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Evaluation of Antimicrobial Activity of Different Aquatic Extracts Against Bacterial Isolates from UTI in Babylon Province, Iraq

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There are incrementsto the need ofthe usage of new non-chemical medicines, pharmaceuticalsand health products. Plantsare traditionallyused as medicine over the world for their pharmacological values. They effectively used as anti-fungal,antiviral,anti-bacterial, anti-inflammatory, anti-diabetic, anti-oxidant anti-ulcer, andantitussive. Thisstudy aimsto investigate the effects of plant extracts (*Glycyrrhiza glabra*, *Cuminum cyminum*, *Zingiber officinale*,*Origanum majorana* and *Petroselinum crispum*) against different types of gram-positive and negative bacterial isolates. Antimicrobial activity of tested aqueous extracts by well-diffusion methodagainst various bacterial isolates were done, to estimate their antibacterial activity. Results showed that the aquatic extracts of different plants produce a good antibacterial effects when compared with the synthetic antibiotic ciprofloxacin. As all bacterial isolates in this study where sensitive to these extracts with variable ranges of inhibition zones ranging from 18-32mm in diameter. Thus we can concluded that the aquatic extracts of *Glycyrrhiza glabra*, *Cuminum cyminum*, *Zingiber officinale*,*Origanum majorana* and *Petroselinum crispum* can be beneficial as treatment of UTI-causing bacteria.

Keywords:*Glycyrrhiza glabra, Cuminum cyminum, Zingiber officinale, Origanum majorana,Petroselinum crispum.*

Plants are rich sources of natural products that are extractedfrom them due to their content of active substances that used for centuries.These extracts can be used as antimicrobial activity to treat various diseases caused by pathogenic organisms in addition to their numerous pharamaceutical effects such as antibacterial, antifunfial,antiparasitic and anticarcinogenic[1].The use of crude plant extracts inherbal medicine for treatment of diseasescan be seen in different form like infusion or tincture[2]. Bacterial resistance to different types of antibiotics is growing because of genetic variability and mutation therfore we must understand the genetic mechanisms of resistance to develop new drugs from plants that have natural products with safety and efficiency for human being[3].

Plant extracts widly used to reduce the bacterial growth of most infections.The antimicrobial activity of them dependent on their methodes of extraction and chemical structures of plants. *Glycyrrhiza glabra* has important chemical components called licorice extracted from its roots that have medical benefits as antibacterial,antiviraland anti-ulcerative activity,it used for treatment of upper respiratory and liver diseases[4,5].

Cuminum cyminum has anticholesterol and antimicrobial avtivity and the active compnents

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of it is cumin oil or cuminol, these extracts are important in stimulation of digestive system enzymes secration to treat different types of diseases and used as bactericides against plant bacterial diseases [6,1].

Ginger (*Zingiber officinale*) has anti-microbial activity that can be used in treatment of bacterial infectionsthrough its aromatic and medicinal properties**.**Both ethanolic and aqueus extraction of Ginger have bactericidal effects on some types of pathogenic organismis[7,8]. *Petroselinum crispum* extractes have antibacterial activity,damage bacterial cell wall and inhibit the bacterial growth in addition to their cytotoxic activity at higher concentration[9].

Many researches found the *Origanum majorana* has an essential oils that affect the bacterial growth through altering cell membrane permeability, and causing a distortion of the membrane structure,also the extractes of this plant has agricultural, pharmaceutical and interfer with cosmetic industries[10].

The antimicrobial activity of plant extracts have been comparad with the effects of different types of antibiotics through measuring the inhibition zones of bacterial growth, ciprofloxacin has a wide antibacterial sepectrum against the pathogenic gram-positive and -negative bacteria that caused different types of urinary tract, respiratory tract,skin infections and other types of infections through inhibition the action of enzymes that interfer with bacterial repliction[11].

Aim

This study aims to investigate the action of plant extracts(*Glycyrrhiza glabra*, *Cuminum cyminum*, *Zingiber officinale*,*Origanum majorana* and *Petroselinum crispum*)against a number of positively- and negatively-gram stained bacterial isolates and copmarad them with the effects of ciprofloxacin.

MATERIAL AND METHODS

Extraction Methods

Plants were collected fromthe market. Aqueous extracts were prepared by soaking 30 grams of powder in 100ml of distillated water, leave them to stand for 72 hours, and sterilized by using Millipore 0.45 filter paper. 50% concentration of the extract was obtained by this way[12].

