

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 methanolic 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 ,endocarditis and bacteremia .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 ophthalmia , 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 ³ it 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 faecalis* 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 faecalis* 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 <i>Enterococcus faecalis</i> (VREfs)	Sensitive	Sensitive	Resistant
<i>Escheriachia coli</i>	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 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. ferruginea*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, flavonoids, carbohydrates, proteins and amino acids. Methanolic successive extraction yielded the presence of alkaloid, tannins, saponins, flavonoids and carbohydrates^{20,21}

The rich content of flavonoids, 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 in agreement 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 subtilis* , *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 .

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

الخلاصة:

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

تهدف الدراسة لتقييم التأثير التثبيطي لخلاصة الاثيل والمثيل لأوراق نبات *Albizia lebbeck* على جرثومة (VREfs) المقاومة لل فانكوميسين.

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

اظهرت النتائج التي حصلت من دراسة الفعالية ضد 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 تظهر الدراسة اي تأثير تثبيطي على جرثومة *Escherichia coli* التي استخدمت للمقارنة .

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