

## Evaluation of Antibacterial Activity of *Zizyphus Jujuba* (Jujube)

**Dr. Dhanapal Venkatachalam\***

\*Principal, Sree Sastha Pharmacy College, Sree Sastha Nagar, Chembarambakkam, Chennai-600123, India

\***Corresponding author:** Dr. Dhanapal Venkatachalam, Principal, Sree Sastha Pharmacy College, Sree Sastha Nagar, Chembarambakkam, Chennai - 600123, India Email: [vddpaul@gmail.com](mailto:vddpaul@gmail.com); Tel: +919443952113

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### Abstract

**Objective:** The objective of the study was 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. *Z.jujuba* commonly called, Red date or Chinese date or Bera (Pushto), belonging to family Rhamnaceae, 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.

**Methodology:** Crude methanol, n-hexane, chloroform, ethyl acetate and aqueous extracts of *Zizyphus jujuba* were used for antibacterial screening. Antibacterial activity was tested against pathogenic bacterial strains of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhi*, *Bacillus Pumilus*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Streptococcus pneumonia*. Antibacterial and antifungal activities of *Zizyphus jujuba* extract was carried out by using disc diffusion method.

**Results:** The n-hexane and aqueous fractions showed significant activity of 60 and 66.66% against *Bacillus pumalis* and *Pseudomonas aeruginosa*, respectively. Activity of ethyl acetate fraction was 65.38, 62.96, 62.96 and 72% against *Staphylococcus epidermidis*, *Salmonella typhi*, *P. aeruginosa* and *B. pumalis*, respectively. On the contrary, no activity of this fraction was recorded against *Streptococcus pneumoniae*. 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.

**Conclusion:** The results conclude that n-hexane, ethyl acetate and aqueous extracts of *Zizyphus jujuba* possess

antibacterial activity.

**Keywords:** *Zizyphus jujuba*; Antibacterial activity; *Pseudomonas aeruginosa*; *Escherichia coli*; *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Salmonella typhi*; *Bacillus Pumalis*; *Klebsiella pneumonia*; *Enterobacter aerogenes*

## Introduction

About 60% of the world's populations exclusively rely on traditional medicine (plant extracts) for their primary health-care needs [1]. 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 [2]. 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 subdeciduous tree with dense spreading crown, commonly 0.6 m. girth and 6 m. high. The bark is blackish to grey or brown, rough, regularly and deeply furrow, the furrowed, the furrows are at about 1.2 cm apart. Blaze 9-13mm. Branches usually armed with spines, mostly in pairs, one straight, the other curved. Leaves 3-6.3 by 2.5-5 cm. oblong or ovate, usually minutely serrulate or apex distinctly toothed, obtuse, base oblique and 3nerved, nerves depressed on the glabrous shining upper surface. Petiole 2.5-10 mm long. Flowers 3.8-5 mm. In Diam., greenish, in dense axillary tomentose cymes or fascicles 1.2-1.9 cm Long. Drupe 1.2-2.5 cm. Diam., globose, first yellow then orange and finally reddish brown, containing a single stone surrounded by fleshy pulp. 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 plaster. The dried fruits are anticancer, anodyne, refrigerant, sedative, styptic, pectoral, tonic and stomachic. They help in digestion and blood purification. The present work was to screen the antibacterial effect of aerial parts of *Zizyphus jujuba* using a standard procedure.

## Materials and Methodology

### Collection and authentication

*Zizyphus jujuba* was collected from in and around Chembarambakkam, Chennai India. The plant was identified and authenticated by the taxonomist. The authenticated specimen was deposited in the Department of Pharmacognosy, Sree Sastha Pharmacy College. The authentication specimen number is SSPC/P.COG/001/2019. 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, ethyl acetate 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* [3,4]. 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:

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

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Zone of Inhibition of Standard

