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Assessment of the Antibacterial Effect of the *Oliveria decumbens* vent. Essential Oil on *Escherichia coli*-induced Cystitis in the Rat Model

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Abstract

Antibiotic resistance is among the most concerning issues worldwide. Currently, the use of natural alternatives with improved therapeutic effects and fewer complications than common therapies is considered a novel therapeutic approach to care urinary tract infections (UTI). In this research, we evaluated the prospective activity of *Oliveria decumbens vent* essential oil (ODEO) in the treatment of *Escherichia coli*-induced cystitis. The antibacterial properties of ODEO were investigated using standard microdilution assays against *E. coli*. To induce cystitis, 1.5×10^8 CFU/ml of *E. coli* (ATCC 700928) was injected into the bladder of Wistar rats, and then they were prescribed ODEO and gentamicin. The histopathological parameters of the bladders were tested at the end of the study. The MIC and MBC of the ODEO against *E.coli* were 0.54 µl/ml and 1.024 µl/ml respectively. In the infected group with no treatment, the infiltration of inflammatory cells and the thickness of bladder tissue were increased notably, however in the groups treated with ODEO, especially at higher doses, the parameters were significantly decreased ($p<0.01$). The ODEO efficacy was comparable to gentamicin in the reduction of the bacterial count. In addition, after administration of the ODEO, inflammation, fibrosis, and thickness of epithelium also decreased in a dose-dependent manner ($p<0.01$). In conclusion, treatment with ODEO which added as drinking supplement or injected subcutaneously resulted to the renal clearance of pathogens in study rats.

Keywords: *Oliveria decumbens*; Herbal agent; *E. coli*; Cystitis.

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Introduction

UTIs predominately caused by *E. coli* represent one of the most common bacterial infections worldwide which may raise the risk of renal complications and patient mortality (1). It has been demonstrated that the infecting bacteria such as *E.coli* could attack the urethra and bladder, colonize there, spread into the kidney, and cause inflammation, which leads to insufferable pain, overactive bladder, dysuria, burning cloudy urine, and hematuria (2). While conventional antibacterial agents are widely used in the treatment of severe cystitis, it has been shown that highly resistant strains of *Uropathogenic E. coli* (UPEC) are common in countries with less monitored antibiotic consumption (3)**,** which also result in the development of drug-related adverse effects. So, because of maintaining similar efficacy and lowering the side effects, an alternative treatment option is unavoidable. Resistance to common antibiotics necessitates the progression of novel antimicrobial agents to prevent multidrug-resistant microorganisms' growth and pathogenicity (4).

Recently, herbal-based drugs have changed the medicinal market, and there is a remarkable increase in the number of these medications. Essential oils taken from medicinal and aromatic plants that contain aromatic compounds are extensively considered an indispensable resource in the health, food, and pharmaceutical fields (5). Many studies have shown several favorable activities of herbal agents in the prevention and treatment of UTIs (6). *Oliveria decumbens Vent* is a beneficial medicinal plant that is grown in western and southern parts of Iran (7). In folk medicine of Iran, *O. decumbens* has been widely used for fever, treatment of gastrointestinal problems, and relief of abdominal cramping pain (8). This plant is a prominent source of essential oil that has already been described to demonstrate outstanding lipid peroxidation suppressing, radical scavenging effects, and antioxidant activity in living cells as well as chemical assay (9, 10). In addition, its cytotoxic effects on cancer cell lines have been reported previously (11) (12). It has been shown that the *O. decumbens* essential oil has considerable antibacterial activity against many pathogens, including *E. coli* (4).

The herb is a well-known member of the Umbelliferae family which has global distribution. Most plants of this family, produce bioactive volatile compounds. In previous studies, it has been revealed that thymol and carvacrol were the major components of the *O. decumbens* essential oil (4). Essential oils have been studied as natural sources of lipophilic and volatile mixtures of several bioactive compounds that has anti-quorum sensing activity, can disrupt cytosol and trans-membrane enzymes, and the energy production system of the bacterial or fungal cells or increase the cell wall permeability.

The purpose of this experiment was to evaluate the potential antibacterial effects of *O. decumbens* essential oil (ODEO) in the treatment of *E. coli* induced cystitis in the rat model. We assessed the antibacterial activity and the histopathological effects of the essential oil on the bladder were also determined to evaluate the activity of ODEO on these parameters.

Materials and Methods

Essential Oil

The ODEO was purchased from Barij-Essence Pharmaceutical Company (Kashan, Iran). Doses of ODEO were selected based on the results of the MIC test.

