Imperial Journal of Interdisciplinary Research (IJIR)
Vol-2, Issue-10, 2016
ISSN: 2454-1362, http://www.onlinejournal.in

In Vitro AntibiofilmAnd Antibacterial Activity Of *HyssopusOfficinalis* Extract And Its Oil Against *Pseudomonas Aeuroginosa*

Aseel Mohammed Hamzah
Zoonotic Disease Unit / Veterinary Medicine College / Baghdad University – Iraq

Abstract *:This study aimed to evaluate the antibacterial and antibiofilm activity of Hyssopus officinalis extract and its oil against Pseudomonas aeuroginosa.Hyssopus officinalis oil was obtained using hydro distillation whileHyssopus officinalis extract was obtained by an ethanolic extraction method and both extracts submitted to the antimicrobial assay by disc diffusion method and tube broth dilution assays (minimum inhibitory (MIC) and bactericidal (MBC) concentrations) against Pseudomonas aeuroginosa. Biofilms formed were examined using tube assay .*
The results exhibited significant activity against Pseudomonas aeuroginosabacteria with inhibition zone and minimal inhibitory concentration value in the range of 7–16 mm and 0.5–1 µl/ml, respectively.

Key words: *Hyssopus officinalis extract, Hyssopusofficinalisoil,Pseudomonas uroginosa,antibiofilm,antibacterial*

Introduction

In the last century the search for antimicrobial drugs from medical herbals and their essential oils has been of great interest, the infectious diseases are known to have been treated with texture derived from plants, however, antibiotic resistance has growing ultimately in the recent years and is discomposing an ever rising therapeutic trouble(1).

Pseudomonas aeruginosa is a periodic causative agent in healthcare associated infections (2) furthermore *P. aeruginosa* is the most common Gram-negative pathogen causing nosocomial pneumonia in Iraqi hospitals (3,4), and have beenassign to contamination of inanimate objects in the hospitalsetting and simplify by healthcare workers who may carry thispathogen via direct person-to-person contact Today, fullyactive antibiotic options available to treat nosocomial infectionsdue to multidrug-resistant (MDR) *P. aeruginosa* are extremelylimited (5,6), Furthermore, diverse resistance mechanisms were found in these MDR isolates.

Bacteria more resistant to manyantimicrobial agents when itprovides a biofilmon a surface than the same bacteria growing in afree - swimming state(7). The active ingredient isolated frommedicinal herbs have been studied for theirantibacterial effects against planktonic bacteria. Furthermore, some plants have been studied their ability to prevent the formation of biofilm in some pathogens such as *Listeria monocytogenes* (8) and *Pseudomonas aeruginosa* (9).

The aims of this study were to evaluate the antibacterial activity of *Hyssopus officinalis* plant extracts (crude and oil) as the minimal inhibitory concentration (MIC) by diffusion and dilution methods for *P. aeruginosa* and to study the antibiofilm activity of *Hyssopus officinalis* against *P. aeruginosa* .

Material and method

1. Preparation of *Hyssopus officinalis* extracts

The plants were purchased from markets in Baghdad.Grinding the plant and the powdered plant material (250 g) was extracted in a 1000 ml conical flask with 500ml solvents(ehanol,w:v ,1:2) for 9 days in freeze after that filtered using Whattman No 4 filter paper. The filtrate obtained was concentrated by evaporated to dryness to obtain the crude extract. The crude extracts were kept at 4°C until further uses.

2.Preparation of *Hyssopus officinalis* oil by Steam distillation

Thirty gram of the plant put in a pressure cooker and add water about three litter then closed the pressure cooker and we put the Gasoline transfer hose on Slot steam out fixed it with dough until dry, after that closed the pressure cookerand put cooker on fire then put the rest of Gasoline transfer hose on acontainor fulled with water to allow condence the steam in Gasoline transfer hose and collected the oil at the end of hose in sterile bottle glass, the crude extracts were kept at 4°C until further uses.

