

**Evaluation the antibacterial effects of *Echinophora platyloba* extracts against some *Salmonella* species**

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**Type of article:** Original**Abstract**

**Introduction:** Salmonellosis, which is caused by nontyphoid salmonella bacteria, is one of the most common foodborne diseases, and it causes gastrointestinal infections worldwide, most of which are limited gastroenteritis that requires antimicrobial treatment. The aim of this study was to investigate the effects of *echinophora platyloba* extract on inhibiting the growth of *Salmonella typhi*, *Salmonella enteritidis*, and *salmonella choleraesuis*.

**Methods:** *Echinophora Platyloba* extract was collected in the East Azarbaijan Province in Iran in June 2015. Weeds, infected plants, and dried roots were separated and removed. After drying and grinding the plant, 100 grams of powder were weighed, and the extraction of the plant was carried out by percolation. This study tested the Minimum Inhibitory Concentration (MIC) by the broth micro dilution method and Minimum Bactericidal Concentration (MBC). All of the data were analyzed by SPSS statistical software, version 22.0. One-Way ANOVA and the Duncan test were used to compare the effect of various concentrations of the extract on each type of bacteria.

**Results:** Our results indicated that, in 250 mg/ml of extracts discs, the largest growth inhibition zones were formed, and they were  $26.11 \pm 1.16$ ,  $21.23 \pm 0.89$ , and  $19.65 \pm 0.60$  in *S. enteritidis*, *S. typhi*, and *S. choleraesuis* groups, respectively. The statistical results indicated that, in each type of bacteria, there was a statistical difference ( $p < 0.01$ ) between the various concentrations of the extracts and the chloramphenicol discs. Also, it was indicated that this extract at a concentration of 150 mg/ml had a germicidal effect on *S. enteritidis* and *S. typhi* bacteria and that 250 mg/ml had a bactericidal effect on *S. choleraesuis*.

**Conclusion:** The results of this study indicated that *E. platyloba* extract has potential effects as antimicrobial agents.

**Keywords:** antimicrobial action, *echinophora platyloba*, salmonella

**1. Introduction**

The use of herbal products for the treatment of diseases has increased in recent years, especially for the treatment of infectious diseases. Clinical microbiologists are interested in using plants for the treatment of infections, because their side effects are significantly lower than those of chemical antibiotics (1). It is estimated that about 500,000 plant species have been identified in the world (2), with less than 1000 species being known as a medicinal plants (3). Currently, about 30 to 50% of the medicinal products available in the U.S. are from plants (4), and the use of natural products in the United Kingdom has increased with the extraction of different supplements (5). Medicinal plants have a major therapeutic role in Iran, and several plants have been used for centuries to treat various diseases. One of the native Iranian plants is *Echinophora platyloba* D.C, which has various saponins, flavonoids, and alkaloid compounds (6). In addition, one of the most important terpene compounds identified in *Echinophora* essence was trans- $\beta$ -Ocimene (7). It was indicated that plants have a wide variety of secondary metabolites, with *in vitro* antimicrobial activity, and these metabolites include tannins, terpenoids, alkaloids, and flavonoids (1). The different species of *Echinophora* have been defined as *Echinophora tenuifolia*, *E. platyloba* DC., *Echinophora sibthorpiana* Guss, *Echinophora anatolica* Boiss, *Echinophora cinera*, *Echinophora vadiaus* Boiss, *Echinophora orientalis*

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*Hedge and Lamond, Echinophora tournefortii joub, Echinophora trichophylla Sm, E. spinosa*. Four of these species are found in Iran, i.e., *Echinophora orientalis, Echinophora sibthorpiana, Echinophora cinerea* and *E. platyloba* (8). In Iran, *E. platyloba* is used mainly for flavoring foods (7) and to prevent mold from growing in tomato paste and pickles. Due to the anti-fungal, carminative, and digestive properties of various species of *Echinophora* genera, they also have been used to treat wounds and gastric ulcers in traditional medicine (9). Previously, it was reported that plants can have anti-microbial, anti-cancer, and stimulant effects on the stomach (10). Acute gastroenteritis is widespread in low hygienic and weak economy regions (11), and, in developing countries, such as Iran, salmonellosis has particular importance (12). Researchers have reported that the most prevalent isolated salmonella from children's diarrhea was *S. enteritidis* (12). In recent years, human infectious and food contamination with *S. enteritidis* have increased throughout the world (13), and salmonellosis has caused extensive deaths, especially in developing countries (14). Salmonellas cause different disease syndromes and, according to their antigenic profiles, they have host specificity (15). The results of previous Iranian studies indicated that 6.8% of the poultry source samples were infected by different Salmonella serovars, and it was indicated that current prevalence of *S. enteritidis* in Iran is more than that of *S. typhimurium* (16-19). The results of a study on a total of 139 Salmonella isolates demonstrated that Salmonella Enteritidis, Infantis, and Typhimurium included 84.9% of the isolates, and high rates of antibiotic resistance were documented (20, 21). There are a few Iranian reports on the anti-bacterial effects of some herbal plants on salmonella spp. (22). The objective of this research was to investigate the effect of *Echinophora platyloba* extracts on inhibiting the growth of *Salmonella enteritidis, typhi, and choleraesuis* and to compare comparison it with chloramphenicol antibiotic.

