

Antibacterial Study of Medicinal Plant *Trigonella foenum*

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Abstract – The present study was conducted to evaluate the antibacterial study of the medicinal plant *Trigonella foenum* crude extract and its subsequent solvent soluble fractions against six bacterial strains like *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, *Erwinia carotovora* and *Agrobacterium tumefaciens* by determining zone of inhibition. The results revealed that all fractions except water fraction showed promising inhibitory activity against *Escherichia coli* and *Staphylococcus aureus*. Chloroform fraction showed activity of 19mm and ethyl acetate fraction show activity of 17mm against *Escherichia coli* while the same fractions showed activity of 18mm each against *Staphylococcus aureus*. The chloroform fraction show excellent activity of 19mm against *Erwinia carotovora*. The chloroform and ethyl acetate fractions were active against all bacterial strains and show excellent activity.

Keywords – *Trigonella foenum*, extraction, fractionation and antibacterial activity

1. Introduction

Bacteria, fungi, Viruses and other microorganism are potentially pathogenic to humans and animals. Antibiotic resistance bacteria outbreaks have been reported in hospitals throughout the world. Therefore, discovery of new antimicrobial agents to combat such diseases is very important and essential [1]. Medicinal plants represent a rich source of these microbial agents. Plants are the richest resources of drugs of traditional systems of modern medicines, folk medicines, nutraceuticals, food supplements and chemical entitled for synthetic drugs. The Potential of higher plants as source for new drugs is still largely unexplored. Only a small percentage of plant has been investigated phytochemically [2].

Trigonella foenum is an annual herb which is cultivated in India, Africa, Pakistan and in Egypt. Various medicinal properties like anti-inflammatory, cardio tonic, carminative, anticholesterolemic, demulcent, diuretic, expectorant, hypotensive and laxative have been attributed to this plant in the traditional system of Indian medicine. It is much used in herbal medicine, especially in North Africa, the Middle East and India [3]. This present study was designed to explore the antibacterial activity of *Trigonella foenum* on selected human pathogens.

2. Materials and Methods

2.1. Collection of Sample

Plant *Trigonella foenum* was collected from Lachi, District Kohat, Khyber Pakhtunkhwa, Pakistan in flowering season (5Kg). The plant was authenticated by the herbarium staff of Department of Botany, Kohat University of Science and Technology. It was rinsed with water to remove dust particles. Then crushed to fine powder and weighed 1.5 kg.

All studies were carried out at Department of Pharmacy, Kohat University of Science and Technology (KUST), Kohat, Khyber Pakhtunkhwa, Pakistan.

2.2. Preparation of Crude Extract and Fractions

The shade dried plant powder of weight 1500 gm was soaked in Methanol (5 liter) for three weeks. The plant extract was prepared by using Dildar *et al.* 2009 method with slight modifications [4]. The methanolic extract was evaporated under reduced pressures to obtain a gummy residue of weight 180 gm with the help of rotary evaporator. The crude residue was suspended in water and defatted with *n*-hexane (500ml) to obtain fraction weighed (10 g). It was again fractionated with chloroform (500ml) to obtain fraction weighed (19 g). Similarly it was fractionated with ethyl acetate (500ml) to afford fraction weight (24 g). Water fraction was obtained of weighed (70.5g). The crude extract and fractions were tightly packed and stored in refrigerator at 4°C.

2.3. Preparation of Solution

The test compound was prepared by dissolving 64mg of the fractions in 32 mL Di-Methyl Sulfoxide (DMSO) and make solutions of concentration 2µg/µL and 4µg/2µL. DMSO was selected as a solvent for the present study [5].

2.4. Antibacterial Assay

In this study, extract and fractions of *Trigonella foenum* were evaluated for antimicrobial activities against gram positive and gram negative. The bacterial strains such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and clinically isolated *Salmonella typhi*, *Erwinia carotovora* and *Agrobacterium tumefaciens* were tested by Disc Diffusion Susceptibility Method.

The antibacterial bioassay was done by (agar disc diffusion method) by measuring the zone of inhibition against the test microorganisms by using Kirby-Bauer method [6]. In separate conical flask 28g/L nutrient agar media was prepared. All the apparatus as well as the media was sterilized for half an hour at 1.5 pounds pressure and 121°C. The nutrient agar media was poured in Petri plates in laminar flow cabinet and allowed to solidify for 20 minutes. A hoop

full of cells from the stock culture was transferred to flasks of nutrient agar which were incubated in shaking water bath for 24 hr at 37°C. With the help of glass spreader, 50µl inoculums suspension were spread on each nutrient agar plate and allowed to dry for 15min in refrigerator. Sterile discs of 6mm in diameter were placed on the surface of agar media. Various fractions and extract in concentration of 2µg/µL and 4µg/2 µL were loaded on each disc. DMSO (6µL/disc) was taken as negative control. Antibiotics such as Azithromycin (50µg/ 6µl) and Ciprofloxacin (30µg/ 6µl) were used as positive controls against gram +ive bacteria and gram -ive bacteria respectively. Then incubate these Petri plates at 37 °C for 24hrs. Zones of inhibition were measured around the discs and record the antimicrobial potential of various fractions and extract.

