

## Anti-hyperglycemic and antioxidant potential of *Croton bonplandianus*. Bail fractions in correlation with polyphenol content

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ARTICLE INFO	ABSTRACT
<p><b>Article type:</b> Short communication</p> <p><b>Article history:</b> Received: Dec 19, 2016 Accepted: Sep 28, 2017</p> <p><b>Keywords:</b> Anti-hyperglycemia <math>\alpha</math>-Amylase <math>\alpha</math>-Glucosidase Polyphenols Postprandial hyperglycemia</p>	<p><b>Objective(s):</b> Diabetes mellitus, a carbohydrate metabolic disorder, occurs due to absolute or relative deficiency of insulin. Current treatment strategies involve either preventing or delaying the intestinal absorption of glucose to lower the levels of postprandial hyperglycemia (PPHG). Herbal remedies have been since ancient times for treating diabetes mellitus. Therefore, identifying novel phytochemicals with <math>\alpha</math>-amylase and <math>\alpha</math>-glucosidase inhibitory activity that would reduce the glucose absorption as well as the rise in postprandial blood glucose level is vital. Consequently, the present study was aimed to investigate the anti-hyperglycemic activity of <i>Croton bonplandianus</i> against these pancreatic enzymes.</p> <p><b>Materials and Methods:</b> The methanol extract of <i>C. bonplandianus</i> leaf was prepared and further fractionation was performed with n-hexane, ethyl acetate and chloroform. The antioxidant activity and anti-hyperglycemic activity of the extracts and its fractions were determined. Further, GC-MS analysis was performed for the leaf extract.</p> <p><b>Results:</b> The chloroform fraction (ChF) was found to contain highest quantity of polyphenols (114.28 <math>\mu</math>g/ml of GAE), flavonoids (95.68 <math>\mu</math>g/ml of quercetin) and tannins (63.80 <math>\mu</math>g/ml of GAE) and also possessed effective inhibitory activity against <math>\alpha</math> amylase (IC<sub>50</sub> 95.78 <math>\mu</math>g/ml) and <math>\alpha</math> glucosidase (IC<sub>50</sub> 126.81 <math>\mu</math>g/ml). The antioxidant activity of ChF was also higher when compared to other fractions. Further, GC-MS analysis of ChF showed the presence of various components that may be responsible for the above mentioned activities.</p> <p><b>Conclusion:</b> The study findings suggest that the components present in the leaves of <i>C. bonplandianus</i>, may provide a potential therapeutic source in developing treatment for hyperglycemia. Further bioassay guided fractionation procedure is required to identify the active constituents.</p>

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### Introduction

Diabetes mellitus, a metabolic disorder, is caused either by insufficient insulin secretion (Type-1) or insulin resistance (Type-2) leading to carbohydrates, proteins and fat metabolic disturbance (1). According to an evaluation in 2016 by World Health Organization (WHO), an estimated 422 million adults live with diabetes. Type 2 diabetes is more prevalent than type 1 diabetes, comprising of 90% of the world population (WHO 2013)(2) and it is predicted that diabetes would be the 7th leading cause of death by 2030 (3). India is among the top three countries with highest diabetic population, with over 60 million new cases and with over 0.9 million deaths in 2015 (4). Postprandial hyperglycaemia (PPHG) is the major risk factor for the development of type 2 diabetes mellitus. Glycated end products, formed due to PPHG, are the major contributors for diabetes complications and aging (5). Regulation of blood glucose level to lower PPHG is a recent therapeutic approach to the diabetes management

Oral antidiabetic drugs delay the production or retard the absorption of glucose through inhibition on carbohydrate metabolizing enzymes, alpha- amylase and alpha- glucosidase, which are the key enzymes involved in hydrolyzing polysaccharides. This results in slow metabolism that indirectly lowers the postprandial increase in plasma glucose level (3). Current therapies available for diabetes include rapid acting, long acting short acting insulin and various oral antidiabetic agents such as voglibose, miglitol and acarbose which are known to inhibit glucosidase enzymes. However, the above treatment methods are proven to cause side effects like low blood sugar, abdominal distention, bloating, meteorism, flatulence and diarrhea (6).

Thus the present approach requires identifying and exploring for  $\alpha$ -amylase and glucosidase inhibitors with less or no side effects. Indian system of traditional medicine is in practice since thousands of years and has reports of various plants with hypoglycaemic property with fewer side effects (3). In recent times, several

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secondary metabolites produced by the plants for self-defence, have received increased attention in the treatment of hyperglycaemia, which is proven to possess different modes of action including delaying glucose absorption through their inhibition on the carbohydrate metabolizing enzymes (7).

*Cbonplandianus* Bail, an exotic weed found in riverbanks and other waste lands (8), commonly known as Bantulsi in Northern India. It was known to have an exemplary antimicrobial activity. The plant was also shown to possess antioxidant and wound healing properties (9). The methanol extract was shown to exhibit cytotoxic effect and the dichloromethane extract was shown to possess inhibitory effect against alpha glucosidase enzyme (10). The ethanolic extract of the leaf was already reported to possess alpha amylase inhibitory activity (11); thus, the present study was carried out to study the inhibitory effect of the methanolic extract of the plant leaves and its fraction on alpha amylase and alpha glucosidase.

