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# USE PLANT EXTRACTS AGAINST SOME BACTERIAL SPECIES AND COMPARE THEM WITH SEVERAL ANTIBIOTICS

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## ABSTRACT

The aim of this study was to evaluate the biological activity of organic solvent extracts of *Euphorbia prostrate* on growth inhibition of *pseudomonasaeruginosa* and *staphylococcus aureus* and compare them with several antibiotics. The results of testing alcohol extracts showed high efficiency in inhibition of growth of s. aureus in concentration of 50mg / ml, but for *p. aeruginosa*, growth inhibition began at 70mg / ml. Results also showed that hexane extract had no effect on both bacteria. on the other hand, Ethyl acetate extract had inhibitive effects on *s. aureus* at 70mg / ml and on*p. aeruginosa* at 90mg / ml. When the activity of the three organic extracts on inhibition of test bacteria were compared with several antibiotics, identity of inhibition degree was found between them and even superiority on some others.

**Keywords:** Plant Extracts, Biological Activity of Organic solvent extracts.

## INTRODUCTION

The plant *(E .prostrata*) back to the family( milk Euphorbiaceae) characterized by production of a juice milky Milk work in Iraq resides this plant from the south to the north, especially in public parks, also resides in the tropics warm, and characterized this Gender owning small papers and a wooden leg [(1)]. Most types are Eaphorbia spp. Toxicity so used in medical treatments as the genus *E. resinifera* drug called Euphorbium which uses highly antiseptic effectiveness of its ability to kill contaminated microbes. *E. neriifolia*. highly toxic and increases the toxicity up boiling it was used material milk to treat rheumatism and general weakness and pain of the teeth Chinese people have been using material milk to treat diseases above [(2,3)], either *E .antiquorurn*

  

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has been used since ancient times in the preserving of meat and Based on the high efficiency of this plant extracted the effect of dissolved studied within the organic solvents toward the two types of bacteria:

**STAPHYLOCOCCUS AUREUS**

The cell has a diameter of approximately 1µm gather bunches of grape like colonies after the antenna cuddling be soft and shiny circular high, either in the optional anaerobic conditions, the optimum growth temperature is $370C^0$ and PH = 7.4-7.6 [4,5]. *Staphylococcus aureus* virulence factors have assisted in pathogenicity and include protein-A, which is linked covalently layer peptidoglycan. Some isolates contain capsules which consist of glucosaminuronic acid and lose this capsule during the growing. *Staphylococcus aureus* ability to produce enzymes Proteinase Hyaleurondase, Nuclease, phosphatase, Fibrinolysin coagulase [6].*S. aureus* bacteria and is one of the relevant medical and public health importance of microbiology and to their capacity to produce many virulence factors that enable it to penetrate the body and the ability togenerateaseptic infection and resistance to many antibiotics [7].

**PSEUDAMONAS AERUGINOSA**

Bacteria bacillary negative gram, airbags mandatory producing pigments such as (Pyocyanin) and Florin[8]. This bacteria throws nutrition and do not require complex food materials and can multiply in wet environments as well as on being one of the causes of infections secondary infection in those who are asleep patients in hospitals which gave its importance in terms of bacteriological studies of these patients are exposed to surgery and blood transfusions and some cases that require a hole stalk hobby and other situations that facilitate the entry of bacteria into the patient's body and then occurrence of infection [9,10,11].

**MATERIALS AND METHODS OF WORK**

**1. Sampling plant Euphorbia prostrate:**

Plant samples were collected under study from different areas in the provinces of Anbar and Mosul and transferred to the laboratory and cleaned of impurities and then washed and left to dry and conducted by stirring constantly to prevent rot and after the sample is grinded by electric grinder to get the powder, which put in a dry nylon bags and save it in the refrigerator until use the plant were diagnosed by lush, Faculty of Science, University of Mosul. It was obtained bacterial isolates from the Central Health Laboratory after confirmation of which were conducted diagnostic tests to make sure the races.

**2. Prepare an implanted bacterial**

  

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For the purpose of airborne bacterial preparation was activated bacteria work a new culture medium at the center of Agar nutritious and incubated for 18-24 hours a degree 370C then moved 3-4 colonies to test tubes containing 4 ml of nutrient broth Nutrient broth and incubated for 4-5 hours a degree 370C then was stuck equation by bacterial physiological saline solution Normal Saline (attended by dissolving 0.85 g of sodium chloride in 100 ml of distilled water) for tube equal to 0.5 McFarland used number in a sensitivity test.

