In *vitro* Antibacterial and Anti-Fungal Activities of Methanolic Extract of *Mentha pulegium*

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ABSTRACT

The aims of our research were analysis of the secondary metabolite products and in *vitro* antibacterial and anti-fungal activities. Infectious diseases which are one of the main causes of morbidity and mortality worldwide represent a critical problem to health. Pharmacological industries have produced number of new-antibiotics in the last three decades, but microbial resistance to these antibiotics has increased because of genetic ability of the bacteria to acquire and transmit the resistance against therapeutic agents. *Mentha pulegium* is a species of flowering plant in the family Lamiaceae. The FTIR analysis of *Mentha pulegium* leaves proved the presence of alkenes, alkyl halides, aromatic, and amide which shows major peaks at 958.62, 985.62, 1012.63, 1026.13, 1093.64, 1242.16, 1255.66, 1593.20, 1606.70 and 2362.80. In the current study, the anti-microbial activity of *Mentha pulegium* methanolic extract was evaluated by determining the zone of inhibition against bacteria, fungi and yeast. Maximum zone formation was against *Staphylococcus aureus* (5.89±0.20). *Mentha pulegium* was very highly active against *Aspergillus terreus* (6.37±0.22).

Keywords: *Mentha pulegium, In vitro, FT-IR, Anti-Bacterial, Anti-Fungal Activity*

INTRODUCTION

The present study involves an assessment using FT-IR spectroscopic techniques to investigate the authenticity of commercial sample of the herbal drug by analyzing their fingerprints. The presence of antimicrobial activity in a particular part of a particular species may be due to the presence of one or more bioactive compounds such as alkaloids [1-10], glycosides, flavonoids, steroids, saponins etc.. Recently, a number of plants have been reported for antibacterial properties across the world. Based on the present study, it is concluded that the whole plants of A. lanata contains various bioactive components with high degree of antibacterial activity against various pathogens. It is hoped that this study would direct to the establishment of some compounds that could be used to invent new and more potent antibacterial drugs of natural origin [11-16]. Further work will emphasize the isolation and characterization of active principles responsible for bio-efficacy and bioactivity. The Lamiaceae family contains wide variety of aromatic plants mainly in temperate countries which is native in Europe, North Africa, Asia Minor, and near East. This plant is a perennial aromatic herb with 10 to 45 cm height and a 4 angles stem with small green long-stalked leaves that grows in moist damp streams. Medicinal aromatic plants like as *Mentha pulegium* due to their chemical components with therapeutic effects are important for treating human diseases[17-23]. This plant has been traditionally used due to its antiseptic effect for treatment of cold, sinusitis, bronchitis, cholera, food poisoning and tuberculosis. Microorganisms including gram positive and gram negative bacteria have been recognized as the main causes of various human infections. With regard to the occurrence of multidrug resistant bacteria, it is necessary to discover new antibiotic sources and plants can be potential source for this purpose [24-29]. The objectives

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of our study were analysis of the secondary metabolite products and in *vitro* antibacterial and anti-fungal activities.

MATERIALS AND METHOD

Collection and preparation of plant material

The leaves were purchased from local market in Hilla city, middle of Iraq. After thorough cleaning and removal foreign materials, the leaves were stored in airtight container to avoid the effect of humidity and then stored at room [30-36] temperature until further use.

Preparation of sample

About 20 grams of the plant sample powdered were soaked in 100 ml methanol for 16 hours in a rotatory shaker. Whatman No.1 filter paper was used to separate the extract of plant. The filtrates were used for further phytochemical analysis [38-42]. It was again filtered through sodium sulphate in order to remove the traces of moisture.

Fourier transform infrared spectrophotometer (FTIR)

The powdered sample of *Mentha pulegium* was treated for FTIR spectroscopy (Shimadzu, IR Affinity, Japan). The sample was run at infrared region between 400 nm and 4000 nm.

