Eurasian J. Sci. Eng., 2023, 9(2), 262-269
https://doi.org/10.23918/eajse.v9i2p21

Published by Tishk International University
Available at: https://eajse.tiu.edu.iq/

RESEARCH ARTICLE Open Access

DETERMINATION OF ANTIFUNGAL ACTIVITY OF ESSENTIAL OIL OF *MENTHA PIPERITA* (MINT)

Peshawa Y. Aziz [1], Shokhan H. Azeez [*2], Nabaz Hamarashid Hama [3], Hevar Nyaz Abdulqadir [3], and Othman Mohammad [1]

[1] Department of Medical Laboratory Science, Technical College of Applied Science, Sulaimani Polytechnic University, Sulaimani, Kurdistan Regional Government, Iraq
[2*] Department of Dental Nursing, Sulaimani Technical Institute, Sulaimani Polytechnic University, Sulaimani, Kurdistan Regional Government, Iraq
[3] Medical Laboratory Science Department, College of Science, Komar University of Science and Technology, Sulaimani, Kurdistan Region, Iraq

Article History
Received: 25.03.2023
Revised: 28.05.2023
Accepted: 22.06.2023
Communicated by: Dr. Orhan Tug
Email address:
shokhan.azeez@spu.edu.iq
Corresponding Author

Abstract:
Fungal infections are a major global health problem, particularly in vulnerable populations such as immunocompromised individuals and those living in resource-limited settings. Traditional anti-fungal agents are often associated with high costs, side effects, and the emergence of drug-resistant strains. Therefore, there is a need for the development of new, safe, and effective anti-fungal agents. This study aimed to investigate the anti-fungal activity of mint essential oil against selected fungal strains. The leaves of mint were gathered, dried and powdered. After that, hydro distillation method was used for isolation of essential oil. The anti-fungal activity of the essential oil was evaluated against three fungal strains, including *Aspergillus niger*, *Cryptococcus neoformans*, and *Trichophyton interdigitale*, using a two-fold dilution. The results showed that the mint essential oil had a significant effect on the growth of the fungi, with two of the three strains being highly sensitive. The concentrated essential oil was found to be most effective at preventing fungal growth. These findings suggest that Mint essential oil has potential as an anti-fungal agent, particularly against *Cryptococcus neoformans*. The use of different types of plant essential oil such as mint essential oil could provide a promising approach for the development of new anti-fungal agents, particularly in the context of drug-resistant infections.

Keywords: *Antifungal; Hydro Distillation; Mentha Piperita; Multi Drug Resistance*

1. Introduction

Natural compounds from both plant and animal sources have traditionally been used in medicine due to their broad therapeutic spectrum. Many scientists believe that our world's natural resources will be the key to developing anti-fungal medicine. Although anti-fungal medications are generally effective in treating fungal infections, there are instances where certain fungal infections may be resistant to specific medications, requiring alternative treatment options, and as a result, people are suffering from highly resistant fungal strains [1]. Because of advances in medicine, treating a fungal infection is far more difficult than treating a bacterial infection. Fungi, like bacteria, can develop multi-drug

resistance, making them considerably more hazardous when they cause infection. According to a recent study on fungal infections, there are 150 million cases of severe fungal infections per year, resulting in 1.7 million deaths, and the figure is still rising [1]. Scientists believe that *Mentha piperita* can be employed in the medical area to treat microorganism infections because it is a natural resource from our environment [2].

Cryptococcus neoformans, Candida albicans, and Trichophyton interdigitale are fungi that can cause serious infections in humans. *Cryptococcus neoformans* can cause meningitis, particularly in immunocompromised individuals, and is treated with anti-fungal agents such as fluconazole and amphotericin B [3]. *Candida albicans* can cause a range of infections, including thrush, systemic candidiasis, and invasive candidiasis, and is treated with anti-fungal agents such as fluconazole, echinocandins, and amphotericin B [4]. *Trichophyton interdigitale* can cause various dermatophytosis infections such as athlete's foot and nail infections, and is treated with topical anti-fungal agents such as terbinafine and azoles [5].

