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Comparative anti microbial studies of aqueous, methanolic and saponins extract of seeds of *Trigonella Foenum-Graecum* on human vaginal pathogens causing UTI infection

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ABSTRACT

The aqueous, methanolic and saponin extracts of Trigonella foenum-Graecum seeds were screened for antimicrobial activities against some human vaginal pathogens Staphylococcus aureus, Pseudomonas aeruginosa, streptococcus facecalis, klebsiella pneumoniae, Escherichia coli, Enterobacter faecalis, Enterobacter faecium and Proteus mirabilis isolated from patient samples. Extracts were found to produce significant inhibition against all the pathogens. Saponin extract were observed to be more active than methanolic and aqueous fraction. Extracts are found to be more active against klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa and Enterobacter faecalis strains.

Key Words: Trigonella foenum- Graecum, Human Vaginal Pathogens, Saponin.

INTRODUCTION

Medicinal plants have always been integral to the traditional healthcare system all over the world. In India, from ancient times, different parts of medicinal plants have been used to cure specific ailments. Today, there is widespread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects. Natural antimicrobials can be derived from plants,

animal tissues, or microorganisms. The shortcomings of the drugs available today, propel the discovery of new pharmacotherapeutic agents in medicinal plants. [1-3]

Fenugreek also known as Methi-dana is seeds of Trigonella foenum –graecum belongs to the family Leguminosae. The seeds are hard, yellow to reddish brown in color, oblong, rhomboidal, with deep furrow running obliquely from the side which divides the seed in unequal parts. The seeds are 2- 5mm long and 1.5-3 mm wide have pleasant odor and bitter taste.[4-7]

Urinary tract infections (UTIs) are a leading cause of morbidity and health care expenditures in persons of all ages. Sexually active young women are disproportionately affected, but several other populations, including elderly persons and those undergoing genitourinary instrumentation or catheterization, are also at risk. An estimated 40 percent of women report having had a UTI at some point in their lives. Urine located within the urinary tract, excluding the distal region of the urethra is considered sterile in healthy individuals, as indicated by the absence of cultivable bacterial cells. A urinary tract infection (UTIs) describes a condition in which there are micro organisms established and multiplying within the urinary tract. It is most often due to bacteria (95%), but may also include fungal and viral infection. [8-11]

In the present study methanolic, aqueous and saponin Extracts of seeds of Trigonella Foenum-Graecum plants were screened for potential antibacterial activity toward vaginal pathogens causing urinary tract infections (UTIs).

MATERIALS AND METHODS

Plant materials

Seeds of Trigonella foenum –graecum were collected from Local Market of Indore, Madhya Pradesh and were identified by the Botany Department, Janata PG College, A.P.S. University, Rewa (M.P.). The seeds were stored in an air-tight container for further use.

Preparation of extracts

Seeds were shattered and screened with 40 mesh. It was soxhlet extracted three times with petroleum benzene for 4hr at 60^{o}C. After drying and levigation, the residues were inverse flow extracted 10 times with 70% methanol for 4hr at 85^{o}C, then were filtrated and the residue was extracted with distilled water for 48hr under reflux condition. The alcohol solution (Filtrate) was evaporated to dryness with reduced pressure at 60 ^{o}C, and dissolved with water. After filteration and discarding the extraneous components, the solution was extracted by adding water-saturated n-butanol (1:1v/v), the n-butanol phase was then treated by 1M KOH, alkaline–water phase was removed. The n-butanol phase evaporated to dryness under pressure and the raw saponin was obtained. All extracts were screened for phytochemical analysis.

Preparation of microorganisms for experiment

All the microorganisms were isolated from in & outpatients samples from Chotiram hospital and research centre Indore. For use in experiments, the organisms were sub-cultured in nutrient broth, nutrient agar, Macconky agar and Blood agar media. Muller Hinton agar was used in antibiotic sensitivity testing.

Preparation and application of disks for experiment [12-21]
Different concentration of the extracts (10-60 µg/ml) was prepared by reconstituting with DMSO. The test microorganisms were streak to Muller Hinton agar medium by streaking plate method. After streaking the autoclaved filter paper discs (5 mm in diameter) impregnated with the extracts were placed on plates using flame-sterilized forceps. The antibacterial assay plates were incubated at 37°C for 24hr. For positive control Amoxycillin/cefitaxime/Ampicillin (60µg/ml) and for negative control solvent DMSO was used.

Observation of results
Results were recorded as presence or absence of zone of inhibition. The inhibitory zone around test paper disks indicated absence of bacterial growth and it was reported as positive (growth inhibition observed) and absence of zone as negative. The test was repeated thrice in interday interval to insure reliability of the results. The diameters of the inhibition zones were measured in mm (after subtraction the diameter of disc i.e 5mm), shown in table 1.The concentration of extract showing inhibition were further diluted and experiment was repeated to identify the minimum inhibitory concentration (MIC), shown in table 2. The Percentage of relative inhibition zone diameter (% RIZD) as compare to inhibition obtained from standard drug at same concentration was calculated, shown in table 3.

