

Antioxidant, α -glucosidase and 15-lipoxygenases inhibitory activities of *Cassia singueana* Del. and *C. sieberiana* DC. (Fabaceae)

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Summary

The present study investigates the antioxidant, α -glucosidases, and anti-inflammatory properties of leaf extract of *Cassia singueana* and *C. sieberiana*. *Cassia singueana* and *C. sieberiana* are restorative plants utilised for the treatment of diabetes, ulcer, malaria, and wound healing. The extracts were evaluated for antioxidant activities via DPPH radical scavenging, ABTS⁺ radical cation scavenging, FRAP, β -carotene bleaching, phenolic content, and flavonoid content. Meanwhile, α -glucosidases and anti-inflammatory properties were also evaluated against α -glucosidase enzyme and 15-LOX inhibitory activities. The results for *C. singueana* extract revealed potent activities for DPPH (IC₅₀: 9.35 μ g/mL), phenolic contents (120.01 mg GAE/g dry extract), and α -glucosidases inhibition (I%: 90.47%). On the other hand, the findings for *C. sieberiana* recorded significant activities for FRAP (2120 μ mol Fe²⁺/g dry extract) and 15-LOX inhibition (I%: 80.93%). The extract of *C. singueana* and *C. sieberiana* contain possible antioxidant, α -glucosidases, and anti-inflammatory agents that are responsible for the popular use of these plants in the treatment of diabetes and ulcer.

Key words

Cassia singueana, *Cassia sieberiana*, antioxidant, anti-inflammatory, anti-diabetic, Fabaceae

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Introduction

Free radicals or reactive oxygen species (ROS) are produced as by-products of metabolic processes by all aerobic organisms (Ogino and Wang, 2007). However, it is important to note that antioxidants are defence mechanism that are accountable of protecting the cells and tissues against damages caused by the free radicals. Oxidative stress tends to result in oxidative damage to cellular components, including proteins, lipids, DNA, and sugars, which is mainly caused by the imbalance between free radicals and antioxidants in the human body (Durackova, 2010). Meanwhile, free radicals that are normally produced in limited quantity by the body are important for the regulation process that involves the sustenance of cell functions such as signal transduction, activation of receptors, and gene expression (Goossens et al., 2009). On the other hand, it should be noted that excessive production of free radicals in cells initiates the release of inflammatory signals (Salzano et al., 2014). In particular, inflammation can occur as a result of the overproduction of leukotriene (LT) via the enzymatic pathway such as 15-lipoxygenase pathway (El-Guendouz et al., 2016). On a similar note, the overproduction of free radicals has also been discovered to induce severe brain cell damage that leads to diabetes (Reagan et al., 1999). Hence, oxidative stress and chronic inflammatory cell damage have been connected in a wide range of human diseases, which include diabetes, neurodegenerative disorder, atherosclerosis, cancer, and inflammatory diseases (Aruoma et al., 2006).

Diabetes mellitus (DM) is caused by the dysfunction of β -cells that causes an abnormal rise in glucose level in the blood. In response to this matter, one of the strategies adopted to stabilise the abnormal rise in blood glucose is the inhibition of the enzyme known as α -glucosidase that is responsible for both the release and absorption of free glucose into the blood system. Nevertheless, it is crucial to note that the use of most drugs in the treatment of DM and other inflammatory-related diseases have been identified with side effects (Biswas et al., 2016). A considerable amount of studies has reported the relationship between oxidative stress, DM, and inflammation by stating that the antioxidants can significantly inhibit or delay the oxidation process of substances (Prabhakar, 2013). Hence, antioxidants that are capable of inactivating free radicals may be an effective strategy for the prevention and treatment of various oxidative stress-related diseases such as DM and inflammatory diseases. Moreover, synthetic antioxidants have been reported with potential health risks, which further highlights the importance of searching for effective antioxidants that originate from a natural product (Stephanie et al., 2009).

Cassia singueana Del. and *C. sieberiana* DC. are medicinal plants native to Africa that have been diversely applied as traditional medicine. In particular, they are used in the treatment of diabetes mellitus, ulcer, malaria, constipation, diarrhoea, fever, and skin disorder (Schmelzer and Gurib-Fakim, 2008; Onakpa and Ode, 2010; Shahidul Islam and Mohammed, 2017; Kelechi and Favour, 2015).

