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In vitro α-Amylase, α-Glucosidase and β-Galactosidase Inhibitory Activity of the Extract and Solvent Fractions of *Blumea oblique* (L.) Druce.

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Abstract

OBJECTIVE: To investigate the therapeutic effects of *Blumea oblique* methanolic extract and its fractions for α-amylase, α-glucosidase and β-Galactosidase inhibitory activity by *in vitro* assays. **METHODS**: Aerial parts of *Blumea oblique* were extracted with methanol by maceration. And its fractions were prepared by dispersing uniformly in distilled water and subjected to solvent fractionation with toluene, ethyl acetate and butan-2-one. The fractions were concentrated under reduced pressure. The inhibitory effect of these BO extract and its fractions on α-amylase, α-glucosidase and β-galactosidase enzymes was determined by *in vitro* assays. **RESULTS**: The results revealed that the methanolic extract and its fractions of *Blumea oblique* were inhibited α-amylase, α-glucosidase and β-galactosidase enzymes in a dose dependent manner. Among all these fractions, ethyl acetate fraction of *Blumea oblique* has shown the prominent α-amylase, α-glucosidase and β-galactosidase enzyme inhibitory activity with IC_{50} values of 5.74±0.18, 6.16±0.27 and 11.81±0.13mg/ml and it was well comparable with standard IC_{50} value 5.25±0.41, 5.39±0.28 and 10.72±0.23mg/ml respectively. And Phytochemical reports of *Blumea oblique* revealed that it contains alkaloids, carbohydrates, steroids, saponins, flavonoids and phenolic compounds. These results substantiate the use of *Blumea oblique* as medicine for the treatment of diabetes by controlling postprandial hyperglycemia. **CONCLUSION:** The results suggest that ethyl acetate fraction of aerial parts of *Blumea oblique* shown significant α-amylase, α-glucosidase and β-galactosidase enzyme inhibitory activity, which may be helpful to reduce the postprandial blood glucose levels. However, the chemical compounds responsible for the inhibitory action of these enzymes need to be further identified and characterized. This may be useful for the development of new antidiabetic agents from plant sources.

Keywords
α-amylase, α-glucosidase and β-galactocidase, *Blumea oblique*

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1. INTRODUCTION:

Blumea oblique (L.) Druce, commonly called as Obliqua Blumea [Synonym: *Erigonon obliquus* L.] belongs to family *Asteraceace* (Compositae), native to tropical America grows in sandy or clayey areas especially near the sea in dry regions of India and Pakistan [1].

Many plants of genus Blumea are used in folk medicine for the treatment of various ailments, such as bronchitis, asthma, influenza and also remedial in

malaria [2]. It is occasionally used as sudorific and juice of its leaves is considered to be insect repellent [3].

Description of the plant: *Blumea oblique* (BO) is an annual erect or woody, deep-rooted, villous, a herb, growns up to 75cm tall. Leaves are sessile, base sub-amplexicaul, spiral, oblong or obovate; lower dentate, the upper often entire, alternate. Heads terminal, disciform, solitary or two, heterogamous. Ray florets pink. Disc florets yellowish. Achenes oblong, hairy, pappus creamy. And bear flowers and fruits in the month of August to January [4].

The phytochemical study of the hexane soluble fraction of the methanolic extract of *Blumea oblique* which led to the isolation of six acetylenic thiophenes [5]. Acetylenic thiophenes have been found to possess very interesting biological activities. They have been found to show nematocidal, insecticidal, antibacterial, and antiviral activity. And two new bioactive bithienylacetylenes isolated from *Blumea oblique* showed antifungal activity against *Epidermophyton floccosum* and *Pleurotus ostreatus* [1,6].

