Basrah Journal of Veterinary Research,Vol.15, No.3,2016
Proceeding of 5th International Scientific Conference,College of Veterinary Medicine
University of Basrah,Iraq

ANTIBACTERIAL AND ANTI- INFLAMMATION ACTIVITY OF *Salix* EXTRACTION *on staphylococcus aureus* ISOLATED FROM DIFFERENT CUTANEOUS INFECTION

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Key words : *Salixa acmophylla* , Anti inflammation , Aspirin

ABSTRACT

This study was conducted to elevated the ethanol and methanol of *Salixa acmophylla* extraction *on* bacteria *Staphylococcus aureus* isolated from skin wound of cattle, about 100(66.66%)from 150 sample isolated , and made the biochemical test , also sensitivity of antibiotic .The loaded antibiotic discs namely: gentamicin (CN-10 µg), levofloxacin (LEV -5µg), amoxicillin (Ax -25 µg) , tetracycline (TE -30 µg) ,vancomycin(30µg),erythromycin (25µg)and streptomycin(25 µg) were placed on the surface of the medium and left for 30 min at room temperature for complete diffusion. The plates were incubated for 24 hrs. at 37°C. also made antimicrobial against isolated bacteria disk diffusion techniques , Minimum inhibition concentration (MIC) with different concentration of plant extracts (25mg /ml ,50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg /ml and 300, . Minimum bacterial concentration (MBC) by Macro-dilution broth method were done . Anti-inflammation test albumin denaturation Maximum inhibition in case of ethanol extract about (65.25±1.2 %) and in methanol extract about (58.135±1.23) was observed at concentration 300 µg/ml., Aspirin, a standard anti-inflammatory drug showed the maximum inhibition ، (67.35±0.56%) at the concentration of (100µg/ml **) .**

INTRODUCTION

Since the beginning of civilization, humans have used natural products for healing of different diseases. Plants are biochemical labs that produce inside their cells a variety of complex substances with numerous biological and pharmacological active compounds. Therefore, many plants become the primary source of substance for drug development (1, 2).

It is a large shrub or small tree widely distributed in Turkey ,Syria , Palestine ,Iraq, Iran, Pakistan, Kashmir , India and Central Asia .(3)

Salixa cmophylla belonged to family *Salicaceae* which contain 450 species (4)

It is a large tree over 30 m high, but most are shrubs or small trees. White is also known as the salicin willow, has been used for its health benefits for thousands of years (5) .

The most famous chemical constituents of *Salix* leaves are Salicyl alcohol , Linolenicacid, Galactose, 4,6-O-nonylidene, 4-Acetoxy-3-methoxycinnamic acid, Stearic acid, Stearylaldehyde (6,7) .

The *Salix* was the anti-inflammatory and anti-nociceptive properties (8,9). It used to treated traumatism ,skin disease especially viruses and fungus infection , antipyretic, decreased high pressured .Tonic for the work of the kidneys (10).

Recent study was proved that used of watery extraction of *salix* in treatment of Acute myeloid leukemia (11). *Salix* plant was used in the treatment of many conditions, including arthritis, menstrual , dental and back pain reduce fevers. and it is used as an anti-inflammatory drug (12).

Staphylococcus aurous causes a wide range of infections from a variety of skin, wound and deep tissue infections to more life-threatening conditions such as pneumonia ، endocarditis, septic arthritis and septicemia. This bacterium is also one of the most common species in nosocomial infections. However, little is known about the virulence factors behind all these conditions. In addition, *S. aurous* may also cause food poisoning, scalded-skin syndrome and toxic shock syndrome through production of different toxins (13).

Chronic wounds harbor aerobic (gram -positive cocci *Staphylococcus aurous* is reported to be present in frequencies varying from (43% to 88%)of the ulcers (14,15).

Inflammation is a complex process, associated with pain and increase of vascular permeability, increase of protein denaturation and membrane alteration. Inflammation is one of the body's non-specific internal systems of defense, there sponge of a tissue to an accidental cut is similar to the response that results from other types of tissue damage, caused by burns due to heat, radiation ،bacterial or viral in vision (16).

The present investigation was aimed to evaluate the antimicrobial effect of ethanol and methanol extraction against *Staphylococcus aureus* isolated from cutaneous wound in the cattle ,also involves determination of anti-inflammatory activity by Inhibition of albumin denaturation(17).

MATERIAL AND METHODS

Sample collection:

One hundred fifty samples (wounds , skin infections) were collected, using sterile cotton swabs from‹hangars rearing calves and cattle. Brain heart infusion broth used as transport medium until reached to the laboratory then incubated at 37C^{o} for 24hours (18).

