SHORT REPORT

A C G publications

Rec. Nat. Prod. 11:4 (2017) 406-410

records of natural products

Bioassay-Guided Isolation of Antioxidants and *α*-Glucosidase Inhibitors from the Root of *Cassia sieberiana* D.C. (Fabaceae)

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(Received January 03, 2017; Revised March 01, 2017; Accepted March 07, 2017)

Abstract: Bioassay-guided isolation was used to investigate the antioxidants and α-glucosidase inhibitors from extract of root of *Cassia sieberiana*. The ethyl acetate fraction demonstrated strong free radical scavenging (DPPH and $ABTS^+$) and potent α-glucosidase inhibition. The subsequent fractionation and purification of the ethyl acetate fraction using silica gel chromatography and Sephadex LH-20, yielded; islandicin (**1**), chrysophanol (**2**), physcion (**3**), emodin (**4**), quercetin (**5**), kaempferol (**6**), dihydrokaempferol (**7**), and piceatannol (**8**). Quercetin (**5**) showed the most active antioxidant activity with IC_{50} values of 1.58 mM and 1.30 mM against DPPH and $ABTS^+$ radicals, followed by piceatannol (**8**) with IC_{50} values of 3.96 mM and 3.28 mM, which is better than the standard BHT (with IC_{50} value 8.93 mM) and trolox (with IC_{50} value 8.25 mM), for DPPH and $ABTS^+$ radicals scavenging activities, respectively. For the *α*-glucosidase inhibitory assay, quercetin (**5**) and piceatannol (**8**) showed higher potency against *α*-glucosidase with IC_{50} values of 5.73 µM and 7.37 µM respectively, than standard quercetin with IC_{50} value of 9.20 µM and acarbose with IC_{50} value of 14.12 µM. This study presents the first report on the *α*-glucosidase inhibitors from root of *C. sieberiana* and all the compounds are isolated from this source for the first time.

Keywords: *Cassia sieberiana;* antioxidant; phytochemicals; *α*-glucosidase. © 2017 ACG Publications. All rights reserved.

1. Plant Source

Cassia is one of the genera of Fabaceae, well-known for excellent medicinal values in phytochemical and pharmacological researches [1]. *Cassia sieberiana* is native to sub-Sharan Africa and it is popular in the treatment of diabetes and other diseases by traditional herbalist of the region [2]. The flower, fruit, root, leaf, or stem-bark are used as decoction to cure diabetes and other diseases such as hypertension, malaria and convulsion [2-4]. Herein we report the isolation and characterization of chemical constituents from the root of *C. sieberiana*, responsible for the treatment of diabetes and other oxidative stress related diseases. The antioxidant and α-glucosidase activity of the isolated compounds were also reported.

The plant was collected in January, 2016, from Bauchi State, Nigeria. It was identified by Mr. Baha'uddeen Said Adam of the Department of Plant Biology, Bayero University Kano.

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 05/17/2017 EISSN: 1307-6167

A voucher specimen (BUKHAN 0065) has been deposited at the Herbarium of Department of Plant Biology, Bayero University Kano, Nigeria.

2. Previous Studies

Compounds isolated from *C. sieberiana* included myricetin, quercetin-3-*O*-rhamnoside, leucopelargonicol, epicatechol, *β*-sistosterol, stigmasterol [4] and epiafzelechin [5]. Pharmacologically, it has been reported that various extracts of *C. sieberiana* have demonstrated antimicrobial, antifungal, analgesic, anti-ulcerogenic, anti-inflammatory, antiparasitic and antispasmodic effects [6-7]. However, the investigation of root extract of *C sieberiana* for *α*-glucosidase inhibitory activity and isolation of compounds responsible for its α-glucosidase activity have not been reported to date.

