

Antibacterial Effects of *Citrus aurantium* on Bacteria Isolated from Urinary Tract Infection

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Abstract

Background: Emerging antibacterial resistance rates and beta-lactamase producing bacteria recovered from UTI is an increasing problem in different regions, limiting therapeutic options. Therefore, this study aimed at using the extract and essence of *Citrus aurantium* (abundantly found in Iran) and assessing their effect on bacterial agents causing urinary tract infections, and compare the effect with common antibiotics used in treatment of UTI.

Materials and Methods: In an experimental design the *E. coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae* and *Enterococcus faecalis* were isolated from UTI and the antibacterial effect of *Citrus aurantium* against these bacteria were determined using subculture. Antibacterial effects of the herb extract were evaluated by well diffusion assay and nalidixic acid and Co-trimoxazole were evaluated by agar disc diffusion.

Results: *Enterococcus faecalis* showed 100% sensitivity to extract, essence and Co-trimoxazole, and 80% to nalidixic acid. *E. coli* had 100% sensitivity to Co-trimoxazole, and nalidixic acid and was totally resistant against extract and essence. *Klebsiella Pneumoniae* had 80% sensitivity to Co-trimoxazole, 75% to nalidixic acid and resistance against extract and essence. *Streptococcus agalactiae* showed 100% sensitivity to essence and Co-trimoxazole and 90% sensitivity against nalidixic acid and shown 80% sensitivity to extract. *Staphylococcus aureus* MRSA showed 100% sensitivity to Co-trimoxazole and 70% sensitivity to essence, extract and nalidixic acid.

Conclusion: Detection of antibiotic resistance among isolates is important in prevention and control of infections. In this study, the extracts of *Citrus aurantium* were found with high antibacterial effects on gram positive bacteria compared with gram negative bacteria.

Keywords: *Citrus aurantium*; Antibacterial; Antibiotic resistance; Urinary Tract Infection

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Introduction

Urinary tract infection is one of the most common infections in humans. It is more common in women than men which this difference is mostly due to anatomical sex differences (1). Most cases of human urinary tract infections are caused by *E. coli*. Other bacteria such as *Enterococcus*, *Proteus*, *Pseudomonas*, *Enterobacter*, and staphylococcal are mainly responsible for secondary infections urinary tract,

because of secondary actors and hospital infections (2). Plants with medicinal effects are important compounds of traditional medicine in virtually all cultures. Medicinal plants are of great benefit in the field of curing diseases and act as important sources of materials for a wide variety of human ailments (3). Some researchers have shown that using plants with medical effects is better than common antibiotics

used for treatment of patients with different diseases (4). Herbal compounds have been used numerously as fabulous source of a wide range of essential oils and nutrients required by the body. Fresh fruits and edible plants and industrially processed juices, contain mostly flavanones and flavones (5, 6). *Citrus aurantium* extract and its primary proto alkaloidal constituent p-synephrine are extensively used in weight management products and as thermogenic agents. Citrus plants (*Citrus aurantium*) is in Rutales order. Orange flowers, are called *Citrus aurantium* which is dried, used as an anti-seizure in neurological diseases such as hysteria and neurasthenia and also is used to relieve hiccups. Moreover, *Citrus aurantium* has a mild sedative effect, appetizer and fix palpitations (7). In general microorganisms such as bacteria have the genetic ability to acquire resistance to many drugs, which are utilized as therapeutic agents (8). The clinical efficiency of many antibiotics

Materials and Methods

Plant Material

This experimental study was conducted between February 2014 and March 2015. The leaves of *Citrus aurantium* were collected from the local areas in Shiraz, Iran. The plant material was authenticated by the department of Microbiology, Shahid Beheshti University of Medical Sciences.

Preparation of extracts

The dried and powdered peel materials (20 gm) were extracted with 200 ml of each solvent separately using soxhlet extractor for 2 to 5 h at a temperature not exceeding the boiling point of the solvent (8). The solvents used for the study were Acetone. The extracts and essence were filtered and then concentrated to dryness. They were transferred to microtubes and kept at 4°C before use. The extracts were dissolved in 2% aqueous dimethyl sulfoxide (DMSO) to produce a stock solution of 100 mg/ml (8).