More importantly, previous studies have shown that both *C. singueana* and *C. sieberiana* possess antioxidant, antimalarial, anti-diarrheal, and anti-ulcer activities (Saidu et al., 2011; Ifeanyi and Ode, 2012; Abdulrazak et al., 2015; Gideon et al., 2015; Bello et

al., 2016; Jibril et al., 2017a). On another note, phytochemicals that include anthraquinones, triterpenes, sterols, and flavonoids have been discovered in both *Cassia* species, while stilbene was only found in the root of *C. sieberiana* (Jibril et al., 2017b; Ode and Asuzu, 2014). In addition, it is crucial to note that these phytochemicals often display different biological activities such as antioxidant, anti-inflammatory, antimicrobial, anticholinesterase, and anticancer properties (Cai et al., 2006). Furthermore, the overall pharmacological effect of the plant extract might be caused by the individual or synergistic effects of the phytochemicals. As a result, it is necessary to screen the plant extract on various assays in order to avoid losing some other potential bioactivities of the plant. More importantly, it should be noted that research on α -glucosidase and 15-lipoxygenases inhibitory activity of the leaf extracts from both *Cassia* species have not been reported in the literature despite the wide application of *C. singueana* and *C. sieberiana* leaf extract as a remedy to treat diabetes, ulcer, skin disorder, constipation as well as wound healing.

Regarding this matter, several methods have been developed to evaluate the antioxidant potentials of plant extracts that include 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging, ferric reducing antioxidant power (FRAP), and β -carotene bleaching assays (Huang et al., 2005). In the current research, the methanol leaf extract of *C. singueana* and *C. sieberiana* were evaluated for antioxidant properties through different biochemical assays: DPPH scavenging, ABTS, FRAP, β -carotene bleaching, total phenolic content, and total flavonoid content. Apart from that, the present study also examined the α -glucosidase and 15-lipoxygenase enzymes inhibitory activities of the methanol leaf extracts of both plants.

Material and Methods

General experimental procedures

β -carotene was purchased from Fluka, ascorbic acid was purchased from GCE Laboratory Chemicals. 2,2'-Diphenyl-1-picrylhydrazyl (DPPH), 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), linoleic acid, 2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ), were purchased from Calbiochem. Folin-Ciocalteu's phenol, gallic acid, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), acarbose, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), *p*-nitrophenyl- α -D-glucopyranoside (*p*-PNG), and quercetin were all purchased from Sigma Aldrich. α -Glucosidase was obtained from Megazyme, while 15-lipoxygenase was purchased from Cayman Chemical Co.

Plant materials

The leaves of *C. singueana* and *C. sieberiana* were collected in January 2016, from Bauchi State, Nigeria. Both plants were identified by Mr. Baha'uddeen Said Adam of the Department of Plant Biology, Bayero University Kano. Voucher specimen, BUKHAN0316, and BUKHAN0065 have been deposited for *C. singueana* and *C. sieberiana* respectively, at the Herbarium of Department of Plant Biology, Bayero University Kano, Nigeria.

Extraction and isolation

The air-dried and powdered leaf of *C. singueana* (500 g) and *C. sieberiana* (400 g) were extracted in methanol (each 3×4 L) at room temperature. The extract was filtered and concentrated under reduced pressure to yield the methanol leaf extract for *C. singueana* (CALM, 12 g, 2.40%) and *C. sieberiana* (CBLM, 7 g, 1.75%). The crude extracts were stored in the refrigerator before screening for antioxidant, α -glucosidase, and 15-lipoxygenases inhibitory activities.

Antioxidant capacity assay

DPPH free radical scavenging

The DPPH radical scavenging ability of the extract was estimated as previously reported in the literature with minor modification (Wu et al., 2015; Salleh et al., 2015a). The methanolic DPPH radical solution (80 mg/mL) was prepared in the dark and diluted to an absorbance of 0.07 (± 0.02) at 517 nm. An aliquot (100 μ L) of each test sample (eight serial dilutions) was added to the DPPH radical solution (100 μ L) in a 96-well plate. The sample was allowed to react with DPPH radical for 30 min in dark and the absorbance value (A) was recorded against a blank DPPH at 517 nm from a spectrophotometer. Butylated hydroxyanisole and BHT were used as a positive control. The experiment was performed in three replicates. The inhibition of radical scavenging activity in percent (I%) was calculated according to the equation below:

$$I\% = [A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}] \times 100$$

A_{blank} = absorbance of the blank solution (containing radical solution without sample); A_{sample} = absorbance of a sample solution. The IC_{50} value is the effective concentration that could scavenge 50% of the two radicals against the concentration of the samples.