Table 1. Zone of inhibition for extracts, Standard & Control

Con in µg/ml		Zone of Inhibition (mm)*							
		EC	**PA**	**EFa**	**EFi**	**KP**	**SF**	**SA**	**PM**
ME	**10**	-	-	-	-	-	-	-	-
	20	8.16±0.16	7.0±0.28	-	3.16±0.33	8.16±0.440	-	5.33±0.33	-
	40	11.5±0.28	11.66±0.16	8.86±0.16	6.83±0.33	14.16±0.44	6.5±0.5	8.16±0.16	-
	60	16.16±0.16	16.16±0.16	11.66±0.33	11.66±0.16	18.83±0.16	10.83±0.16	14.16±0.16	9.833±0.16
AE	**10**	-	-	-	-	-	-	-	-
	20	7.16±0.16	6.83±0.33	-	3.16±0.16	7.16±0.44	-	2.66±0.33	-
	40	11.33±0.16	11.33±0.16	9.33±0.33	6.83±0.33	12.33±0.33	7.83±0.16	5.83±0.16	-
	60	15.33±0.33	15.83±0.40	12.16±0.16	10.66±0.16	16.83±0.44	11.5±0.5	10.5±0.28	7.83±0.44
SE	**10**	-	-	-	-	-	-	-	-
	20	7.16±0.16	7.0±0.28	-	3.83±0.16	11.16±0.16	-	7.5±0.28	-
	40	13.5±0.28	11.83±0.16	10.0±0.50	7.33±0.16	14.66±0.33	12.00±0.28	10.66±0.33	-
	60	17.16±0.16	17.16±0.16	13.0±0.28	12.5±0.28	21.16±0.44	15.16±0.16	15.66±0.33	11.16±0.16
SD	**60**	22.5±0.763 (a)	24.16±0.726 (a)	19.5±0.28 (b)	21.16±0.60 (a)	24.83±0.60 (b)	23.83±0.16 (a)	25.16±0.726 (b)	19.0±0.288 (a)
Con	**-**	-	-	-	-	-	-	-	-

** mm= Mean of three replicates±SEM*
Met: Methanolic extract AE: Aqueous Extract SE: Saponin Extract Con: Control (DMSO) SD: Standard (a = cefitaxime, b= Amoxycillin)
EC= Escherichia coli, PA= Pseudomonas aeruginosa, EFa= Enterobacter faecalis, EFi= Enterobacter faecium, KP= klebsiella pneumoniae, SF= Streptococcus facecalis, SA= Staphylococcus aureus and PM= Proteus mirabilis

Table 2 Minimum Inhibitory Concentration (MIC) for extracts

	Zone of inhibition and Minimum Inhibitory Concentration (MIC) for extracts							
Organism	**EC**	**PA**	**EFa**	**EFi**	**KP**	**SF**	**SA**	**PM**
ME	2.66±0.16 (8µg/ml)	3.16±0.33 (18µg/ml)	2.5±0.288 (20µg/ml)	3.16±0.33 (36µg/ml)	2.66±0.66 (14µg/ml)	2.66±0.44 (30µg/ml)	3.16±0.16 (18µg/ml)	2.83±0.16 (46µg/ml)
AE	2.5±0.28 (8µg/ml)	3.16±0.16 (18µg/ml)	2.16±0.16 (20µg/ml)	3.16±0.16 (38µg/ml)	2.5±0.28 (16µg/ml)	3.5±0.28 (30µg/ml)	2.66±0.33 (20µg/ml)	3.16±0.16 (48µg/ml)
SE	3.33±0.16 (8µg/ml)	3.83±0.16 (18µg/ml)	2.33±0.16 (20µg/ml)	3.83±0.16 (34µg/ml)	2.83±0.16 (12µg/ml)	2.16±0.15 (26µg/ml)	2.83±0.44 (14µg/ml)	3.0±0.288 (44µg/ml)

Met: Methanolic extract AE: Aqueous Extract SE: Saponin Extract
EC= Escherichia coli, PA= Pseudomonas aeruginosa, EFa= Enterobacter faecalis, EFi= Enterobacter faecium, KP= klebsiella pneumoniae, SF= Streptococcus facecalis, SA= Staphylococcus aureus and PM= Proteus mirabilis

Table 3 Percentage of relative Inhibition Zone diameter (% RIZD) for extracts as compare to standard at 60µg/ml

Organism	**Percentage of relative Inhibition Zone diameter (% RIZD) at 60µg/ml**							
	EC	**PA**	**EFa**	**EFi**	**KP**	**SF**	**SA**	**PM**
ME	71.82%	66.88%	59.79%	55.10%	75.83%	45.44%	56.27%	51.73%
AE	68.13%	65.52%	62.35%	49.21%	67.78%	48.25%	41.73%	41.21%
SE	76.26%	71.02%	66.66%	59.07%	85.21%	63.61%	62.24%	58.73%

Met: Methanolic extract AE: Aqueous Extract SE: Saponin Extract
EC= Escherichia coli, PA= Pseudomonas aeruginosa, EFa= Enterobacter faecalis, EFi= Enterobacter faecium, KP= klebsiella pneumoniae, SF= Streptococcus facecalis, SA= Staphylococcus aureus and PM= Proteus mirabilis

RESULTS AND DISCUSSION

In this study the results of the investigations show that all the extracts from the bark possess antimicrobial activities against mentioned test organisms. The minimum inhibitory concentration lies in the range from 08µg/ml to 48µg/ml.

Saponin extract were observe to be more active than ethanol and aqueous extracts. As compare to the standard, extracts were observed to be less active at concentration 60µg/ml. The percentage of relative inhibition zone diameter (% RIZD) observed to be in the range 41.73%-85.21% shown in table 3. Results clearly indicate that further purification of this compounds can leads to isolation of potent antibacterial compound active against some urinary pathogens.

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