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Evaluation of the Antibacterial, Anthelmintic Activities of Leaves of *Ziziphus oenoplia* (L.) Mill.

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ABSTRACT:

Herbal Medicine, sometimes referred to as Herbalism or Plant Medicine, is the use of herbs for their therapeutic Value. Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures all over history. It was an integral part of the development of modern development. In recent years, there is a tremendous interest in the possible role of nutrition in the inhibition of disease. Helminth infections are among the most widespread in humans, affecting a large population worldwide. Although the majority of infections due to helminths are generally restricted to tropical regions and cause enormous hazard to health and contribute to the occurrence of undernutrition, anaemia, eosinophilia and pneumonia, parasitic diseases. Alcoholic extract of Z.oenoplia exhibited anthelmintic activity in a dose-dependent manner, giving the shortest time of paralysis (P) and death (D) with 400mg/ml concentration. The alcohol and water extracts of *Ziziphus oenoplia* leaves showed significant antioxidant activity, while the alcohol extract demonstrated notable antibacterial and anthelmintic activities

Keywords: Herbal medicine; *Ziziphus oenoplia*; Antibacterial activity; Anthelmintic activity; Antiplasmodial activity; Medicinal plants.

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INTRODUCTION

Many drugs commonly used nowadays are of herbal origin. Indeed, about 25 percent of the prescription drugs dispensed in the United States hold at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesised to mimic a natural plant compound. The US herbal medicine consumption alone was worth US$ 17 billion in the year 2000 and the global market for herbal medicines nowadays is estimated to be a whopping US$ 60 billion. The World Health Organisation (WHO) estimates that 4 billion people, 80 per cent of the world population, presently use herbal medicine for some aspect of primary health care. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rainforests and other places for their potential medicinal value.

PLANT PROFILE

Table 1. Vernacular names of *Ziziphus oenoplia*

VERNACULAR NAMES	
English	*Ziziphus oenoplia* (L.) Mill.
Hindi	Makkay, Kokal ber
Telugu	Parigi
Sanskrit	Karkandhu
Tamil	Curia
Bengali	Siyakul
Kannada	Barige, Challe
Konkani	Burgi
Malayalam	Vanthutali
Marathi	Burgi, Chini bor, Maastodi
Oriya	Kontaikoli
Nepalese	Aule bayar, Boksi bayar

Figure 1. *Ziziphus oenoplia* plant

Ziziphus oenoplia (L.) Mill. is a thorny, climbing shrub that belongs to the family **Rhamnaceae**. The genus Ziziphus includes about 40 species of spiny shrubs and small trees found in tropical and subtropical regions.

The plant has simple, alternate leaves that are oval to lance-shaped. The leaves are slightly uneven at the base, have smooth or finely toothed margins, and are covered with soft hairs. Three prominent veins arise from the base of each leaf.

The fruit is a small, round drupe with a fleshy outer part and a hard inner stone containing a single seed. When fully ripe, the fruit becomes black, shiny, and pleasant to eat.

Ziziphus oenoplia is widely distributed from the Indian subcontinent to southern China, Southeast Asia, and northern Australia. It commonly grows along roadsides, in forests, and among thickets

CHEMICAL CONSTITUENTS

The family Rhamnaceae is of pharmaceutical importance because many of its plants contain biologically active compounds, particularly anthraquinones. Several species in this family are also known to produce benzylisoquinoline alkaloids and cyclopeptide alkaloids, which may contribute to their medicinal properties.

Ziziphus oenoplia has been reported to contain various cyclopeptide alkaloids, including zizyphines (F, I, K, N, O, and P), amphibine-B, amphibine-F, frangufoline, and mauritine-D. These phytochemical constituents are believed to be responsible for several of the plant's pharmacological activities.

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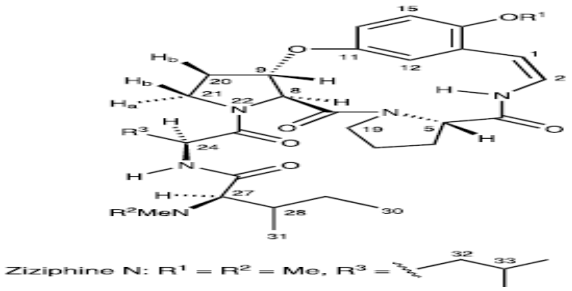


Figure 2. Structure of Zizyphine Structure

Medicinal Uses

Ziziphus oenoplia is a medicinal plant that has been used in traditional systems of medicine for many years. The plant contains cyclopeptide alkaloids known as ziziphines, which are believed to contribute to its therapeutic properties.