ABTS⁺ radical cation scavenging

The ABTS⁺ radical cation was determined according to a reported method (Zhi-Gang et al., 2010; Salleh and Ahmad, 2016). The ABTS⁺ radical cation was prepared by reacting equal volume of the ABTS aqueous solution (7 mM) and $K_2S_2O_8$ (2.45 mM) in the dark and allowed to incubate for approximately 12 - 16 hours. This ABTS⁺ radical cation solution was diluted to give an absorbance of 0.70 (± 0.02) at 734 nm before use. The sample (100 μ L) was added to the ABTS⁺ radical cation solution (100 μ L) in a 96-well plate. The sample mixture was kept for 6 min in dark and the absorbance value (A) was recorded against a blank ABTS⁺ radical cation at 734 nm from a spectrophotometer. Trolox was used as positive control for the ABTS⁺ radical cation. The experiment was performed in three replicates. The inhibition of ABTS radical scavenging activity in percent (I%) was calculated according to the equation below:

$$I\% = [A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}] \times 100$$

A_{blank} = absorbance of the blank solution (containing radical solution without sample); A_{sample} = absorbance of a sample solution. The IC_{50} value was taken as the effective concentration that could scavenge 50% of the two radicals against the concentration of the samples.

Ferric reducing assay

The ferric reducing antioxidant power (FRAP) was measured as described in the literature with slight modification (Salleh et al., 2015b). The working FRAP reagent was prepared with TPTZ (5 mL, 10 mM), $FeCl_3$ (5 mL, 20 mM) and sodium acetate buffer (50 mL, 300 mM, pH = 3.6). The freshly prepared FRAP reagent (3.0 mL) was mixed with a sample solution (100 μ L) and deionized water (300 μ L). The reaction mixture was incubated for 30 min at 37°C in a water bath. The absorbance was recorded at 595 nm. A standard solution of Fe^{2+} was used to prepare a calibration curve. The FRAP values were expressed as μ mol Fe^{2+} /g dry extract. In this assay, all solutions were used on the day of preparation.

β -Carotene bleaching assay

The antioxidant activity of the extract was evaluated using the method described in the literature with slight modification (Salleh et al., 2016a). β -carotene (2.0 mg) was dissolved in chloroform (10 mL). This β -carotene solution (1.0 mL) was pipetted into a flask (100 mL) containing linoleic acid (20 mg) and tween 40 (0.2 g). Chloroform was removed under vacuum at 40°C for 10 mins. After evaporation, distilled water (100 mL) was slowly added to the mixture with vigorous shaking to form an emulsion. An aliquot (2 mL) of the emulsion was added to the sample solution (0.2 mL) in a test tube and absorbance was immediately measured at 470 nm against a blank. The blank consist of solvent (0.2 mL) instead of the sample solution. The test tubes were placed on a water bath at 50°C for 2 h. the absorbance of the solution was monitored every 20 mins up to 120 min at 470 nm. The results were based on two different parameters of the antioxidant activity (AA%) and the oxidant rate ratio (R_{OR}). The antioxidant activity (AA%) and the oxidant rate ratio (R_{OR}) was calculated according to the equation below:

$$AA\% = [R_{\text{blank}} - R_{\text{sample}} / R_{\text{blank}}] \times 100$$

where R_{sample} and R_{blank} = bleach rate of β -carotene with or without sample, respectively. Degradation rate (R_D) was calculated according to the following equation:

$$R_D = \ln [A_{t=0} / A_{t=x}] \times 1/t$$

where $A_{t=0}$ = absorbance at 470 nm and $t = 0$ and $A_{t=x}$ is the absorbance at 470 nm at $t = 20, 40, 60, 80, 100, 120$ min respectively. The oxidation rate ratio (R_{OR}) was calculated by the equation;

$$R_{OR} = (R_{\text{sample}} / R_{\text{blank}}).$$

Total phenolic content

The total antioxidant content was determined according to a previously published method (Salleh et al., 2015c). Briefly, 10 mg of each extract were dissolved separately in 10 mL of distilled water. Diluted sample (100 μ L) and Folin-Ciocalteu reagent (50 μ L) were mixed together properly. The mixture was kept for five minutes after which 150 μ L of Na_2CO_3 (20%, w/v) was added. After incubation of the mixture, for 60 minutes at room temperature, the absorbance was measured at 765 nm by a spectrophotometer. A standard solution of gallic acid was used to prepare the calibration curve. The total phenolic content was calculated and expressed as milligram gallic acid equivalent/ g dry extract (mg GAE/ g dry extract).

