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Antibacterial activity of cold and hot aqueous Extract of *Mentha piperita*

Asma M. Aloraibi [1*], Amna Ali Alhadad [2], Amal Fitouri Belgasim [3] , Amira Mosbah Issa [4]
[1,3,4] Department of Botany, Faculty of Sciences, University of Ajdabiya, Ajdabiya, Libya
[2] Department of Biology Faculty of Education, Bani Waleed University, Bani Walied, Libya
[*]Corresponding author: asma.elmhdi@uoa.edu.ly

النشاط المضاد للبكتيريا للمستخلص المائي البارد والساخن على نبات النعناع

أسماء امهدي العريبي [1*]، آمنه علي الحداد [2]، أمل فيتوري بالقاسم [3]، أميرة مصباح عيسى [4]
[1،3،4] قسم النبات، كلية العلوم، جامعة اجدابيا، اجدابيا، ليبيا
[2] قسم الأحياء، كلية التربية، جامعة بني وليد، بني وليد، ليبيا

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Abstract:

The present study investigates the antibacterial potential of Mentha piperita L. leaves (family Lamiaceae) aqueous extract against Staphylococcus, Pseudomonas, and Escherichia coli using the disc diffusion method. The goal was to evaluate the effectiveness of different concentrations (20% and 50%) of the extract compared to a control group (water). Both cold and hot aqueous extracts of Mentha piperita exhibited significant antibacterial activity against the tested bacterial strains. The highest inhibition zone for the cold aqueous extract was observed at concentrations of 20% and 50%, particularly against Pseudomonas (0.165 mm and 0.5 mm, respectively). In contrast, the hot aqueous extract showed the most pronounced antibacterial effect at a concentration of 20% against Staphylococcus (0.22 mm) and at 50% against Pseudomonas (0.11 mm). These findings suggest that the aqueous extract of Mentha piperita has notable antibacterial properties, with the highest activity observed against Pseudomonas at higher concentrations. The results indicate the potential of Mentha piperita as a natural antibacterial agent that could be further explored for medicinal applications. This study also emphasizes the need for additional research to explore the bioactive compounds in Mentha piperita and their mechanisms of action against bacterial pathogens. While promising, further investigations into its efficacy, safety, and potential as a therapeutic agent are necessary. Overall, the study contributes to the growing interest in plant-based antibacterial agents and their potential to combat antibiotic-resistant bacteria in the future, which is increasingly important for addressing public health concerns globally.

Keywords: Antibacterial activity, *Escherichia coli*, Mentha *piperita*, *Pseudomonas*.

الملخص :

تُعنى الدراسة الحالية بالتحقيق في الإمكانيات المضادة للبكتيريا لاستخلاص الأوراق المائية لنبتة النعناع الفلفلي *Mentha piperita* L. (التي تنتمي إلى عائلة الشفوية) ضد أنواع البكتيريا *Staphylococcus* ، *Pseudomonas*، *Escherichia coli* باستخدام طريقة الانتشار على الأطباق الهدف من الدراسة هو تقييم فعالية تركيزات مختلفة (20% و50%) من الاستخلاص مقارنة مع مجموعة التحكم (الماء). أظهرت كل من الاستخلاصات المائية الباردة والساخنة لنبتة النعناع نشاطًا مضادًا للبكتيريا بشكل ملحوظ ضد السلالات البكتيرية التي تم اختبارها. تم ملاحظة أعلى منطقة تثبيط للاستخلاص المائي البارد عند التركيزين 20% و50%، وخاصة ضد ب*sPseudomonas* (0.165 مم و0.5 مم على التوالي). بالمقابل، أظهر الاستخلاص المائي الساخن أكبر تأثير مضاد للبكتيريا عند التركيز 20% ضد *Staphylococcus* (0.22 مم) وعند 50% ضد ب*Pseudomonas* (0.11 مم). تشير هذه النتائج إلى أن الاستخلاص المائي لنبتة النعناع يمتلك خصائص مضادة للبكتيريا بارزة، حيث لوحظت أعلى فعالية ضد *Pseudomonas* عند التركيزات الأعلى. تشير النتائج إلى إمكانية استخدام *Mentha piperita* كعامل مضاد للبكتيريا طبيعي يمكن استكشافه بشكل أكبر للتطبيقات الطبية. كما تؤكد هذه الدراسة على الحاجة إلى مزيد من البحث لاستكشاف المركبات البيولوجية النشطة في *Mentha piperita* وآليات عملها ضد مسببات الأمراض البكتيرية. على الرغم من هذه النتائج المشجعة، فإن المزيد من التحقيقات حول فعاليته وسلامته وإمكاناته كعامل علاجي ضروري. بشكل عام، تساهم الدراسة في زيادة الاهتمام بالعوامل المضادة للبكتيريا المستخلصة من النباتات وإمكاناتها في مكافحة البكتيريا المقاومة للمضادات الحيوية في المستقبل، وهو أمر أصبح ذا أهمية متزايدة لمعالجة قضايا الصحة العامة عالميًا.

