Original Article

The Antimicrobial Activity of Ferula Gummosa on Bacterial Strains Isolated from Patients with Gastroenteritis

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Abstract

Objective: Ferula gummosa grows as a native plant in different parts of Iran and has been used for treatment of some infections such as UTI since ancient times. Present study is planned to show the antibacterial effect of this herb on bacteria isolated from infectious diarrhea.

Patients and Methods: Extracts of the plant were achieved using maceration method. The concentration of 0.2gr/ml was used against pathogenic bacteria causing gastroenteritis; isolated from patients admitted to Shahid Beheshti University of Medical Sciences Hospitals and also minimum inhibitory concentration (MIC) of the extract was determined.

Results: The highest value of MIC was due to *Proteus mirabilis, Escherichia coli, Klebsiella pneumoniae* and even Pseudomonas with 1/1024 dilution liter. Three antibiotics of tetracycline, trimethoprim and ciprofloxacin were also tested against the bacteria. The highest MIC was in 1/1000 mg/ml of ciprofloxacin and was against Pseudomonas aeruginosa and Serratia marcescens with MIC dilution of $\frac{1}{2}$.

Conclusion: According to our findings, it is recommended to use Ferula gummosa extract, as a herbal alternative treatment, in more investigations to prove its treatment effects on infections.

Key words: MIC, pathogenic bacteria, infectious diarrhea, Ferula gummosa

Introduction

One of the most important problems regarding chemical medications in comparison with traditional medicine is the increasing trend in consumption of chemical agents which gradually has led to resistance in viruses and bacteria species and ,thereby, use of stronger types of antibiotics, which may have more side effects (1).

More than 20 species of genus Ferula grow in Iran. Our study was aimed to introduce the antibacterial effect of this plant on pathogenic bacteria, isolated from patients with diarrhea.

Ferula gummosa is a perennial plant with 1 to 2 meters height that has grayish green leaves with 30 cm length and short stems covered with fine cords. Its flowers are yellow with multiple clusters and the root is glandular and conical. (2)

Ferula gummosa can grow in different parts of Iran such as the provinces of Mazandaran, Zanjan, Ghazvin, the city of Gachsar, Mount Damavand, and the Zagros Mountains. So it can be found mostly in northern and western Iran (3).

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The results of the current study might lead physicians to a new alternative for treatment of infectious agents.

Patients and Methods

Ferula gummosa was collected and, in order to prepare the root for extraction, the herb was completely dried and then crushed into a soft powder.

To obtain the ethanol extract, the herb root powder was soaked into ethanol for three successive days and the solution achieved was filtered and then concentrated by a rotary evaporator device. After obtaining the extract, it was dried and as no weight reduction was observed in laboratory temperature during two consecutive days, the extract was devoid of ethanol. We exploited different dilutions of the extract for bacterial tests.

First we prepared 1gr/lit dilutions from the extract with sterile distilled water in sterile containers, which was used as batch and the primary source for microbial tests. We also prepared different dilutions of this extract in order to reduce the possibility of error and to obtain all the possible dilutions from the original source.

Microbial strains were isolated from patients diagnosed with infectious diarrhea and hospitalized in different wards of hospitals affiliated to Shahid Beheshti Medical School. The fecal samples were taken based on the diagnosis of infectious diarrhea, hence stool samples were examined microscopically and cultured by use of media such as blood agar, Salmonella-Shigella Agar (S.S), eosin methylene blue (EMB) Nutrient Agar followed by culturing on specific biomedical media such as Triple Sugar Iron-Agar (TSI), Indole, citrate and urea.

For MIC determination, dilution of bacteria is a significant criterion, since it is important to know what