3. Present Study

The air-dried and powdered root of *C. sieberiana* (1.0 kg) was extracted with MeOH three times (3 × 4 L) at room temperature. The solvent was removed from the extract under vacuum at 40^{o}C to yield 50 g of crude MeOH extract. The MeOH extract (50 g) was suspended in distilled water and partitioned successively with hexane, ethyl acetate (EtOAc), and butanol (BuOH) to obtain 2.5 g, 20.8 g, and 15.7 g fractions, respectively. The fractions were screened for their total phenolic contents, total flavonoid contents, free radical scavenging (DPPH and ABTS) ability and *α*-glucosidase inhibitory activity.

The EtOAc fraction (20 g) was subjected to VLC (silica gel, 300 g) and eluted with *n*-hexane, *n*-hexane-EtOAc and EtOAc in a gradient manner to give 6 fractions (F1-F6). F2 (5 g) was purified on silica gel CC (110 g) using gradient of *n*-hexane and *n*-hexane-EtOAc to give islandicin (**1**) (10 mg) as reddish powder [8], chrysophanol (**2**) (50 mg) as yellow powder [9] and an orange-yellow solid, physcion (**3**) (30 mg) [9]. F3 (3 g) was subjected to silica gel CC (70 g), then further purified on preparative TLC using *n*-hexane - EtOAc (4:1) to obtain emodin (**4**) (17 mg) as orange-red solid [10]. F5 (4 g) was loaded onto a silica gel CC (100 g), eluted with *n*-hexane-EtOAc-MeOH in gradient manner and further purified on Sephadex LH-20 with MeOH (100%) to afford quercetin (**5**) (22 mg), a yellow solid [11] and kaempferol (**6**) (35 mg) as yellow powder [12]. Purification of F6 (3 g) over silica gel CC using *n*-hexane-EtOAc, (1:4) as eluting solvent and then on Sephadex LH-20 (MeOH, 100%) yielded dihydrokaempferol (**7**) (35 mg), as a yellow powder [11] and piceatannol (**8**) (40 mg), as a light brown solid [13]. The isolated compounds (**1**-**8**) (Figure 1) were identified using spectroscopic techniques and the data obtained are in agreement with those from literature.

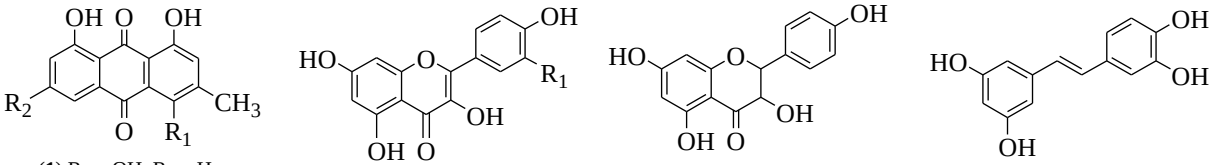

Figure 1. Chemical constituents isolated from the root of *Cassia sieberiana*

Total phenolic and total flavonoid assay: Phenolic compounds are natural source of antioxidants and possess many other bioactive properties [9]. Contents of total phenolic (TPC) and total flavonoids (TFC) of various fractions from the MeOH extract of *C. sieberiana* were evaluated and the highest value was obtained in the EtOAc fraction, 927 ± 0.7 mg gallic acid/g dry extract and 346.5 ± 0.4 mg quercetin/g dry extract, for total phenolic and total flavonoid contents, respectively as shown in Table 1.

Table 1. Total phenolic and total flavonoid contents of fractions from root of *C. sieberiana*

Fractions	Total phenolic content (mg GAE/g dry extract)	Total flavonoid content (mg QE/g dry extract)
Hexane	340 ± 1.4	76.5 ± 1.2
EtOAc	927 ± 0.1	346.5 ± 0.4
BuOH	640 ± 1.4	184.4 ± 1.0
Aqueous	135 ± 0.4	44.1 ± 2.4

Values are means ± SD of three determinations. $P < 0.05$ compared to the control.