## Materials and Methods

### Materials

Alpha amylase (A3176) from porcine pancreas and alpha glucosidase were obtained from Sigma Aldrich Co, St Louis, USA. The standards –quercetin, ascorbic acid and gallic acid, were procured from Sigma Aldrich Co, St Louis, USA. The solvents methanol, hexane, ethyl acetate, chloroform and dinitrosalicylic acid (DNS) reagent was purchased from SRL Chemicals and DPPH from Himedia.

### Plant material

The plant was collected from Chennai and was authenticated (No:PARC/2011/1021) by Dr Jayaraman, Director, Plant Anatomy Research Center, Medicinal Plant Research Unit, Tambaram, Tamil Nadu. A voucher specimen is deposited at the Department of Biomedical Sciences, Sri Ramachandra University, Chennai for reference. The healthy leaves were thoroughly washed in running water, shade dried and made into coarse powder.

### Extraction and fractionation

Leaf powder (500 g) was weighed and extracted with 2.5 l of methanol (1:5) by cold percolation method for three days and further fractionated with solvents of increasing polarity i.e. hexane, ethyl acetate and chloroform (12). The fractions were filtered using Whatmann's No.1 filter paper and kept for evaporation. All the fractions were stored at -20 °C.

### Phytochemical screening

The presence of phytoconstituents such as phenols, flavonoids, alkaloids, tannins, saponins, terpenoids in methanol extract and its fractions was determined according to the standard protocols (13).

### Quantification of secondary metabolites

#### Estimation of total polyphenol

The total phenol content of the crude extract and fractions was quantified using Folin–Ciocalteu reagent (14). Briefly, 50 µl of 25% sodium carbonate and 20 µl of Folin-Ciocalteu reagent were added to 100 µl of the sample. This mixture was shaken thoroughly and the volume was made up to 1 ml with distilled water. The samples were incubated in dark for 1 hr at RT and absorbance was read at 765 nm using gallic acid as standard. The total phenol content was expressed as mg of gallic acid equivalents.

#### Estimation of total flavonoids

The total flavonoid was quantified using aluminium chloride (14). The reaction mixture containing 50 µl of the sample, 50 µl of aluminium chloride, 750 µl of 95% ethanol and 50 µl of potassium acetate was made up to 3 ml with distilled water and the tubes were incubated for 30 min at RT. The absorbance of the sample was measured at 415 nm using quercetin as the standard. The total flavonoid content was expressed as mg of quercetin equivalents.

#### Estimation of tannin

The tannin content was estimated using Folin's phenol reagent (15). To 500 µl of the sample, 0.5 ml Folin's phenol reagent and 5 ml of 35% sodium carbonate were added. The samples were incubated for 5 min at RT and the absorbance was read at 640 nm using gallic acid as the standard.

### In-vitro antioxidant assays of various fractions

#### DPPH Free radical scavenging activity

The DPPH free radical scavenging activity of the extract is based on reduction of stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) in the presence of a hydrogen donor. 190 µl of DPPH and 10 µl of the sample (62.5, 125, 250, 500, & 1000 µg/ml) was incubated in dark for 30 min. The absorbance of the sample was read at 517 nm using ascorbic acid as the control (16).

#### Hydroxyl radical scavenging assay

The hydroxyl radicals scavenging activity of the crude extracts and fractions was estimated using deoxyribose method (17). The sample (62.5-1000 µg/ml) was mixed with FeCl<sub>3</sub> (100 µM), EDTA (104 µM), H<sub>2</sub>O<sub>2</sub> (1 mM) and 2-deoxy-D-ribose (2.8 mM) and made up to 1 ml with potassium phosphate buffer (20 mM, pH 7.4). The samples were incubated for 1 hr at 37 °C and 1 ml each of TCA (2.8%) and TBA (0.5% in 0.025 M NaOH) were added to the above solution and the absorbance was measured at 532 nm using ascorbic acid as the standard.

**Table 1.** Phytochemical analysis of extract and fractions of *C. bonplandianus*

Phytoconstituents	Crude extract (CE)	Hexane fraction (HeF)	Ethyl acetate fraction (EaF)	Chloroform fraction (ChF)
Polyphenols	+	+	+	+
Flavanoids	+	+	+	+
Tannin	+	+	+	+
Saponin	-	-	-	-
Steroids	-	-	-	-
Terpenoids	+	-	-	+
Quinone	-	-	-	-

**Reducing power capacity**

The reducing power of plant extract and its fractions was determined by their property to reduce ferric (III) to ferrous (II) (18). To 1 ml of the sample (62.5-1000 µg/ml), 2.5 ml of phosphate buffer (0.2 M, pH=6.6) and 2.5 ml of 1% potassium ferricyanide were added and incubated at 50 °C for 20 min. Subsequently, 2.5 ml of 10% TCA was added to the above mixture and mixed thoroughly. To 2.5 ml of above reaction mixture, 2.5 ml of distilled water and 0.5 ml of 0.1% FeCl<sub>3</sub> were added and allowed to stand for 10 min. Then, the absorbance was read at 700 nm using ascorbic acid as the standard.

