Study of the effect of antibiotics and "Grape seed extract" on *Staphylococcus aureus* isolated from clinical sample

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Abstract:

The present study included a collection of 10 isolate from patients (urine, blood, and swabs) from Al-kindi teaching hospital in Baghdad city during the 3 months from 1/11/2017 to 1/12/2017, All bacteria isolated in this study were identified based on colonial morphology, and microscopic examination.

The standard disk diffusion method was used to determine the antibiotic sensitivity pattern of *S. aureus i*solates, seven antibiotic disks were used which include different groups of antibiotics . all *S.aureus* isolates were resistant to penicillin (100%) and (100%), Vancomycin , (75%),Tobramycin(33%),Azithromycin(50%)Oxacillin(100%),chloramphenicol (75%)**,** Tetracycline (12%).

Grape is a phenolics rich plants, Black Grape seed extract was reported to have many pharmalogically benefits including antioxidant, antimicrobial, anticancer and antiaging and anti Alzheimer's properties, Well diffusion method was used to study the activity of plant extracts was showed activity against *S.aureus*, result show higher activity than Antibiotics .

Introduction

Staphylococcus:

Staphylococcus is a group of bacteria that can cause a number of diseases as a result of infection of various tissues of the body. *Staphylococcus* is more familiarly known as staph (pronounced "staff"). Staph-related illness can range from mild and requiring no treatment to severe and potentially fatal .

The name *Staphylococcus* comes from the Greek *staphyle*, meaning a bunch of grapes, and *kokkos*, meaning berry, and that is what staph bacteria look like under the microscope, like a bunch of grapes or little round berries. (In technical terms, these are gram-positive, Over 30 different types of staphylococci can infect humans,

but most infections are caused by *Staphylococcus aureus*. Staphylococci can be found normally in the nose and on the skin (and less commonly in other locations) of around 25%-30% of healthy adults and in 25% of hospital workers. In the majority of cases, the bacteria do not cause disease. However, damage to the skin or other injury may allow the bacteria to overcome the natural protective mechanisms of the body, leading to infection **(1)**

Staphylococcus aureus:

Staphylococcus aureus is a type of bacteria. It stains Gram positive and is non-moving small round shaped or non-motile cocci. It is found in grape-like (staphylo) clusters. This is why it is called Staphylococcus.

Staphylococcus aureus belongs to the family *Staphylococcaceae*. It affects all known mammalian species, including humans. Further due to its ability to affect a wide range of species, *S. aureus* can be readily transmitted from one species to another. This includes transmission between humans and animals.

S. aureus may occur commonly in the environment. *S. aureus* is transmitted through air droplets or aerosol. When an infected person coughs or sneezes, he or she releases numerous small droplets of saliva that remain suspended in air. These contain the bacteria and can infect others.

Another common method of transmission is through direct contact with objects that are contaminated by the bacteria or by bites from infected persons or animals. Approximately 30% of healthy humans carry *S. aureus* in their nose, back of the throat and on their skin.

Around one third of healthy individuals carry this bacteria in their noses, pharynx and on their skin. In normal healthy and immunocompentent person, *S. aureus* colonization of the skin, intestinal tract, or nasopharynx does not lead to any symptoms or disease.

When *S. aureus* is isolated from an abscess or boil or other skin lesion, it is usually due to its secondary invasion of a wound rather than the primary cause of disease. *S. aureus* may similarly be isolated from abscesses, breast absecesses or mastitis, dermatitis or skin infections and genital tract infections**(2).**

Grape seed :

In the past decade interest on the topic of antimicrobial plant extracts has been growing **(3).**Plants used for traditional medicine contain a wide range of substaneous

that can be used to treat chronic as well as infectious diseases. The medical values of plants lie in some chemical substances that produce a definite physiological action on the human body **(4).**Substantial attention is presently focused on the potential health effects of grapes and grape products **(5).** The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds **(4).**Grape fruit contains various nutrient elements such as: vitamins, minerals, carbohydrates, edible fibers and phytochemicals. Polyphenols are the most important phytochemicals in grape because they posses many biological activities and health promoting benefits **(6)** .Grape is a phenol-rich plant and these phenolics are mainly distributed in the skin, stem, leaf and seed of grape rather than their juicy middle section **(4).** Polyphenols grape seeds are mainly flavonoids **(5).** Most phenolics in muscadines are located in the seeds, gallic acid, catechin and epicatechin are the main phenolic founds in muscadine seed **(3)** .Grape seed extract(GSE) is reported to have many pharmalogical benefits including: antioxidant ,anti-inflammatory ,anticarcinogenic and antimicrobial properities,slowing aging, Alzheimer's disease and other neurodegenerative disorders and diabetes Also GSE shows promise for application in the food industry as an. inexpensive novel natural alternative to reduce viral cotamination and enhance food safety **(7)**. Also grape l were found to exert antimicrobial activities on 11bacterial species associated with Catfish Clarias gariepirus spoliage **(8**).GSE high in proanthocyanidins, positively affected the in vitro demineralization and / or remineralization processes of artificial root caries lesions , suggesting it potential as a promising natural agent for noninvasive root caries therapy (**9**) The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants (**3**) .