concentration of the herb is effective on every concentration or number of bacteria. In MIC method, turbidity is dependent on number of bacteria in a suspension McFarland method (4).
First, we selected 9 test tubes and poured 1ml of culture medium into each, then we poured 1ml of Ferula gummosa extract in tubes 1 and 2. The dilution method was conducted in tubes 2 to 7 and 1/2, 1/4, 1/8, 1/32, 1/64 and 1/128 dilutions were respectively achieved in tubes number 2 to 7.
We poured 1ml of the plant extract in tube 9 for negative control, which showed no growth rate. For positive control, 0.5 ml of microbial suspension was poured into tube 8, which demonstrated microbial activity and growth.
Finally 0.5 ml microbial suspension was poured into tubes 1 to 7 and all the tubes were incubated in autoclave for 24 hours at 37 C and the results were recorded.
Reviewing samples was done from tube 1 to 2 and sample transparency and growth rate were compared to tubes 9 as negative control.
The MIC is considered as the lowest concentration which resulted in a significant decrease in inoculum viability (>90%). In order to confirm MIC, we established MBC considered as the concentration which 99.9% or more of the initial inoculum was killed. To obtain MBC, we cultured the lowest MIC tube (4).
Gram negative and Gram positive pathogenic bacteria isolated from stool of patients with bacterial diarrhea were isolated. The 0.5 McFarland standards were used to adjust the turbidity of the inculum to obtain a concentration of 1.5×10^8 cfu/ml.
In order to review research process and determination of bacterial activity against antimicrobial agents, antibiotics such as ciprofloxacin, trimethoprim and tetracycline were used to achieve MIC turbidity.

Results

Ferula gummosa extract was achieved by using maceration method. As antibiotics efficacy on pathogenic bacteria are obtained in quantities of milligrams and micrograms and less use of antimicrobial products (smaller MIC) leads to more effectiveness, we used the least amount of extract with 0.2 gr/ml consistency and the satisfactory results obtained. Each experiment repeated three times. Although there are many methods such as diffusion method to determine antimicrobial activity of a substance, we used turbidity method in order to achieve more accurate results. (5)
The results are shown in table 1.

Table 1: MIC determination of Ferula gummosa extract with 0.2 gr/ml on bacteria causing infectious diarrhea

Bacteria	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$	$\frac{1}{256}$	$\frac{1}{512}$	$\frac{1}{1024}$	$\frac{1}{2048}$
Serratia marcescens	-	-	-	-	-	-	+				
Proteus mirabilis	-	-	-	-	-	-	-	-	-	-	+
Enterococcus faecalis	-	-	-	-	-	-	-	-	-	+	
Salmonella typhi	-	-	-	-	-	-	-	-	-	+	
Escherichia coli	-	-	-	-	-	-	-	-	-	-	+
Klebsiella pneumoniae	-	-	-	-	-	-	-	-	-	-	+
Yersinia enterocolitica	-	-	-	-	-	-	-	-	+		
Pseudomonas aeruginosa	-	-	-	-	-	+					
Shigella dysenteriae	-	-	-	-	-	+					

$\frac{1}{2}, \frac{1}{4}, \frac{1}{8}$, …, extract dilution coefficient

(-) dilution of extract which indicates growth inhibition and MIC determination (+) dilution of extract which indicate growth

Table 2: Ciprofloxacin with $^1/_{1000}$ mgr/ml concentration MIC determination on bacteria causing infectious bacterial diarrhea

Bacteria	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	**Positive Control**	**Negative Control**
Serratia marcescens	-	-	-	-	+	+	+	--
Proteus mirabilis	-	-	-	+	+	+	+	--
Enterococcus faecalis	-	-	-	+	+	+	+	--
Salmonella typhi	-		+	+	+	+	+	--
Escherichia coli	-	+	+	+	+	+	+	--
Klebsiella pneumoniae	-	-	+	+	+	+	+	--
Yersinia enterocolitica	-	-	+	+	+	+	+	--
Pseudomonas aeruginosa	+	+	+	+	+	+	+	--
Shigella dysenteriae	-	-	-	-	+	+	+	--

-- $\frac{1}{2}, \frac{1}{4}, \frac{1}{8}$, …, extract dilution coefficient

(+) dilution of extract which indicate growth (-) dilution of extract which indicates growth inhibition and MIC determination

Table 3 : MIC determination of Trimethoprim with 1/1000 mg/ml concentration on bacteria causing bacterial diarrhea

