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In-vitro anti-denaturation and antibacterial activities of *Zizyphus oenoplia*

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Abstract

Zizyphus oenoplia mill belongs to the family Rhamnaceae. The fruits of the plant are used as stomach-ache pills and root bark decoction is used to promote the healing of fresh wounds. Plant has shown the presence of cyclopeptide alkaloids. Aerial parts of the plant were reported to have anti-inflammatory and anticholinergic activities. Bark and leaves of the plant were reported for antibacterial activity. No biological and antibacterial activities were reported on root part of *Zizyphus oenoplia.* The aim of the study was to investigate the Anti-denaturation and antibacteriial activities for various extracts of the *Zizyphus oenoplia*. Root part of *Zizyphus oenoplia* was extracted with ethyl acetate, 90% ethanol and water using soxhlet apparatus. The solvents were evaporated under reduced pressure below 40^0C using rotary evaporator to obtain dry extracts. These crude extracts were subjected to investigation for anti-denaturation study and antibacterial study by using a method designed by William's et al and agar ditch diffusion method respectively. Phytochemical analysis revealed the presence of carbohydrates, alkaloids, phenolic compounds, tannins and saponins. All the extracts of *Zizyphus oenoplia* protected Bovine Serum Albumin (BSA) from denaturation more than 97% at 1µg/ml concentration. Only ethanolic extract showed antibacterial activity against *Staphylococus aureus*.

Key words: *Zizyphus oenoplia*, Anti-denaturation, Bovine serum albumin and Antibacterial activity.

Introduction

Zizyphus oenoplia mill belonging to the family Rhamnaceae is a thorny straggling shrub found throughout the hotter parts of India, Ceylon, Tropical Asia and Australia. Chemical investigation of this plant has shown the presence of cyclopeptide alkaloids such as Zizyphine- A, B, C, D, E, Abyssinine-B and A in stem bark of the plant. These cyclopeptide alkaloids were screened as possible growth inhibitors for bacteria, fungi and at the same time no antibiotic activity was

observed [1]. The plant is also reported to contain the other cyclopeptide alkaloids like, Zizyphine (F, I, K, N, O, P), amphibine-B, Amphibine-F, frangufoline, Mauritine-D. This plant is used traditionally as a folk medicine in Thailand for its antiinfectious, antidiabetic, antidiuretic activities [2]. The root part is used for the treatment of epilepsy by traditional users [3]. Ethanolic extracts of the aerial parts of the plant exhibit hypotensive effect and low diuretic activity. Ethanolic extracts of the bark showed anti-inflammatory and anticholinergic activities [4]. Chloroform and methanolic extracts of the bark and leaves showed antibacterial activities [5]. Stem bark is used as mouth wash for sore throat, dysentery and inflammation of uterus [6]. The fruits are edible and used as one of the ingredient in the preparation of stomach-ache pills. Decoction of the root bark is used to promote the healing of fresh wounds [7, 8].

Anti-denaturation study was performed by using Bovine Serum Albumin (BSA).BSA assay seeks to eliminate the use of live specimens as far as possible in the drug developmental process. When BSA is heated, it undergoes denaturation and expresses antigens associated with type III hypersensitive reaction and which are related to diseases such as serum sickness, glomerulonephritis, rheumatoid arthritis and systemic lupus erythematosus. Thus the assay applied for the discovery of those drugs which can stabilize the protein from denaturation process. Several nonsteroidal anti-inflammatory drugs such as Indomethacin, Ibufenac, Diclofenac sodium, salicylic acid and flufenamic acid prevent denaturation of BSA at pathological pH (6.2-6.5). Extract of natural product like methanolic extract of *Boehmeria jamaicensis* (Urticaceae) and *Gliricida sepium* (Papilionaceae) prevent denaturation of BSA. Ethanolic extract of the dried green leaves of *Artocarpus altilis* protected BSA from denaturation at a concentration less than 1 µg/ml. Other compounds like phenyl propanoid and eugenol which prevent the denaturation of BSA were found to have the anti- inflammatory activity. The above stated examples are supporting the validity of the use of anti-denaturation effects induced by plant extracts in heat treated BSA, as potential therapeutic parameters for finding anti-inflammatory compound without the use of animal for preliminary pharmacological screening [9]. No previous report on biological activity and antibacterial activity of root part is available. Therefore the present study is conducted on root part of *Zizyphus oenoplia*.

Materials and Methods

Plant material

The root part of *Zizyphus oenoplia* was collected from Kottakkal of Malappuram district, kerala, India and identified by Dr.P.S.Udayan, Senior Scientist, Taxonomy division, Centre for Medicinal Plants Research, Arya Vaidyasala, Kottakkal, kerala, India. The roots were cut, air dried and ground into powder.

