Anti-diabetic and anti-obesity activity of Caralluma adscendens var. gracilis and Caralluma pauciflora

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Research Article

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Abstract

The plants fractions of *Caralluma adscendens var. gracilis* and *Caralluma pauciflora* were evaluated for their total phenolic content in relation to their antioxidant activity and inhibitory effect of starch and lipids digestive enzymes. Among all fractions of *C. adscendens var. gracilis* and *C. pauciflora*, diethyl ether fractions showed highest phenolic content (36.23 ± 1.51 mg of GAE g^{-1} DW, 28.21 ± 3.61 mg of GAE g^{-1} DW), DPPH radical scavenging activity (27.96 ± 3.45 µg ml^{-1} and 37.23 ± 0.92 µg ml^{-1}), inhibition of α-glucosidase (59.13 ± 1.31 µg ml^{-1} and 73.03 ± 2.04 µg ml^{-1}), α-amylase (78.1 ± 3.47 µg ml^{-1} 110.5 ± 4.1µg ml^{-1}) and pancreatic lipase (41.91 ± 3.51 µg ml^{-1} and 49.12 ± 3.89 µg ml^{-1}) compared to butanone and *n*-butanol fractions which showed little or no significant activities compared to standards. To the best of our knowledge, the present study provides the first evidence that these two plants (*C. adscendens var. gracilis* and *C. pauciflora*) are potent inhibitors of key enzymes in type 2 diabetes and obesity studies *in vitro*.

1. Introduction

Starch and triglycerides respectively from carbohydrates and lipids are the major dietary source of glucose, monoglycerides and free fatty acids. They are produced by the partial hydrolysis of starch and triglycerides and monoglycerides respectively by lingual and/or gastrointestinal α-amylase, α-glucosidase and lipase or pancreatic lipase (Dhital et al., 2013). Glucose absorbed via small intestinal mucosa influences postprandial blood glucose levels and hyperglycaemic condition in type 2 diabetes. On the other hand, free fatty acids and monoglycerides form mixed micelles with bile salts, cholesterol and lysophosphatidic acids are absorbed into enterocytes where resynthesis of triglycerides takes place (Birari and Bhutani, 2007). The synthesized triglycerides stored in adipose tissue and the excess of triglycerides in adipose tissue leads to overweight and obesity. Thus, limiting absorption of glucose, free fatty acids and glycerides can significantly suppress the hyperglycaemia and obesity as well as other complications associated with these chronic metabolic diseases. Although, acarbose and orlistat are potent drugs for the inhibition of carbohydrate and lipid digesting enzymes, research reports revealed the severe side effects of these drugs in long term usage (Birari and Bhutani, 2007). Recently, there has been growing interest on plant-based natural medicines for the treatments of non-communicable disease not only due their no or lesser side effects but also for their cheaper cost compared to conventional drugs.

Caralluma adscendens var. gracilis and *Caralluma pauciflora* belongs to the family of Asclepiadaceae which is native to India and some other Asian and African countries. Traditionally, *Caralluma* species has been claimed to have antidiabetic and anti-obesogenic properties. *C. Fimbriata* (Latha et al., 2004, Kuriyan et al., 2007, Dutt et al., 2012, Sudhakara et al., 2014, Gujjala et al., 2016), C. *sinaica* L. (Habibuddin et al., 2008) *C. umbellata* (Bellamakondi et al., 2014), C. *tuberculate* (Ahmad et al., 2014), *C. edulis* (Singh et al., 2016) have been reported for their appetite suppressing, anti-obesogenic, reno-protective, antidiabetic, anti-oxidative, anti-hyperglycemic, effects. However, anti-diabetes and/or anti-

obesity activities of the *Caralluma adscendens var. gracilis* and *Caralluma pauciflora*, which are actually used for traditional management of the diabetes and obesity, have yet not been scientifically evaluated.

Hence, the present study was designed to ascertain the anti-diabetic and anti-obesity effects of *Caralluma adscendens var. gracilis* and *Caralluma pauciflora* by investigating their inhibitory activity against key enzymes linked to type 2 diabetes and obesity.

2. Materials And Methods

2.1 Chemicals and reagents

Porcine pancreatic lipase, α-amylase and yeast α-glucosidase, orlistat, *p*-nitrophenylbutyrate (PNPB), *p*-nitrophenyl-α-D-glucopyranoside (pNPG), acarbose, gallic acid, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and ascorbic acid were purchased from Sigma-Aldrich, Germany. Starch, dinitrosalicylic acid (DNS), diethyl ether, butanol, n-butanone, dimethylsulfoxide (DMSO) were obtained from Merck Chemicals Company, Germany.