Acetylenic thiophenes have shown very good antimicrobial activity against Candida albicans and Staphylococcus albus only when irradiated with UV-A light. Thiophenic compounds having 1-alkynyl groups linked to a thiophene in the α-position, or to a 2,2'-bithienyl system in the 5-position have exhibited the most potent UV-mediated antibiotic activity. These compounds were also found to possess photoactive antiviral and cytotoxic activities. A naturally occurring α-terthiophene, has shown anti-HIV activity on irradiation with UV- A light [6].

Certain thienopyrimidine derivatives exhibit antidiabetic, antiallergic, antibacterial, antidepressant, analgesic, and anti-inflammatory activities [7]. Hence, the present study is focused on the antidiabetic activity of the BO by *in vitro* studies.

Diabetes Mellitus is the world's fastest growing metabolic endocrine disorder with compromised carbohydrate and lipid metabolism. The imperfect metabolism can be attributed to the impaired insulin secretion, insulin action or both. India has an estimated 77million people with diabetes, which makes it the second most affected in the world, after china. One in six people (17%) in the world with diabetes is from India. The number is projected to grow by 2045 to become 134million per the international diabetes federation (IDF)[8]. Traditionally, a number of plants have been used in the management of diabetes and only few of them have been proven scientifically [9].

2. MATERIALS AND MATERIALS:

2.1. Collection of Plant material:

For the present investigation, the plant *Blumea oblique* was collected from Station Ghanpur, after proper identification by an expert taxonomist, Dr.V.S. Raju, Department of Botany, Kakatiya University, Warangal. And a specimen of the plant was deposited in the Department of Pharmacognosy, University College of Pharmaceutical Sciences, Warangal, India.

The collected plant material was thoroughly checked for foreign organic matter and removed. For preparation of crude drug powder, aerial parts of plant were taken separately and dried under shade. The completely shade dried leaves were powdered and extracted with methanol by maceration in a round bottom flask at room temperature. Then methanolic extract of BO (MEBO) was dispersed uniformly in distilled water and subjected to solvent fractionation with toluene (TLBO), ethylacetate (EABO), and butan-2-one (BNBO). The fractions were concentrated under reduced pressure (by Rotavapour) and phytochemical investigations were done.

2.2. Drugs, chemicals, and solvents:

DPPH (1,1-Diphenyl-2-picryl-hydrazyl), α-amylase(porcine pancreas), α-glucosidase (*Saccharomyces cervisiae*) and β-galactosidase (E.coli), P-nitro phenyl β-D-glucopyranoside (PNPG), O-nitrophenyl-β-galactosidase (ONPG), 3,5- Dinitro salicylic acid, Acarbose, Quercetin, Sodium potassium tartarate, Sodium dihydrogen phosphate, Potassium dihydrogen phosphate, Di sodium hydrogen phosphate and Di potassium hydrogen phosphate, Dimethyl sulfoxide, Sodium nitrate, Aluminium chloride, reagents and buffers were procured from Gamut Scientifics (SRL), Secunderabad, Telangana, India. All other chemicals and solvents used are of analytical grade.

2.3. Phytochemical screening:

The dried extract and fractions of *Blumea oblique were* subjected to phytochemical screening for the presence of different classes of organic compounds like alkaloids, carbohydrates, steroids, saponins, flavonoids, tannins, steroids, proteins and phenolic compounds according to standard methods [10]. Any change of colours or the precipitate formation was used as indicative of positive response to these tests.

2.4. *In vitro* antidiabetic activity

2.4.1. α-Amylase Enzyme Inhibitory Assay:

This assay was carried out by using a modified procedure of McCue and Shetty [13]. Stock solution of BO extract and its fractions were prepared by dissolving up to 10mg of each extract in 1ml of

DMSO. A total of 250µL of extracts (1.25-10mg/mL) was placed in a tube and 250µL of 0.02M sodium phosphate buffer (pH6.9) containing α-amylase solution (0.5mg/ml) was added. This solution was pre-incubated at 25°C for 10min, after which 250µL of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at time intervals and then further incubated at 25°C for 10min. The reaction was terminated by adding 500µL of Dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5min and cooled to room temperature. The reaction mixture was diluted with 5mL distilled water, and the absorbance was measured at 540nm using ELICO SL159 UV-Visible Spectrophotometer. The assay was performed in triplicates. Acarbose was used a standard and the inhibitory activity of α-amylase were calculated by using the following formula.