**Nitric oxide scavenging assay**

Sodium nitroprusside at physiological pH spontaneously generates nitric oxide upon reaction with oxygen produces nitrite ions, which are estimated using Griess reagent (14). Half ml of sodium nitroprusside was added to 0.5 ml of the sample (62.5-1000 µg/ml) and incubated at 25°C for 180 min. Then 0.5 ml of Griess reagent was added to the above solution and the absorbance was read immediately at 546 nm. The control sample was prepared without plant extract.

**Alpha amylase inhibitory assay**

The assay was carried out according to the standard protocol with slight modifications (19). To 1 ml of the sample (200-1000 µg/ml), 1 ml of enzyme solution (0.5 mg/ml) was added and incubated at 25°C for 30 min. One ml of 1% starch solution was added to all the tubes and incubated again for 15 min. Finally 1 ml of the DNS solution was added and the samples were incubated in boiling water bath for 15 min. The tubes were then cooled and the reaction mixture was diluted with distilled water (1:5) and the orange-yellow colour developed was recorded at 540 nm using spectrophotometer. Acarbose was used as a standard drug.

**Alpha glucosidase inhibitory assay**

α-Glucosidase inhibitory activity of extract and frac-

tions was studied with minor modification (20). Ten µl of sample (200-1000 µg/ml) was mixed with 50 µl of 0.1 M phosphate buffer (pH 7.0), 25 µl of 0.5 mM 4-nitrophenyl α-D-glucopyranoside (dissolved in 0.1 M phosphate buffer, pH 7.0), and 25 µl of α-glucosidase solution (1 mg/ml in 0.01 M phosphate buffer, pH 7.0) and incubated at 37 °C for 30 min. The reaction was terminated by adding 100 µl of 0.2 M sodium carbonate solution. The amount of *p*-nitrophenol released was measured at 410 nm using microplate reader. Acarbose was used as a standard.

**GC-MS analysis**

Among the different fractions, ChF displayed better results against alpha amylase and alpha glucosidase, hence, a GC-MS analysis was performed to identify the compounds present in this fraction. GC-MS analysis was carried out under following condition. The carrier gas, helium was passed at a constant flow rate of 1.51 ml/min. A sample volume of 2 µl was used and the column temperature was programmed to 70°C with increasing temperature of 10 °C/min to 300°C. The mass spectra were obtained through ionization energy of 70 eV in the EI mode. The total running time for the GC-MS study was 30 min. By comparing with the mass spectra, the organic compound present in the ChF was identified using inbuilt library [NIST-11].

**Results****Phytochemical screening and quantification of secondary metabolites**

In the present study, the preliminary phytochemical screening was performed for the crude extract and all the fractions. The results showed the presence of polyphenols, flavonoids, tannins and terpenoids (Table 1). Terpenoids were present in crude methanol extract (CE) and chloroform fraction (ChF). The total amount of phenols, flavonoids and tannins were estimated using the absorbance of respective standards (Table 2). The total phenols (114.28±0.05 µg/ml of GAE), flavonoids (95.68±0.03 µg/ml of Quercetin) and tannins (63.8±0.009 µg/ml of GAE) were found to be in the order of ChF>EaF>HeF>crude extract.

**Table 2.** Quantification of secondary metabolite content of extract and fractions of *C. bonplandianus*

Phytoconstituents	Crude extract (CE) (µg/ml)	Hexane fraction (HeF) (µg/ml)	Ethyl acetate fraction (EaF) (µg/ml)	Chloroform fraction (ChF) (µg/ml)
Polyphenols	12.3±0.01	23.27±0.06	28.57±0.03	114.28±0.05
Flavonoids	23.6±0.6	12.56±0.05	43.08±0.01	95.68±0.03
Tannins	4.57±0.009	10.87±0.08	28.9±0.007	63.8±0.009

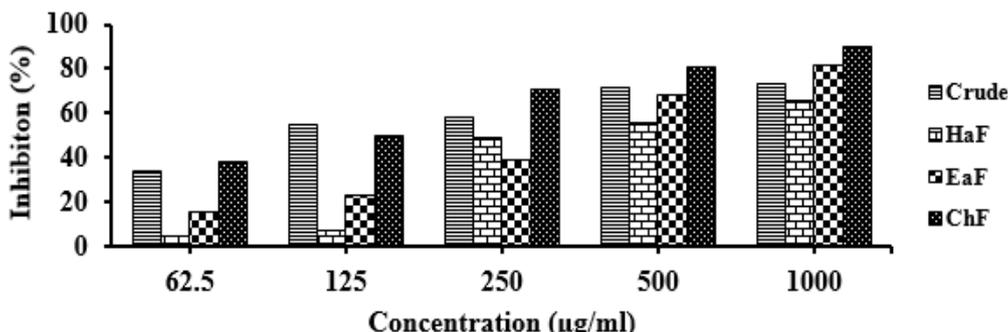


Figure 1. DPPH radical scavenging assay of extract and fractions of *C. bonplandianus*

**DPPH free radical scavenging activity**

The DPPH radical scavenging activity of crude extract and fractions was found to be concentration dependent as shown in Figure 1. The standard ascorbic acid showed 98% inhibition with an IC<sub>50</sub> 3.5µg/ml. The ChF showed excellent scavenging activity with an IC<sub>50</sub> 72.13 µg/ml followed by CE (133.79 µg/ml), EaF (450.21 µg/ml) and HeF (602.34 µg/ml).

