Evaluation of activity of alcoholic extract of *Bauhinia variegate* against some G+ve & G-ve

Emad M. Rasheed .Madiha H. Hussain Janet A. Misak
technology Almansur

Abstract :

The antibacterial activity of methanolic extract of Bauhinia variegate

The present study was carried out to investigate the antibacterial activity of methanolic extract of *Bauhinia variegate* The antibacterial activity was evaluated according to the well diffusion assay using different isolates of gram G+ve and gram G-ve bacteria which were isolate from Urain , septum and wound , and identified by bio chemical and api20E system.

Two isolates were represented gram positive bacteria and twelve isolates of gram negative bacteria The plant extract was more active against G+ve isolates than G-ve isolates . The most susceptible G+ve isolates to the extract was *Staphyllococcus* aureus while *klebseilla pneumoiae* (G-ve) showed highest susceptibility to extract than other G-ve isolates . The resistant isolates were Entero *Bacter aeroginosa*, *Enterobacter cloacae* , *Morexella catarrhalis* and *Serratia marcescens*. The antibacterial activity of extract was observed as broad spectrum activity against G+ve and G-ve isolates .

Key words: Bauhinia variegata, anti bacterial, well difussion assay.

تقويم فعالية المستخلص الكحولي لنبات خف الجمل Bauhinia variegata, ضد بعض انواع البكتريا الموجبة والسالبة لصبغة كرام

عماد محمد رشيد مديحة حمودي جانيت ارشاك

الخلاصة :

اجريت هذة الدراسة لمعرفة الفعالية ضد الجرثومية لمستخلص نبات خف الجمل الكحولي (***Bauhinia variegate)*** .قيمت الفعالية ضد الجرثومية للمستخلص اعتمادا على طريقة الانتشار بالحفر وذلك ضد عزلات محلية مختلفة شخصت بالطرق البايوكيمياوية واستخدم نظام Api20Eوقد شملت العزلات عزلتان موجبتان لصبغة كرام واثنا عشر عزلة سالبة لصبغة كرام اظهر المستخلص الكحولي للنبات فعالية عالية تجاه العزلات الموجبة الصبغة كرام اكثر من العزلات السالبة لصبغة كرام حيث كانت المكورات العنقودية الذهبية اكثر العزلات تحسسا للمستخلص في حين اظهرت جرثومة الكلبسيلا الرئوية تحسسا اكثر من غيره من الجراثيم السالبة لصبغة كرام ، اما الجراثيم المقاومة لفعالية المستخلص فكانت *E.*aeroginosa ,*E.*cloaca*eM.catarrhalisS.marcescens*

لوحظ ان فعالية المستخلص المضادة للجراثيم المستخدمة في الدراسة ذات طيف واسع ضدالجراثيم الموجبة والسالبة لصبغة كرام.

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Introduction:

Plant have played a key role in day –to –day life support system of human beings from times immemorial with the present day urge together knowledge of nature resources for their scientific and economic exploitation for various uses , the urgency of assessing botanical information at micro level has received special attention and thus require afresh surveys to be conducted to know not only the floristic richness area but also the ethno –medicinal practices (Kumari *et al*.,2011).

Plants are the richest resource of drugs of traditional systems of medicine , modern medicines ,nutraceuticals ,food supplements ,folk medicines , pharmaceutical intermediates and chemical entities for synthetic drugs .(Joshi *et al*., 2011).

The existence of potent antibiotics , resistant or multi-resistant strains are continuously appearing . Thus , there is an urgent need to systemically evaluate the plant used in traditional medicine as green medicine which is safe .(Sharma *et al* .,2010). There is a growing trend in the use of medicinal plant because of their medical effectiveness ,low toxicity and many natural anticancer agents derived from these plants (Kobeasy *et al*.,2011) .

The relatively large Bauhinia genns (family:fabaceae) consisting of trees , climbers and shrubs is distributed in awide rang of geographic location , certain Bauhinia species have along histroy of traditional medicinal applications .(Valdir,2009).

Bauhinia variegata known as kachnara ,is an herbaceous plant , Its powdered bark is used for tonic

,ulcers and skin disease.(Manandher,2002). It is also used as antigioterogenic (Singh *et al* .,2006), and as antitumor(Balajirao *et al*.,1995).However the above account , it is abvious that there is no information available about the antibacterial activity of the methanolic extract of flower of *Bauhinia variegate*. The present investigation was designated to explore the antibacterial activity of methanolic extract of the leaves of *B.variegata* on growth of different local bacterial isolates obtained from different clinical specimens .

