Antibacterial activity of the volatile oils from Mentha piperia L. and Vitex agnus-castus L on Pseudomonas aeruginosa isolated from burn injuries

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Abstract

This study was aimed to extract the volatile oils from two medicinal plants (*Mentha piperia,* and *Vitexagnus-castus*)and evaluation of biological activity against the bacteria *Pseudomonas aeruginosa* isolated from burn injuries that collected from patients in Al Hussein Hospital / Karbala .The present experimental in *vitro* study was carried out to evaluate the antibacterial activities of essential oils on the isolates was determined by disc diffusion method . Microdilution broth susceptibility assay was used in order to determine the MICs of essential oils. The essential oils of *M. piperia* had strong antimicrobial activity (inhibition zone22.5–10 mm) when 0.5µl and 0.25 µl where as *V. agnus castus* had moderate antibacterial activity (inhibition zone reached 12.5mm at 0.5 µl) .According to the results, essential oils exhibited moderate to strong antibacterial activity against the tested bacteria .

الفعالية ضد المايكروبية للزيوت الطيارة لنبات النعناع الفلفليM. piperita ونبات كف مريم V . agnus castus على بكتريا الزائفة الزنجارية المعزولة من خمج الحروق

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الكلمات المفتاحية : الحروق , الزائفة الزنجارية , نبات النعناع , نبات كف مريم .

الخلاصة

هدفت الدراسة استخلاص الزيوت الطيارة من نباتي النعناع وكف مريم ومن ثم تقييم الأثر التثبيطي ضد بكتريا الزائفة الزنجارية المعزولة من خمج الحروق من المرضى الراقدين في مستشفى الحسين (عليه السلام) في كربلاء. تم اختبار هذه العزلات وتحديد التركيز المثبط الادنى لزيت النعناع وزيت كف مريم . من خلال النتائج لوحظ ان تلك الزيوت اعطت فعالية ضد ميكروبية عالية الى متوسطة وقد بلغت اقطار التثبيط لزيت النعناع 22.5-10ملم عند تركيز 0.5 مايكروليتر في حين بلغت 12.5 ملم عند تركيز 0.5 مايكروليتر لزيت كف مريم . وعليه فان زيت النعناع يكون ذو تأثير اقوى من زيت كف مريم .

Introduction

The skin is the largest human organ, that's protect body against intruders such as heat, cold ,regulation body temperature and other. Also can provides body shape (1).Many of bacterial groups find in the surface of the skin ,like this microbe called Normal flora such as :*Diptheriods*, *Staphylococcus* spp. (coagulase negative), *Micrococcus* spp., *Bacillus*spp. & fungi(2,3).

Pseudomonas aeruginosa is an opportunistic pathogen causing severe, acute and chronic nosocomial infections in immunocompromised, catheterized or burn patients. The organism is generally resistant to numerous antimicrobial agents due to natural resistance in particular impermeability or mutations and acquisition of resistant determinants (4).
Medicinal plants have been used for centuries in traditional medicine because of their therapeutic value (5). Therefore use plant extract as drug to treat infection because it have active constituents (6),such as essential oils were widely used as antibacterial agent , have a strong active against *P.aeruginosa* like *Eucalyptus* spp. its content essential oils(7).*Mentha piperita*, is a plant found in many parts of the world which has an economical value for its flavoring, odor, and therapeutic properties in foods and cosmetic industrial products. In addition, the leaves and flowers of *M. piperita* have medicinal properties(5).The essential oil in the dried leaves of peppermint (2.5%) is mostly made up from menthol (50%), menthone (10 to 30%), menthyl esters (up to 10%) and further monoterpene derivatives (pulegone, piperitone, menthofurane) (8). Moreover ,menthol has bactericidal effects (9). *Vitexagnus-castus* L. is a perennialgrey shrub, with a strong aromatic odour. α-Pinene, β-Pinene, Limonene, Sabinene, 1,8-Cineole and Terpineol were identified as the principal constituents ofthe leaf oil(10) .