2.2 Collection of plant materials

Caralluma adscendens var. gracilis and *Caralluma pauciflora* plants were collected from Satyamangalam village of Pudukottai district, Tamil Nadu, India. The plants were identified by Prof. V. S. Raju, Department of Botany, Kakatiya University, Warangal, India. Voucher specimens of the plants were deposited at the herbarium of College of Pharmaceutical Sciences, Kakatiya University, Warangal, India (AVN-CG-1-07 and AVN-CP-1-07). All plant materials were washed, shade dried, blended and stored in air tight containers for subsequent analysis.

2.3 Preparation of extracts

The fresh 1 kg of whole plant powder of *C. gracilis* was chopped, crushed, and macerated in 3 L ethanol at room temperature for 7 days. The extract was filtered in Whatmann filer paper no 1 and concentrated in rotary evaporator (Buchi Rotavapor II, Switzerland) to give a dark greenish semi-solid residue. The above ethanolic extract was dispersed in 1 L of water and fractionated successively with diethyl ether, butanone and *n*-butanol. All fractions were dried by using above-mentioned rotary evaporator when aqueous extract was dried at 50^{o}C in a water bath till dryness. All extracts and fractions were transferred to microtubes and stored at 4ºC until further analysis.

2.4 Estimation of total polyphenol content

The total polyphenol content of each extract was estimated as gallic acid equivalent according to the method of McDonald et al. (2001) with slight modifications. Briefly, a 200 µL of extract was dissolved in 10% DMSO (500 µg mL^{-1}) and incubated with 1 mL of Folin Ciocalteau reagent (diluted 10 times) and 800 µL of 0.7 M Na_2CO_3 for 30 min at room temperature. The absorbance of the solution was then

measured at 765 nm on a Shimadzu UV mini 1240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). All measurements were done in triplicate.

2.5 In vitro antioxidant or free radical scavenging activity

The total free radical inhibition activity of the extracts was determined using a slightly modified method of Tuba and Gulcin (2008) when ascorbic acid was used as a standard. Briefly, a 0.3 mM solution of DPPH was prepared in methanol and 500 µL of this solution was added to 1 mL of the extract (dissolved in 10% DMSO) at different concentrations (30–150 µg mL^{-1}). These solutions were mixed and incubated at dark for 30 min at room temperature. The absorbance was then measured at 517 nm against a blank sample lacking scavenger. The antioxidant or free radical inhibition activity was calculated according to the following formula:

% Inhibition = [(AB-AA) / AB] x 100 (1)

where AB = Absorbance of the blank DPPH solution and AA = Absorbance of the tested extract.

2.6 In vitro α-glucosidase assay

The α-glucosidase inhibitory activity of extracts was determined according to the method described by Ademiluyi and Oboh (2013) with slight modifications. Briefly, a 250 µL of each extract or acarbose at different concentrations (30–150 µg mL^{-1}) was incubated with 500 µL of 1.0 U mL^{-1} α-glucosidase solution in 100 mM phosphate buffer (pH 6.8) at 37°C for 15 min. Thereafter, 250 µL of 4-Nitrophenyl β-D-glucopyranoside (pNPG) solution (5 mM) in 100 mM phosphate buffer (pH 6.8) was added and the mixture was further incubated at 37°C for 20 min. The absorbance of the released *p*-nitro phenol was measured at 405 nm and the inhibitory activity was expressed as a percentage of the control without inhibitor. All assays were carried out in triplicate and the calculation was done according to the following formula.

% Inhibition = $[(Abs_{Control} - Abs_{test})/ Abs_{control}] \times 100$ (2)

2.7 In vitro α-amylase assay

The α-amylase inhibitory activity of extracts was determined according to the method described by Shai et al. (2010). A 250 µL of each extract dissolved in 10% DMSO or acarbose at different concentrations (30–150 µg mL^{-1}) was incubated with 500 µL of porcine pancreatic amylase (2 U mL^{-1}) in 100 mM phosphate buffer (pH 6.8) at 37°C for 20 min. A 250 µL of 1% starch dissolved in 100 mM phosphate buffer (pH 6.8) was then added to the reaction mixture and incubated at 37°C for 1 h. A 1 mL of DNS colour reagent was then added and boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm and the inhibitory activity was expressed as a percentage of the control without inhibitor. All assays were carried out in triplicate and the calculation was done according to the following formula.