2. Organism and growth conditions

Microorganism wasmedical isolate collectedfrom the culture collections of the zoonotic diseases unit/veterinary medicine college at The University of Baghdad. *Pseudomonas aeruginosa* was maintained on brain heart infusion broth Overnight culture was prepared by inoculating approximately 2 ml Mueller Hinton broth (MHB) (Unipath) with 2–3 colonies of organism taken from BA. Broths were incubated overnight at 35 °C. Inocula were prepared by diluting overnight cultures in saline to approximately 10^8 cfu ml for bacteria.

3.Assay for antibacterial activity

a. Disk diffusion method

For the disk diffusion assay (10) 1 mL of each bacterial suspension was adjusted to 0.5 McFarland turbidity standards and inoculated (0.2 ml each) onto Mueller Hinton agar (MHA, Oxoid) plates (diameter: 15 cm) then uniformly spread on a solid growth medium in a Petri dish. A sterilized stainless steel borer was then used to make four wells (6 mm diameter) for different concentrations of the *Hyssopus officinalis* extract were separately redissolved in sterile distilled water at concentrations of 20, 10, 5 and 2.5 mg/ml and 0.5 ml of each of the extracts was then introduced into the wells . Plate cultured with *P.aeruginosa* after that were incubated for 24 h under appropriate cultivation conditions, antibacterial activity was determined by measurement of diameter zones of inhibition (mm) (against the test organisms) around the extracts (11).

3.Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MICs) of plant extracts were determined using Mueller–Hinton broth tube dilution(12). The *Hyssopus officinalis* were determined by diluting the various concentrations (50, 20, 10, 5, 2.5 and 1.25mg/ml) of theextract. MIC determination was performed by a serial dilution technique using a six sterile glass test tube. Plant extract (100 µL) of each concentrate was placed into the test tube and (100 µL) muller hinton broth was mixed in the test tube. Then, 100 µL of 0.5 McFarland turbidity standard 10^8 cfu/ml bacterial cell suspensions were placed in each tubes after that the tubes were incubated for 24 h at 37 C while the control tube was containing the growth muller hinton medium, and the inoculums (organism control) after incubation, after incubation put (50 µL) of tetrazolium salts as indicators, since bacteria convert them to colored formazan derivatives that can be quantified. The lowest concentrations without visible growth completely inhibited the bacteria (MICs). The assay was repeated twice with three replicates per assay.

4. Minimum Bactericidal Concentration (MBC)

The MBCs were determined by first selecting tube that showed no growth during MIC determination and a loop ful from each tube was subculturing the test dilution on to a fresh brain heart agar plate and incubating further for 18-24 h. The highest dilution that yielded no single bacterial colony on a solid medium was taken as MBC.

6.The effect of *Hyssopus officinalis*extract on the bacterial biofilm formation

Biofilm formation was assessed in glasses sterile test tube seven appropriate concentrations (20,10,5,2.5,1.25,0.625,0.312 and 0.156 mg/ml) of extract were prepared from a serial two-fold dilutions method in muller hinton medium, 1ml of each concentration was transferred into a sterile glass tube and then 1ml of the 0.5 McFarland turbidity standard was added to each tube. All the tubes were incubated for 24 hours at 37°C, one control tube containing bacteria + muller hinton broth (negative control).

7. Assessment of biofilm biomass

Cell adherence was indirectly assessed by the modified crystal violet (CV) assay (13). The crystal violet assay is based on the dye penetrates and stains cell membranes of attached cells thereby providing information on the density of the attached cells (14), after that the absorbed stain is quantified by de-staining with ethanol then reading the absorbance on spectrophotometer. The amount of stain absorbed is directly proportional to the amount of biofilm on the surface so the higher the absorbance reading indicated for the greater biomass , The procedure was washing the glass tube after incubation, three times with sterile distilled water to remove loosely associated cells, after that glass tube were air-dried and then oven-dried at 60 C^o for 45 min then the glass tubes were stained with 1mll of 1% crystal violet and incubated at room temperature for 15 min then the glass tubes were washed 5 times with sterile distilled water to remove unabsorbed stain after that the semi-quantitative assessment of biofilm formation was performed by adding 1ml of ethanol to destain the tubes after which 1ml of the destaining solution was then transferred to a new tube and the absorbance determined at 590 nm using a spectrophotometer.

