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HUMAN VAGINAL PATHOGEN INHIBITION STUDIES ON AQUEOUS, METHANOLIC AND SAPONINS EXTRACTS OF STEM BARKS OF *ZIZIPHUS MAURITIANA*

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ABSTRACT

The aqueous, methanolic and saponin extracts of *Zizyphus mauritiana* barks 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 *Staphylococcus aureus* and *Escherichia coli* strains.

INTRODUCTION: From ancient times, different parts of medicinal plants have been used to cure specific ailments. 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].

Ziziphus belongs to the kingdom plantae, order- roasles, division- magnoliophyta, class- magnoliopsida, family- rhamnaceae, and 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 can reach up to 12 m tall and 30 cm diameter at breast height, but is highly variable in size and general appearance. The bark is dark grey, dull black or reddish with long vertical fissures, reddish and fibrous inside. The branches are spreading and droop at the ends. Stipules are mostly spines, in pairs with one hooked and one straight, or both hooked [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.. 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).

MATERIALS AND METHODS:

Plant materials: Barks of *Ziziphus mauritiana* 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 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 filtration and discarding the extraneous components, the solution was extracted by adding water-saturated n-butanol (1:1 v/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 (10-60µg/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 was 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	-	-	-	-	-	-	2.33±0.16	-
	40	7.66±0.16	6.66±0.16	4.16±0.16	5.33±0.16	-	5.0±0.28	8.66±0.16	2.33±0.33
	60	10.33±0.16	10.66±0.16	7.33±0.16	8.16±0.16	8.5±0.288	8.33±0.16	13.0±0.28	4.66±0.16
AE	10	-	-	-	-	-	-	-	-
	20	-	-	-	-	-	-	2.16±0.16	-
	40	5.16±0.16	5.83±0.16	4.16±0.16	3.83±0.16	-	4.83±0.16	8.33±0.16	2.16±0.16
	60	8.0±0.28	9.5±0.288	6.83±0.16	6.16±0.16	6.33±0.16	8.16±0.16	11.66±0.16	4.16±0.16
SE	10	-	-	-	-	-	-	-	-
	20	-	-	-	-	-	-	2.83±0.16	-
	40	7.83±0.166	8.0±0.288	5.5±0.288	7.0±0.288		6.33±0.16	9.16±0.16	3.0±0.28
	60	11.66±0.16	11.83±0.16	9.16±0.16	10.33±0.16	10.16±0.16	10.66±0.16	13.83±0.33	6.16±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 CONCENTRATIONS (MIC) FOR EXTRACTS

Organism	Zone of inhibition and Minimum Inhibitory Concentration (MIC) for extracts							
	EC	PA	EFa	EFi	KP	SF	SA	PM
ME	2.33±0.33 (26µg/ml)	2.66±0.16 (36µg/ml)	2.5±0.288 (38µg/ml)	2.33±0.33 (36µg/ml)	2.16±0.16 (46 µg/ml)	2.16±0.16 (38µg/ml)	2..33±0.16 (20µg/ml)	2.33±0.33 (40µg/ml)
AE	2.16±0.16 (28µg/ml)	2.16±0.16 (36µg/ml)	2.33±0.16 (38µg/ml)	2.16±0.16 (38µg/ml)	2.33±0.16 (50µg/ml)	2.16±0.16 (38µg/ml)	2.16±0.16 (20µg/ml)	2.16±0.16 (40µg/ml)
SE	2.33±0.16 (26µg/ml)	3.16±0.16 (36µg/ml)	2.83±0.16 (38µg/ml)	2.33±0.16 (34µg/ml)	2.33±0.33 (46µg/ml)	2.5±0.28 (34µg/ml)	2.83±0.16 (20µg/ml)	3.0±0.288 (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 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	45.91%	44.12%	37.55%	33.08%	34.23%	34.95%	51.66%	24.52%
AE	35.55%	39.32%	35.02%	29.11%	25.49%	34.24%	46.34%	21.89%
SE	51.82%	48.96%	46.97%	48.81%	40.91%	44.73%	54.96%	34.36%

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 20µg/ml to 50µg/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 21.89%-54.96% 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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