**Hydroxyl radical scavenging assay**

The hydroxyl radical scavenging activity of the extract and fractions showed concentration dependent activity. The hydroxyl radical scavenging activity of the crude extract and different fractions is shown in Figure 2. Ascorbic acid showed 98% inhibition with an IC<sub>50</sub> value 6.8 µg/ml. The ChF and EaF were found to

possess higher hydroxyl radical scavenging activity with IC<sub>50</sub> of 85.92 µg/ml and 152.91 µg/ml respectively compared to other fractions. The CE (295.25 µg/ml) and HeF (774.81 µg/ml) possessed less hydroxyl radical scavenging activity.

**Reducing power activity**

The radical scavengers in the sample cause reduction of Fe<sup>3+</sup> ions to Fe<sup>2+</sup> and the intensity of formed Prussian blue determine the amount of Fe<sup>2+</sup> read at 700 nm. The samples showed concentration dependent activity. Reducing power activity of the standard, ascorbic acid, at 50µg/ml was found to be 0.700. The order of activity were found to be EaF (0.79) > ChF (0.68) > CE (0.60) > HeF (0.45) (Figure 3).

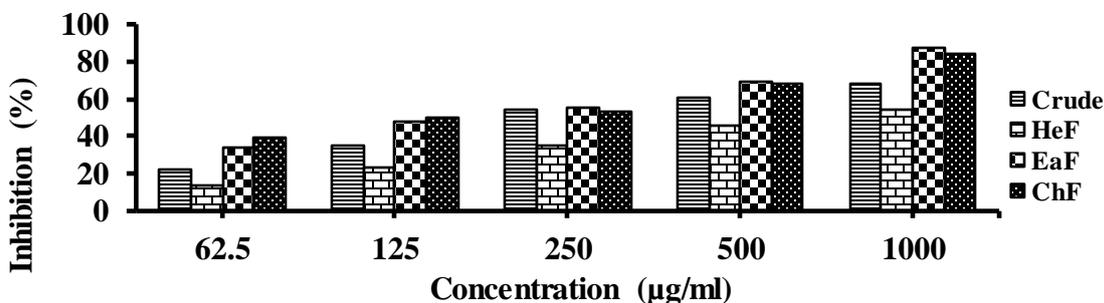


Figure 2. Hydroxyl radical scavenging assay of extract and fractions of *C. bonplandianus*

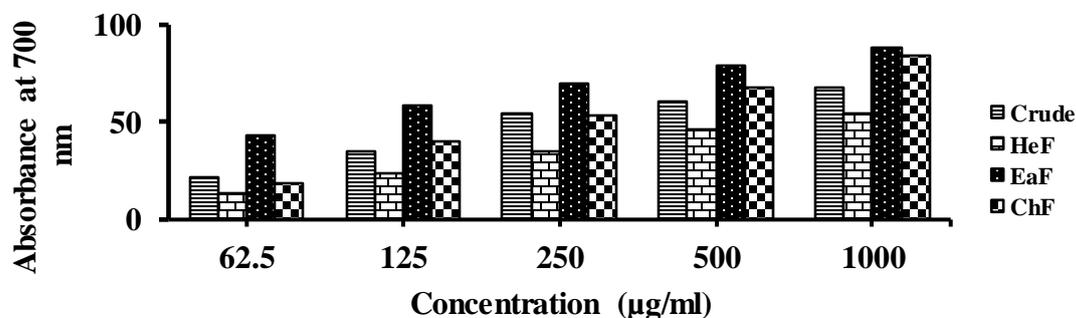


Figure 3. Reducing power capacity of extract and fractions of *C. bonplandianus*

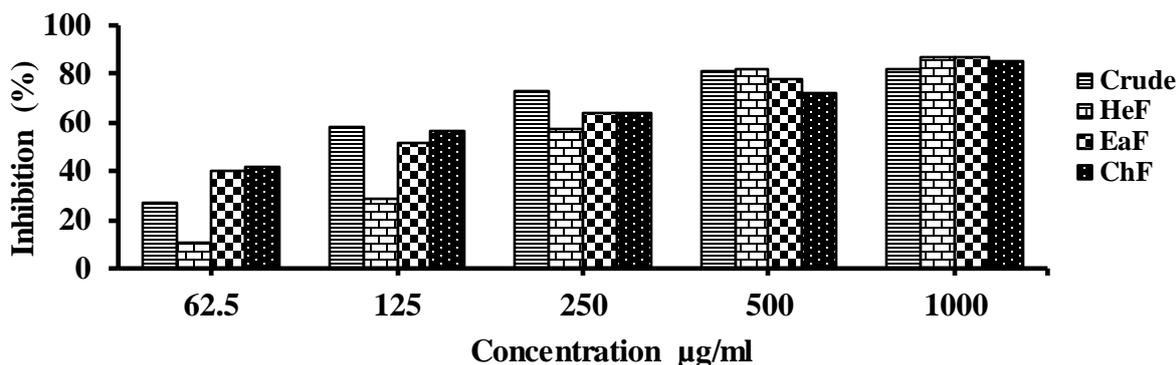


Figure 4. Nitric oxide scavenging assay of extract and fractions of *C. bonplandianus*

**Nitric oxide scavenging assay**

Sodium nitroprusside spontaneously generates nitric oxide at physiological pH, while the nitric oxide scavengers compete with oxygen to reduce the production of nitric oxide. The nitric oxide scavenging activity of the extract and fractions showed concentration dependent activity (Figure 4). The IC<sub>50</sub> value of the standard was found to be 12.6 µg/ml. The ChF (33.80 µg/ml), EaF (57.25 µg/ml) and CE (75.3 1 µg/ml) possessed higher nitric oxide scavenging activity and the HeF (344.81 µg/ml.) had less scavenging activity.

