

Study the Antibacterial Activity of *Bauhinia variegata* Linn. plant Leaf Extracts Against Some Species of Pathogenic Bacteria

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Abstract

Microbial resistance to antibiotics is highly increasing during the three last decades. Evaluation of natural products to access new and effective antimicrobial agents is one of the scientific strategies to combat drug-resistant pathogens. With this perspective, leaf extracts of *Bauhinia variegata* which had documented uses in traditional medicine, were investigated for antimicrobial activity against four bacterial strains, two gram-positive; *Staphylococcus aureus*, *Streptococcus pyogenes*. and two gram-negative; *Escherichia coli*, *Proteus mirabilis*. The antimicrobial activity was evaluated by measuring inhibition zone diameters in agar well diffusion assay. The aim of the present study was to evaluate the antimicrobial potential of *B. variegata* leaves extract as compared with antibiotics such as ampicillin, so that this medicinal plant could serve as a good candidate to treat various infectious diseases. The crude extracts of *Bauhinia variegata* showed high antibacterial activity against all bacterial strains as compared with the antibiotic ampicillin which recorded inhibition zone ranged from 21 mm to 34 mm. Ethanol diluted extract revealed no activity at the dilutions from 2% to 16%, while it showed less activity at the dilutions of 32% and 64% against *staphylococcus aureus*, *Streptococcus pyogenes* and *Proteus mirabilis*. *Escherichia coli* showed resistance against all extract dilutions. It was noted that the extracts were more active against *Proteus mirabilis*, as compared with other bacterial species. Finally, all the bio-extracts were well stable at room temperature during the duration of the study and did not show any reduction of activity against the bacterial strains used in this study experiments.

Keywords: *Bauhinia variegata*, Antibacterial activity, Ethanol extracts.

Introduction

Antibiotic resistance has become a global concern in recent years. This problem is of great significance especially in developing countries because infectious diseases are one of the major causes of mortality in these countries. The screening of natural products has been the source of innumerable therapeutic agents. Higher plants, as a source for new potential drugs is still largely unexplored and only a small percentage of them have been subjected to phytochemical investigation [1]. Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases. The plant extracts have been developed and proposed for use as antimicrobial substances. Many of the plant materials used in traditional medicine are

readily available in rural areas at relatively cheaper than modern medicine. Thus it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential [2, 3].

Bauhinia variegata (leguminosae) is a deciduous tree, the various parts of the plant viz., flower buds, flowers, stem, stem bark, leaves, seeds and roots are practiced in various indigenous systems of medicine and popular among the various ethnic groups for the cure of variety of ailments. The plant *B. variegata* Linn. commonly known as Mountain Ebony is a medium-sized, deciduous tree, found throughout India. It has been used in dyspepsia, bronchitis, leprosy, ulcer, to prevent obesity, as an astringent, tonic and anthelmintic [4, 5]. Various researchers have reported that *Bauhinia variegata* has

antidiabetic activity [6, 7], good insecticidal [8, 9], antigoiterogenic [10], and better antioxidant [11]. It is also reported as an anti-inflammatory [12]. The saline extract of *B. variegata* seed exhibited haemagglutination activity against erythrocytes of man, monkey, rabbit, goat, rat, buffalo, sheep, cow, horse, mule and fowl [13].

The aim of the present study was to evaluate the antibacterial potential of *B. variegata* leaf ethanol extract as compared with the watery extract, so that this medicinal plant could serve as a good candidate to treat various infectious diseases.

Materials and Methods

B. variegata leaves were collected from College of Science-Baghdad University at the period 25/10/2011, and it was authenticated by Horticulture Dept.-College of Agriculture-Baghdad University, Iraq.

Extract preparation

The leaves of *B. variegata* were washed with water, dried at 40 °C over night using oven apparatus, then powdered coarsely. Crude extract was obtained by mixing 5 g of the powder with 250 ml of 70% ethanol or distilled water at room temperature and shaken for 24 hrs using stirrer apparatus. The crude extract was evaporated under reduced pressure, and then diluted with distilled water or/and with ethanol 70% to obtain 6 dilutions which were 2, 4, 8, 16, 32, or 64% of *B. variegata* leaf extracts. Dilutions prepared by dissolving 2 ml of the extract in 100 ml of water or/and ethanol to obtain conc. of 2% and so on for other dilutions [14].