الكلمات المفتاحية: النشاط المضاد للبكتيريا، *Escherichia coli*، النعناع.

Introduction:

Numerous plants are used to heal various illnesses and ailments, and their usage in medicine dates back thousands of years and is being practiced today. antibacterial properties. Plants as new natural bacteriostat or bacteriocide agents using of antimicrobial agents derived from medicinal used for treatment of bacterial melena, ,joint pains and then 700 species ,among them at least 500 species have(2) toothpastes and also as tlavor in many medicines antimicrobial drugs is medicin plants which are used plants were helpful against many diseases,these plants relief

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of tuberculosis symptoms in western and easter essences which are available in aromatic plants are one of the valuable compounds having different therapeutic characteristics including antimicrobial effect increeasing the development of resistance to antibiotics has created the need for new antimicrobial agents, and medicinal plants have been found to be a good source. These plants have been in use in traditional medicine for centuries and have cured many diseases effectively (3). They, and their derivatives, may be able to bring about a new mode of treatment for resistant bacteria. With the increasing trend towards healthy eating, demand for natural, plant-based additives has been escalating. Among the most renowned families of medicinal plants is Lamiaceae, which is not only applied in traditional and mainstream medicine but also in food technology. The members of this family are spread throughout Europe, Asia, North America, and North Africa. Dried and fresh Mentha leaves are sought the most. (4) Peppermint (piperita) is a vital herb of the Lamiaceae family that has been regarded as one of the primary sources of antimicrobial compounds. (9) Peppermint is a native non-indigenous perennial herb that measures 100 mm (approximately 40 inches) in height, with a distinct four-sided stem. Its stalked, serrated leaves are opposite. Its flowers are of irregular form with pink and purple tints. Importantly, peppermint leaves contain approximately 0.5-4% volatile oil, which consists primarily of 50-78% free menthol, together with monoterpene, mentholfurane, and the presence of traces of jasmine (0.15%), which significantly enhances the quality of the oil (6) It is well suited for growth in full sun and tolerates acidic, neutral, or basic soils, but will also grow in heavy clay soils (7). It produces false spikes with small inconspicuous bracts with denticulate margins. The flowers are pink or purple. (7).

Mints have been used for hundreds of years globally and were introduced to India in 1952 from Japan. Corn mint plants exhibit prominent overground stems, bigger leaves, tiny flowers, and creeping underground rhizomes (8).

Mentha plants, having aromatic activity, are traditionally applied in traditional medicine and also in food preservation, showing effectiveness against bacteria, fungi, and yeasts. Peppermint is rich in menthol content and is widely utilized in teas, ice creams, confections, chewing gums, and toothpaste (9).

These flowering plants have stoloniferous, branched stems that may be purplish in color, and leaves that are short-petioled, oblong-ovate, and serrated. Leaves may also appear grayish in color (10). The peppermint yields a variety of compounds such as carotenes, tocopherols, betaine, and choline and exhibits a complex and variable profile. The flowers are typically purple or pink and predominantly comprised of menthol (29-48%) and mentholne (20%), though differing based on climate, cultivar, and region (11). Peppermint can yield 0.1-1% volatile oil, and a large majority of parts of Mentha species, including their extracts, are commonly utilized in aromatherapy, pharmacology, and nutrition. Mint's essential oils and extracts are employed as flavoring for most food products like cheese, chocolate, soft drinks, jellies, syrups, candies, and chewing gums in the food industry (12). Peppermint oil is calming in nature that propagates to topical use, serving as a counterirritant and analgesic, capable of alleviating pain and enhancing circulation to the affected area (6).

