

**Antimicrobial effect of some herbal plants crude extracted on
Staphylococcus spp and E.coli and mix with some antibiotics**

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Summary:

Antibacterial activity of hot aqueous extract prepared from five plants against two pathogenic bacteria staphylococcus spp and E.coli .In this study taken five plants Leaves and flowers were , Allium sativum ,Dianthus carphyllus, Mentha, Elettaria cardamomum and Coriandrum sativum. These sample taken from Al-sader hospital and the plants taken from market in misan city. We use the disk diffusion methods as the antimicrobial susceptibility test also mix plants extract crude with antibiotics Ciprofloxacin, Amikacin, Imipenem, Lomfloxacin, Penicillin and Amoxicillin. The study was aimed to evaluated the inhibitory effect of aqueous plant extract against two isolates staphylococcus spp and E.coli in vitro and synergism with some antibiotics.

key words : Plants extract ,staphylococcus spp , Ecoli ,synergism , antibacterial activity.

Introduction:

In the past, antibiotics have been critical in the fight against infectious disease caused by bacteria and other microbes. Antimicrobial resistance is a worldwide growing problem, isolation of microbial agents less susceptible to regular antibiotics and recovery of resistance regulate during antimicrobial therapy is increase throughout the world, part of the problem is due to increasing use, and misuse, of existing antibiotics in human and veterinary medicine[1,2]. Herbs and spices are generally considered safe and proved to be effective against certain ailments. They are also extensively used, particularly, in many Asian, African and other countries[3].

Louis Pasteur was the first describe the antibacterial effect of onion and garlic juices[4,5].Garlic is a strong antibacterial agent against both Gram–positive and Gram–negative bacteria such as E.coli, Salmonella spp, Streptococcus spp, Staphylococcus aureus, Klebsiella spp, Proteus mirabilis, Shigella dysenteriae ,Pseudomonas aeruginosa and Helicobacter pylori,[6–7] also it's effective even against those strains that have become resistant to antibiotics[8].

Carlic(Allium sativum) is one of the most extensively researched medicinal plants and its typical order and antibacterial activity depend on allicin produced by enzymatic activity of allinase (a cysteine sulfoxide lyase) on alliin after crushing or cutting garlic clove [3–9]. Clove belongsto a tree Eugenia caryophyllata (syzgium aromaticum is used as a spice in almost all the world fare. Bud oil of clove has natural behavior and the main properties include antioxidants, insecticidal ,antifungal and antibacterial properties[10] . The most important constituent of clove is the phenyl propene eugenoldue to which it has strong characteristics aroma. Major parts of clove consist of eugenol comprises 70 to 90% and remaining 15%consist of dry weight [11].Mold,yeast and bacterial growth could be inhibited by the application of clove essential oil [12].Cardamom (Elettarta cardamomum) is dried fruit of the tall perennial herbaceous plant,Elettarta cardamomum belonged to the family Zingiberaceae.It is small cardamom

well known as the green of species is a rich spice obtained from the seeds of a perennial plant [13]. *Mentha piperita* oil has antibacterial effects against both gram positive and gram negative bacteria [14]. Peppermint extracts are bacteriostatic against *Streptococcus pyogenes*, *Streptococcus aureus*, *E. coli*, *Serratia marcescens* and *Mycobacterium ovium* [15]. *Coriander sativum* contains antioxidant, which can delay or prevent the spoilage of food seasoned with this spice. Chemicals derived from *coriander sativum* leaves were found to have antibacterial activity against *Salmonella choleraesuis* [16]. *Syzygium aromaticum* commonly called cloves and locally called *kenepeli* is a common spice as antibacterial activity of clove against *E. coli* and *Pseudomonas* spp gram negative and positive bacteria [17]. Synergism is defined as a positive interaction created when two agents are combined and together they exert an inhibitory effect that is greater than the sum of their individual effects. The synergism is a new concept in developing agents for antibacterial, antioxidant and also for anticancer activity [18].

Materials and methods:

Microorganisms:

Use two types of isolates pathogenic from wound infection *E. coli* as gram negative and *Staphylococcus* spp as gram positive. These samples taken from Al-Sader hospital and the plants taken from market in Misan city.

Preparation of the plant extracts:

The aqueous extracts fifty gm from the dry powder of the medicinal plants were put in conical flask size 1000ml, 450ml of distilled water were added, left on hot plate with magnetic stirrer at 25°C for two days. Suspension was filtrated by filter paper 0.45mm diameter type Whatman No.2. Extract was dried in oven at 45°C, then weighted by using sensitive balance, kept in the refrigerator at 4°C for usage in another time [19].

Preparation of Bacterial Suspension:

Bacterial suspension obtained from wound infection diagnosed isolated(E coli, staphylococcus aureus , prepared on Muller Hinton Broth and incubated at 37c for 24 hours, then 1micron was taken from each bacterial suspension and diffused on Muller Hinton agar by using L-shape spreader, then 3 equal distant wholes were mode inside the plates for putting different plant extract concentration plate were incubated at 37c for 24 hours.

Antibiotic sensitivity test:

Antibiotic sensitivity test was performed by Kirby–Bauer’s disk diffusion technique as following: A sterile cotton swab was dipped into bacterial suspension matching to 0.5McFarland tube for each isolates and streaked it in three directions on the surface of Muller–Hinton agar plates then left 5–10 minutes on room temperature. By using a sterile forceps, the selected antibiotics (7 antibiotics) was put on the surface of plate and left it for five minutes, incubated at 37 ° c for 24 hours then the zones of inhibition were measured in millimeter by using ruler.

