Effect of Mint extract on some pathogenic Bacteria

Assist Lec.Abdulqadr Khdir Hamad
Assist Lec Shawqi Muhammad Hassan,
Dr. Ahmed Farhan Shallal

Department of Biology
College of Science
Raparin University

پوختە

باکگراوەند : نە خۆشیە بەکتریایەکان بە تایبەتی نە خۆشیاکانی وەکو دلە کزیێ ، رشانەوەو، قورعە ی گەدەو کە لە نە خۆشخانە توشیان دەبین دەبنە هۆی کێشە لە بواری تەندروستی.

ئاما نج: ئامانجی ئەم توێژینە وە بۆ زانینی کاریگەری :پێکهاتەکانی نە عنا لە سە رگەشەی هە نـدێک بە کتریـای زیـان بە . (*Staphylococcus sp, Streptococcus sp,* *Escherichia coli* and *Pseudomonas* خشی وەکو

ڕێگاکا نی کـارکردن:پێکهاتەکـانی نـا و نە عنـا بە ڕێـگەی سـتاندەرد ئامـادەکرا. سـامپەلەکان وەرگیران لە نە خۆشخانەی رزگاری لە هەولێر.خەستی جیاواز لە پێکهاتەی نەعنا توێنرایەوە لە ناو ١٠ ملی ٩٨٪ ئیسانۆڵ وپاشان هەڵگیرا لە پلەی گەرمـی ژوور. کـاریگەری دژەمیکرۆبەکـانی ناو نە عنا دیـاری کـرا بە بەکارهێنـانی ڕێگەی دیسـک دیفیـوژن لە ڕێگەی پێوانی تیرەی زۆنە ڕوونەکە(ئە و زۆنە ئەوە پیشان دەدات بە کتریا گەشەی نەکردووە) بە دەوری دیسکەکە.

ئەنجـام: ئە نجـامی تـوێژینەوە کە ئەوە پیشـان دەدات کە پێکهاتەکـانی نـاو نەعنـاکە کـاریگەریـان هەبـووە لە سـەر گەشـەی بەکتریاکـان . نە عنـاکە کـاریگەری زیـاتری هە بـووە لە سـەرگرام پۆزەتیـف بەکتریاکـان بە بەراورد لە گەڵ گـرام نێگەتیـف بە کتریاکـان بە پشـت بە سـتن بە پێوانی تیرەی زۆنە ڕوونەکە.

دەرئەنجام:ئە م توێژینەوە ئە وە پیشان دەدات کە پێکهاتەکانی ناو نەعنا لە وا نەیە دەرفەتێکی زۆ باش دابین کات بۆ زیاتر بەکارهێنانی دژە تەنە سروشـتیە کـان و کە م کـردنەوی دژە تەنە کیمیاوی یەکان دژی نە خۆشیەکان.

ڕاسپاردەکان: ئەنجام دانـی تـوێژینەوەی زیـاتر لە سـەر ڕووەکە دەرمانیەکـان (نەعنـا) پیویسـتە بۆزیاتر ڕوون کردنەوەی کاریگەرەکانیان لە سـەر زیندەوەرە ووردەکان.

الخلاصة:
الهدف: لمعرفة مدى تأثيرمستخلص نبات النعناع (Mentha piperita) على نمو أنواع البكتريا المرضية التالية (Staphylococcus sp, Streptococcus sp, Escherichia coli and Pseudomonas sp)
المنهجية:حضر مستخلص نبات النعناع بطريقة قياسية. جُمعتْ العينات من مستشفى رزكاري في هولير. حضر أربع تراكيز مختلفة من نبات النعناع (0.5mg, 2mg, 3mg and 5 mg) وأذيبت في 10 مل من كحول الايثانول بتركيز(96%) وحفظ المستخلص بدرجة حرارة الغرفة لحين الأستخدام. قيست الفعالية المضادة لنمو البكتريا للمستخلص بطريقة(Mullar Hinton agar) وذلك بقياس قطر المنطقة المثبطة حول قرص الانتشار.
النتائج:أظهرت نتائج الدراسة الحالة بأنّ مستخلص نبات النعناع كان له تأثيرا واضحا على نمو البكتريا. واظهرت الدراسة معنوية عالية بالتأثيرللمستخلص على نمو البكتريا الموجبة لصبغة غرام(Staphylococcus sp. and Streptococcus sp) مقارنتا بالبكتريا السالبة لصبغة غرام (Escherichia coli and Pseudomonas sp) وذلك من خلال الأعتماد على قطر المنطقة المثبطة حول قرص الانتشار.
الأستنتاجات: أظهرت هذه الدراسة بأنّ مستخلص نبات النعناع ربما يوفر الفرصة الملائمة لزيادة استخدام الأنواع الطبيعية للمضادات الحيوية وتقليل استخدام للمضادات الحيوية الكيميائية.
التوصيات: الد راسات الإضافية ضرورية لتَوضيح تأثير النباتات الطبية (مثل النعناع) على انواع اخرى من الاحياء المجهرية.