% Inhibition = $[(Abs_{Control} - Abs_{test})/ Abs_{control}] \times 100$ (3)

2.8 In vitro pancreatic lipase assay

The method for measuring pancreatic lipase activity was modified from that of Kim et al. (2009). Briefly, an enzyme buffer was prepared by the addition of a solution of porcine pancreatic lipase [2.5 mg mL^{-1} in 10 mM MOPS (morpholinepropanesulphonic acid) and 1 mM EDTA, pH 6.8]. Thereafter 169 µL of Tris buffer (100 mM Tris-HC1 and 5 mM $CaCl_2$, pH 7.0) was added to 100 µL of the extract (30–150 µg mL^{-1}) at the test concentration, or orlistat, after which 20 µL of the enzyme buffer was added and incubated for 15 min at 37°C. A volume of 5 µL of the substrate solution [10 mM p-NPB (p-nitrophenyl butyrate) in dimethyl formamide] was then added and incubated for 30 min at 37°C. The lipase activity was determined by measuring the hydrolysis of p-NPB to *p*-nitrophenol at 405 nm.

$$\% \text{ Inhibition} = [(Abs_{Control} - Abs_{test})/ Abs_{control}] \times 100 \quad (4)$$

2.9 Statistical analysis

Data were analysed using a statistical software package (SPSS for Windows, version 23, IBM Corporation, USA) using Tukey's-HSD multiple range *post-hoc* test. Values were considered significantly different at $p < 0.05$.

3. Results

3.1. Total polyphenol content

Results of the total polyphenol content of the *Caralluma adscendens var. gracilis* and *Caralluma pauciflora* are shown in table 1. We found highest polyphenolic content in diethyl ether fraction of *Caralluma adscendens var. gracilis* (36.23 ± 1.51 mg GAE/g DW) and *Caralluma pauciflora* (28.21 ± 3.61 mg GAE/g DW).

3.2 In vitro antioxidant or free radical scavenging activity

The results of *in vitro* antioxidant or free radical scavenging activity of different fractions of *C. adscendens var. gracilis* and *C. pauciflora* are depicted in Fig. 1. Diethyl ether fraction of *C. adscendens var. gracilis* and ascorbic acid showed significantly ($p < 0.05$) higher antioxidant activity compared to butanone and n-butanol fractions, while no significant difference was observed between butanone and *n*-butanol fractions. Similarly, antioxidant activity of diethyl ether fraction of *C. pauciflora* and ascorbic acid was significantly higher than other fractions when the activity of diethyl ether fraction was lower than ascorbic acid in most concentrations studied. The IC_{50} value of diethyl ether fraction was also significantly ($p < 0.05$) lower (27.9 µg ml^{-1}, 37.21 µg ml^{-1}) compared to butanone (74.02 µg ml^{-1}, 74.19 µg ml^{-1}) and *n*-butanol (81.65 µg ml^{-1}, 64.95 µg ml^{-1}) fractions (Fig. 1).

3.3 In vitro α-glucosidase activity

Data of the α-glucosidase inhibitory activity of the fractions derived from alcoholic extract of *C. adscendens var. gracilis* and *C. pauciflora* are shown in Fig. 2. It was observed that the diethyl ether fraction of *C. adscendens var. gracilis* and *C. pauciflora* exhibited comparable and even significantly ($p < 0.05$) higher α-glucosidase inhibitory activity in some concentrations compared to acarbose and other factions. Further, IC_{50} values of the factions also showed that the diethyl ether fraction exhibited significantly lowest IC_{50} value for *C. adscendens var. gracilis* (59.13 μg ml^{-1}) and *C. pauciflora* (73.03 μg ml^{-1}) compared to other factions as well as acarbose (Table 2).

3.4 *In vitro* α-amylase activity

The data for *in vitro* α-amylase activity are presented in Fig. 3 and Table 2. The data showed an increasing inhibitory activity of diethyl ether fraction of *C. adscendens var. gracilis* and *C. pauciflora* on α-amylase on a dose dependent manner from 30–150 μg ml^{-1} with corresponding IC_{50} values of 78.1 μg ml^{-1} and 110.5 μg ml^{-1} respectively (Table 2).

3.5 In vitro pancreatic lipase activity

Data of the *in vitro* pancreatic lipase inhibitory activity of the fractions derived from alcoholic extract of *C. adscendens var. gracilis* and *C. pauciflora* are shown in Fig. 4. It was observed that the diethyl ether fraction of *C. adscendens var. gracilis* and *C. pauciflora* exhibited a significantly ($p < 0.05$) higher lipase inhibitory activity compared to other factions. Further, IC_{50} values of the fractions also showed that the diethyl ether fraction exhibited significantly lowest IC_{50} value for *C. adscendens var. gracilis* (41.92 μg ml^{-1}) and *C. pauciflora* (49.13 μg ml^{-1}) compared to other factions (Table 2).