Screening for Antibacterial activity

a) Bacterial Isolates

The bacteria were isolated from urinary tract infected sample of patients. The antibacterial activity of *Citrus aurantium* extract were tested against *E. coli* and *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae* and *Enterococcus faecalis*. The isolates were stored at -20 °C in trypticase soy broth supplemented with 20% glycerol. Urine samples were collected from 60 patients with UTI who were not taking antibiotics and then cultures were made on Blood agar, Macconkey, Eosin methylene blue agar and Bile esculin agar. After appear in colonies of bacteria, gram staining was performed and was detected in specific media.

used to treat the patients in existence is being treated by multi drug-resistant pathogen such as beta lactamase producing *pseudomonas aeruginosa* (9). To overcome the antibiotic resistance of bacteria, researchers are much interested in finding and producing new drugs from plants with medicinal effects. Many infectious diseases are treated with herbal remedies. The natural herbal products used as standardized plant extracts provide unbounded opportunities for new drug leads due to having rich chemical properties. This calls for discovering new antibacterial compounds with different structure and new mechanisms of action and treat for new-emerging and re-emerging infectious diseases (10). The aim of this study was to assess the antimicrobial effects of *Citrus aurantium* essence and its extract on bacteria isolated from patients with urinary tract infection compared with common antibiotics used in UTI treatment.

Antimicrobial assay

Prime petri plates of sterile fused Mueller Hinton Agar (Hi-Media, Mumbai) at temperature of 40 °C. After solidification 6 millimeter wells were primed. In these wells solvent extracts were increased. The plate was incubated overnight at 37 °C. After incubation the zones of growth inhibition were measured and recorded. These studies were done in triplicate. Antibacterial essence and extract were investigated on bacteria isolated from 60 patients with urinary tract infection by agar disk diffusion method according to 2014 CLSI guidelines.

Disc Diffusion Method

Agar disc diffusion method was performed by soaking the blank discs in essence and extracts of *Citrus aurantium* and putting them on Mueller Hinton agar medium. In this study the antimicrobial effect of the extract and essence of *Citrus aurantium* were compared with commonly used antibiotics in treatment of UTI (Cotrimoxazole and nalidixic acid). Inhibition zone of antibiotic discs was compared with essence and extract of *Citrus aurantium* and results were reported as susceptible, intermediate and resistant to extract, essence and antibiotics.

Ethics Statement

All patients were accepted to participate in this study and it was approved by the Shahid Beheshti University of Medical Sciences Ethics Committee.

Results

In this study *Enterococcus faecalis* had 100% sensitivity to extract and Co-trimoxazole, and 80% to nalidixic acid. *E. coli* showed 100% sensitivity to Co-trimoxazole, nalidixic acid and it was totally resistant

against the extract and essence. *Klebsiella pneumoniae* had 80% sensitivity to Co-trimoxazole, 75% to nalidixic-acid and resistance against concentration unit extract and essence of *Citrus aurantium*. *Streptococcus agalactiae* exhibited 100% sensitivity

to essence and Co-trimoxazole and 90% to nalidixic acid and 80% sensitivity to extract. *Staphylococcus aureus* MRSA showed 100% sensitivity to Co-trimoxazole and 70% sensitivity to essence, extract and nalidixic acid (Chart 1).