Since ancient times Mentha has been used by different cultures for a variety of purposes. Mint has numerous health advantages [6]. For example, it is a nutrient-dense food high in vitamin A, as well as an antioxidant that aids the human body in eliminating cells harmed by free radicals [7]. Nowadays one of the most frequent digestive system disorders is irritable bowel syndrome (IBS). According to research, peppermint oil has a significant impact on the treatment of IBS. This is thought to be due to methanol, a chemical in the oil that can help relieve IBS symptoms by relaxing the muscles of the digestive tract [8]. A study by West et al, of the impact of peppermint oil in treatment of 700 IBS patients, found that 75% of those who took peppermint oil showed signs of improvement in their IBS symptoms [8].

Mint can also help with dyspepsia, Indigestion can occur when food remains in the stomach for an extended period of time [9]. It has been found that using peppermint oil with meals helps food flow through the stomach faster, which may alleviate symptoms of this form of indigestion [10]. Another research project conducted a clinical experiment in which peppermint oil and caraway oil administered as capsules demonstrated therapeutic effects similar to those seen with indigestion medication, providing relief from stomach discomfort and other digestive issues [11].

Some researchers have stated that plant essential oil can increase brain function; however, the accuracy of this claim has yet to be determined. In one of these researches, 144 adults who smelt peppermint oil scent five minutes before testing found that it boosted their memory considerably [12]. Another study discovered that sniffing peppermint oil while driving improved attention and reduce levels of frustration, anxiety, and exhaustion among study participants [13].

Influenza is one of the most common winter ailments. Most flu medications contain methanol, which is also present in peppermint oil. Hence, there are numerous studies indicating that mint can truly benefit patients with cold symptoms [14]. Flu medication can be used as a nasal decongestant, to remove congestion and improve airflow and breathing [15]. In addition, one recent study found that mint helped participants to overcome cold symptoms by making them appear to go away [16].

Mint essential oil has been widely utilized as an anti-bacterial agent. As is well known, more bacteria strains are developing resistance to drugs and because humanity is running out of options, scientists are turning to natural medicines like mint oil to treat these illnesses. In one study, volatile oil extracted from the leaves of Mentha piperita L. was used to treat multi drug resistance (MDR) strains from hospitalized patients [17]. The researchers discovered that the minimum inhibitory concentration

(MIC) was lower for *Staphylococcus aureus*, *Escherichia coli*, and *Proteus mirabilis* strains (20 mg/mL) and higher (40 mg/mL) for *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* strains. The anti-bacterial qualities of mint essential oil are thought to be derived from the components found in mint leaves, which are l-menthol, menthone, menthyl acetate, and limonene [18]. These components within the leaf make it a good candidate for use as an anti-bacterial, fungal, or viral treatment. More studies and investigations are now needed to determine the true potencies of these medicinal aromatic herbs. The aim of the present study is to determine the antifungal effect of the essential oil of *Mentha piperita*.

2. Methodology

2.1 Collection And Preparation Of Plant Essential Oil

The fresh mentha leaves were gathered in April 2022 from Chawg village, 4 kilometers from Halabja city in the Kurdistan Region of Iraq. The University of Sulaimani's College of Agriculture supplied voucher specimens that were used to identify the plant. Next, the leaves were dried and powdered using a low-temperature (28°C) drying method. The drying process took place over a period of two days and to minimize the loss of volatile compounds, the powdered leaves were sealed in an airtight container. After that, hydro distillation procedure was used to isolate the essential oil. In accordance with a documented protocol, 100g of powder was soaked in 350 ml of distilled water for 3 hours in a conical flask [19]. After filtering, the essential oil was subsequently collected.

2.2 Microbial Strains

The Mentha essential oils were tested against *Cryptococcus neoformans*, *Candida albicans* and *Trichophyton interdigitale*. These microorganisms were obtained from the Pasteur Institute of Iran in Tehran. The fungal strains were stored at 4°C.