Synergy test:

To test the combined plant crude extract with equal volume antibiotics (0.1:0.1) were mixed by [18].

Results:

Result illustrated here indicate that both of crude extracts from some plants leaves showed anti–bacterial activity against either G +ve bacteria staphylococcus aureus, and G–ve Escherichia coli) however the crude extract of allium sativum,syzgium aromaticum,Elettarta cardamomum,Mentha piperita and dianthus carphyllus were more potent antibacterial agent against gram+ve bacteria than G–ve bacteria.In table (1) show the plant crude extract inhibition zone the high concentration

in *Allium sativum* in case of *staphylococcus* spp 10^{-1} was 21mm and lower concentration in 10^{-2} , 10^{-3} was 19mm, 15mm and in *E.coli* was 10^{-1} , 19mm, 10^{-2} , 10^{-3} was 15, 14 and the lower concentration in *elettaria cardamomum* *E.coli* 10^{-1} , 10^{-2} , 10^{-3} was 10mm and in *staphylococcus* spp 10^{-1} , 10^{-2} , 10^{-3} was 17mm, 17mm, 10 mm respectively. In table (2) the antibiotics antagonism with plant extracted the high concentration inhibition zone .Effect of ciprofloxacin 5 mg mix with *allium sativum* in *staphylococcus* spp was (32mm) and *E.coli* (33mm) and the low in penicillin. Some plant extract mix with *Allium sativum* some antibiotic in table (3) show the high inhibition zone in *staphylococcus* spp was (20mm) ciprofloxacin and in *E.coli* was 11mm and the low effect with penicillin *staphylococcus* spp (10mm) and no effect in *E.coli* .

Discussion:

In a constant attempt to improve their quality of life human have used plant as source of food clothing , medicine and cosmetics in life .Some plant as are know as medicinal because they contain active substances that course certain reactions to cure of disease on the human organism. In our study the effect of *Allium sativum* warty hot extract crude against *staphylococcus* spp was (19mm) and *E.coli* (17mm) in concentration 10^{-1} these study agree with [20] that show the concentration of (*Allium sativum*) effect on *staphylococcus* spp in different sample like in sputum 33.3%the MIC was 0.75, 15, 22.5, 30, 45 was positive test .In table(2) ciprofloxacin(5mg)give 20mm or sensitive and 21mm in *E.coli* The antimicrobial activity of plant extract on *staphylococcus* spp strains were conference and synergism were possible with all the antimicrobial drugs tests ciprofloxacin presented synergism with *Allium sativum* was (30mm) *staphylococcus* spp and *E.coli* (33mm) also this study agree with [21] that show effect of raw extract of *Allium sativum* antagonism with ciprofloxacin in *staphylococcus* spp 42mm and in *E.coli* 30mm. In case effect of antibiotics ciprofloxacin

with aqueous garlic extract range from 40mm to 45mm) therefore agreement with our study[22].

Table (1): Plants crude extract inhibition zone effected on staphylococcus spp and E.coli

E.coli			Staphylococcus spp			Plants crude extract inhibition zone (mm)
10^{-3}	10^{-2}	10^{-1}	10^{-3}	10^{-2}	$C=10^{-1}$	
14	15	19	15	19	21	1- Allium sativum
10	14	17	8	17	19	2- Dianthus carphyllus
10	14	15	8	9	19	3-Mentha
10	10	10	10	17	17	4-Elettaria cardamomum
9	10	11	15	17	18	5-Coriandrum sativum

C=Concentration

Table (2): Effect some plants extracts mix with some antibiotics on staphylococcus spp and E.coli.

Inhibition zone E.coli	Inhibition zone Staphylococcus spp	Antibiotics mix with plant extract
33mm	32mm	Ciprofloxacin+Allium sativum
27mm	30mm	Ciprofloxacin+syzygium aromaticum
23mm	26mm	Amikacin +Allium sativum

30mm	28mm	Amikacin syzgium aromaticum
32mm	30mm	Imipenem +Allium sativum
25mm	26mm	Imipenem +syzgium aromaticum
32mm	22mm	Lomfloxacin +Allium sativum
28mm	26mm	Lomfloxacin syzgium aromaticum
10mm	25mm	Lomfloxacin +Allium sativum
9mm	22mm	Methicillin syzgium aromaticum
11mm	21mm	Penicillin+ Allium sativum
12mm	23mm	Penicillin +syzgium aromaticum
13mm	22mm	Amoxicillin+Allium sativum
18mm	28mm	Amoxicillin +syzgium aromaticum

Table (3): Antibiotics sensitivity test for Staphylococcus spp and E.coli

E.coli	Staphylococcus spp	Antibiotics
21mm S 17mm S	20 mm S 18.5mm S	Ciproflacin (5mg) Amikacin (10mg)
21mm S	22mm S	Imipenem (5mg)
20mm S ----- -----	19mm S ----- 10mm R	Lomfloxacin (5mg) Methicillin (10mg) Penicillin

		(10mg)
10mm R	11mm R	Amoxicillin
		(10mg)

S =sensitivity R= Resistance

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