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Original Article

Evaluation of *in vitro* Hypoglycemic Activity and Mechanism of Action of *Olax scandens* Roxb. Stem Bark Extracts

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ABSTRACT

Background: *Olax scandens* Roxb. (Olacaceae) is a scandent thorny shrub used by many ethnic tribes of Asia to cure stomach ache, diarrhoea, fever, cough, mouth ulcers, anaemia, filaria, joint pains, intestinal and liver diseases and diabetes. **Objectives:** The present study was undertaken to investigate the *in vitro* hypoglycemic activity and mechanism of action of *O. scandens* stem bark extracts in five different *in vitro* models at different glucose concentrations. **Materials and Methods:** Various extracts of *O. scandens* stem bark was studied for their effect on glucose adsorption, glucose diffusion, glucose uptake by yeast cells, alpha glucosidase and alpha amylase inhibition at different glucose concentrations. **Results:** In the glucose adsorption method, the butanolic extract showed statistically significant increase in glucose bound concentration of 7.35 mM/L at 100 mM glucose concentration used. All extracts showed a significant retardation in glucose diffusion across a dialysis membrane into the external medium, at different time intervals. GDRI of ethyl acetate extract was 84.471 at 30 min whereas the butanolic extract and aqueous ethanolic extract showed highest GDRI of 80.267 and 70.386 at 120 min respectively. In the method of glucose uptake by yeast cells, aqueous ethanolic and butanolic extracts both showed significantly higher uptake of glucose of 65% at 5 mg/mL concentration. The ethyl acetate extract and standard acarbose showed 26.25% and 35% inhibition of alpha glucosidase respectively at 100 µg/mL concentration. Whereas at the same concentration, ethyl acetate extract and acarbose showed 48.922% and 63.093% inhibition of alpha amylase. **Conclusion:** Significant *in vitro* hypoglycemic activity was seen in glucose diffusion method suggesting a possible role of fibre which may retard glucose release across the dialysis membrane. This mechanism can be correlated with retardation in the transport of glucose across the intestinal lumen thus causing a reduction in post prandial hyperglycemia.

Keywords: Hypoglycemia, *in vitro*, *Olax scandens*, Stem bark.

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INTRODUCTION

Herbal medicine is the oldest form of healthcare known to mankind. It owes its existence much to the painstaking labour of traditional folk healers of India and the world who were the pioneers in discovering the curative effects of several herbs.[1] Food habits and man's desire for a comfortable lifestyle have greatly influenced our health in an unfavourable manner. Consequently chronic fatigue syndrome, hypertension and diabetes have become the most common health problems.[2] Diabetes mellitus ranks highly among the top ten disorders causing mortality throughout the world. It is a chronic condition characterised by high glucose due to an absolute or relative lack of insulin.[3] At present treatment for diabetes includes oral hypoglycemic agents, insulin supplements, lifestyle modifications including diet and exercise.[4] A very large ethnobotanical and medicinal plant literature is available relating to diabetes mellitus. Compounds from such antihyperglycemic herbal medicines can be of great utility to treat diabetes mellitus or its complications once their efficacy, mechanism of action and safety are established.[3]

Olax scandens Roxb. is a member of the family Olacaceae and traditionally it is known as Dheniaani in Hindi.[5] It is a scandent thorny shrub which grows upto 5m height and is often found in the wild.[6,7] It is widely distributed across India and Asia and reported to be used by several ethnic tribes of India. Traditionally the root, flower and bark of the plant are used to treat stomach ache, diarrhoea, fever and cough.[8] Fresh young leaves are used to treat mouth ulcers,[9] warm leaf paste is used to treat sores of filaria,[10] crushed leaves along with mustard oil are used to treat joint pains.[11] The bark is used to treat anaemia in Ayurveda and as a supporting drug to treat diabetes.[12] Phytochemical analysis of various extracts of stem bark were found to possess steroid

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and/ triterpenoids and their glycosides, saponins and phenolic compounds.[13] Pharmacologically it is reported as an anti-pyretic, anti microbial, antioxidant and to prepare metallic nanoparticles for anti cancer activity.[14]

Aqueous methanolic leaf extract of Olax acuminata has been reported to show anti-hyperglycemic effects at a dose of 250 mg/kg in alloxan induced diabetic mice.[15] Hexane, ethyl acetate and acetone fractions of leaf extract of *Olax subscorpioidea* were reported to show remarkable inhibition of α-amylase and α-glucosidase which are the carbohydrate digesting enzymes.[16] There are many reports available on various species of genus *Olax* for their antihyperglycemic activity but similar reports on *O. scandens* are not available and scientifically not evaluated. To provide scientific evidence to traditional claim of *O. scandens* bark being used as a supporting drug to treat diabetes. we have attempted to carry out scientific research, to study the effect of different extracts of the stem bark of the plant on various *in vitro* hypoglycemic models.