Discussion

The present study revealed that diethyl ether fraction of *C. gracilis* and *C. pauciflora* show potential antioxidant activity and inhibitory activities of carbohydrate and lipid digesting enzymes. Generally, antioxidants are very important to the body to protect the organs from the free radical damage (Kasote et al., 2015). Studies suggested that plants with rich content of polyphenols contain higher antioxidant activity (Shahidi, 2012). In our findings, the observed highest antioxidant activity of diethyl ether fraction of *C. gracilis* and *C. pauciflora* can be attributed to higher polyphenolic content as reflected in the total phenol content (Table 1). Similar reports are documented for aerial parts of *C. edulis* (Ansari et al., 2005), C. *Arabica* (Khasawneh et al., 2014), C. *adscendens* var. *fimbriata* (Maheshu et al., 2014), C. *diffusa* (Chandran et al., 2014) and *in vitro* propagation of *C. tuberculata* (Rehman et al., 2014).

Obesity and type 2 diabetes are associated with increased oxidative stress. Excessive generation of free radical has been associated with tissue damage and complication in diabetic and obese patients (Giugliano et al., 1996, Rahimi et al., 2005 Newsholme et al., 2007, Marseglia et al., 2015). Several scientific reports showed that carbohydrate and fat rich foods cause increased absorption of glucose, triglycerides and free fatty acids from the intestine (Birari and Bhutani, 2007). This leads to hyperglycemic condition, insulin resistance, excess of abdominal adipose tissue, overweight and obesity (Birari and Bhutani, 2007). The observed *in vitro* inhibitory activities of the diethyl ether fraction of both

plants against α-glucosidase, α-amylase and pancreatic lipase activities in this study indicate its antidiabetic and anti-obesity activity. Similar results were also reported for the methanolic extract of *C. umbellata*, a plant from same genus, on α-amylase and pancreatic lipase activity (Bellamakondi et al., 2014). Few other species from the *caralluma* genus such as *C. fimbriata* have been reported for their anti-diabetic, insulin sensitizing and antioxidative effects in rats (Latha et al., 2004, Sudhakara et al., 2014). The antidiabetic activity of *C. sinaica* has also been reported (Habibuddin et al., 2008). The effect of *C. Fimbriata* on appetite, food intake and anthropometry has been reported in adult Indian men and women (Kuriyan et al., 2007). In spite of these reported antidiabetic activities of different species of Caralluma genus, to the best of our knowledge this is the first study where we reported the antidiabetic and anti-obesity activity of *C. gracilis* and *C. pauciflora*.

Conclusions

The data of this study suggest that the diethyl ether fraction of *C. gracilis* and *C. pauciflora* showed the strong antioxidant, anti-diabetic and anti-obesity activities via free radical scavenging activity, *in vitro* α-glucosidase, α-amylase and pancreatic lipase inhibition. Further studies are needed to identify the possible bioactive compounds from the most effective diethyl ether fraction and investigate their anti-diabetic, ant-obesity and toxicological effects in experimental animals and humans.

Declarations

Acknowledgments

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Authors' contributions: Mopuri; conducted the literature search, drafted the manuscript and data analysed. Mopuri, Dowlathabad, Kommidi and Islam; reviewed the manuscript and provided contributing ideas. All authors had a role in approving the final version of the manuscript

Data availability: The dataset generated for this study are available on request to the corresponding author.

Competing interests: The authors declare no competing interests.

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Tables

Table.1 Total polyphenolic content of the fractions of *Caralluma adscendens var. gracilis* and *Caralluma pauciflora*.

	Total Polyphenols (mg GAE/g DW)	
Plant fractions	*Caralluma adscendens var. gracilis*	*Caralluma pauciflora*
Diethyl ether	36.23±1.51^{c}	28.21±3.61^{c}
Butanone	14.23±2.20^{b}	17.17±2.5^{b}
n-Butanol	18.72±7.36^{a}	11.67±4.0^{a}

Data are presented as mean ± SD of triplicate determinations. $^{a-c}$ Different letters mentioned as superscripts in a given fraction were significantly different from each other (Tukey's-HSD multiple range *post hoc* test, $p < 0.05$, IBM, SPSS, version 23). AA: Ascorbic acid

