint was traditionally used to treat flatulence, digestion problems, gall bladder problems and coughs. The oil was extracted and rubbed into the skin for aches and pains. The Native Americans also used it in several traditional ways. Nowadays it is used in many countries for various ailments. Mint extracts and menthol-related chemicals are used in food, drinks, cough medicines, creams and cigarettes. Chemical substances that can be extracted from wild mint include menthol, menthone, isomenthone, neomenthol, limonene, methyl acetate, piperitone, beta-caryophyllene, alpha-pinene, beta-pinene, tannins and flavonoids. The present study was aimed at investigating the antibacterial activity and phytochemical analysis of various solvent extracts of Mint.

MATERIALS AND METHODS

Collection of Plant Material

Healthy disease free, indigenously grown mature leaves of Mint was purchased from local market of Solapur (M.S.). The identification of plant material was confirmed by a Botanist in the Dept. of Botany, Walchand College of Arts and Science, Solapur (M.S.).

Test Pathogens

Nine strains of enteric pathogenic bacterial cultures were used in this study. These are *E.coli*, *Salmonella typhi*, *Salmonella paratyphi A*, *Salmonella paratyphiB*, *Shigella dysenteriae*, *Shigella sonnei*, *Enterobactor spp.*, *Citrobactor spp.* and *Klebsiella spp.* The pure pathogenic bacterial strains were collected from Dept. of Microbiology, V.M.Govt.Medical College, Solapur (M.S.). The collected pure pathogenic bacterial strains were isolated from hospitalized patients at Govt.Civil Hospital, Solapur (M.S.) The cultures were maintained on nutrient agar slants at 4⁰C and subcultured for 24hr. before use.

Preparation of Solvent Extracts

Thoroughly washed mature leaves were shade dried and then powdered with the help of electric blender. Twenty five grams of the powder was filled in the thimble and extracted successively with Ethanol, Methanol, Diethyl Ether and Acetone using a Soxlet extractor for 48hr. All the extracts were concentrated using rotary flash evaporator and preserved at 5⁰C in airtight bottle until further use. All the extracts were subjected to antibacterial activity assay and phytochemical analysis.

Antimicrobial Activity Assay

Antimicrobial activity of the Mint solvent extracts was determined by agar well diffusion method on Muller- Hinton agar medium¹¹. Cups are made on Muller- Hinton agar plates using cork borer and inoculum containing 10⁶ CFU/ml of pathogenic bacteria were spread on the solid plate with the help of sterile glass rod. Then 100ul of solvent extract was placed in the cups made in inoculated plates. All the plates were incubated for 24hr. at 37⁰C and after incubation period zone of inhibition was measured in mm. Antimicrobial activity of Standard antibiotics Ampicillin and Tetracycline were observed in comparison with Mint leaves solvent extracts.

Determination of Minimum Inhibitory Concentration (MIC)

MIC was determined by both agar and broth dilution methods¹². For broth dilution tests, 0.1ml of standardized suspension of bacteria (10⁶ CFU/ml) was added to each tube containing different concentrations of solvent extracts (05-50ul/ml) and incubated for 24hr at 37⁰C. In agar plating method dilutions having 05-50ul of solvent extracts was placed in the cups on the inoculated plates and incubated as mentioned above.

Phytochemical Analysis

Qualitative Phytochemical Analysis

The Mint leaves solvent extracts was tested for the presence of bioactive compounds by using standard method¹³.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was used to identify the characteristic functional group in the crude Mint leaves powder. A small quantity (5mg) of the powder was dispersed in dry potassium bromide (Kbr). The mixture was thoroughly mixed in a mortar and pressed at pressure of 6 bars within 2 min. to form a Kbr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using Perkin Elmer 2000 infrared spectrometer. The sample was scanned from 4000 to 400 cm^{-1} for 16times to increase the signal to noise ratio.

RESULTS AND DISCUSSION

In the present study significant antibacterial activity is observed by all solvent extracts of Mint leaves. The antimicrobial activity of Mint leaves solvent extracts are represented in table 1. The result revealed that the ethanol extract of Mint shows highest antibacterial activity against *Shigella sonnei*. The methanol extract of Mint shows highest antibacterial activity against *Enterobactorspp.* The diethyl ether extract of Mint shows highest antibacterial activity against *Salmonella typhi* while acetone extract of Mint shows highest antibacterial activity against *Klebsiellaspp.* The antimicrobial activity of standard antibiotic Ampicillin were found to be maximum against *Citrobactor spp.* while standard antibiotic Tetracycline showed highest antibacterial activity against *Sal.para.B.*