MATERIALS AND METHODS

Collection of plant material and authentication

The aerial parts and roots of plant *O. scandens* were collected in January 2019 from Hyderabad, Telangana. A voucher specimen of *O. scandens* GPRCP/NAZ/OS/250119 is being maintained in the Department of Pharmacognosy, GPRCP, Hyderabad. Plant authentication was done by Dr. P.V. Prasanna, Scientist, Botanical Survey of India, Hyderabad, Telangana.

Extraction and fractionation

The plant stem bark was cut, air dried and grounded into a powder and extracted with 80% aqueous ethanol for 7 days by maceration. After 7 days the crude alcoholic solution was filtered and concentrated by vacuum distillation using rotary flash evaporator. The extract was concentrated, dried, weighed and stored in a dessicator.

Fractionation of the aqueous ethanolic extract

125 g of crude aqueous ethanolic extract (mother extract) was soaked in 500 mL distilled water overnight and a solution was prepared. The solution was fractionated with different solvents like chloroform, ethyl acetate, butanol in the increasing order of polarity, each time using 250 mL of the solvent (4 x 250 mL). All the fractions were filtered, concentrated and stored in air tight containers and named as AEEOS, CEOS, EAOS, BEOS for aqueous ethanolic extract, chloroform extract, ethyl acetate extract and butanolic extract respectively.

Methods to detect *in vitro* Hypoglycemic Activity

Glucose adsorption capacity

To 25 mL of each of 5,10, 20, 50 and 100 mM glucose solutions, required amount of 1 ml of 1% extract solution was added and mixed properly (250 mg of the extract in 25 mL distilled water and sonicated when necessary for proper mixing). The mixture thus obtained was then incubated on a shaker water bath maintained at 37°C for 6 hr. After 6 hr, centrifugation of mixture was done at 4000 rpm for 20 min. The glucose content in the supernatant was analysed using GOD-POD kit.[17] The glucose concentration was determined in mg/dL as below.

$$Glucose\ bound\ (mg/dl) = \frac{G1 - G6}{Weight\ of\ the\ sample}\ x\ Volume\ of\ the\ solution$$

G_1–Original concentration of glucose solution.

G_6 – Glucose concentration after 6 hr.

Glucose bound in mM/L = glucose concentration in mg/dl x 0.05551

Effect on *in vitro* glucose diffusion

Various extracts of *O. scandens* were tested for their effect on diffusion of glucose using Franz's diffusion cell. The acceptor compartment of the Franz's diffusion cell was filled with water up to the brim. 25 mL of glucose solution (20 mM) was mixed with required quantity of test extract such that it constitutes 1% concentration. A dialysis membrane was placed between the donor and acceptor compartments. Above mixture was placed in the donor compartment of the diffusion cell and the whole assembly was placed on a magnetic stirrer maintained at 37°C for 3 hr. At different time intervals (30, 60, 120 and 180 min) samples were withdrawn and glucose content was determined. A control was also performed simultaneously.[18] Glucose Dialysis Retardation Index (GDRI) value was calculated by the below formula.

$$GDRI = 100 - \frac{Glucose\ content\ with\ addition\ of\ sample\ (mg/dl)}{Glucose\ content\ of the\ control\ (mg/dl)}\ X\ 100$$

Glucose uptake by yeast cells

1 mL of different concentrations of extracts (1-5 mg/mL) was mixed with 1 mL glucose solution (10 mM) and incubated for 10 min at 37°C. The reaction was started by adding 100 µL of yeast suspension, mixed by vortexing and again incubated at 37°C for 1 hr. After incubation, the mixture was centrifuged at 2500 rpm/g for 5 min. Supernatant was collected and glucose content was determined. A control was also performed simultaneously without the addition of sample.[19,20] Percentage increase in glucose uptake by yeast cells was calculated as follows.

$$\%\ Increase\ in\ glucose\ uptake = \frac{Abs\ of\ \ control - Abs\ of\ sample}{Abs\ \ of\ control}\ x\ 100$$