**Alpha amylase inhibitory assay**

Diabetic patients have increased plasma blood glucose level after food consumption. Thus inhibiting the activity of alpha amylase could reduce the glucose absorption in the body, thereby lowering the plasma glucose level. Apart from the CE, different fractions of *C. bonplandianus* were assessed for the alpha amylase inhibitory activity. Acarbose, standard alpha amylase inhibitor drug, showed 92% inhibitory activity with an IC<sub>50</sub> value of 84.50 µg/ml. The ChF (IC<sub>50</sub> value of 95.78 µg/ml and CE (IC<sub>50</sub> of 105.26 µg/ml) were shown to have higher inhibitory activity with 92% and 90% respectively (Figure 5). The EaF (IC<sub>50</sub> of 176.48 µg/ml) and HeF (IC<sub>50</sub> of 242.48 µg/ml) was

shown to possess less inhibitory effect on the amylase.

**α-glucosidase inhibitory activity**

The *in vitro* α-glucosidase inhibitory activity of the crude extract and other fractions exhibited concentration dependent activity (Figure 6). A carbose showed the highest inhibitory effect of 97% (IC<sub>50</sub>107.24 µg/ml). The ChF was shown to possess the highest activity with an IC<sub>50</sub> value of 126.81 µg/ml followed by EaF (157.89 µg/ml). CE and HeF showed the least inhibitory activity with IC<sub>50</sub> 679.04 µg/ml and 463.05 µg/ml respectively.

**GC-MS analysis**

The GC-MS study revealed the presence of a wide range of phytochemicals which may be responsible for the antioxidant and anti-diabetic activity of ChF. ChF was found to contain phenol 2,4-bis(1,1-dimethyl) (4.94%), cyclotetracosane (2.54%), Z-5-nonadecene (3.50%), N-Nonadecenol-1 (2.54%) and 3-eicosene. Phenol,2,4-bis(1,1-dimethyl) was reported to possess antioxidant activity. Cyclotetracosane has been found to have an efficient α- amylase inhibitory activity (Table 3).

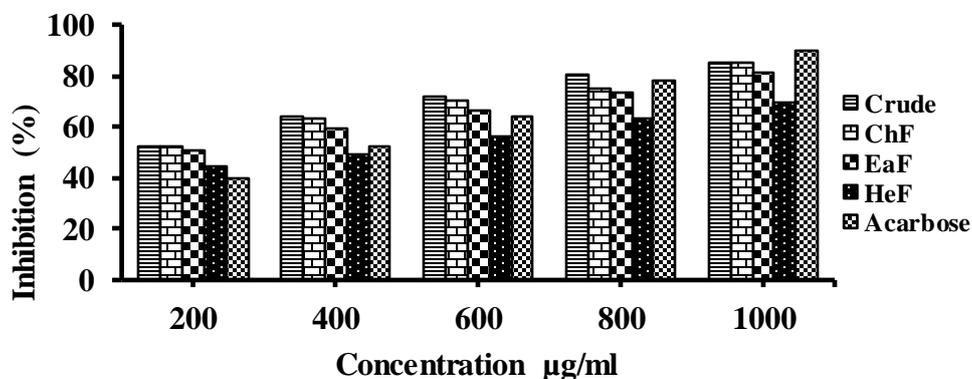


Figure 5. Alpha amylase inhibitory assay of extract and fractions of *C. bonplandianus*

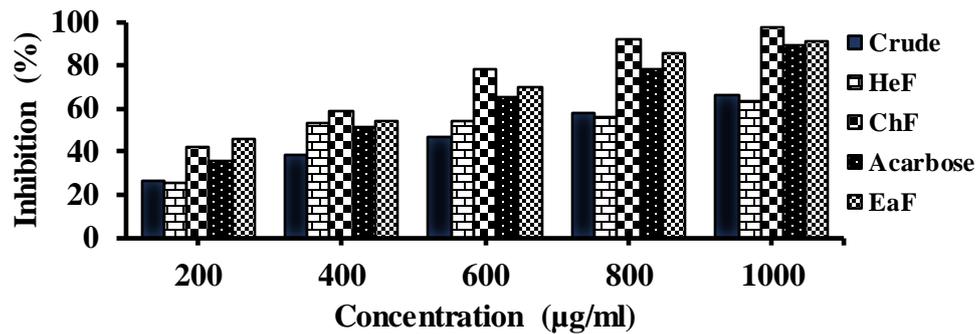
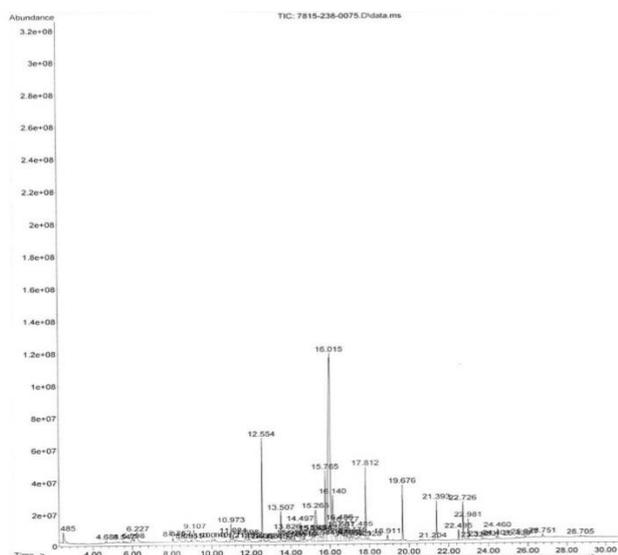


