

Available online on 25.12.2017 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

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Research Article

Anti-Diabetic Potential of Aqueous, Methanolic and Saponin Extract of Leaves of *Ziziphus nummlaria* Linn.

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ABSTRACT

The aqueous and methanolic extract of the leaves of *Zizyphus nummularia* were obtained from successive solvent extraction. The methanolic extract was further solvent extracted with water saturated n-butanol solvent and organic layer was acidified with 1 N KOH to obtain the raw saponin extract. Different concentrations of extracts were treated with alpha amylase enzyme in phosphate buffer (pH 6.9), their spectroscopic estimation was done at 540 nm after stopping the reaction with DNS. All the extracts have produced significant enzyme inhibition and their IC₅₀ value was observed to be 114.16 µg/ml ± 1.30 to 137.87 µg/ml ± 1.82.

Cite this article as: Dubey K, Dubey R, Gupta R, Gupta A, Anti-Diabetic Potential of Aqueous, Methanolic and Saponin Extract of Leaves of *Ziziphus nummlaria* Linn., *Journal of Drug Delivery and Therapeutics*. 2017; 7(7):173-174

INTRODUCTION:

Screening of the herbals for the safe and effective treatment of the disease is bottleneck in current research. Medicinal plants have always been integral to the traditional healthcare system all over the world to cure specific ailments. Plants of many medicinal values helps to overcome many chronic disorders and simultaneously the drawbacks of allopathic drugs such as severe side effects, cost effectiveness etc has boosted the use of herbal medicines to be an excellent remedy for diseases like cancer, diabetes, liver diseases and arthritis and due to the same the herbal drug extraction & isolation is of prime importance in present research. The specific phytochemical category based herbal extraction is widely used for the screening, identification and isolation of pharmacological active compound^{1,2,3}.

Diabetes mellitus is one of the very common chronic diseases across the world and exploring the therapeutic value of natural ingredients in such chronic disease by the researchers can be helpful in incorporating into everyday life of common people which may be an effective approach in the management of diabetic complications. This will also be helpful to decrease the socio economical burden on the middle class family of the society.

The present work is aimed for the successive extract of leaves of *Ziziphus nummularia*, family rhamnaceae which will be subjected to specific saponin extraction. The extracts were pharmacologically screened for

antidiabetic potential using alpha-amylase inhibition assay.

MATERIALS AND METHODS:

Plant Materials Leafs of *Ziziphus nummularia* were collected from Malwa region of Madhya Pradesh in the month of March-April, 2017 and were identified by the Department of Pharmacognosy, College of Pharmacy, Dr. A.P.J. Abdul Kalam University, Indore (M.P.). The leaves were later air-dried, powdered and stored in an air-tight container for further use.

Shimadzu UV 1800 UV Visible spectrophotometer, Continue soxhlet extractor and Chemicals from Sdfine, Loba Chem and HiMedia Lab were used

Preparation of Extracts

Leaves were shattered and screened with 40 no. mesh. It was soxhlet extracted three times with petroleum ether for 4hr at 60°C. After drying and levigation, one part of the residues were inverse flow extracted 10 times with 70% methanol for 4hr at 85°C, then were filtrated and the other part was extracted with distilled water for 48hr under reflux condition. The alcohol solution (Filtrate) was evaporated to dryness and dissolved with water. After filtration and discarding the extraneous components, the solution was extracted by adding water-saturated n-butanol (1:1v/v), the n-butanol phase was then treated by 1M KOH, alkaline-water phase was removed. The n-butanol phase evaporated to dryness and

the raw saponin was obtained. All extracts were screened for phytochemical analysis.

α -Amylase Inhibition Assay

Aqueous, methanolic and saponin extract of different concentrations from 80-160 μ g/ml were prepared. About 0.5 ml extract was then treated with 0.5 ml of alpha amylase (0.5 mg/ml). The solution was then incubated at 25°C for 10 minutes. About 0.5 ml of 1% starch solution in 0.02 M sodium phosphate buffer of pH 6.9 was added to all the tubes and was incubated at 25°C for 10 minutes. The reaction was stopped by adding 1.0 ml of DNS and the reaction mixture was kept in boiling water bath for 5 minutes and cooled to room temperature. The solution

was made up to 10 ml with distilled water and the absorbance was read in the UV-Visible Spectrophotometer at 540 nm against phosphate buffer as blank solution. Maltose is used as positive control.

Absorbance was calculated by using following formula

$$\alpha\text{-Amylase Inhibition Activity} = \frac{(Ac+) - (Ac-) - (As - Ab)}{(Ac+) - (Ac-)} \times 100$$

Where, Ac+, Ac-, As, Ab are defined as the absorbance of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme), a test sample (with enzyme) and a blank (a test sample without enzyme) respectively³⁻⁶.

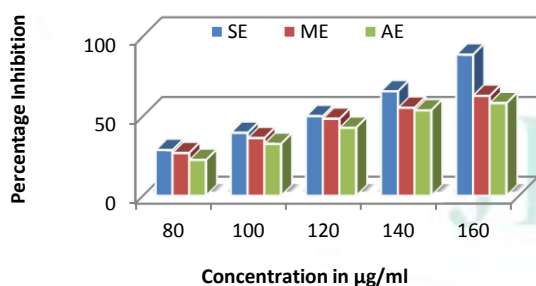
RESULTS AND DISCUSSION:

Table 1: The Percentage Alpha Amylase Inhibition of different extract of *Zizyphus nummularia* Linn.

Extract of ZNL	Percentage Inhibition					IC ₅₀
	80 μ g/ml	100 μ g/ml	120 μ g/ml	140 μ g/ml	160 μ g/ml	
AE	22.3 \pm 1.2	32.4 \pm 1.05	42.5 \pm 1.71	53.4 \pm 0.31	58.03 \pm 0.47	137.87 \pm 1.82
ME	26.6 \pm 1.6	36.6 \pm 1.80	48.2 \pm 0.55	55.06 \pm 0.75	62.5 \pm 0.80	129.65 \pm 1.10
SE	28.53 \pm 1.02	39.4 \pm 1.31	49.8 \pm 1.86	65.04 \pm 0.11	88.38 \pm 1.20	114.16 \pm 1.30

ZNL: Leaves of *Zizyphus nummularia* Linn, AE: Aqueous Extract, ME: Methanolic Extract, SE: Saponin Extract, IC₅₀: 50% Inhibitory Concentration

The aqueous, methanolic & saponin extract of *Zizyphus nummularia* Linn leaves has confirmed the antidiabetic potential via alpha amylase inhibition assay. The inhibition percentage of aqueous, methanolic & saponin extract was observed to be 20.2 -58.3%, 26.66-62.53% and 28.53-88.38 % respectively as indicated in table Table 1 & Figure 1.



SE: Saponin Extract, ME: Methanolic Extract, AE: Aqueous Extract

Figure 1: Graphical Plot of Alpha Amylase Inhibition of different extract of *Zizyphus nummularia* Linn.

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