Isolation and Cultivation of *Staphylococci*:

The collected samples were cultured onto nutrient agar ,blood agar and Mannitol salt agar. The inoculated plates were incubated for 24-48 hours at 37°C.Thesuspected colonies were picked up and propagated in nutrient agar slope for further examinations.

Biochemical test

All isolates on mannitol salt agar medium, were identified as *S. aureus* by conventional biochemical tests (gram stain, catalase positive, coagulase test, oxidation test)

Antibiotic Susceptibility test .

Antibacterial activity was carried out using a disc-diffusion method (19). Petri plates were prepared with 10 ml of sterile Mueller Hinton Agar. The test cultures were swabbed on the top of the solidified media and allowed to dry for 10min . The loaded antibiotic discs namely: gentamicin (CN-10 µg), levofloxacin (LEV -5µg), amoxicillin (Ax -25 µg) , tetracycline (TE -30 µg) ,vancomycin(30µg),erythromycin (25µg)and streptomycin(25 µg) were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. The plates were incubated for 24 hrs. at 37°C. Zones of inhibition were recorded in millimeters and interpreted by referring to zone diameter interpretive standard from (Bioanalyze sensitivity discs Ankara/Turkey).

Extraction of *Salix acmophylla*

Samples were collected from perfumer in the Basrah market, at this time the leaves are completely grown and modified. The samples were stored in the laboratory for the further reference. The leaves were washed, dried and grinded to make the powder about 20g extracted with100 ml of each methanol and ethanol in the Soxhlet apparatus for the period of 72h, then it was filtered with the Whitman filter paper and filtrate was then evaporated to dryness .The extract was used for anti-microbial and ant inflammation (20).

Antimicrobial activity

Preparation of inoculum

The inoculum of the bacterial strains were prepared by suspending one isolated colony from Muller Hinton(MH) agar plates in 5 ml of MH broth and overnight broth cultures. The suspensions were adjusted in 0.5 McFarland standard turbidity to obtain final inoculums of (1. 5×10^8CFU/ml) after 24 h of growth at 37°C and confirmed using a spectrophotometer. The essential oils were dissolved in dimethyl sulfoxide (DMSO, 25 mg/ml) and diluted to MH broth for antibacterial tested. All strains were tested by broth micro- dilution (BMD) and disk diffusion (DD) techniques according to the National Committee for Clinical Laboratory Standards (NCCLS) (21,22) .

Minimum inhibitory concentration (MIC) of methanol and ethanol *Salix* extracts

The MIC of the plant extracts was determined on Mueller Hinton Agar medium following the method of (23, 24) . *Staphylococcus aureus* culture was made on Mueller Hinton Agar plates and the disc impregnated with different concentration of plant extracts (25mg /ml ,50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg /ml and 300 mg/ml)were placed on the medium and incubated at 37°C for 24 hrs. The lowest concentration inhibiting the visible growth of the organism was considered as MIC.

Determination of minimal bactericidal concentration of (MBC)

Macro-dilution broth method In the macro-dilution broth method, a two-fold serial dilution of each methanol and ethanol *Salix* extracts were prepared in sterile Mueller-Hinton broth to achieve a decreasing concentration ranging from 0.50 1.25mg/ml in six sterile tubes labeled 1 to 6. Each dilution was seeded with 100µl of the standardized bacterial inoculum (1.5X 10^8CFU/ml). The inoculated culture tubes were incubated at 37°C for 18 to 24 hrs. A set of tubes containing only seeded broth (i.e. without plant extract) was kept as control. The lower concentration that did not permit any visible growth when compared with the control was considered as the MIC.The minimum bactericidal concentration (MBC) is the lowest concentration of antimicrobial agent that will prevent the growth of an organism after subculture on to antibiotic-free media. To determine the MBC, a 100µl aliquot from the tube showing MIC was placed on MHA plate antibiotic free and was spread over the plate. After incubation at 37°C for 24hrs, the plates were examined for the growth of a bacterium to determine the concentration of the extract at which 99.9% killing of bacteria was achieved.

Anti- inflammatory activity in- vitro

Anti- inflammatory activity was done by inhibition of albumin denaturation .The reaction mixture was consisting of test extract at different concentrations and 1% aqueous solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of 1N HCl. The samples were incubated at 37 ^{0}C for 20 min and then heated at 57 ^{0} C for 20min. After cooling the samples, the turbidity was measured by spectrophotometer at 660 nm (25,26).