MATERIALS AND METHODS

Plant samples collection

The arial parts of plant samples including the leaves and buds of *M.piperia* and *V.agnus-castus*L. , were collected at Pre- flowering stage from their natural habitat in Iraq (Table 1). The samples were cleaned from impurities, such as contaminating plants, dust, and other pollutants. The collected plants were air dried and were cut to pieces

Table 1: Plants and their families, collection sites, and parts used

No	Scientific name	Plant family	Collection site	Part
1	*Menthapiperia*	Labiatae	Karbala	Leaves
2	*Vitecagnus-castus*	Verbenaceae	Karbala	Leaves

Essential Oils Extraction

The aerial parts of the *M. piperita* and *V. agnus- castus*were hydrodistillated for 2.5 h , using an all-glass Clevenger-type apparatus, according to the method (11). The sample oils were stored in sealed vials at 4◦C .

Minimum inhibitory Concentration (MIC):-

Under aseptic conditions, 96 well microtitre plates were used for Resazurin based Microtitre Dilution Assay. The first row of microtiter plate was filled with 100 µl of test materials in 10% (v/v) DMSO or sterile water. All the wells of microtitre plates were filled with 100 µl of nutrient broth. Two fold serial dilution (throughout the column) was achieved by starting transferring 100 µl test material from first row to the subsequent wells in the next row of the same column and so that each well has 100 µl of test material in serially descending concentrations. Finally, a volume of 10 µl was taken from bacterial suspension and then added to each well to achieve a final concentration of 5×10^6 CFU/ml. To avoid the dehydration of bacterial culture, each plate was

wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Each microtitre plate had a set of 3 controls: (a) a column with Tetracyclin as positive control, (b) a column with allsolutions with the exception of the test extract and (c) a column with all solutions except bacterial solution replaced by 10 µl of nutrient broth. The plates were incubated in temperature controlled incubator at 37° C for 24 h.At the end incubation period 10 µl of resazurin solution as indicator was added in each well . Then the plate was incubated for two hours. The colour change in the well was then observed visually.Any colour change observed from purple to pink or colourless was taken as positive. The lowest concentration of plant leaf extract at which colour change occurred was recorded as the MIC value. All the experiments above were performed in triplicates. The average values were calculated for the MIC of test material (12,13).

Disc diffusion method

The test isolates was grown in Muller-Hinton Broth (MHB, Merck) medium at 37°C for 18h. The bacterial number in the final inoculums was adjusted to 106 CFU/ml. A bacterial lawn was prepared by pouring 0.1 ml of bacterial suspension onto each plate of Muller-Hinton Agar medium (MHA, Himedia), spread by a sterile cotton swab, and allowed to remain in contact for 1 min. Two fold Serial dilution 1/2, 1/4,1/8 , to 1/256 of each essential oil were prepared in order to impregnate the paper discs. The sterile filter paper discs containing tested essential oils (6-mm diameter) were then placed on the bacterial lawn. The Petri dishes were subsequently incubated at 37°C for 24 h and the inhibition zone around each disc was measured in mm. As positive controls, discs tetracycline 0.2mgwere used (14,15).

Results and Disscusion

Bacterial isolation and characterization:

Clinical samples comprising of burn swabs from Patient in Al Hussein hospital ,they were cultured on blood agar and MacConkey agar .Colonies that were suspected to be *P. aeruginosa* were confirmed using biochemical tests and Api 20 E system .As well as a standard strain from the Central Public Health Laboratory Baghdad / Iraq , it's have symbol ATCC 27853 was obtained.

