

Antibacterial Activity of Essential Oil of *Saturejahortensis* Against Multi-Drug Resistant Bacteria

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Received: November 24, 2013; **Revised:** December 8, 2013; **Accepted:** December 16, 2013

Background: Development of resistance to many of the commonly used antibiotics is an impetus for further attempts to search for new antimicrobial agents.

Objectives: In the present study, the antibacterial activity of *Saturejahortensis* essential oil against multi-drug resistant bacteria isolated from the urinary tract infections was investigated.

Materials and Methods: During the years 2011 to 2012 a total of 36 strains of pathogenic bacteria including 12 *Klebsiellapneumoniae*, 12 *Escherichia coli* and 12 *Staphylococcus aureus* species were isolated from urine samples of hospitalized patients (Amir Al-Momenin Hospital, Zabol, South-eastern Iran) suffering from urinary tract infections. After bacteriological confirmatory tests, the minimum inhibitory concentrations of the essential oil of *Saturejahortensis* were determined using micro-dilution method.

Results: The antibiotic resistance profile of the *E. coli* isolates were as follows: ceftazidime (50%), cefixime (41.6%), tetracycline (75%), erythromycin (58.3%). However *K. pneumoniae* isolates showed resistance to ceftazidime (33.3%), cefixime (58.3%), erythromycin (75%) and *S. aureus* isolates were resistant to cefixime (33.3%), trimethoprim-sulfamethoxazole (41.66%), penicillin (50%), oxacillin (83.3%), ceftazidime (66.6%) and vancomycin (8.3%). The essential oil of this plant had inhibitory effect against most isolates. More than 1/3 of the *E. coli* isolates showed the lowest MIC (10 ppm) whereas more than 1/3 of the *K. pneumoniae* isolates showed the highest (250 ppm) MIC values. In contrast, equal number of *S. aureus* isolates showed the low MIC values (10 and 50 ppm), while the highest MIC (250 ppm) was seen in 1/3 of isolates and moderate MIC (100 ppm) was seen in one isolate only.

Conclusions: The *Saturejahortensis* essential oil has a potent antimicrobial activity against multi-drug resistant bacteria. The present study confirms the usefulness of this essential oil as antibacterial agent but further research is required to evaluate the practical value of this plant before proving its therapeutic applications.

Keywords: Drug Resistance, Multiple; *Escherichia coli*; Bacteriological Technique

1. Background

The development of resistance to many of the commonly used antibiotics provides an impetus for further attempts to search for new antimicrobial agents to combat infections and overcome problems of resistance and side effects of the currently available antimicrobial agents. The search for compounds with antimicrobial activity has gained increasing importance recently, due to the growing worldwide concerns about the alarming increase in the rate of infections by antibiotic-resistant microorganisms (1). Medicinal properties of aromatic plants and their extracts have been recognized since ancient times and they are still used in medicine, food and cosmetic industries (2). More so, many of these plants

have been known to synthesize active secondary metabolites such as phenolic compounds found in essential oils with established potent insecticidal and antimicrobial activities, which indeed has formed the basis for their applications in some pharmaceutical products, alternative medicine and natural therapies (3-6). The genus *Satureja* is comprised of some 200 species of often aromatic herbs and shrubs are widely distributed in the Mediterranean Area, Asia and boreal America. Many members of this genus have aromatic and medicinal properties. The essential oil constituents and antimicrobial activities of some *Satureja* species have been studied (7, 8). *Satureja hortensis*, one of these species, is an annual plant (10-35 cm tall) and aromatic herb with lilac, purplish or white flowers and linear to linear oblanceolate leaves. It grows wild on

Implication for health policy/practice/research/medical education:

Antimicrobial agents are important for treatment of infection diseases.

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rocky or eroded slopes, screes, gravelly places and coastal dunes, fallow fields and roadsides in Turkey (9).

2. Objectives

In the present study, the antibacterial activity of *Saturejahortensis* essential oil against multi-drug resistant bacteria isolated from the urinary tract infections was investigated.

3. Materials and Methods

3.1. Bacterial Isolates

A total of 36 strains including 12 *Klebsiella pneumoniae*, 12 *E. coli*, and 12 *Staphylococcus aureus* isolated from urine culture of hospitalized patients (Amir Al-Momenin Hospital, Zabol, south-eastern Iran) suffering from urinary tract infections during the years 2011-2012 were evaluated. Isolated bacteria were identified by Gram's stain and standard biochemical tests (10).

