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Evaluation of antibacterial activity of *Zizyphus jujuba*

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Abstract
Red date or Bera (Pushto), is used primarily for its fruits. Jujube, a delicious fruit, is an effective herbal remedy improving stamina and muscular strength and aids weight gain, strengthens liver function and increases immune system resistance. The aim of this study is to evaluate in-*vitro* efficacy of antibacterial activity of crude methanol, n-hexane, chloroform, ethyl acetate and aqueous extracts of *Zizyphus jujuba* against some human pathogenic bacterial strains. Antibacterial and antifungal activities of *Zizyphus jujuba* extract was carried out by using disc diffusion method. The crude methanol, n -hexane, chloroform, ethyl acetate and aqueous fractions showed 41.37, 44.82, 41.37, 55.17 and 44.82% activity against *Enterobacter aerogenes* respectively. The crude methanolic extract and n-hexane fractions were inactive against *Escherichia coli, S. pneumoniae* and *Klebsella pneumonia* respectively.
From this study, the extracts namely n-hexane, ethyl acetate and aqueous extracts of *Zizyphus jujuba* possess antibacterial activity.

Keywords: *Zizyphus jujuba*, Antibacterial activity, *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus Pumalis*

Introduction
About 60% of the world's populations exclusively rely on traditional medicine (plant extracts) for their primary health-care needs (Farnsworth, 1994) [6]. Currently, there are increasing incidents of infections due to evolution of new pathogens and resistance of the present pathogens to the existing antibiotics, for example, multi-drug resistant tuberculosis (MDR-TB) is resistant at least to isoniazid and rifampicin, the two most powerful first-line anti-TB drugs (Leimane, 2005) [17]. Plants are rich sources of bioactive compounds. Of the world's 25 bestselling pharmaceutical agents, 12 are naturally derived products. In view of this, an attempt has been made to study the antibacterial activity of herbal drug. *Zizyphus jujuba* is also called as Badari, Baer, Bogari, Barihannu belonging to family Rhamnaceae. The plant is distributed throughout India, Iran, Afganistan, and in China. It is a small sub deciduous tree with dense spreading crown, commonly 0.6 m. girth and 6 m. high. It is used primarily for its fruits. Jujube, a delicious fruit, is an effective herbal remedy improving stamina and muscular strength and aids weight gain. It strengthens liver function and increases immune system resistance. It functions as antidote, diuretic, emollient and expectorant. The leaves are febrifuge, astringent and said to promote the hair growth. In the treatment of strangury they are used to form a plaster. The dried fruits are anticancer, anodyne, refrigerant, sedative, styptic, pectoral, tonic and stomachic. They help in digestion and blood purification. The objective of the present study determines the evaluation of the antibacterial activities of red date using various organic compounds.

Materials and Methods
Collection and authentication
Zizyphus jujuba was collected from in and around Chembarambakkam, Chennai, Tamil Nadu, India and the plant was identified, authenticated by the taxonomist. The authenticated specimen was also deposited in the Department of Pharmacognosy, Sree Sastha Pharmacy College. The aerial parts were dried in room temperature for 2 months. Dried specimen was powdered using mechanical grinder and passed through 60 mesh sieve to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

Preparation of extract
The powdered material of *Z. jujuba* (7 kg) was soaked in methanol for 15 days, twice, at room temperature, with occasional shaking. Each time, the material was filtered and the filtrate was concentrated at 40 °C under vacuum, by rotary evaporator. A blackish crude methanolic extract of *Z. jujuba* (850 g) was obtained.

Fractionation

The crude methanol extract of *Z. jujuba* (1000 g) was suspended in distilled water (5000 ml) and partitioned with n-hexane (3 x 500 ml), chloroform (3 x 500 ml) and ethyl acetate (3 x 500 ml), respectively, to yield the n- hexane (200 g), chloroform (160 g), ethyl acetate (110 g) and aqueous (240 g) fractions. 90 g of the crude methanolic extract of *Z. jujuba* was left for biological/pharmacological activities. All the fractions will only contain their particular compounds based on the solubility from the crude extract. For example, the n-hexane fraction will contain only those compounds which are non-polar, and so an.

