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Antimicrobial Activity of Methanol Extract of Microalgae (Hapalosiphon *welweschii*a) Against Some Pathogenic Bacteria

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Abstract:

The present study targets three bacterial species namely, *Listeria sp.*, *Escherichia. coli* and *Pseudomonas. aerugenosa* that. This creates health issues for women and rarely for men, through the use of natural products extracted from the microalgae *Haplosiphonwelweschii* secondary metabolites for the second time, in Iraq. High activity was clarified by the extract of methanol against three bacterial species recording, the ratio of 100% sensitivity for all in comparison with three types of antibiotics namely Ceftriaxone (30 µg) Amikacin(30µg) and Gentamicin (10 µg). It was found that the *listeria* resistant to Ceftriaxone while sensitive to the other (Amikacin and Gentamicin) and bacteria *E. coli* also resistant to Ceftriaxone and Amikacin while 20% sensitive to gentamicin the laste bacteria *P. aeruginosa*is resistant to Ceftriaxone and 20% is sensitive to Amikacin and 10% to Gentamicin. This study confirmed that plant extract more effect against several bacterial is adds compared with usage antibiotic

Key words: methanol extract, resistances antibiotic, Bacteria

Introduction:

Infections triggered by drug-resistant, bacteria were not a medical issue until the early 1980s due to the multiplicity of antibiotics. However, the development of the selective pressure exerted by the resistance bacteria was considerably accelerated by over-prescribing medicines clinical practice. because of the notable bacteria, ability to develop resistance to every antibiotic, we can anticipate that even bacteria species like Listeria *sp*, *Escherichia. coli* and Pseudomonas. *aerugenosa* which are still considered to be susceptible to almost all antibiotics. The enhanced use of antibiotics in animals and humans for therapeutic reasons has resulted to the evolution of antibiotic resistance, a significant concern for public health (1)

The Listeria genus involves several species of which one, *Listeria monocytogenes*, is the only human pathogen (15 presently identified). *L. Monocytogenes*, an optional intracellular pathogen, can trigger serious foodborne illness (listeriosis) in people at danger of pregnant females and their fetuses, elderly people and patients with immunocompromise. (2) *Outbreaksand* Sporadic instances of listeriosis involve multiple contaminated foods such as milk, raw meat, cheese, meat products, fish and vegetables (3) Generally species of *Listeria* are prone to a broad spectrum of antimicrobials, but the first, multi-resistant *L. monocytogenes* was isolated in 1988 (4). Since this year, antibiotic-resistant *L. monocytogenes* isolates have been recovered from food, environment and human listeriosis cases (4)

Nosocomial or hospital-acquired, infections are, defined as infections, which are acquired, during the hospital stay (5). In developing nations, as well as developed countries, nosocomial infections are also significant public health issues (6). Urinary tract infection is the most, common type of nosocomial infection (UTI)

surgical-wound infection, pneumonia, blood stream infection (BSI) (7,8). Urinary tract infections (UTIs) are serious health affecting problems world wide. *Escherichia. coli*, *Enterococcus. faecalis, Serratia. Marcescens, Pseudomonas. aeruginosa, Klebsiella. pneumoniae, Staphylococcus. aurous, Staphylococcus. saprophyticus,* and *Proteus mirabilis* are the most frequent bacteria that cause UTIs in human beings. (9,10).

Escherichia coli is, the most prevalent cause infections in human urinary tract and is a major cause of systemic and enteric diseases (11)

Bacteremia, nosocomial pneumonia, cholecystitis and infectious arthritis are included in systemic disease s. Also *E coli* is a major cause of neonatal meningitis (12).

A wide scope, antimicrobial specialists adequately repress development of *E. coli*, the β-lactams, aminoglycosides, fluoroquinolones and sulfamethoxazole trimethoprim- are regularly used to treat network and medical clinic contaminations related of *E. coli* (13). Investigations clinical of antimicrobial treatment and theoutcome of patients tainted with carbapenemase, -creating *E. coli* contrasted and patients contaminated with helpless strains are limited and propose more terrible clinical results for patients with diseases because of resistant isolates (14).

Pseudomonas aeruginosa resistant to high concentration of salts and dyes, weak antiseptics and commonly used antibiotics (15). *Pseudomonas aeruginosa* is an opportunistic human pathogen (16). Although it is not generally considered as the causing of urinary tract infection several cases of UTI probably caused by *Pseudomonas aeruginosa* (17).