Table 2: IC50 values for antioxidant and enzyme inhibition activities

Plant/fractions	Antioxidant	α-Glucosidase	α-Amylase	Lipase
Caralluma adscendens var. gracilis	IC_{50} values (μg ml^{-1})			
Diethyl ether	27.9 ±3.46[a]	59.13±1.32[a]	78.1±3.47[a]	41.92±3.51[b]
Butanone	74.02±3.84[bc]	85.21±12.4[b]	108.8±5.59[b]	106.48±23.07[c]
n-butanol	81.65±2.76[bd]	115.36±12.18[c]	146.9±9.31[c]	118.58±14.78[c]
Caralluma pauciflora				
Diethyl ether	37.21±.92[b]	73.03±2.056[a]	110.5±4.1[a]	49.13±3.83[b]
Butanone	74.19±3.36[c]	104.32±6.58[b]	172.79±2.1[b]	95.83±9.02[c]
n-butanol	64.95±5.79[b]	131.14±10.47[c]	216.78±26.3[c]	127.21±20.34[d]
Ascorbic acid	27.12±2.77[a]	ND	ND	ND
Acarbose	ND	62.31±3.93[ab]	90.88±7.2[a]	ND
Orlistat	ND	ND	ND	2.70±0.84[a]

Data are presented as mean ± SD of triplicate determinations. [a-c] Different letters mentioned as superscripts in a given fraction were significantly different from each other (Tukey's-HSD multiple range *post hoc* test, $p < 0.05$, IBM, SPSS, version 23).