Figure 6. Alpha glucosidase inhibitory assay of extract and fractions of *C. bonplandianus*

Table 3. Constituents of chloroform extract determined by GC-MS

Name of the compound	Retention time	Area (%)
	17.813	3.50
Z-5-Nonadecene	21.394	2.10
	19.678	2.54
	22.984	1.05
Cyclotetracosane	21.394	2.10
	22.984	2.54
N-Nonadecenol-1	19.678	2.54
Cycloeicosane	17.813	3.50
3-Eicosene	17.813	3.50
Z-8—Hexadecene	15.763	4.55
6-5Heptadecenal	21.394	2.10
Phenol,2,4-bis(1,1-dimethyl)	12.554	4.94



## Discussion

Herbal formulations have been in practice since time immemorial and offer effective treatment against several diseases including diabetes. There exists excellent literature in Ayurveda to cure diabetes with medicinal plants. A large number of medicinal plants have already been studied for their anti-diabetic properties. Controlling blood sugar level is

one of the effective approaches currently used to reduce the post-prandial hyperglycaemia (PPHG). One therapeutic approach to reduce glucose absorption involves the inhibition of carbohydrate digesting enzymes,  $\alpha$ -amylase and  $\alpha$ -glucosidase.  $\alpha$ -Amylase catalyses the hydrolysis of 1,4-glycosidic linkage in polysaccharides while  $\alpha$ -glucosidase hydrolyses disaccharides to monosaccharides. Hence, retardation of starch digestion, by inhibiting these enzymes, prevents glucose entry into the systemic circulation and helps in controlling diabetes (21). In the present study, anti-hyperglycaemic and antioxidant activities of HeF, EaF and ChF of the *C. bonplandianus* leaves were studied. Among the different fractions studied, ChF and EaF have been shown to possess potent inhibitory activity against the pancreatic enzymes,  $\alpha$ -amylase and  $\alpha$ -glucosidase. This could be correlated with the content of secondary metabolites such as polyphenols ( $114.28 \pm 0.05 \mu\text{g/ml}$  &  $28.57 \pm 0.03 \mu\text{g/ml}$ ), tannins ( $63.8 \pm 0.009 \mu\text{g/ml}$  &  $28.9 \pm 0.007 \mu\text{g/ml}$ ) and flavonoids ( $95.68 \pm 0.03 \mu\text{g/ml}$  &  $43.08 \pm 0.01 \mu\text{g/ml}$ ). The  $\text{IC}_{50}$  value for  $\alpha$ -amylase and  $\alpha$ -glucosidase was found to be  $95.78 \mu\text{g/ml}$  and  $126.808 \mu\text{g/ml}$  (ChF) and  $105.26 \mu\text{g/ml}$  and  $157.887 \mu\text{g/ml}$  (EaF) respectively. Acarbose, used as a positive control revealed 50% inhibition at  $84.50 \mu\text{g/ml}$  for  $\alpha$ -amylase and  $107.24 \mu\text{g/ml}$  for  $\alpha$ -glucosidase. The  $\alpha$ -amylase inhibitory activity of the fraction and the extract was found to be concentration dependent. Polyphenols in plants have been reported to exert antioxidant activity and carbohydrate enzyme inhibiting activity, which aid in lowering PPHG in the management of diabetes (22, 23). The formation of hydrogen bonds between the residues of the active site of amylase with the hydroxyl groups in polyphenols is believed to be in-charge of the inhibitory activity of the metabolite (24). The hypoglycaemic effect of polyphenols could be attributed to the reduction in dietary carbohydrates absorption, by modulating the glucose metabolizing enzymes, increased  $\beta$ -cell function and secretion of insulin (25). These polyphenols also scavenge reactive oxygen species and prevent cell death (26) by increasing the endogenous antioxidative system, thereby improve oxidant/antioxidant balance and attenuate oxidative stress (27).

Since an ideal diabetic drug should possess antihyperglycaemic and antioxidant properties (23), the fractions were also tested for their *in vitro* antioxidant properties through DPPH, nitric oxide scavenging, hydroxyl radical scavenging and reducing power assay. Oxidative stress due to free radical generation and reduced antioxidant activity leads to tissue damage that may further result in atherosclerosis, cancer, coronary heart disease and diabetes (28). Reducing the free radical generation with scavengers will further protect against the diabetes-induced complications. Based on the study, ChF showed excellent free radical scavenging property ( $IC_{50}$ -72.13  $\mu$ g/ml) followed by CE ( $IC_{50}$ -133.79  $\mu$ g/ml) towards DPPH. Likewise, ChF showed good activity against hydroxyl radical ( $IC_{50}$ -85.92  $\mu$ g/ml), nitric oxide ( $IC_{50}$ -33.80  $\mu$ g/ml) and reducing activity (0.88 OD at 700 nm). This property may be attributed to the presence of polyphenols and flavonoids present in the ChE.