2.3 Agar Well Diffusion Method

In December 2022, at Komar University of Science and Technology's Microbiology lab, Department of Medical Laboratory Science, antifungal activity against three different strains of fungi was investigated. Each fungus was suspended in 100 microliters of water and adjusted to the McFarland density standard of (0.7) [20]. Using sterilized cotton swabs, the suspensions were dispersed over potato dextrose agar, and 75 microliters of mint essential oil at one concentration, 1/4, were added directly to the wells using the agar well diffusion method. The essential oil was diluted with water prior to the experiment. The plates were then incubated at a temperature of 25°C for a duration of six days to allow for the diffusion of the essential oil components. After the incubation period, the diameter of the inhibitory zone (mm) surrounding the wells was measured as an indicator of antimicrobial activity [20].

3. Results

According to the findings, the Mint essential oil is highly sensitive to two of the three strains tested. In the study, the mint essential oil was shown to be most effective in preventing fungal growth. The effect of the essential oil differed based on the strain. Figure 1 shows that *Trichophyton interdigitale* had the smallest inhibitory zone, while *Cryptococcus neoformans* had the biggest, measuring 24mm for the 1/4 concentration, and *Candida albicans* had a medium sized inhibitory zone, measuring 17mm (Figures 2 & 3 and Table 1).

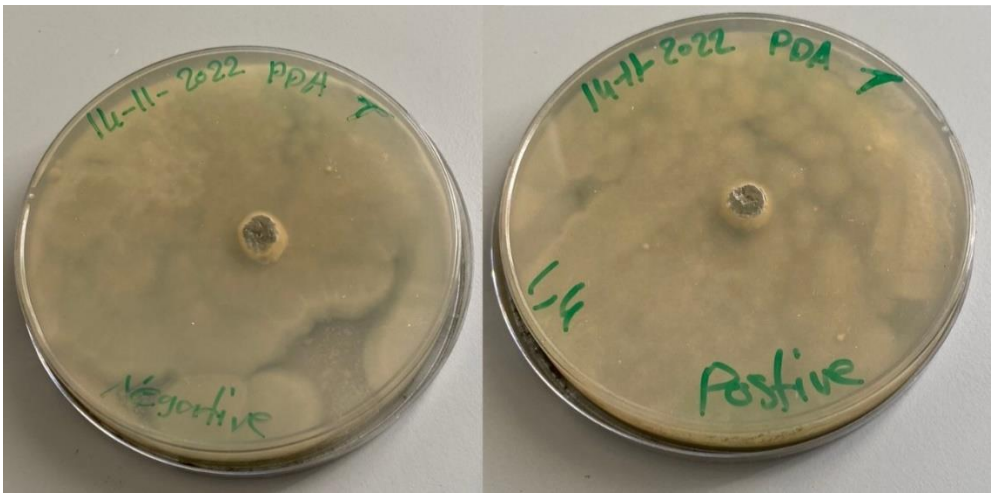

Figure 1: Shows anti-fungal activity of mint essential oil against *Trichophyton interdigitale*.

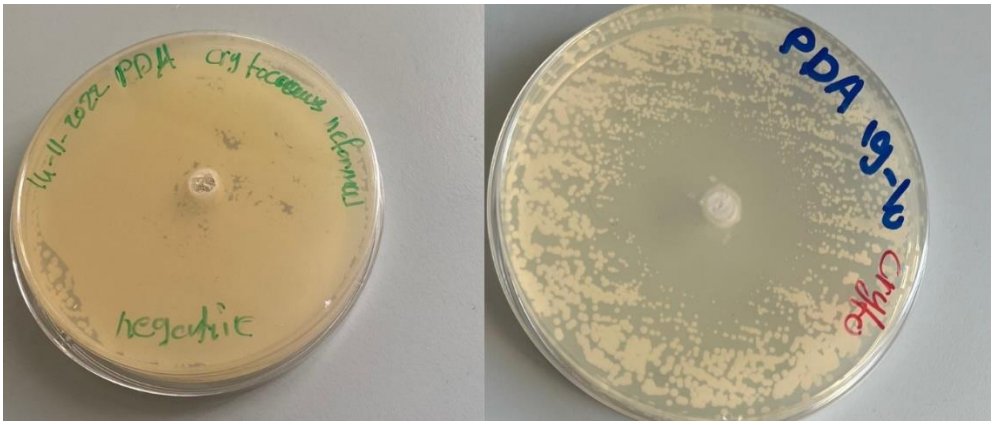

Figure 2: Shows anti-fungal activity of mint essential oil against *Cryptococcus neoformans***.**