Antibacterial activity

Determining percent inhibition

Antibacterial activity of the crude methanol, n-hexane, chloroform, ethylacetate and aqueous extracts of *Z. jujuba* were determined against *Escherichia coli, Pseudomonas aeruginosa, S. aureus, Staphylococcus epidermidis, Salmonella typhi, Bacillus pumalis, Klebsella pneumoniae, Streptococcus pneumoniae and Enterobacter aerogenes* (Ahmad *et al*., 2009) [1, 2]. Eighteen hours old culture of the test organism from the nutrient broth was transferred to sterile nutrient agar plates to make bacterial lawn. After 30 min, using a sterile 6 mm borer, wells were dug in plates. Stock solutions (3 mg/ml) of the test samples were prepared in sterile dimethyl sulfoxide (DMSO, less than 1%). 100 µl of crude methanolic extract and fractions were loaded to their respective wells. Amoxicillin and DMSO (less than 1%) were used as positive and negative controls, respectively. Zone of inhibition was measured (in mm) in comparison with positive control using the following formula

$$\% \text{ Inhibition} = \frac{\text{Zone of Inhibition of Sample}}{\text{Zone of Inhibition of Standard}} \times 100$$

Determination of minimum inhibitory concentration (MIC)

After determining the percent inhibition, the MIC50 of the test samples at the concentration of 0.9, 1.5, 2.1, 2.7 and 3.2 mg/ml were measured against the test organisms (Banso, 2009) [5]. To sterile nutrient broth in the test tubes (4 ml), test samples and test organisms were inoculated, incubated for 24 h at 37 °C. Results were recorded after 24 h based on the percent clarity against negative control. Negative control in this case was nutrient broth media.

Results and Discussion

The interest regarding the research on medicinal plants has increased over the last few decades due to onset of new infection, in particular, infections by *Enterococcus* and *Staphylococcus* species, which are agents of many intra-hospital infections and antibiotic resistance to available drugs, e.g. *S. aureus* has become resistant to several antibiotics to which it was previously susceptible. Some of the antibiotics to which it is now resistant are pencillin G, macrolides, lincosamides, tetracyclines and gentamicin (Ayliffe, 1997) [4]. With the intent of exploring new bioactive compounds from plant origin, we have selected *Z. jujuba*, which is locally used as an analgesic, tranquilizer, convulsant and have been prescribed for the treatment of insomnia and anxiety (Peng and Zhu, 2001) [21]. The results of antibacterial activity of the crude methanol, n-hexane, chloroform, ethylacetate and aqueous extracts of *Z. jujuba* are presented in Table-1.

Table 1: Antibacterial activity of crude methanolic extract & various fractions of *Zizyphus jujube*

Name of Bacteria	Zone of Inhibition of standard (Amoxicillin) 10µg/Disc	Crude Extract Methanol		*n*-hexane		$CHCl_3$		EtOAc		Aqueous	
		Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)
S.epidermidis	26	9	34.61	14	53.84	10	38.46	17	65.38	10	38.46
S.typhi	27	10	37.03	15	55.55	14	51.85	17	62.96	15	55.55
S.pneumoniae	29	9	31.03	0	0	8	27.58	0	0	12	41.37
S.aureus	26	8	30.76	10	38.46	13	50.00	8	30.76	9	34.61
P.aeruginosa	27	15	55.55	10	37.03	12	44.44	17	62.96	18	66.66
K.pneumoniae	21	6	28.57	0	0	8	38.09	6	28.57	7	33.33
B.pumalis	25	13	52.00	15	60.00	8	32.00	18	72.00	10	40.00
E.aerogens	29	12	52.00	15	60.00	8	32.00	18	72.00	10	40.00
E.coli	27	0	0	8	29.62	8	29.62	13	48.14	11	40.74

Table 2: MIC_{50} values (mg/ml) of crude methanolic extract and various fractions of *Zizyphus jujube*

Name of Bacteria	Crude extract	n-hexane	$CHCl_3$	EtOAc	Aqueous
S.epidermidis	3.7	2.9	3.2	2.4	3.2
S.typhi	3.2	2.9	3.0	2.4	2.9
S.pneumonia	3.2	0	3.2	0	2.7
S.aureus	2.9	3.0	2.7	3.1	3.0
P.aeruginosa	2.5	3.0	2.7	2.3	1.9
K.pneumoniae	3.4	0	3.1	3.7	3.2
B.pumalis	2.5	2.1	3.2	2.1	1.0
E.aerogens	2.7	2.7	3.0	2.7	2.9
E.coli	0	4	3.8	3.0	3.2