Figures

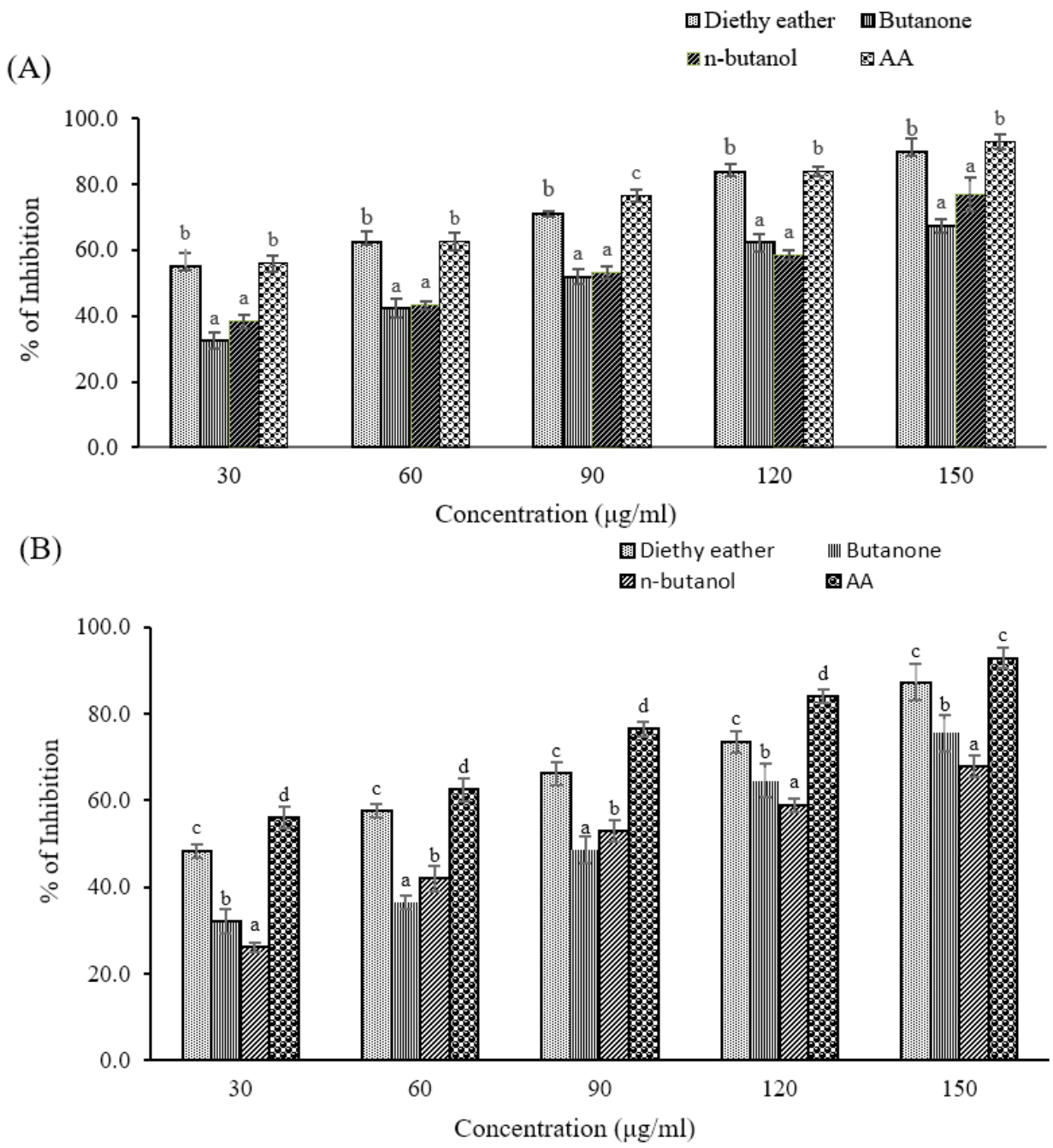

Figure 1

In vitro antioxidant activity of the fractions of (a) *Caralluma adscendens var. gracilis* and (b) *Caralluma pauciflora*. Data are presented as mean ± SD of triplicate determinations. [a-d] Different letters above the bars for a given fraction were significantly different from each other (Tukey's-HSD multiple range *post hoc* test, $p < 0.05$, IBM, SPSS, version 23). AA: Ascorbic acid

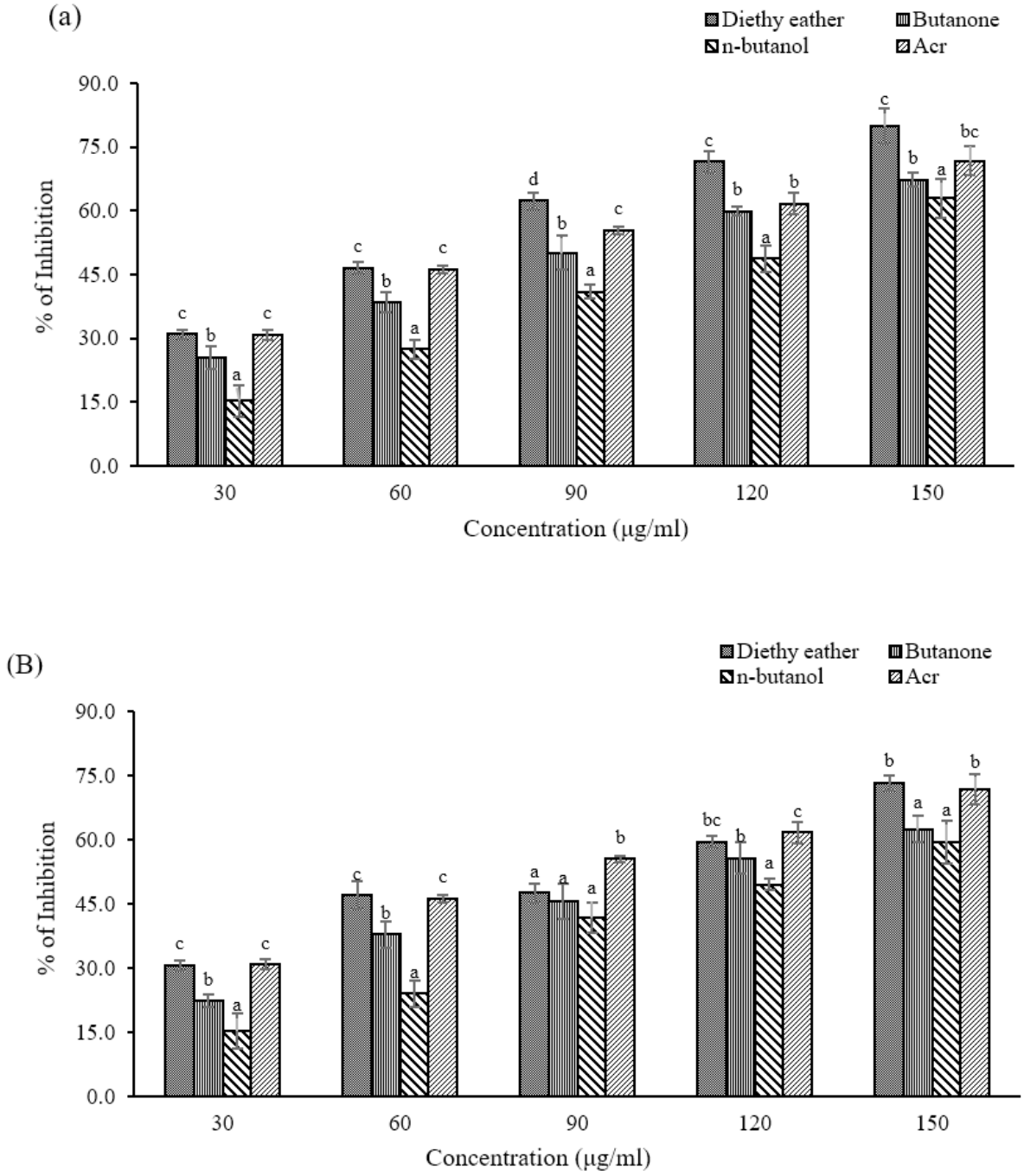

Figure 2

In vitro α-glucosidase activity of the fractions of (a) *Caralluma adscendens var. gracilis* and (b) *Caralluma pauciflora*. Data are presented as mean ± SD of triplicate determinations. [a-d] Different letters above the bars for a given fraction were significantly different from each other (Tukey's-HSD multiple range *post hoc* test, $p < 0.05$, IBM, SPSS, version 23). Acr: Acarbose.

