Asian Journal of Pharmaceutical Research and Development
(An International Peer-Reviewed Journal of Pharmaceutical Research and Development)
www.ajprd.com
ISSN 2320-4850

Research Article

ANTIBACTERIAL ACTIVITY OF AERIAL PARTS OF DIFFERENT SPECIES OF ZIZIPHUS GENUS

*Anil Midha, Sudhir Kumar Singh**
Dept. of Pharmaceutical Science, OPJS University, Churu, Rajasthan, India

ABSTRACT

The leaves and stem powder alcoholic extracts of Ziziphus ziziphus and Ziziphus xyloporus were selected from Ziziphus genus are tested for their antibacterial potential against E. coli ATCC 8739, S. typhi ATCC 23564, S. aureus ATCC 29736, K. pneumoniae ATCC 10031, S. fecalis ATCC 8043, B. subtilis ATCC 6633,S. boydi ATCC 9207, P.mirabilis ATCC 2124, B. cereus, M. luteus ATCC 29736.The alcoholic extracts of Ziziphus xyloporus shown more anti-microbial activity than Ziziphus ziziphus.

Keywords: *anti-microbial activity, Ziziphus ziziphus, Ziziphus xyloporus*.

INTRODUCTION:[1,2,3,4]

In developing countries like India, facing poor air quality, lack of fresh drinking water, poor sanitation habits. As the World Health Organization (WHO) points out, outdoor air pollution contributes as much as 0.6 to 1.4 percent of the burden of disease in developing regions, and other pollution, such as lead in water, air, and soil, may contribute 0.9 percent. all plants share certain features, out of which 199,350 species belongs to flowering angiosperm plants. *ziziphus* is a genus of about 40 species of spiny shrubs and small trees in
the buckthorn family, rhamnaceae.
The rhamnaceae are a large family of flowering angiosperm plants, mostly trees, shrubs, and some vines, commonly called the buckthorn family the family contains about 55 genera and 950 species. the rhamnacee have a worldwide distribution, but are more common in the subtropical and tropical regions. Zizyphus is used for improving muscular strength and weight, for preventing liver diseases and stress ulcers, and as a sedative.

Correspondence, Author
Sudhir Kumar Singh *
Dept. of Pharmaceutical Science,
OPJS University, Churu,
Rajasthan, India

Jujube is also used for various skin conditions including dry and itchy skin, purpura, wounds, and ulcers; digestive problems including lack of appetite and diarrhea; and circulatory problems including high blood pressure and anemia. Other uses are for fatigue, hysteria, fever, inflammation, asthma, and eye diseases.

MATERIAL AND METHODS

Collection, authentication and preparation of plant material:

Ziziphus ziziphus (L) Karst and Ziziphus xyloporus wild were collected and authenticated by qualified botanist. After authentication, around 1 kg of twigs and leaves were collected; after collection , aerial plant`s parts and dust was removed and plant`s parts were kept for shade drying, after complete drying the drug was stored in a well closed container away from sun light. Drying bark was totally peeled off from branches and dried stem part was converted into pieces manually after that these pieces were exposed to grinding and coarse powder was stored in well closed container away from sun light.

Extraction:[9]

Leaves and stems of *Ziziphus ziziphus (l) karst* and *Ziziphus xyloporus*, were carefully washed under running tap water and dried in shade for two weeks. Dried leaves and stem were

powdered, sieved (#40) and stored in an air tight container at room temperature. Dried powder leaves and stem of both plant was then extracted alcoholic, and water by using soxhlation method. The extracts were concentrated to dryness using Rotary evaporator. followed by subjected to different qualitative chemical tests to establish the presence of a mixture of phytoconstituents i.e. alkaloids, glycosides, carbohydrates, phenolics and tannins, phytosterols, fixed oils, fats, proteins an amino acids, flavonoids, saponins, gums and mucilage by means of detection methods.

Preliminary Anti-Microbial Investigation: [5,10]

Antibacterial Activity Assa

The different concentrations of the extracts were tested for antibacterial activity using agar disc diffusion assay. The strains of microorganisms obtained were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 37^0 C for 24h and were referred to as seeded broth. Media were prepared using Muller Hinton Agar (Himedia), poured on Petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs. Sterile discs of six millimeter width had been impregnated with 20 µl of test extract and introduced onto the upper layer of the seeded agar plate. The plates were incubated overnight at 37^0C. Antibacterial activity was assigned by measuring the inhibition zone formed around the discs. The experiment was done three times and the mean values were presented. Streptomycin (10µg/disc)\ and penicillin (10µg/disc) were used as standards.