Most *Pseudomonas aeruginosa*, are resistant, especially in hospital-acquired infections, to antibiotics used in the therapy of UTIs primarily in patients with immunocompromise (18). Antimicrobial resistance is a natural widespread phenomenon (19)

Aim of study:

Due to unwanted side effects connected with this classic drug, such as resistance growth, therefore present study focused on activity of natural, products from algae Microalgae (Hapalosiphon *welweschii*a) as antibacterial because of their accessibility and use in traditional, medicine in particular

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Materials and Methods:

Sample collection:

Listeria species were collected from the laboratory of the Bnt-ALhuda hospital in Nassiriyah Government /Iraq, from woman vagina. *E. coli and Pseudomonas aeruginosa* samples collected from persons infected with urinary tract infections in the microbiology laboratory of Bnt-ALhuda hospital Nassiriyah Government /Iraq during the period March 2017 to May 2018.

Isolation and Identification:

After samples were purified on different growth media, bacteria were identified on basis of morphological and culture characteristics like morphology of colony, color of colony and colony characters like elevation, surface, density and biochemical tests (21). The process of isolation and identification was carried out in laboratory of the pharmacy fluctulity, department of microbiology, pharmacy collage in University of Thai-Qar, Nassiriyah province.

Biological activity:

According to the standard operational procedures, biological activity **test**, were done on Mueller-Hinton-agar (Oxoid, Hampshire, England,) using Kirby Bauer diffusion disk method (22).

The biological agents tested were: gentamicin (10 ug), ceftriaxone (30ug) and Amikacin (30ug) (Oxoid, England). The antimicrobial susceptibility test has been done by diffusion disk method according to Bauer *et al*. (1966) as the following:

Three to five well isolated colonies were suspended in an infusion broth of 5 ml of brain core, incubated for 8 h at 37 C. In comparison with the standards 0.5 McFarland (equal to 1.5 X108 cell / ml), the turbidity of the increasing culture was adapted

A sterile cotton swab was immersed in the adapted suspension and rotated several times strongly on the tu be's inner wall above the broth stage to remove surplus inoculum from the swab. The entire surface of a Mueller – Hinton agar plate has been streaked with the dipping swab. The streaking has been repeated more times and the plate was rotated approximately 60˚each time to ensure from the distribution of inoculum. As a final step the rim of the Mueller – Hinton agar has been swabbed. Inoculated Mueller – Hinton agar Was permitted to dry at room temperature for 15- 20 minutes before antimicrobial disks were applied. The antimicrobial disks were placed on the surface of the inoculated agar plate and each disk was carefull y pushed to guarantee to ensure compete, contact with, the agar surface by using sterilized, forceps. The plates were then inverted for overnight in an incubator at 37 C. Using the ruler transparent, the diameter of growth inhibition areas has evaluated. The Clinical and Laboratory Standard Institute criteria (23) determined the sensitivity and strength.

Preparation of Plant extracts

Methanol extract preparation of microalgae was followed the way to get the extract from *Hapalosiphon welweschii* based on (20)

Studying the effect of plant extracts on the pathogenic bacteria:

The well diffusion method was applied according, to (24) for assessing the activity of the of plant extracts concentration 750 mg /1ml. Standardized bacterial suspensions stock (1.5×10^{8})cell/ml of *L. monocytogenes* , *P. aeruginosa* and *E. coli* thoroughly mixed to each 25 ml of sterile Muller- Hinton - agar for each plate ,this inoculated Muller - Hinton- agar divided into sterile petri dishes, well, 6 mm in diameter was trimmed using a sterile Pasteur pipette and the agar disk was separated afterwards wells filed with 0.1ml of each concentration of 750mg/ml of plant extract use microtiter pipette to spread for two hours at room temperature. Then the plates were incubated upright position at 37c for 24 hours. Each concentration of the antibiotics and the activity of these plant extracts was determined by evaluating the inhibition area diameter (millimeter) against the tested or ganism around each well.

Result:

The results of bacterial isolation showed that *listeria* sp. found in 10 samples (100%) of cases from woman. *P. aureganosa* and *E. coli* were isolated from 20 (100%) samples isolated from same hospital. The results of bacterial isolation were presented in table, 1.

Table (1) The presence of *Listeria sp.*, *E. coli* and *P. aeruginosa* in collected clinical samples

Bacteria	Sample	No. Sample	Positive %
Listeria	Vagina discharge	10	100%
E. coli and	Urine	20	100%
P. aeruginosa			
Total		30	100

The susceptibility of isolated *listeria* from women, Pseudomonas *aeruginosa and E. coli* from urine to antibiotics is shown in table 2. The highly susceptible *Listeria* isolates susceptible to Gentamicine and Amikacin while resistances to Ceftriaxon Pseudomonas *aeruginosa* is used Amikacin and Gentamicin while resistances to ceftriaxone, while *E. coli* isolates resistance to all antibiotics. These results for the sensitivity of bacteria isolation to the antibiotic either in relation to the effect of plant extracts on the types of isolated bacteria isolation observe all bacteria highly susceptible to plant extracts.