Amongst the polyphenols investigated, flavonoids have been shown to possess highest inhibitory activity, which could be attributed to the number of hydroxyl groups on the B ring of the flavonoid skeleton. Sales *et al.* and Robert K *et al.* demonstrated that the antioxidant activity of flavonoids is mainly due to its reaction with nitric acid and reduction of formation of peroxynitrite, which otherwise oxidizes directly resulting in irreversible damage to cell membrane (29, 30). Hence, it could be speculated that flavonoids present in the plant can be used in the treatment of oxidative stress induced diseases. Therefore, the relationship between this antihyperglycaemic effect and antioxidant activity can be correlated with the polyphenol content ( $114.28 \pm 0.05$   $\mu$ g/ml) of the ChF. In the present study result, ChF fraction was found to contain  $95.68 \pm 0.03$   $\mu$ g/ml of flavonoids, showing potent nitric oxide scavenging activity. The potent inhibitory activity of ChF fraction against  $\alpha$ -amylase and  $\alpha$ -glucosidase compared to other fractions could be due to high polyphenols content and the antioxidant potential.

Since ChF exhibited potent activity against  $\alpha$ -amylase and  $\alpha$ -glucosidase, GC-MS analysis was carried out which revealed the presence of compounds with numerous biological properties. For instance, phenol, 2, 4-bis(1,1-dimethyl) a phenolic compound has shown good antioxidant activity (31) and cyclotetracosane present in ChF showed  $\alpha$ -amylase inhibitory activity in other studies (32). Eicosene, a fatty acid in the *Allium atrovioleaceum* flower was found to possess antimicrobial activity and antioxidant activity (33).

In diabetic condition, hyperglycaemia is a result of high liver glycogenolysis and gluconeogenesis which reduces the glucose uptake by cells. The glucosidase inhibitors inhibit the glycogen-debranching enzymes,  $\alpha$ -1,6-glucosidase, in the liver which lowers the rate of glycogenolysis thereby increasing the accumulation of glycogen stores in the liver (34).

The plant derived  $\alpha$ -amylase and  $\alpha$ -glucosidase blockers may offer a potential therapeutic approach for the control of PPHG and also diabetes. In this study, the ChF and EaF fraction of *C. bonplandianum* showed significant  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity compared to acarbose. This plant could be a prospective alternative complementary medicine in the management of diabetes.

## Conclusion

The present study aimed to determine the anti-hyperglycaemic and antioxidant activity of the crude methanol extract and its sub-fractions of *Croton bonplandianus*. The ChF was shown to possess potent  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity with antioxidant activities compared to other fractions. These potent activities could be related with the phenolic content of the chloroform extract. In future, isolating and identifying the lead compound from the fraction could make a significant contribution in the treatment of diabetes mellitus.

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## References

1. Chauhan A, Sharma PK, Srivastava P, Kumar N, Dudhe R. Plants having potential antidiabetic activity: a review. *Der Pharm Lett* 2010; 2:369-387.
2. World Health Organization. World health statistics. Geneva: World Health Organization; 2013.
3. Bhutkar MA, Bhise SB. *In vitro* assay of alpha-amylase inhibitory activity of some indigenous plants. *Int J ChemSci* 2012; 10:457-462.
4. Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus in India. *Australas Med J* 2014; 7:45-48.
5. Sangeetha R, Vedaasree N. *In vitro*  $\alpha$ -amylase inhibitory activity of the leaves of *Thespesiapopulnea*. *ISRN Pharmacol* 2012; 2012:515634.
6. Gomathi D, Kalaiselvi M, Uma C. *In vitro*  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory effects of ethanolic extract of *Evolvulusalsinoides* (L.). *Int Res J Pharmacy* 2012; 3:226-229.
7. Mohamed EA, Siddiqui MJ, Ang LF, Sadikun A, Chan SH, Tan SC, *et al.* Potent  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activities of standardized 50% ethanolic extracts and sinensetin from *Orthosiphonstamineus* Benth as antidiabetic mechanism. *BMC Complement Altern Med* 2012; 12:176-182.
8. Vadlapudi V, Naidu KC. *In vitro* antimicrobial screening of selected Indian medicinal plants. *Int J Appl Biol Pharm* 2010; 1:302-307.
9. Divya S, Naveen Krishna K, Ramachandran S, Dhanaraju MD. Wound healing and *in vitro* antioxidant activities of

