

Antimicrobial Activities of *Ferula assafoetida* Oil Against Gram Positive and Gram Negative Bacteria

¹Mujeeb Ur Rahman, ²Shereen Gul and ¹Ejaz Ali Odhano

¹PCSIR Laboratories, Quetta, Balochistan, Pakistan

²Department of Botany, University of Balochistan, Quetta, Pakistan

Abstract: The antibacterial activity of *Ferula assafoetida* oil against Gram positive (*Bacillus megaterium*, *B. subtilis*, *Lactobacillus acidophilus*, *Micrococcus luteus*, *Staphylococcus epidermidis*, *S. aureus*, *Vibrio cholerae*) and negative bacteria (*Escherichia coli*, *Salmonella typhi*, *Shigella flexneri*) was investigated and compared with antibacterial reference drugs, amoxicillin, streptomycin and kanamycin. The activities of oil and drug varied from organism to organism. The oil showed activity even at 50 µg amount. The oil was least active against *Vibrio cholerae* even at 200 µg amount. The antibacterial activity was comparable with reference antibiotics at same amount against some of the Gram positive and negative bacteria. At higher amounts (150/200 µg) all the bacterial strain were more sensitive to oil than the reference antibiotics (100 µg) except *Vibrio cholerae*. The MIC of oil ranged from 80-200 µg/mL against the susceptible bacteria. All the MBC (Minimum Bactericidal Concentration) were the same as the MICs.

Key words: *Ferula assafoetida* • Oil • Antibacterial activity • Gram positive • Gram negative • MIC • MBC

INTRODUCTION

The antimicrobial activities of essential oils have been known since the dawn of early medicinal practices to the present time of investigation [1]. About 163 plants of different families have been evaluated by Naqvi *et al.* [2, 3] for their antimicrobial activities and found 30% of them active. Other workers, Morris *et al.* [4], Syed *et al.* [5-7] and Rahman and Gul [8,9] have also reported antibacterial and antifungal activities of essential oils of different plant species against various microorganisms. Rahman and Gul [9] and Qasim and Rafiq [10] have reported the effectiveness of *Psammogeton canescens* and *Carum carvi* oil against Gram positive and negative pathogenic bacteria.

The genus *Ferula* of the plant family Umbelliferae consists of 140 species which are widespread from Mediterranean region to central Asia. Out of which only 15 species are identified in Pakistan [11]. *Ferula assafoetida* is one of the important species of *Ferula*. This species is native to Afghanistan and Iran and is locally known as 'Hing'. Its cabbage like raw tops are consumed as salad by the locals. The gum like exude of *Ferula assafoetida* finds application as flavour in Indian vegetarian cooking [12]. It is a useful for bronchitis,

hysteria, stomach pain, insect bite and headache. It is also used as antidote for flatulence. The gum is also recommended for pharmaceutical preparations as local stimulant to mucous membrane of the alimentary canal [13].

The present work deals with the investigation of the antimicrobial activity, MIC and bactericidal/bacteriostatic activities oil of *Ferula assafoetida* against seven gram positive and three gram negative bacteria.

MATERIALS AND METHODS

Oil Isolation: The plants of *Ferula assafoetida* were collected from Quetta region of Balochistan, Pakistan. The oil of 6 month old seeds was obtained by water distillation. Crushed seeds (500g) were water distilled using a Dean Starke apparatus [13].

Cultures: Standard cultures of Gram positive (*Bacillus megaterium*, *B. subtilis*, *Lactobacillus acidophilus*, *Micrococcus leuteus*, *Staphylococcus epidermidis*, *S. aureus*, *Vibrio cholerae*) and negative bacteria (*Escherichia coli*, *Salmonella typhi*, *Shigella flexneri*) obtained from Drug Testing Laboratories, Government

of Punjab, Pakistan, were used for the present studies. These cultures were maintained on nutrient agar slants, stored in refrigerator at 5°C and sub cultured after every 15 days.

Antibacterial Studies: Antibacterial activities of the essential oil were studied by using agar diffusion method [14]. Mueller Hinton agar of Oxide was used for the evaluation of the sensitivity of different strains towards the volatile oil of *Ferula assafoetida*. A stock solution (1mg/mL) of the essential oil and the dilutions of the stock solution containing 50, 100, 150 and 200 µg were prepared in dimethyl sulfoxide (DMSO). 100 µL of each dilution was added in the respective well and 100 µL DMSO for control. Amoxicillin, Streptomycin and Kanamycin were used as standard reference drugs (100 µg). DMSO was also used for the preparation of antibiotic solutions. The inhibition zones were recorded after incubating the plates at 37°C for 24 hrs.