Results

1. Antimicrobial Activities of the *Hyssopus officinalis* and its oil

The present research work investigated the antimicrobial activity of crude extract of*Hyssopus officinalis* and its oil were evaluated according to their zone of inhibition against *P.aeruginosa*and the results (zone of inhibition) . The results revealed that the oil extract is potent antimicrobials against *P.aeruginosa*. Among the different concentrations extract studied 50 and 20 mg/ml showed a high degree of inhibition followed by 10 mg/ml concentrated extract, Oil and ethanolic extract at 50mg/ml show maximum inhibition zone diameter obtained against*P. aeruginosa* with diameter 20.5±0.32 mm and 18.3±0.22 respectively (Table 1; Fig.1 and Fig.2).

Table 1: Antimicrobial activity (zone of inhibition, mm) of plant extract *Hyssopus officinalis* and its oil against *P.aeruginosa*.

Extract	**Zone of inhibition of *P.aeruginosa***			
	50 mg/ml	20 mg/ml	10 mg/ml	5 mg/ml
Oil extract	20.5±0.32	18.5±0.25	13±0.25	-
Ethanolic extract	18.3±0.22	15±0.15	10±0.05	-

Values are mean of triplicate readings (mean ± S.D)

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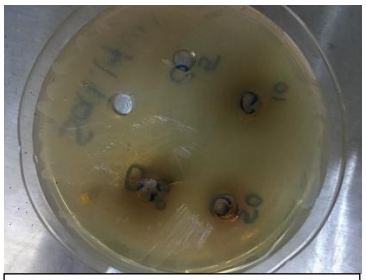

Fig. 1: Antimicrobial activity of ethanolic extract against

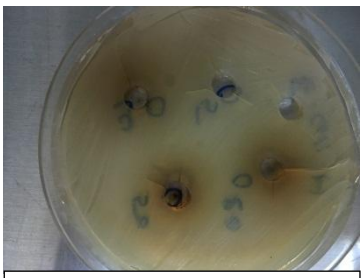

Fig. 2: Antimicrobial activity of oil extract against *P.aeruginosa*

2. Determination of MIC, MBC values

The antimicrobial activity of *Hyssopus officinalis* extract and its oil studies their potency were quantitatively assessed by determining the MIC and MBC, respectively, as given in Table 2.

The MIC of ethanolic extract and its oil against *P.aeruginosa* had been shown in Figure 3and Figure4. From the data in Figure 3and 4, it was clarify that oil extract showed higher minimum value of MIC than extract for 1.25 mg/ml extract was observed visualize using 0.5 ml(0.04mg/ml) of *p*-iodonitrotetrazolium violet(trazolium salt)((INT) after that the tubes were examined for color change and the MIC was indicated by the first clear tube that not changed to red color when compared with the control tubes or none inhibited concentrations.

Table 2. MIC and MBC values for crude extract of *Cassia acutifolia* against three microorganisms

Microorganisms	**Antimicrobial activity of Ethanolic extract**		**Antimicrobial activity of Oil extract**	
	MIC mg/ml	**MBC mg/ml**	**MIC mg/ml**	**MBC mg/ml**
p.aeruginosa	2.5	5	1.25	1.25

Fig.3. The minimum inhibition concentration of oil extract against *P. aeruginosa*

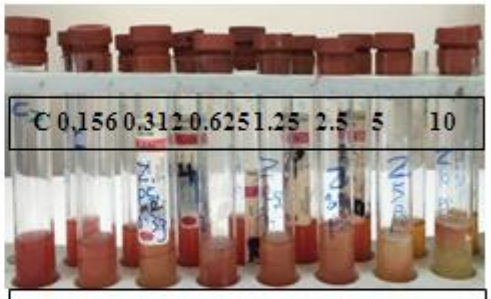

Fig.4. The minimum inhibition concentration of ethanolic extract against *P. aeruginosa*

3. Effect of ethanolic and oil extract on bacterial biofilm

Biofilm inhibition studies carried out using oil and ethanolic extract and at all the tested concentration have successfully inhibited biofilm formation of *p.aeruginosa* as dose dependent manner.

biofilm inhibition percentage was observed in the following order as oil > ethanolic extract against *p.aeruginosa* at the lowest concentration of 1.25 mg/ml.