**3. Perpetuating grown fresh**

A new culture medium by ( Sub culturing) every 4-6 weeks amid Agar nutritious and lap degree 370C temperature for 24 hours kept the isolates in the fridge degree 40C for the purpose of long-term conservation transmits bacterial growth aged 18-24 an hour to the center of broth infusion brain and heart Brain heart infusion broth and add 5% of glycerol and saves degree $20C^0$

**4. Prepare organic solvent extracts**

Were prepared organic solvent extract powder plant Euphorbia prostrata dry according to the method [12]. Where were three solvents tested different polar organic which is ethyl alcohol (Ethyl Alcohol) as a solvent poles and Ethyl Acetate (Ethyl acetate) as a solvent medium polar and hexane (n-Hexane) as a solvent does not poles [13] .take 10 grams of powder dry matter of the plant were extracted materials which are sequential device extraction (Soxhelt) by 200 ml of each solvent for 24 hours after it has been the focus of material extracted rotary evaporator temperature 40-450C and then dried by oven and 400C degree heat for the purpose of preparation of the concentrations used in the experiment for the purpose of testing the effectiveness of inhibitory extract organic solvents to test bacteria were prepared stocks Stock solution to extract organic solvents [14].

**A.** extract ethanol: Taking 1 g of dry extract and melted in 2 ml of ethyl alcohol 99% and then complete the volume to 10 ml with distilled water, but for the control consists of 2 mL ethyl alcohol and he finished size with distilled water to 10 ml.

**B.** extract hexane: Taking 1 g of dry extract and melted in 1 ml hexane and then melted in 1 ml ethyl alcohol 99% and fuller size with distilled water to 10 ml tube and control be from 1 ml hexane with 1 ml ethyl alcohol has completed volume with distilled water to 10 ml.

**C.** Acetate extract ethyl: Taking 1 g of dry extract and melted in 1 ml Ethyl Acetate and then melted in 1 ml ethyl alcohol 99% and fuller size with distilled water to 10 ml and control included 1 ml Ethyl Acetate with 1 ml ethyl alcohol 99% and completed the volume to 10 ml.

**5. Testing effectiveness anti of plant extracts in vitro**

  

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Followed by Agar diffusion method by drilling wells [15] to test the sensitivity of bacteria for plant extracts and this method by formation 4 ml digging equal the dimensions in the center of Mueller Hinton steel Muller Hinton agar and a diameter of 8mm by cork borer to contain solutions of the plants by 0.4 ml per hole after the publication of 0.1 ml of airborne bacteria at the center and then put dishes in the refrigerator for 24 hours for the spread of solutions of the plants in the culture media [16] and then incubated degree 370C for 18-24 hours and read the result measured the diameter of inhibition zone [17].

**6. Minimal inhibitory concentration (MIC)**

Applied for this purpose way relieve Agar dilution method[18] where he was mixing 2 ml of each concentration of plant extract with 18 ml of central Muller Hinton melted at a temperature of 500C in addition to a dish of control which does not contain the extract. Then airborne bacterial transplant Comparative turbid tube McFarland

No .0.5 by cotton swab Cotton swab on media containing concentrations of theextract in addition to the central control, left counterpoint few minutes to dry and then incubated normal conditions.

**7. Macfarland Standard Solution**

It consists of :

solution A: dissolving 1.75 g of barium chloride H2O .BaCl2 in 100 ml of distilled water

solution B: adding 1 ml of concentrated sulfuric acid H2SO4 to 100 ml of distilled water

When you use 0.5 ml Added solution -A- to 99.5 ml of -B- lotion and use the solution for comparison to give an approximate number of bacterial cells $1.5 \times 10^8$ cells / ml and when to check the sensitivity to antibiotics and plant extracts in determining the value of the minimum inhibitory concentrations MIC plant extracts [19].

**8. Testing the sensitivity of bacteria to antibiotics**

Used group of antibiotics processed from Oxoid Company Table (1) and followed the way counter the spread of the Agar[17] where he was the center of Mueller transplant Hinton solid bacterial Comparative a tube McFarland No. 0.5 and left the dishes for a quarter of an hour to dry then put antibiotic tablets to the center steel and then incubated in the incubator degree 18-24saah 370C and for a period, then the results were compared with the results of plant extracts, it has been using a range of antibiotics, as in table 1.

  

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| Company | cod | µg/Disantibiotic |
|---|---|---|
| Oxoid (England) | OB | Cloxacilin (5µg) |
| Oxoid (England) | C | Chloramphenicol (30µg) |
| Oxoid (England) | P | Penicillin (10µg) |
| Oxoid (England) | CAR | Gentamycin (100µg) |
| Oxoid (England) | RD | Refadin (30µg) |
| Oxoid (England) | Amo | Amoxylin (25µg) |

