



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

Dubey Raghvendra^{1*}, Dubey Kushagra², Janapati Yasodha Krishna³, Sridhar C.⁴ and Jayaveera K.N.⁵

1. Chordia Institute of Pharmacy, Indore (M.P.)
2. Ujjain Institute of Pharmaceutical Sciences, Ujjain (M.P.)
3. P.Rami Reddy Memorial College of Pharmacy, Kadapa (A.P.)
4. CES College of Pharmacy, Kurnool (A.P.)
5. Director, Oil Technology & Research Institute, Anantapur (A.P.)

Abstract

Preliminary phytochemical screening of the leaves extract of *Zizyphus nummularia* revealed the presence of alkaloids, flavonoids, glycosides, saponins. The aqueous, methanolic and saponin extracts of *Zizyphus mauritiana* leaves has been screened for antimicrobial activities against some human vaginal pathogens *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *streptococcus faecalis*, *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; rosales, 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,

***Correspondence Author:**

A12/16, Basant Vihar colony, Ujjain (M.P.) 456001
Mob. +919893422022,
E.mail: raghuji22@rediffmail.com

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 (UTI) 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 nummularia* 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 60°C. After drying and levigation, the residues were inverse flow extracted 10 times with 70% methanol for 4hr at 85°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 °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 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 Amoxicillin/ceftriaxime/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.

Acknowledgment

Sincerely thanks to Dr. S.N. Dwivedi, Principal Investigator, Department of Botany, Janata College, APS University (M.P.), Dr. Chitins, Head, Department of Microbiology, Choitram Hospital & Research Centre, Indore. Dr. (Mrs.) V. Kothari, Head, Pathology Department, CHL Apollo, Indore for their valuable support.

References

1. Stuffness, M.; Douros, J. Current status of the NCI plant and animal product program. *J Nat Prod* 1982, 45, 1-14.
2. Baker, J. T.; Borris, R. P.; Carte, B. Natural product drug discovery and development: New perspective on international collaboration. *J Nat Prod* 1995, 58, 1325-1357.
3. Farombi, E. O. African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. *African J Biotech* 2003, 2, 662-671.
4. Liu, M. J.; Cheng, C. Y. A taxonomic study of the genus *Ziziphus*. *Acta Horti*. 1995, 390, 161-165.
5. Bhansali, A. K. Monographic Study of the family Rhamnaceae of India. Ph.D. thesis, University of Jodhpur, India, 1975
6. Singh, J. P.; Singh, I.S. Some promising varieties of ber. *Indian Horti*. 1973, 18(2), 3-28.
7. Jawanda, J. S.; Bal, J. S.; Josan, J. S.; Mann, S. S. Ber cultivation in Punjab. *Punjab Horti. Journal* 1981, 21, 17-22.
8. Srivastava, S. K.; Srivastava, S. D. Structure of Zizogenin, a new saponin from *Ziziphus mauritiana*. *Phytochemistry* 1979, 18(10), 1758-1759.
9. Huang, R.L.; Wang, W-Y.; Kuob, Y-H.; Lina, Y-L. Cytotoxic Triterpenes from the Fruit of *Ziziphus jujube*, *The Chinese Pharmaceutical Journal* 2001, 53, 179-184.
10. Jiang, J-G.; Huang, X-J.; Chen, J.; Lin, Q-S. Comparison of the sedative and hypnotic effect of flavanoids, saponins and polysaccharides extracted from semen *Ziziphus jujube*. *Natural Product Research* 2007, 21(4), 310-320.
11. Kunin C. M. Urinary tract infections in females. *Clin Infect Dis* 1994, 18, 1-12.
12. Patton, J. P.; Nash, D. B.; Abrutyn, E. Urinary tract infection: economic considerations. *Med Clin North Am* 1991, 75, 495-513.
13. Stamm, W. E.; Hooton, T. M. Management of urinary tract infections in adults. *N Engl J Med* 1993, 329, 1328-34.
14. Sobel, J. D. Pathogenesis of urinary tract infection. Role of host defenses. *Infect Dis Clin North Am* 1997, 11, 531-49.
15. Binutu, O. A.; Lajubutu, B.; Antimicrobial potentials of some plant species of the Bignoniaceae family. *Afr J Med Med Sci* 1994, 23, 269.
16. Oyedeji, O. A.; Adeniyi, B. A.; Ajayi, O.; Konig, W. A. Essential oil composition of *Piper guineense* and its antimicrobial activity. Another Chemotype from Nigeria. *Phytother Res* 2005, 19, 362-364.
17. Alade, P. I.; Irobi, O. N. Antimicrobial activities of crude leaf extracts of *Acalypha wilkensiana*. *J Ethnopharmacol* 1993, 39, 171-174.
18. Rabe, T.; Van, J. Antibacterial activity of South African plants used for medicinal purposes. *J Ethnopharmacol* 1997, 56, 81-87.
19. Perez, C.; Pauli, M.; Bazevque, P. An antibiotic assay by the agar well diffusion method. *Acta Biol Med Exp* 1990, 15, 113-115.
20. Fleischer, T. C.; Ameade, E. P. K.; Mensah, M. L. K.; Sawyer, I. K. Antimicrobial activity of the leaves and seeds of *Bixa orellana*. *Fitoterapia* 2003, 74, 136-38.
21. Taylor, R. S. L.; Manandhar, N. P.; Hudson, J. B.; Towers, G. H. N. Screening of selected medicinal plants of Nepal for antimicrobial activities. *J. Ethnopharmacol*. 1995, 546, 153-159.
22. Samy, R. P. Antimicrobial activity of some medicinal plants from India. *Fitoterapia* 2005, 76, 697-699.

23. Alzoreky, N. S.; Nakahara, K. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *International Journal of Food Microbiology* 2003, 80, 223–30.
24. Bauer, A. W.; Kirby, W. M. M.; Sherris, J. C.; Turck, M. Antibiotic susceptibility testing by standardized single disc method. *American Journal of Clinical Pathology* 1966, 36, 493–6.

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= Amoxicillin)

EC= *Escherichia coli*, PA= *Pseudomonas aeruginosa*, Efa= *Enterobacter faecalis*, EFi= *Enterobacter faecium*, KP= *klebsiella pneumoniae*, SF= *Streptococcus faecalis*, 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 faecalis*, 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 faecalis*, SA= *Staphylococcus aureus* and PM= *Proteus mirabilis*