Bacterial Isolates

Anoverall of eightnegatively-gram stainedand threepositively-gram stainedbacterial isolates were collected from urine clinical samples (UTI) and used in this study. These bacterial isolates were; *S. saprophyticus, S. epidermidis,S. aureus, P. fluresence,P.aeruginosa,E. aerugenes, E.coli, K. pneumoniae, P. vulgaris, Acinetobacter,Proteus mirabilis.* All these bacterialisolates were cultivated for activation and re-cultured as three successive times on nutrient agar plates.Identification and diagnosis of these isolates were applied by conventional biochemical procedures[13].

Agar Well Diffusion Assay for*In vitro*Testing the Antimicrobial Activity (14)

Bacterial growths of Loopfull from each bacterial isolates were taken and applied into nutrient broth,incubation at 37°C for 18 hours. Normal saline was used to dilute the bacterial suspensions to adjust the turbidity and comparing with standard McFarland tube number 0.5. Cotton swab dipped into the tube of suspension and streaked Mueller-Hinton agar plates,left them to dry for 5-15 minutes at room temperature. Four wells of about 5mm in diameterwere made in the media by cork borer, 0.1ml of theextracts were added. Then incubationovernight at 37 C°. Zone of inhibition was measured,disks of ciprofloxacin were add in the center of agar plate to compare the results of bacterial inhibition[13].

RESULTS AND DISCUSSIONS

The evaluation of bacterial inhibitory activity of aquatic extracts of *Glycyrrhizaglabra* was processed using theagar diffusion test,as in Figure (1). This figure shows that *Glycyrrhizaglabra* exhibited an inhibitory activity against all the bacterial isolates of the study with inhibition zones ranging from 18-25mm, with the largest inhibition against *E.coli* (25mm) followed by *Pseudomonasaerogenosa* and *Staphylococcussaprophyticus* (24 and 23mm) respectively. Lowest inhibition against *Klebsiellapneumonia* and*Enterobacteraerogenes* (18mm) for each.

Figure (2) shows the inhibitory activity of aquatic extracts of *Cuminumcyminum* against different bacterial isolates in the study. Results demonstrated that inhibition zones were the