Antimicrobial activity

Minimum Inhibitory Concentration

On the basis of the primary screening results (Fig. 1), *M.piperia* essential oils showed a good antibacterial activity against *P.aeruginosa.* whereas, weak antibacterial activity was demonstrated by the essential oils *of V. agnus- castus*. Minimum inhibitory concentration for *V. agnus- castus* against *P. aeruginosa* isolates were shown in the figure (**1A**). The strains Pse-1,Pse-3, Pse-4 and Pse-5 ,were similar in their susceptible to those essential oil MIC 1/8 , but Pse-2 and S the standard were give a weak susceptible to oil 1/16 MIC. While the peppermint oils had strong activity shown in figure (**1B**)The isolates Pse-1 , Pse-2 and Pse-5 ,were similar in their susceptible to those essential oil MIC 1/64 ,but Pse-4 and Pse-3 were less resistant than another isolates , S is the standard strain high susceptible to essential oil.

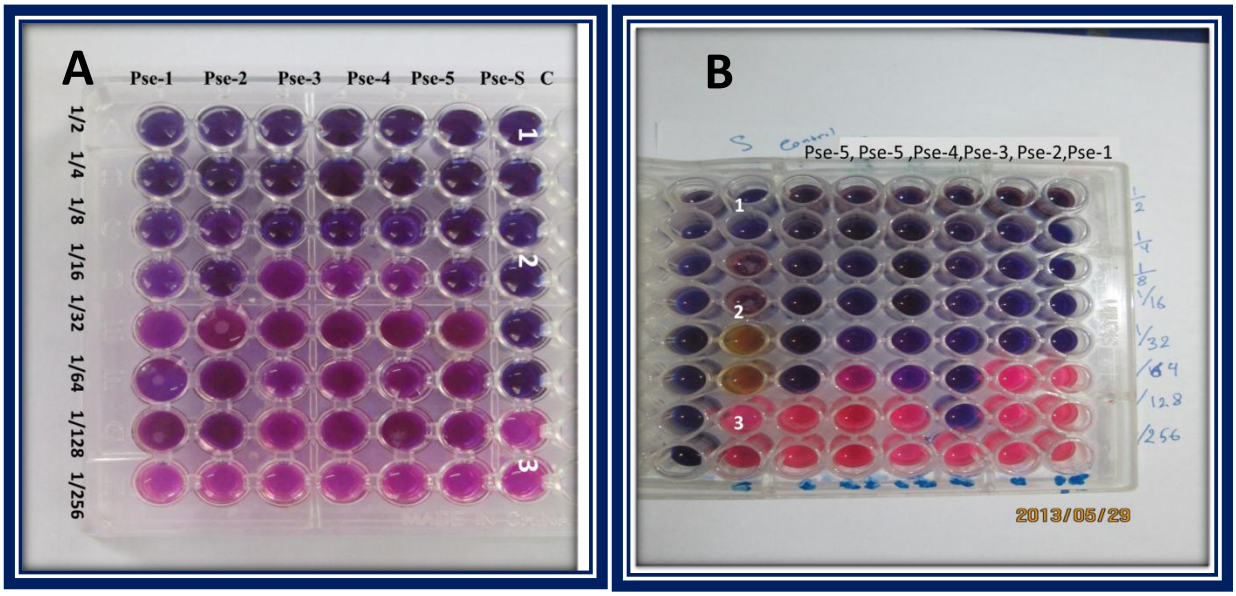

Figure (1): The result of MIC of *V. agnus- castus* (A) and *M. pipperia*(B): the wells that colored with blue it's susceptible to oils but wells pink color that resistant to oils .(Pse-1:*P.aeruginosa* 1,Pse-2: *P. aeruginosa,* Pse-3: *P. aeruginosa* 3 ,Pse-4: *P. aeruginosa* 4,Pse-5 : *P. aeruginosa5 ,S* is *Standard strain of P. aeruginosa*). C: control.

According to the results, essential oils exhibited moderate to strong antimicrobial activity against the tested bacteria (fig. 1). However, the essential oils of *M.piperita* show antimicrobial activity due to active compound in oils like phenols were gave strong action against bacteria .The mechanisms of action of plant essential oils has been increasing due to essential oils can break down the cell membrane and stop the synthesis of proteins and important biological processes in the cell, also change the pH levels inside and outside the bacterial cell. Have resulted in all these operations or one of which leading to death (16).

