Jundishapur J Microbiol. 2012;**5**(3):511-515. DOI: 10.5812/jjm.3665

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Jundishapur Journal of Microbiology
www.jjmicrobiol.com

The Effect of *Peganum harmala* and *Teucrium polium* Alcoholic Extracts on Growth of *Escherichia coli O157*

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ARTICLE INFO

Article type:
Original Article

Article history:
Received: 26 Jul 2011
Revised: 23 Nov 2011
Accepted: 01 Dec 2011

Keywords:
Peganum harmala
Teucrium polium
Escherichia coli O157
Medicinal Plants

ABSTRACT

Background: Several medicinal plants have been used by humans for their vast diversity in chemical compound content and antimicrobial activity. However, their appropriate use remains under investigation by many researchers.

Objectives: This study aimed to determine the stage at which *Escherichia coli O157* growth is more sensitive to certain concentrations of certain medicinal plants.

Materials and Methods: Alcoholic extracts of the aerial parts and seeds of *Peganum harmala* and *Teucrium polium* were prepared using standard methods. The effective concentration (0.15-0.4 mg/ml) was chosen, based on their zones of inhibition on plates that were inoculated with *E. coli O157* and plant extract and used to monitor bacterial growth by spectrophotometry.

Results: All concentrations of the aerial parts of *T. polium* extract and the seeds of *P. harmala* decreased the absorbance in the first several hours after inoculation, after which bacterial growth increased to normal levels. Compared with control, no significant differences on the effect of the aerial parts of *P. harmala* extract on bacterial growth were observed.

Conclusions: Based on the comparison of growth curves, higher concentrations effected greater inhibition. However, the effects of other concentrations of the plant extracts on bacterial growth should be investigated further, although their toxicity must be assessed before use.

▸ *Implication for health policy/practice/research/medical education:*
Two Iranian endemic plants *P. harmala* and *T. polium* were selected and inhibition effects of different concentrations of their alcoholic extract on growth phases of E. coli O157 was investigated.

▸ *Please cite this paper as:*
Mashreghi M, Niknia S. The Effect of *Peganum harmala* and *Teucrium polium* Alcoholic Extracts on Growth of *Escherichia coli O157*. *Jundishapur J Microbiol*. 2012;**5**(3):511-5. DOI: 10.5812/jjm.3665

1. Background

Medicinal plants were the first medicines and have been used since ancient times (1), and they continue to be used by various cultures around the world (2). All drugs from plants contain substances, such as alkaloidal constituents; essential oils; phenols; unsaturated long-chain aldehydes; peptides; and ethanol-, methanol-, and butanol-soluble compounds with specific therapeutic activities (1, 3). The antibacterial activities of several species of plants have been reported by many researchers (1, 4, 5). We investigated 2 plants, *Peganum harmala* and *Teucrium polium,* commonly called “Espand” and “Kalbpoureh,” respectively, in Iran. *Peganum harmala* is a perennial herbaceous, glabrous plant that can grow to 30–100 cm and is distributed throughout the Middle East, North Africa, and Central Asia (2, 6). It is famous for its antimicrobial

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DOI: 10.5812/jjm.3665

activities, due to certain compound, such as alkaloids that are found in its seeds and roots (2, 6, 7). The smoke of its seeds is used traditionally as a disinfectant. Also, alkaloids in *P. harmala* seed have vasorelaxant, antihemosporidian, anticancer, antinociceptive, antitumor and antineoplastic, and antiprotozoal effects (8).

Teucrium polium is a durable, wild-growing, flowering grass plant that can grow to 10-30 cm, has a callous white exterior, and abounds in southwestern Asia, Europe, and North Africa (1, 8). Like *P. harmala*, *T. polium* is famous for its antimicrobial activities, stemming from compounds, such as flavonoids and terpenoids, that are found in its aerial parts (9, 10). *T. polium* is well known for its diuretic, antipyretic, diaphoretic, antispasmodic, tonic, anti-flammatory, antihypertensive, anorexic, analgesic, and antidiabetic properties. It also protects against ethanol-induced gastric mucosal damage (92.8 %) and has been reported to reduce nicotinamide adenine dinucleotide phosphate (NADPH)-initiated lipid peroxidation in rat liver microsomes (11).

