Comparative study of *in vitro* inhibitory effect of Crude ethanolic and methanolic extracts of *Albizia lebbeck* leaves on Vancomycin Resistant *Enterococcus faecalis*(VREfs)

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Abstract:

Background :
Infections with Vancomycin-Resistant Enterococci could result in significant morbidity and mortality particularly in immunocompromised patients .The purpose of present study was to evaluate the antibacterial activity of the *Albizia lebbeck* leaves on Vancomycin Resistant *Enterococcus faecalis* (VREfs) .

Materials and methods:
Absolute ethanol and absolute methanol were used to extract the active compounds of the plant leaves .The antibacterial activity against (VREfs) and *E.coli* (used as a control) was then assessed .

Results :
Both ethanolic and methanolic extracts exhibited antibacterial activity on VREfs but not on *E.coli* . Ethanolic extracts showed greater antibacterial activity on VREfs with an inhibition zone of 24 mm at concentration of 200 mg/ml than the mthanolic extracts (inhibition zone of 21 mm) at same concentration.

Conclusion :
Albizia lebbeck possesses an important antibacterial effect that might be considered in the fight against infectious diseases .

Introduction

Human infections with bacteria that possess resistance to multiple antibiotics and chemotherapeutic agents are becoming an increasing problem all over the world. The use of plant secondary metabolites (phytochemicals) and plant extracts as resistant –

modifying agents has become a promising solution for this problem. These products act through different mechanisms than that used by conventional antibiotics and therefore could be used alone or in combination with other antibacterial agents in the treatment of resistant bacteria [1] .

Enterococci are gram positive cocci that are part of normal flora of human alimentary canal . They can cause a wide spectrum of infections like urinary tract infections ,endocordities and bacteriemia .Other types of infection associated with enterococci are infection of abdomen ,pelvis , biliary tract , wounds and to a less extent bone and joint infections[2,3]. Significant morbidity and mortality can complicate enterobacterial infections particularly in critically ill patients . If these bacteria had caused systemic infections , the treatment may become very difficult [(4,5)].The rates of vancomycin resistant enterococci in serious clinical infection have increased all over the world during the last 15 years[6,7].

Albizia lebbeck is an important medicinal plant .It is a large deciduous tree which can attain a height up to 9 meters and a trunk girth up to 1.8 m. This tree is very common in India and can occasionally be cultivated in Iraq mostly in Baghdad and Basrah . In Basrah particularly, *Albizia lebbeck* is growing quite well and seems to be naturalized . The bark contains about 7-11% tannin and a little saponin . Different parts of the plant were used traditionally in the treatment of various types of conditions . The leaves are regarded useful in opthalmia , the root -bark powder is used to strength tooth gum in cases of ulceration and bleeding, powdered seeds when taken internally were found beneficial in scrofulous swelling and the flowers for same purpose when used externally [8].

The purpose of this study was to assess the antibacterial activity of *Albizia* leaves on Vancomycin Resistant *Enterococcus faecalis* (VREfs). Up to our knowledge this is the first study investigating role of the Albizia against VREfs in our locality .

Materials and methods

Preparations of dilutions of crude extract for antibacterial assay:

The fresh leaves of the plant were washed thoroughly with sterile distilled water and then left to dry in fresh air at room temperature . The dried leaves were crushed into fine powder using a grinder . A measured amount of the powder was then dissolved separately in ethanol and methanol solution to make the extracts according to the standard method [9] as follows:

Two conical flasks were used, to each 50 grams of the dried powdered leaves were transferred separately. Absolute ethanol (300 ml) was added to the first flask and the same volume of absolute methanol was added to the second . Both flasks were allowed to stand for 24 hours at room temperature . The contents of both flasks were agitated vigorously after that using a mechanical shaker in order to mix and dissolve the materials. The resulting suspension from both flasks were separately filtered using No.1 sterile Whitman filter papers to obtain filtrate . Each filtrate was then evaporated successively in a water bath at controlled temperature of 60° till dryness .A sticky mass of each filtrate was obtained at the end of the dryness process which were then utilized in the study[10,11].

Preparation of bacterial inoculums

The microorganisms used in the study were Vancomycin-Resistant *Enterococcus faecalis* (VREfs). The bacteria were obtained from microbiology laboratory in Al-Basrah General Hospital after identification .To a test tube containing 4 ml of 0.9 % normal saline, 3-10 colonies of the VREfs were transferred. An optical density by of 0.1% at wave length of 654 nanometer was measured by a spectrophotometer to reach a concentration of 10^7 [12].The same procedure of broth preparation of *Escherichia coli* (*E.coli*) obtained from same laboratory was performed and used as a control for comparison.