- Croton bonplandianus* leaf extract in rats. *Global J Pharmacol* 2011; 5:159-163.
10. Qaisar M, Chaudhary BA, Sajid MU, Hussain N. Evaluation of  $\alpha$ -glucosidase inhibitory activity of dichloromethane and methanol extracts of *Croton bonplandianum* Baill. *Trop J Pharm Res* 2014; 13:1833-1836.
  11. Keerthana G, Kalaivani MK, Sumathy A. *In vitro* alpha amylase inhibitory and anti-oxidant activities of ethanolic leaf extract of *Croton bonplandianus*. *Asian J Pharm Clin Res* 2013; 6:32-36.
  12. Majekodunmi SO. Review of extraction of medicinal plants for pharmaceutical research. *Merit Res J Med MedSci* 2015; 3:521-527.
  13. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. *J Pharm Sci* 2011; 1:98-106.
  14. Jain A, Sharma P, Sunil K. Evaluation of phenolic and flavonoid profile and screening of antioxidant activity of the plant *Croton sparsiflorus* by bio-autographic method. *J Pharm Res* 2010; 3:1146-1148.
  15. Schanderl SH. In methods in food analysis. New York: Academic Press; 1970. P. 709.
  16. Leong LP, Shui G. An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chem* 2002; 76:69-75.
  17. Halliwell B, Gutteridge JM. The definition and measurement of antioxidants in biological systems. *Free Radica Biol Med* 1995; 18:125-126.
  18. Sre P, Sheila T, Murugesan K. Phytochemical screening and *in vitro* anti-oxidant activity of methanolic root extract of *Erythrina indica*. *Asian Pac J Trop Biomed* 2012; 2:S1696-S1700.
  19. Kazeem MI, Adamson JO, Ogunwande IA. Modes of inhibition of  $\alpha$ -Amylase and  $\alpha$ -glucosidase by aqueous extract of *Morinda lucida* Benth leaf. *BioMed Res Int* 2013; 2013:1-6.
  20. Bachhawat JA, Shihabudeen MS, Thirumurugan K. Screening of fifteen Indian ayurvedic plants for  $\alpha$ -glucosidase inhibitory activity and enzyme kinetics. *Int J Pharm PharmSci* 2011; 3:267-274.
  21. Cheng AY, Fantus IG. Oral antihyperglycemic therapy for type 2 diabetes mellitus. *CMAJ* 2005; 172:213-226.
  22. De Sousa E, Zanatta L, Seifriz I, Creczynski-Pasa TB, Pizzolatti MG, Szpoganicz B, et al. Hypoglycemic effect and antioxidant potential of Kaempferol-3,7-O-( $\alpha$ -D-glucopyranoside) from *Bauhinia forficata* Leaves. *J Nat Prod* 2004; 67:829-832.
  23. Mai TT, Fumie N, Chuyen NV. Antioxidant activities and hypolipidemic effects of an aqueous extract from flower buds of *Cleistocalyx operculatus* (Roxb.) Merr. and Perry. *J Food Biochem* 2009; 33:790-807.
  24. Perera HK, Premadasa WK, Poongunran J.  $\alpha$ -glucosidase and glycation inhibitory effects of *Costus speciosus* leaves. *BMC Complement Altern Med* 2016; 16:2.
  25. Bahadoran Z, Golzarand M, Mirmiran P, Saadati N, Azizi F. The association of dietary phytochemical index and cardiometabolic risk factors in adults: Tehran Lipid and Glucose Study. *J Hum Nutr Diet* 2013; 26:145-153.
  26. Fukuda T, Ito H, Yoshida T. Effect of the walnut polyphenol fraction on oxidative stress in type 2 diabetes mice. *Biofactors* 2004; 21:251-253.
  27. Pérez-Matute P, Zulet MA, Martínez JA. Reactive species and diabetes: counteracting oxidative stress to improve health. *Curr Opin Pharmacol* 2009; 9:771-779.
  28. Gundimeda U, McNeill TH, Elhiani AA, Schiffman JE, Hinton DR, Gopalakrishna R. Green tea polyphenols precondition against cell death induced by oxygen-glucose deprivation via stimulation of laminin receptor, generation of reactive oxygen species, and activation of protein kinase C. *J Biol Chem* 2012; 287:34694-34708.
  29. Sales PM, Souza PM, Simeoni LA, Silveira D.  $\alpha$ -Amylase inhibitors: a review of raw material and isolated compounds from plant source. *J Pharm PharmSci* 2012; 15:141-183.
  30. Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA. Flavonoids: a review of probable mechanisms of action and potential applications. *Am J Clin Nutr* 2001; 74:418-425.
  31. Lee YS, Kang MH, Cho SY, Jeong CS. Effects of constituents of *Amomum xanthioides* on gastritis in rats and on growth of gastric cancer cells. *Arch Pharm Res* 2007; 30:436-443.
  32. Dehpour AA, Babakhani B, Khazaeiand S, Asadi M. Chemical composition of essential oil and antibacterial activity of extracts from flower of *Allium atroviolaceum*. *J Med Plants Res* 2011; 5:3667-3672.
  33. Tundis R, Menichini F, Loizzo MR, Bonesi M, Solimene U, Menichini F. Studies on the potential antioxidant properties of *Seneciostabianus* Lacaita (Asteraceae) and its inhibitory activity against carbohydrate-hydrolysing enzymes. *Nat Product Res* 2012; 26:393-404.
  34. Bhat M, Zinjarde SS, Bhargava SY, Kumar AR, Joshi BN. Antidiabetic Indian plants: a good source of potent amylase inhibitors. *Evid Based Complement Alternat Med* 2011; 2011:810207.