Figure 3: Shows anti-fungal activity of mint essential oil against *Candida albicans.*

Table 1: shows the average of inhibition zone in (mm) for each strain of fungus

Fungus Name	Replicate 1 Inhibition zone (mm)	Replicate 2 Inhibition zone (mm)	Replicate 3 Inhibition zone (mm)	Mean ± Standard deviation
Cryptococcus neoformans	23	24	25	24 ± 1
Candida albicans	16.5	17.5	17	17 ± 0.5
Trichophyton interdigit	0	0	0	0 ± 0

4. Conclusion and Discussion

Essential oils from the Mentha leaves were successfully isolated by hydro distillation. The different types of essential oils are important natural materials. These are used in food production and in industrial applications. Essential oils of various species of Mentha may contain antibacterial and antifungal compounds which are important for preservation. The current study has demonstrated the antifungal activity of Mentha essential oil against several human fungal pathogens.

The data of this study showed that Mentha oil exhibited sufficient antifungal activity against *Cryptococcus neoformans* and *Candida albicans* but had no detectable effect on *Trichophyton interdigitale*. It is the first study in iraqi kurdistan that used mint eesential oil on these specius of fungi. In another study, some important plant extracts (mint, pomegranate and coriander) have been investigated on *Candida glabrata* specius and all three plant extracts showed antifungal properties [21]. In other research, the essential oil of pepperment showed significant antifungal activity against *Alternaria alternaria*, *Fusarium tabacinum*, *Penicillum spp.*, *Fusarium oxyporum*, and *Aspergillus fumigates* [22].

The previous studies showed that the essential oils of mint possess antimicrobial activity [23,24]. The Mentha oils have demonstrated a broad spectrum of effects against the fungal species [25]. In a different study, essential oils of various herbs including *Mentha spicata* and *Mentha piperita* were tested against microbial food poisoning and human pathogens, *M. spicata* oils contained carvone and menthone, these compounds showed antifungal activity even more powerful than that of commercial fungicide bifonazole [26]. It is concluded that the essential oils of Mentha have great antifungal potential against some of the fungal species and could be employed as natural fungicides and preservatives. This study provides an overview on the susceptibility of human fungi towards mint essential oils and their constituents. In another study, the effect of Mentha oil on *Salmonella enteritidis* and *Listeria monocytogenes* in a suitable medium was analyzed, Mint essential oil's antibacterial activity basically relied on its concentration, pH, composition, temperature and type of microbe [27]. In research by Devkatte and his coworkers in 2005, data on peppermint oil against *C. Albicans* showed an inhibition zone of approximately 18 mm [28]. Furthermore, Agarwal and his coworkers in 2010 found a 22.2 mm inhibition zone for Mentha oil against *C. Albicans* [29]. The well diffusion data obtained in the current study showed similar or slightly different results in comparison with the above studies. Thus, essential oils of Mentha might have potential antimicrobial characteristics.

In conclusion, the results of this study demonstrate the anti-fungal activity of Mint essential oil against selected fungal strains. Regarding the use of Mint essential oil as a natural and safe anti-fungal agent,

further investigation is needed to determine the mechanism of action of the essential oil and to evaluate its safety and efficacy in vivo.

5. Author's Contribution

"Authors confirm that the manuscript has been read and approved by all named authors. We also confirm that each author has the same contribution to the paper. We further confirm that the order of authors listed in the manuscript has been approved by all authors."

6. Conflict of Interest

"There is no conflict of interest for this paper"

7. Acknowledgments

The authors would like to thank Komar University of Science and Technology for their help to perform this study.