Determination of Minimal InhibitoryConcentration (MIC) [11,12]

MIC was determined by both agar and broth dilution methods. For broth dilution tests, 0.1ml of standardized suspension of bacteria (106 CFU/ml) was added to each tube containing different concentrations of the active fraction (0-20 µ/ml)and incubated for 24h. at 37^0C. In agar plating method dilutions having 0-20 µ/ml of active fraction was placed in the cups on the inoculated plate and incubated as mentioned above. The lowest concentration of the tube or plate that did not show any visible growth by macroscopic evaluation was considered as the MIC. The data obtained were statistically analyzed using Analysis of Variance (ANOVA).

Determination of Minimum Bactericidal Concentration (MBC) [14]

After culturing the test organisms separately in nutrient broth containing various concentration of the stem bark extract of the plant, the broth was inoculated onto freshly prepared agar plates to assay for the bactericidal effect. The culture was incubated at 37°C for 24h. The lowest concentration of the plant extract that does not yield any colony growth on the solid medium after the incubation period.

RESULT AND DISCUSSION

Plant based drugs are gaining popularity because of several advantages such as fewer side effect, better patient tolerance, relatively less expensive and acceptance due to a long history of use, especially herbal medicines has provide rational means for the treatment of many diseases that are incurable in other system of medicine.Many plants contain non-toxic glycosides that can get hydrolyzed to release phenolics that are toxic to microbial pathogens. Therefore the principle active compounds detected may be responsible for the antibacterial activity of the tested organisms. Which is in The results in Table 5 indicate that the MIC of the leaves extract of the plant ranged between 35 and 50 mg/ml. The effect of the plant extract on the MIC for the test microorganisms is in line with the report that microorganisms varied widely in the degree of their susceptibility. An antimicrobial agent with highly active antimicrobial agent gives a low MIC while a low activity against an organism has a high MIC. The minimum bactericidal concentration (MBC) of the leaves extract of the plant ranged between 45 and 60 mg/ml (Table 6). The MIC and BC is normally used to evaluate the efficacy of the agents such as antiseptics, disinfectants and indeed chemotherapeutic agents under standard conditions also support the sensitivity test results.

Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic
variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus, there has been a continuing search for new and more potent antibiotics. This study therefore provided bases to the folkloric use of this plant as a remedy for urinary tract infection, antipyretic, diaphoretic and abortifacient and other infections caused by the pathogens studied as practiced ethnomedically the world over. *Ziziphus xyloporus* leaves have showed maximum antibacterial activity and so this plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hither unmet therapeutic needs. Such screening of various natural organic compounds and identifying active agents is the need of the hour, because successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development. Suggestion for further studies, purification and characterization of the phytochemicals (principle active compound) that would be obtained with a view to obtaining useful chemotherapeutic agent.

Table No.01 The MIC values of Streptomycin sulphate and Leaves extracts of *Ziziphus xyloporus* and *Ziziphus ziziphus* by broth dilution method

S.No	Name of Bacteria	Streptomycin (µg/ml)	Kanamycin (µg/ml)	*Ziziphus xyloporus* (µg/ml)	*Ziziphus ziziphus* (µg/ml)
1.	*Staphylococcus aureus*	15	12	20.5	19
2.	*Bacillus subtilis*	15	12	19.5	19
3.	*Klebsiella pneumonia*	3	4	5	6
4.	*Escherichia coli*	5	6	7.5	12.5

Table No.02 The MIC values of Streptomycin sulphate and Stem extracts of *Ziziphus xyloporus* and *Ziziphus ziziphus* by broth dilution method

S.No	Name of Bacteria	Streptomycin (µg/ml)	Kanamycin (µg/ml)	*Ziziphus xyloporus* (µg/ml)	*Ziziphus ziziphus* (µg/ml)
1.	*Staphylococcus aureus*	15	12	22.5	20
2.	*Bacillus subtilis*	15	12	19.5	19.5
3.	*Klebsiella pneumonia*	3	4	5.5	7.5
4.	*Escherichia coli*	5	6	8.5	13.5

Table No. 03 Shows *In Vitro* Anti-bacterial activity of Leaves of *Ziziphus xyloporus* using cup- plate method

Conc. Of compound (µg/ml)	***Name of the Microbes***									
	E. coli ATCC 8739	***S. typhi ATCC 23564***	***S. aureus ATCC 29736***	***K. pneumoniae ATCC 10031***	***S. fecalis ATCC 8043***	***B. subtilis ATCC 6633***	***S. boydi ATCC 9207***	***P. mirabilis ATCC 2124***	***B. cereus***	***M. luteus ATCC 29736***
20	4.2	0	8.9	6.2	3.3	7.0	-	-	4.3	2
25	8.2	0	18.3	12.4	8.1	18.1	-	-	16.7	9.2