%Inhibitory = {[Absorbance (control) - Absorbance (sample)] /Absorbance (control)}X100.

A control was prepared using the same procedure replacing the extract with distilled water.

2.4.2 α-Glucosidase Enzyme Inhibitory assay:

The alpha-glucosidase inhibitory activity was determined according to the method described by Apostolidis with some modifications [14]. Stock solutions of extracts were prepared by dissolving up to 10mg of each extract in 1ml of DMSO. A total of 50µL of extracts (1.25-10mg/mL) and 100µL of yeast alpha glucosidase solution in phosphate buffer (pH6.9) were incubated at 25°C for 10minutes followed by the addition of 50µL of 5Mmol/L P-nitrophenyl-β-D-glucopyranoside solution in 0.1M phosphate buffer (pH 6.9). The reaction mixture was then incubated at 25°C for 5min and the reaction was terminated by adding 3mL of 100Mm sodium carbonate solution into the mixture and absorbance of liberated p-nitrophenol was observed at 405nm using ELICO SL159 UV-Visible Spectrophotometer. The assay was performed in triplicates. Acarbose was used a standard and the inhibitory activity of α-glucosidase were calculated by using the following formula.

%Inhibition=(Absorbance(control)–Absorbance(extract))/Absorbance (control) X100.

2.4.3. β-Galactosidase Inhibitory Assay:

β-galactosidase solution was adjusted to 0.2 U/mL by 0.1M phosphate buffer (pH 7.3). 200µl of enzyme solution, 100µl of different concentrations of BO extract/fractions and 2.4mL of 0.1M phosphate buffer (pH 7.3) were mixed and incubated at 37°C for 15 min. After pre-incubation, 100µl of 30Mm, 2-NitroPhenyl *β*-D-galactopyranozide (ONPG) solution in 0.1M phosphate buffer (pH 7.3) was added and incubated at 37°C for 30 min. The reaction was terminated by 2mL of 0.2M sodium carbonate solution. The absorbance of the mixture solution was measured by at spectrophotometer at 410 nm. The assay was performed in triplicates. The %inhibitory of BO extract and its fractions were calculated according to the following equation:

%Inhibitory = {[Absorbance (control) - Absorbance (sample)] /Absorbance (control)}X100

The IC_{50} values defined as the concentration of the extract that inhibited 50% of the enzyme activity were determined from plots of % inhibition versus log inhibitor concentration and calculated by logarithmic regression analysis from the mean inhibitory values [15].

3. RESULTS:

3.1. Preliminary phytochemical screening:

Table 1: Preliminary Phytochemical screening of methanolic leaf extract and its fractions of *B.oblique*.

S.No	Phytoconstituents	MEBO	DIFFERENT FRACTIONS OF EXTRACT		
			TLBO	EABO	BNBO
1	Alkaloids	+ve	+ve	+ve	+ve
2	Carbohydrates	+ve	+ve	+ve	+ve
3	Steroids/triterpenoids	+ve	+ve	+ve	+ve
4	Saponins	+ve	+ve	+ve	+ve
5	Flavonoids	+ve	+ve	+ve	+ve
6	Proteins & Amino acids	-ve	-ve	-ve	-ve
7	Tannins/phenolic compounds	+ve	+ve	+ve	+ve

+; Present, -; Absent.