Alpha glucosidase inhibition assay

Isolation of α– glucosidase enzyme was done using small intestine of the sacrificed rat, washed in a pre-cooled buffer saline. It was thoroughly cleaned, reduced to 2-3 mm small pieces, blot dried, weighed and homogenized in a homogenizer. The homogenate was then centrifuged at 5000 x g for 30 min and the supernatant obtained was collected. The supernatant was used as enzyme source and stored at very low temperature until its use. The assay was performed using 40 µL of the above tissue homogenate. It was mixed with 80 µL of test extracts (20-100 µg/mL) and incubated for 15 min at 37°C. 280 µL of maltose solution (37 mM) was added to the mixture and again incubated for 30 min. The test tubes were then placed in boiling water for 10 min to stop the reaction. Then mixture was centrifuged and the supernatant was collected to determine the glucose concentration. Absorbance was noted at 505 nm and recorded.[21] The inhibitory activity on alpha glucosidase was expressed as percentage inhibition of the enzyme.

$$\%\ Alpha\ glucosidase\ Inhibition\ = \frac{Abs\ of\ control\ -\ Abs\ of\ test}{Abs\ of\ control}\ x\ 100$$

Alpha amylase inhibition assay

Isolation of alpha amylase from wheat was done using 100 gm of malted wheat flour mixed with 200 mL of 0.2% calcium chloride solution, stirred and macerated for 2 hr at room temperature. The mixture was cold centrifuged (4°C) at 9,500 rpm for 10 min. The clear brown supernatant was collected and stored at 2-3°C. The β – amylase present in the supernatant was inactivated by heat treatment at 70°C for 15 min. Alpha - amylase is resistant to inactivation by this treatment between pH ranges of 6.5 to 8. Apart from β - amylase, other heat labile proteins present are also rendered denatured. The process was initiated by adjusting the pH of the liquid extract to 6.6 with cold 4% NH_4OH solution. Heat treatment was provided using two water baths maintained at two different temperatures of 85-90°C and 72-74°C respectively. The liquid extract was placed in a water bath maintained at 85-90°C until the temperature of the liquid rose to 69°C and then transferred to water bath maintained at 72-74°C, stirred continuously to maintain 70°C for 15 min. The solution was stored at 2-3°C until further use.[22]

250 µL of 0.02 M sodium phosphate buffer was mixed with 250 µL of isolated wheat alpha amylase solution and 250 µL of various concentrations of stem bark extracts (20-100 µg/mL) separately. The mixture was incubated for 10 min at room temperature. 250 µL of 1% w/v starch solution (substrate prepared using sodium phosphate buffer) was added and incubated further for 10 min at 37°C. The enzymatic reaction was stopped by adding 500 µL of freshly prepared DNS reagent and placed in boiling water bath for 5 min. The mixture was allowed to cool, diluted with 5 mL distilled water and absorbance was determined at 540 nm. A control (without extracts) was simultaneously performed.[23-25]

The following formula was used to calculate alpha amylase percentage inhibition:

$$\%\ Alpha\ amylase\ Inhibition\ = \frac{Abs\ of\ control\ -\ Abs\ of\ test}{Abs\ of\ control}\ x\ 100$$

RESULTS

Various extracts of *O. scandens* stem bark were investigated for their hypoglycemic activity using different *in vitro* hypoglycemic techniques including glucose adsorption capacity, effect on *in vitro* glucose diffusion, study on glucose uptake by yeast cells, alpha glucosidase and alpha amylase inhibition assays.

In the glucose adsorption method, all the tested extracts exhibited a significant glucose adsorption/binding capacity which was directly proportional to the glucose concentration used. The results are shown in Table 1 and Figure 1A. Relatively higher amounts of glucose were adsorbed with increased glucose concentration. Among all the test extracts, the butanolic extract showed the maximum and remarkable increase in capacity to bind glucose with 7.35 mM/L at 100 mM glucose concentration used. It was followed by chloroform, aqueous ethanolic extract and ethyl acetate extracts showing 4.42, 2.577 and 1.255 mM/L of glucose bound value at 100 mM concentration respectively. Further the butanolic and aqueous ethanolic extracts showed glucose binding even at low glucose concentrations used (5 mM).

Various extracts of *O. scandens* stem bark were studied for their effect on the retardation of glucose diffusion across a dialysis membrane. The results are shown in Table 2 and Figure 1B. In the study, the rate of glucose diffusion was studied at different time intervals of 30, 60, 120, 180 min across a dialysis membrane. When compared to control all extracts showed a significant reduction in glucose diffusion across the dialysis membrane into the external solution which was measured as Glucose Dialysis Retardation Index (GDRI). The GDRI value was 84.471 at 30 min for ethyl acetate extract which was significantly higher than that of the control. It was also significantly high for aqueous ethanolic and butanolic extracts at 120 min with a GDRI value of 70.386 and 80.267 in comparison to control.

