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Anti-microbial studies of aqueous, methanolic and saponins extract of leaf of *Ziziphus mauritiana* on human vaginal pathogens causing UTI infection

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Abstract

Preliminary phytochemical screening of the leaves extract of *Zizyphus mauritiana* revealed the presence of alkaloids, flavonoids, glycosides, saponins. The aqueous, methanolic and saponin extracts of *Zizyphus mauritiana* leafs has been 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 *Enterobacter faecalis*, *Enterobacter faecium, Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus.*

Key Words: Ziziphus Species, Human Vaginal Pathogens, Saponin.

Introduction

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. [1-3]

Ziziphus Species is very common throughout the world with lot of varieties from trees to shrubs. Among different varieties of jujube, Indian jujube (*Ziziphus mauritiana*) is also found to have number of pharmaceutical importance. Ziziphus belongs to the kingdom; plantae, order; roasles, division; magnoliophyta, class; magnoliopsida, family; rhamnaceae, genus; ziziphus. *Z. mauritiana* is a fast growing small to medium-sized, single or multi-stemmed, spiny shrub or tree, which is almost evergreen, but is deciduous during the dry season. It has a round, spreading crown. It can reach up to 12 m tall and 30 cm diameter at breast height, but is highly variable in size and general appearance. The leaves are simple, shining green above and whitish tomentose beneath, due to persistent dense hairs (occasionally glabrous), margins minutely serrulate, leaf shape ranging from almost round to an elongated ellipse, commonly sub-orbicular to ovate-oblong,

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rounded at both ends, highly variable in shape and size but always with three basal nerves and two stipular spines, one long and straight, the other small and curved back, and often brown in colour. The leaves are solitary or in pairs, from 2 to 6 cm in length (occasionally 1.3-12.0 cm) and 1.5-5.9 cm at the widest point (occasionally 0.4-6.5 cm wide). [4-10]

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. [11-14]

In the present study methanolic, aqueous and saponin Extracts of Barks of ziziphus nummmlaria plants were screened for potential antibacterial activity toward vaginal pathogens causing urinary tract infections (UTIs).

Material and methods

Plant materials

Barks of *Ziziphus nummularia* were collected from Malwa region of Madhya Pradesh in the month of Feb-March, 2007 and were identified by the Botany Department, Janata PG College, A.P.S. University, Rewa (M.P.). The bark were later air-dried, powdered and stored in an air-tight container for further use.

Preparation of extracts [9-10]

Sample were shattered and screened with 40 mesh. It was soxhlet extracted three times with petroleum benzene for 4hr at 60ºC. After drying and levigation, the residues were inverse flow extracted 10 times with 70%methanol for 4hr at 85ºC, 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 ºC, 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 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 [15-24]

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.

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 14µg/ml to 60µg/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 22.78%-94.87% 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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Table No 1: Zone of inhibition for extracts, Standard & Control

		Zone of Inhibition (mm)*							
		EC	**PA**	**EFa**	**EFi**	**KP**	**SF**	**SA**	**PM**
	10	-	-	-	-	-	-	-	-
	20	5.83±0.16	7.33±0.33	5.5±0.288	4.6±0.333	-	-	4.5±0.28	-
	40	9.83±0.33	11.5±0.28	9.5±0.288	8.16±0.16	9.16±0.16	9.33±0.16	8.83±0.16	4.16±0.16
	60	14.16±0.16	16.33±0.33	13.66±0.33	12.66±0.16	13.0±0.577	15.5±0.28	13.83±0.16	6.16±0.440
	10	-	-	-	-	-	-	-	-
	20	5.5±0.28	6.5±0.288	3.5±0.288	2.5±0.28	-	-	4.16±0.16	-
	40	8.33±0.16	10.16±0.28	6.5±0.50	6.3±0.33	7.16±0.16	8.33±0.16	7.33±0.16	-
	60	12.83±0.16	13.66±0.16	11.66±0.33	10.16±0.440	11.5±0.28	12.66±0.33	13.33±0.33	4.33±0.33
	10	-	-	-	-	-	-	-	-
	20	08.5±0.28	08.83±0.16	9.83±0.16	7.5±0.28	-	-	6.33±0.44	-
	40	12.0±0.28	13.83±0.44	14.33±0.33	11.66±0.33	10.83±0.44	11.5±0.28	9.33±0.16	4.16±0.16
	60	16.16±0.16	18.16±0.16	18.5±0.288	17.66±0.16	15.33±0.33	17.66±0.44	15.83±0.16	7.33±0.440
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 No 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	2.5±0.28 (16µg/ml)	3.83±0.16 (18µg/ml)	3.16±0.44 (18µg/ml)	2.33±0.33 (18µg/ml)	3.66±0.33 (28 µg/ml)	3.33±0.16 (28µg/ml)	2.5±0.28 (18µg/ml)	4.16±0.16 (40µg/ml)
AE	3.16±0.16 (18µg/ml)	3.5±0.28 (18µg/ml)	2.16±0.16 (18µg/ml)	2.5±0.28 (20µg/ml)	3.16±0.16 (28µg/ml)	3.16±0.16 (28µg/ml)	2.5±0.28 (18µg/ml)	4.33±0.33 (60µg/ml)
SE	2.5±0.28 (14µg/ml)	3.33±0.16 (16µg/ml)	2.5±0.28 (14µg/ml)	2.16±0.16 (16µg/ml)	2.66±0.33 (26µg/ml)	2.16±0.16 (24µg/ml)	2.16±0.16 (16µg/ml)	4.16±0.16 (40µg/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 No: 3 Percentage of relative Inhibition Zone diameter (% RIZD) for extracts as compare to standard at 60µg/ml

	Percentage of relative Inhibition Zone diameter (% RIZD) at 60µg/ml							
	EC	**PA**	**EFa**	**EFi**	**KP**	**SF**	**SA**	**PM**
ME	62.93%	67.59%	70.05%	58.44%	52.35%	65.04%	54.96%	32.42%
AE	57.02%	56.53%	59.79%	48.01%	46.31%	53.12%	52.98%	22.78%
SE	71.82%	75.16%	94..87%	83.45%	61.73%	74.10%	62.91%	38.57%

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*