3. Results and Discussion

In this study the antibacterial activities of the *Trigonella foenum* were observed. The antibacterial study was performed against six bacterial strains i.e. *Escherichia coli*,

Pseudomonas aeruginosa, *Staphylococcus aureus*, *Salmonella typhi*, *Erwinia carotovora* and *Agrobacterium tumifaciens*.

Results show that Plant showed excellent activity against *Escherichia coli*, *Staphylococcus aureus*, *Erwinia carotovora* and *Agrobacterium tumifaciens*. All fractions except water fraction show excellent inhibitory activity against *Escherichia coli* and *Staphylococcus aureus*. The chloroform and ethyl acetate fractions were active against all bacterial strains and show excellent activity. Chloroform fraction show maximum activity against *Escherichia coli* and *Erwinia carotovora*. Ethyl Acetate fraction show maximum inhibition results against *Escherichia coli* and *Staphylococcus aureus*. Water fraction was completely inactive against all bacterial strains. All fractions except chloroform and ethyl acetate were completely inactive against *Salmonella typhi* as shown in Table 1. This study is in closely related with the studies conducted by Kumar *et al.* 2012 [7].

Table 1: Antibacterial activities of extracts and fractions of *Trigonella foenum*

Test Microorganisms	Zone of inhibition (mm)										
	Crude		n-hexane		Chloroform		Ethyl acetate		Aqueous		Std.
Extract µg/ µl	2/1	4/2	2/1	4/2	2/1	4/2	2/1	4/2	2/1	4/2	50 or 30µg/ 6µl
<i>Escherichia coli</i>	12	14	9	11	16	19	1	17	-	-	28*
<i>Salmonella typhi</i>	-	-	-	-	14	16	1	14	-	-	30*
<i>Pseudomonas aeruginosa</i>	13	15	-	-	11	12	1	14	-	-	30*
<i>Staphylococcus aureus</i>	14	17	13	16	16	18	1	18	-	-	25**
<i>Erwinia carotovora</i>	10	13	-	-	18	19	9	12	-	-	24*
<i>Agrobacterium tumifaciens</i>	11	15	-	-	14	16	1	15	-	-	36*

(-): No inhibiton zone, Ciprofloxacin (*): 30µg/6µl, Azithromycin (**):50µg/6µl.

4. Conclusion

The Our present results suggest that each fraction has variable effects in different bioassays. It is recommended that *Trigonella foenum* is an important plant from medicinal point of view and can be a potent for further bio-assays which would lead to synthesis of safe herbal drugs with no or least side effects of global interests.

References

- [1] P. D. Abeysinghe, "Antibacterial activity of aqueous and ethanol extracts of mangrove species collected from Southern Sri Lanka" Asian Journal of Pharmaceutical and Biological Research, 2(1), 2012, pp. 79-83.
- [2] R. Chandra, V. Dwivedi, K. Shivam, A. K. Jha. Detection of Antimicrobial Activity of *Oscimum sanctum* (Tulsi) & *Trigonella foenum graecum* (Methi) against some selected bacterial & fungal strains" Research Journal of Pharmaceutical, Biological and Chemical Sciences, 4, 2011 2, pp. 809-813.
- [3] K. Ritu, C. Atam, S. B. Pal, G. Ashutosh, S. Kalyan, Antimicrobial activity of ethnolic extract of *Trigonella Foenum*, International Research Journal of Pharmacy, 1(1), 2010, 181-183.
- [4] D. Ahmed, M. Mansha, A. Hannan, M. Barkaat and N. Bibi, "Antibacterial Activity Of *Ballota limbata* Against Potential Multidrug Resistant Human Pathogens, Journal of Applied Sciences Research, 5(10), 2009, pp. 1611-1614.
- [5] Y. Kumar Vaghasiya, R. Nair, M. Soni, S. Baluja and S. Chanda, "Synthesis, structural determination and antibacterial activity of compounds derived from vanillin and 4-aminoantipyrine" J. Serb. Chem. Soc. 69 (12), 2004, 991-998.
- [6] A.W. Baur, W .M. Kirby, J. C. Sherris, M. Turck, "Antibiotic susceptibility testing by a standardized single disk method" Am J Clin Path. 45, 1966, 493-496.
- [7] A. Kumar, A. Kumar, P. Thakar, S. Patil, A. kumar, P. Sharma. "Antibacterial activity of green tea (*Camellia sinensis*) extracts against various bacteria isolated from environmental sources" Recent Res. Sci & Tech. 4(1), 2012, 19-23.