In the glucose uptake method, all the stem bark extracts of *O. scandens* showed a linear increase in glucose uptake by yeast cells along with increase in glucose concentration. The results are presented in Table 3 and Figure 1C. All the extracts assisted the transport of glucose and showed different degrees of glucose uptake across the yeast cell membrane. The aqueous ethanolic and butanolic extracts showed significantly higher activity of 65% each at 5 mg/mL concentration.

In the alpha glucosidase inhibition assay, all the extracts studied showed moderate inhibition of alpha glucosidase. Results are shown in Table 4 and Figure 1D. The ethyl acetate extract showed 26.25% and standard acarbose showed 35% inhibition of alpha

Table 1: Effect of *Olax scandens* Stem Bark Extracts on Glucose Adsorption Capacity.

Extract	Concentration of Glucose solution	Glucose Bound (mg/dL)	Glucose Bound (mM/L)
AEEOS	5 mM	3.272 ± 0.085	0.181[a] ± 0.0005
	10 mM	8.68 ± 0.022	0.481[a] ± 0.001
	20 mM	21.89 ± 0.195	1.215[a] ± 0.011
	50 mM	42.522 ± 0.115	2.360[a] ± 0.006
	100 mM	46.443 ± 0.096	2.577[a] ± 0.005
BEOS	5 mM	1.666 ± 0.210	0.092[b] ± 0.012
	10 mM	2.144 ± 0.083	0.119[b] ± 0.005
	20 mM	2.883 ± 0.081	0.160[b] ± 0.005
	50 mM	24.222 ± 0.092	1.344[b] ± 0.005
	100 mM	132.415 ± 0.124	7.350[b] ± 0.007
EAOS	5 mM	0.00 ± 0.000	0.00 ± 0.000
	10 mM	8.411 ± 0.053	0.466[c] ± 0.003
	20 mM	14.301 ± 0.042	0.793[c] ± 0.002
	50 mM	19.459 ± 0.071	1.080[c] ± 0.004
	100 mM	22.615 ± 0.151	1.255[c] ± 0.008
CEOS	5 mM	0 ± 0.00	0 ± 0.000
	10 mM	1.849 ± 0.132	0.102[d] ± 0.007
	20 mM	12.016 ± 0.082	0.666[d] ± 0.005
	50 mM	21.601 ± 0.038	1.199[d] ± 0.002
	100 mM	79.639 ± 0.184	4.42[d] ± 0.010

Values are mean ± SEM of triplicate determinations, all values are significantly different from each other ($p \leq 0.0001$). Data was analysed by two way ANOVA followed by Tukey's multiple comparisons test for significant differences. AEEOS-Aqueous Ethanolic Extract of *O. scandens*, BEOS–Butanolic extract of *O. scandens*, EAOS – Ethyl acetate extract of *O. scandens*, CEOS – Chloroform extract of *O. scandens*.

Table 2: Effect of *Olax scandens* Stem Bark Extracts on Glucose Diffusion.

Extracts	Glucose content in dialysate (mM)			
	30 min	60 min	120 min	180 min
Control	0.80 ± 0.003	1.03 ± 0.001	2.46 ± 0.0006	2.27 ± 0.001
AEEOS	0.32[a] ± 0.0008 (60.04)	0.32[a] ± 0.002 (68.54)	0.73[a] ± 0.001 (70.38)	1.56[a] ± 0.001 (30.99)
BEOS	0.29[b] ± 0.003 (63.39)	0.36[b] ± 0.0003 (64.62)	0.48[b] ± 0 (80.26)	0.96[b] ± 0.002 (57.57)
EAOS	0.12[c] ± 0.001 (84.47)	0.30[c] ± 0.0003 (70.39)	0.53[c] ± 0.0003 (43.36)	1.12[c] ± 0.001 (50.49)
CEOS	0.50[d] ± 0.0005 (36.58)	0.35[d] ± 0.002 (65.51)	1.39[d] ± 0.001 (62.77)	1.65[d] ± 0.010 (27.24)

Values in parenthesis indicate glucose dialysis retardation index (GDRI). Data of all determinations was carried out by two way ANOVA followed by Dunnett's multiple comparisons test for significant differences. Values are mean ± SEM of triplicate determination. ($p \leq 0.001$) Values are significantly different in comparison with the control.

glucosidase at 100 µg/mL concentration each with an IC_{50} value of 2.23 µg/mL and 1.86 µg/mL respectively.

