

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

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There are increments to the need of the usage of new non-chemical medicines, pharmaceuticals and health products. Plants are traditionally used as medicine over the world for their pharmacological values. They are effectively used as anti-fungal, antiviral, anti-bacterial, anti-inflammatory, anti-diabetic, anti-oxidant, anti-ulcer, and antitussive. This study aims to 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 method against various bacterial isolates were done, to estimate their antibacterial activity. Results showed that the aquatic extracts of different plants produce a good antibacterial effect when compared with the synthetic antibiotic ciprofloxacin. As all bacterial isolates in this study were sensitive to these extracts with variable ranges of inhibition zones ranging from 18-32mm in diameter. Thus we can conclude 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*.

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Plants are rich sources of natural products that are extracted from 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 pharmaceutical effects such as antibacterial, antifungal, antiparasitic and anticarcinogenic<sup>1</sup>. The use of crude plant extracts in herbal medicine for treatment of diseases can be seen in different forms like infusion or tincture<sup>2</sup>. Bacterial resistance to different types of antibiotics is growing because of genetic variability and

mutation therefore 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<sup>3</sup>.

Plant extracts widely used to reduce the bacterial growth of most infections. The antimicrobial activity of them dependent on their methods 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, antiviral and anti-ulcerative activity, it used for treatment of upper respiratory and liver diseases<sup>4,5</sup>.

*Cuminum cyminum* has anticholesterol and antimicrobial activity and the active components

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of it is cumin oil or cuminal, these extracts are important in stimulation of digestive system enzymes secretion to treat different types of diseases and used as bactericides against plant bacterial diseases<sup>6,1</sup>.

Ginger (*Zingiber officinale*) has antimicrobial activity that can be used in treatment of bacterial infection through its aromatic and medicinal properties. Both ethanolic and aqueous extraction of Ginger have bactericidal effects on some types of pathogenic organisms<sup>7,8</sup>. *Petroselinum crispum* extracts have antibacterial activity, damage bacterial cell wall and inhibit the bacterial growth in addition to their cytotoxic activity at higher concentration<sup>9</sup>.

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 extracts of this plant has agricultural, pharmaceutical and interfere with cosmetic industries<sup>10</sup>.

The antimicrobial activity of plant extracts have been compared with the effects of different types of antibiotics through measuring the inhibition zones of bacterial growth, ciprofloxacin has a wide antibacterial spectrum 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 interfere with bacterial replication<sup>11</sup>.

#### 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 compare them with the effects of ciprofloxacin.

## MATERIAL AND METHODS

### Extraction Methods

Plants were collected from the market. Aqueous extracts were prepared by soaking 30 grams of powder in 100ml of distilled 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<sup>12</sup>.

### Bacterial Isolates

An overall of eight negatively-gram stained and three positively-gram stained bacterial isolates were collected from urine clinical samples (UTI) and used in this study. These bacterial isolates were; *S. saprophyticus*, *S. epidermidis*, *S. aureus*, *P. fluorescens*, *P. aeruginosa*, *E. aerogenes*, *E. coli*, *K. pneumoniae*, *P. vulgaris*, *Acinetobacter*, *Proteus mirabilis*. All these bacterial isolates 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<sup>13</sup>.

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

Bacterial growths of Loop full 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 diameter were made in the media by cork borer, 0.1ml of the extracts were added. Then incubation overnight at 37°C. Zone of inhibition was measured, disks of ciprofloxacin were added in the center of agar plate to compare the results of bacterial inhibition<sup>13</sup>.

## RESULTS AND DISCUSSIONS

The evaluation of bacterial inhibitory activity of aquatic extracts of *Glycyrrhiza glabra* was processed using the agar diffusion test, as in Figure (1). This figure shows that *Glycyrrhiza glabra* 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 *Pseudomonas aerogenosa* and *Staphylococcus saprophyticus* (24 and 23mm) respectively. Lowest inhibition against *Klebsiella pneumoniae* and *Enterobacter aerogenes* (18mm) for each.