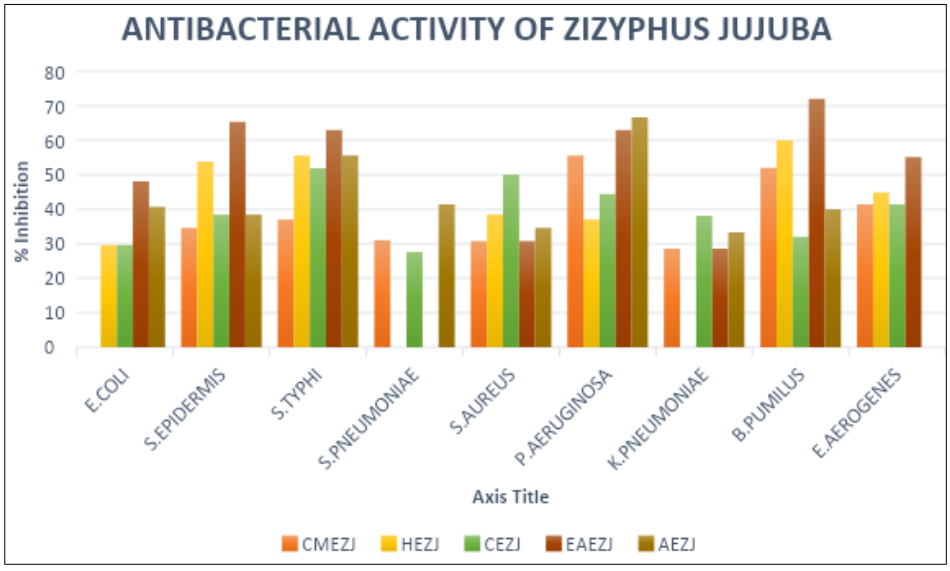

Fig 1: Antibacterial activity of crude methanolic extract & various fractions of *Zizyphus jujube*

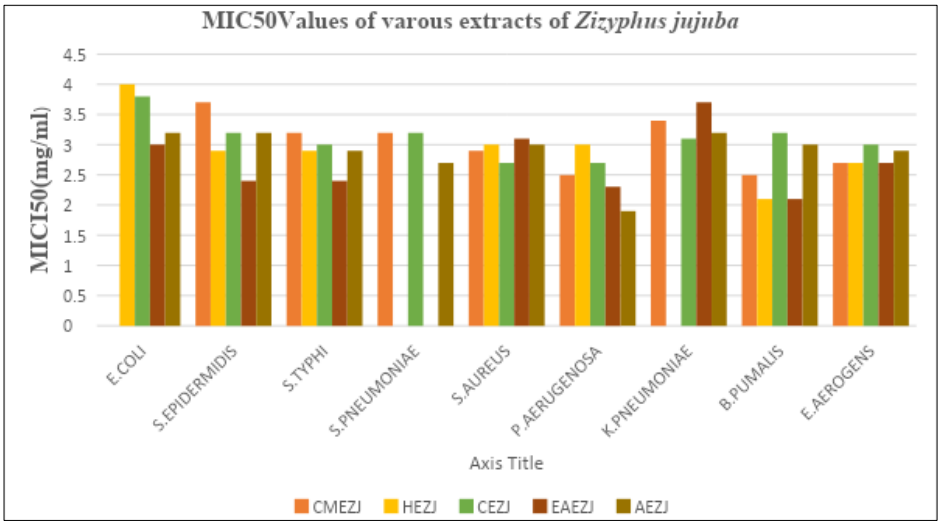

Fig 2: MIC_{50} values (mg/ml) of crude methanolic extract and various fractions of *Zizyphus jujuba*