P. harmala and *T. polium* are traditional medicinal plants that are used for many purposes, particularly in treating gastrointestinal problems. *Escherichia coli* that is an anaerobic bacterium commonly found in the mammalian intestinal tract (12), of which there are pathogenic strains, such as O157: H7, which causes significant human diseases, such as diarrhea, hemorrhagic colitis (HC), and hemolytic-uremic syndrome (HUS) (13, 14).

2. Objectives

The object of this study was to examine the antibacterial activities of alcoholic extracts of *P. harmala* and *T. polium* against *E. coli* O157.

3. Materials and Methods

3.1. Plant Collections and Identifications

P. harmala (Zygophylaceae) (*Figure 1*: left) was collected from hills around Mashhad (northeast of Khourasan state, Iran) between May and August, and *T. poluim* (Labiatae) (*Figure 1: right*) was collected from Birjand (southeast of Khourasan state, Iran) in July. Taxonomic identification was peformed by the Faculty of Science Herbarium, Ferdowsi University of Mashhad, Iran.

3.2. Plant Extract Preparation

The aerial parts of *P. harmala* and *T. polium* were dried for 8-10 days at room temperature and ground to a powder separately, as were *P. harmala* seeds. The powder was first tyndallized, dissolved in ethanol (ethanol:water = 8:2,v/v) and distilled under a vacuum for several hours at 82°C. Finally, the ethanol was removed by evaporation.

3.3. Bacterial Strain

The *E. coli* strain *O157* NCTC1290 was a kind donation of Prof. Penington, University of Aberdeen, UK.

3.4. Well Diffusion Assay

To determine the effective concentration, inhibiton zones of ethanolic extracts of *P. harmala* and *T. polium* were examined against *E. coli O157* by well assay technique (15). Overnight cultures of *E. coli O157* were spread onto Mueller-Hinton agar (Merck, Germany), and various concentrations of extracts (*P. harmala*: seeds: 0.15, 0.2, 0.3, and 0.4 mg/mL; aerial parts: 0.15, 0.2, 0.3, and 0.4 mg/ml; *T. polium*: 0.1, 0.2, 0.3, and 0.4 mg/mL) were added to the pits in the culture with 10 mm diameter and incubated at 37°C for 24 h. Then, the diameter of the inhibitory zone was measured, and the corresponding effective concentration was selected for subsequent experiments.

3.5. Spectrophotometeric Assay

The growth curve of *E. coli O157* was drawn per standard methods (16, 17). Various amounts of plant extract (0.15–0.4 mg/mL) were added to nutrient broth medium (Merck, Germany), containing 1-mL bacterial inocula (10^8 cells/

Figure 1. The Plants of This Study: *Peganum harmala* (Left), *Teucrium polium* (Right)

ml), by shaking the samples. The time–zero absorbance was read at 600 nm on a WPA Lightwave UV/Vis spectrophotmeter (Biochrom, UK). Subsequent absorbance readings were recorded in 1-hour intervals for 10 hrs. The results were transferred to Excel, and after mean absorbance values were calculated, logarithmic graphs were drawn and compared.

4. Results

Based on the inhibitory zone diameters (*Figure 2 and Figure 3*), the extract of seeds of *P. harmala* and *T. polium* at 0.3 mg/ml had a greater inhibitory effect on *E. coli O157* than other concentrations. However, the aerial part extracts of *P. harmala* did not inhibit *E. coli O157* growth.