الكلمات المفتاحية: مستخلص نبات النعناع، البكتريا الموجبة لصبغة غرام ، بالبكتريا السالبة لصبغة غرام

Abstract

Background: Bacterial infections are an emerging public health concern, especially in the nosocomial infections such as such as nausea, flatulence, vomiting, stomach cramps, and parasitosis.

Objective: To know the effect of mint extraction *(Mentha piperita)* on the growth of these pathogenic bacteria(*Staphylococcus sp, Streptococcus sp, Escherichia coli* and *Pseudomonas sp)*

Methods: The Mint extract was prepared by standard procedure. Four samples of bacteria were taken from Rzgary hospital in Hawler. Different concentrations (0.5mg, 2mg, 3mg and 5 mg) of mint were dissolved in (10 mL) of 96% of ethanol and kept a room

temperature. The anti-microbial activity of mint extraction was determined by using disc diffusion method (Mullar Hinton agar) by measuring the inhibition zone diameter.

Results: The results of current study showed that mint extract has effect on the bacterial growth. The study demonstrated that there was more significant effect on growth of gram positive bacteria (*Staphylococcus sp.* and *Streptococcus sp*.) than gram negative bacteria (*Escherichia coli* and *Pseudomonas sp*.) based on the inhibition zone diameter.

Conclusion: This study showed that mint extraction may provide an excellent opportunity to increase using natural types of antibiotics and decrease using chemical antibiotics.

Recommendation: Further studies are necessary to explain the effect of medical plants (Mint) on microorganisms.

Key words: Mint extract, Gram positive bacteria, Gram negative bacteria

Introduction

Higher and aromatics plants have traditionally been used in folk remedy, showing inhibition against bacteria and fungi [12.] *Mint (Mentha piperita)* is an aromatic perennial herb cultivated in most the parts of the world, which have traditionally been used in folk medicine. Mint are rich sources of iron and magnesium, which play important role in human nutrition [3] .The *extraction of* Mint (*Mentha piperita*) is a well-known as an herbal therapy used for a variety of bacterial infections such as nausea, flatulence, vomiting, indigestion, stomach cramps, menstrual cramps and parasitosis. Furthermore, mint has been documented to affect fungal infections [11].

The increasing resistance of microorganisms to chemicals and drugs has encouraged scientists to search for novel sources of biocides with broad-spectrum activities [4]. Leaves of mint plant are frequently used as an herbal tea and for culinary purpose to add flavour and aroma. The distinctive smell and flavor are considered as a characteristic feature of *Mentha* spp.

The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic agents [5] .The *antibacterial activities of M. piperita leaves extracts and essential oil against different bacterial strains have been reported [10] . In addition, mint is quite rich in phenolic compounds, and these have been appeared to have antioxidant activity* [6]. Many investigations have demonstrated the antimicrobial activity of the constituents of some higher plants that which appears the same effects of antibiotics this work gives some cheap and benefit treatments for some bacterial and fungal infections [9].

M. piperita extract shows antimicrobial activity against a variety of bacteria, including *Staphylococcus epidermidis, Pseudomonas syringae, Bacillus cereus* and *Escherichia coli*. It also shows positive effect against a fungi including *Alternaria alternate, Aspergillus niger,* and *Penicillium funiculosum* [7]. The aim of our study was to examine the antimicrobial activity of *M. piperita against Staphylococcus sp, Streptococcus sp*, *E. coli* and *Pseudomonas sp*

Material and method

The test microorganisms *Staphylococcus sp, Streptococcus sp*, *E. coli* and *Pseudomonas sp* were taken from Rzgary hospital in Hawler. Mint leaves washed thoroughly in tap water and dried at dark room temperature. The leaves were powdered and extracted by dissolving in 10 ml of %96 of ethanol and kept at room temperature. The crude extract of mint was dissolved at room temperature (28 ± 1 °C) in the corresponding solvent (10 mL) by simple solution techniques to prepare different stock solution (0.5mg, 2mg, 3mg and 5 mg) of mint extraction.

The organisms were inoculated onto Muller Hinton Agar, and incubated at 37 ºC for overnight. The sterile paper disks (5 mm diameter) were soaked with different extracts (0.5mg, 2mg, 3mg, 5mg) .Then the discs were placed on the surface of plates of microorganisms and incubated at 37 ºC.

Determination of antibacterial activity

The antimicrobial activity of leaf extract was determined by using disc diffusion method by measuring the inhibition zone diameter around disc of mint extract.