The experiment was performed in triplicate . Percent inhibition of protein denaturation was calculated as follows :

Percentage inhibition = (Absorbance control–Absorbance sample) X 100/ Abs. control

RESLUTS AND DISCUSSION

A total of 150 wound skin samples of cattle's were tested and *S .aureus* was isolated from 100 (66.66%) samples based on cultural and biochemical properties. All the 100 isolates Gram-stained smears of the pure cultures exhibited clusters of Gram-positive cocci (Figure 1).

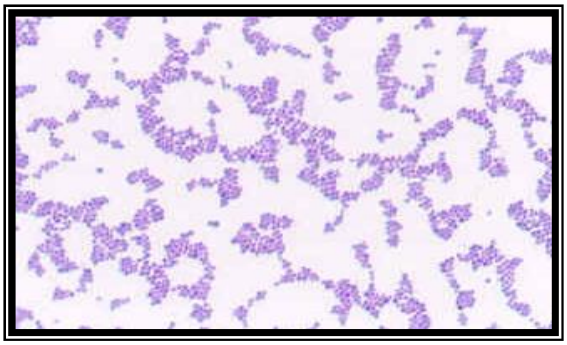
Table (1) : Number and percentage of bacteria isolates.The isolates also fermented mannitol with the color change of MSA (Mannitol Salt Agar) and production of small yellow colonies (Figure 2).

Isolated bacteria	Number	Percentage
Staphylococcus aurous	100	66.66%
Other and no growth	50	33.33%
Total	150	

These isolates were positive for catalase and coagulase test. In catalase test; Hydrogen peroxide was broken-down into water and oxygen. Production of oxygen was indicated by bubble formation, (Figure 3,4).

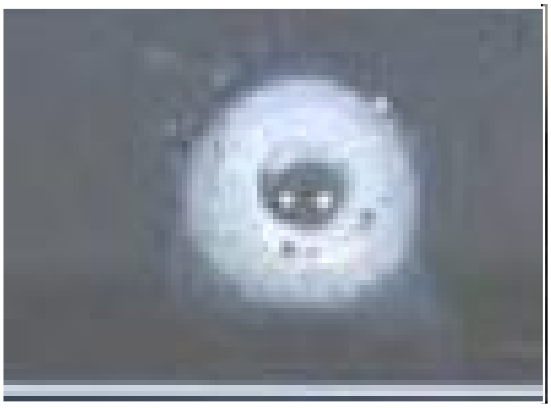
The isolates were identified as *S. aureus* by coagulase test .The positive result of coagulase test was confirmed by the formation of curd like clotting (27) .

Based on antibiotic sensitivity test, . The isolates were found 100% sensitive to Gentamycin and Levofloxin , 75% sensitive to Vancomycin Erythromycin and Tetracycline , the isolates also found 98% resistance to Streptomycin. On the other hand several isolates were found moderated to Oxacillin (Figure 5).