Determination of antimicrobial activity of crude bioactive compounds of *Mentha pulegium*

The test pathogens were swabbed in Müller-Hinton agar plates. Sixty *mL* of plant extract was loaded on the bored wells. Antifungal activity was evaluated by measuring the zone of inhibition against the test microorganisms. Methanol was used as solvent control. Amphotericin B and fluconazole were used as reference antifungal agent. The tests were carried out in triplicate. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation.

RESULTS AND DISCUSSION

Identification of biochemical compounds

Analysis of compounds was carried out in methanolic extract of *Mentha pulegium*, shown in **Table 1.** Chromatogram FTIR analysis of the methanol extract of *Mentha pulegium* showed the presence of ten major peaks and the components corresponding to the peaks were determined as follows. The FTIR analysis of *Mentha pulegium* leaves proved the presence of alkenes, alkyl halides, aromatic, and amide which shows major peaks at 958.62, 985.62, 1012.63, 1026.13, 1093.64, 1242.16, 1255.66, 1593.20, 1606.70 and 2362.80. In the current study, the anti-microbial activity of *Mentha pulegium* methanolic extract was evaluated by determining the zone of inhibition against bacteria, fungi and yeast. Maximum zone formation was against *Staphylococcus aureus* (5.89±0.20). *Mentha pulegium* was very highly active against *Aspergillus terreus* (6.37±0.22). Herbal drugs are being proved as effective as synthetic drugs with lesser side effects. WHO encourages countries to provide safe and effective traditional remedies and practices in public and private health services and it also published two monographs on medicinal plants with information on pharmacopoeial summaries for quality assurance: botanical features, distribution, identity tests, purity requirements, chemical assays, and active or major chemical constituents, clinical applications, pharmacology, contraindications, warnings, precautions, potential adverse reactions, and posology. One important finding in this study was this fact that active components of this plant affect cell wall. As a common rule those antibacterial agents that can disrupt cell wall or inhibit its biosynthesis are of great importance. These agents act as bactericidal agents and so it is unlikely that after their usage the infection relapses. In most medicinal plant studies this step is ignored while finding those medicinal plants that can affect bacterial cell wall can help to find effective bactericidal agents. Finally it should be noticed weather condition is very decisive in producing active substances in plants and hence this plants that grown in different climate area should be screened for bioactive compounds.

Table 1. FT-IR peak values of *Mentha pulegium* methanolic leaves extract.

No.	Peak (Wave number cm-1)	Intensity	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	958.62	77.147	Strong	=C–H	Bending	Alkenes	650-1000
2.	985.62	71.466	Strong	=C–H	Bending	Alkenes	650-1000
3.	1012.63	65.604	Strong	C-F	Stretch	alkyl halides	1000-1400
4.	1026.13	65.029	Strong	C-F	Stretch	alkyl halides	1000-1400
5.	1093.64	72.698	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1242.16	81.648	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1255.66	81.607	Strong	C-F	Stretch	alkyl halides	1000-1400
8.	1593.20	79.968	Medium	C=C	Stretch	Aromatic	1400-1600
9.	1606.70	78.913	Bending	N-H	Stretch	Amide	1550-1640
10.	2362.80	83.382	Unknown	-	-	-	-

CONCLUSION

The FTIR analysis of *Mentha pulegium* leaves proved the presence of alkenes, alkyl halides, aromatic, and amide which shows major peaks at 958.62, 985.62, 1012.63, 1026.13, 1093.64, 1242.16, 1255.66, 1593.20, 1606.70 and 2362.80. In the current study, the anti-microbial activity of *Mentha pulegium* methanolic extract was evaluated by determining the zone of inhibition against bacteria, fungi and yeast. Maximum zone formation was against *Staphylococcus aureus* (5.89±0.20). *Mentha pulegium* was very highly active against *Aspergillus terreus* (6.37±0.22).

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Conflict of Interest: None to declare.

Ethical Clearance: In our research, all protocols were approved under the Department of Biology, College of Science for women, University of Babylon, Hillah city, Iraq and all methods were carried out in accordance with approved guidelines.

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