Table 2. Antimicrobial susceptibility test of some antibiotics and methanol extract of microalgae against *listeria sp.*

listeria sp. strain No.	methanol extract	Gentamicin (10µg)	Amikacin (30µg)	Ceftriaxone (30µg)
NO1	**S**	**S**	**S**	R
NO2	**S**	**S**	**S**	R
NO3	**S**	**S**	**S**	R
NO4	**S**	**S**	**S**	R
NO5	**S**	**S**	**S**	R
NO6	**S**	**S**	**S**	R
NO7	**S**	**S**	**S**	R
NO8	**S**	**S**	**S**	R
NO9	**S**	**S**	**S**	R
NO10	**S**	**S**	**S**	R
TOTAL	**100%**	**100%**	**100%**	0%

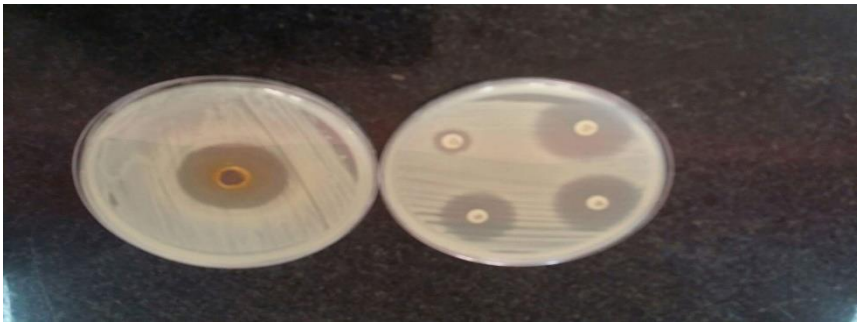

Figure 1. The Susceptibility of Listeria sp. toward (A) plant extract showing high sensitivity, (B) antibiotics showing resistance and sensitivity.

Table 3. Antimicrobial susceptibility test of some antibiotics and methanol extract of microalgae against E. *coli.*

E.coli isolates No.	methanol extract	Gentamicin (10µg)	Amikacin (30µg)	Ceftriaxone (30µg)
NO1	**S**	**R**	**R**	**R**
NO2	**S**	**S**	**R**	**R**
NO3	**S**	**R**	**R**	**R**
NO4	**S**	**S**	**R**	**R**
NO5	**S**	**R**	**R**	**R**
NO6	**S**	**R**	**R**	**R**
NO7	**S**	**R**	**R**	**R**
NO8	**S**	**R**	**R**	**R**
NO9	**S**	**R**	**R**	**R**
NO10	**S**	**R**	**R**	**R**
TOTAL	**100%**	**20%**	**0%**	**0%**

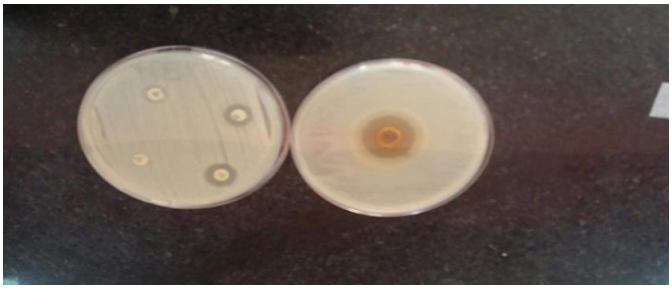

Figure 2. The Susceptibility of E. *coli* toward (A) antibiotics showing resistance and sensitivity; (B) plant extract showing high sensitivity.

Table 3. Antimicrobial susceptibility test of some antibiotics and methanol extract of microalgae against Pseudomonas *aeruginosa.*

P. aeruginosa Strain No.	Methanol extract	Gentamicin (10µg)	Amikacin (30µg)	Ceftriaxone (30µg)
NO1	**S**	**S**	**S**	**R**
NO2	**S**	**S**	**S**	**R**
NO3	**S**	**S**	**R**	**R**
NO4	**S**	**S**	**R**	**R**
NO5	**S**	**R**	**S**	**R**
NO6	**S**	**S**	**R**	**R**
NO7	**S**	**S**	**R**	**R**
NO8	**S**	**S**	**S**	**R**
NO9	**S**	**S**	**S**	**R**
NO10	**S**	**S**	**S**	**R**
TOTAL	**100%**	**10%**	**20%**	**0%**

Figure 3. The Susceptibility of P. *areugenosa* toward (A) plant extract showing high sensitivity, (B) antibiotics showing sensitivity.