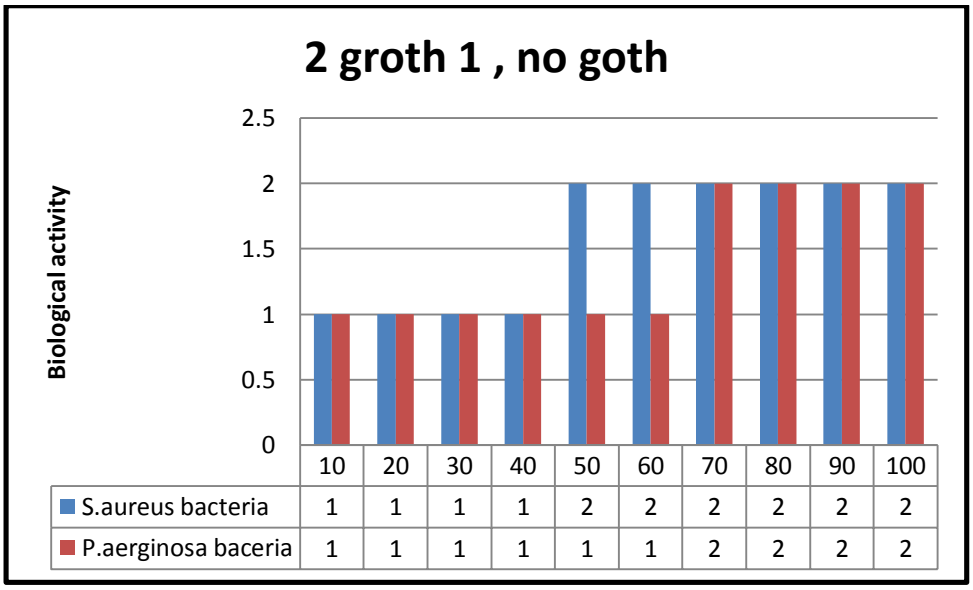
## RESULTS AND DISCUSSION

Results obtained indicated that the anti-effectiveness and the value of minimum inhibitory concentrations increased with increasing concentration of the extract in the center and that's the same results to as pointed out by some studies [16,20], varied effectiveness of extracts contrast organic solvents and accordingly change genus bacterial and this variation is as follows:

### A- Ethyl Alcohol extract 99%:

This was extracted obvious effect towards the bacteria *S. aureus* than against bacteria *P. aerginosa* were affected *S. aureu* within the concentration of 50 mg / ml, while *P. aeruginosa* affected within the concentration of 70 mg / ml, as shown in Figure (1)

**Figure (1) shows the biological activity of Ethyl Alcohol concretions for bacteria sex (MIC).**



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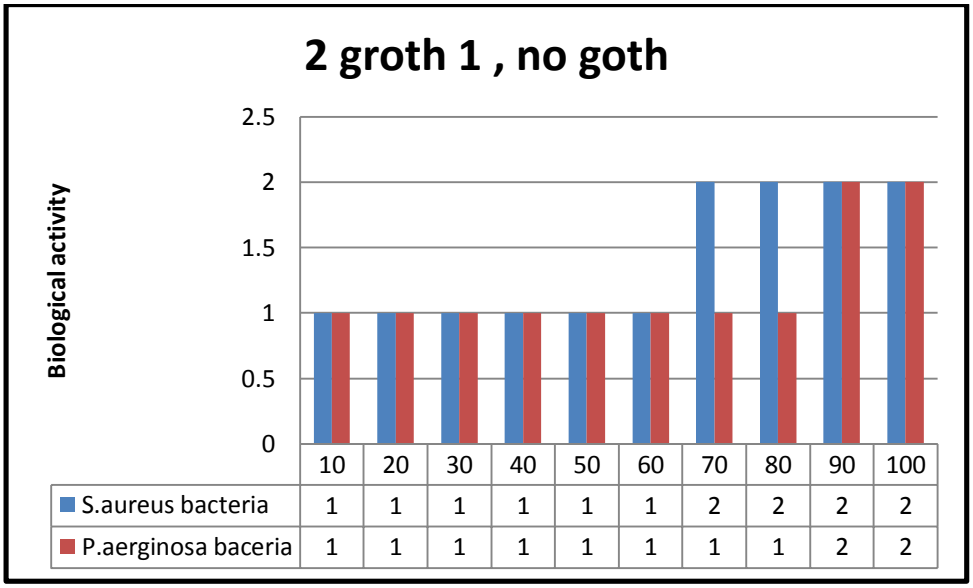
**B**- with respect to hexane extracts it has been observed that this extract does not appear any act inhibitory at any concentration of concentrations for both genus, and may be due largely to lack of permeability of the bacterial cell membrane of these materials or not extracted in the effective influence the success of the enzymes and proteins located inside the bacterial cell, which is one of the necessary and effective for the growth and multiplication of bacteria materials [21.22] showed acetate extracts ethyl actually mixed daunting gender bacteria act inhibitory has appeared in bacteria, *S. aureus* within a concentration of 70 mg / ml in the bacterium *P. aeruginosa* has It appeared within a concentration of 90mg / mL just as in Figure2.

