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Anti-Diabetic and Antioxidant Potential of Saponin Extract of Leaves of *Ziziphus Mauritiana*

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ABSTRACT

Objective: The saponin extract of *Ziziphus mauritiana* was screened for antidiabetic and antioxidant potential.

Method: The methanolic extract of leaves of *Ziziphus mauritiana* was solvent extracted with water saturated n-butanol. Both the layer where separated and the organic layer was acidified with 1 N KOH to obtain the raw saponin extract. The different concentrations of saponin extract was treated with alpha amylase enzyme and 1% starch solution in phosphate buffer (pH 6.9). Spectroscopic estimation of incubated extracts was done at 540 nm after stopping the reaction with di nitro salicylic acid reagent. The antioxidant activity of extract was evaluated by reducing power assay. The saponin extract was mixed with equal volume of phosphate buffer (pH 6.9) and potassium ferricyanide (1%). The mixture was heated and treated with trichloro acetic acid (10%), which was further centrifuged and spectroscopically estimated at 700nm on the addition of freshly prepared ferric chloride solution (0.1%). The increase in absorbance as compare to standard indicates increase in reducing power.

Result: The saponin extract have produced significant alpha amylase enzyme inhibition and reducing capacity potential. The IC_{50} value was observed to be 82.12µg/ml ± 0.60 and the reducing capacity was observed to more than standard Ascorbic acid.

Conclusion: Saponin extract were found to be active towards alpha amylase inhibitory activity and elicit the reducing potential which significantly indicates their potent antioxidant activity.

Keywords: Ziziphus mauritiana, Antidiabetic activity, Antioxidant activity, Saponin extract.

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INTRODUCTION

The specific phytochemical category based herbal extraction and isolation for the safe and effective treatment of the disease is bottleneck in current research. Saponins are the one of the major bioactive phytochemical constituent found in large number of herbals. These plant derived phytomedicines have different pharmacological actions and have been the excellent remedy for the number of diseases like cancer, diabetes, hypertension, liver diseases and many more. [1-7]

Diabetes mellitus a chronic diseases across the world is a key disease for the exploring the therapeutic value of natural ingredients. The bioactive molecules are also investigated for inhibiting free radical oxidation due to chronic diseases which causes damages to the normal cells. In the present work an attempt is made for the saponin extraction of the ziziphus mauritiana leaves their evaluation for antidiabetic and antioxidant potential. [7-10]

MATERIALS AND METHODS

Plant Materials

The Leaves of Ziziphus Mauritiana[11-13] were collected, authenticated, dried, powdered and stored in an air-tight container. Shimadzu (UV 1800) UV Visible spectrophotometer, continue soxhlet extractor, borosil & Asgi glassware's and Chemicals from Sdfine, Loba Chem, HiMedia Lab were used.

Preparation of Extracts

The dried leaves were screened with 40 mesh sieve and soxhlet extracted with petroleum ether followed with 70% methanol and distilled water for 48hr under reflux condition. The methanolic extract is dried and solvent extracted by

adding water-saturated n-butanol (1:1v/v).The aqueous phase and n-butanol phase was separated and organic phase was treated with 1M KOH solution. The raw precipitates of saponins was obtained, which was removed and evaporated to dryness. The extract was screened for phytochemical analysis. [11-16]

Alpha Amylase Inhibition Assay

Different dilutions of the saponin extract in the range 50-150 µg/ml were prepared. About 0.5 ml of different concentration of the extract was treated with 0.5 ml of the enzyme alpha amylase (0.5 mg/ml). The above solution was 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 between enzyme and substrate was stopped by adding 1.0 ml of DNS reagent. The reaction mixture was then 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 recorded in the UV- Visible Spectrophotometer at 540 nm against phosphate buffer as blank solution. Acarbose is used as positive control.