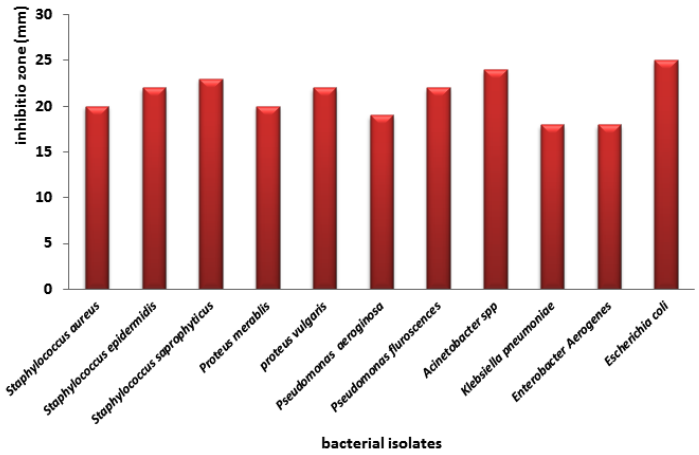

Fig. 1. Antibacterial activity of *Glycyrrhizaglabra* against bacterial isolates

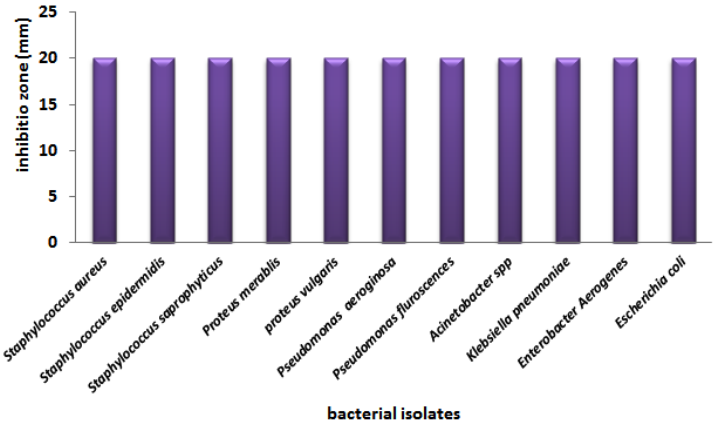

Fig. 2. Antibacterial activity of *Cuminumcyminum* against bacterial isolates

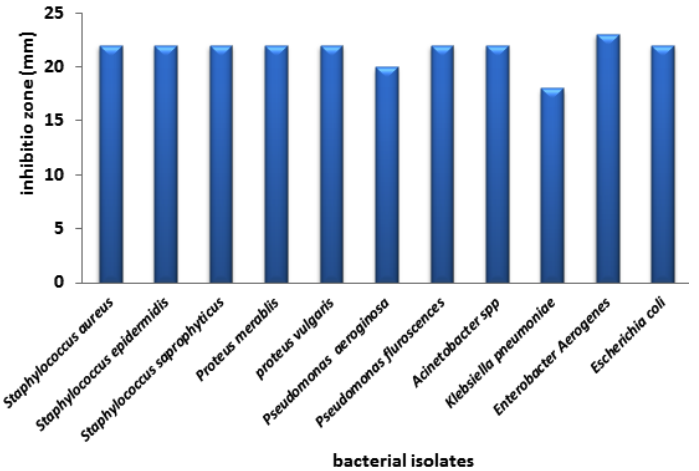

Fig. 3. Antibacterial activity of ginger against bacterial isolates

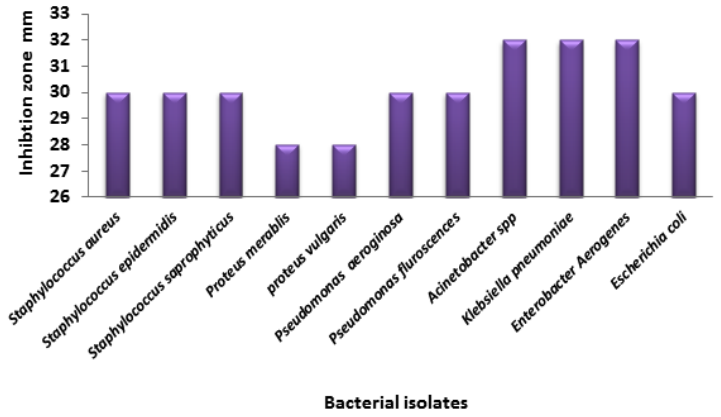

Fig. 4. Antibacterial activity of *Petroselinumcrispum* against bacterial isolates