### Determination of Minimum Inhibitory Concentration (MIC)

After determining the percent inhibition, the MIC<sub>50</sub> 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 [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 penicillin G, macrolides, lincosamides, tetracyclines and gentamicin [6]. With the intent of exploring new bioactive compounds from plant origin, we have selected *Z. jujuba*, which is locally used as an analgesic, tranquilizer and convulsant have been prescribed for the treatment of insomnia and anxiety [7]. The results of antibacterial activity of the crude methanol, n-hexane, chloroform, ethyl acetate and aqueous extracts of *Z. jujuba* are shown in (Table-1). 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. MIC<sub>50</sub> of the test samples ranged from 1.9 - 3.8 mg/ml (Table 2). This work is continuation of my effort on exploring new bioactive compounds. In my previous work, I have utilized similar approach and explored various fractions of *Onosma griffithii* for antibacterial activity against *E. coli*, *B. subtilis*, *S. aureus*, *Shigella flexenari* and *S. typhi* [3,4]. Same strategy was followed by Ajaiyeoba et al., in which the n-hexane, ethyl acetate, ethanol and water extract of *Parkia bicolor* A. Chev were tested for antimicrobial activity against *S. aureus*, *B. cereus*, *E. coli*, *P. aeruginosa* [8]. The crude methanolic extracts of different plants were tested against gram positive and negative bacteria for new bioactive compounds [9]. 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 MIC<sub>50</sub> [10-26].

Name of Bacteria	Zone of Inhibition of standard(Amoxicillin) 10µg/Disc	Crude Extract Methanol		n-hexane		CHCl <sub>3</sub>		EtOAc		Aqueous	
		Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition	Inhibition (%)	Zone of Inhibition	Inhibition (%)	Zone of Inhibition	Inhibition (%)	Zone of Inhibition	Inhibition (%)
<i>S.epid ermidis</i>	26	9	34.61	14	53.84	10	38.46	17	65.38	10	38.46
<i>S.typhi</i>	27	10	37.03	15	55.55	14	51.85	17	62.96	15	55.55
<i>S.pne umoniae</i>	29	9	31.03	0	0	8	27.58	0	0	12	41.37
<i>S.aureus</i>	26	8	30.76	10	38.46	13	50	8	30.76	9	34.61
<i>P.aeruginosa</i>	27	15	55.55	10	37.03	12	44.44	17	62.96	18	66.66
<i>K.pne umoniae</i>	21	6	28.57	0	0	8	38.09	6	28.57	7	33.33
<i>B.pumalis</i>	25	13	52	15	60	8	32	18	72	10	40
<i>E.aerogens</i>	29	12	52	15	60	8	32	18	72	10	40
<i>E.coli</i>	27	0	0	8	29.62	8	29.62	13	48.14	11	40.74

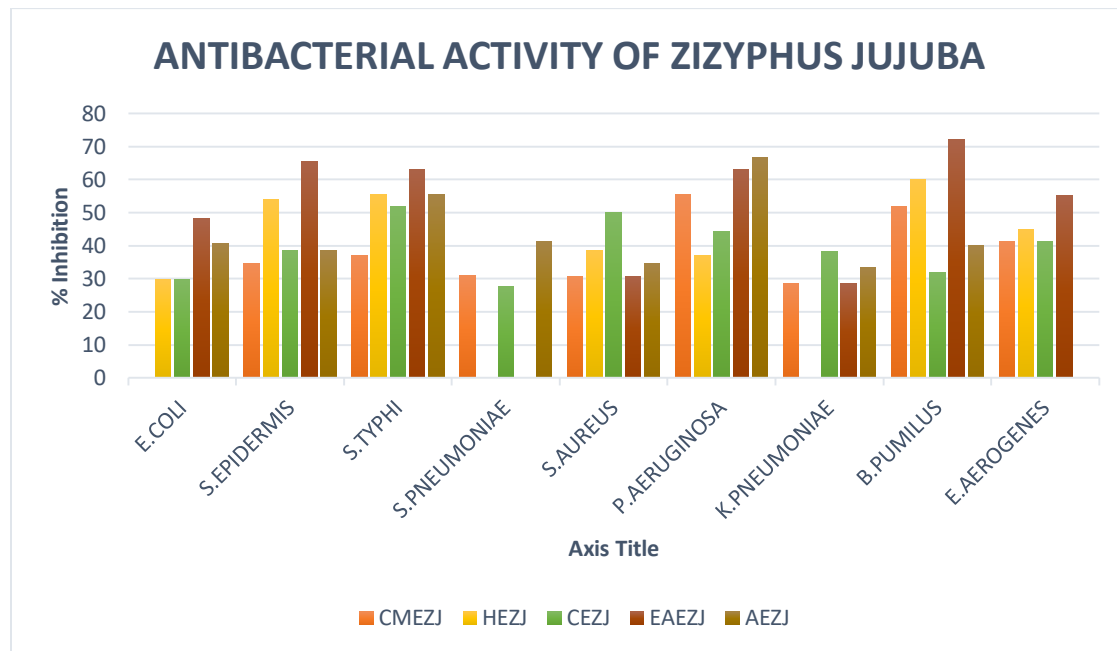
**Table 1:** Antibacterial activity of crude methanolic extract & various fractions of *Zizyphus jujube*.