Total flavonoid content

The total flavonoid content was determined by the aluminium chloride colorimetric method with slight modification (Zhi-Gang et al., 2010). The test sample (0.1 mL) was diluted with 0.4 mL of distilled water in a volumetric flask and 0.03 mL of NaNO_2 (5.0%, w/v) was added. After five minutes, 0.03 mL of AlCl_3 (10%, w/v) was added. The mixture was incubated for six minutes, followed by addition of 0.2 mL of NaOH (1.0 M) and 0.24 mL of deionized water. The mixture was shaken vigorously and the absorbance was measured against a blank at 510 nm. A standard solution of quercetin was used to prepare a calibration curve. The results were expressed as milligram quercetin equivalent/ g dry extract (mg QE/g dry extract).

α -Glucosidase inhibitory activity

The α -glucosidase inhibitory assay was conducted according to the previously described methods with slight modifications (Lee et al., 2014; Aminudin et al., 2015). Briefly, 10 μL of the sample with a concentration of 100, 80, 60, 40, 20, 10 and 1 μM was added to 130 μL of potassium diphosphate buffer (30 mM, pH 6.5) and 10 μL α -glucosidase enzyme solution (Maltase-2 U/mL) in the 96-well plate. The mixture was incubated for 20 min at 37°C before the addition of *p*-nitrophenyl- α -D-glucopyranoside (*p*-NPG) (50 μL , 1 mM). The mixture was incubated again for 20 min at 37°C in the dark. The reaction was quenched by adding of sodium carbonate solution (50 μL , 0.2 M) and the absorbance was then recorded at 405 nm by measuring the quantity of *p*-nitrophenol released from *p*-NPG. Acarbose and quercetin were employed as the positive control for this assay. The percentage of inhibition was calculated as a percentage relative to a control. Inhibition concentration was calculated according to the following equation:

$$\text{I\%} = [A_{\text{Control}} - (A_{\text{Sample}} - A_{\text{Blank sample}})] / A_{\text{Control}} \times 100$$

where A_{Sample} is the absorbance of the reaction mixture of the test sample, $A_{\text{Blank sample}}$ is the absorbance of the reaction mixture containing all reagents except enzyme and A_{Control} is the absorbance of the reaction mixture containing all reagents except the test sample.

15-Lipoxygenase inhibitory assay

The reagents for 15-lipoxygenase were prepared according to the standard protocol (Lipoxygenase inhibitor screening assay kit, Item No. 760700: Cayman Chemicals) (Shaveta et al., 2014; Salleh et al., 2016b). The assay buffer (0.1 M Tris-HCl, pH 7.4) was prepared by diluting the stock buffer solution in tenfold. Chromogen which served as the indicator was prepared by mixing equal volume of the developing reagents 1 and 2 followed by vortexing. The solution was used within one hour of preparation. The 15-lipoxygenase (soybean) (10 μL) was diluted with the assay buffer (990 μL) and stored on ice. It was used within one hour. Arachidonic acid (25 μL), potassium hydroxide (25 μL , 0.1 M) and HPLC-grade water (950 μL) were all mixed thoroughly and used as the substrate. This prepared arachidonic acid solution (1 mM) was used within 30 minutes. Test samples were prepared in methanol to obtain a working concentration of 0.1 mg/mL in the respective wells. The sample solution (10 μL) was added to the lipoxygenase enzyme (90 μL), then the reaction was initiated by the addition

of arachidonic acid (10 μL). The plate was then shaken for five minutes on an orbital shaker, and chromogen solution (100 μL) was added to all wells in a dark condition to stop the action of enzyme catalysis. The plate was incubated for five minutes and read at 495 nm. Quercetin was used as a positive control in this assay. Results were expressed as a percentage relative to the initial activity.

$$\text{I\%} = [A_{\text{initial}} - (A_{\text{sample}} - A_{\text{blank sample}})] / A_{\text{initial}} \times 100$$

where A_{initial} activity is the absorbance of reaction mixture containing all reagents except the test sample, A_{sample} is the absorbance of the reaction mixture of the sample and $A_{\text{blank sample}}$ is the absorbance of reaction mixture containing all reagents except enzyme.