Experimental Model

All tests were organized following the Guiding Principles for the Care and Use of Research Animals, endorsed by Islamic Azad University, Science and Research Branch. Thirty-five male Wistar albino rats (three to four months old), weighing 180-220 g and pathogen-free, were prepared from the Pasteur Institute of Iran and segregated randomly into seven groups (5 rats in each group). Animals were kept in separate cages and provided food and water ad libitum. The animals were kept until the end of the experiment in controlled conditions of temperature (24-22 °C) and relative humidity (56. 5%) and 12 hours light, 12 hours dark cycles. *E. coli* (ATCC 700928) strains were obtained from the Pasteur Institute of Tehran, Iran.

For cystitis induction, 1.5×10^8 CFU/ml of *E. coli* (ATCC 700928) was injected into the bladder of the rats in groups 2-7. Groups were designated as follows: group 1, sterile saline injected into the bladder (control); group 2, the infected group with no treatment (control); group 3, the infected group with Gentamicin treatment (2 mg/kg, Intramuscular (IM) injection); group 4, the infected group with ODEO treatment (0.5 µl/ml, added into drinking water); group 5, the infected group with ODEO treatment (1 µl/ml, added into drinking water); group 6, the infected group with ODEO treatment (0.5 µl/ml, subcutaneous injection (SC)); group 7, the infected group with ODEO treatment (1 µl/ml, SC). The treatments were administered twice daily and continued for 14 days. During the study, urine samples of all groups were collected on days 3, 7, and 10, and cultured to determine the bacterial count. In the last part of the study, the urine samples were collected and then rats

were euthanized with ether, and their bladders were carefully dissected and were examined for pathological evaluation.

Microbiological evaluation

MIC values of the experimental and control groups were recognized using a 96-well sterile microtiter plate as previously described (4). ODEO was serially diluted in Mueller Hinton Broth (Merck, Germany) at different concentrations (from 1 to 2048 µl/ml). Ten microliters of the prepared bacterial suspension (1.5×10^8 CFU/ml) were subjected into a final volume of 100 microliters in each well. Three wells containing non-treated bacterial suspension were considered as controls. A multi-detection micro-plate reader (Labtech, UK) was employed to record the optical density of each well at 600 nm every two hours for 24 h at 37 °C. Minimal bactericidal concentration (MBC) was measured by plotting five µl of clear-well samples into nutrient agar plates and incubation them at 37 °C for 24 hours. The MBC was considered as the concentration of ODEO at which there was no microbial growth. All the tests were performed in triplicate.

The bacterial quantitative colony counts (log CFU/ml) of *E. coli* in different groups were determined by culturing the rat's urine. Urine cultures were performed by inoculating the 0.01 ml of rat urine on Mac Conkey agar media for 24 h at 35°C (13).

Histopathological examination

Following euthanasia, bladders were dissected, fixed in formalin, and underwent histopathological examination. All samples were stained with hematoxylin and eosin (Gibco, Germany) and observed under light microscopy. The level of inflammation, thickness of the bladder epithelium, fibrosis and changes in the transitional epithelium were recorded (0 to 4; 0 = absent, 4 = severe; n = 5 per treatment group) to reflect the effects of agents on severity of cystitis (14).

Statistical Analysis

Statistical analyses were performed using SPSS version 17 and GraphPad Prism, with the one-way, two-way analysis of variance tests, and Student's t-test. P values of <0.05 were considered statistically significant.

Results and Discussion

Antibacterial effect of O. decumbens essential oil

Bacterial cystitis is one of the UTIs, which predominantly affects women (15). The most common causative agent is *E. coli* causing infiltration of inflammatory cells, edema and fibrosis are some