3.2. α-Amylase Enzyme Inhibitory Assay:

Aerial parts of BO Methanolic extract and its fractions inhibited the catalysis of α-amylase at 1.25, 2.5, 5, 10 mg/ml concentration and the percentage inhibition were dose-dependent. The percentage inhibition of MEBO ranging from 8.51 to 65.76% with

IC_{50} value 7.05±0.32mg/ml, TLBO ranging from 10.73 to 72.90% with IC_{50} value 6.64±0.13mg/ml, EABO shown percentage inhibition ranging from 11.34 to 86.7% with IC_{50} value 5.74±0.18mg/ml and BNBO has shown percentage inhibition ranging from 12.83 to 78.66% with IC_{50} value 5.94±0.22mg/ml. Among all the test samples, *Blumea oblique* ethyl acetate fraction has shown remarkable α-amylase enzyme inhibition i.e., 86.7% at 10mg/ml concentration, and it was well comparable with the standard drug acarbose 14.37 to 92.46% inhibition at 10mg/ml concentration with IC_{50} value 5.25±0.41 mg/ml.

α-Amylase enzyme Inhibitory of BO methanolic extract, its fractions and standard (acarbose) were shown in table 2 and figure 1.

Table 2: α-Amylase inhibitory activity and IC_{50} values by *BO* methanolic extract fractions and Acarbose

Test sample	Concentration (mg/ml)	% Inhibition	IC_{50}
MEBO	1.25	8.51±0.30	7.05±0.32
	2.5	21.83±0.16	
	5	42.84±0.22	
	10	65.76±0.43	
TLBO	1.25	10.73±0.51	6.64±0.13
	2.5	21.31±0.24	
	5	40.82±0.16	
	10	72.90±0.37	
EABO	1.25	11.34±0.64	5.74±0.18
	2.5	22.52±0.53	
	5	41.25±0.18	
	10	86.71±0.26	
BNBO	1.25	12.83±0.25	5.94±0.22
	2.5	26.80±0.14	
	5	49.51±0.62	
	10	78.66±0.54	
Acarbose	1.25	14.37±0.32	5.25±0.41
	2.5	28.41±0.28	
	5	50.32±0.40	
	10	92.46±0.56	

Data expressed as mean ± SD, (n=3);

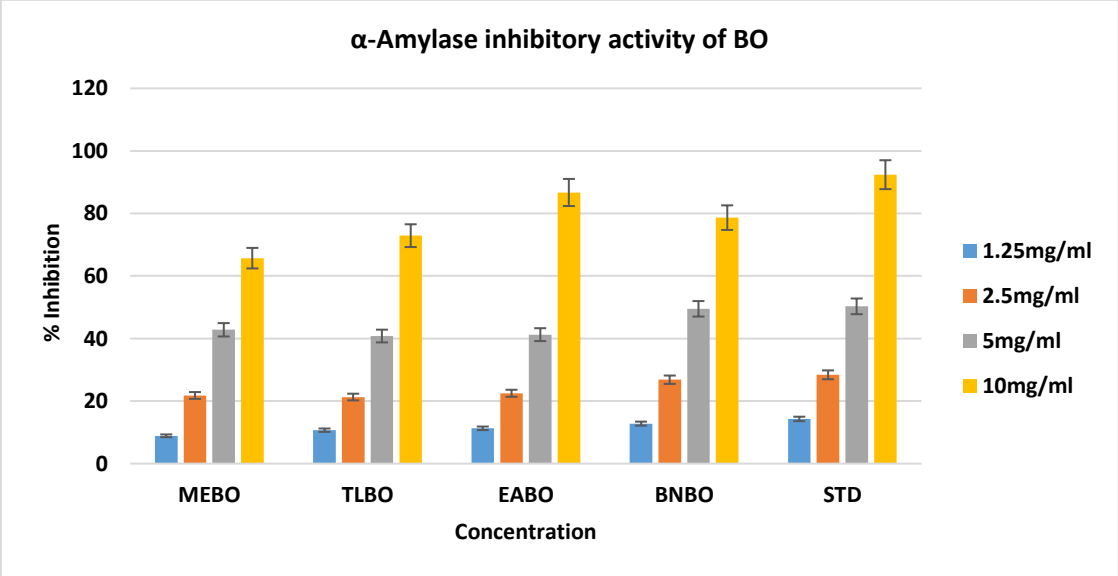

Figure 1: Effect of methanolic extract and different fractions of BO and STD (Acarbose) on α-amylase enzyme inhibition. Data expressed as mean±SD (n=3).