Agar disc diffusion assay: The susceptibility test of the isolates to different antibiotics was carried out using disc diffusion method on Mueller-Hinton agar as recommended by CLSI (11). Briefly, 10 µL of a suspension equivalent to 0.5 McFarland standards was spread over the Mueller-Hinton agar plate and suitable antibiotic discs were aseptically placed on the surface of the inoculated plates. Antibiotics and their concentrations were as follows: tetracycline (30 µg), erythromycin (15 µg), ceftazidime (30 µg), trimethoprim-sulfamethoxazole (1.25+23.15 µg), penicillin (10 µg), oxacillin (30 µg) and vancomycin (10 µg).

3.2. Plant Materials and Distillation of Essential Oil

The leaves of *Saturejahortensis* plants were collected from Kerman (south-eastern, Iran) and dried at room temperature in the herbarium of Kerman Azad University. Dried materials were crashed and transferred to glass containers and preserved until extraction procedures were performed in the laboratory. For distillation process, 300 g of the ground materials was subjected to water distillation for 4 hours using a Clevenger apparatus. The distilled essential oil was dried over anhydrous sodium sulfate, filtered and stored at 4°C. Minimum Inhibitory Concentration (MIC) of essential oil: The broth micro-dilution method was used to determine MIC of the essential oil using Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/v). Briefly, serial twofold dilutions of the extract were prepared in a 96-well microtiter plate ranged 250 ppm, 100 ppm, 50 ppm and 10 ppm, respectively. To each well, 10 µL of indicator solution (prepared by dissolving a 250 ppm essential oil in 2 mL of DMSO as solvent) and 10 µL of Mueller Hinton broth were added. Finally, 10 µL of bacterial suspension (106 CFU/mL) was added to each well to achieve a concentration of 104

CFU/mL. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37°C for 18-24 hours. The color change was then assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of three values was calculated as the MIC values for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate any visible growth. The microorganism growth was assessed by turbidity.

4. Results

4.1. Antibiotic Susceptibility

The antibiotic resistance profiles of the *E. coli* isolates were as follows: ceftazidime (50%) cefixime (41.6%), tetracycline (75%), erythromycin (58.3%). However *K. pneumoniae* isolates showed resistance to ceftazidime (33.3%), cefixime (58.3%), erythromycin (75%) and *S. aureus* isolates were resistant to cefixime (33.3%), trimethoprim-sulfamethoxazole (41.66%), penicillin (50%), oxacillin (83.3%), ceftazidime (66.6%) and vancomycin (8.3%).

4.2. Assessment of MIC for Essential Oil

Different inhibitory effects of essential oil against most *E. coli*, *K. pneumoniae* and *S. aureus* isolates are demonstrated in Table 1. The essential oil of plant had inhibitory effect against most isolates. More than 1/3 of the *E. coli* isolates showed the lowest MIC (10 ppm), while moderate (100 ppm) and highest (250 ppm) MIC values were seen in 1/4 and 1/3 of *E. coli* isolates respectively. Beside this, more than 1/3 of the *K. pneumoniae* isolates showed the highest (250 ppm) MIC values, while moderate (100 ppm) and lowest (50 ppm) MIC values were seen in 1/4 and 1/3 of the isolates respectively. In contrast, equal number of *S. aureus* isolates (4 of 12) showed the low MIC values (10 and 50 ppm), while the highest MIC (250 ppm) was seen in 1/3 of isolates and moderate MIC (100 ppm) was seen in one isolate only.

5. Discussion

In the present study, between 1/4 to 1/3 of isolates showed the highest MIC values (250 ppm). The genus *Satureja* presents great variability in the concentration of the major components in its essential oils composition due not only to the existence of different species and subspecies, but also because of a numerous of other parameters, mainly the environmental and climatic conditions (12-14). Different pharmaceutical properties such as anti-diarrheal and antispasmodic (15), anti-inflammatory (16) as well as antimicrobial properties (17) were reported in the literature.