Materials and methods:

1- Collection of bacterial samples

Fourteen samples of urine ,septum and wound swabs were collected from patients of baghdad teaching hospital .The samples were transported to laboratory in an ice cold condition . Loopful of urine samples and swabs of septum and wound were inoculated on nutrient broth and incubated at 37c for 24 hrs .

Then the growth of each samples were maintained on nutrient agar slants at 4ċ.

2- Plant materials

B.variegata plant were obtain from Syria , leaves were washed under tap water and then dried in room temperature at shade . The dried leaves were crushed to affine powder

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by an electrical grinder . The plant classification was done in the plant protection department , agriculture college , baghdad university.

3- Identification of bacterial isolates

The 14 isolates were characterized on the basis of morphological , cultural and biochemical characteristics and were identified according to Bergeys manual of Systemic Bacteriology (kreig and holt , 1984) and this identification was confirmed by using Api 20E(bio merieux, france).

4- Extraction of plant

organic solvent extraction of B. variegata leaves was carried out by using Methanol 95% according to the method described by (Effraim et al., 2000) . This was done by using soxhlet apparatus ,the extracting unit contains the solvent and cellulose(thumble) located inside it that contains the dry plant powder . A distiller unit is fitted on to the extraction unit for condensation of solvent vapor ,50gm . of plant powder was put inside the thumble and 500 ml of 95% ethanol was put inside the flaks . The extraction was carried until aclear solvent appeared in extracting unit . The extract was dried by electric oven at 40-45ċ . The find extract was kept frozen at -20ċ until use.

5- Anti bacterial assay

A) bacterial media: mullar –hinton media was sterilied and 20 ml were poured into strile petridishes , the solidified plates were bored with 5mm diameter cork borer . The plates with wells were used for anti bacterial study.

B) antibacterial activity of plant extract

The Methanloic extract of B. veriegata was tested for anti bacterial activity against different isolates of bacteria using agar well diffusion assay (Lasta et al.,2008).

Petridishes containing muller – hinton media were seeded with 24hrs old nutrient broth culture of each bacterial isolate , inoculum size was adjusted to achieve find concentration of 10 c cfu /ml by matching with 0.5 Mcferland nephlometer standards 0.1 ml of Mehanolic extract were poured into the wells by micropipette. The plates were kept in refrigerator for one hour to facilitate the diffusion of extract into the media , then were incubated at 37c for 24 hrs . The plates were observed for the zone of inhibition of growth around the wells . Average of triplicate was considered and compared with control by using Methanol in the one well instead of extract .

The results:

1- Identification of bacterial isolates

Atotal of 14 seputum , urince and wound swab samples were analyzed for isolation and identification as per standard methods The fourteen samples showed

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prominent bacterial count , the most common isolates were 2G+ve isolates (S.aurens and S.viridans) and 12G-ve isolates (S.marcesens , 2 isolates of k . Pneumoniae,Acinito bacter ssp., 2 isolates of Enterobacter (E.aeroginosa and E. cloacae) , C.freundii , M.catarrhalis , E.coli and 3 isolates of Pseudomonas aurogenosa). Table(1) .

2- Antibacterial activity of plant extract

The result of antibacterial assay reveald that Methanolnic extract of Bauhinia exhidted broad spectrum activity against tested isolates . The highest activity was against G+ve isolates compared with G-ve isolates . Our results shown that S.aureus was the most suspectible bacteria followed by S.viridans with inhibition zone of (14,8) mm respectivity , while for G-ve isolates k.pneumoniae showed the highest suspectibility for 12mm diameter zone of inhibition followed E.Coli,pseudomonas , A.cinito bacter and Citrobacter with (10,10,7,6) mm diameter zone of inhibition respectivly , and has no effect on E . Aeroginosa E . Cloacae , Morexella and Serratia with 5mm diameter for each . Table 2 and 3 .