In the alpha amylase inhibition assay, the plant extracts showed moderate inhibition of alpha amylase whereas the ethyl acetate extract showed inhibition almost similar to standard acarbose as shown in Table 5 and Figure 1E. The ethyl acetate extract showed 48.922% inhibition at 100 µg/mL concentration and IC_{50} value of 97.253 µg/mL whereas the standard acarbose showed 63.093% inhibition at the same concentration and IC_{50} value of 81.98 µg/mL respectively.

DISCUSSION

Olax scandens Roxb. is a scandent thorny shrub widely distributed across India and traditionally used by several ethnic tribes for its ethnomedical uses. The bark is used in Ayurveda to treat anaemia and as a supporting drug to treat diabetes. Several reports are available on different species of the genus *Olax* for their antihyperglycemic properties, but similar research has not been investigated for the plant *O. scandens*. Hence an attempt was made to scientifically investigate the traditional use of *O. scandens* as a supporting drug to treat diabetes. Five different *in*

Table 3: Effect of *Olax scandens* Stem Bark Extracts on Percentage Increase in Glucose Uptake by Yeast Cells.

Extracts	% Increase in Glucose Uptake by Yeast Cells				
	1 (mg/mL)	2 (mg/mL)	3 (mg/mL)	4 (mg/mL)	5 (mg/mL)
AEEOS	$17.58^{a} \pm 0.126$	$28.94^{a} \pm 0.173$	$35.54^{a} \pm 0.464$	$49.13^{a} \pm 0.167$	$65.60^{a} \pm 0.168$
BEOS	$15.17^{b} \pm 0.144$	$23.26^{b} \pm 0.168$	$29.04^{b} \pm 0.219$	$58.95^{b} \pm 0.143$	$65.70^{b} \pm 0.210$
EAOS	$4.04^{c} \pm 0.220$	$9.24^{c} \pm 0.220$	$14.30^{c} \pm 0.144$	$25.57^{c} \pm 0.083$	$28.38^{c} \pm 1.08$
CEOS	$13.58^{d} \pm 0.220$	$16.90^{d} \pm 0.220$	$17.19^{d} \pm 0.083$	$17.91^{d} \pm 0.144$	$18.97^{d} \pm 0.752$

Values are mean ± SEM for triplicate determination; Data was analysed by two way ANOVA followed by Tukey's multiple comparisons test for significant differences. ($p \leq 0.0001$).

Table 4: Effect of *Olax scandens* Stem Bark Extracts on Alpha Glucosidase Inhibition.

Extracts	Concentration (µg/mL)							IC_{50} (µg/mL)
	5	10	20	40	60	80	100	
Acarbose	$18.95^{a} \pm 0.751$	$21.25^{a} \pm 0.721$	$22.5^{a} \pm 1.653$	$27.08^{a} \pm 0.751$	$29.58^{a} \pm 0.416$	$32.5^{a} \pm 0.360$	$35.0^{a} \pm 0.360$	1.86
AEEOS	$6.25^{b} \pm 0.721$	$7.5^{b} \pm 0.360$	$8.75^{b} \pm 0.360$	$10.20^{b} \pm 0.908$	$10.62^{b} \pm 1.082$	$11.87^{b} \pm 0.721$	$12.5^{b} \pm 1.443$	7.12
BEOS	$10.0^{c} \pm 1.08$	$10.62^{c} \pm 1.082$	$14.37^{c} \pm 0.721$	$16.87^{c} \pm 0.360$	$18.12^{c} \pm 0.721$	$18.75^{c} \pm 0.721$	$19.37^{c} \pm 0.721$	3.97
EAOS	$8.75^{d} \pm 1.443$	$10.0^{d} \pm 0.721$	$13.12^{d} \pm 0.360$	$16.25^{d} \pm 0.360$	$20.62^{d} \pm 0.721$	$23.75^{d} \pm 1.082$	$26.25^{d} \pm 0.082$	2.23
CEOS	$10.0^{e} \pm 0.360$	$11.25^{e} \pm 0.721$	$13.12^{e} \pm 1.082$	$14.37^{e} \pm 1.082$	$15.0^{e} \pm 1.443$	$15.62^{e} \pm 0.721$	$17.5^{e} \pm 0.721$	5.76

Values are mean ± SEM for triplicate determination; Data was analysed by two way ANOVA followed by Dunnett's multiple comparisons test for significant differences. All values are significantly different in comparison to standard Acarbose. ($p \leq 0.0001$).