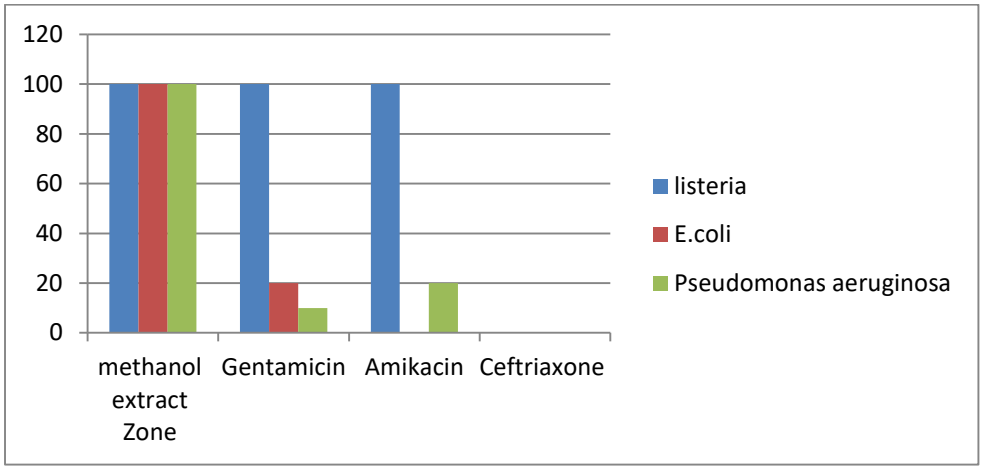

Figure 4: Graphical representation between the methanol plant extract, Gentamicin, Amikacin and Ceftriaxone on the in vitro growth of bacteria.

(0-14) Resistances bacterial to extract and antibioitic
(14-20) Sensivitybacterial to extract and antibioitic

Discussion:

Genitourinary tract infections or diseases of the reproductive tract are a significant issue of the sexual health of women. They are commonly seen in reproductive-age women and are usually found with vaginal discharge. The The World Health Organization (WHO) estimates that 80% of the world's residents rely for primary health care primarily on traditional medicine (25). Once methanol
extract levels. In this research, welweschii were used and it had many bacteria (*Listeria, E. coli* and Pseudomonas *aeruginosa*) and time has played a major part in therapy since the decline. It is difficult to speculate the mechanism by which these bioactive, compounds, act as bactericidal.

Where we note in this study that the types of bacteria isolated sensitive to the phytosanitary and high in order to compare with the types of antibiotics, where they used three antibiotics and found that the bacteria isolated from uterine fluids (*listeria* spp were sensitive to Amikacin and Gentamicin 100% and it is resistances Ceftriaxone100% while *E. coli* 20% sensevite to Gentamicin and resisranes to Amikacin and Ceftriaxone. *Pseudomonas aeruginosa.* 10% sensevite to Gentamicin and 20% to Amikacin but resisranes to Ceftriaxone. That is why we can replace the use of plant extracts or alcoholic instead of antibiotics where the bacteria found in this study found 100% sensitivity to the extract compared to the type of antibiotic 0% of the three types of bacteria.

References:

1. **Poyart-Salmeron, CCarlier, C Trieu-Cuot, P Courvlain, PAL, Courtieu, P.1990.Transferable** plasmid –mediated antibiotic resistance in listeria monocytogenes. Lancet; 335:1424-1426.

2. **Azizoglu,R.O and Kathariou,S.** 2016.*Listeria*: Properties and OccurrencesEncyclopedia of Food and Health ,Pages 567-570

3. **Gomez, D., Azon, E., Marco, N., Carraminana, J. J., Rota, C., Arino, A., and Yangüela, J**. 2014.Antimicrobial resistance of Listeria monocytogenes and Listeria innocua from meat products and meat-processing environment. Food Microbiol. 42, 61-65.

4. **Zhang, Y., Yeha, E., Hallb, G., Cripeb, J., Bhagwatc, A. A., and Menget, J.** 2007. Characterization of Listeria monocytogenes isolated from retail foods. J. Food Microbiol. 113, 47-53.