Mint phytochemicals can function as natural food preservatives by inhibiting spoilage microorganisms. Using mint extracts as additives can prolong product shelf-life and reduce the need for synthetic preservatives and flavorings. Peppermint oil and its constituents, such as flavonoids (12%) and polymerized polyphenols, are commercially used in food, pharmaceuticals, and cosmetics. Peppermint oil and menthol display moderate antibacterial effects against both gram-positive and gram-negative bacteria. They are commonly incorporated in breath fresheners, drinks, antiseptic mouth rinses, chewing gums, candies, and tobacco. Clinical trials have explored peppermint oil's roles in managing irritable bowel syndrome, and its moderate antibacterial and antioxidant activities have been noted. Peppermint is also considered for its effects against cancers, colds, and cramps (4). Both fresh and dried mint leaves are regularly used as a culinary source (7; 6).

Menthol and peppermint oil exhibit fungicidal activity against Candida albicans, Aspergillus albus, and dermatophytic fungi. However, there remains limited investigation into the antibacterial properties of *Mentha piperita* (6).

The present study established the antimicrobial activity of *Mentha piperita* against pathogenic bacteria. Both aqueous and organic solvent leaf extracts demonstrated strong antibacterial properties against various pathogens, including *Pseudomonas* aeruginosa, *Escherichia coli*, and *Staphylococcus* species.

Figure 1. *Mentha piperita*

Aim of this study:

- Studying the effect of cold and hot aqueous extracts of leaves of plant (mint) on the bacteria (-) to determine the extent of its sensitivity and resistance against these extracts.
- Antibiotics Sensitivity test of *Staphylococcus*, *E. coli* and *Pseudomonas*

Methods and Methods:

Bacterial isolates:

Mint leaves were washed thoroughly using tap water and air dried in the dark room at room temperature prior to powdering.

Preparation of leaf extract:

Mint leaves washed thoroughly in tap water and dried at dark room temperature the leaves were powdered. Extraction: Cold extract: a weight of 20 grams of dry powder was taken from the used parts of mint plants, and placed in a conical flask with a capacity of 1000 ml, and 100 ml of distilled water was added to it, after which the samples were left; To settle, then filtered with three layers of gauze cloth , To separate the large particles, then a final filtration was carried out using a centrifuge, for 15 minutes to separate the small particles and obtain a clear solution (1) then this clear solution was sterilized using cellulosic filters; To obtain a sterile solution, and thus the concentration of the filtrate is 20%. Hot extract: Take 20 grams of dry powder of the used parts, from mint plants, and put it in a conical flask with a capacity of 1000 ml, and add to 100 ml of boiled distilled water at 100° C . The samples were then left; In order to settle, it was filtered with three layers of gauze cloth to separate the large particles, then the final filtration was carried out using a centrifuge for 15 minutes to separate the small particles, and obtain a clear solution (1) then this clear solution was sterilized using filters Cellulosic, to be Obtaining a sterile solution, and Thus the concentration of the filtrate 20 %.(1).

Figure 2. Preparation of extracts

Testing the efficiency of aqueous extract (cold and hot) leaves of mint in inhibiting the growth of the bacteria Three colonies of bacteria pure culture were taken by sterile Loop and incubated to the tube containing 10 ml of normal saline. The tube shaked by rocking before 0.100 ml of bacterial culture was placed on the surface of the plate containing Nitrate agar and distributed by swab on the surface of the media The plates were left for 15 minutes to absorb the stuck in the culture medium. Three replicates were made for each isolate. Then discs of filter paper with a diameter of 6 mm were taken. Each group of discs was placed in a kind of extract and left for ten minutes to be imbibed with the immersed extract, then lifted and using forceps and distributed On the dishes grown with bacterial isolates, then dishes they were incubated at 37°C for 24 hours, then the results were recorded by measuring the diameters of the inhibition area in milliliters, and the method was repeated for all the isolates under study the plates were left for 15 minutes to absorb the stuck in the culture medium.