Table 5: Effect of *Olax scandens* Stem Bark Extracts on Alpha-Amylase Inhibition.

Extract	Concentration (µg/mL)							IC_{50} (µg/mL)
	5	10	20	40	60	80	100	
Acarbose	$4.21^{a} \pm 1.5$	$16.12^{a} \pm 0.578$	$18.40^{a} \pm 3.554$	$26.60^{a} \pm 1.461$	$31.29^{a} \pm 2.639$	$49.17^{a} \pm 1.131$	$63.09^{a} \pm 1.891$	81.98
AEEOS	$3.90^{a} \pm 1.912$	$7.70^{b} \pm 1.457$	$12.73^{b} \pm 0.413$	$15.92^{b} \pm 1.409$	$19.58^{b} \pm 1.206$	$25.91^{b} \pm 1.358$	$33.37^{b} \pm 0.807$	163.52
BEOS	$2.56^{a} \pm 0.988$	$5.58^{c} \pm 1.338$	$7.00^{c} \pm 0.741$	$14.57^{c} \pm 1.380$	$19.15^{c} \pm 0.995$	$20.24^{c} \pm 0.956$	$23.45^{c} \pm 0.739$	213.10
EAOS	$3.82^{a} \pm 1.581$	$12.64^{a} \pm 0.972$	$20.14^{a} \pm 1.213$	$26.57^{a} \pm 1.367$	$33.37^{a} \pm 1.176$	$43.82^{a} \pm 1.533$	$48.92^{a} \pm 1.701$	97.25
CEOS	$3.27^{a} \pm 2.134$	$6.69^{d} \pm 2.001$	$11.03^{d} \pm 1.545$	$17.98^{d} \pm 1.407$	$21.91^{d} \pm 1.148$	$27.80^{d} \pm 2.131$	$35.53^{d} \pm 0.796$	146.56

Values are mean ± SEM for triplicate determination; values bearing the same superscript in comparison to Acarbose are not statistically different ($p \geq 0.05$). Data analysis was done by two way ANOVA followed by Dunnett's multiple comparison's test.

vitro hypoglycemic models were studied to evaluate the *in vitro* hypoglycemic activity and the possible mechanism of action of the stem bark extracts of *O. scandens*.

Glucose adsorption or glucose binding capacity is one of the methods to determine the *in vitro* hypoglycemic activity by studying the extent of glucose adsorption by the extract. The butanolic extract showed maximum capacity to bind glucose with 7.35 mM/L at 100mM glucose concentration. The adsorption capacity may be due to fibers, soluble and insoluble constituents present in the extracts which are reported to adsorb glucose.[26] These fibres adsorb glucose and prevent its transport across the intestinal lumen thus causing reduced post prandial hyperglycemia.

In the glucose diffusion model, effects of various stem bark extracts on glucose diffusion across a dialysis membrane were measured. Glucose Dialysis Retardation Index (GDRI) is an