Bacteria	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	Positive Control	Negative Control
Serratia marcescens	-	-					+	--
Proteus mirabilis	-	-	-	+	+	+	+	--
Enterococcus faecalis	-	-	+	+	+	+	+	--
Salmonella typhi	-	-	+	+	+	+	+	--
Escherichia coli	-	-	+	+	+	+	+	--
Klebsiella pneumoniae	-	-	+	+	+	+	+	--
Yersinia enterocolitica	-	-	+	+	+	+	+	--
Pseudomonas aeruginosa	+	+	+	+	+	+	+	--
Shigella dysenteriae	+	+	+	-	+	+	+	--

-- $\frac{1}{2}, \frac{1}{4}, \frac{1}{8}$, …, extract dilution coefficient

(+) dilution of extract which indicate growth (-) dilution of extract which indicates growth inhibition and MIC determination

Table 4: MIC determination of tetracycline with 1/1000mg/ml on bacteria causing bacterial diarrhea

Bacteria	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	Positive Control	Negative Control
Serratia marcescens	-	+	+	+	+	+	+	--
Proteus mirabilis	-	-	+	+	+	+	+	--
Enterococcus faecalis	+	+	+	+	+	+	+	--
Salmonella typhi	-	+	+	+	+	+	+	--
Escherichia coli	-	+	+	+	+	+	+	--
Klebsiella pneumoniae	-	-	+	+	+	+	+	--
Yersinia enterocolitica	-	-	+	+	+	+	+	--
Pseudomonas aeruginosa	-	+	+	+	+	+	+	--
Shigella dysenteriae	-	-	+	+	+	+	+	--

$\frac{1}{2}, \frac{1}{4}, \frac{1}{8}$, …, extract dilution coefficient

(+) dilution of extract which indicate growth

(-) dilution of extract which indicates growth inhibition and MIC determination

Discussion

Many plants are being annually studied around the world to detect their health benefits. Part of these researches are focused on herbal antimicrobial effects which have been taken into consideration due to increased use of antibiotics and bacterial resistance (6) , (10).

Iran by having abundant native plant is an appropriate place to find and use medicinal herbs. Ferula gummosa is a native plant that grows in different parts of Iran and it has been used by native Iranians as a medicinal plant. So we decided to study the antimicrobial activity of this herb on bacteria causing infectious diarrhea (2,7).

Investigating efficacy of Ferula gummosa extract on dysentery diarrhea and also, comparing it with common antibiotics, (e.g. effectiveness of Ferula gummosa extract with 1/512 dilution on Enterococci as a gram positive bacteria causing enteritis) shows Ferula gummosa can play a major role as an inhibitor medicine on this pathogen .

Other bacteria causing infectious diarrhea such as proteus, klebsiella and Escherichia coli are gram negatives and herb extract with 1/1024 turbidity was completely effective on proteus and klebsiella as opportunistic agents in children and elderly infectious diarrhea. Furthermore, Escherichia coli is one of the most important bacterial agents causing infectious diarrhea in travelers which leads to 10% of intestinal infections in children under 10 years of age (8).

Effectiveness of Ferula gummosa extract with 1/1024 dilution on Escherichia coli is extremely important especially in comparison with other plant extract such as madder and cherry resin (2,9).

Although Salmonella typhi and Shigella dysenteriae demonstrated same sensitivity toward ciprofloxacin, however, Ferula gummosa extract is reported as a more effective agent on them (3). Salmonella typhi is an important cause of gastroenteritis particularly in Iran and it was sensitive toward Ferula gummosa with 1/512 dilution. Shigella dysenteriae was sensitive to Ferula gummosa with 1/64 dilution as well.

However, Yersinia enterocolitica causing infectious

diarrhea is not isolated from patient stool samples routinely, but in this research, the extract efficacy on this pathogen with 1/226 MIC is considerable.
Pseudomonas aeruginosa is one the most resistant bacteria to various antibiotics, hence the solution can be finding an alternative herbal antibacterial agent. (7) Pseudomonas sensitivity to Ferula gummosa extract with 1/32 dilution was significant in this research while it was resistance to ciprofloxacin and trimethoprim. Pseudomonas' sensitivity to Ferula gummosa extract is a positive indicator and its one of the significant findings of this research.

Conclusion

In conclusion and according to our findings, since the Ferula gummosa extract on bacteria causing infectious diarrhea, even on pseudomonas, is very effective, it is recommended to utilize Ferula gummosa extract as a herbal alternative in more investigations to prove its treatment effects on infections.

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