In India, the roots are used in Ayurvedic medicine for various ailments. The Konkani communities of Maharashtra use chewed leaves as a dressing for wounds. In Myanmar (Burma), the stem bark is traditionally used as a mouthwash for sore throat, dysentery, and inflammation of the uterus.

The fruits are edible and are used as a tonic, aphrodisiac, and remedy for fever and coryza. The roots are traditionally used as an anthelmintic agent and for the treatment of hyperacidity and epilepsy. The bark is also used in the tanning industry.

Studies have reported that extracts of Ziziphus oenoplia possess antiplasmodial activity against Plasmodium falciparum, the parasite responsible for malaria. Ethanolic extracts of the aerial parts have shown hypotensive and mild diuretic effects. Ethanolic bark extracts have demonstrated anti-inflammatory and anticholinergic activities, while

chloroform and methanolic extracts of the bark and leaves have shown antibacterial activity.

The fruits are also used as an ingredient in traditional remedies for stomach pain. A decoction prepared from the root bark is used to promote the healing of fresh wounds.

Collection and Authentication of Plant Material

Fresh leaves of Ziziphus oenoplia (L.) Mill. (Family: Rhamnaceae) were collected from the surroundings of Nalgonda, Telangana, India, during January. The plant material was identified and authenticated by Dr. P. S. Udayan, M.Sc., M.Phil., Ph.D., Senior Scientist (Taxonomy), Centre for Medicinal Plants Research (CMPR), Arya Vaidya Sala, Kottakkal.

A voucher specimen of the plant was prepared and deposited in the Department of Pharmacognosy under Voucher No. 06709 for future reference.

Extraction of Plant Material

The collected leaves were shade-dried and coarsely powdered. The powdered material was subjected to successive extraction using a Soxhlet apparatus with solvents of increasing polarity, namely benzene, ethyl acetate, and alcohol. In addition, a separate aqueous extraction was carried out using distilled water.

The major extraction process was performed using alcohol and distilled water. The extracts obtained were concentrated using a rotary vacuum evaporator to obtain a thick mass. The percentage yield of each extract was calculated separately.

Evaluation of Antibacterial Activity

The antibacterial activity of the leaf extracts of Ziziphus oenoplia was evaluated using the agar well diffusion method described by Kumar et al. Test microorganisms were obtained from IMTECH (MTCC), Chandigarh.

The bacterial strains used in the study were:

- Staphylococcus aureus (MTCC 3160)
- Bacillus subtilis (MTCC 736)
- Escherichia coli (MTCC 739)
- Klebsiella pneumoniae (MTCC 3384)

The bacterial cultures were subcultured in nutrient broth and incubated at 37°C. The inoculated nutrient agar medium was poured into sterile Petri plates and allowed to solidify. Wells of 6 mm diameter were made using a sterile cork borer.

Different concentrations of the extracts were added to the wells. Gentamycin (5 µg/mL) was used as the standard antibiotic, while water or alcohol served as the vehicle control. The plates were incubated at 37°C for 24 and 48 hours. After incubation, the diameter of the inhibition zones was measured and compared with those of the standard and control groups.

Evaluation of Anthelmintic Activity

The anthelmintic activity was evaluated according to the method described by Ajaiyeoba et al., with slight modifications. Adult Indian earthworms (Pheretima posthuma) were selected for the study because of their anatomical and physiological similarity to human intestinal roundworms.

Five different concentrations (25, 50, 100, 200, and 400 mg/mL) of the alcoholic leaf extract were prepared in distilled water. Six earthworms were placed in each treatment group. The time required for paralysis and death of the worms was recorded. Albendazole was used as the reference standard.

Results

Antibacterial Activity of Alcohol Extract of Ziziphus oenoplia Leaves

The alcoholic leaf extract of Ziziphus oenoplia exhibited significant antibacterial activity. The extract was effective against Escherichia coli, Staphylococcus aureus, and Bacillus subtilis. However, it did not show any inhibitory activity against Klebsiella pneumoniae.

These findings indicate that the alcoholic extract possesses promising antibacterial properties against both Gram-positive and selected Gram-negative bacteria.