Figure 3. Antimicrobial activity of Mantha against bacteria

Antibiotic sensitivity test:
Three replicates were made for each isolate.Then discs of filter paper with a diameter of 6 mm were taken Each group of discs was placed in a kind of extract and left for ten minutes to be imbibed with the immersed extract,then lifted and using forceps and distributed on the dishes were incubated at 37C for 24 hours then the results were recordad by measuring the diameters of the inhibition zone in milliliters. Three colonies of bacteria pure culture were taken by sterile loop and incubated to the tube containing 10 ml normal sline thetube shaked by rocking before 0.100ml of bacterial culture was placed on the surface of the plate containing Mueller hington agar and distributed by swab on the surface of the media disks of antibiotics were placed on surface of the media spaced by distances and incubated in the incubator at 37c for 24-48 hours inhibit on zone diameter to each antibiotic disk were measured.

Results:
In Table 1 demonstrates that the 20% cold water extract of *Mentha piperita* leaves resulted in the largest zone of inhibition against various bacterial strains, including *Pseudomonas*. This table highlights the effectiveness of the cold aqueous extract in inhibiting bacterial growth, with the zone of inhibition being used as an indicator of the extract's antimicrobial properties. A larger zone of inhibition indicates a stronger antimicrobial effect, which suggests that the 20% cold water extract of *Mentha piperita* has potent antibacterial activity against these strains.

Table 1. The mean of inhibition zone of cold aqueous extract of mentha leaves (20%,50%) against *Staphylococcus pseudomonas* and *Escherichia coli*

Bacterial isolates	20%	50 %
Staphylococcus	0.077	0.17
Pseudomonas	0.165	0.5
E. coli	0.14	0.17

- At the 20% concentration, the extract exhibited moderate inhibition against all three bacterial strains, with the largest zone of inhibition observed for *Pseudomonas* (0.165 mm).
- At the 50% concentration, the extract showed enhanced antibacterial activity, especially against *Pseudomonas*, which demonstrated the widest zone of inhibition (0.5 mm).

Figures 4 and 5 visually compare the antimicrobial activity of the 20% and 50% cold aqueous extracts of *Mentha piperita* leaves. These figures illustrate the size of the inhibition zones and provide a clear visual representation of the differences in antibacterial activity at the two concentrations. The data suggest that increasing the concentration of the extract leads to stronger antibacterial effects, particularly against *Pseudomonas*.

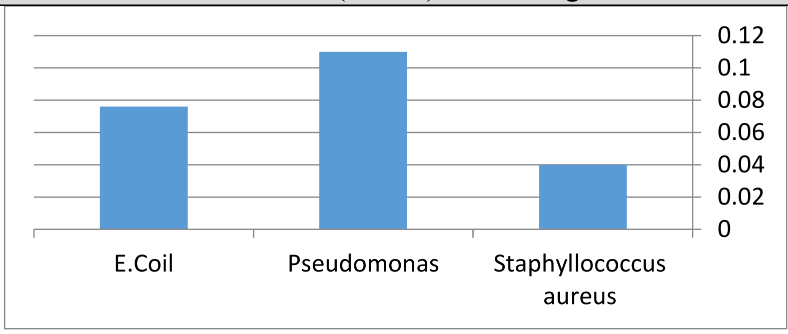

Figure 4. Comparison of antimicrobial activity of cold aqueous extract of Mentha leaves with concentration 20% against the bacteria.

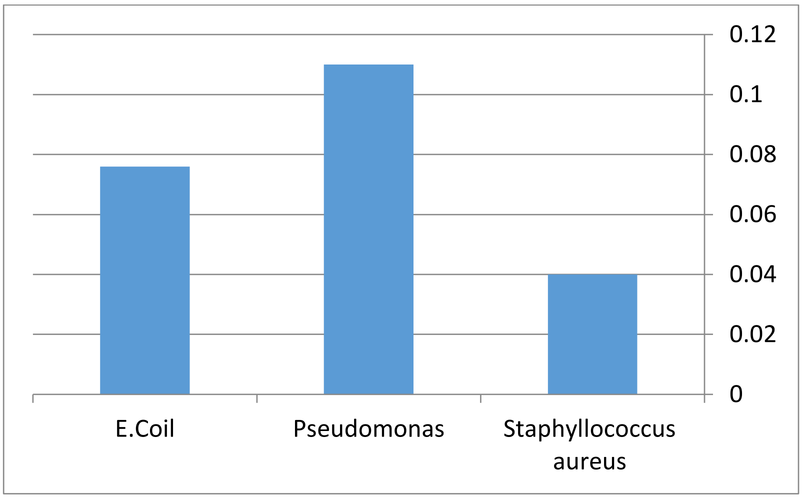

Figure 5. Comparison of antimicrobial activity of cold aqueous extract of menthe leaves with concentration 50%aganist the bacteria