The crude methanolic extract showed moderate activity against *P. aeruginosa, B. pumalis and E. aerogens* with 55.55, 52 and 41.37%, low against *S. typhi, S. epidermidis, S. pneumoniae, S. aureus and K. pneumoniae* with 37.03, 34.61, 31.03, 30.76 and 28.57% inhibition, respectively. It was inactive against *E. coli*. The n-hexane fraction was significantly active against *B. pumalis* (60%), moderately active against *S. typhi, S. epidermidis* and *E. aerogens* with 55.55, 53.84 and 44.82%, low against *S. aureus, P. aeruginosa* and *E. coli* with 38.46, 37.03 and 29.62%, respectively. In contrast, the n-hexane fraction presented no activity against *S. pneumoniae* and *K. pneumoniae*. The chloroform fraction of the plant was moderately active against *S. typhi, S. aureus, P. aeruginosa* and *E. aerogens* having percentage inhibition of 51.85, 50, 44.44 and 41.37. Low activity was observed against *S. epidermidis, K. pneumoniae, B. pumalis, E. coli* and *S. pneumoniae* with 38.46, 38.09, 32, 29.62 and 27.58% inhibition, respectively. The EtOAc fraction was significantly active against *B. pumalis, S. epidermidis, S. typhi and P. aeruginosa* with 72, 65.38, 62.96 and 62.96, moderately active against *E. aerogens, E. coli* with 55.17 and 48.14% inhibition. It showed low activity against *S. aureus* and *K. pneumoniae* with 30.76 and 28.57 percentage inhibition and inactive against *S. pneumoniae*. The aqueous fraction showed significant activity against *P. aeruginosa* with 66.66% and moderate activity against *S. typhi, E. aerogens, S. pneumoniae, E. coli* and *B. pumalis* with 55.55, 44.82, 41.37, 40.74 and 40% inhibition, respectively. It conferred low activity on *S. epidermidis, S. aureus* and *K. pneumoniae* with inhibition percentage of 38.46, 34.61 and 33.33, respectively.

MIC50 of the test samples ranged from 1.9 - 3.8 mg/ml was depicted in Table 2. The similar approach was used and explored various fractions of *Onosma griffithii* for antibacterial activity against *E. coli, B. subtilis, S. aureus, Shigella flexenari and S. typhi* (Ahmad *et al*., 2009) [1, 2]. Same strategy was followed by Ajaiyeoba, 2002 [3], in which the n-hexane, ethyl acetate, ethanol and water extract of *Parkia bicolor* were tested for antimicrobial activity against *S. aureus, B. cereus, E. coli, P. aeruginosa*, (Ajaiyeoba, 2002) [3]. The crude methanolic extracts of different plants were tested against gram positive and negative bacteria for new bioactive compounds (Shahidi, 2004). The n-hexane and EtOAc fractions of *Z. jujuba* were significantly active against *B. pumalis, S. epidermidis, S. typhi and P. aeruginosa* with low values of MIC50.

Conclusion

The findings of the present study revealed that *Z. jujuba* (Rhamnaceae) contain potent antimicrobial property. The n-

hexane fraction of *Z. jujuba* was significantly active against *B. pumalis* (60%, MIC50 =2.1 mg/ml), EtOAc was active against *B. pumalis* (72%, MIC50 = 2.1 mg/ml), *S. epidermidis* (65.38%, MIC50 = 2.4 mg/ml), *S. typhi* (62.96%, MIC50 = 2.4 mg/ml), *P.aerugenosa* (62.96%, MIC50 = 2.3 mg/ml) and aqueous fraction against *P.aerugenosa* (66.66%, MIC50 = 1.9 mg/ml). So the research should be extended for the isolation of active compounds.