Minimum Inhibitory Concentration (MIC) of the sixteen different solvent extract varied against different test pathogens. The MIC of Mint leaves solvent extract required for test pathogens are represented in table 2. Lowest MIC of 5ul was observed against *Shigella sonnei* while highest MIC of 50ul was observed against *Citrobactor spp.* by Ethanol extract of Mint. Lowest MIC of 5ul was observed against *Sal.para.B* while highest MIC of 40ul was observed against *Klebsiella spp.* by Methanol extract of Mint. Lowest MIC of 10ul was observed against *Sal.thphi* while highest MIC of 35ul was observed against *Citrobactor spp.* by Diethyl ether extract of Mint. Lowest MIC of 15ul was observed against *Klebsiella spp.* while highest MIC of 50ul was observed against *Enterobactorspp.* by Acetone extract of Mint.

The aim of FTIR analysis is to determine the existence of functional group that exists on isolate. The IR spectrum of the Mint leaves extract in the form of Kbr pallet is shown in figure 1.

The absorption at 3389 cm^{-1} is due to the stretching of hydroxyl group that are present in the extract. The bond at 1654 cm^{-1} is due to c=c stretching .The absorption at 1262 cm^{-1} is due to the C-N stretching vibration. The absorption at 1064 cm^{-1} is due to olefinic C-H out of plane bending modes.