3.3. α-Glucosidase Enzyme Inhibitory Assay:

Aerial parts of BO methanolic extract and its fractions inhibited the catalysis of α-glucosidases at 1.25, 2.5, 5, 10mg/ml concentration and the percentage inhibition were dose-dependent. The percentage inhibition of MEBO ranging from 10.38 to 62.23% with IC_{50} value 7.28± 0.03mg/ml, TLBO ranging from 11.35 to 68.40% with IC_{50} value 7.00±0.10mg/ml, EABO shown percentage inhibition ranging from 11.62 to 78.47% with IC_{50} value 6.16±0.27mg/ml and BNBO has shown percentage inhibition ranging from 12.85 to 76.21% with IC_{50} value 6.56 ±0.32mg/ml.

Among all the test samples of Blumea *oblique,* ethyl caetate fraction has shown remarkable α-amylase enzyme inhibition i.e., 78.47% at 10mg/ml concentration, and it was well comparable with the standard drug acarbose 14.83 to 92.71% inhibition at 10mg/ml concentration with IC_{50} value 5.39±0.28 mg/ml.

α-glucosidase enzyme Inhibitory of BO methanolic extract, its fractions and standard (acarbose) were shown in table 3 and figure 2.

Table 3: α-Glucosidase inhibitory activity and IC_{50} values by *BO* methanolic extract, fractions and Acarbose

Test sample	Concentration (mg/ml)	% Inhibition	IC_{50}
MEBO	1.25	10.38±0.24	7.28±0.03
	2.5	22.65±0.56	
	5	42.64±0.12	
	10	62.23±0.63	
TLBO	1.25	11.35±0.49	7.00±0.10
	2.5	21.65±0.28	
	5	39.37±0.34	
	10	68.40±0.56	
EABO	1.25	11.62±0.33	6.16±0.27
	2.5	24.71±0.42	
	5	43.53±0.17	
	10	78.47±0.50	
BNBO	1.25	12.85±0.37	6.56±0.32
	2.5	22.93±0.42	
	5	42.72±0.63	
	10	76.21±0.29	
Acarbose	1.25	14.83±0.24	5.39±0.28
	2.5	25.62±0.52	
	5	44.34±0.43	
	10	92.71±0.16	

Data expressed as mean ± SD, (n=3).