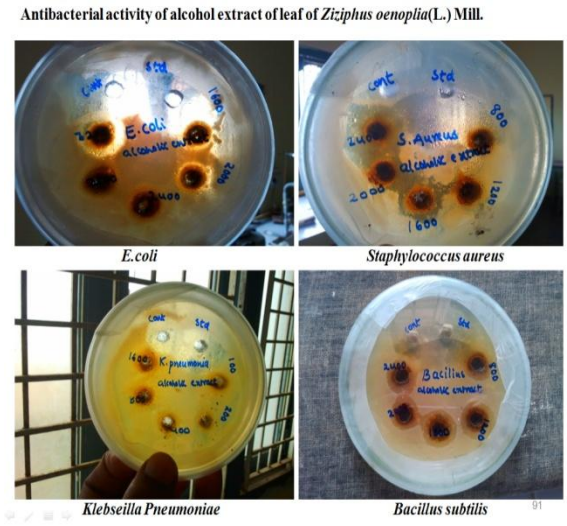


Figure 3.

Table 2.

Organism	Concentration	Ethanol extract	Water extract
	Zone of inhibition in diameter (mm) (mean±SD)		
E.coli	100 mg/ml	4.0±0.5	-
	200 mg/ml	4.5±0.2	-
	400 mg/ml	5.0±0.36	-
	800 mg/ml	6±0.8718	-
	1600 mg/ml	8±0.7211	-
	2000 mg/ml	10 ±0.1	-
	2400 mg/ml	12±0.7211	-
	2800 mg/ml	13±0.5292	-
	3200 mg/ml	14±1.217	-
	Each value is expressed as mean±SD (n=3).		

Table 3.

Organism	Concentration	Ethanol extract	Water extract
	Zone of inhibition in diameter (mm) (mean±SD)		
Staphylococcus aureus	100 mg/ml	4.0±0.5	-
	200 mg/ml	4.5±0.2	-
	400 mg/ml	5.0±0.36	-
	800 mg/ml	6±0.8718	-
	1600 mg/ml	8±0.7211	-
	Each value is expressed as mean±SD (n=3).		

Antibacterial activity of alcohol extract of leaf of *Ziziphus* against *S. aureus*

Table 4.

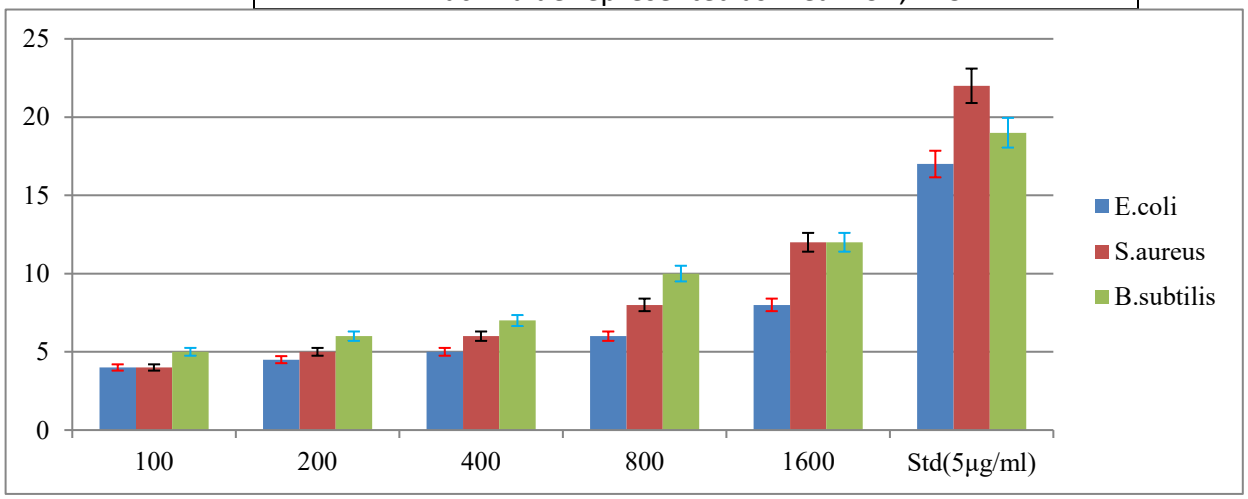
Organism	Concentration	Ethanol extract	Water extract
	Zone of inhibition in diameter (mm) (mean±SD)		
B. subtilis	100 mg/ml	4.0±0.5	-
	200 mg/ml	4.5±0.2	-
	400 mg/ml	5.0±0.36	-
	800 mg/ml	6±0.8718	-
	1600 mg/ml	8±0.7211	-
	2000 mg/ml	10 ±0.1	-
	2400 mg/ml	12±0.7211	-
	2800 mg/ml	13±0.5292	-
	Each value is expressed as mean±SD (n=3).		

Antibacterial activity of alcohol extract of leaf of *Ziziphus* against *B. subtilis*

Table 5.