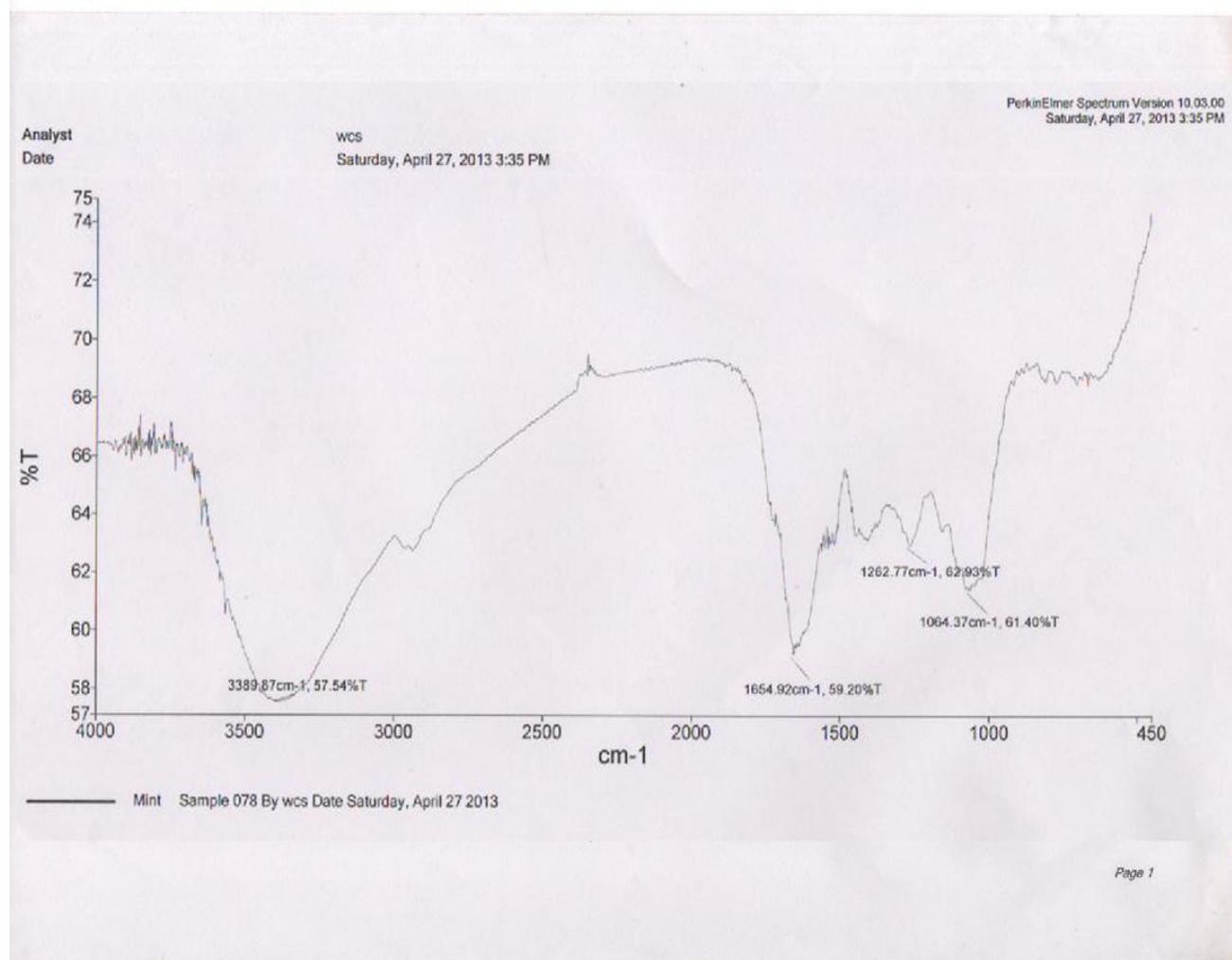


Figure1. Fourier transform infrared spectroscopy (FTIR) analysis of crude Mint leaves.

The results of phytochemical analysis of various solvent extracts of Mint leaves are represented in table 3. The phytochemical analysis showed presence of flavonoid, alkaloid, tannin, phenol, glycosides etc.

The results obtained in this study suggest that the identified phytochemical compounds may be the bioactive compounds and these various solvent extracts of Mint leaves can be used as potential source of drugs in the treatment or control of intestinal disorders.

Various medicinal plants are valuable and radially available resources for primary health care and complementary healthcare system. Undoubtedly medicinal plants containing substances of medicinal value that has yet to be discovered, though large numbers of plants are constantly being screened for their antimicrobial activity. These plants may prove to be a rich source of compounds with possible antimicrobial activities.

Table 1. Antibacterial activity of different solvent extracts of Mint.

M/O	Zone of Inhibition (mm)					
	Ethanol	Methanol	D.E.	Acetone	Ampi.	Tetra.
<i>E.coli</i>	7mm	5mm	6mm	5mm	13mm	14mm
<i>Sal.typhi</i>	3mm	4mm	7mm	4mm	14mm	11mm
<i>Sal.para A</i>	4mm	5mm	3mm	4mm	10mm	12mm
<i>Sal.para B</i>	4mm	6mm	5mm	3mm	11mm	15mm
<i>Shigella sonnei</i>	8mm	6mm	5mm	4mm	6mm	12mm
<i>Shigella dysenteriae</i>	6mm	3mm	3mm	2mm	12mm	11mm
<i>Enterobactor spp.</i>	5mm	7mm	5mm	4mm	12mm	14mm
<i>Citrobactor spp.</i>	2mm	3mm	4mm	5mm	15mm	13mm
<i>Klebsiella spp</i>	4mm	4mm	5mm	6mm	9mm	11mm

(Ampi- Ampicillin, Tetra- Tetracycline)

Table 2. MIC of different solvent extracts of Mint.

M/O	Minimum Inhibitory Concentration (ul/ml)			
	Ethanol	Methanol	D.E.	Acetone
<i>E.coli</i>	10	35	20	40
<i>Sal.typhi</i>	40	20	10	25
<i>Sal.para A</i>	20	15	25	20
<i>Sal.para B</i>	25	05	15	35
<i>Shigella sonnei</i>	05	10	20	40
<i>Shigella dysenteriae</i>	10	20	20	30
<i>Shigella dysenteriae</i>	25	10	30	50
<i>Citrobactor spp.</i>	50	40	35	20
<i>Klebsiella spp.</i>	30	45	25	15

(D.E. - Diethyl ether)

Table 3. Phytochemical analysis of Mint leaves solvent extracts.

Test	Ethanol	Methanol	D.E.	Acetone
Carbohydrate	+	+	-	+
Phenols	+	-	+	+
Alkaloid	+	-	+	-
Flavonoids	+	+	+	+
Saponins	+	+	-	-
Glycosides	+	+	-	-
Steroid	-	+	+	-
Reducing sugar	-	-	-	-
Tanin	+	+	-	-

(+ = Present, - = Absent, D.E. - Diethyl ether)

CONCLUSION

The present study suggested that, the various solvent extracts of Mint leaves have a great potential as antimicrobial agents against selected enteric pathogens and they can be used as an alternative medicine in the treatment or control of enteric bacterial infections. The antimicrobial activity and MIC assays showed promising evidence for the antimicrobial activity of Mint leaves solvent

extracts against selected enteric pathogens. Phytochemical analysis showed presence of antimicrobial substances in the studied extracts. The results revealed the presence of medicinally important constituents in these solvent extracts. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Therefore, the Mint leaves solvent extracts could be seen as a good source for useful drugs.

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