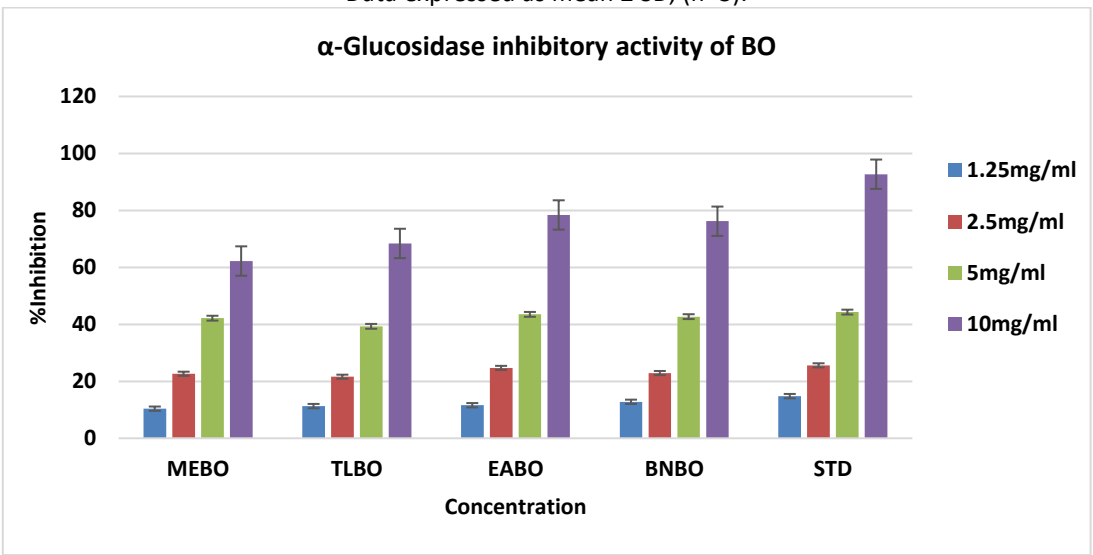

Figure 2: Effect of methanolic extract and different fractions of BO and STD (Acarbose) on α-glucosidase enzyme inhibition. Data expressed as mean±SD (n=3).

4.4. β-Galactosidase Enzyme Inhibitory Assay:

Aerial parts of BO Methanolic extract and its fractions inhibited the catalysis of α-glucosidase at 1.25, 2.5, 5, 10, 20 mg/ml concentration and the percentage inhibition were dose-dependent. The percentage inhibition of methanolic extract of MEBO ranging from 10.65 to 62.97% with IC_{50} value 13.28± 0.32mg/ml, TLBO ranging from 9.63 to 63.67% with IC_{50} value 12.80±0.43mg/ml, EABO shown percentage inhibition ranging from 10.86 to 72.15 % with IC_{50} value 11.81±0.13 mg/ml and BNBO has shown percentage inhibition ranging from 11.21 to 67.54% with IC_{50} value 12.48 ±0.16 mg/ml.

Among all the test samples, Ethyl acetate fraction of BO has shown remarkable α-amylase enzyme inhibition i.e., 72.15% at 10mg/ml concentration, and it was well comparable with the standard drug acarbose 13.62 to 78.97% inhibition at 10mg/ml concentration with IC_{50} value 10.72±0.23mg/ml.

β- galactosidase enzyme Inhibitory of BO methanolic extract, its fractions and standard (quercetin) were shown in table 4 and figure 3.

Table 4: β-galactosidase inhibitory activity and IC_{50} values by BO methanolic *extract*, fractions and Quercetin.

Test sample	Concentration (mg/ml)	% Inhibition	IC_{50}
MEBO	1.25	10.65±0.13	13.28±0.32
	2.5	24.65±0.61	
	5	37.42±0.48	
	10	48.32±0.26	
	20	62.97±0.21	
TLBO	1.25	9.63±0.28	12.80±0.43
	2.5	23.64±0.57	
	5	42.53±0.61	
	10	52.36±0.42	
	20	63.67±0.54	
EABO	1.25	10.86±0.28	11.81±0.13
	2.5	24.62±0.36	
	5	43.19±0.14	
	10	52.36±0.43	
	20	72.15±0.52	
BNBO	1.25	11.21±0.32	12.48±0.16
	2.5	25.41±0.61	
	5	36.21±0.16	
	10	52.48±0.34	
	20	67.54±0.43	
Quercetin	1.25	13.62±0.52	10.72±0.23
	2.5	30.42±0.16	
	5	44.26±0.41	
	10	58.94±0.26	
	20	78.97±0.33	

Data expressed as mean ± SD, (n=3).