Materials and Methods:

Specimens:

The present study included a collection of 10 isolate from patients (urine, blood, and swabs) from Al-kindi teaching hospital in Baghdad city during the 3 months from 1/11/2017 to 1/12/2017, All bacteria isolated in this study were identified based on colonial morphology, and microscopic examination.

Antibiotics sensitivity :

Antibiotic discs were placed on the surface of a Mueller-Hinton agar that has been inoculated with test microorganisms (0.5 MacFrland). During incubation, the

antibiotics diffuse outward from the discs creating a concentration gradient. After 18-24 hours, the zone diameter of inhibition is measured and reference tables are used to determine if the bacteria are Sensitive (S), Intermediate (I) or Resistant (R) to the antimicrobial drugs (**10)**. Results were interpreted in according to Clinical Laboratories Standards Institute (CLSI) **(11)**

Plant extracts activity:

plant extract's preparation :

Gape fruit were purchased from local markets in Baghdad. After opening the fruit, the seeds were cleaned and let to dry at room temperature . Then the peels grinding and 50 mg of plant's powder were dissolved in 500ml of ethanol 70% and extract for 7 hour by Soxhlet. Then the mixture was filtrated and concentrated by oven at 40 °C. These crude extracts were kept at 4 °C until use. of **(12)**

Bacterial Media (Agar Media)

Muller Hinton Agar prepared according to manufacturer's instruction which involved the suspension of 38 gm. in one liter of de-ionized water, after being completely dissolved with boiling, it was sterilized by autoclave at 15 lb. pressure for 15 minutes, then left to cool at 45-50oC, poured and left to solidify then put them in incubator at 37°C for 24 hours then stored in refrigerator until being used.

Antimicrobial Screening (in vitro):

The antimicrobial activity of seed Grape were measured by well diffusion method(**13,14**). The prepared culture plates were inoculated with different selected strains of *S.aureus* using spreading method. Wells were made on the agar surface with 6 mm cork borer. The position of the wells for each extract was marked at the outside walls of plates before application of plant extracts The extracts were poured into the well. Each well was filled with 100µl with corresponding extract with the help of a micropipette. The plates were incubated at 37±2 ºC for 24 hours for bacterial The plates were observed for the zone clearance around the wells. The resulting inhibition zones were uniformly circular. The diameters of the zones of inhibition were measured, including the diameter of the well. Inhibition zones are measured to the nearest millimeter, using a ruler, which is held on the back of the inverted petri plate.

Results and Discussion:

This study entails the important antimicrobial activity of the Grape seeds in inhibition of growth of Staphylococcus *S.aureus*.

The standard disk diffusion method was used to determine the antibiotic sensitivity pattern of *S. aureus i*solates. seven antibiotic disks were used which include different groups of antibiotics. It was revealed that all *S.aureus* isolates were resistant to penicillin (100%) and (100%), Vancomycin , (75%), Tobramycin (33%), Azithromycin (50%) Oxacillin (100%),chloramphenicol (75%), Tetracycline (12%).

Development of antibiotic resistance may be explained by different mechanism; it may either due to possing of β-lactamase by the isolate which may be encoded by transferable plasmids or could be due to apparent of tolerant strains. Our results were agreement with previous study**(15)** who found that all *S. aureus* isolates were resistant to penicillin and amoxillin and Staphylococcal hospital cultures produce high amounts of β-lactamase enzymes **(15)**

Alcoholic plant extracts showed antibacterial activity against *S.aureus* which may reflect the antibacterial activity of plant active ingredients that inhibit bacterial growth, as show in figure **(3).**

Well diffusion method was used to study the activity of plant extracts was showed activity against *S.aureus*, result show higher activity than Antibiotics