Name of Bacteria	Crude extract	n-hexane	CHCl <sub>3</sub>	EtOAc	Aqueous
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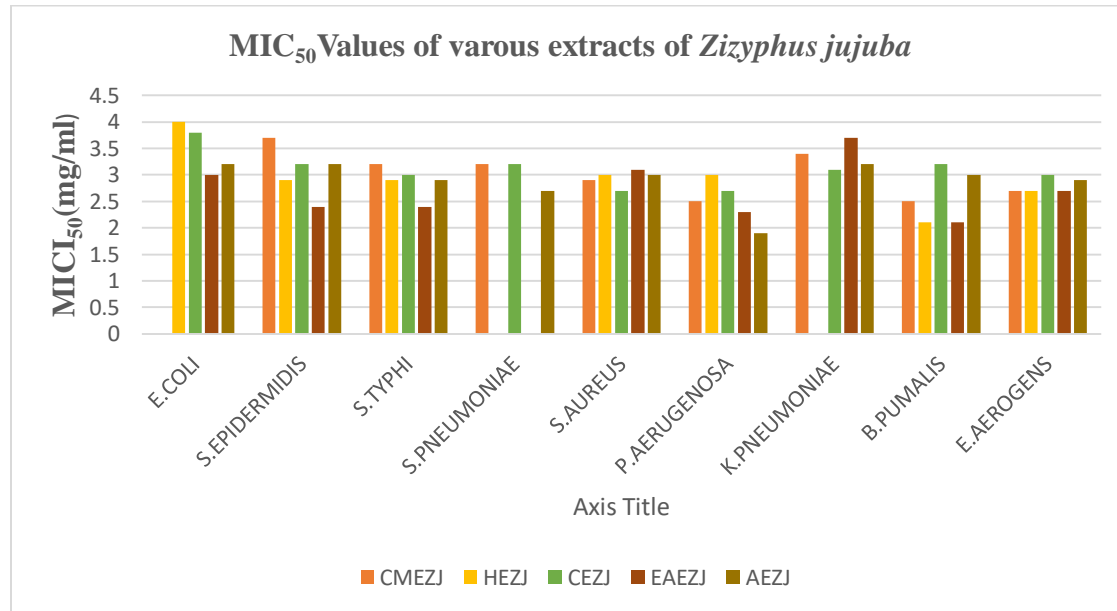
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<i>S.epidermidis</i>	3.7	2.9	3.2	2.4	3.2
<i>S.typhi</i>	3.2	2.9	3.0	2.4	2.9
<i>S.pneumonia</i>	3.2	0	3.2	0	2.7
<i>S.aureus</i>	2.9	3.0	2.7	3.1	3.0
<i>P.aeruginosa</i>	2.5	3.0	2.7	2.3	1.9
<i>K.pneumoniae</i>	3.4	0	3.1	3.7	3.2
<i>B.pumalis</i>	2.5	2.1	3.2	2.1	1.0
<i>E.aerogens</i>	2.7	2.7	3.0	2.7	2.9
<i>E.coli</i>	0	4	3.8	3.0	3.2

**Table 2:** MIC50 values (mg/ml) of crude methanolic extract and various fractions of *Zizyphus jujube*.



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



**Figure 2:** MIC<sub>50</sub> values (mg/ml) of crude methanolic extract and various fractions of *Zizyphus jujube*.

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### Ethics Approval and Consent to Participate

Not Applicable.

### Human and Animal Right

No animals/Humans studies that are base of were used for this research.

### Consent for Publication

Not applicable.

### Conflict of Interest

The author confirmed that this article content has no conflict of interest.

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