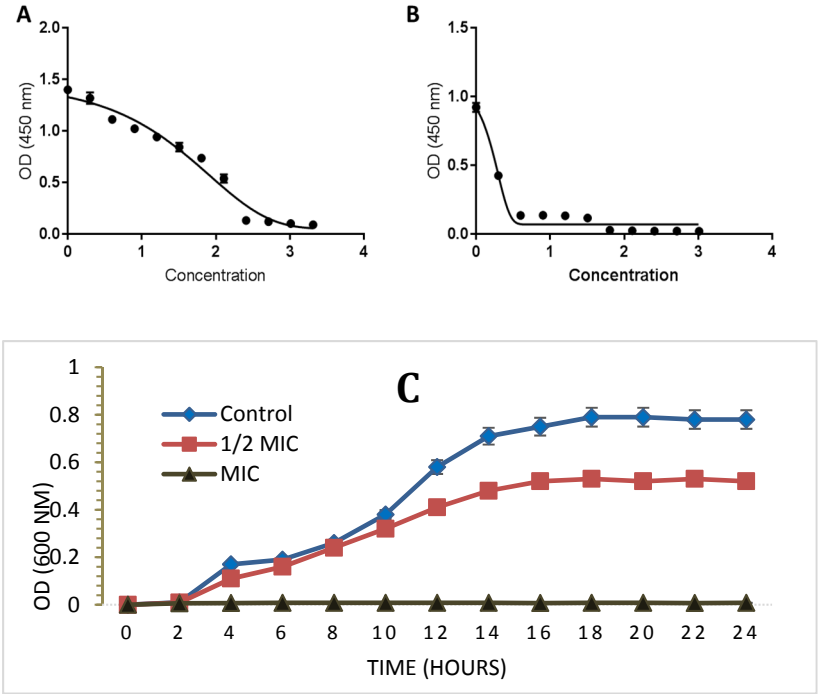

Figure 1. The bacterial growth curve resulting from the incubation of various concentrations of *O. decumbens* essential oil compared to gentamicin. Growth curves of *E.coli* exposed to A) *Oliveria decumbens* essential oil during 24 h compared to B) Gentamicin. Effect of different concentrations of ODEO on the growth of E. coli (C). *p<0.05 vs control.

Table 1. Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of *Oliveria decumbens* essential oil compared to *gentamicin*.

	***Oliveria decumbens* essential oil**		***Gentamicin***	
Bacteria	MIC (µl/ml)	MBC (µl/ml)	MIC (µg/ml)	MBC (µg/ml)
E. coli	0.54	1.024	2.89	4

histopathological changes resulting in this infection (16). Moreover, interruption of the protective flora of the urethra and vaginal region are some side effects of the antimicrobial compounds, which increase the risk of recurrent infections. Besides, following long-term use of antibiotics, general adverse effects are unavoidable (16) (17). The presence of a natural alternative, which may result in improved therapeutic consequences and fewer aftereffects than typical therapies, is preferable by far to any other treatment (17). One of the well-studied alternatives with documented efficacies is herbal agents. The antimicrobial effects of ODEO have been previously documented in many studies. In this study, the antimicrobial property of ODEO was determined by MBC and MIC. Figure 1 shows the bacterial growth curve resulting from the incubation of various concentrations of *O. decumbens* essential oil compared to gentamicin. MBC is defined as the lowest concentration of the agent required to kill a particular bacterium. Table 1 shows the MIC and MBC values for ODEO. It has been demonstrated that some natural agents like cranberry could decrease antibiotic usage and symptomatic UTI episodes compared to the placebo group (18). It has also been shown that Cranberry juice (15 mL) twice daily for one month resulted in decreased rates of bacteriuria in UTI patients (19). The ethanol extract of some herbs such *as Ocimum sanctum, Cinnamomum cassia, Zingiber officinale, Terminalia chebula, Punica granatum,* and *Azadirachta indica* have potential sources of antimicrobial compounds against UTI pathogens including *E. coli* (20). Also, Tuken et al showed that long-term (14 days) treatment with an herbal agent added to the drinking water resulted in the complete clearance of urine from these bacteria (13). Our study is another study that confirms the antimicrobial effects of traditional medicines.

Effects of Oliveria decumbens essential oil on the rat model

The bacterial count of the treatment and control groups are detailed in Figure 2. There was not statistically significant difference between bacterial count in the control groups (groups 1 and 2) count on first and seventh day of experiment. However, the administration of ODEO resulted in a significant reduction in the bacterial count after ten days (P<0.05). Statistically significant differences were noted in the bacteria count between orally and parenterally administered animals, especially at high dosages. The reduction of bacterial count by higher doses of ODEO was also significant compared to the lower doses. Gentamicin was superior to *Oliveria decumbens* essential oil in reducing bacterial count (1.14 ± 0.08 $\log^{10}$). The mean bacterial count reduced by ODEO at a higher dose (SC) was 2.12 ± 0.74 $\log^{10}$. In the current survey, due to the financial constraints, the essential oil components were not examined, but in many previous studies, small differences have been observed in the amount of the main components. Mahboubi et al. showed that the main components of ODEO were thymol (22%), carvacrol (22%), and p-cymene (19%) (21). These results were also confirmed by Amin et all who claimed that the ODEO contains high amount of oxygenated monoterpene elements of which carvacrol and thymol were the major factors (8). Strong antibacterial effects of many herbal essential oils in which thymol and carvacrol are the main components have also been demonstrated in many studies (22, 23). Limonene, another component in the ODEO, has also