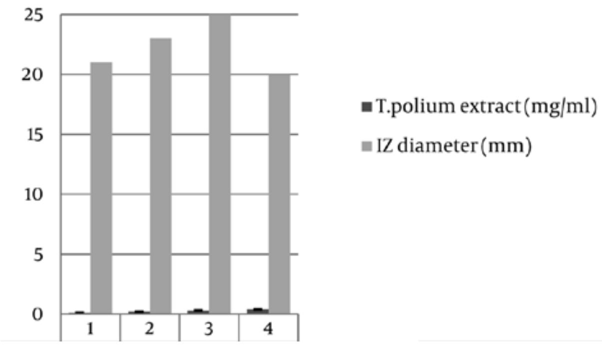

Figure 2. IZ Diameter of the Different Concentrations of *T. polium* Extract

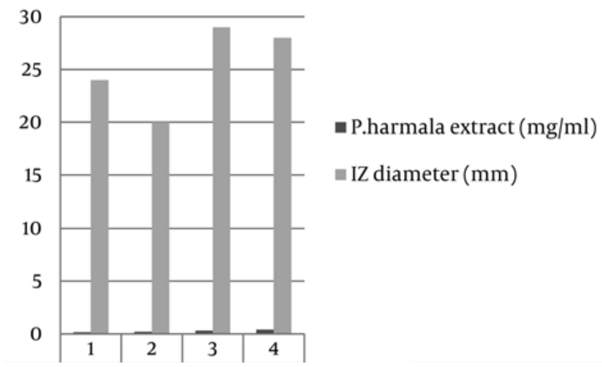

Figure 3. IZ Diameter of the Different Concentrations of *P. harmala* Extracts (Seeds)

By spectrophotometry, all concentrations (0.1, 0.2, 0.3 mg/ml) of the aerial parts of *T. polium* extract, reduced the absorbance (ie, growth) during the first several hours of inoculation, especially at Hour 2; the in the greatest decrease was effected by 0.3 mg/ml extract (*Figure 4*). From Hours 4 to 6, the effects of the various concentrations were the same. At Hour 7, the greatest decline was observed with 0.2 mg/ml extract. At Hours 8 and 9, the effects of 0.2 and 0.3 mg/ml extract were equal, and at Hour 10, the absorbance increased with all concentrations (0.1, 0.2, 0.3 mg/ml) of the extract of the aerial parts of *T. polium* .

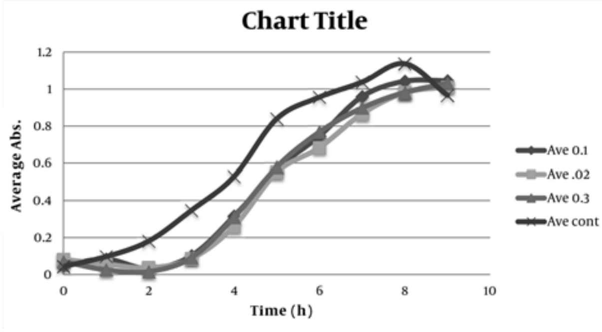

Figure 4. Changes in *E. coli O157* Growth (Abs) at Presence of Different Concentrations of Alcoholic Extract of *Teucrium polium*

With all concentrations (0.15, 0.2, 0.3 mg/mL) of the extract of *P. harmala* seeds, the absorbance (*Figure 5*) decreased in the first several hours of inoculation, especially Hour 2; the greatest declined occurred at 0.15 mg/mL. The effect of 0.3 mg/ml extract was more robust than with other concentrations at Hours 3 to 10. In the 11th hour, the absorbance did not decrease at any concentration (0.15, 0.2, 0.3 mg/mL) of *P. harmala* seed extract and was similar to that of the control.

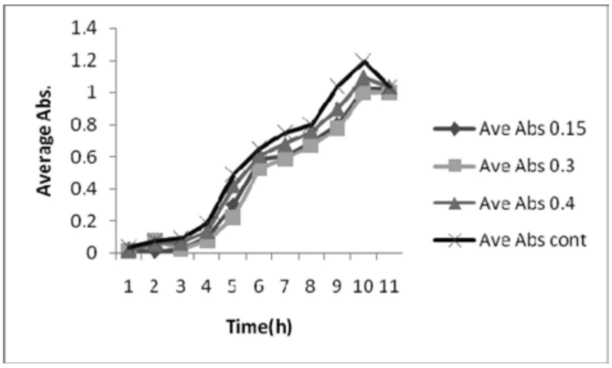

Figure 5. Changes in *E. coli O157* Growth (Abs) at Presence of Different Concentrations of Alcoholic Extract of *Peganum harmala* (Seeds)

By spectrophotometry, there was no inhibition with 0.2, 0.3, or 0.4 mg/ml of the extract of the aerial parts of *P. harmala* against *E. coli O157* (*Figure 6*).