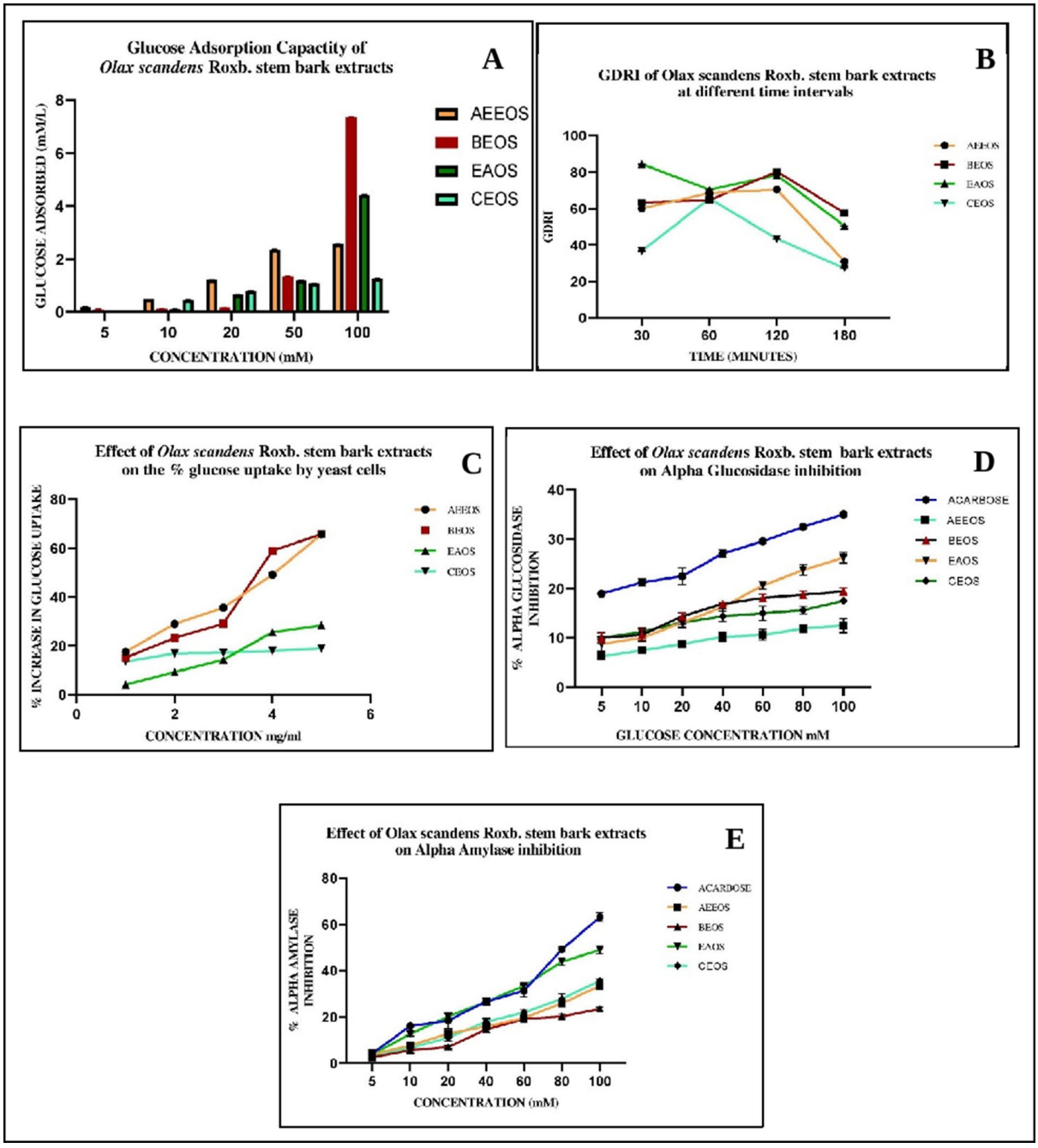

Figure 1: AEEOS-Aqueous Ethanolic Extract of *O. scandens*, BEOS–Butanolic extract of *O. scandens*, EAOS – Ethyl acetate extract of *O. scandens*, CEOS – Chloroform extract of *O. scandens*, GDRI – Glucose Dialysis Retardation Index, [A] Glucose adsorption capacity, [B] GDRI values – *In vitro* Glucose Diffusion, [C] Glucose uptake by yeast cells, [D] % inhibition of alpha glucosidase, [E] %inhibition of alpha amylase.

in vitro index used to calculate extent of retardation of glucose diffusion into the external medium. It is useful to determine the effect of fiber on extent of delay in glucose diffusion in the GI tract.[27] Higher the GDRI, higher is the retardation index of glucose diffusion by the sample. The ethyl acetate extract showed the maximum GDRI of 84.471% at 30 min whereas the aqueous ethanolic and butanolic extracts showed maximum GDRI of 70.386 and 80.267% respectively at 120 min.

Glucose transport is extremely complex in yeast (Sachharomyces cerevisiae) cells and carried out by a facilitated diffusion process. Studies have suggested that the transport of sugar across the cell membrane of yeast is mediated through stereospecific membrane carriers which facilitate the diffusion process of non-metabolizable sugars and metabolizable glycosides.[28,29] Specific carriers facilitate the transport of solutes down the concentration gradient indicating that glucose transport across the yeast cells can occur only if intracellular glucose is effectively utilised.[30] In this method, all the extracts assisted the transport of glucose and

showed different degrees of glucose uptake across the yeast cell membrane suggesting that they are capable of enhancing effective utilisation of glucose by the yeast cells intracellularly. After a specific time, the amount of glucose left in the medium indicated the amount of glucose uptake by yeast cells. The aqueous ethanolic and butanolic extracts showed significantly higher glucose uptake of 65% at 5 mg/mL concentration of glucose.