Disc diffusion method

The antimicrobial activities of *M. piperita* and *V. agnuscastus*essential oil against microorganisms were examined by the presence or absence of inhibition zones and zone diameter .As shown in Table 2, the essential oils of *M.* piperitahad strong antimicrobial activity (inhibition zone 22.5–10 mm) when 0.5 and 0.25µl were applied against all bacteria tested .Where as *V . agnus castus* had moderate antibacterial activity (inhibition zone reached 12.5mm at 0.5 µl) . Essential oils are potential sources of novel antimicrobial compounds, especially against bacterial pathogens. In vitro studies in this work showed that the essential oils inhibited bacterial growth but their effectiveness varied. The antimicrobial activity of many essential oils has been previously reviewed and classified as strong, medium or weak.

Conclusion

From the results of this study ,the wealth of medicinal plants are one of the vital resources having important bearing on human health and the region's economy .Both in *vitro* and in *vivo* works are needed to utilize the excellent antibacterial properties of these highly efficient and beneficial plant extracts. According to the results, essential oils exhibited moderate to strong antibacterial activity against the tested bacteria .

Table (2) rates of inhibition diameters (mm) of peppermint oil and *V. agnus castus* against *P. aeruginosa*

Symbol of strain / concentration µl/ml	Rates of inhibition diameters (mm) of *Vitex agnus castus* oil							Rates of inhibition diameters (mm) of *Mentha piperita* oil						
	Pse-s	Pse-5	Pse-4	Pse-3	Pse-2	Pse-1	Effect of concentration average	Pse-s	Pse-5	Pse-4	Pse-3	Pse-2	Pse-1	Effect of concentration average
0.5	6	12.5	11.5	10	8.5	9.75	9.70± 0.75 A	10.5	15.5	17.5	10.5	10.5	22.5	14.5± 0.5 A
0.25	5.5	10.5	11	10	7.5	9	8.91± 0.00 AB	10	14.5	16	10.5	10	21	13.66 ±3.0 AB
0.125	5.5	9.5	9	9.5	7	8.5	8.16± 0.50 AB	10	13.5	13	10	10	20	12.75 ±7.5 AB
0.0625	5.5	8	8.5	9	7	7.5	7.58± 0.50 ABC	9.5	10	11	9.5	9.5	17.5	11.25± 5.0 BC
0.0312	5	8	7.5	8.5	6.5	7.5	7.16± 0.50 BC	8	10	10	9	8.5	14	9.91± 1.0 AB
0.0156	3.5	5.5	7.5	4	4.5	7	5.33± 1.00 CD	8	10	9.5	8	7	11	8.91 ± 3.0 CD
0.0078	3.5	5.5	6.5	4	3	6	4.75± 0.00 D	3	9.5	9.5	7	6	8.5	7.25± 0.5 C
Cont.(-)	0	0	0	0	0	0	0.00± 0.00 E	0	0	0	0	0	0	0.00 ± 0.0 E
Tetracycline 0.2mg/ml (+)	10	0	9	8	9	7.5	7.25± 0.50 BC	15	0	10	8	9	9	8.5 ± 1 AB
The effect rate of isolates	4.94± 0.74 C	6.61± 1.26 ABC	7.83± 082 A	7.00± 0.92 AB	5.88± 0.82 BC	6.97± 0.66 AB		8.22± 1.11 C	9.27 ±1.43 BC	10.72± 1.27 B	8.05 ±0.77 C	7.83 ±0.79 C	13.72± 1.91 A	
Level of significance	Bacteria = P ≤ 0.05 Concentration= P ≤ 0.0001 Interference = P≤ 0.0001							Bacteria = P ≤ 0.0001 Concentration= P ≤ 0.0001 Interference = P≤ 0.001						
L.S.D	Bacteria= 1.854 Concentration =2.2707 Interference =5.5621							Bacteria= 2.1817 Concentration= 2.672 Interference= 6.5451						

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