## 2. Material and Methods

### 2.1. Plant material

*Echinophora Platyloba* was collected in the East Azarbaijan Province in June 2015. Weeds, infected plants, and dried roots were separated and removed for preparation of the plant; aerial parts were gathered, dried, and powdered, and 100 grams of the powder were prepared by percolation methods; the extraction of the plant was conducted, and the net weight of extracts was 10 grams per 100 grams of plant. In this study, we prepared 50, 100, 150 and 250 mg/ml extracts.

### 2.2. Microorganisms

Cultures of *Salmonella enteritidis* ATTC 4931, *Salmonella typhi* ATTC 6539, and *Salmonella choleraesuis* ATTC 10708 were obtained from the Iranian Research Organization for Science and Technology. Five different isolates of each bacterium that was provided by the Bacteriology Department at Islamic Azad University of Tabriz were evaluated and compared with standard isolates.

### 2.3. Micro-well dilution assay

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the extracts were studied for the bacterial strains in micro plates. MIC values of extract against bacterial strains were determined based on a micro-well dilution method. First, 100  $\mu$ L Muller Hinton broth was added to all wells except the first one, and, then, 100  $\mu$ L of the extracts were added to each well. We transferred 100  $\mu$ L from the second well into the consecutive wells. The inocula of the bacterial strains were prepared from 24-hr cultures, and the suspensions were adjusted to 0.5 McFarland standard turbidity. In the later stage, 30  $\mu$ L of 0.1% resazurin were added to all of the wells. To perform MBC, in the MIC plates where there was intermediate color change, samples were taken and cultured on BHI agar medium. Twenty-four hours after incubation, the growth of bacteria was evaluated, and in plate wells which bacteria growth was negative considered as MBC result.

### 2.4. Statistical analysis

All data were analyzed by SPSS and were expressed as mean  $\pm$  standard error (SE). Data analysis was performed using SPSS version 22.0, One-Way ANOVA, and the Duncan test.

## 3. Results

Our results indicated that the *Echinophora Platyloba* extracts had a great effect on the prevention of salmonella growth. In addition, they showed that the most effect of extract occurred in *salmonella enteritidis* (Table 1). It was demonstrated that the 150-mg/ml extracts had bactericidal effects on *Salmonella enteritidis* and *typhi*, and the 250-mg/ml extracts had germicidal effects on *Salmonella choleraesuis* (Table 2). It was shown that, in 250 mg/ml, the largest growth inhibition zone was formed in all studied salmonellas in this experiment (Table 3). In addition, there

was no significant difference between Chloramphenicol disc and *E. platyloba* in inhibition of growth ( $p > 0.05$ ), although in some cases, such as *S. enteritidis*, the *E. platyloba* was more effective than chloramphenicol ( $p < 0.01$ ). The results showed that, in 250 mg/ml of extracts, the largest growth inhibition zones were formed, and they were  $26.11 \pm 1.16$ ,  $21.23 \pm 0.89$ , and  $19.65 \pm 0.60$  in *S. enteritidis*, *S. typhi*, and *S. choleraesuis* groups, respectively (Table 3). It was demonstrated in the case of *S. enteritidis* that there was statistical difference between the inhibition zone of the 250-mg/ml concentration of extract and chloramphenicol disc ( $p < 0.05$ ), although in *S. typhi* and *S. choleraesuis* inhibition zone in 250-mg/ml concentration of extract was higher than the chloramphenicol disc, but the difference was not significant ( $p > 0.05$ ) (Table 3). Our results indicated that some isolates of the salmonella were insensitive and resistant to chloramphenicol, but, in all cases, there were no resistant isolates to *E. platyloba*.

**Table 1.** Comparison of the minimum inhibitory concentration of the bacteria that were studied

Isolate	<i>S. enteritidis</i>				<i>S. typhi</i>				<i>S. choleraesuis</i>			
	50	100	150	250	50	100	150	250	50	100	150	250
Standard	+	+	+	+	-	+	+	+	-	-	+	+
Isolate 1	-	+	+	+	-	+	+	+	-	-	-	+
Isolate 2	+	+	+	+	-	-	+	+	-	-	-	+
Isolate 3	-	-	+	+	-	-	-	+	-	+	+	+
Isolate 4	-	+	+	+	-	+	+	+	-	-	+	+
Isolate 5	+	+	+	+	-	-	-	+	-	-	-	+