Table(1): identification of bacterial isolates from sample sources

Sample sourse	Seputum	Urine	Wound swab
Bacterial isolates	**Staphycoccus aureus** **Streptococcus viridans** **Pseudomonas aurogenes** **Serratia marcescens** **Klebseilla pneumoniae** **Acinitobacter spp** **Entrobacter aeroginosa** **Citrobacter freundii** **Morexella catarrhalis**	**Escherishia coli** **Pseudomonas aurogenosa** **Klebseilla pneumoniae** **Entrobacter cloacae**	**Pseudomonas aurogenosa**

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Table (2) The effect of methanol extract of Bauhinia variegate on G-ev isolate bacteria (24) hours of incubation under 37c°

Bacterial isolates	Mean of inhibition zone (mm)	
	Extract	Control
E. coli	10	
Pseudomonas	10	5
Serratia mercescens	5	5
Klebsiella pneumoniae	12	5
Klebsiella pneumoniae	12	5
Acinitobacter	7	5
Entrobacter auroginosa	5	5
Citrobacter freundii	6	5
Morexella catarrhalis	5	5
Entrobacter cloacae	5	5
Psendomonas aurogenes	10	5
Psendomonas aurogenes	10	5

Table(3) The effect of methanol extract of Bauhinia variegate on G-ev isolate bacteria (24) hours of incubation under 37c°

Bacterial isolates	Mean of inhibition zone (mm)	
	Extract	Control
Staphyllo coccus aureus	14	5
Streptococcus viridans	8	5

Discussion:

Bacterial infection is one of the most serious global health issues in 21st century (Morris and Masterton ,2002) . The emergance of bacterial resistance to antibiotics is the major health problem and therefore , it is critical to develop new antibiotics with novel mechanism of action to overcome these problem (Wang et al .,2003) . Plant have traditionally provided assures of hope for novel drug compounds , the use of great significance for therapeutic treatment (Jwu , et al.,1999).

Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure . Methanol extract showed degrees of the antibacterial activity . Further trails using solvent of various polarities will explore the effects of solvent composition on extract efficacy (Romero et

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al,. 2005) . In this study we had screened the methanolic extract of Bauhinia plant which is of promising biological activity as shown in result in agreement with jingo and Sumatra ,(2006) .

Result of antibacterial assay revealed that the extract exhibited broad spectrum activity against tested isolates , the highest activity was against G+ve isolates compared with G-ve , it had already shown that G+ve bacteria are more susceptible towards plant extract as compared to G-ve bacteria (Parekh and Ghanda , 2006)

These differences may be attributed to fact that the cell wall at G+ve bacteria is of a single layer , whereas the G-ve cell wall is multilayered structure (Yao et al ., 1995), though the passage of active compound through the G-ve cell wall may be inhibited , in the other hand ,the presence of prion of the outer membrane of G-ve bacteria restricted the diffusion of many antibiotics and the multidrug efflux pumps at the transmembrans would also pump out the antibacterial agent through the active efflux processes which would hence create a higher intrinsic resistance for G-ve bacteria (Nikaido ,1998) . Our results also shown that S.aurens was the most susceptible bacteria followed by S..viridans with inhibition zone of 14 and 8 mm respectively . For G-ve isolates

K. Pneumoniae showed the highest susceptibility for 12 mm in diameter of inhibition zone followed by E.coli , Pseudomonas , Acinitobacter and Citrobacter with (10,10,7,6)mm diameter of inhibition zone respectively , and it has no effect on Entro coccuse, Areoginosa , E.cloacae , Morexella and Serratia with 5mm diameter for each . These result were shown in table 2 and 3 with promising broad spectrum activity of methanolic extract of Bauhinia compared with control . A good number of research papers documented the anti microbial potency of some species of Bauhinia and the activity is attributed to presence of flavonoids and phenolic compounds(knmar et al.,2005).

Flavonoids are known to exhibit antimicrobial activity through information of a complex with the bacterial cell wall . The probable mechanism of phenolic compounds activity includes enzyme inhibition by oxidizing compounds , possibly through reaction with sulphedral groups or through more nonspecific interaction with proteins (Mosan and Wasserman 1987) . The Investigated plant did not show strong antibacterial activity , however negative results do not mean absence of bioactive constituents nor is that the plant inactive . Active compounds may be present in insufficient quantities in the crude extract to show activity with the dose levels employed (Taylor et al .,2001) . If the active principle is present in high enough quantities , there could be other constituents exerting antagonistic effects or negating the positive effects of bioactive , agents (Jayer et al.,1996). With no antibacterial activity , extracts may be active against other bacterial species which were no tested . (Shale et al., 1999) .

The present study provides an important basis for the use of extracts of Bauhinia for the treatment of infection associated with the studied microorganism . Isolates and characterization of bioactive compounds former this plant is being crud in this study to

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allow the scientific community to recommend their use as accessible alternative to synthetic antibiotics.

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