Statistical analysis

Statistical analysis was done by using one way of analysis of variance (ANOVA). For all analysis a value of ($p < 0.05$) was considered significant. All statistical analysis were perform of statistical packed for social science (SPSS) V20.

Result

The change in the inhibition zone of bacterial growth around mint discs are given in our tables (1,2,3,4) respectively and Fig 1. The (Table1) showed significant ($p< 0.05$) effect on *Escherichia coli* growth when used (*M. piperia)*. The result of (table 2) demonstrated significant effect of mint extract on the *Pseudomonas sp* growth. However, the table (3) displayed more significant effect of mint on *Streptococcus sp* compared to its effect on *E. coli* and *Pseudomonas sp*. The results of (table 4) illustrated that mint extract has more effect on *Staphylococcus sp* compared than*E. coli, Pseudomonas sp. and streptococcus sp*, respectively. The maximum activity against all pathogenic bacteria has been detected at 5 mg concentration of mint extract.

Table (1) shows the effect of mint extract on *Escherichia coli* growth

Mint extract(mg)	N	Zone of inhibition (mm) Means ±St. Error	Significant
0.5	3	7.1±0.4	0.007
2	3	8.3±1.6	
3	3	8.3±2.8	
5	3	8.4±2.5	

Table (2) shows the effect of mint extract on *Pseudomonas sp.* growth

Mint extract(mg)	N	Zone of inhibition (mm) Means ±St. Error	Significant
0.5	3	8.1 ±1.6	0.001
2	3	9.4 ±1.5	
3	3	9.6 ± 1.4	
5	3	10.09 ±2.3	

Table (3) shows the effect of mint extract on *Streptococcus sp* growth.

Mint extract(mg)	N	Zone of inhibition (mm) Means ±St. Error	Significant
0.5	3	9.4 ± 1.1	0.001
2	3	10.3 ±1.8	
3	3	10.6 ± 1.8	
5	3	11.05 ± 1.4	

Table (4) shows effect of mint extract on *Staphylococcus sp* growth.

Mint extract (mg)	N	Zone of inhibition (mm)Means ±St. Error	Significant
0.5	3	10.09 ± 1.5	0.001
2	3	11.1 ±1.8	
3	3	11.08 ± 1.4	
5	3	12.4 ± 0.2	

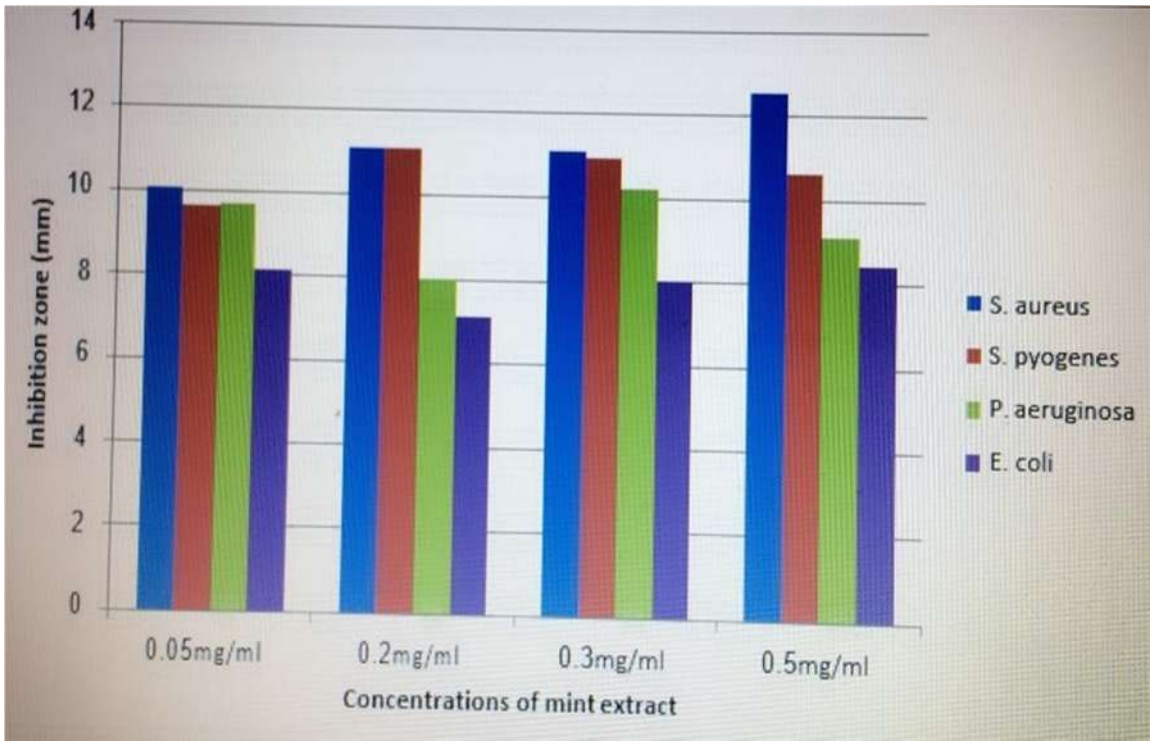

Fig1 .Antibacterial effect of various extracts of *M. piperita* on S.*aureus, S. pyogenes, E. coli and Pseudomonas aeroginosa.*