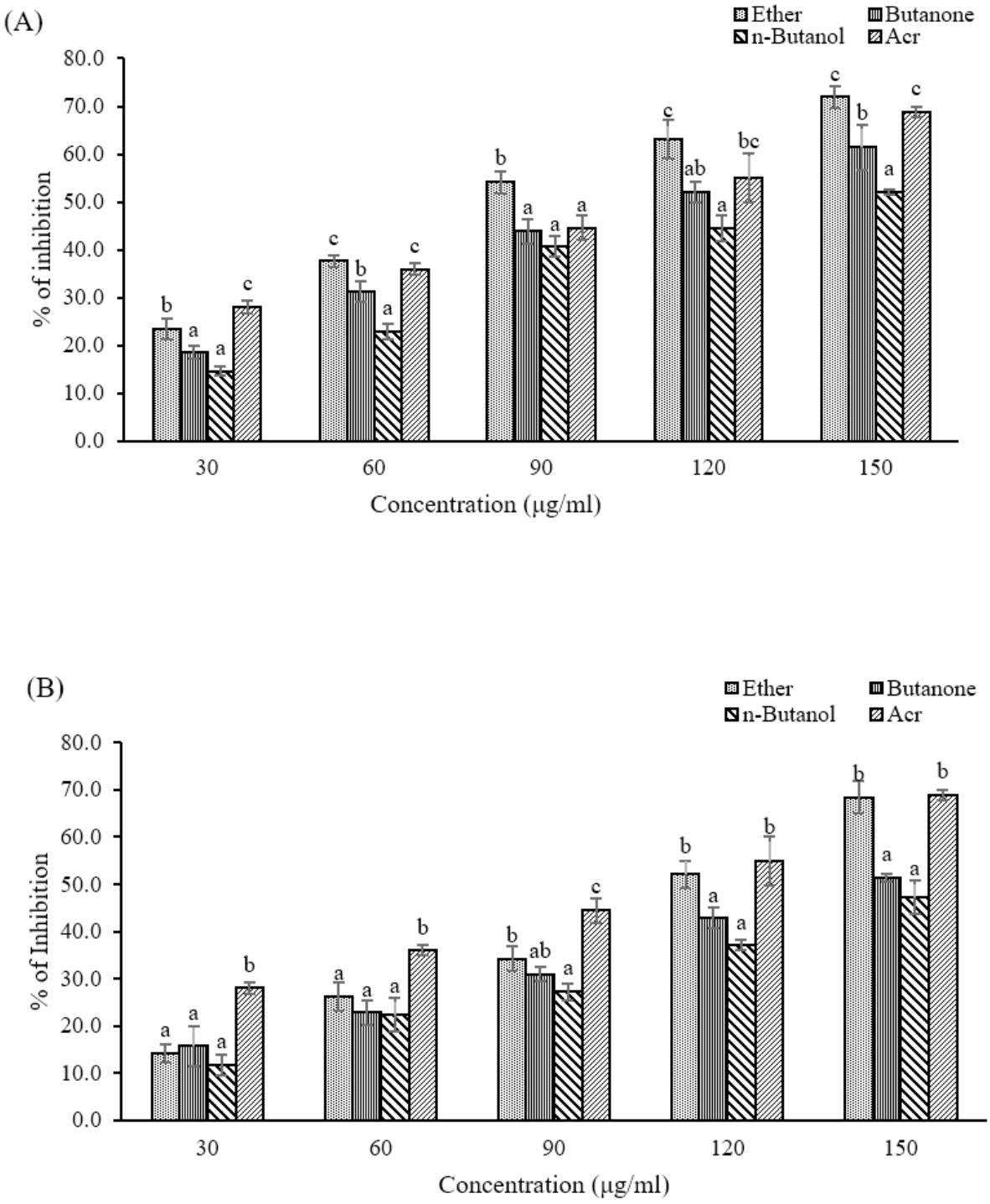

Figure 3

In vitro α-Amylase activity of the fractions of (a) *Caralluma adscendens var. gracilis* and (b) *Caralluma pauciflora*. Data are presented as mean ± SD of triplicate determinations. [a-c] Different letters above the

bars for a given fraction were significantly different from each other (Tukey's-HSD multiple range *post hoc* test, $p < 0.05$, IBM, SPSS, version 23). Acr: Acarbose.