Extraction procedure

The root powder was extracted with solvents of increasing polarity such as ethyl acetate, ethanol 90% and water, for 24hrs with each solvent by hot extraction method using soxhlet apparatus. The extracts were decanted, filtered through Whatman filter paper No.1 and concentrated under reduced pressure below 40^0C using rotary evaporator to obtain dry extracts. The dried extracts were collected and preserved in a desiccator until used for further studies.

Phytochemical analysis

A portion of residue from each extracts was subjected to phyto-chemical analysis in order to see the presence of carbohydrates, proteins, amino acids, alkaloids, phenolic compounds, tannins and saponins according to the standard prescribed methods. [10].

***In-vitro* anti-denaturation studies**

A solution of 0.2% W/V of BSA was prepared in Tris buffer saline and pH was adjusted to 6.8 using glacial acetic acid. Stock solutions of 10,000 µg/ml of all extracts were prepared by using methanol as a solvent. From these stock solutions 3 different concentrations of 100, 200 and 500 µg/ml were prepared by using methanol as a solvent. 50 µl of each extract was transferred to Eppendorf tubes using 1ml micro pipette. 5ml of 0.2% W/V BSA was added to all the above Eppendorf tubes. The control consists of 5ml 0.2% W/V BSA solution with 50 µl methanol. The standard consists 100 µg/ml of Diclofenac Sodium in methanol with 5ml 0.2% W/V BSA solution. The test tubes were heated at 72° C for 5 minutes and then cooled for 10 minutes. The absorbance of these solutions was determined by using spectrophotometer at a wavelength of 660nm. The % inhibition of precipitation (denaturation of the protein) was determined on a % basis relative to the control using the following formula [9].

$$\% \text{ inhibition of denaturation} = \frac{(\text{Abs of control} - \text{Abs of treated})}{\text{Abs of control}} \times 100$$

***In-vitro* antibacterial activity**

Antibacterial activity of solvent extracts was determined against 2 gram +ve bacteria *Bacillus subtilis, Staphylococcus aureus* and 2 gram –ve bacteria *Escherichia Coli* and *Pseudomonas aeruginosa* by agar ditch diffusion method. The agar media along with the bacterial culture was poured into the Petri plate. After homogenization and solidification, a well was prepared in the plates with a cup-borer. 0.1ml of the extract dissolved in respective solvent was pipetted directly into the well. Control for each bacterial strain was maintained by using respective solvent. The plates were incubated for 24hrs at 37°C. The control zones were subtracted from test zones and the resulting zone diameter is considered as zone of inhibition. The extracts were compared with standard antibiotic Pencillin G at 5mg/10ml concentration [11, 12].

Results and Discussion

The color and the percentage yield of each successive extraction were shown in Table 1.

Table-1: % Yield of various extracts of *Zizyphus oenoplia*

Weight of root powder taken (g)	solvent	Color of extract	% yield
200	Ethyl acetate	Wine red	12.5
164	Ethanol	Orange red	1
138	water	Reddish brown	3

Ethyl acetate, Ethanol and water had a yield of 12%, 1% and 3% with wine red, orange red and reddish brown mass respectively. The results of photochemical analysis were shown in Table 2.

Table-2: Preliminary qualitative analysis of various extracts of *Zizyphus oenoplia*

Plant constituents	Aqueous	Ethanolic	Ethyl acetate
Carbohydrate Molish test	+	+	+
Proteins Biuret test	-	-	-
Amino acids Nin hydrin test	-	-	-
Alkoloids Mayer's test Dragendroff test Wagner's test Hager's test	+	+	+
Phenolic compounds Ferric chloride test Lead acetate test	+	+	+
Tannins	+	+	+
Saponins Foam test	+	-	+

+ indicates the presence and – indicates the absence

Ethyl acetate, ethanol and water extracts were found to contain carbohydrates, alkaloids, phenolic compounds and tannins. Ethyl acetate and water extracts showed the presence of saponins.

Table-3: Anti denaturation effect of various extracts of *Zizyphus oenoplia*

Type of Extract	Concentration (µg/ml)	% Anti denaturation
Aqueous	500	57.0 ± 0.4921
Aqueous	200	60.1 ± 0.8178
Aqueous	100	68.6 ± 0.6599
Aqueous	1	99.6 ± 0.2549
Ethanolic	500	57.4 ± 0.6018
Ethanolic	200	60.9 ± 0.6342
Ethanolic	100	68.2 ± 0.2867
Ethanolic	1	98.7 ± 0.6259
Ethyl acetate	500	48.1± 0.3265
Ethyl acetate	200	71.1 ± 0.2054
Ethyl acetate	100	77.0 ± 0.3681
Ethyl acetate	1	97.2 ± 0.4599
Diclofenac Sodium	100	97.4 ± 0.3681

All the extracts showed negative result for Biuret and Ninhydrin tests indicating the absence of proteins and amino acids respectively. The results of anti-denaturation were given in the Table 3 and Figure 1.