References

[1] K Kainz K, Bauer MA, Madeo F, Carmona-Gutierrez D. Fungal infections in humans: the silent crisis. Microbial Cell. 2020 Jun 6; 7(6): 143. https://doi.org/10.15698/mic2020.06.718

[2] Tafrihi M, Imran M, Tufail T, Gondal TA, Caruso G, Sharma S, Sharma R, Atanassova M, Atanassov L, Valere Tsouh Fokou P, Pezzani R. The wonderful activities of the genus Mentha: Not only antioxidant properties. Molecules. 2021 Feb 20; 26(4): 1118. https://doi.org/10.3390/molecules26041118

[3] Ngan NT, Flower B, Day JN. Treatment of Cryptococcal Meningitis: How Have We Got Here and Where are We Going?. Drugs. 2022 Aug; 82(12): 1237-49. https://doi.org/10.1007/s40265-022-01757-5

[4] Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, Reboli AC, Schuster MG, Vazquez JA, Walsh TJ, Zaoutis TE. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clinical Infectious Diseases. 2016 Feb 15; 62(4): e1-50. https://doi.org/10.1093/cid/civ933

[5] Brescini L, Fioriti S, Morroni G, Barchiesi F. Antifungal combinations in dermatophytes. Journal of Fungi. 2021 Sep 5; 7(9): 727. https://doi.org/10.3390/jof7090727

[6] Shan B, Cai YZ, Sun M, Corke H. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. Journal of agricultural and food chemistry. 2005 Oct 5; 53(20): 7749-59. https://doi.org/10.1021/jf051513y

[7] West KP, Christian P, Labrique AB, Rashid M, Shamim AA, Klemm RD, Massie AB, Mehra S, Schulze KJ, Ali H, Ullah B. Effects of vitamin A or beta carotene supplementation on pregnancy-related mortality and infant mortality in rural Bangladesh: a cluster randomized trial. Jama. 2011 May 18; 305(19): 1986-95. https://doi.org/10.1002/14651858.CD008666.pub3

[8] Khanna R, MacDonald JK, Levesque BG. Peppermint oil for the treatment of irritable bowel syndrome: a systematic review and meta-analysis. Journal of clinical gastroenterology. 2014 Jul 1; 48(6): 505-12. https://doi.org/10.1097/mcg.0b013e3182a88357

[9] Inamori M, Akiyama T, Akimoto K, Fujita K, Takahashi H, Yoneda M, Abe Y, Kubota K, Saito S, Ueno N, Nakajima A. Early effects of peppermint oil on gastric emptying: a

crossover study using a continuous real-time 13C breath test (BreathID system). Journal of gastroenterology. 2007 Jul 1; 42(7): 539. https://doi.org/10.1007/s00535-007-2067-3

[10] Madisch A, Heydenreich CJ, Wieland V, Hufnagel R, Hotz J. Treatment of functional dyspepsia with a fixed peppermint oil and caraway oil combination preparation as compared to cisapride. Arzneimittelforschung. 1999 Nov; 49(11): 925-32. https://doi.org/10.1055/s-0031-1300528

[11] Chumpitazi BP, Kearns GL, Shulman RJ. the physiological effects and safety of peppermint oil and its efficacy in irritable bowel syndrome and other functional disorders. Alimentary pharmacology & therapeutics. 2018 Mar; 47(6): 738-52. https://doi.org/10.1111/apt.14519

[12] Moss M, Hewitt S, Moss L, Wesnes K. Modulation of cognitive performance and mood by aromas of peppermint and ylang-ylang. International Journal of Neuroscience. 2008 Jan 1; 118(1): 59-77. https://doi.org/10.1080/00207450601042094

[13] Moss M, Ho J, Swinburne S, Turner A. Aroma of the essential oil of peppermint reduces aggressive driving behaviour in healthy adults. Human Psychopharmacology: Clinical and Experimental. 2023 Mar; 38(2): e2865. https://doi.org/10.1002/hup.2865

[14] Eccles R, Jawad MS, Morris SA. The effects of oral administration of (—)-menthol on nasal resistance to airflow and nasal sensation of airflow in subjects suffering from nasal congestion associated with the common cold. Journal of Pharmacy and Pharmacology. 1990 Sep; 42(9): 652-4. https://doi.org/10.1111/j.2042-7158.1990.tb06625.x

[15] Meamarbashi A. Instant effects of peppermint essential oil on the physiological parameters and exercise performance. Avicenna journal of phytomedicine. 2014 Jan; 4(1): 72. https://doi.org/10.22038/ajp.2014.1170

[16] Kenia P, Houghton T, Beardsmore C. Does inhaling menthol affect nasal patency or cough?. Pediatric pulmonology. 2008 Jun; 43(6): 532-7. https://doi.org/10.1002/ppul.20797