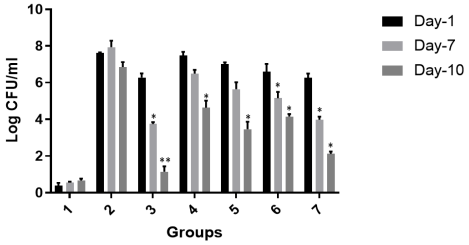

Figure 2. *E. coli* bacterial count in treatment and control groups of the rat model of *E.coli*-induced Cystitis on different days. Group 1, the control group: sterile saline injected into the bladder; group 2, the infected group with no treatment; group 3, the infected group with Gentamicin treatment (2 mg/kg, Intramuscular (IM) injection); group 4, the infected group with *O. decumbens* essential oil treatment (ODEO) (0.5 µl/ml, added into drinking water); group 5, the infected group with ODEO treatment (1 µl/ml, added into drinking water); group 6, the infected group with ODEO treatment (0.5 µl/ml, subcutaneous (SC) injection); group 7, the infected group with ODEO treatment (1 µl/ml, subcutaneous (SC) injection). The P-value was determined using the two-way analysis of the variance test (**p< 0.01, *p<0.05 vs group 2).

revealed anti-inflammatory effects (24). Besides, thymol and carvacrol are reported to be included in inhibition of inflammatory edema and white blood cells migration (25). The data from this study showed that oral and intramuscular administration of ODEO has a potent antibacterial effect. It has been revealed that the variation in chemical composition detected among plants collected from different areas with different climate and geographic conditions can affect the level of biological assets of a plant species. In our study, the company produced the essential oils of *O. decumbens* collected from the Nour Abad Mamasani region (at the flowering stage, June 2021) which demonstrated strong and reliable antimicrobial activity when compared to plants from other regions by Khoshbakht et al (12).

Effects of Oliveria decumbens essential oil on histopathological parameters

Hematoxylin and eosin stain revealed a significant decrease in fibrosis, inflammation, and thickness of epithelium in the bladder tissues of rats treated with *O. decumbens* in a dose-dependent manner in comparison to the control tissues (Table 2). In group 2, the infiltration of inflammatory cells and the thickness of bladder tissue were increased notably, while in the groups treated with ODEO, especially at higher doses,

Table 2. The pathological findings of cystitis treatment with *Oliveria decumbens* essential oil in all the groups

Parameters	G1	G2	G3	G4	G5	G6	G7
Inflammation	1.16±0.4 ^{a}	3.6± 0.5 ^{b}	2.7±0.4 ^{b}	2.8±0.46 ^{b}	2.8±0.46 ^{bc}	3±0.46 ^{b}	2.5±0.5 ^{b}
Fibrosis	1.0±0.0 ^{a}	3.6±0.5 ^{b}	1.3±0.46 ^{c}	2.5±0.5^{cd}	1.5±0.5 ^{ac}	1.3±0.5 ^{ac}	1.16±0.4 ^{ac}
Epithelial thickness	1.0±0.0 ^{a}	2.50±0.5 ^{a}	1.8±0.46 ^{c}	1.66±0.51 ^{cd}	1.3 ±0.5 ^{ac}	1.3 ±0.5 ^{ac}	1.1 ±0.4 ^{ac}

Recorded pathological scores. G1, the control group: sterile saline injected into the bladder; G2, the infected group taking no treatment; G3, the infected group taking Gentamicin treatment (2 mg/kg, Intramuscular (IM) injection); G4, the infected group with *Oliveria decumbens* essential oil (ODEO) treatment (0.5 µl/ml, added into drinking water); G5, the infected group with ODEO treatment (1 µl/ml, added into drinking water); G6, the infected group taking ODEO treatment (0.5 µl/ml, subcutaneous (SC) injection); G7, the infected group taking ODEO treatment (1 µl/ml, subcutaneous (SC) injection). *Means±SD with different letters superscripts (a, b,c,d) in the same column are statistically significant using one-way ANOVA test. n=5; Values are statistically significant at $p<0.05$.