Antibacterial activity of Gentamycin (standard)

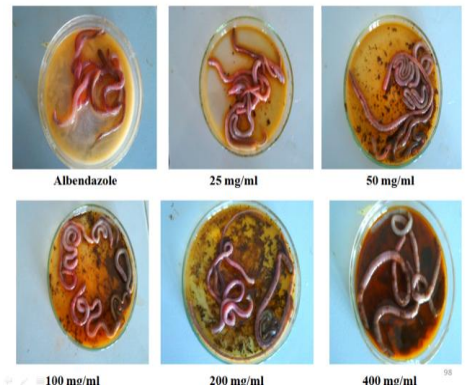
Organism	Concentration	Zone of inhibition in diameter(mm) (mean±SD) Standard : Gentamycin
S.aureus	5µg/ml	22±0.88
E.coli	5µg/ml	17±1.11
B.subtilis	5µg/ml	19±1.81
Each value represented as mean±SD, n=3		



Comparison of antibacterial activity of alcohol extract of *Ziziphus oenoplia* against different bacterial strains.

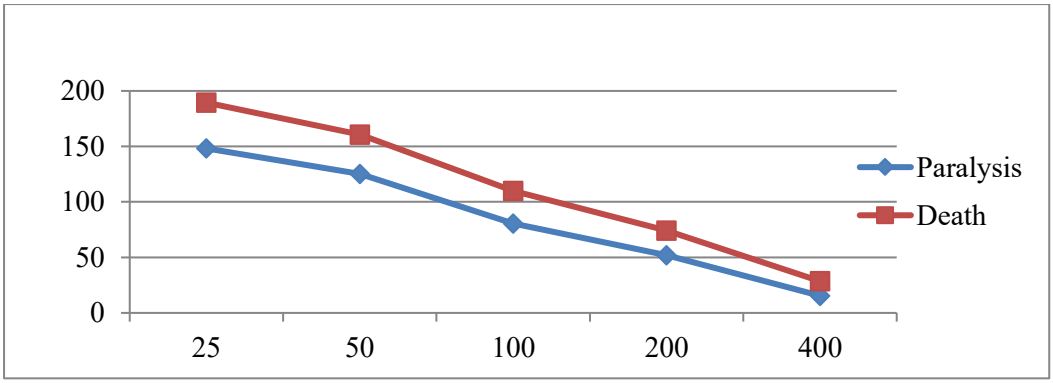
Figure 6. ANTHELMINTIC ACTIVITY of alcohol extract of leaf of *Ziziphus oenoplia* (L.) Mill. [77]

The Alcohol extract of Leaf of *Ziziphus oenoplia* (L) Mill., was an effective Anthelmintic substance. Albendazole was included as reference compound. The test results were compared with Reference compound Albendazole (10mg/ml) treated samples.



Group	Treatment of Extracts	Con mg/ml	Time taken for paralysis(min)	Time taken for death(min)
1	Experimental control	-	-	-
2	Albendazole (Reference)	10	42.47± 0.4557	69.32±0.8702
3	Ethanol	25	148.2±0.910	189.43±3.075
		50	125.1±1.86	160.56±3.273
		100	80.6±0.7550	109.8±1.952
		200	52±1.0	74.06±3.574
		400	15.42±2.468	28.56±2.512
Each value represent mean±SD, n=3.				

Figure 7. In vitro anthelmintic activity of alcohol extract of leaf of *Ziziphus oenoplia*



DISCUSSION

The alcohol extractive values of the plant were found to be higher than the water extractive values. The antibacterial activity exhibited by the alcohol extracts may be due to the presence of phenols and flavonoids. Previous studies have shown that certain synthetic phenols disrupt energy production in helminth parasites by uncoupling oxidative phosphorylation (Martin, 1997). Therefore, it is likely that the alcohol extracts may produce a similar mechanism of action.

CONCLUSION

The Antibacterial potentials of alcohol extract of leaf this species were determined using four bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* & *Bacillus subtilis*) by agar well diffusion method. Alcoholic extract of *Z.oenoplia* exhibited anthelmintic activity in dose-dependent manner giving shortest time of paralysis (P) and death (D) with 400mg/ml concentration. The alcohol and water extracts of leaves of plant of *Ziziphus oenoplia* (L) Mill., has significant antioxidant and the alcohol extract has anthelmintic activity, antibacterial potentials against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis.*

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AUTHOR CONTRIBUTIONS

K. Sudheer Kumar: Conceptualization, study design, collection and authentication of plant material, experimental work, data analysis, interpretation of results, and manuscript preparation.

Ch. Harini: Literature review, experimental assistance, data compilation, manuscript editing, critical review, and final approval of the manuscript.

Both authors have read and approved the final version of the manuscript.

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No external funding was received for this research work.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript..

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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