Table 2. The average size of the inhibition zone from the hot aqueous extract of Mentha leaves.20%,50%) against *Staphylococcus*, *pseudomonas* and *Escherichia coli*

Bacterial isolates	20%	50 %
Staphylococcus	0.22	0.04
Pseudomonas	0.17	0.11
E. coli	0.037	0.076

- At the 20% concentration, the hot aqueous extract showed moderate inhibition against *Staphylococcus* (0.22 mm) and *Pseudomonas* (0.17 mm), but weak activity against E. coli (0.037 mm).
- At the 50% concentration, the antimicrobial effect decreased for all bacterial strains, with the smallest zones of inhibition observed, particularly for *Staphylococcus* (0.04 mm) and *E. coli* (0.076 mm). This suggests that the hot extract may not be as effective at higher concentrations, potentially due to the breakdown of antimicrobial compounds at elevated temperatures.

Next Figures 6 and 7 provide a visual comparison of the antimicrobial activity of the 20% and 50% hot aqueous extracts of *Mentha piperita* leaves. These figures highlight the reduced antimicrobial efficacy at the 50% concentration, especially for *Staphylococcus* and *E. coli*, emphasizing the potential impact of heat on the extract's effectiveness.

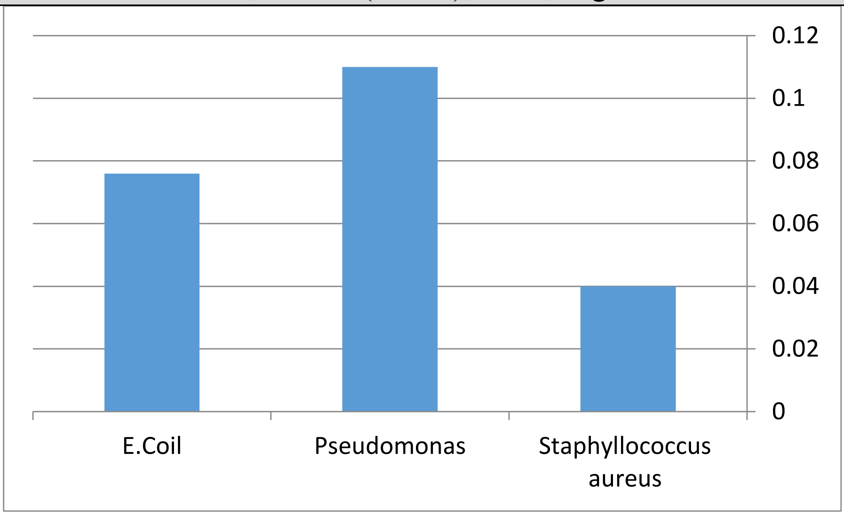

Figure 6. comparison of antimicrobial activity of hot aqueous extract of Mentha leaves with concentration 20%aganist the bacteria

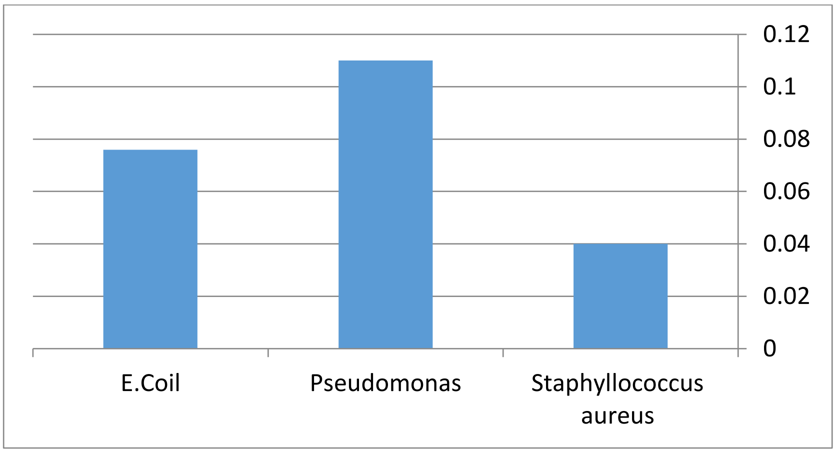

Figure 7. comparison of antimicrobial activity of hot aqueous extract of Mentha leaves with concentration 50%aganist the bacteria