Determination of MIC and Bactericidal/ Bacteriostatic Activity: Minimum inhibitory amounts (MIC) were determined by the tube dilution method using Mueller Hinton broth [14]. The bactericidal/ bacteriostatic activity of the minimum inhibitory amount was determined by spreading 0.1 mL of the appropriately diluted broth culture of the MIC positive tube as well as above and below the MIC tubes, on nutrient agar plates without oil or antibacterial reference drugs. The Petri plates were incubated at 37°C for 18-24 hrs. The dilutions that showed no growth were termed as bactericidal and vice versa.

Statistical Analysis: The statistical analysis were carried out by using computer program SPSS.

RESULTS AND DISCUSSION

The essential oil of *Ferula assafoetida* was found active against all Gram positive (*Bacillus megaterium*, *B. subtilis*, *Lactobacillus acidophilus*, *Micrococcus luteus*, *Staphylococcus epidermidis*, *S. aureus*, *Vibrio cholerae*) and negative (*E. coli*, *Salmonella typhi*, *Shigella flexneri*) bacterial strains. The activity of the essential oil and standard antibiotics varied with their amount and kind of organism (Table 1). Siddiqui *et al.* [15] have also reported the dose dependence of antibacterial activity of Umbelliferae members.

The antibacterial activity of oil in 50µg amount was not significantly different against all the Gram negative bacteria. However, the higher amounts of the oil became

more injurious to *E. coli* than *S. typhi* and *S. flexneri*. At 100µg amount, the oil showed significantly better activity against *E. coli* than amoxicillin and kanamycin. When the amount of oil was doubled i.e., 200 µg, the activity of oil was far better than the standard antibiotics (100 µg). The activity of oil against *S. typhi* in 200 µg amount was 8, 34 percent better than the standard antibiotics Amoxicillin and kanamycin, respectively. The same magnitude of antibacterial activity of oil (150µg), Amoxicillin (100 µg) and Kanamycin (100 µg) was observed against *Shigella flexneri*. Siddiqui *et al.* [15] have also reported the significant antibacterial activities of Umbelliferae plants against *E. coli* and *Shigella dysenteriae*. *F. assafoetida* oil was least active against *V. cholerae*.

The antibacterial activities of the oil against Gram-positive bacteria were also comparable with standard drugs. The 50 µg amount of oil showed 21% better activity against *B. megaterium* as compared to Amoxicillin (100 µg). *B. subtilis*, *L. acidophilus*, *M. luteus* and *S. epidermidis* showed more or less same sensitivity to oil (100 µg) and amoxicillin (100 µg).

The activity of oil and streptomycin against *B. subtilis* was same in 100 µg amount. In 150 µg amount, the oil showed 14-44 % better activity against *B. subtilis*, *M. luteus*, *S. epidermidis* and *S. aureus* than streptomycin. The oil (200 µg) had shown far better antibacterial activities against all the susceptible Gram-positive bacteria with respect to streptomycin antibiotic (100 µg conc.) except *V. cholerae*. *V. cholerae* showed same sensitivity against Streptomycin (100 µg) and oil (200 µg conc.).

The activity of kanamycin (100µg) was 13 to 70 % better than oil (100 µg) against different bacterial strains. 150µg amount of oil had 32, 12 % better activity against *B. subtilis* and *S. albus*, respectively, than kanamycin (100µg). The antibacterial activity of oil at 200µg was 11, 30, 8 percent higher than the reference drug kanamycin (100µg) against *B. megaterium*, *L. acidophilus* and *M. leuteus*, respectively. The activity of oil (200 µg) and kanamycin (100 µg) was the same against *S. aureus*. In case of *V. cholera*, the activity of oil (200µg) was even lower (28%) than Kanamycin (100 µg) in case of *V. cholera*. Rahman and Gul [8] have reported the activity of *Psammogeton canescens*, a member of Umbelliferae against Gram positive bacteria *Bacillus megaterium*, *B. subtilis*, *Lactobacillus acidophilus*, *Micrococcus leuteus*, *Staphylococcus albus*, *S. aureus* and *Vibrio cholerae*. Likewise the oil of *Carum carvi* was reported to be active against *S. aureus* [9].