Reference

1. Ahmad B, Ali N, Bashir S, Choudhary MI. Biological activities of aerial parts of *Tylophora hirsuta* wall. African J Biotechnol. 2009; 8:46274631.
2. Ahmad B, Ali N, Bashir S, Choudhary MI. Parasiticidal, antifungal and antibacterial activities of *Onosma griffithii* Vatke. Afr. J Biotechnol. 2009; 8:5084-5087.
3. Ajaiyeoba E. Phytochemical and antibacterial properties of Parkia Biglobosa and Parkia Bicolor leaf extracts. Afr. J Biomed. Res. 2002; 5:125-129.
4. Ayliffe G. The progressive intercontinental spread of methicilinresistant Staphylococcus aureus Clin. Infect. Dis. 1997; 24:74-79.
5. Banso A. Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*. J Med. Plants Res. 2009; 3:82-85.
6. Farnsworth NR. Ethnopharmacology and drug development, in: Ciba Foundation Symposium. 1994; 185:42-59.
7. Gul J, Mir AK, Farzana G. Ethno medicinal plants used against Jaundice in Dir Kohistan Valleys (NWFP), Pakistan. Ethnobotanical Leaflets. 2009; 13:1029-1041.
8. Hameed I, Dastagir G, Hussain, F, Shams-un-Nisa. Cytotoxicity and Phytotoxicity of some selected medicinal plants of Family Polygonaceae. Afr. J Biotechnol. 2009; 9(5):770-774.
9. Inas SG, Atta S, Mosaad A, Abdel W. Zizyphus jujuba and *Origanum majorana* extracts protect against hydroquinone-induced clastogenicity. Environ. Toxicol. Pharmacol. 2008; 25:10-19.
10. Ivancheva S, Stantcheva B. Ethnobotanical inventory of medicinal plants in Bulgaria. J Ethnopharmacol. 2000; 69:165-172.
11. Jean W, Wooten Stella DE. Comparisons of Potential Allelopathy of Seven Freshwater Species of Spikerushes (Eleocharis). J Aquat. Plant Manage. 1991; 29:12-15.
12. Jee-Hwan Y, Il-Kwon P, Kwang-Sik C, Sang-Cheol S, Young-Joon A. Toxicity of medicinal plant extracts to *Lycoriella ingenua* (Diptera: Sciaridae) and *Coboldia fuscipes* (Diptera: Scatopsidae). J Asia-Pac. Entomol. 2008; 11:221-223.
13. Jeong HP, Hyun JL, Sang BK, Ju YB, Yeon HS. Protection of NMDA-induced neuronal cell damage by methanol extract of Zizyphi Spinosi Semen in cultured rat cerebellar granule cells. J Ethnopharmacol. 2004; 95:39-45.
14. Jun WJ, Han BK, Yu KW, Kim MS. Antioxidant effects of *Origanum majorana* L. on superoxide anion radicals. Food Chem. 2001; 75:439-444.
15. Khare CP. *Zizyphus jujuba*, Encyclopedia of Indian Medicinal Plants. Springer New York, 1995, 493-498.
16. Kuroki T, Kubota A, Miki Y, Yamamura T, Utsunomiya. Lectin staining of neoplastic and normal background colorectal mucosa in nonpolyposis and polyposis patients. J Dis. Colon Rectum. 1991; 34:679684.
17. Leimane V. Clinical outcome of individualised treatment of multidrug-resistant tuberculosis in Latvia: a retrospective cohort study. Lancet. 2005; 365:318-326.
18. Lis H, Sharon NP. The lectins: Properties, functions and applications. Liener IE, Sharon N, Goldstein IJ (eds). Academic Press, New York, 1986, 294-327.
19. Naqvi SB, Sheikh D, Usmanghani K, Shameel M, Sheikh R. Screening of marine algae of Karachi for haemagglutination activity. Pak. J Pharm. Sci. 1992; 5:129-138.
20. Neto VF, Andrade Brandao MGL, Nogueira F, Rosário VE, Krettli AU. *Ampelo zyziphus* amazonicus Ducke (Rhamnaceae), a medicinal plant used to prevent malaria in the Amazon Region, hampers the development of *Plasmodium berghei* sporozoites. Int. J Parasitol. 2008; 38:1505-1511.
21. Peng ZC, Zhu J. Research advances in chemical constituents and pharmacological effects of semen Ziziphi Spinosae. Lishizen Med. Mat. Med. Res. 2001; 12:86-87.
22. Rehman AU. Studies in Natural Product Chemistry. Elsevier Science Publishers, Netherlands. 1991; 9:383-409.
23. Shahidi BGH. Evaluation of antibacterial properties of Iranian Medicinal plants against Micrococcus luteus, Serratia marcescens, klebsiella pneumoniae and *Bordetella bronchoseptica*. Asian J Plant Sci. 2004; 3:82-86.
24. Sharon N, Lis H. Lectins: Cell agglutinating and sugar-specific proteins. Science. 1972; 177:949-959.
25. Tsutomu H, Miwako K, Kazutoshi I, Tomo-omi O, Sumiko S, Tomofusa T *et al*. Effects of tannins and related polyphenols on methicillin-resistant Staphylococcus aureus. Phytochemistry. 2005; 66:2047-2055.
26. Wei CH, Koh C, Crystalline Ricin D. A toxic anti-tumor lectin from seeds of *Ricinus communis*. J Biol. Chem. 1978; 253:2061.
27. Yilin C, Carsten S. Flora of China. 2007; 12:115-355.