Statistical analysis

Experiments were performed in three replicates for each sample and statistical analysis values are reported as mean \pm SD. Standard curves were generated and calculation of the 50% inhibitory concentration (IC_{50}) values was carried out using GraphPad Prism for Windows (version 5.02) software. The Student's t-test was performed using SPSS (version 22) software to observe the comparison between the treatment of samples and untreated control. A value of $p < 0.05$ was considered significantly different.

Results and Discussion

Considering the various medicinal benefits of *Cassia* species, an effort has been made to establish the scientific validity through screening for antioxidant, α -glucosidases, and anti-inflammatory inhibitory activities of methanol leaf extracts of two *Cassia* species. The present study employed various antioxidant assays, which include DPPH, ABTS, FRAP, and β -carotene bleaching assays in order to evaluate the antioxidant capacity of the methanol leaf extract of *C. singueana* and *C. sieberiana*. In particular, the total antioxidant contents were estimated based on the total phenolic content assay and total flavonoid content assay by using Folin-Ciocalteu reagent and aluminium chloride reagent, respectively. Furthermore, it should be noted that DPPH radicals are considered as stable radicals because they have been widely used in the study of free radical-scavenging capacity in natural antioxidant (Yin et al., 2016). The results of the present study revealed that the methanol leaf extract of *C. singueana* contains very strong DPPH radical scavenging activity in a dose-dependent manner with IC_{50} (9.35 $\mu\text{g/mL}$), which is comparable to the standard BHA (IC_{50} 9.50 $\mu\text{g/mL}$) used in the assay. A moderate DPPH radical scavenging ability recorded for the methanol leaf extract of *C. sieberiana* (IC_{50} 59.73 $\mu\text{g/mL}$) is presented in Table 1.

The ABST⁺ radical scavenging significant activity (IC_{50} 5.34 $\mu\text{g/mL}$) ($p < 0.05$) of methanol leaf extract of *C. singueana* was similar with DPPH activity, while *C. sieberiana* showed only moderate ABTS⁺ activity (IC_{50} 68.67 $\mu\text{g/mL}$). In addition, the radical scavenging ability recorded for methanol leaf extract of *C. singueana* and *C. sieberiana* seems to suggest the presence of phenolic compounds in these extracts (Cai et al., 2004). The FRAP assay was performed based on the measurement of the capacity of the extract in reducing Fe^{3+} to Fe^{2+} (Benzie and Strain, 1996).

Table 1. Antioxidant activity of methanol leaf extract of *C. singueana* and *C. sieberiana*

Samples	CALM	CBLM	BHT	BHA	Trolox
DPPH ^a	9.35 ± 0.40	59.73 ± 0.40	8.93 ± 0.20	10.84 ± 0.40	-
ABTS ^a	5.34 ± 0.01	68.67 ± 0.01	-	-	8.03 ± 0.30
FRAP ^b	1302 ± 0.10	2120 ± 0.04	-	-	-
β -Carotene	87.05 ± 0.01	59.27 ± 0.07	85.61 ± 0.03	58.37 ± 0.07	-
R _{OR}	0.1294 ± 0.08	0.4072 ± 0.08	0.1438 ± 0.01	0.4162 ± 0.09	-
TPC ^d	120.10 ± 0.04	78 ± 0.01	-	-	-
TFC ^e	105.8 ± 0.01	84.80 ± 0.20	-	-	-

^aIC₅₀ value in μ g/mL; ^b μ mol Fe²⁺ equivalent per g dry extract; ^c percent (%); ^d mg GAE/g dry extract; ^e mg QE/g dry extract; CALM - *C. singueana* extract; CBLM - *C. sieberiana* extract; BHT - butylated hydroxytoluene; BHA - butylated hydroxyanisole. All values of each data were mean \pm SD (n = 3); p < 0.05; the AA% and R_{OR} values were calculated at time t = 120 min

The FRAP value expressed as μ mol Fe²⁺ equivalents per g dry extract ($y = -0.001x + 2.605$, R² = 0.9976) were obtained by comparing the absorption change in the test sample mixture with those obtained from Fe²⁺ standard calibration curve. Furthermore, the analysis of the metal-chelating properties of the extracts from the methanol leaf extract of *C. singueana* and *C. sieberiana* shows a relatively high metal chelating ability (1302 and 2120 μ mol Fe²⁺ equivalent per g dry extract) for methanol leaf extract of *C. singueana* and *C. sieberiana*, respectively (Table 1).