Microorganisms

Microorganisms were obtained from stock cultures of department of Medical Biology, College of Medicine- Baghdad University. Gram positive bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*) and gram negative (*Escherichia coli*, *Proteus mirabilis*) were used in this study for antibacterial assay. The organisms were sub cultured on to nutrient agar in order to determine their viability. Stock cultures were maintained on nutrient agar slants at 4 °C and then sub-cultured in nutrient broth at 37 °C prior to each antibacterial test. Inoculants of the test organisms were standardized by suspending

5 colonies of a 24 hrs culture in 5 ml of nutrient broth and comparing the turbidity with that of Macfarland standards (1.5×10^8 CFU) after incubating at 35 °C for 2 hrs [15].

Antibacterial Assay

Muller Hinton Agar was prepared according to the manufacturer's instructions. The medium was sterilized by autoclaving at 121 °C for 15 minutes at 15 psi pressure and was used for tests. Sterile agar was poured aseptically into sterile petridishes (10 ml each) and the plates were allowed to solidify at room temperature in sterile condition. After that the plates were seeded with bacterial strains by streaking evenly on to the surface of the medium with a sterile cotton swab. The discs of 6 mm diameter were prepared from Whatmann filter paper No. 1 and were sterilized, and then impregnated with the extracts, solvent DMSO and streptomycin (5 µg/disc) was used as standard. Sterile filter paper with different test concentrations ranging from 2% to 64%. Disks were placed on to the agar with flamed forceps and gently pressed down to ensure contact along with the diluted extract, one appropriate control dry disc also placed at the center. The plates were incubated at 37 °C over night, and then the zones of inhibition were measured with a measuring tape. This experiment was carried out in triplicate for their confirmation. The results were read by the presence or absence of zone of inhibition [16].

Results and Discussion

Antibacterial activity

Table (1) shows the antibacterial effect of the crude ethanol extract of *Bauhinia variegata* on bacterial strains used in this study. Result revealed that the crude ethanol extract possess potential antibacterial activity against all the tested bacterial organisms (*S.aureus*, *strep. pyogenes*, *E.coli*, *P. mirabilis*). The ethanol extract showed a broad spectrum of activity against all the bacterial strains at the tested concentration of 200 mg/ml.

Ethanol extract showed antibacterial activity against gram positive and negative strains of bacteria used in this study which were inhibited at 32% and 64% concentration of the ethanol extract except *E. coli* which showed resistance to all concentrations used

(Table (2)). Ethanol extract recorded inhibition zones for *Strep. Pyogenes* (12 mm, 13 mm) at the concentration of 32% and 64% respectively, followed by *S. aureus* which recorded (11 mm, 12 mm) at the concentrations 32% and 64% of the ethanol extract respectively as compared with antibiotic effect shown in Table (3).

Table (1)

Effect of crude extract of *Bauhinia variegata* (at concentration of 200 mg/ml) on bacterial strains as inhibition zone diameters (mm).

Bacterial strain	Type of extract	Inhibition zone diameter(mm)
<i>Staphylococcus aureus</i>	Water	21
	Ethanol	34
<i>Streptococcus pyogenes</i>	Water	29
	Ethanol	32
<i>Escherichia coli</i>	Water	21
	Ethanol	33
<i>Proteus mirabilis</i>	Water	22
	Ethanol	29

The least activity was observed for *P. mirabilis* (5 mm, 6 mm) at the same concentrations. Water extract showed no effect on all bacteria used in this study.

Table (2)

Effect of six dilutions of leaf ethanol extract of *Bauhinia variegata* on bacterial strains as inhibition zone diameters (mm).

Extract dilution %	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>	<i>Proteus mirabilis</i>	<i>Escherichia coli</i>
2	-	-	-	-
4	-	-	-	-
8	-	-	-	-
16	-	-	-	-
32	11	12	5	-
64	12	13	6	-

These results showed that crude extracts at the highest concentrations (32, 64)% of *B. variegata* (ethanol and water) are more effective than ethanol and water extracts after dilution. Many plants and their extracts used against microbial infections due to the presence of secondary metabolites such as

phenols, essential oils, terpenoids, alkaloids and flavanoids. *Bauhinia variegata* leaves were a potential source of phenols, tannins, flavonoids, steroids and of cardiac glycosides [17-23]. The antibacterial activity of ethanol extract might reside in their phytochemical content. This effect may be due to the appropriate concentrations of the active compounds that found in *Bauhinia* leaf. This activity have been screened because of their great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics, becomes an ever increasing therapeutic problem [24, 25].