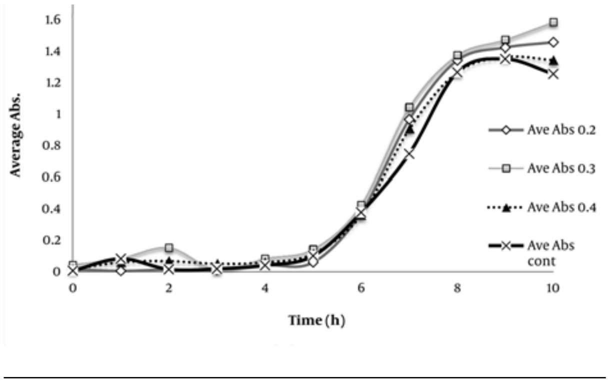

Figure 6. Changes in *E. coli O157* Growth (Abs) at Presence of Different Concentrations of Alcoholic Extract of *Peganum harmala* (the Aerial Parts)

At 0.3mg/ml, the extracts of the aerial parts of *T. polium* and the seeds of *P. harmala* extract effected the greatest inhibition of various growth phases of *E. coli O157*.

5. Discussion

Historically, plants have been a good source of new drug compounds (18). Today, in many parts of the world, the extracts of medicinal plants are used for their antibacterial, antifungal, and antiviral properties (4, 5, 19). In Iran, the use of these natural resources goes back to its origins, and many reports on this topic exist (20). Some groups have reported that there is a relationship between the chemical compounds and the antimicrobial activity of plants (1). Yet, the antibacterial properties of such plants are poorly understood and remain under debate (21, 22). Therefore, an investigation of the antibacterial properties of plants, especially endemic plants, could have a benefit finding identifying an effective species that controls the growth of an important bacterial pathogen.

An ethanolic *P. harmala* extract has been shown to have high antibacterial activity against *MRSA* (methicillin-resistant *Staphylococcus aureus*) (23) and *CRSA* (cefixime-resistant *S. aureus*) (24).

Also, an ethanolic *T. polium* extract has high antibacterial activity against certain bacteria, such as *TRBM* (tetracycline-resistant *Brucella melitensis*) (25), *E. coli,* and *Salmonela Typhi* (26).

The antibacterial effects of 3 plants (*Vitex angus castus*, *Eucalyptus globules,* and *Juglans regia*) (27) have been examined, wherein *E. coli* was killed by *Vitex angus castus* extract at 0.2 mg/ml. Of 38 commonly used medicinal plant species in Thailand, the aqueous and ethanolic extracts of *Quercus infectoria* and the aqueous extract of *Punica granatum* were highly effective against *Escherichia coli O157* with peak MIC and MBC values of 0.09 and 0.19 mg/ml, respectively (14).

Based on these findings, 2 medicinal plants—*P. harmala* and *T. polium*—were chosen for this study. Traditionally, they are used to treat gastrointestinal problems. *P. harmala* has some alkaloids and harmalol—quinazoline derivatives that provide its pharmacological activity (2). *T. polium* has flavonoids that are widespread in the plant kingdom and have many functions, including anti-inflammatory, antimicrobial, enzyme inhibitory, antioxidant, and antitumor activities (10, 28, 29). We use spectrophotometry, because cell biomass (turbidity) is gives a better and more rapid indication of the concentrations of plant extracts that have a greater effect on bacterial growth. The absorbance of turbid samples, such as cell cultures, is based on to light scatter, not molecular absorption. In the first hour of bacterial growth, the absorbance decreased and T % increased at all concentrations, demonstrating that the alcoholic extracts of *P. harmala* and *T. polium* inhibit the growth of *E. coli O157* in the early stages of its growth.

Cells were more vulnerable to the inhibitory effects of plant extracts during exponential growth versus other growth phases. In the comparison of growth curves, higher concentrations had a greater effect.

A comparison of the 3 extracts showed that *T. polium* effected greater inhibition of *E. coli O157*,a major gastrointestinal pathogenic microbe. However, with regard to medical prescription, the toxicity of higher concentrations must be checked before administration.

In this study, the absorbance and T % curves of the extracts of *T. polium* and the seeds of *P. harmala* showed their effective antimicrobial effects against bacteria that cause gastrointestinal problems, especially at the early stage of growth.

Acknowledgements

The authors would like to thank the Microbiological laboratory of Ferdowsi university of Mashhad and Mrs. Sedighe Molaei and Mrs. Hanie Shariatmadari for their assistances to complete the work and Miss Masoume Esmaeili nezhad and Miss Soraya Ebrahimi for their assistances to complete the graphs.

Financial Disclosure

None Declared.

Funding/Support

This research was supported by Research Deputy of Ferdowsi University of Mashhad, Iran (grant no: 40839).

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