**Figure (2) The biological activity for concentrations the ethyl acetatextract to bacteria MIC**




The results show that there is variation range in the inhibition of extracts) and also varied for genus of bacteria under study came in agreement as stated from the results [23.24], and this difference is due to the degree of influence the types of plant extracts in microbiology returns to various factors, the most important type of vegetable extract and extraction method as well as the polar solvent used type..

The figure -3- shows the results of examination of the sensitivity of the bacteria to a range of antibiotics used within the life of this experience is clear that the bacteria*s. aureus* cationic dye Cram was more sensitive to the anti-life, compared with*p. aeruginosa* may be due to differences in the composition of the cell membrane in both groups as characterized by bacteria *s. aureas* as pungent most antibiotics compared to pigment Cram [25].

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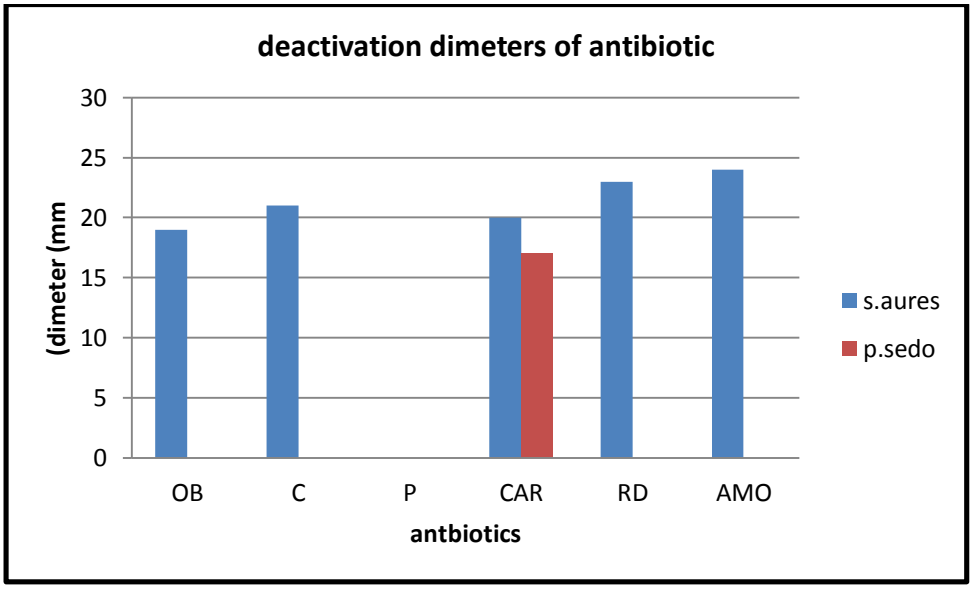
Note from Figure (3) resistant bacteria *s. aureus* to penicillin because they are capable of producing enzymes B-Lactamase which analyzes loop B-Lactamas in the penicillin and its derivatives and then converting the latter to the compound is effective [26], while the bacteria *p. aeruginosa*, resistant to the antibiotics penicillin group showed as provided for in [27] that cause resistance to bacteria *p. aeruginosa* is the change that occurs in the permeability of the membrane of bacteria and the production of enzymes B-Lactamase which show resistance to penicillin [28] through the previous results and Figure (3) note that negative bacteria to dye cram *p. aeruginosa* were resistant to most antibiotics used and the life of this variant of the bacterium s. This may explain aureus bacteria possess *p. aeruginosa* membrane is self-barrier membrane is composed of Lipopolysaccharide associated with complex proteins act to prevent a lot of life to the passage of antibiotics within the bacterial cell [25.29], and may be mutations and genetic cause of life to prevent the passage of antibiotics into the cell [25.29, 30].

**Figure 3 shows the inhibition diameters in mm antibiotic used against genus of bacteria**




There are many factors that affect the success of the assay sensitivity, including the amount and type of center used in the experiment, where the use of central Mueller Hinton which is characterized by being appropriate for the growth of bacteria under study, as well as a constant pH value that can contribute to the success of the antibiotic action [19], and must be to point out other factors that contribute to the success of the examination of the sensitivity of the size of the vaccine user and the circumstances of the lap from the heat and through the current study notes that the bacteria have shown a clear sensitivity to the group extracts organic plant may be greater

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when compared to the sensitivity of some of the life of antibiotics, which has affected within the concentration of 50 mg / mL (MIC) organic extract (ethanol 99%) of the bacteria s. aureus at a concentration of 50 mg / mL, and it can be explained by the fact that these bacteria these extracts were not familiar with before so I did not show resistance to them, and there is another explanation is that the material learned intimacy chemical to interact with the bacterial cell components and the presence of recipients on the bacterial cell wall and vectors suitable for molecules to inside the cell in order to help stop doing enzymes and molecules effective (14.13)

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