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In vitro evaluation of Aldose reductase inhibitory potential of *Bougainvillea spectabilis*

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Abstract

The dried Leaves powder of *Bougainvillea spectabilis Linn* was extracted by successive solvent extraction method to obtained hydroalcholic and aqueous extract. Water saturated n-butanol was used for further extraction of the dried hydroalcoholic extract. The layers were separated and n-butanol layer was acidified with 1 N KOH to obtain the raw saponin extract. All the extracts were screened for *invitro* aldose reductase inhibitory activity in purified goat lens using Hayman and Kinoshita method in which decrease in NADPH concentration was estimated at 340nm using UV Visible spectrophotometer. From the result it was observed that all the three extracts inhibit AR activity, but at different extent. From dose response curve it was found that saponin extract (SE) is more effective followed by aqueous extract (AE) and methanolic extract (ME) with IC_{50} values of 32.66 ±0.33 µg/ml , 73.48 ±1.13 µg/ml and 131.0 ±1.65 µg/ml respectively. In the end it was concluded that among the three extracts, saponin extract of *Bougainvillea spectabilis* is potent in inhibiting the aldose reductase enzyme which contribute major role in the diabetes complication.

Key-words: Aldose Reductase, Goat Eye Lens, NADPH, Saponin Extract, Methanolic Extract.

Introduction

Diabetes mellitus is a chronic disorder of carbohydrate, lipid and protein metabolism characterized by increase in blood glucose level in the body. It is a major risk factor of cataract which is the one of the diabetes complication. The enzyme aldose reductase is the key enzyme of the polyol pathway that reduces glucose into sorbitol with the conversion of NADPH into NADP+ due to which accumulation of polyol in lens fibers occurs this leads to cataract. The leaves of B. spectabilis are reported to have antidiabetic, hepatoprotective, antiviral, antibacterial and insecticidal properties. Reported constituents are flavonoids, betacyanine, alkaloids and tannins which are used as a medicine for variety of disorders[1].

In the present study an attempt is made for evaluation of antidiabetic potential of extracts of Leaves of *Bougainvillea Spectabilis*, family malvaceae by using aldose reductase inhibition assay.

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Material and Methods

Preparation of Extracts

The Leaves were dried and powdered and extracted in a soxhlet extractor with water and 70% methanol and distilled water. The methanolic extract was further solvent extracted with water-saturated n-butanol (1:1v/v). Separation of n-butanol phase was performed and it was treated with 1M KOH solution. The raw precipitates of saponin was obtained, which was dried and all extracts were screened for phytochemical analysis. [2]

Aldose reductase inhibitory assay

The lenses were quickly removed from goat eyeballs which were obtained from local abattoir soon after slaughtering and homogenized in cold deionized water. The homogenate was centrifuged at 10,400 RPM at 0-4°C for 15 min to remove insoluble material. To the supernatant fraction saturated ammonium sulfate solution was added to form a 40% saturation. To ensure the complete precipitation the thick suspension was allowed to stand for 15 minutes with occasional stirring then it was centrifuged and the precipitate was discarded. The above procedure was repeated and the salt saturation was increased to 50% then to 75%. The precipitate obtained was used for the enzymatic assay. For determining the aldose reductase inhibitory activity a sample cuvette was taken containing mixture of 0.3mL of enzyme

extract, 0.5mL NADPH (0.104mM), 0.75mL sodium phosphate buffer (pH 6.2, 0.1M), 0.1 ml extracts (AE, ME and SE) and 0.7 mL of deionized water. The above mixture was incubated at 30ºC for 10 min. and 0.75mL D,L-glyceraldehyde (10mM) was added to substrate and the absorbance was recorded at 340 nm for 3 minutes at 30 sec. interval. The assay was performed in triplicate. IC_{50} value and Percentage inhibitions was calculated from a dose–response curve. [3]

Results and Discussion

All the three seed extracts of *Bouaainvillea Spectabilis* were potentially inhibit goat lens aldose reductase activity to various extents with IC_{50}values ranging from 32.66 µg/mL to >100 µg/mL, as showed in Table 1. Highest percentage of inhibition was shown by Saponin extract [IC_{50} (32.66 ±0.33 µg/mL)] followed by aqueous extract [IC_{50}(73.48 ±1.13 µg/mL)] and methanolic extract [IC_{50} (131.0 ±1.65 µg/mL)]. From the result it was clearly indicated that the saponin extracts is more active than other extracts.

Table 1: Aldose reductase inhibitory activity of different extracts of *Bougainvillea spectabilis*

Extract of LBS	Percentage Inhibition						IC50
	50 µg/ml	75 µg/ml	100 µg/ml	125µg/ml	150 µg/ml	175µg/ml	
ME	19.28±0.23	26.84±1.34	37.46±0.54	48.075±0.89	59.69±1.45	65.30±0.12	131.0 ±1.65
AE	34.24±1.23	51.36±0.56	65.48±2.34	82.60±1.89	99.72±1.09	117.84±1.89	73.48 ±1.13
SE	74.75±0.23	108.62±0.45	149.5±1.89	188.37±0.67	225.25±0.21	263.12±0.87	32.66 ±0.33

LBS: Leaves of *Bougainvillea Spectabilis*. AE: Aqueous Extract, ME: Methanolic Extract, SE: Saponin Extract, IC_{50}: 50% Inhibitory Concentration

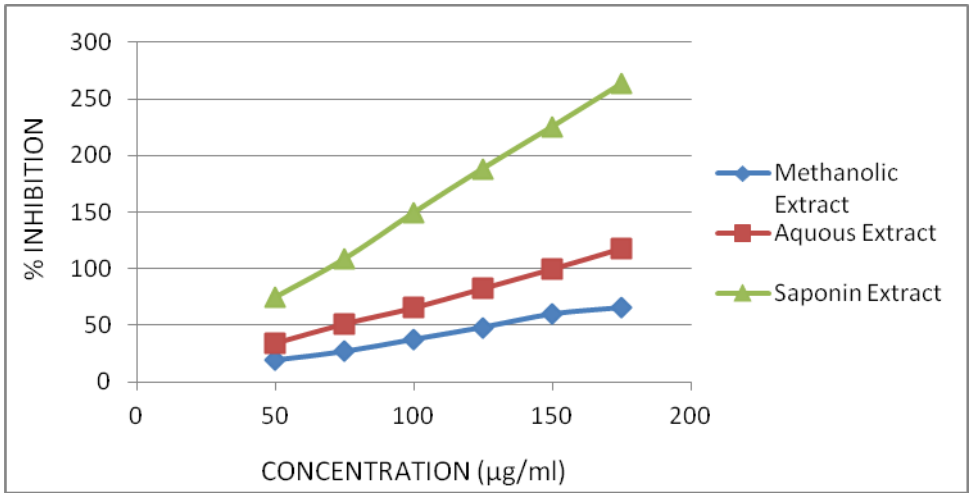

Fig. 1: Effect of extracts of *Bougainvillea spectabilis* on aldose reductase activity

Conclusion

Result of this study indicates that all the extracts of *Bougainvillea Spectabilis* Leaves showed significant aldose reductase inhibitory activity. The ARI capacity was seen to be strongest with saponin and methanolic extrct but weakest with aqueous extract. From the result it can be concluded that the plant seeds could be helpful in the treatment of cataract and can be used effectively for the management of diabeties complications.

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