Table 1: Antibacterial activity of *Ferula assafoetida* oil compared with Amoxycillin, Streptomycin and Kanamycin

(Average of 10 zones)

Name of organism	Inhibition zone (diameter mm) in control(DMSO)	Inhibition zone (diameter mm) against different amounts of <i>Ferula assafoetida</i> oil (µg)				Inhibition zone (diameter mm) against standard antibiotics(100 µg)			L.S.D.At	
		50	100	150	200	Amoxycillin	Streptomycin	Kanamycin	P=0.05	P=0.01

Gram Negative Bacteria										
<i>Escherichia coli</i>	4	10.3	23.0	34.0	42.0	19.5	28.5	21.00	1.24	1.86
<i>Salmonella typhi</i>	4	11.5	19.0	24.0	30.0	27.5	33.5	20.0	1.34	2.02
<i>Shigella flexneri</i>	4	10.3	18.0	25.0	30.5	25.0	28.5	25.5	0.95	1.43
Gram Positive Bacteria										
<i>Bacillus megaterium</i>	4	16.0	20.0	30.0	37.0	12.6	34.0	33.0	2.45	3.70
<i>Bacillus subtilis</i>	4	12.0	16.5	28.0	33.0	15.3	16.0	19.0	3.81	5.73
<i>Lactobacillus acidophilus</i>	4	08.5	13.5	25.0	32.0	14.0	31.5	22.0	3.15	4.75
<i>Micrococcus luteus</i>	5	17.0	23.5	24.0	30.0	21.0	21.0	27.0	2.45	3.70
<i>Staphylococcus epidermidis</i>	5	16.5	20.0	30.0	37.0	21.0	26.0	26.0	4.07	6.13
<i>Staphylococcus aureus</i>	4	09.0	14.0	23.0	29.0	17.0	19.5	24.0	3.15	4.75
<i>Vibrio cholerae</i>	4	05.0	08.0	12.0	19.0	25.0	19.0	26.0	2.69	4.05

Table 2: Minimum inhibitory concentration (MIC) of *Ferula assafoetida* oil and reference antibiotics (Amoxycillin, Streptomycin and Kanamycin)

(Average of 10 replicates)

Name of organism	Ferula assafoetida oil MIC µg/mL	Amoxycillin MIC µg/mL	Streptomycin MIC µg/mL	Kanamycin MIC µg/mL	L.S.D.At	
					P=0.05	P=0.01

Gram Negative Bacteria						
<i>Escherichia coli</i>	110.00	125.00	072.00	085.80	09.71	15.71
<i>Salmonella typhi</i>	145.00	075.00	105.00	090.00	15.85	25.66
<i>Shigella flexneri</i>	200.00	080.00	110.00	095.00	24.43	39.54
Gram Positive Bacteria						
<i>Bacillus megaterium</i>	125.00	205.00	085.50	090.00	33.16	53.67
<i>Bacillus subtilis</i>	165.00	220.00	082.20	072.50	34.89	56.47
<i>Lactobacillus acidophilus</i>	175.00	145.00	072.50	075.30	48.46	78.44
<i>Micrococcus luteus</i>	115.00	070.00	080.50	125.00	25.07	40.57
<i>Staphylococcus epidermidis</i>	080.00	200.00	095.00	105.00	19.42	31.43
<i>Staphylococcus aureus</i>	180.00	180.00	080.00	092.50	30.05	48.64
<i>Vibrio cholerae</i>	195.00	100.00	075.00	085.30	35.45	57.38

The minimum inhibition concentration (MIC) and bactericidal/ bacteriostatic activities of oil and standard antibiotics are given in Table 2. The data showed that the MIC value of oil against Gram positive and negative strain varies from 110-200 and 80-195 µg/mL, respectively. The MIC value for amoxicillin was 75-125 & 70-220, streptomycin 72-110 & 72-95 and kanamycin 85-95 & 72-125 µg/mL against Gram positive and negative susceptible bacteria, respectively. Although the MIC

amounts of reference drugs were less than *Ferula assafoetida* oil against most of the microorganisms used in the present studies. However, the MIC of *F. assafoetida* oil was less than all the three drugs in case of *S. epidermidis*, amoxicillin in case of *E. coli*, *B. magaterium*, *B. subtilis* and kanamycin in case of *M. luteus*. All the minimum inhibitory amounts of reference drugs as well as of *Ferula assafoetida* were bactericidal.

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