Antibacterial activity of leaf extracts

The antibacterial efficacy of ethanolic and methanolic extracts of the *Albizia lebbeck* leaves were tested on the two microorganisms, (Vancomycin-Resistant *Enterococcus faecalis* and *Escherichia coli*) using the agar well diffusion method [13] .The cultures from the standardized broths were swabbed on the corresponding sterile Mueller Hinton Agar plates using sterile cotton swabs. Using a sterile borer, four wells of 6 mm were punched in each inoculated plates. The bottom of each plate was sealed with molten agar [illegible] t was used to fill the base of each ditch. The crude ethanolic and methanolic extracts were dissolved separately in 30% Dimethyl sulphoxide (DMSO) to obtain 200 mg/ml, 150 mg/ml, 100 mg/ml and 50 mg/ml concentrations of each . To each labeled well ,0.1 ml from these concentrations were transferred according to the methods of [14,15]. Same concentrations of the DMSO solvent alone were also prepared and used in the plates for comparison all plates were then incubated overnight . The zone of inhibition measured in mm was then recorded for each microorganisms.

Results

The crude ethanolic and methanolic extracts of *Albizia lebbeck* leaves showed inhibitory effects of the in vitro growth of Vancomycin Resistant *Enterococcus feacalis* but not on *E. coli*. The control solution DMSO showed no any inhibitory effect on the types of tested bacteria(table 1).

Table1:Effect of different extracts and DMSO solvent on bacterial growth

For specific type of *Albizia lebbeck* leaves extract , the crude ethanolic extract showed greater inhibitory effect on the growth of Vancomycin Resistant *Enterococcus feacalis* than the crude methanolic extract as shown in tables 2 and 3.

Table 2: Effect of ethanolic extract on the growth of VREfs

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Microorganisms	Ethanolic extract	Methanolic extract	DMSO
Vancomycin Resistant *Enterococcus faecalis*(VREfs)	Sensitive	Sensitive	Resistant
Escheriachia coli	Resistant	Resistant	Resistant

Concentration of ethanolic extract	Inhibition zone of(VREfs)
50 mg/ml	5mm
100 mg/ml	12mm
150 mg/ml	19mm
200 mg/ml	24mm

Table 3: Effect of methanolic extract on the growth of VREfs

Concentration of methanolic extract	Inhibition zone of(VREfs)
50 mg/ml	3mm
100 mg/ml	9mm
150 mg/ml	15mm
200 mg/ml	21mm

For concentrations of ethanolic extracts of 50, 100, 150 and 200 mg/ml resulted in inhibition zones of 5, 12, 19, 24 mm respectively. For methanolic extracts of 50, 100, 150 and 200 mg/ml the inhibition zones recorded were 3, 9, 15, 21 mm respectively.
For ethanolic extract the highest concentration of 200mg/ml resulted in maximum inhibition zone of VREfs growth with an inhibition zone of 24 mm, while the lowest concentration of 50mg/ml lead to lowest inhibition zone 5mm . For methanolic extract ,the inhibition zones recorded for the same concentrations were 21mm and 3mm respectively.

Discussion

Various extracts of different species[5] of the genus *Albizia lebbeck* were reported by different authors to have a variety of pharmacological activities . Some of these activities were the antimicrobial activity of A. ferrugenia[16] and *A. lebbeck* [17], the anti-diabetic activity of *A. odoratissima*[18] and the anti-depressant activity of *A. julibrissin* [19].
The present study was aimed at scientific validation of antibacterial activity of the leaves of *Albizia lebbeck* on Vancomycin Resistant *Enterococcus faecalis* . The results indicate that the plant leaves are a good source of antibacterial effect against this type of gram positive bacteria which are the cause of many types of infection in medical practice .The ethanolic extract of the plant leaves showed greater inhibitory activity against VRSfs than the methanolic extract .

Many studied performed phytochemical screening of ethyl successive extraction of the leaves *Albizia lebbeck* and showed the presence of glycosides ,tannins, saponins, flavoniods, carbohydrates, proteins and amino acids. Methanolic successive extraction yielded the presence of alkaloid, tannins, saponins, flavonoids and carbohydrates [20,21]

The rich content of flavoniods, tannins, and saponins in the leaves of *Albizia lebbeck* could be the main components that are responsible for the antimicrobial activity. These compounds were documented by many studies to have medicinal and physiological activities [22,23].

Ashwaq AS. and Abbas DM.[24] in 2013 attributed the antimicrobial activity of the leaves of *Albizia lebbeck* against pathogenic bacteria of urinary tract infections (*Proteus* sp., *Klebsiella* sp., *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*) to three alkaloidic components which were isolated and purified by gas chromatography technique .