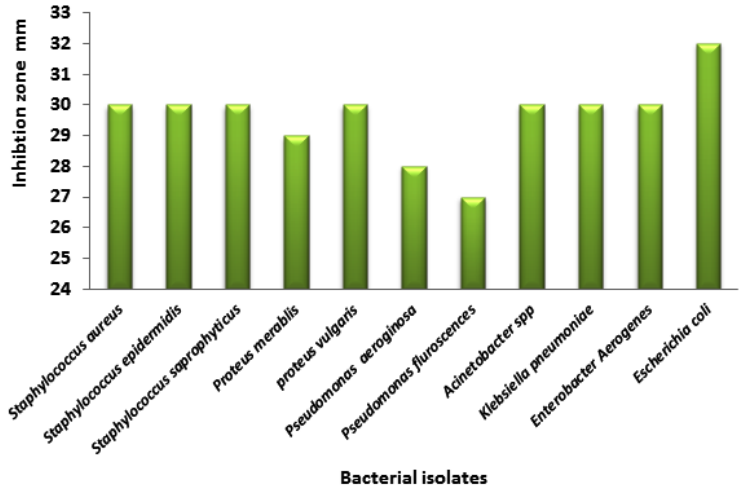

Fig. 5. Antibacterial activity of *Origanummajorana* against bacterial isolates

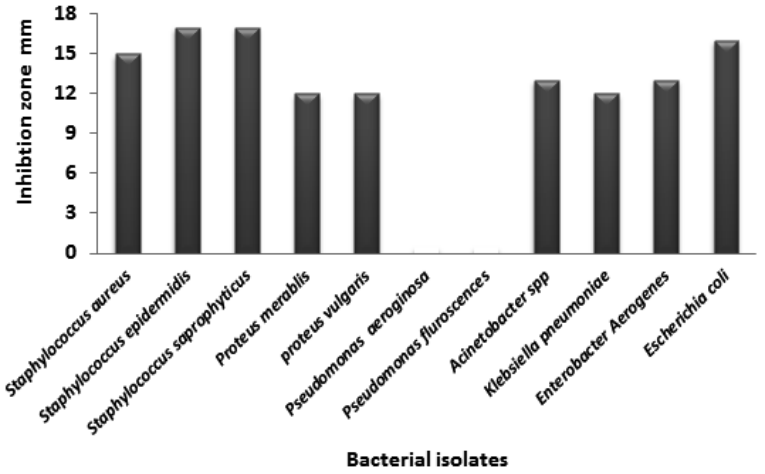

Fig. 6. Antibacterial activity ofCiprofloxacin against bacterial isolates

same (20mm) against all the tested bacterial isolates. While the diameter of inhibition zones produced by Ginger were ranging from 23mm against *Enterobacteraerogenes* to 18mm with *Klebsiellapneumonia*; and 22mm against most of isolates, figure (3).

The antibacterial effect of *Petroselinumcrispum* exhibited as a high inhibition level as compared with other extracts, as the inhibition zone diameters range from 32-28mm; higher effects against *Klebsiellapneumonia, Enterobacteraerogenes* and *Acinetobacterspp* (32mm), followed by 30mm as an inhibition against 6 bacterial isolates, and the lowest one (28mm) with *Proteus mirabilis* and *Proteus vulgaris* (4).

Results in table (5) revealed the antibacterial inhibitory effect of *Origanummajorana*Gram positive and negative bacteria with the highest inhibition 32 mm against *E. coli*, 30 mm against seven bacterial isolates, while the least inhibition against *Pseudomonas aeruginosa*and *Pseudomonas fluroscence*(28) and 27mm respectively. In contrast to all these aquatic extracts, Ciprofloxacin antibiotic is used as a synthetic antibacterial reagent; figure (6). This showed resistance of bacteria to this reagent; especially *Pseudomonas aeruginosa*and *Pseudomonas fluroscence* 0 mm, while the largest effect were only reach to about (17 mm) with staphylocci and *E.coli*, smallest zones were 12 mm with Proteus spp.

Extract of Licorice root has been used traditionally for a long period od history asmedicines and folk preparations, and being used in many diseases from hundredyears before[15]. It contains a large number of active components. About 40-50% of its total dry material weight is accounted of water-soluble, biologicallyactive complexes.

Glycyrrhizin represents about 10-25% of liquorice root extract. It is considered to be the most commonly used of the folkmedicines in Asia.Its structure is a saponin compound[16, 17]. Thus due of the existence of these different secondary metabolites such as flavonoids, alkaloids, and saponins in its aquatic-root extract, it exhibits potent antibacterial activity[18, 19], also it has a role as antiviral agent against many viruses[20, 21].

*In vitro*works haddemonstrated that aquaticand ethanolic extracts of liquorice displayan inhibitory action onthe cultures of *Strept. pyogenes*and *Staph. aureus*(22), and many pathogenic organisms[23].

In vitro testing of the extracts from liquorice root was carried out against[13] bacterial isolates by the use of agar diffusion method, that showed variousantibacterial activities. The inhibitory antibacterial properties of this extract to prevent the growth of*S. flexneri, Shigella sonnei,S. paratyphi BSalmonella typhi*and *ETEC E. coli* was revealed[24].

Glycyrrhizin had been studied by Alonso and Tratado[22], as it is regularly used amongthe orally administered foods,and it obstructs the growth of some bacterial sorts, also can prevent dental caries formation, it demonstratedantimicrobial effects against both Gram-positive and -negative bacteria by Gupta*et al*[25].

Cuminspice is usedpopularly as a traditional therapy,for the reason thataromatic substancesare presentin this herb, it isprescribedfor the mild digestive sicknesses, and used in the treatment of diseases caused by *K. pneumoniae;*especially the essential oil of its seeds that haddisplayedto have a significant *in vitro*antibacterial activity[26,27].Cuminaldehyde and para-cymene are the main two active components of cumin; Cuminaldehyde has made knowndivers eactivitieslikeantifungal,antibacterial,anti-diabetic and anti-platelet[28]. Antimicrobial effect of both aqueous and oil extracts of it, hadjudged against a wide collection of valuable, pathogenic microbial strainsof both gram-positive and -negative, as it is reported to obstruct*Salmonella spp, E.coli, Aspergillus niger*and*Bacillus cereus* growth[29,30].