Absorbance was calculated by using following formula

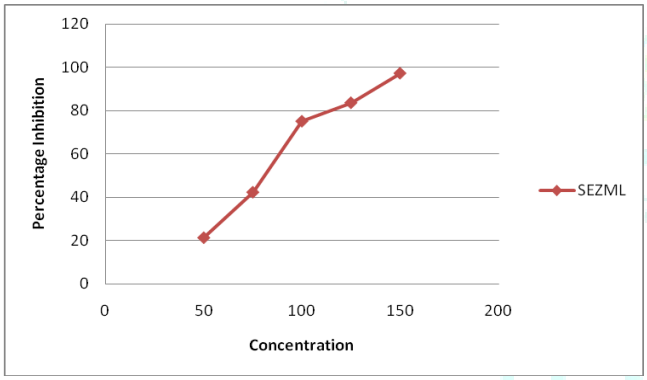
$$\alpha\text{-Amylase Inhibition Activity} = (Ac+) - (Ac-) - (As-Ab) / (Ac+) - (Ac-) \times 100$$

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) respectively. [17-22]

Table 1: Result of alpha amylase inhibition and reducing potential of extract of Zizyphus Mauritiana.

SE ZML	Concentration					IC50
	50 µg/ml	75 µg/ml	100µg/ml	125 µg/ml	150µg/ml	
Percentage inhibition of Alpha Amylase	21.12±0.433	42.20±0.36	74.95±3.73	83.47±1.34	97.09±0.93	82.1243± 1.811
Absorbance for Reducing Power Assay	0.083±0.057	0.156±0.01	0.276±0.015	0.416±0.025	0.596±0.020	-

SEZML: Saponin extract of Leaves of Zizyphus Mauritiana, IC50: 50% Inhibitory Concentration



SEZML: Saponin extract of Leaves of Zizyphus Mauritiana,

Figure 1 Alpha Amylase Inhibition Curve of Saponin Extract of *Ziziphus Mauritiana.*

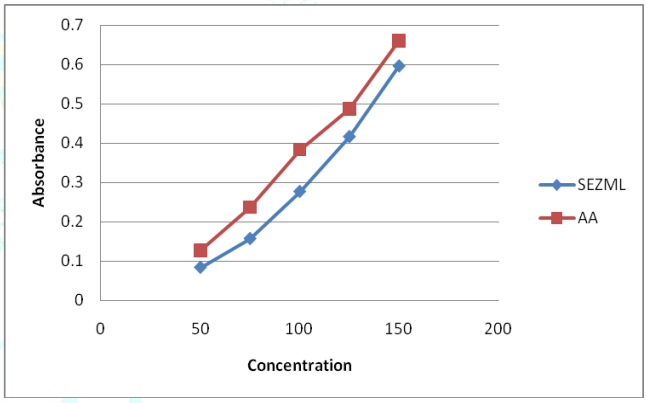


Figure 2 Reducing Potential Curve of Saponin Extract of *Ziziphus Mauritiana.*

Reducing Power Assay

The assay was performed for the evaluation of antioxidant potential of extract. About 2.5ml of different concentration of saponin extract was mixed with 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5ml of potassium ferricyanide (1%). This mixture was kept at 50ºC in water bath for 20 minutes. After cooling, 2.5 ml trichloro acetic acid (10%) was added and centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml) was taken in a test tube and mixed with 2.5 ml distilled water and 0.5 ml freshly prepared ferric chloride solution (0.1 %). The absorbance of sample was measured at 700 nm. All the tests were performed in triplicates. Ascorbic acid and Phosphate buffer (pH 6.6) was used as standard and blank, respectively. [22-24]

RESULTS AND DISCUSSION

The saponin extract of Ziziphus mauritiana leaves has confirmed the antidiabetic and antioxidant potential via alpha amylase inhibition assay and reducing capacity assay respectively. In alpha amylase inhibition assay the inhibition percentage of extract was observed between 21.2- 97.09 % as showed in Table 1 & Figure 1. The IC_{50} were observed to be 82.12 g/ml which indicates the concentration for 50% inhibition of enzyme activity. The reducing power assay was done to check the electron donating nature of the extract to reduce oxidative free radicals or intermediates. In the presence of the different concentrations of extract the yellow color of the solution is changes to light green and blue. The absorbance of same was recorded and the Table 1 and Figure 2 indicate the absorbance curve of the extract for reducing potential.

CONCLUSION

The result indicated that the extract is active towards alpha amylase inhibition activity and elicit the reducing potential which significantly indicates potent antioxidant activity.

It was concluded from the result that the extracts have antidiabetic potential and can effectively contribute for assistance in the metabolism of carbohydrates. Increased in the absorbance of the reaction mixture for reducing potential assay indicates increase in reducing power and clearly conclude the antioxidant potential of extract. In future the individual compounds from extract can be isolate and pharmacologically evaluate for providing insight of the molecular mechanism.

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