[17] Muntean D, Licker M, Alexa E, Popescu I, Jianu C, Buda V, Dehelean CA, Ghiulai R, Horhat F, Horhat D, Danciu C. Evaluation of essential oil obtained from Mentha× piperita L. against multidrug-resistant strains. Infection and Drug Resistance. 2019 Sep 13: 2905-14. https://doi.org/10.2147/IDR.S218141

[18] Liang R, Xu S, Shoemaker CF, Li Y, Zhong F, Huang Q. Physical and antimicrobial properties of peppermint oil nanoemulsions. Journal of agricultural and food chemistry. 2012 Aug 1; 60(30): 7548-55. https://doi.org/10.1021/jf301129k

[19] Azeez SH, Gaphor SM. Evaluation of antibacterial effect against Porphyromonas gingivalis and biocompatibility of essential oil extracted from the gum of Pistacia atlantica kurdica. BioMed research international. 2019 May 29; 2019. https://doi.org/10.1155/2019/9195361

[20] Magaldi S, Mata-Essayag S, De Capriles CH, Pérez C, Colella MT, Olaizola C, Ontiveros Y. Well diffusion for antifungal susceptibility testing. International journal of infectious diseases. 2004 Jan 1; 8(1): 39-45. https://doi.org/10.1016/j.ijid.2003.03.002

[21] Jayan L, Priyadharsini N, Ramya R, Rajkumar K. Evaluation of antifungal activity of mint, pomegranate and coriander on fluconazole-resistant Candida glabrata. Journal of oral and maxillofacial pathology: JOMFP. 2020 Sep; 24(3): 517. https://doi.org/10.4103/jomfp.JOMFP_355_19

[22] Desam NR, Al-Rajab AJ, Sharma M, Mylabathula MM, Gowkanapalli RR, Albratty M. Chemical constituents, in vitro antibacterial and antifungal activity of Mentha× Piperita

L.(peppermint) essential oils. Journal of King Saud University-Science. 2019 Oct 1; 31(4): 528-33. https://doi.org/10.1016/j.jksus.2017.07.013

[23] Ferdeş M, Ungureanu C. Antimicrobial activity of essential oils against four food-borne fungal strains. University Politehnica of Bucharest Scientific Bulletin. 2012; 74(2): 8. https://doi.org/10.2174/22127984076661506151121 53

[24] Haddouchi F, Chaouche TM, Zaouali Y, Ksouri R, Attou A, Benmansour A. Chemical composition and antimicrobial activity of the essential oils from four Ruta species growing in Algeria. Food chemistry. 2013 Nov 1; 141(1): 253-8. https://doi.org/10.1016/j.foodchem.2013.03.007

[25] Mickienė R, Ragažinskienė O, Bakutis B. Antimicrobial activity of Mentha arvensis L. and Zingiber officinale R. essential oils. biologija. 2011 Jul 1; 57(2). https://doi.org/10.6001/biologija.v57i2.1834

[26] İşcan G, Kirimer N, Kürkcüoğlu M, Başer HC, Demirci F. Antimicrobial screening of Mentha piperita essential oils. Journal of agricultural and food chemistry. 2002 Jul 3; 50(14): 3943-6. https://doi.org/10.1021/jf011476k

[27] Tassou CC, Drosinos EH, Nychas GJ. Effects of essential oil from mint (Mentha piperita) on Salmonella enteritidis and Listeria monocytogenes in model food systems at 4 and 10 C. Journal of Applied Bacteriology. 1995 Jun; 78(6): 593-600. https://doi.org/10.1111/j.1365-2672.1995.tb03104.x

[28] Devkatte AN, Zore GB, Karuppayil SM. Potential of plant oils as inhibitors of Candida albicans growth. FEMS yeast research. 2005 Jun 1; 5(9): 867-73. https://doi.org/10.1016/j.femsyr.2005.02.003

[29] Agarwal V, Lal P, Pruthi V. Effect of plant oils on Candida albicans. Journal of Microbiology, Immunology and Infection. 2010 Oct 1; 43(5): 447-51. https://doi.org/10.1016/s1684-1182(10)60069-2