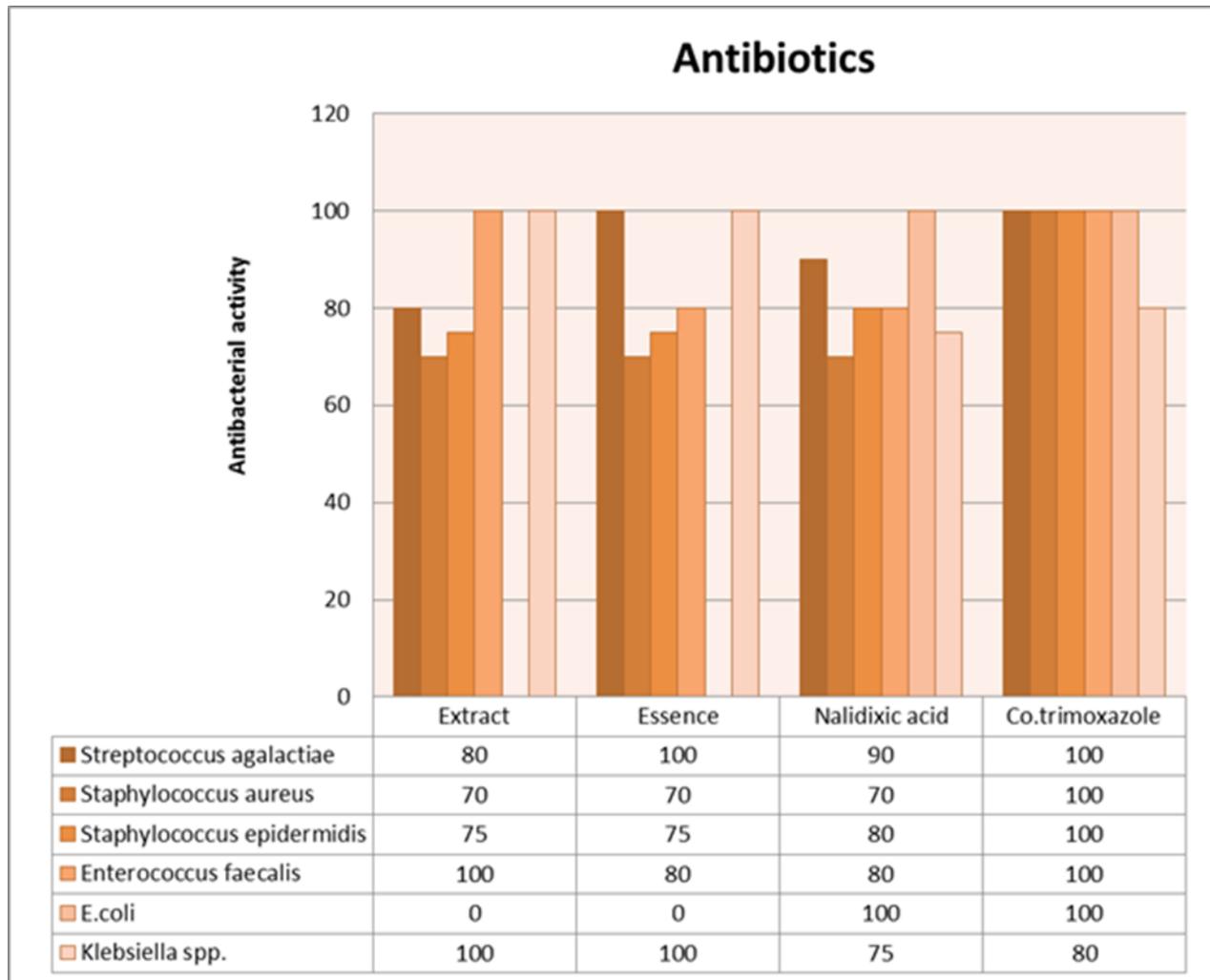


Chart 1. Percentage of antibacterial activity of *Citrus aurantium* and common antibiotics against clinical isolates from UTI.

Discussion

The present study was performed on 60 samples obtained from patients with urinary tract infection and found that the essence and extract of *Citrus aurantium* has antibacterial activity against bacteria causing UTI. Melendez et al determined the antibacterial activity of common plants. They examined the antibacterial effects of 172 plant species against *E. coli* and *Staphylococcus aureus* by disc diffusion method. Among the 172 species of examined plants, methanol extracts of 14 plant species including *Citrus aurantium* were found with antimicrobial effect (11). Hazzset al used *Citrus aurantium* in the treatment of the weight increase

disease (obesity) (12). Moreover, the efficacy of *Citrus aurantium* and diazepam was observed in reducing anxiety before surgery (13). Maruti et al. found *Citrus lemon* L. effective against *Pseudomonas aeruginosa* and compounds like coumarin and tetrazene present in lemon peel extract (14). These results in comparison with our study with the same test organism and solvent shows that *Citrus aurantium* essence and extract showed relatively similar results. The aforementioned results prove the medicinal properties of *Citrus aurantium*, which could be used as an effective pre-drug in reducing anxiety and inflammation in patients with urinary tract infections. Based on the antimicrobial effect of

essence and extract of *Citrus aurantium* against *Streptococcus agalactiae* and *Enterococcus faecalis* bacteria, they can be of great benefit in treatment of patients with UTI besides effective drugs. According to this reality that extracts is taken from a plant with different methods and solvents can be Show the different antibacterial effects on a particular bacteria, we suggest to be investigated on other extracts and essences with different solvents and be measured the antibacterial effects of them. In addition, the Enterococcus is an important factor in the development of heart diseases, digestive diseases and tract-genital diseases, so, the antibacterial effect of extracts and essences of plant should be evaluated in vivo condition for treatment of these diseases.

Conclusion

The extract and essence of *Citrus aurantium* had a greater effect on gram-positive bacteria compared with gram negative bacteria examined in this study. Based on these results, *Citrus aurantium* essence and extract could be used as an adjunct to medication in treatment of urinary tract disease caused by gram-positive bacteria.

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Authors' Contributions

DA contributed to researching data and writing the draft, EG and GH making study design, FF and HA discussing the content, DH contributed to editing the manuscript. TS contributed to discussing of the content, AN contributed to searching data. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest in this work.

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