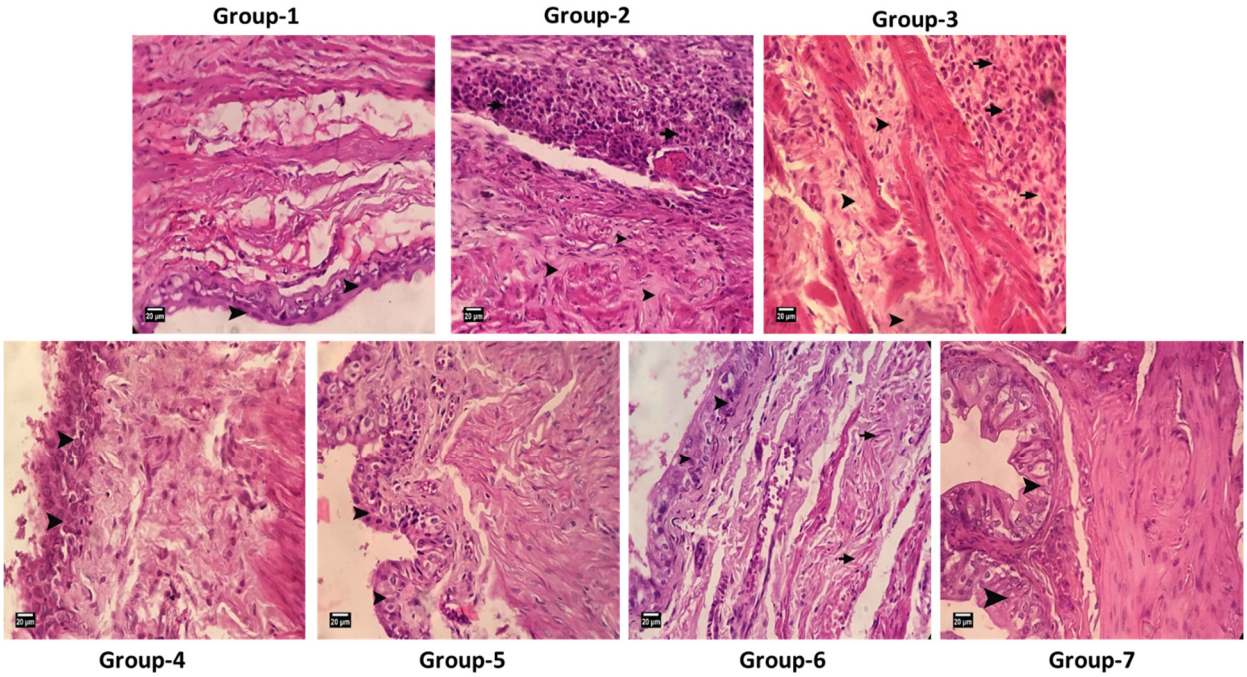

Figure 3. Staining of bladder (B-D) tissues of all groups showing pathological changes. Arrowheads point at sites of fibrosis and inflammation. Group 1, the control group: sterile saline injected into the bladder; Group 2, the infected group with no treatment; Group 3, the infected group with Gentamicin treatment (2 mg/kg, IM); Group 4, the infected group with *Oliveria decumbens* essential oil (ODEO) treatment (0.5 µl/ml, added into drinking water); Group 5, the infected group with ODEO treatment (1 µl/ml, added into drinking water); Group 6, the infected group with ODEO treatment (0.5 µl/ml, subcutaneous injection); Group 7, the infected group with ODEO treatment (1 µl/ml, subcutaneous injection).

the parameters were significantly decreased ($p<0.01$) (Figure 3). Our findings are in line with earlier surveys that documented the effectiveness of *O. decumbens* against various bacteria which has been attributed to its thymol and carvacrol compounds (12). The essential oil of *O. decumbens* not only eradicated the pathogenic bacteria but also decreased the inflammation and fibrosis resulting from the infection of the bladder. It has been revealed that bioactive compounds of the herb disrupt the bacterial membrane and inhibits the ergosterol biosynthesis and bacterial growth resulting in cell death due to ATP and ions leakage (4).

Conclusion

The results of the present study shed light on the antibacterial activity of the *O. decumbens* essential oil on *E.coli*-induced cystitis. The essential oil might be studied more as an alternative agent for the treatment of urinary tract infections. In addition, to eradicate the causative bacteria, the essential oil can also protect the uroepithelial cell layers. Further, *in vivo* tests and clinical trials are needed to evaluate the efficacy and safety of ODEO treatment for the management of UTIs in humans.

Acknowledgments

Not applicable

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