Table 1. The Effects of Plant Essential Oil against Three Human Pathogens^a

Bacterial Code	MIC of Essential Oil, ppm	Resistance Pattern for <i>E. coli</i>	Resistance Pattern for <i>E. coli</i>	Resistance Pattern for <i>K. pneumoniae</i>	Resistance Pattern for <i>S. aureus</i>
1	250	A ₁ , A ₂	A ₁ , A ₂	A ₁ , A ₂	A ₁ , A ₂ , A ₃ , A ₄ , A ₅ , A ₆
2	250	A ₁ , A ₂	A ₁ , A ₂	A ₁ , A ₂	A ₁ , A ₂ , A ₄ , A ₅ , A ₆
3	250	A ₁	A ₁	A ₁	A ₁ , A ₂ , A ₄ , A ₅ , A ₆
4	50	-	-	-	A ₄ , A ₆
5	100	A ₁	A ₁	A ₁	A ₄ , A ₆
6	50	A ₁ , A ₂ , A ₃	A ₁ , A ₂ , A ₃	A ₁ , A ₂ , A ₃	A ₆
7	50	A ₁ , A ₂ , A ₃	A ₁ , A ₂ , A ₃	A ₁ , A ₂ , A ₃	-
8	50	A ₁ , A ₂ , A ₃	A ₁ , A ₂ , A ₃	A ₁ , A ₂ , A ₃	-
9	100	-	-	-	A ₆
10	100	A ₁ , A ₂ , A ₃	A ₁ , A ₂ , A ₃	A ₁ , A ₂ , A ₃	A ₂ , A ₄ , A ₅ , A ₆
11	250	A ₁	A ₁	A ₁	A ₁ , A ₂ , A ₄ , A ₅ , A ₆
12	250	A ₂	A ₂	A ₂	A ₄ , A ₅ , A ₆

^a A₁, cefixime; A₂, trimethoprim-sulfamethoxazol; A₃, Vancomycin; A₄, Cefazidime; A₅, penicillin; A₆, Oxacillin.

Based on the study of Adiguzel et al. the essential oil of *Saturejahortensis* exhibits activity against 25 bacteria, 8 fungi, and yeast, *C. albicans*; exerting the minimum inhibitory concentration values ranging from 15.62 to 250 µL/mL (18). In a more recent study, Sahin et al. have found that essential oil and methanol extract of *Saturejahortensis* show have a strong inhibitory effect on a wide range of bacteria and fungi (17). Many of the previous studies demonstrated that the members of the genus *Satureja* show a high antimicrobial activity due to the presence of thymol, carvacrol, and their precursors (19). Testing of the antibacterial activities of essential oils in vitro tested against *E. amylovora* showed that *S. hortensis* could inhibit growth of this bacterium (20). The results obtained in the study of Razzaghi-Abyanehet al. clearly showed a new biological activity for *S. hortensis* L. as strong inhibition of aflatoxin production by *A. parasiticus* (21). Carvacrol and thymol, the effective constituents of *S. hortensis* L., may be useful to control aflatoxin contamination of susceptible crops in the field (21). Oil of *Saturejahortensis* at 70, 80, 90 and 100 µg concentrations, was more effective against *Salmonella enteritidis*, *Streptococcus pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Streptococcus mutans* (22). Even though *S. hortensis* essential oil has been evaluated in terms of antimicrobial activity against a limited number of microorganisms by Deans and Svoboda, this is the first study confirming that the essential oil from *S. hortensis* possesses a wide antimicrobial activity spectrum, since it inhibits the growth of all of the human and plant pathogenic and/or food spoilage bacteria, fungi, and yeast species tested (23). In the study of Mahboubi and Kazempour, the MIC values of savory oils against different kinds of microorganisms were in the ranges of

0.06-16 µL/mL (24). As the work for the development of herbal medicines is in progress worldwide, the present report will help in discovery of new products. Besides, the same substances may also be used for the treatment of plant pathogenic fungi as conventional method. In conclusion, the *Saturejahortensis* essential oil has potent antimicrobial activity against multi-drug resistant bacteria. The present study suggests the use of this essential oil as antibacterial agent but further research is required to evaluate the practical value of this plant in clinical applications.

Acknowledgements

This study was supported by the Institute of Plant Biotechnology, University of Zabol, Zabol, Iran.

Author's Contribution

All authors had equal role in design, work, statistical analysis and manuscript writing.

Financial Disclosure

There is no conflict of interest.

Funding/Support

Institute of Plant Biotechnology, University of Zabol.

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