A substantial controversy is still present regarding the antimicrobial activity of the leaves extract related to type of extraction solvent. While Z. Sheyin *et al.*[25] who used Ethyl acetate , Absolute ethanol and Aqueous solvents to extract *Albizia lebbeck* leaves, found that only the ethyl acetate extract showed antimicrobial activity against the tested microorganisms (namely *pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia, Escherichia coli ,Shigella spp.* and *Salmonella typhi.* Absolute ethanol and Aqueous extracts showed no any inhibitory action on these bacteria. They concluded that the effectiveness of the extracts largely depends on the type of solvent used for the extraction .

Our result of the inhibitory antibacterial activity of both ethanolic and methanolic extracts of *A. lebbeck* leaves are ˙ greement with the study of Mohammed Nazeen Bobby *et al*.[17] who demonstrated comparable inhibitory activities of both methanolic and ethanolic extracts against the

growth of all tested microorganisms (*E. coli, Bacillus subtilus , proteus vulgaris, pseudomonas aeruginosa,Salmonella typhi)* only *Staphylococcus aureus* was not inhibited by ethanolic but not methanolic extracts in their study . Contrary to the study of Rahul *et al* in 2010, who reported potent in vitro activity of *Albizia lebbeck* leaves against *Escherichia .coli ,Staphylococcus aureus ,Pseudomonas aeruginosa* and *Bacillus cereus* , the current study didn't reveal any inhibitory effect against the growth of (*E.coli*). This might be attributed to the complex structure of cell wall in gram negative bacteria protecting them from the inhibitory activity of the leaves extracts [21,23].Another explanation of the contrary might be attributed to the insolubility of the active ingredients of the *A. lebbeck* leaves in different solvents.

It can be concluded from this study that the traditional plant *A. lebbeck* is an important medicinal plant with antimicrobial effect that can be considered in the treatment of bacterial infection . Its further purification and studies to justify its use in the therapeutic fields are required .

References

1-Abreu,A.C. and *et al*., (2012). Plants as Sources of New Antimicrobials and Resistance-Modifying Agents. Nat. Prod. Rep., 29(9): 1007–1021.

2-Fridkin SK, Gaynes RP. Antimicrobial resistance in intensive care units. Clin Chest Med 1999;20:303-16, viii.

3- Cereda R.F., Sader H.S., Jones R.N. Sejas L., Machado A.M., Zanatta,Y.P.Rego S.T. and Medeiros E.A. (2001). *Enterococcus faecalis* resistant to vancomycin and teicoplanin (VanA phenotype) isolated from a bone marrow Transplanted patient in Brazil ;5(1): 40-46.

4- Sastry V., Brennan P.J., Levy M.M., et al. Vancomycin-resistant enterococci: an emerging pathogen in immunosuppressed transplant recipients. Transplant Proc 1995;27:954-5.

5- Leclerq R., Courvalin P. Resistance to glycopeptides in enterococci. Clin Infect Dis 1997;24:545-56

6-Delmas, J., F. Robin, C. Schweitzer, O. Lesens, and R. Bonnet. 2007. *Evaluation of a new chromogenic medium, ChromID VRE, for detection of vancomycin-resistant enterococci in stool samples and rectal swabs.* J. Clin. Microbiol. 45:2731-*2733*

7-Top, J., R. Willems, and M. Bonten. 2008. Emergence of CC17 Enterococcus faecium: from commensal to hospital-adapted pathogen. FEMS Immunol. Med. Microbiol. 52:297-308.

8- Chakravarty H.L. (1976).*Albizia lebbeck* Benth. Plant wealth of Iraq (a dictionary of economic plants) . vol. (1),P 11-12.

9-Mamman, P., Mahelia, W., Susbratus, S., Sanbo, K. (2012). Antibacterial effect of crude Extract of *azadiracha indica* against
Escherichia coli, Salmonella species and *Staphylococcus aureus*.
International Journal of Medicine and Medical Science.5(1):14-18.

10-Falodun, A., Okunrobo , L.O., and Uzoanmake, N. (2006). Phytochemical Screening and anti-inflammatory Evaluation of methanolic and aqueous extract of Euphorbia heterophylla linn (Euphorbiaceae) .African Journal Biotechnology 5(6): 529-53.

11-Ponnusamy S.,Gnanaraj W.E., Selvakumar V. and Nelson J. (2010). The effect of leaves extracts of Clitoria ternatea Linn against the fish pathogens. Asian Pacific Journal of Tropical Biomedicine ;3(9):723-726.

12- Alade,P.I;and Irobi,O.N.(1993).Antimicrobial activities of crude leaf extracts of *Acalypha wilkensian* .J.of thnopharm :39:171-174

13- Oluduro, A. (2012). Evaluation of Antimicrobial Properties and Nutritional Potentials of Moringa oleifera Leaf in South-West Nigeria. *Malaysian Journal of Microbiology* 8(2): 59-67.