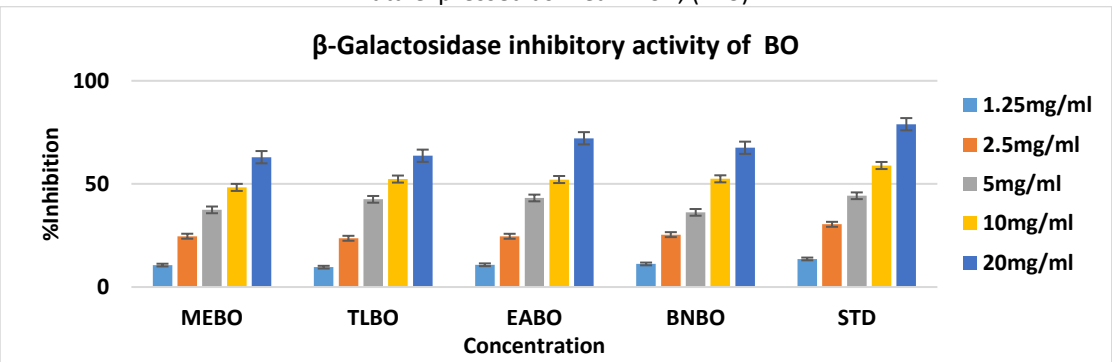

Figure 3: Effect of methanolic extract and different fractions of *BO* and STD (Quercetin) on β-galactosidase enzyme inhibition. Data expressed as mean±SD (n=3).

DISCUSSION:

Management of the blood glucose level is a critical strategy in the control of diabetic complications. Inhibitors of saccharide hydrolysing enzymes (alpha-amylase and alpha-glucosidase) have been useful as oral hypoglycemic agents for the control of hyperglycemia especially in patients with type-2 diabetes mellitus [17]. Inhibition of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently reducing the postprandial plasma glucose rise [18].

The enzymes α-amylase and α-glucosidase are linked to postprandial high blood glucose levels (BGL). α-amylase is linked to breaking the polysaccharides into disaccharides and oligosaccharides. α-glucosidase digest the disaccharides and polysaccharides and break them into glucose monomers aiding carbohydrate digestion. Inhibition of these enzymes can lead to a control on postprandial BGL by controlling carbohydrate digestion and hence controls diabetes significantly. Even though plenty of medications are available for the treatment of diabetes, they have limitations due to their adverse side effects and high costs. Hence it was found to be very difficult to manage and cure this disease effectively by using the available oral hypoglycemic drugs or insulin. Therefore, the focus of scientists shifted towards the development of the natural herbal medicine with high therapeutic potential and less or no toxic effect.

The inhibitory effects of methanolic extract of *Blumea oblique* against porcine pancreas α-amylase, yeast α-glucosidase and E.coli β-galactosidase were evaluated in comparison with the anti-diabetic drugs.

Preliminary phytochemical screening of methanolic extracts and its fractions of BO revealed the presence of alkaloids, carbohydrates, steroids, saponins, flavonoids and phenolic compounds [16].

Among all the BO methanolic extract and its fractions, EABO showed significant against α-amylase, α-glucosidase and β-galactosidase inhibitory activity, with IC_{50} values of 5.74±0.18, 6.16±0.27 and 11.81±0.13mg/ml, BNBO showed mild inhibition IC_{50} values of 5.94±0.22, 6.56±0.32 and 12.48±0.16mg/ml, TABO showed moderate inhibitory effect IC_{50} values of 6.64±0.13, 7.00±0.10 and 12.8±0.43mg/ml and MEBO showed weak enzyme inhibition IC_{50} values of 7.05±0.32, 7.28±0.03 and 13.28±0.32mg/ml, against α-amylase, α-glucosidase and β-galactosidase when compared with standard drug (acarbose) IC_{50} value 5.25±0.41, 5.39±0.28mg/ml and (quercetin) 10.72±0.23mg/ml respectively. Ethylacetate fraction of methanolic extract of BO aerial parts has shown a significant percentage of inhibitory effect on α-amylase, α-glucosidase and β-galactosidase enzymes.

The inhibitory effect of BO extract and fractions on α-amylase, α-glucosidase and β-galactosidase enzymes were found to be in the order EABO>BNBO>TLBO>MEBO.

CONCLUSION

The results achieved from this study elaborated scientific support regarding the use of *Blumea oblique* to treat diabetes through a mechanism based on its α- amylase, α-glucosidase and β-galactosidase inhibitory activity. However, further investigations are recommended to validate these effects *in-vivo.*

CONFLICTS OF INTEREST

We declare that there are no conflicts of interest.

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