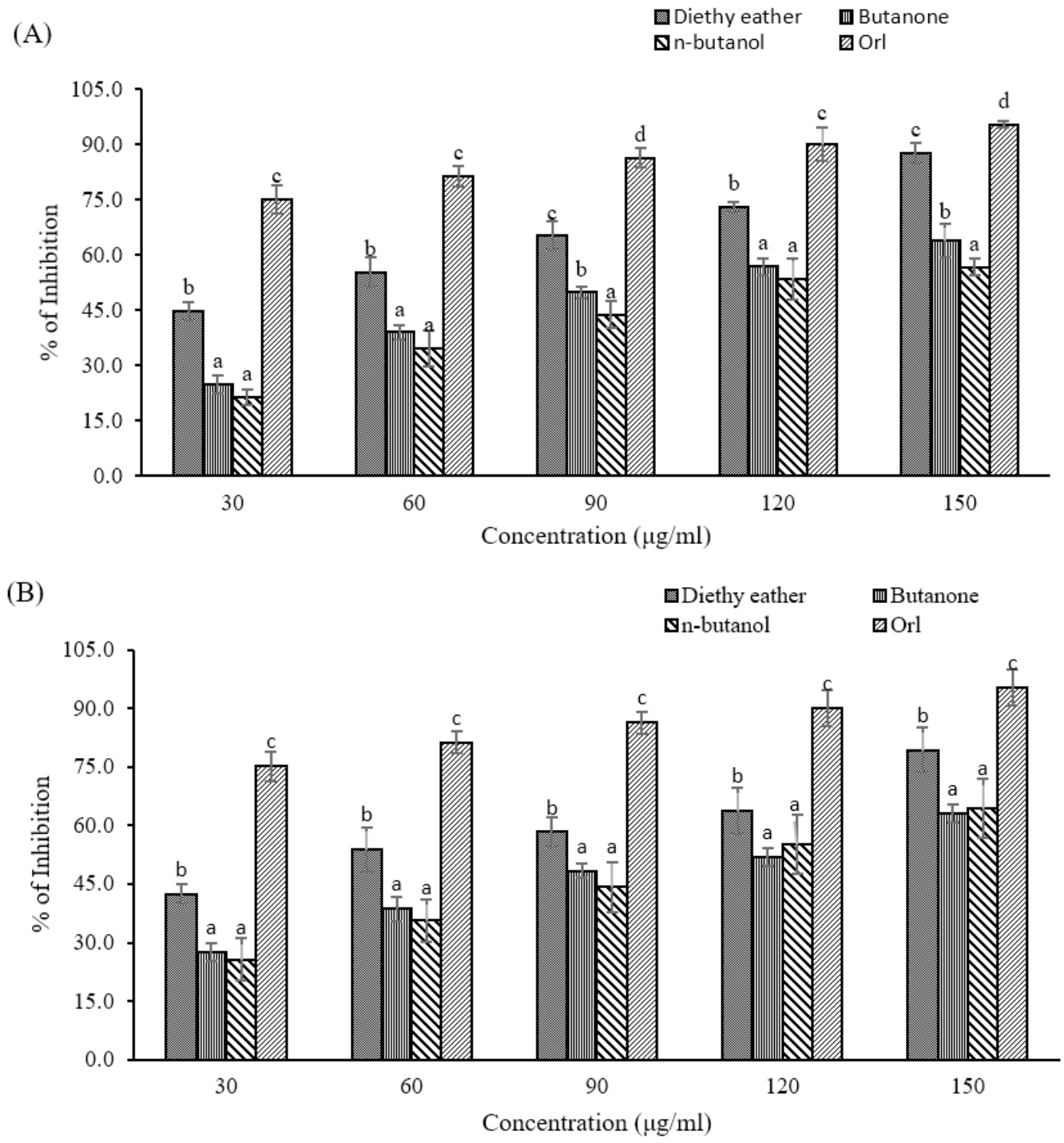

Figure 4

In vitro pancreatic lipase activity of the fractions of (a) *Caralluma adscendens var. gracilis* and (b) *Caralluma pauciflora*. Data are presented as mean ± SD of triplicate determinations. [a-d] Different letters

above the bars for a given fraction were significantly different from each other (Tukey's-HSD multiple range *post hoc* test, $p < 0.05$, IBM, SPSS, version 23). Orl: Orlistat.