Discussion

The results of present study indicated that the mint extract has effect on the growth of *E.coli, Pseudomonas sp*, *Streptococcus sp* and *Staphylococcus sp.*

The current study showed that the antimicrobial activity of mint has more effect on growth of gram positive bacteria (*Staphylococcus sp.* and *Streptococcus sp)* as compared to its effect on gram negative bacteria (*E.coli, Pseudomonas sp)* as shown in the tables (1,2,3,4) respectively. Gram-negative bacteria are more resistant to mint extract than Gram-positive bacteria. This is may be due to the different structures of the cell walls of Gram-positive and Gram-negative bacteria. Approximately 90%–95% of the cell wall of Gram-positive bacteria consists of peptidoglycan, to which other molecules, such as teicoic acid and proteins. Peppermint oil and menthol have moderate antibacterial effects against both gram-positive and gram negative bacteria [8]. 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, menthone, menthyl acetate, menthofuran, and limnone[2]. 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[1]**.** Another study demonstrated that the mint extract showed moderate inhibition against *Bacillus subtilis, Pseudomonas aureus* and *Streptococcus aureus* but less inhibition against *Bacillus subtilis, Pseudomonas aureus* and *Serratia marcesens*

Conclusion

M. piperita extracts have a major antimicrobial activity against pathogenic bacteria, mostly on Gram positive bacteria (*Staphylococcus sp.* and *Streptococcus sp).* Mint extract with 5 mg concentration has the highest effect 12.4mm zone of inhibition *on Staphylococcus sp* growth. While same concentration has the minimum effect on *E.coli* 8.4 mm of zone inhibition. Due to the strong interest in natural drugs obtained from medicinal plants, the *M. piperita* extracts against these pathogens are recommended. The results from the present study are very encouraging and demonstrate that in the future, Mint should be studied more to explore its potential in the treatment of most bacterial infections.

References

1. Deans, SG and Baratta, MT (1998). Antimicrobial & Antioxidant properties some essential oils. *Flau.Fragrance* ; 235-44.

2. Hirobe, C.,et al . 1994: Screening for antitumour activity of crude drugs (IV): Studies on cytotoxic activity of Israeli medicinal plants. *Natural Medicine,* 48: 168-170.
3. Pramila, D., et al. (2012). "Phytochemical analysis and antimicrobial potential of methanolic leaf extract of peppermint (Mentha piperita: Lamiaceae). *J Med Plants* Res 6(2): 331-335.

4.Neeraj, T., et al. (2013). "Antimicrobial activity and medicinal values of essential oil of Mentha piperita L. *Int J Eng Innov Technol* 2: 214-218.

5.Harika, V. C., et al. (2010). "In Vitro Anti Microbial Activity of Leaf Powder." International Journal of Research in Pharmaceutical and Biomedical Sciences 1(2): 128-131.

6.Turkoglu, S. (2014). Assessment of wild mint from Tunceli as source of bioactive compounds, and its antioxidant Activity. *Cellular and molecular biology* (Noisy-le-Grand, France0 61(8): 63-68

7. Leila Farzaei., et al (2015). Bioactive Potential of Peppermint Extract Against Some Human Bacterial and Fungal Pathogens. *Avicenna J Clin Microb Infec* 2(4):e29693.

8. Saeed. S,A. Nain and P. Tariq(2006). In Vitro Antibacterial Activity Of Peppermint. *Pak. J. B ot*., 38(3): 869-872.

9.M.S.R.Al-Juboory(2008). Effect of some plant extracts on some pathogenic bacteria. *Diala Jour*

10. Mimica Dukic, N., Bozin, B., Sokovic, M., Mihailovic, B. Matavulj, M. 2003: Antimicrobial and antioxidant activities of three Mentha species essential oils. *Planta Medica*. 69: 413-419.

11.Škrinjar, M. M., et al. (2009). "Effect of Mint (Mentha piperita L.) and Caraway (Carum carvi L.) on the growth of some toxigenic Aspergillus species and Aflatoxin B1 production. *Zbornik Matice srpske za prirodne nauke* (116): 131-139.

12.Bupesh, G., Amutha, C., Nandagopal, S., Ganeshkumar, A., Sureshkumar, P. and Murali, K., 2007. Antibacterial activity of Mentha piperita L.(peppermint) from leaf extracts-a medicinal plant. *Acta Agriculturae Slovenica*, *89*(1), p.73