5. **Ajantha. G.S., *et al*.2011** Urinary tract infection in a tertiary care hospital with special reference to Methicillin Resistant S. *aureus*(MRSA). *Ind J of Research and Reports in Medical Sciences*. 1(1), 22-26.

6. **EhinmiduJ.O.,** 2003. Antibiotics susceptibility patterns of urine bacterial isolates in Zaria, Nigeria. *Trop J Pharm Res*, 2 (2) 223-28.

7. **Alka N, Priti S, Shanta SN .**2012. Bacterial pathogens in urinary tract infection and antibiotic susceptibility pattern. *J Pharm Biomed Sci*, 21 (12).

8. **Joseph GangouéPiéboji*, Sinata and Koulla-Shiro, Pierre Ngassam, DieudonnéAdiogo, Thomas Njine, Peter Ndumbe.**2004**. Antimicrobial** resistance of Gram-negative bacilli isolates from inpatients and outpatients at Yaounde Central Hospital, Cameroon. *International Journal of Infectious Diseases.* 8, 147-54.

9. **Manikandan S, Ganesapandian S, Singh M, Kumaraguru AK.**2011**. Antimicrobial** Susceptibility Pattern of Urinary Tract Infection
Causing Human Pathogenic Bacteria. *Asian J Med Sci*.3(2):56-60.

10. **Afzal S, Naemullah S**.2008. Spectrum of the Microorganisms in Children with Urinary Tract Infection. J Rawalpindi Med Coll; 12:44-46.

11.**Sarac¸li, M.A., Baysallar, M., and Gun**, **H.**1999 “Nosocomial Uropathogens and their antibiotic susceptibilities in a Turkish Military Hospital: a prospective and microbiological study,” *Turkish Journal of Medical Sciences*, 29(2): 165–168.

12. Celik, I., Inci, N., Denk, A., Sevim, E., Yasar, D. and M. Yasar, M.,2005 "Prevalence of Hospital acquired infections in Anesthesiology intensive care unit," *Fırat Tıp Dergisi*, 10(3): 132– 135.

13. Eckburg, PB., Bik, EM., Bernstein, CN., Purdom, E., Dethlefsen., L .2005. Diversity of the human intestinal microbial flora. Science 308: 1635- 1638.

14. Schultsz, C. Gearlings., S .2012. Plasmid-mediated resistance in Enterobacteriaceae: changes landscape and implications for therapy.
Drugs 72: 1-16.

15.Todar, Kenneth .2004. Todar, s Online Textbook of Bacteriology. " The Good, the Bad, and the Deadly ". (SCIENCE Magazine – June 4, Vol 304: p 1421.

16.Vianelli, N.; Giannini, M.B. & Quartic, C.2006. Resolution of a *Pseudomonas aeruginosa*outbreak in a haematology unit with the use of disposable sterile water filter. Haematol J. 91 (7): 983 – 985.

17.Orenstein, R. and Wong, E. S.1999. Urinary tract infections in adults. American Family Physician. www. aafp.org

18.Mohammed, H. K.1998. Urinary tract infection in patients hospitalized at Al – Rasheed military hospital. M Sc. Thesis in Microbiology. College of Medicine. Baghdad University.

19. Price, L.B., Johnson, E., Vailes, R. and Silbergeld, E.2005. Fluoroquinolone – resistant *Campylobacter* isolated from conventional and antibiotic – free chicken products. Environ. Hlth. Perspect. ,113: 557 – 560. 24.

20. Amal, KH. Khalaf., and Ahmed, H. Mohammad.*2015*. In vitro activity of Triazolo[1,5-a] pyrimidine carboxylic acid extracted from*microalgae Hapalosiphonwelweschiiagainst*Trichomonasvaginalis. *Journal.Thi-Qar Sciences*
: 5 (2)

21. Kenneth, L.K., 1958. Prepared research paper RP 2911, central nations for the revised ISCC-NBS color name blocks. *Journal*. Res. NBS, 16: 427-427

22. Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. 1996.Antibiotic susceptibility testing by standard single disc method. *Americana Journal. Clinical Pathology; 45: 493-496.*

23. Clinical and Laboratory Standards Institute Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A10 ,2009 *Wayne*, PA Clinical and Laboratory Standards Institute.

24.Kavanagh, F.1972, Analytical Microbiology. F. Kavanagh(ED), Vol: II, Academic press, New York, and London,11.

25.Taran, M.; Azizi, E.; Shikhvaisi, A. and Asadi, N. 2009. The Anthelmintic Effect of *Pistaciakhinjuk*AgainstProtoscoleces of *Echinococcusgranulosus.* World J. Zoo., 4: 291-295.