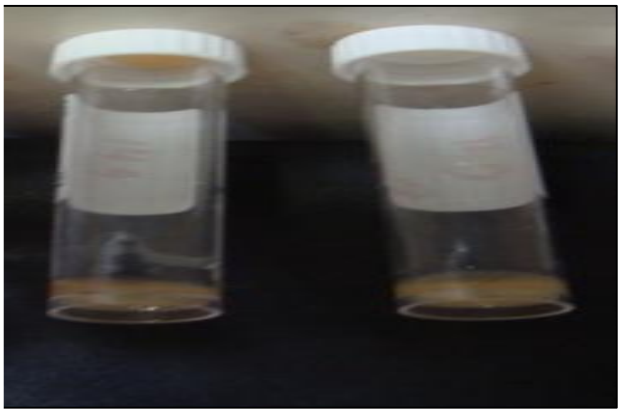


Figure(1) Gram positive cocci of of *S. aureus*

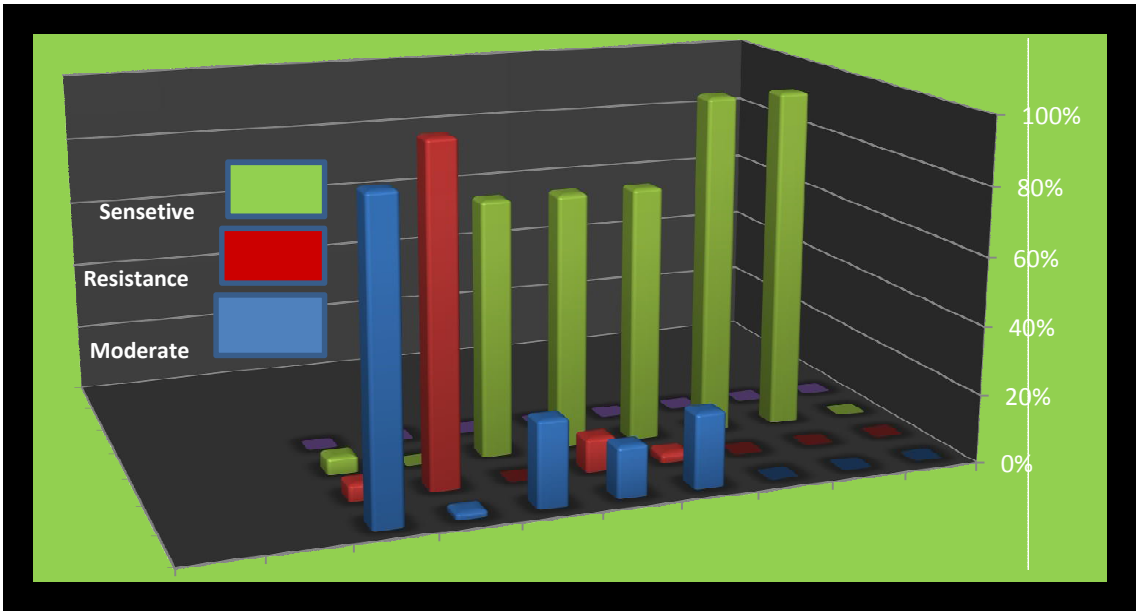
Figure (2) :*S. aureus* on the manitol salt agar



Figure(3): Catalase test



Figure(4): coagulase test



Figure(5) : Antibiotic sensitivity test against *staphylococcus aureus*

The antimicrobial activities of ethanol and methanol extract of plant , gave different zones of inhibition on the isolated organism tested (Table 2) . In this study, the antimicrobial activity was investigated and exhibited better antimicrobial activity against Gram-positive bacteria *Staphylococcus aureus* . The explanation of Gram-positive bacteria is more susceptible than Gram-negative bacteria may attribute to the differences in their cell wall structure. so the external membrane of Gram-negative bacteria renders highly hydrophilic surfaces whereas the negative charge of the surface of the Gram-positive wall may reduce their resistance to antibacterial compounds (28) . However, the higher result in about (18mm) in concentration 300mg/ml . The activity of ethanol extract against *S. aureus* more than methanol extract due to contained tannins (29) , flavonoids(30) , saponins (31), terpenoids (32), and alkaloids (33) .

Table (2): Inhibition zone of ethanol and methanol extraction of *Salix acmophylla* against *staph. aureus*

Bacteria	Ethanol extract						Methanol extract					
	Concentration											
	25	50	100	150	200	300	25	50	100	150	200	300
Staphylococcus aureus	10mm	10mm	13mm	13mm	15mm	18mm	8mm	10mm	10mm	10mm	15mm	15mm

The results appear that the MIC for *Staph aurous* in ethanol extraction about (0.0625mg/ml) and MBC about (0.25mg/ml), in methanol MIC (0.25mg/ml) and MBC (0.5mg/ml).Table(3). Figure 6