Table 3. Antibiotic sensitivity test

Bacterial isolates	**Antibiotics**				
	Ampicillin	Gentamycin	Cefixime	Daptomycin	Ceftazidime
Staphylococcus	0.31	0.43	-	0.2	-
Pseudomonas	-	4.36	-	-	0.9
E. coli	0.21	2.28	0.65	0.43	0.36

- *Staphylococcus* was sensitive to Gentamycin (0.43 mm) and Ampicillin (0.31 mm), but less sensitive to Daptomycin (0.2 mm).
- *Pseudomonas* showed significant resistance to most antibiotics, with the largest zone of inhibition against Gentamycin (4.36 mm).
- *E. coli* demonstrated moderate sensitivity to Gentamycin (2.28 mm) and Ampicillin (0.21 mm), with a more moderate response to Cefixime (0.65 mm) and Daptomycin (0.43 mm).

Figures 8, 9, and 10 compare the inhibition zones of the cold and hot aqueous extracts of *Mentha piperita* leaves and antibiotics for *Staphylococcus*, *Pseudomonas*, and *Escherichia coli*. These figures provide a clear visual comparison, indicating the relative strength of the extracts versus antibiotics in inhibiting bacterial growth. They also illustrate how the extracts compare to standard antibiotic treatments.

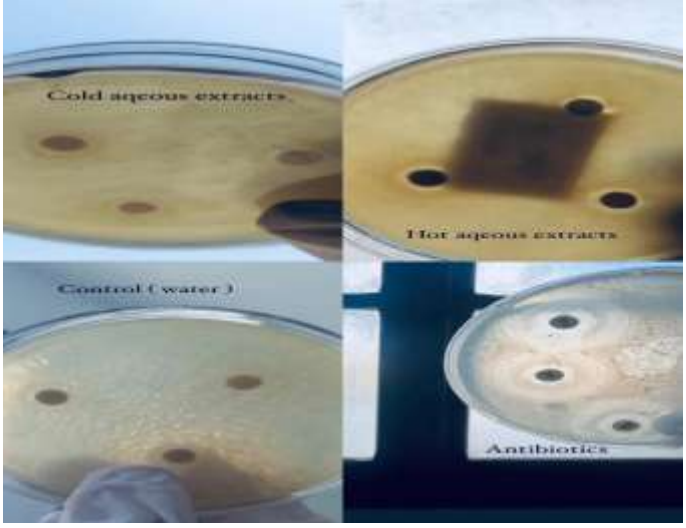

Figure 8. Inhibition zone of cold and hot aqueous extracts of Mentha and antibiotics on Staphylococcus comparison with control

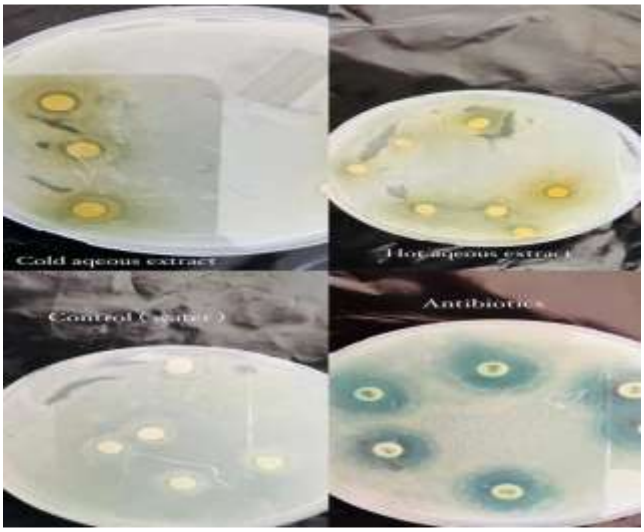

Figure 9. Inhibition zones of cold and hot aqueous extracts of Mentha and antibiotics on *Pseudomonas* comparison with control