Table (3) shows the effect of the antibiotic ampicillin on bacterial strains recorded as inhibition zone diameters which was in harmony with the effect of crude extracts of *Bauhinia variegata* leaf. Plant based antimicrobial represents the vast untapped source for medicine. Plant based antimicrobials have enormous therapeutic potential as they can solve the purpose without any side effects that are often associated with synthetic antimicrobials, continued further research and exploration of plant derived antimicrobials is needed today. Medicinal plants were important source for the development of potential, new chemotherapeutic drugs and the *in vitro* antibacterial test form the basis [26, 27].

Table (3)

Effect of Ampicillin antibiotic (100 µl/mg) on bacterial strains as inhibition zone diameters (mm).

Bacterial strain	Gram staining	Inhibition zone diameter(mm)
<i>Staphylococcus aureus</i>	+	30
<i>Streptococcus pyogenes</i>	+	26
<i>Escherichia coli</i>	-	22
<i>Proteus mirabilis</i>	-	28

The polarity of the solvent seems to play an important role in exhibiting potential antibacterial activity. Ethanol extracts of *B. variegata* L. showed remarkable activity against some pathogenic bacterial strains. In

addition such results justify the traditional use of *B. variegata* L. [28].

It was concluded that the antibacterial activity of the crude extracts of *B. variegata* may be used for various human ailments especially for various infectious diseases. Thus this plant could be utilized as an alternative source of useful antimicrobial drugs. Further studies are needed to isolate, characterize and elucidate the structure of the bioactive compounds of this plant for antimicrobial drug formulation.

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الخلاصة

ازدادت مقاومة المايكروبات للمضادات الحيوية بشكل عالي خلال العقود الثلاثة الاخيرة. وان تقييم المنتجات النباتية الطبيعية للوصول الى عوامل جديدة ومؤثرة كان احد الاستراتيجيات العلمية لمواجهة مقاومة الكائنات المرضية للدواء. مع هذا التوجه، شملت الدراسة استخدام مستخلصات آذان الفار *Bauhinia variegata* Linn. والذي تم توثيق استعماله في العلاج التقليدي، فقد فحصت الفعالية المايكروبية ضد اربعة سلالات بكتيرية اثنان موجبة لصبغة كرام : *Streptococcus* و *Staphylococcus aureus* و *Escherichia coli*, *Proteus pyogenes* واثان سالبة *mirabilis*, تم تقييم الفعالية ضد المايكروبية للمستخلصات بقياس قطر مناطق التثبيط بطريقة فحص الانتشار على الاكار. كان الهدف من هذه الدراسة لتقييم قوة الفعالية المايكروبية لمستخلص الاوراق لنبات آذان الفار مقارنة بالمضادات الحيوية كالمبسلين، لهذا فان هذا النبات الطبي يخدم كعامل جيد لمعالجة مختلف اصابات الامراض. اظهرت المستخلصات الخام لنبات خف الجمل فعالية مضادة للبكتريا عالية تجاه جميع السلالات البكتيرية بالمقارنة مع المضاد الحيوي الامبسلين، والتي سجلت مناطق تثبيط ضمن المدى ٢١ ملم الى ٣٤ ملم. المستخلص الايثانولي المخفف لم تظهر اي فعالية عند التخفيف ٢% الى ١٦% ، بينما اظهرت فعالية قليلة عند التخفيف ٣٢% و ٦٤% تجاه البكتريا *Streptococcus* ، *Staphylococcus aureus* ، *Proteus mirabilis* و *pyogenes*. اظهرت البكتريا *E. coli* مقاومة تجاه جميع التخفيف. لوحظ ان المستخلصات كانت اكثر فعالية تجاه بكتريا *Proteus mirabilis* بالمقارنة مع الانواع الاخرى من البكتريا. واخيراً، كانت جميع المستخلصات ثابتة بشكل جيد عند درجة حرارة الغرفة اثناء فترة الدراسة ولم تظهر اي انخفاض في الفعالية تجاه السلالات البكتيرية المستخدمة في تجارب الدراسة.