**Table 2.** Comparison of the minimum bactericidal concentrations of the bacteria that were studied

Isolate	<i>S. enteritidis</i>				<i>S. typhi</i>				<i>S. choleraesuis</i>			
	50	100	150	250	50	100	150	250	50	100	150	250
Standard	-	-	+	+	-	-	+	+	-	-	-	+
Isolate 1	-	-	-	+	-	-	+	+	-	-	-	+
Isolate 2	-	-	+	+	-	-	-	+	-	-	-	+
Isolate 3	-	-	-	+	-	-	-	+	-	-	+	+
Isolate 4	-	-	+	+	-	-	+	+	-	-	-	+
Isolate 5	-	-	+	+	-	-	+	+	-	-	-	+

**Table 3.** Comparison of the growth inhibition zones of *E. platyloba* and chloramphenicol disc

Disc Concentration, mg/ml	<i>S. enteritidis</i>	<i>S. typhi</i>	<i>S. choleraesuis</i>
250	$26.11 \pm 1.16^{*c}$	$21.23 \pm 0.89^c$	$19.65 \pm 0.60^b$
125	$16.36 \pm 0.97^b$	$14.18 \pm 0.78^b$	$10.01 \pm 1.21^a$
62.5	$6.47 \pm 1.14^a$	$6.32 \pm 1.15^a$	---
Chloramphenicol disc	$17.70 \pm 0.93^b$	$18.97 \pm 0.55^c$	$17.12 \pm 1.08^b$
p-value	0.001	0.001	0.001

\*The different alphabetic characters in each column demonstrate significant statistical differences.

#### 4. Discussion

The therapeutic properties of herbs have had a prominent role in the evolution of most natural plant therapies. Because of low income in conventional medicine during a lapse of time, the knowledge of traditional medicine started to evanesce, although in the latest years due to lesser side effects of plants, a wide range of studies have been conducted on their various effects in the control and treatment of animal and human disease. Because of natural medicine's low side effects compared to chemical drugs, they have been used for a long time to treat diseases, and, in recent years, there has been a growing trend of such treatment (23). It was previously reported that the main components of essential oil and extract, such as ocimene,  $\alpha$ -pinene, myrcene, and  $\alpha$ -phellandrene have antibacterial activity (9). In Iran, *E. platyloba* is used mainly as a food flavor (7) and for the preservation of tomato paste and pickles. The objective of this study was to investigate the effect of *Echinophora platyloba* extracts on inhibiting the growth of *Salmonella enteritidis*, *typhi*, and *choleraesuis* and to compare it with chloramphenicol antibiotic. Different researchers have reported the fungicidal properties of *E. platyloba* plants (24-29). According to previously done surveys, *E. platyloba* is a source of saponin, alkaloid, and flavonoid (6), while the results of various studies demonstrated that these substances have significant antifungal activity (25, 30-32). Additionally, it was reported that gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*) were more sensitive than gram-positive bacteria (*Staphylococcus aureus*) and it was reported that the most active concentration

was 10 mg/ml, which was completely effective on all of the gram-negative bacteria and inhibited their growth (33). Other researchers have demonstrated that gram-negative bacteria generally are more resistant than gram-positive bacteria (34). It was reported that evaluation of minimum inhibitory concentration and MBC values of *E. platyloba* D.C essential oil on different bacteria demonstrated the notable sensitivity of gram-positive bacteria and the relative susceptibility of gram-negative bacteria (35). Researchers indicated that methanolic extract of *E. platyloba* D.C can inhibit the growth of *S. aureus* and *P. aeruginosa* (36) and *S. aureus* (35). M. Avijgan et al. reported that there was a 50% reduction in MIC and a 75% reduction in minimum lethal concentration values of the mixture of Amphotericin B and 5% ethanolic extracts against *C. albicans* in comparison to Amphotericin B alone (37). Our results indicated that *E. Platyloba* extracts had a great effect in the prevention of salmonella growth. It was demonstrated that the MIC of extracts was initiated from 50 mg/ml in *S. enteritidis* and 100 mg/ml in *S. typhi* and *S. choleraesuis* and 150 mg/ml and 250 mg/ml of extracts were absolutely inhibited from the growth of bacteria, respectively. The MBC of extracts in case of all examined *salmonellas* was begun from 150 mg/ml, and the complete bactericidal effect was observed for 250 mg/ml. Our results indicated that the *E. Platyloba* extracts have antibacterial effects on *salmonella*.

### 5. Conclusions

The results of this study indicated that *E. platyloba* extract have potential usage as antimicrobial agents. Regarding the results of this study, clearly *E. platyloba* demonstrated antibacterial activity. Its antibacterial activity against *Salmonella enteritidis* was the highest, followed by *S. typhi* and *S. choleraesuis*. Finally, for therapeutic or industrial utilization, it can be suggested that the active substances of *E. platyloba* be purified and evaluated for their antibacterial effects.

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### Conflict of Interest:

There is no conflict of interest to be declared.

### Authors' contributions:

Both authors contributed to this project and article equally. Both authors read and approved the final manuscript.

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