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

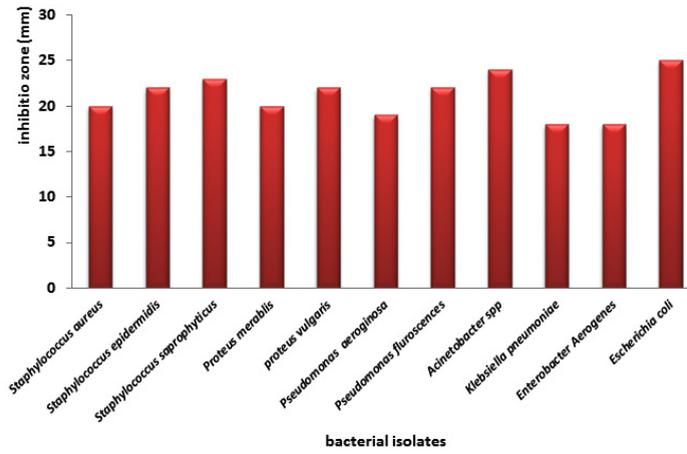


Fig. 1. Antibacterial activity of *Glycyrrhizaglabra* (ÚÑÐ ÓæÓ) against bacterial isolates

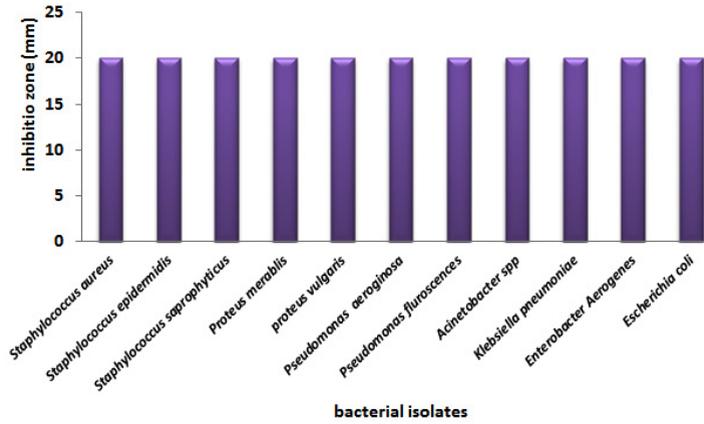


Fig. 2. Antibacterial activity of *Cuminumcyminum* (Ûæä) against bacterial isolates

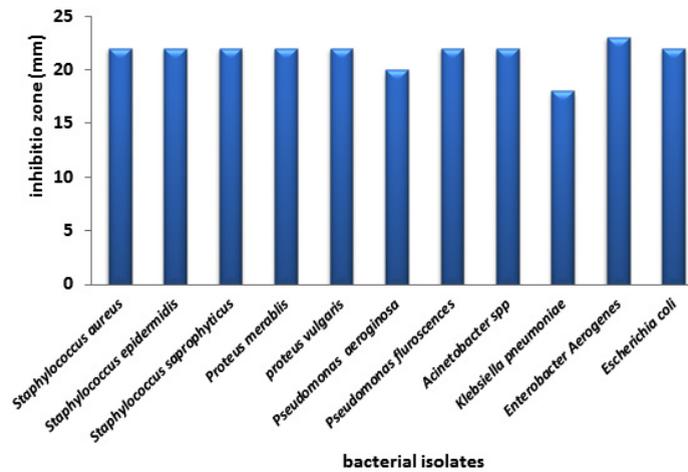


Fig. 3. Antibacterial activity of ginger(ÒäËËá) against bacterial isolates

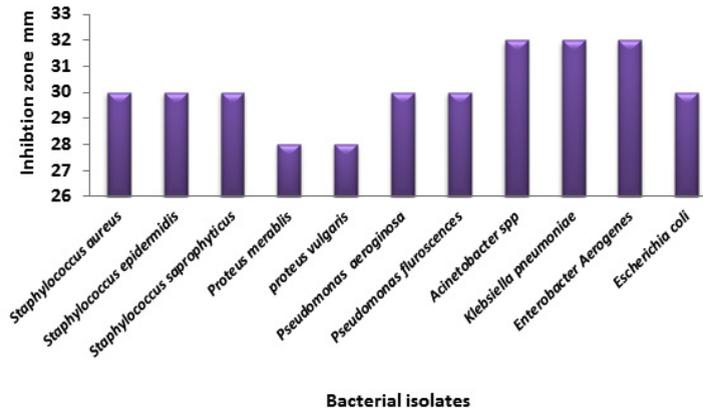


Fig. 4. Antibacterial activity of *Petroselinum crispum* against bacterial isolates