Fig.5 and Fig.6 validated that the oil and methanolic extract have promising antibiofilm effect against *p.aeruginosa.*

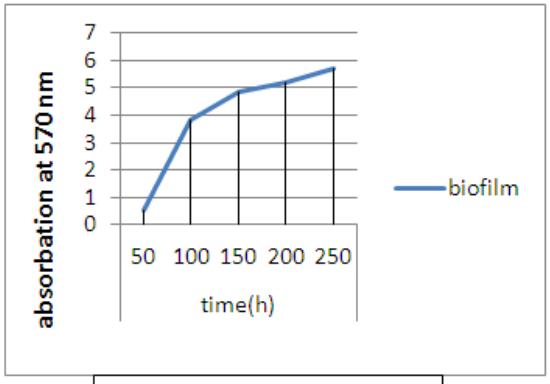

Fig.5 biofilm of oil extract

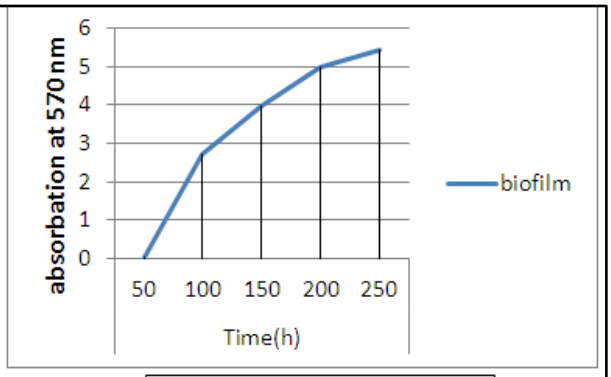

Fig.6 biofilm of ethanolic

Discussion

on account of the increase of the resistance of most microbial infections to conventional therapy, researchers have been encourage to identify alternates for the treatment of diseases. Herbal extracts and other biologically active compounds isolated from herbs have acquiredglobally interest in this consideration as they havebeen known to recovaryinfections and illness since past times (15,16). Inthe present study, anti biofilm effect of plant extracts against*P.aeruginosa*has been studied adopting biofilm inhibitionspectrophotometric assay, the oil of plant more potent and effect on biofilm bacteria formation than ethanolic extract . All the plant extracts (oil and ethanolic) tested inhibitedbiofilm as dose dependent manner therefore the high concentrate of both oil and extract were very effective against biofilm formation as well as low concentrated untile 1.25 mg/ml. pathogen in biofilm have been become moreresistant to antibiotics than their planktonic form (17),The natural compounds of plant play an important role in inhibiting cellattachment due to promising tool for reducing microbialcolonization on various surfaces (18). Application ofanti-adhesion agents seem to be a very interestingapproach in the prevention of microbial diseases(19,20).

Based on our present results, oil and ethanolicextract exhibited a antibacterial effect against *P.aeruginosa* with low MICs values.

This study revealed significant differences in displaying the antimicrobialpotential for the herbal oil and ethanolic extract tested. All the herbal extract manifestedantibacterial properties gainst *P.aeruginosa* but the level of bacterial growth inhibition induced by plantconcentration, as determined by the disc diffusion assay, proved to be dependentmostly on herbal type of extract and on extract concentrated.By using disc method we observed that oil determined differentinhibition zones on *P.aeruginosa*. also possessing the lowest MIC and MBCs values.Comparison of theethanolicextract.Plant antimicrobial agent depend on the methods of extraction.