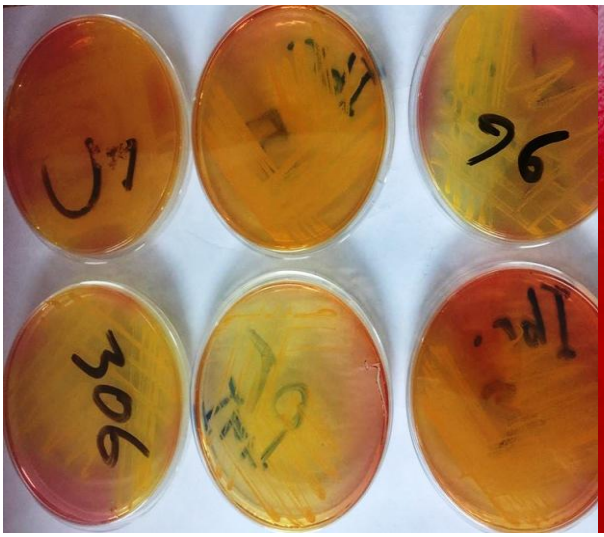
(Table 1) The inhibition zones (mm) of GSE against *S. aureus*

No. sample	Stock	80%	60%
80	20cm	15cm	11cm
16	19cm	8cm	10cm
5	20cm	10cm	15cm
9	19cm	10cm	15cm
4	Zero	Zero	Zero
2	23cm	15cm	20cm
3	24cm	10cm	22cm

This study agree with other researchers , they found that G+ve bacteria more effective than G-ve bacteria but with different concentrations. The different in antimicrobial activities of plant extracts depends on the solvents of extraction **(16)**

also **(17)** found that black grape seed extract (*Vitis vinifera*) was more effective against gram positive bacteria than gram negative bacteria and its effect also against pathogenic *C.albicans*.

Other researcher [showed that the partial hydrophobic nature of their phenolic compounds is responsible for the antimicrobial activity Accumulation and attachment of these phenolics to the bacterial cytoplasmic membrane eventually lead to the cell death.



Figure(1) *Staphylococcus aureus* on mannitol

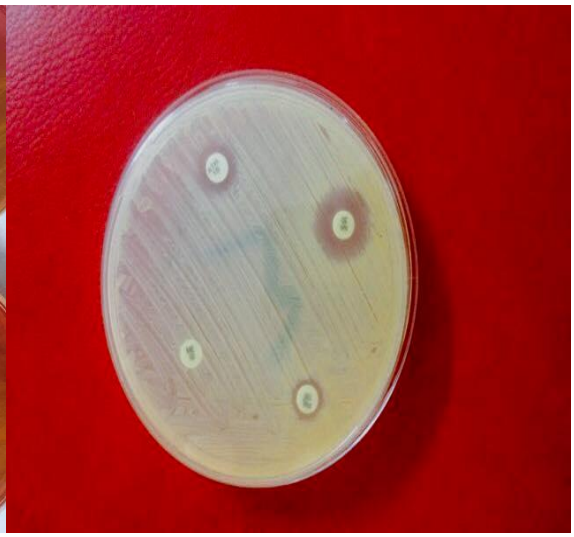


Figure (2) Antibiotic sensitivity

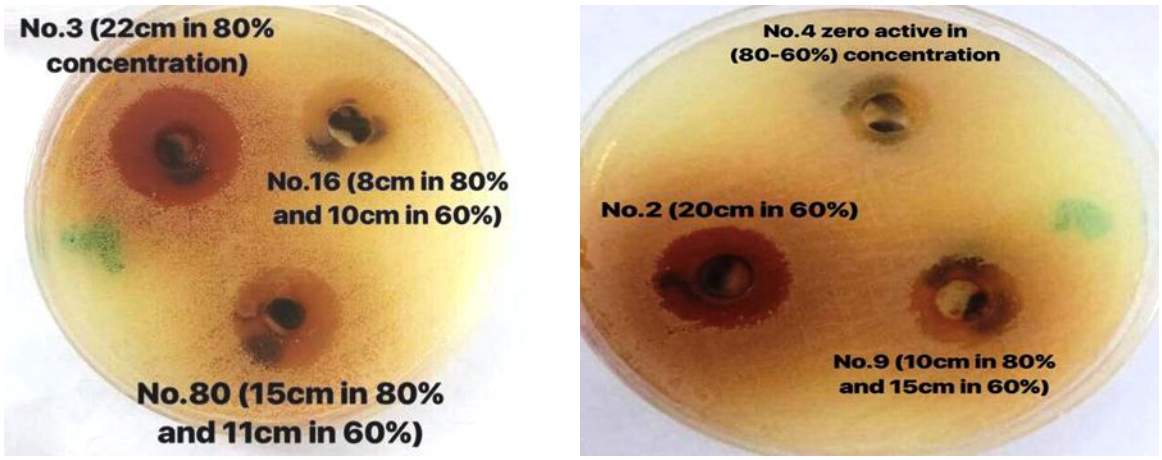


Figure (3) antimicrobial activity of GSE (Grape seeds extract)

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