14-Akujobi, C., Anyanwu, B. N., Onyeze, C. and Ibekwe, V. I. (2004). Antibacterial Activities and Preliminary Phytochemical Screening of Four Medicinal Plants. Journal of Applied Sciences. 7(3); 4328 – 4338.

15-Esimone, C. O., Adiukwu, M. 8 . and Okonta, J. M. (1998). Preliminary Antimicrobial Screening of the Ethanolic Extract from the Lichen Usnea subfloridans (L). Journal of Pharmaceutical Research and Development. 3(2); 99 – 102.

16-Sarkiyayi S, Karago J and Hassan R (2011) Studies on anti typhoid properties of aqueous methanol leaves extract of *Albizia ferruginea* (Musase). International Journal of Biochemistry research & Review 1: 24-30.

17-Bobby, M.N., Wesely E.G. and Johnson M.N. (2012). *In vitro* anti- bacterial activity of leaves extracts of *Albizia lebbeck* Benth against some selected pathogens. Asian Pacific Journal of Tropical Biomedicine 2: 859-862.

18-Kumar D., Kumar S., Kohli S., Arya R., Gupta J. (2011) Antidiabetic activity of methanolic bark extract of *Albizia odoratissima* Benth. in alloxan induced diabetic albino mice. Asian Pac. J. Trop. Med. 4: 900-903.

19-Kim JH, Kim SY, Lee SY, Jang CG (2007) Antidepressant-like effects of *Albizzia julibrissin* in mice: involvement of the 5-HT1A receptor system. Pharmacol Biochem Behav 87: 41-47.

20- Sittiwet C. Niamasa N. Puangpronpitag D. (2009). Antimicrobial activity of *Acanthus ebracteatus* vahl. Aqueous extract: The potential for skin infection treatment. Int. J. of biological chemistry; 3(2): 95-98.

21- Rahul C, Pankaj P, Sarwan SK, Mahesh JK(2010). Phytochemical screening and antimicrobial activity of *Albizzia lebbeck*. J. Chem. Pharm. Res. 2(5):476-484

22- Kapoor BBS, Bhumika, Khatri JS.(2007).Antimicrobial activity of some medicinal tree species of Hanumangarh district of Rajasthan .Journal of phytological Research ,20:325-326.

23-Collee,J.:Fraser ,A.: Harmion ,B. and Biomon ,A.,(1996). Practical medical microbiology .Makie and MC Carteney 14 ,Churchil liv :ngsto, New york , p. 978.

24- Shenta A.A. and Al-Maliki A.D. (2013). Isolation and identification of three alkaloids compounds from *Albizia lebbeck* L.leaves and study of their antimicrobial activity against pathogenic bacteria of urinary tracts inflammatory in vitro. J. Thi-QA Sci., 3(4) : 99-111.

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25- Sheyin Z., Maimako J., Shindang J., Essien C.U., Bigwan E.I. and Ede F. R. (2015). Antimicrobial activity of *Albizia lebbeck* leaf extract on some medically important bacteria. Int. J. Curr. Microbiol. App.Sci 4(9): 473-477.

دراسة مقارنة التأثير التثبيطي لخلاصة الاثيل والمثيل لأوراق نبات *Albizia lebbeck* على بكتريا **Vancomycin Resistant *Enterococcus feacalis* (VREfs)**

الخلاصة:

تعد الاصابة بجرثومة Vancomycin Resistant *Enterococcus feacalis* (VREfs) من المسببات الرئيسية للوفيات وخاصة في مرضى العوز المناعي وغيرهم .

تهدف الدراسة لتقييم التاثير التثبيطي لخلاصة الاثيل والمثيل لاوراق نبات *Albizia lebbeck* على جرثومة (VREfs) المقاومة للفانكومايسين.

من المواد التي استعملت في هذه الدراسة الايثانول النقي والميثانول لاستخلاص المركبات الفعالة لاوراق نبات *Albizia lebbeck* ثم تقييم التاثير التثبيطي للخلاصات على الجرثومة.

اظهرت النتائج التي حصلت من دراسة الفعالية الضد الجرثومية على بكتريا (VREfs) ان خلاصة الاثيل كان لها تأثير تثبيطي اكبر على الجرثومة عند التركيز 200mg/ml بمنطقة تثبيط 24mm في حين كان تأثير خلاصة المثيل اقل من الاثيل بمنطقة تثبيط 21mm عند نفس التركيز. لكن لم تظهرالدراسة اي تاثير تثبيطي على جرثومة *Escherichia coli* التي استخدمت للمقارنة .

نستنتج من هذه الدراسة ان نبات *Albizia lebbeck* يمتلك اهمية تثبيطية على الجراثيم المسببة لأمراض مختلفة .