References

1. (2014) Antimicrobial resistance: global report on surveillance. World Health Organization.
2. Chatzinikolaou I, Abi-Said D, Bodey GP, Rolston KV, Tarrand JJ, Samonis G: Recent experience with Pseudomonas aeruginosa bacteremia in patients with cancer: Retrospective analysis of 245 episodes. Arch Intern Med. 2000, 160: 501-509.
3. Mohammed JA, Inam JL, Aseel MH. Bacterial isolation from burn wound infections and studying their antimicrobial susceptibility. Kufa Journal For Veterinary Medical Sciences., 2011; 2(1): 121-131.
4. Hamzah,AM,AL-Rajehi,SH,AL-Zubaidy,IA.plasmid isolation from Pseudomonas aeruginosa that isolated from burning patient. Intern J Agri Sci Res.2013,3(3):185-188.
5. Gales AC, Jones RN, Turnidge J, Rennie R, Ramphal R. Characterization of *Pseudomonas aeruginosa*isolates: occurrence rates, antimicrobial susceptibility patterns, and molecular typing in the global SENTRY Antimicrobial Surveillance Program, 1997–1999. Clin. Infect. Dis. 2001;32(Suppl. 2):S146–S155.
6. Tam VH, Chang KT, Abdelraouf K, et al. Prevalence, resistance mechanisms, and susceptibility of multidrug-resistant bloodstream isolates of *Pseudomonas aeruginosa*. Antimicrob.Agents Chemother.2010;54(3): 1160–1164.
7. Donlan RM and Costerton JW. Review biofilms survival mechanisms of clinically relevant microorganisms. *Clin Microbial Rev*. 2002; 15: 167-193.
8. Sandasi M, Leonard CM and Vilioen AM. The effect of five common essential oil components on *Listeria monocytogenes* biofilms. *Food Control.* 2008; 19(11): 1070-1075.
9. Adonizio A, Kong KF and Mathee KI. Inhibition of quorum sensing – controlled virulence factor production in *Pseudomonas aeruginosa* by south Florida plants extracts. *Antimicrobial Agents and chemotherapy*. 2008; 52(1): 198-203.
10. Quinn PJ, Carter ME, Markey B, and Carter CR.(2006). Clinical Veterinary Microbiology.M.Wolfe.London.
11. Opara, Ansa. 1993. Antimicrobial activity of plant Mukia maderaspatuna. Int. J. Pharm. Pharma. Sci., vol. 5: 200–202.
12. Hamzah AM. Antibacterial of *Cassia acutifolia*extract against some bacteria and anti-biofilm formation activities against *Pseudomonas aeruginosa*.BEST J.,vol.4(8).
13. Djordjevic, D., Wiedmann, M., & Mclandsborough, L.A. 2002. Microtitre plate assay for assessment of *Listeria monocytogenes* biofilm formation. *Applied andEnvironmental Microbiology*, 68:2950–2958.
14. Mendez, C., Garza, E., Gulati, P., Morris, P.A. and Allen, C.A. 2005. Isolation and identification of micro-organisms in JSC Mars-1 simulant soil. *Lunar and Planetary Science*, XXXVI:1–2.
15. Tamilvanan S, Venkateshan N, Ludwig A. The potential of lipidand polymer-based drug delivery carriers for eradicatingbiofilm consortia on device-related nosocomial infections. JControl Release. 2008;128:2-22.
16. Zhang XB, Zou CL, Duan YX, Wu F, Li G. Activity-guided isolationand modification of juglone from Juglansregia as a potentcytotoxic agent against lung cancer cell lines.BMC Complementary Altern Med 2015;15:396.
17. Lewis K: Riddle of biofilm resistance. Antimicrob Agents Chemother 2001,45:999-1007.
18. Bavington C, Page C: Stopping bacterial adhesion: a novel approach totreating infections. Respiration 2005, 72:335-344.
19. Ofek I, Hasty DL, Sharon N: Anti-adhesion therapy of bacterial diseases:prospects and problems. FEMS Immunol Med Microbiol 2003, 38:181-191.
20. Steinberg D, Feldman M, Ofek I, Weiss EI: Effect of a high-molecularweight component of cranberry on constituents of dental biofilm.J Antimicrob Chemother 2004, 54:86-89