



Screening of antibacterial effect of the *Scrophularia Striata* against *E. coli in vitro*

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ABSTRACT

Introduction: This study was aimed to evaluate the antimicrobial activity of the ethanol and aqueous extracts of *Scrophularia striata* plant on *E. coli O157:H7* in vitro.

Methods: In this experimental study the ethanol and aqueous extract of the plant was prepared and their antibacterial effects were determined using sink diffusion and broth macrodilution methods against the bacterium *E. coli O157:H7*.

Results: The ethanol extract of *Scrophularia striata* plant had inhibitory effect on the *E. coli O157:H7* in two methods of sink diffusion and macrodilution, but the aqueous extract of this plant had not antibacterial effect. The MIC and MBC amounts were obtained 90mg/ml and 100 mg/ml, respectively.

Conclusion: Based on the present results that the ethanol extract of the *Scrophularia Striata* plant showed inhibitory effect on bacterium, more researches are recommended to evaluate its in vivo effects and to identify active compounds.

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

Scrophularia striata plant showed antibacterial properties in *in vitro* conditions which may provide an insight for future research in order to identify its effective compounds in *in vivo* condition.

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Introduction

Gastrointestinal disorders specially diarrhea are important causes of mortality in developing countries. The Enterohemorrhagic *E. coli* is one of six groups of *E. coli* bacterium and an etiologic agent of the diarrhea. This bacterium produces cytotoxins known as verotoxin or Shiga-like toxins that are the main causes of the hemorrhagic colitis (1). This organism was first reported in 1982 in a nursing home located in Ontario, Canada, as agent of the Ulcerative colitis (1,2). Also, there are reports of sporadic cases of the enterohemorrhagic *E. coli* disease from 1982 onward (3). Appearance of bloody diarrhea and high fever in the infection resulted from *E. coli* serotype *O157:H7* is prognosis of HUS syndrome that is usually followed by diarrhea and hemolytic anemia, thrombocytopenia and acute renal injury (3). Different types of undercooked sandwiches and hamburgers are

known as causes of the disease. Humans are known as main resource of enteroinvasive and enterotoxin producing *E. coli* strains that this resource contaminates the food through contact with contaminated production devices or water contaminated with the human feces (3). Vice versa about enterohemorrhagic strains (*O157:H7*) animals (cow and maybe poultry, sheep and pig) are resource of the microbe. Therefore, animal source foods can become contaminated after slaughter or production. On the other hand, antibiotic resistance has been reported in many humans contaminated to *E. coli O157:H7* who have used undercooked or raw contaminated meats (4). The Center for Disease Control and Prevention (CDC) estimates that the *E. coli O157:H7* strain causes disease in more than 73000 persons and 60 deaths each year in the USA. The human contamination to this bacterium is associated with contamination of foods, milk, plants and person-to-

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person transmission (5). The plant *Scrophularia striata* is of the family scrophulariaceae that is often gramineous and rarely arboreal with opposite, alternate or simple leaves and without stipel. This plant has five petals and zygomorpha flowers and corolla has lobe and the fruit is a capsule with numerous seeds. Branches of this plant are used as stomach nourishing (6). With regard to different references to antimicrobial effects of local pharmaceutical plants of Iran (7,8), including *Scrophularia striata* plant on *Staphylococcus aureus* and *Pseudomonas aeruginosa* (9), and with respect to increase of the antibiotic resistance in result of consuming antimicrobial drugs for preventing and threatening infections and also side effects and their different adverse effects, research on medicinal plants has been considered in order to discover new pharmaceutical resources against bacterial infections (10,11). In recent years it has been emphasized to replace natural materials in control and treatment of infections and different diseases instead of chemical drugs that have undesirable side effects (9,12). Plants synthesize some materials against attack of insects, herbivorous animals, and microorganisms and antimicrobial effects of these materials are well known. Antimicrobial properties of many plant extracts are due to existence of materials like tannins, phenolic compounds and suchlike (13). The genus *Scrophularia striata* contains compounds like alkaloids, resin glycosides, iridoid and cryptophilic acid (14,15) that usually are found in different parts of plants like leave, skin, stem, bud and scion. The extract of some edible spices and plants like *Entada africana*, *Terminalia avicenoides*, *Mitragina stipulosa*, *Lannae acida* and *Avicennia marina* can prohibit some intestinal pathogens including *E. coli* (16-18). Since brewed *Scrophularia striata* plant is used in west area of Iran in order to treat skin, internal, and deep infections (9), this research was conducted experimentally with the aim of studying the antibacterial effect of aqueous and alcoholic extracts of this plant on the *E. coli* O157:H7 bacterium.