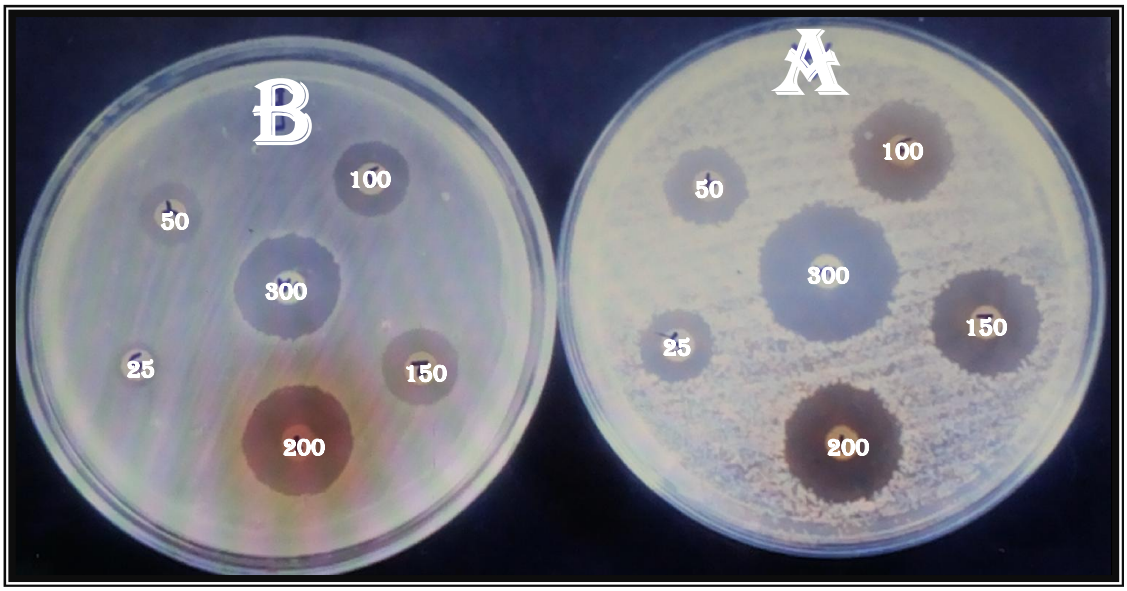


Figure (6): The antimicrobial activities of : (a)ethanol (b) methanol extract of *Salixa acmophyll*

Table (3) : MIC and MBC of ethanol and methanol extraction of *Salixa acmophyll a*against *staph. aureus*

Type of extraction	MIC	MBC
Ethanole extraction	0.0625	0.25
Methanole extraction	0.25	0.5

Most biological proteins lose their biological function when denatured . Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity,(anti-inflammatory drugs Aspirin) have shown dose dependent ability to thermally induced protein denaturation (34).

Maximum inhibition in case of ethanol extract about (65.25±1.2 %)and in methanol extract about (58.135±1.23) was observed at concentration 300 µg/ml., Aspirin, a standard anti- inflammatory drug showed the maximum inhibition ، (67.35±0.56%)at the concentration of 100µg/ml. Table(4).

Table 4 :Effect of ethanol and methanol extract of *Salixacmophylla* on albumin denaturation

Type of test	**Concentration**					
	25	50	100	150	200	300
Albumin denaturation in Ethanol extraction	21.7 ± 1.2	31.7.2 ± 1.1	45.25 ± 1.2	55.25 ± 1.1	58.05 ± 1.1	65.25 ± 1.2

Albumin denaturation in Methanol extraction	20.01 ± 1.1	28.01 ± 1.1	32.25 ± 1.2	40.25 ± 1.23	45.12 ± 1.32	58.13 ± 1.23
Standard drug (Aspirin)			67.35 ± 0.57			

Phenolic and Flavonoids are contained in the plants had several biological activities such as antioxidant, anti-mutagenic and anti-inflammatory. Flavonoids capable of inhibiting the expression and activation of nitric oxide , and therefore could be used additionally during inflammatory therapy. The importance of nitric oxide inflammation may indicate that drugs which modulate nitric oxide production could be successfully used in the management of inflammatory diseases(35).

الفعالية ضد الجرثومية وضد الالتهابية لمستخلص الصفصاف على المكورات العنقودية المعزولة من مختلف الاصابات الجلدية في الابقار

رنا عدنان فائز

فرع الاحياء المجهرية والطفيليات البيطرية ،جامعة البصرة ،كلية الطب البيطري ،البصرة العراق

الخلاصة

أجريت هذه الدراسة لتحديد تاثير المستخلص الميثانولي والايثانولي لنبات الصفصاف ضد بكتريا المكورات العنقودية المعزولة حيث عزلت 100 عينه من 150 من جروح الجلدية للابقار واجريت عليها الاختبارات البيوكيماوية كما اجري اختبار الحساسية الدوائية باستخدام اقراض مضادات حيوية مثل(CN-gentamicin 10 µg), levofloxacin (LEV -5µg), amoxicillin (Ax -25 µg) , tetracycline (TE -30 µg) vancomycin(30µg),erythromycin (25µg)and streptomycin(25 µg), وضعت على سطح

الوسط ثم حضنت لمدة 24ساعه وبدرجه 37 . كما اجري التركيز المثبط الادنى بتراكيز مختلفة (mg/ml 25mg /ml ,50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg /ml and 300) واجري التركيز البكتيري الادنى وكما اجري اختبار ضد الالتهاب وكانت قيمه التثبيط العليا في حالة المستخلص الايثانولي ا (65.25±1.2 %) وفي حالة المستخلص الميثانولي (58.135±1.23) بتركيز 300 µg/ml بوجود دواء الاسبرين كمادة قياسيه وكانت قيمته(67.35±0.56%) بتركيز 100µg/ml

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