Alcoholic extract and oil of Cumin seedshave ability to inhibit growth of *Klebsiella pneumoniae,*especially its clinical isolates, with improvement in the morphology of cells, decrement of urease activity and impeding capsule expression. Additionally, have capability to prevent properties of *Streptococcus pyogenes*and *Streptococcus mutans*in biofilm-formation.Moreover the cumin extracts has anti-fungal properties against human, soil, food and animal pathogens, yeasts, mycotoxin and aflatoxins producers[31,32,33].

Ginger exhibiteda robust antibacterial and to a lessdegree antifungal properties. Main activeingredients of ginger have been studied*in vitro*to inhibit multiplication and growth of colon bacteria; as ginger can counteractbacterial

ability to produceflatulence due to fermention of undigested carbohydrates. It constrains growth of *Staphylococci, Salmonella, Proteus sp,E. coli* and *Streptococci*(34).Gingerol, Ingenol and Shogaol, isolated from ginger rhizome are the main active components that exhibited antiviral and antibacterial activity against many pathogens; as *M. avium* and *M. tuberculosis in vitro*,*H. pylori* and many periodontal bacteria[35,36,37].

Hindi *et al.*[12] showed that the aquatic extract of ginger give a significant influenceto inhibit most of the studied sixteen bacterial isolates both gram positive and negative, also in a study of Suhad and her coworkersdisplayed that the ginger aquatic extract gave the peakeffect against *Staphylococcus aureus, Streptococcus pyogenes, Proteus vulgaris,* and *Klebsiella pneumonia*. A number of antibiotics likechloromphenicol, erythromycin,gentamicin,nalidixic acid and trimethoprim were used as a comparison with this extract of ginger, usingantibiogram test. Aa resultsheinstituted that the inhibitory action of gingerwassuperior than that to Gentamicin and Chloromphenicol against *Proteus vulgaris, Klebsiellapneumoniae, Staphylococcus aureus* and*Streptococcus pyogenes*[38].

Various compounds from different phytochemicalcategories have been identified in Parsley. Also,different pharmacological activities have been attributedto it. In Iran Parsleyas seeds or leaves has many usages for renal system, as inflammation, kidney stone,renal and urinary tract disease, and urinary tract infections[39,40,41]. Also, extracts from its leaves are widely used in Serbia as an antimicrobial agent[42].

As Parsley leaves and stems possess antibacterialactivity on *B. subtilis* and *E. coli*. Both hot and coldwater extract from them demonstratedantibacterial activity against *pseudomonas aeruginosa*,*S. aureus, S. epiderm*and *S. pyogenes*,with higherinhibition zone in hot waterextract [43].Leaf extracts showed higher celldamage on both bacteria withhigher activity with methanol extract, Coumarins are responsible components for thisproperty[44,45].

Microbial analysiscarried out by Nessrien *et al*[46] and Gutierrez*et al*[47] showed that, essential oils of marjoram has antimicrobial properties,as it is rich in phenolic compound that being particularly active as antimicrobials against both bacteria and fungi.

The essential oils from its leaves showed antibacterial effects on various bacteria (*Bacillus cereus*, *E. coli*, *Staphylococcus aureus*, *Proteus* spp., *Enterobacter* spp., *Klebsiella* spp.,*Acinetobacter* spp. and *Pseudomonas* spp.) in agar diffusion assay. Also the ethanol and water extracts of majorana have shown antimicrobial activity against both gram positive and negative bacteria and its possible food applications by minimum inhibitory concentration estimation[48].

Farooqi and Sreeramu[49] have reported the antimicrobial activity ofmajoranaagainst *Bacillus anthracis, Proteus vulgaris,Salmonella spp, Streptococcus agalactiae*,*Streptococcus spp* and *Aspergillus fumigatus*.Shahidi[50] screened some used medicalIranian traditional plants for antibacterialproperties against two *E. coli*strains and found thatMajorana showed anti-*E.coli* activity.Leelavathi[51] has conducted a comparative study of the antibacterialactivity of crude extract of *in vivo* and *in vitro* leaves of majoranaagainst *Staphylococcus aureus* and reported that *in vitro* leafextract showed better antibacterial activity.

At present, most pathogenic bacteria developan antibioticresistance. Thus tooverwhelm this frightening problem, it is an urgency to discover a numerof novel active compounds.Organic solvents and water are used to prepare extracts from these spices which are biologically active compounds, and can be applied for thesynthesis of potent drugs. Thus spices, that consideredas ausualcomponentsof our routine food preparations, which may givedefense to a certainextent against bacterial pathogens the natural enemies.

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