Materials and Methods

This experimental study was designed in the Medical Plants Research Center, Shahrekord University of Medical Sciences. To perform this study the standard strain of *E. coli* O157:H7 (ATCC 43895) was used. To achieve this aim a suspension with turbidity equivalent to 0.5 McFarland in normal saline was prepared from culture of the bacterium on the Eosin-methylene blue (EMB) agar. In this condition number of bacteria is about 1.5×10^8 CFU/ml (colony forming unit/ml). This suspension was used in the next stages of experiments (19).

The plant extract

Aerial organs of the *Scrophularia striata* plant of the family Scrophulariaceae with the local name of "Teshneh Dari" were collected from Zagros mountain ranges (around the Ilam city) in spring of the year 2012 and the herbarium

was prepared after they were identified and approved by the Research Institute of Jihad-e-Agriculture in Ilam province (Code=384). The collected plant materials were first cleaned and then were powdered by the mill and were maintained in the suitable and proper conditions. To prepare the aqueous extract, 10 ml distilled water was poured in the beaker per gram powder and after it was boiled the plant powder was added and boiled for 15 minutes. The obtained extract was entered to the rotary evaporator to remove solvent (9). The aqueous extract with 15% efficiency after filtration using filters with diameter of 0.45 μ was subjected to the antimicrobial testing in different concentrations by diffusion method. To prepare the ethanol extract, 100 gram of the plant dried powder was mixed with 500 cc of 80% ethanol and was maintained for 24 hours at the room temperature (22 °C). After removing the solvent by the rotary evaporator the obtained alcoholic extract was powdered in incubator at a temperature of 37 °C. Then 1 gram of the plant alcoholic extract powder was added to the 5 ml of dimethyl sulfoxide solvent (DMSO) and became sterile by the filtration.

Studying the microbial sensitivity

To determine the microbial sensitivity, the amikacin antibiotic was used as positive control and the DMSO solvent was used as negative control. To ensure the effect of ethanol and aqueous extracts on the desirable bacterium, the bacterium was influenced with different concentrations of the extracts. To achieve this end two sinks were provided in the Mueller Hinton agar medium with a diameter of 7 mm that after the bacterium culture from bacterial suspension equivalent to 0.5 McFarland on the medium, the amikacin antibiotic (concentration 30 μ g) was added to one of the sinks and the plant extract with concentrations of 20-50-100-200-400 mg/ml was added to the another sink. Then plates were incubated for 24 hours at a temperature of 37 °C and the results were recorded based on measuring diameter of the inhibition zone provided by the plant extract (all experiments were repeated 5 times) (20).

Determining the minimum inhibitory concentration and minimum bactericidal concentration

Experiments were conducted in two stages and the corrected macrodilution method recommended by the National Committee for Clinical Laboratory Standards (NCCLS) was used (21). According to this method a suspension with the concentration equivalent to 0.5 McFarland was prepared from the bacterium in the TSB (Trypticase Soy Broth) and then was affected by successive dilutions in the concentration range from 20-200 mg/ml (20-40-60-80-100-120-140-160-200) at a temperature 37 °C and after 24 hours the MIC was determined by observing tubes turbidity. To determine MBC, contents of the tubes that the turbidity was not seen in them were subcultured onto the EMB medium and the serial dilution

method was used to count the colony. The first tube that amount of decrease in bacteria number growth was more than the thousandth compared with the zero time of the control tube was selected as MBC. Sometimes the MBC concentration is equal to the MIC concentration. In the next stage to determine the MBC concentration exactly intermediate concentrations, between the MBC concentration and lower concentrations, also were tested by the similar method (20). Results obtained from experiments were compared statistically with each other.