Inhibition of carbohydrate digesting enzymes such as alpha amylase and alpha glucosidase is one of the mechanisms to cure diabetes mellitus. Alpha glucosidase inhibitors delay the breakdown of carbohydrates in the small intestine thus decreasing the amount of glucose available for transport across the intestinal membrane.[31] These inhibitors detected in plants show a strong alpha glucosidase inhibition which can be used as an effective therapy for reduction in post prandial hyperglycemia with minimal side effects.[32] In the present study, all the extracts showed moderate inhibition of alpha glucosidase. The ethyl acetate extract showed 26.25% inhibition with an IC_{50} value of 2.23 µg/mL whereas the standard acarbose showed 35% inhibition with an IC_{50} value of 1.86 µg/mL. In the alpha amylase inhibition assay, the ethyl acetate extract showed 48.922% inhibition of alpha amylase which was nearer to the inhibition value of standard acarbose at 63.093%. Previous reports suggest that mild alpha amylase inhibition is desirable as excessive inhibition can increase the amount of undigested carbohydrates in the colon which can undergo bacterial fermentation abnormally.[33]

With the help of various *in vitro* antidiabetic techniques, we can determine the mechanism of action through which plant extracts show hypoglycemic potential. In this investigation all the *O. scandens* stem bark extracts showed hypoglycemic effect mediated by different mechanisms like increased glucose adsorption, decreased rate of glucose diffusion, promotion of glucose transport across the cell membrane and inhibition of carbohydrate digesting enzymes alpha glucosidase and alpha amylase. Among all the methods, the extracts showed maximum activity by decreasing the rate of glucose diffusion across the dialysis membrane. This mechanism can be correlated with retardation in the transport of glucose across the intestinal lumen and hence causing a reduction in post prandial hyperglycemia.

In our preliminary phytochemical investigation, it was observed that various extracts of stem bark were found to possess steroids and/ triterpenoids and their glycosides, saponins and phenolic compounds. The presence of a triterpenoid acid known as oleanolic acid is reported to possess antidiabetic activity.[34] In our phytochemical analysis, we found the presence of a triterpenoid acid named Ursolic acid in the stem bark which is the isomer of oleanolic acid which maybe the phytoconstituent responsible for its antidiabetic activity.[35] Further during our phytochemical investigation for crude fibre content of various parts of *O. scandens* including leaf, root, stem and stem bark, the stem bark was found to be rich in crude fibre at 23%. The presence of fibres may also be responsible for its hypoglycemic activity. However, a detailed scientific evaluation is required. This is our attempt to justify the use of *O. scandens* in the treatment of diabetes based on traditional claims.

CONCLUSION

O. sandens has been reported traditionally to be used as a supporting drug in the treatment of diabetes. To establish its use in the treatment of diabetes and justify the traditional claim, the present study has been undertaken using five different *in vitro* hypoglycemic models. Various extracts of the stem bark showed hypoglycemic activity in all the models studied with significant action seen in the glucose diffusion model. Presence of fibres, soluble and insoluble constituents in the extracts has been attributed to their hypoglycemic activity. There is a need to further explore this plant for its antidiabetic activity. To strengthen its use in the treatment of diabetes and to justify the traditional claims, further research can be carried out in various *in vivo* experimental diabetic models and cell studies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

***O. scandens*:** *Olax scandens*; **GPRCP:** G. Pulla Reddy College of Pharmacy; **GDRI:** Glucose Dialysis Retardation Index; **AEEOS:** Aqueous Ethanolic Extract of *O. scandens*; **BEOS:** Butanolic extract of *O. scandens*; **EAOS:** Ethyl acetate extract of *O. scandens*; **CEOS:** Chloroform extract of *O. scandens*.

SUMMARY

Various extracts of the stem bark of *O. scandens* were evaluated for their effect on *in vitro* hypoglycemic activity in different models including effect on glucose adsorption, glucose diffusion, glucose uptake by yeast cells, inhibition of carbohydrate digesting enzymes like alpha glucosidase and alpha amylase. The extracts showed hypoglycemic action in all models and significant activity was seen in the glucose diffusion model. The butanolic extract showed a significant increase in glucose adsorption. The aqueous ethanolic and butanolic extract showed a significant increase in glucose uptake by yeast cells and a significant retardation in the diffusion of glucose across a dialysis membrane at 120 minutes. Moderate inhibition of alpha glucosidase and alpha amylase was shown by the ethyl acetate extract.

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