Table No. 04 Shows *In Vitro* Anti-bacterial activity of Stem of *Ziziphus xyloporus* using cup- plate method

Conc. Of compound (µg/ml)	***Name of the Microbes***									
	E. coli ATCC 8739	***S. typhi ATCC 23564***	***S. aureus ATCC 29736***	***K. pneumoniae ATCC 10031***	***S. fecalis ATCC 8043***	***B. subtilis ATCC 6633***	***S. boydi ATCC 9207***	***P. mirabilis ATCC 2124***	***B. cereus***	***M. luteus ATCC 29736***
20	4.9	0	9.4	6.9	4.1	7.9	-	-	5.1	2.9
25	9.1	0	19.5	13.6	9.2	19.3	-	-	17.2	10.6

Table No. 05. Shows *In Vitro* Anti-bacterial activity of Leaves of *Ziziphus ziziphus* using cup-plate method.

Conc. Of compound (µg/ml)	**Name of the Microbes**									
	E. coli ATCC 8739	*S. typhi ATCC 23564*	*S. aureus ATCC 29736*	*K. pneumoniae ATCC 10031*	*S. fecalis ATCC 8043*	*B. subtilis ATCC 6633*	*S. boydi ATCC 9207*	*P. mirabilis ATCC 2124*	B.cereus	*M.luteus ATCC 29736*
20	5.2	3.2	5.2	4.3	-	6.0	4.3	-	6.8	-
25	15.3	8.5	14.1	10.2	-	12.2	10.4	-	18.9	-

Table No. 06. Shows *In Vitro* Anti-bacterial activity of Leaves of *Ziziphus ziziphus* using cup-plate method.

Conc. Of compound (µg/ml)	**Name of the Microbes**									
	E. coli ATCC 8739	*S. typhi ATCC 23564*	*S. aureus ATCC 29736*	*K. pneumoniae ATCC 10031*	*S. fecalis ATCC 8043*	*B. subtilis ATCC 6633*	*S. boydi ATCC 9207*	*P. mirabilis ATCC 2124*	B.cereus	*M.luteus ATCC 29736*
20	5.9	4.4	6.5	5.4	-	7.4	5.2	-	8.4	-
25	16.4	9.2	15.2	11.1	-	13.4	11.2	-	20.1	-

REFERENCES

1. *http://www.factmonster.com/dk/science/encyclopedia/classifying-plants.html*
2. *https://en.wikipedia.org/wiki/Rhamnaceae*
3. *https://en.wikipedia.org/wiki/Ziziphus*
4. *http://www.webmd.com/vitamins-supplements/ingredientmono-62zizyphus.aspx?activeing red ientid = 62&activeingredientname=zizyphus*
5. *Khandelwal. Practical Pharmacognosy. 1st ed. Pune: Nirali Publications; 1995.*
6. *Wallis.T.E. Textbook of Pharmacognosy. 5th ed. New Delhi: CBS Publishers & Distributors; 1985*
7. *World health organization guidelines of "Quality control methods for medicinal plant materials"A.I.T.B.S publisher and distributors Delhi: Geneva; 1998.*
8. *http://www.ccras.nic.in/PharmacopoeialWork/vol %20-%205/3.%20Appendices %20(Vol% 205).pdf*
9. *Dinesh Kumar, Sunil Kumar, Satyender Kumar et al, 'antimicrobial and preliminaryphytochemical screening of crude leaf extract of pandanus odoratissimus' Pharmacologyonline 2: 600-610 (2010)*
10. *Kokate CK. Practical Pharmacognosy, 4th ed., Vallabh Prakashan, New Delhi. 2005:107-111.*
11. *Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M. . Antibiotic susceptibility testing by a standardized single disk method. American journal of clinical pathology. 1966: **45**; 493-496*

12. *Collins. C. H, Lyne PM, Grange JM (1995). Microbiological methods (7th edn) Butterwort . Heinemann Ltd. Britain. 175. 190.*
13. *Snedecor G.W. and Cochran W.G.. A statistical method. Iowa State University Press, Ames, Iowa, USA. (s)1967*
14. *Banso A. Phytochemical and antibacterial investigation of bark extracts of Acacia nilotica Journal of Medicinal Plants Research 2009:**3(2);** 082-085*