Results

According to the sink diffusion method results showed that the ethanol extract of the *Scrophularia striata* plant had the inhibitory effect on the growth of the bacterium *E. coli O157:H7* in concentrations 100, 200 and 400 mg/ml with the average inhibition zone of 12 ± 0.8 , 14 ± 0.8 and 16 ± 0.8 mm, respectively but the inhabitation zone was not observed by any of concentrations of the plant's aqueous extract and the aqueous extract of this plant had not the antibacterial activity against the bacterium *E. coli O157:H7* in this study. Results obtained from determining the MIC amount showed that no turbidity was observed in the concentration 90 mg/ml after passing 24 hours. Also The MBC amount was equal to the concentration of 100 mg/ml.

Discussion

In the recent years many attention has been paid to the *E. coli O157:H7* as the most prevalent agent of the HUS syndrome. Ulcerative colitis and TTP (Thrombotic Thrombocytopenia Purpura) diseases are developed in the human by this bacterium and can be effective in the humans contamination through raw livestock products like (cows as the first resource) (22). To study and compare antimicrobial effects of the *Scrophularia striata* extract on the *E. coli O157:H7* bacterium, aqueous and ethanol extracts of the plant aerial organs were tested by the sink diffusion method. No inhibitory effect of aqueous extract of the plant against the *E. coli O157:H7* bacterium was observed and the aqueous extract of this plant had not the antimicrobial activity against the *E. coli O157:H7* bacterium. But the ethanol extract of the plant had the inhibitory effect on the bacterium growth in concentrations 100,200 and 400 mg/ml. The antimicrobial effect of the *Scrophularia striata* extract on *Staphylococcus aureus* and *Pseudomonas aeruginosa* was studied in the study conducted in the Medical Science University of Ilam and it was concluded that the obtained aqueous extract can be used as antiseptic product in treatment of external infections resulted from these two microorganisms (9). In the present study also the antimicrobial effect of the *Scrophularia striata* extract was proved with this difference that the aqueous extract of this plant unlike the ethanol extract was not effective on the *E. coli O157:H7* and this

shows that effective antimicrobial materials against the *E. coli O157:H7* has been extracted by the ethanol solvent. In the other study antimicrobial the effects of the aqueous and ethanol extracts of the skin of four plants namely *Entada africana*, *Mitragina stipulosa*, *Terminalia avicennoides* and *Lanmae acid* were studied on 10 enterohemorrhagic *E. coli* strains (*E. coli O157:H7*) and MIC amount equal to 1.56-50 mg/ml and MBC amount equal to 6.25-25 mg/ml was reported (1). Also in the study of the Tajbakhsh and colleagues the antibacterial effects of the *Avicennia marina* extract on three strains of *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa* were studied and the MBC amounts were reported to be 7/9,15/8 and 33/8, respectively (18). In the present study the MIC amount for microorganism *E. coli O157:H7* was 90 mg/ml and the MBC amount was 100 mg/ml that may indicate less antimicrobial effects of this plant rather the above mentioned plants. Also results of this research showed that the inhabitation zone have been increased when the extract amount in all samples has increased. This might resulted from increase in the microbial sensitivity of the *E. coli O157:H7* against higher amounts of the plant extract or increase of the extract antimicrobial property in high amounts. It should be noted that by increase of concentration and the effect an increase can be provided in creation of the toxicity (9,23). In other studies antimicrobial effects of the *Silene multifida* extract and extract of the walnut leave on *Staphylococcus aureus*, *E. coli*, *Candida albicans*, and *pseudomonas aeruginosa* bacteria have been showed (24,25). Also antimicrobial effects of the *Peperomia tertaphyalla* extract on *Staphylococcus aureus*, *E. coli* and *Candida albicans* have been assessed and the possibility of using this plant in treatment of Cystitis and Cutaneous infections resulted from above bacteria has been suggested (26). In the present study also the effect of the *Scrophularia Striata* extract on the *E. coli O157:H7* was observed that with regard to the possibility of existence of biologic effective compounds in this plant, its antibacterial effect is a reasonable issue and can smooth the way in order to study antimicrobial effects of this plant in animal models for possible using in the treatment of enteritis caused by this bacterium.

Conclusion

Results of this study shows that the *Scrophularia striata* plant has antibacterial properties in *in vitro* conditions and these findings can provide more insights for future research in order to identifying, determining the amount and purification of its effective compounds in the *in vivo* condition.

Authors' Contributions

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

Conflict of Interests

The authors declare no conflict of interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the author.

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