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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. The minimum inhibitory concentration lies in the range from 08µg/ml to 48µg/ml. The percentage of relative inhibition zone diameter (% RIZD) observed to be in the range 41.73%-85.21%. Extracts are found to be more active against *klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterobacter faecalis* strains.

KEYWORDS: Trigonella foenum- Graecum, Human Vaginal Pathogens, Saponin.

INTRODUCTION

Recently, there is an increasing tendency toward traditional medicine due to occurrence of harmful effects of chemical drugs on human health and various deficits of the modern medicine in treating some diseases.[1] Medicinal plants have a long history of usage[2] with low side effects.[3,4] Recent studies have shown promising results for these plants in prevention[5,6] and treatment[7,8] of a wide variety of diseases such as diabetes,[9,10] hypertension,[11,12] atherosclerosis,[13,14] cardiovascular disease,[15,16] and cancer.17,18 Medicinal plants have also the capacities to diminish drug-induced adverse effects[19,20] and

even heavy metals or other toxicities toxicities.[21,22] Therefore, they might be considered as reliable sources for development of new drugs. One of the medicinal plants that has been used since antiquity in the traditional medicine of Iran and for which significant therapeutic properties have been mentioned is fenugreek.

The health benefits and medicinal properties of herbal food products are known since antiquity. Fenugreek [Trigonella foenum-graecum Linn. (Fabaceae)], a seed spice used to enhance flavor, color and texture of food, is employed for medicinal purposes in many traditional systems. A number of epidemiological studies and laboratory research have unraveled the biological actions of fenugreek.[23]

Urinary tract infections (UTIs) are a leading cause of morbidity and health care expenditures in persons of all ages. It 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.

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).

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.

MATERIALS AND METHODS

Plant materials & Preparation of extracts

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.).

Seeds were shattered and screened with 40 meshes and defatted with petroleum benzene followed by inverse flow extraction 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 filtration 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 and application of disks

Different concentration of the extracts (10-60 µg/ml) was prepared by reconstituting with DMSO. The test microorganisms (Procured from in & outpatient's samples from CHRC, Indore) 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 experiment

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.

RESULTS AND DISCUSSION

In this study the results of the investigations show that all the extracts from the Seeds 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.

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

ME: Methanolic extract AE: Aqueous Extract SE: Saponin Extract Con: Control (DMSO) SD: Standard (a = cefitaxime, b= Amoxycillin)

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)

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%

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.*

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