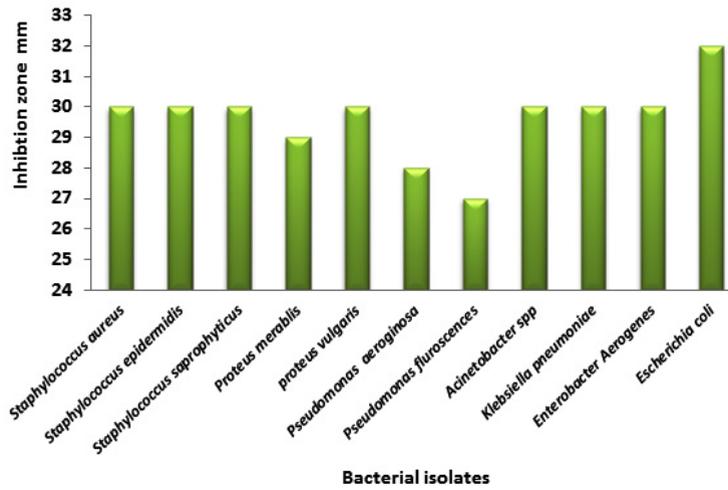


Fig. 5. Antibacterial activity of *Origanum majorana* against bacterial isolates

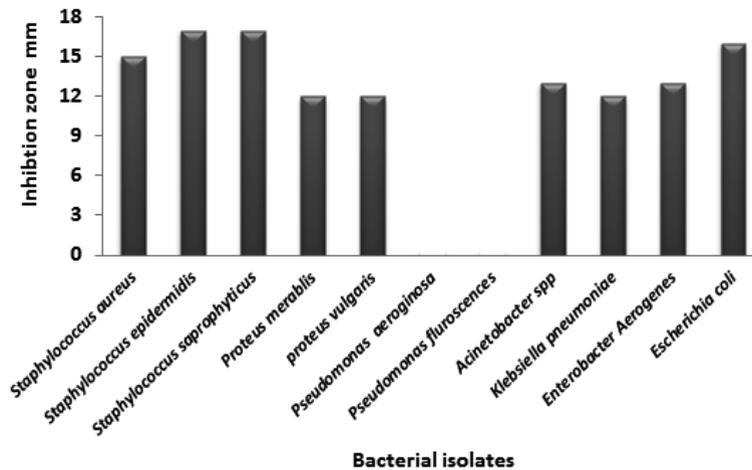


Fig. 6. Antibacterial activity of Ciprofloxacin against bacterial isolates

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

The antibacterial effect of *Petroselinum crispum* exhibited as a high inhibition level as compared with other extracts, as the inhibition zone diameters range from 32-28mm; higher effects against *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Acinetobacter* spp (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 *Origanum majorana* 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 fluorescens* (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 fluorescens* 0 mm, while the largest effect were only reach to about (17 mm) with staphylococci and *E. coli*, smallest zones were 12 mm with *Proteus* spp.

Extract of Licorice root has been used traditionally for a long period of history as medicines and folk preparations, and being used in many diseases from hundred years before<sup>15</sup>. It contains a large number of active components. About 40-50% of its total dry material weight is accounted of water-soluble, biologically active complexes.

Glycyrrhizin represents about 10-25% of licorice root extract. It is considered to be the most commonly used of the folk medicines in Asia. Its structure is a saponin compound<sup>16,17</sup>. 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<sup>18,19</sup>, also it has a role as antiviral agent against many viruses<sup>20,21</sup>.

*In vitro* works had demonstrated that aquatic and ethanolic extracts of licorice display an inhibitory action on the cultures of

*Strept. pyogenes* and *Staph. aureus* (22), and many pathogenic organisms<sup>23</sup>.

*In vitro* testing of the extracts from licorice root was carried out against<sup>13</sup> bacterial isolates by the use of agar diffusion method, that showed various antibacterial activities. The inhibitory antibacterial properties of this extract to prevent the growth of *S. flexneri*, *Shigella sonnei*, *S. paratyphi B*, *Salmonella typhi* and *ETEC E. coli* was revealed<sup>24</sup>.