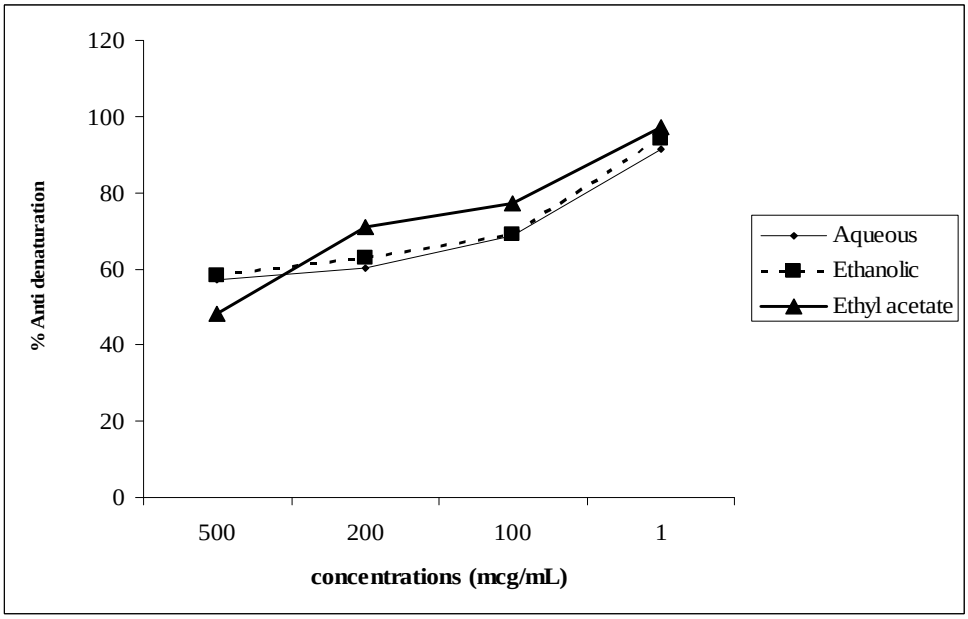

Figure-1: **Anti denaturation activity of various extracts of *Zizyphus oenoplia***

All the extracts protected the BSA against heat induced denaturation. The percentage of BSA protection against heat was increased with decreasing concentration Aqueous and ethanolic extracts of *Zizyphus oenoplia* protected the Bovine Serum Albumin against heat denaturation by 57% at 500 µg/ml.

Table-4: Antibacterial activities of various extracts of *Zizyphus oenoplia* root

Type of Extract	**Concentration (µg/ml)**	**Zone of inhibition in mm**			
		B. subtilis	*S. aureus*	*E. Coli*	*P. aeruginosa*
Aqueous	10,000	*	*	*	*
	5,000	*	*	*	*
	500	*	*	*	*
Ethanolic	10,000	*	5.1± 0.52	*	*
	5,000	*	3.2± 0.81	*	*
	500	*	2.1± 0.96	*	*
Ethyl acetate	10,000	*	*	*	*
	5,000	*	*	*	*
	500	*	*	*	*
Control (Pencillin G)	5mg/10ml	12.5± 0.96	13.2± 0.96	12.8± 0.96	14.2± 0.96

* indicates no measurable zone.

Ethyl acetate protected the Bovine Serum Albumin against heat denaturation less than by 9% when compared to other extracts at 500 µg/ml. At 200µg/ml and 100µg/ml, aqueous and ethanolic extracts showed greater percentage of protection than ethyl acetate. At 1 µg/ml, aqueous, ethanolic and ethyl acetate extracts protected BSA against heat by 99.6%, 98.7% and 97.2% respectively. All the extracts were found to have the good % of anti-denaturation at lowest concentrations. This result is coinciding with the statement given by William et. al., that the anti-denaturation action of extract is more when the concentration is less. The control Diclofenac sodium showed 97.4% of anti-denaturation effect at 100 µg/ml.

The data presented in Table 4 revealed that none of the extracts except ethanolic extract against *Staphylococcus aureus* showed antibacterial activity. Ethanolic extract of *Zizyphus oenoplia* showed 8 mm of zone of inhibition. This indicates that root part of *Zizyphus oenoplia* has minimal antibacterial activity against *S. aureus*.

Conclusion

Phytochemical analysis reveals the presence of carbohydrates, alkaloids, phenolic compounds, tannins and saponins. So the secondary metabolites of *Zizyphus oenoplia* may be responsible for the anti-denaturation effects. All the extracts of *Zizyphus oenoplia* demonstrated the anti-denaturation effect at lower concentration therefore the root extracts may possess anti-inflammatory activity. Further *in-vivo* animal experiments and phytochemical investigation are required to prove the biological activity like anti inflammatory and responsible secondary metabolite respectively.

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