On another note, the extent of β -carotene bleaching by the linoleate free radical in a system can be hindered and neutralised by the presence of antioxidants in the system (Clarke et al., 2003). Hence, the presence of antioxidants enables the β -carotene to retain their colour due to the slow degradation of β -carotene. On the other hand, the absorbance rapidly decreases as a result of the fast degradation of β -carotene in the sample due to the absence of antioxidant. In the present study, the methanol leaf extract of *C. singueana* demonstrates a low rate of β -carotene degradation (R_{OR}) (0.1294), but higher antioxidant activity (AA%) (87.05%) compared to the standards, BHT (AA% 85.61%) and BHA (AA% 58.37%) antioxidant activities as shown in Table 1. More importantly, this result suggested the possibility of secondary metabolites with abstract-able hydrogen in these extracts. Meanwhile, phenols such as flavonoids are the major constituents that are responsible for the antioxidant property in most plant-derived antioxidants, thus making the estimation of such groups to be informative (Cai et al., 2006). The present study evaluated the total phenolic content (TPC) and total flavonoid content (TFC) of extracts obtained from *C. singueana* and *C. sieberiana*. The standard calibration curve of gallic acid and quercetin were constructed in order to calculate both the TPC and TFC. Specifically, TPC is expressed in mg gallic acid equivalent (GAE) per gram of extract ($y = 0.0003x + 0.0476$, R² = 0.9970), while the TFC is expressed as mg quercetin equivalent (QE) per gram of extract ($y = 0.0002x + 0.0513$, R² = 0.9977). As can be observed in Table 1, methanol leaf extract of *C. singueana* shows higher total phenolic content value (120.1 mg GAE/g dry extract) and total flavonoid content (105.8 mg QE/g dry extract) greater than the methanol leaf extract of *C. sieberiana*. In particular, as shown in Table 1, the significantly higher antioxidant potentials recorded from DPPH, ABTS, FRAP, and a β -carotene assay of the methanol

leaf extract of *C. singueana* could be attributed to the higher TPC and TFC values observed in the leaf extract of *C. singueana*.

Recently, a toxicological study found that the methanol extract of the leaves from this plant is safe (Ode and Asuzu, 2014). Data from the present study suggest that the leaves of both *Cassia* species possess anti-oxidative activities and can be used as a potential alternative medicine for oxidative stress that is related to non-communicable chronic diseases. Meanwhile, it is important to note that α -glucosidase is the key enzyme responsible for the release and absorption of free glucose into the blood. A delay in the release of glucose into the blood could serve as the best means of avoiding postprandial hyperglycaemia (Schneider and Bucar, 2005). In addition, the methanol leaf extract of *C. singueana* and *C. sieberiana* demonstrate stronger α -glucosidase inhibitory activity (90.47% and 88.89%, respectively) compared to the standard acarbose (81.82%) used in the assay. Regarding this matter, the significant activity suggests that the extracts contain a compound(s) with potent α -glucosidase enzyme inhibitory activity. Moreover, the products of 15-LOX (15-lipoxygenase) has been implicated as one of the reasons that cause various human diseases such as diabetes mellitus, ulcer, asthma, skin cancer, rheumatoid arthritis, and Alzheimer's diseases (Schneider and Bucar, 2005). In the 15-LOX assay, the methanol leaf extracts of *C. singueana* and *C. sieberiana* inhibitory activities were 79.10% and 80.93%, respectively, which display significant inhibition against 15-LOX. These activities are comparable to the inhibitory activity for the standard quercetin (83.04%) used in the assay as presented in Table 2.

Table 2. Enzyme inhibitory activity of methanol leaf extract of *C. singueana* and *C. sieberiana*

Samples	α -Glucosidase (I%)	15-LOX (I%)
CALM	90.47 ± 0.01	79.10 ± 0.01
CBLM	88.89 ± 0.01	80.93 ± 0.01
Quercetin ^b	91.24 ± 0.01	83.04 ± 0.01
Acarbose ^b	81.82 ± 0.01	-

^a Data represents mean \pm SD of three replicates; (p < 0.05); ^b positive control; CALM - *C. singueana* extract; CBLM - *C. sieberiana* extract

Conclusions

A considerable amount of studies have agreed about the effectiveness of natural antioxidants in the treatment of diabetes, cancer, cardiovascular, and inflammatory diseases. More importantly, the results of the current research showed that the leaf of *C. singueana* and *C. sieberiana* contain possible antioxidants as well as possess α -glucosidase and 15-lipoxygenase inhibitory potential. Overall, it can be concluded that the bioactivities observed in the present study support the ethnomedicinal uses of both plants in the treatment of diabetes, ulcer, and wound healing.

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