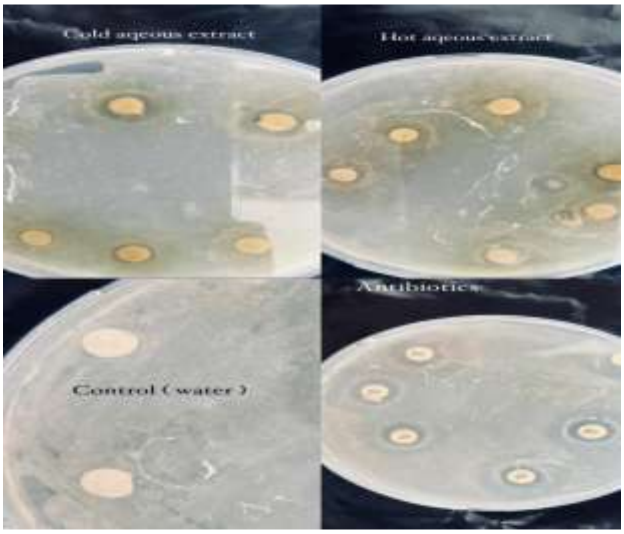

Figure 10. Inhibition zones of cold and hot aqueous extracts of Mentha and antibiotics on *E. coli comparison* with control**.**

Discussion:
The results of present study indicated that the mint extract has effect on the growth of *E. coli*, *Pseudomonas* sp and *Staphylococcus* sp.
The current study showed that the antimicrobial activity of mint has more effect on growth of (*Staphylococcus* sp) as compared to its effect on (E. coli. Pseudomonas sp) as shown in the figure (4,5,6,7) respectively Peppermint oil and menthol have moderate antibacterial effects against both gram-positive and gram-negative bacteria this study showed that used crude extract of mint (Mentha piperita) significantly affected against all bacteria tested .this is may be because of the leaf contains many potent compounds such as menthol mentholne,menthyl acetate (correct spelling),mentholfuran and,limnone.our results are in agreement with what was reported by deans and Baratta who stated that M.piperita has shown antibacterial activity against the pathogenic bacteria (12) the varying degree of sensitivity of the bacteria and the nature and combinations of phytocompounds present in the extracts as observed by(11).
Antibacterial activity of M. piperita oil and its various extracts were examined with the help of an agar diffusion method, in which we measured the growth inhibition zone diameter for various concentrations. The findings, as seen from Figs. 1 and 2 and described in Table 3, depict that the antimicrobial activity may be attributed to a high concentration of menthol present in the pet. ether and chloroform extracts, phenols or flavonoids in the chloroform and ethyl acetate extracts. In conclusion, the possible active chemical constituents of M. piperita can be an important source of drug for the elimination of bacterial infection. The results of this study are highly promising and underscore the need for further in-depth studies to further investigate the potential of the plant in the prevention of infectious diseases.
Moreover, leaves of the herb peppermint have various active compounds such as steroids, tannins, glycosides, saponins, soaps, and volatile nature oils, all of which contribute to its inhibitory activity.
Saponins can disrupt plasma membranes of bacterial cells, and tannins inhibit protein synthesis, exhibiting a high ability to inhibit bacterial growth. Our study of the activity of some Iraqi plants' volatile oils, like mint, revealed them to have very good properties in inhibiting the growth of microorganisms. Phenolic compounds are also significant in preventing the growth of bacteria by inhibiting enzymes that regulate crucial metabolic reactions, thus disrupting protein functioning and preventing the bacteria from surviving. (10)

Conclusion:
Their use in the cure of disease dates to 460-730 BC when Hippocrates utilized plant drugs in his practice. Among them is M. piperita, or peppermint, and contains a vast array of secondary metabolites including tannins, phenols, steroids, flavonoids, and volatile oils. These were reported to exhibit antimicrobial activity by in vitro studies. Our current research centered on assessing the antibacterial capacity of M. piperita extracts. The results indicate that the extract inhibited bacterial growth of strains such as *E. coli*, *Pseudomonas*, and *Staphylococcus* significantly. The antibacterial test reconfirmed that M. piperita extract was active against all the three strains, pointing to its potential as a source for novel antibacterial compounds.(12)
This research is a preliminary assessment of the antimicrobial potential of M. piperita, highlighting its ability to produce new metabolites. The antibacterial activities shown in plant extracts can possibly open the door to the discovery of new antibacterial medicines for medicinal use. Moreover, such extracts are also widely utilized for self-treatment by families (13).

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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