Glycyrrhizin had been studied by Alonso and Tratado<sup>22</sup>, as it is regularly used among the orally administered foods, and it obstructs the growth of some bacterial sorts, also can prevent dental caries formation, it demonstrated antimicrobial effects against both Gram-positive and -negative bacteria by Gupta et al<sup>25</sup>.

Cumin spice is used popularly as a traditional therapy, for the reason that aromatic substances are present in this herb, it is prescribed for the mild digestive sicknesses, and used in the treatment of diseases caused by *K. pneumoniae*; especially the essential oil of its seeds that had displayed to have a significant *in vitro* antibacterial activity<sup>26,27</sup>. Cuminaldehyde and para-cymene are the main two active components of cumin; Cuminaldehyde has made known diverse activities like antifungal, antibacterial, anti-diabetic and anti-platelet<sup>28</sup>. Antimicrobial effect of both aqueous and oil extracts of it, had judged against a wide collection of valuable, pathogenic microbial strains of both gram-positive and -negative, as it is reported to obstruct *Salmonella* spp, *E. coli*, *Aspergillus niger* and *Bacillus cereus* growth<sup>29,30</sup>.

Alcoholic extract and oil of Cumin seeds have 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, mycotoxins and aflatoxins producers<sup>31,32,33</sup>.

Ginger exhibited a robust antibacterial and to a less degree antifungal properties. Main active ingredients of ginger have been studied *in vitro* to inhibit multiplication and growth of colon bacteria; as ginger can counteract bacterial

ability to produce flatulence due to fermentation 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<sup>35,36,37</sup>.

Hindi *et al.*<sup>12</sup> showed that the aquatic extract of ginger give a significant influence to inhibit most of the studied sixteen bacterial isolates both gram positive and negative, also in a study of Suhad and her coworkers displayed that the ginger aquatic extract gave the peak effect against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Proteus vulgaris*, and *Klebsiella pneumoniae*. A number of antibiotics like chloramphenicol, erythromycin, gentamicin, nalidixic acid and trimethoprim were used as a comparison with this extract of ginger, using antibiogram test. As a result she instituted that the inhibitory action of ginger was superior than that to Gentamicin and Chloramphenicol against *Proteus vulgaris*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Streptococcus pyogenes*<sup>38</sup>.

Various compounds from different phytochemical categories have been identified in Parsley. Also, different pharmacological activities have been attributed to it. In Iran Parsley seeds or leaves has many usages for renal system, as inflammation, kidney stone, renal and urinary tract disease, and urinary tract infections<sup>39,40,41</sup>. Also, extracts from its leaves are widely used in Serbia as an antimicrobial agent<sup>42</sup>.

As Parsley leaves and stems possess antibacterial activity on *B. subtilis* and *E. coli*. Both hot and cold water extract from them demonstrated antibacterial activity against *Pseudomonas aeruginosa*, *S. aureus*, *S. epidermidis* and *S. pyogenes*, with higher inhibition zone in hot water extract<sup>43</sup>. Leaf extracts showed higher cell damage on both bacteria with higher activity with methanol extract, Coumarins are responsible components for this property<sup>44,45</sup>.

Microbial analysis carried out by Nessrien *et al.*<sup>46</sup> and Gutierrez *et al.*<sup>47</sup> 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<sup>48</sup>.

Farooqi and Sreeramu<sup>49</sup> have reported the antimicrobial activity of majorana against *Bacillus anthracis*, *Proteus vulgaris*, *Salmonella spp.*, *Streptococcus agalactiae*, *Streptococcus spp.* and *Aspergillus fumigatus*. Shahidi<sup>50</sup> screened some used medicinal Iranian traditional plants for antibacterial properties against two *E. coli* strains and found that Majorana showed anti-*E. coli* activity. Leelavathi<sup>51</sup> has conducted a comparative study of the antibacterial activity of crude extract of *in vivo* and *in vitro* leaves of majorana against *Staphylococcus aureus* and reported that *in vitro* leaf extract showed better antibacterial activity.

At present, most pathogenic bacteria develop an antibiotic resistance. Thus to overcome this frightening problem, it is an urgency to discover a number of 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 the synthesis of potent drugs. Thus spices, that considered as usual components of our routine food preparations, which may give defense to a certain extent against bacterial pathogens the natural enemies.

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