Free radical scavenging: DPPH and $ABTS^+$ radicals are widely used for the study of the free radical-scavenging ability of natural antioxidants [13]. The fractions were tested for their antioxidant property using radical scavenging (DPPH and ABTS) method and for their α-glucosidase inhibitory activity (Table 2). The EtOAc fraction showed higher DPPH radical scavenging (1.88 ± 0.4 µg/mL) and $ABTS^+$ radical scavenging (8.49 ± 1.3 µg/mL) compared to other fractions. Similarly, the potency of the fractions against α-glucosidase was the highest in the EtOAc fraction with IC_{50} value of 68.26 ± 2.4 µg/mL.

Table 2. Free radical scavenging (DPPH and ABTS) and α-glucosidase inhibitory activity of fractions from root of *C. sieberiana*

	IC_{50} (µg/mL)		IC_{50} (µM)
Sample	**DPPH radical**	**$ABTS^+$ radical**	**α- glucosidase**
Hexane fraction	64 ± 0.5	71.6 ± 0.9	NA
EtOAc fraction	1.88 ± 0.4	8.49 ± 1.3	68.26 ± 2.4
BuOH fraction	8.9 ± 0.3	30.7 ± 1.2	115.33 ± 3.7
Aqueous fraction	120 ± 3.0	100 ± 1.6	NA
AA	3.19 ± 0.2	-	-
BHT	17.21 ± 1.3	-	-
BHA	9.50 ± 0.4	-	-
Trolox	-	8.03 ± 0.3	-
Quercetin	1.50 ± 0.2	0.80 ± 0.4	10.0 ± 2.6
Acarbose	-	-	11.79 ± 3.5

Values are means ± SD of three determinations; NA = not active; AA = ascorbic acid; BHT = butylated hydroxytoluene; BHA = butylated hydroxyanisole. $P < 0.05$ compared to the control.

Based on the high TPC, and TFC values; strong free radicals scavenging and α-glucosidase inhibitory activities of EtOAc fraction, it was subjected to fractionation and purification using silica gel chromatography and Sephadex LH-20 to obtain eight compounds (**1**-**8**). Table 3 shows the activities of compounds (**1**-**8**) against free radicals (DPPH and $ABTS^+$) and α-glucosidase enzyme. It was found that, quercetin (**5**) (IC_{50} = 1.58 ± 0.1 mM) exhibited a stronger DPPH radical scavenging activity than ascorbic acid (IC_{50} = 2.44 ± 0.3 mM), BHT (IC_{50} = 8.93 ± 0.2 mM) and BHA (IC_{50} = 10.84 ± 0.4 mM). Piceatannol (**8**) (IC_{50} = 3.96 ± 0.2 mM) and kaempferol (**6**) (IC_{50} = 7.75 ± 0.3 mM) demonstrated better DPPH scavenging activity than the standards, BHA and BHT. Quercetin (**5**) and piceatannol (**8**) demonstrated very potent $ABTS^+$ radical scavenging (with IC_{50} values of 1.30 ± 0.4 mM and 3.28 ± 0.2 mM, respectively), compared to the standard, Trolox (IC_{50} = 8.25 ± 0.2 mM). Compounds **1**-**4** were found to be inactive against the radicals. The presence of hydroxyl groups and the *ortho*-dihydroxyl substitution in the structure of quercetin (**5**) and piceatannol (**8**) enhanced their activity towards DPPH and $ABTS^+$ radicals [15]. Furthermore, the presence of double bond at position 2 and 3 in the structure of quercetin (**5**) and piceatannol (**8**), enhanced their radicals scavenging ability, than in dihydrokaempferol (**7**), lacking a double bond in the structure [15].

α-Glucosidase assay: In the α-glucosidase assay, quercetin (**5**) and piceatannol (**8**) possess high potency, with IC_{50} values of 5.73 ± 3.6 µM and 7.37 ± 2.9 µM, respectively against α-glucosidase than the standard, quercetin (IC_{50} = 9.20 ± 5.4 µM) and acarbose (IC_{50} = 14.12 ± 1.5 µM) used in the assay. Kaempferol (**6**) (IC_{50} = 65.0 ± 5.6 µM) and dihydrokaempferol (**7**) (IC_{50} = 92.8 ± 7.4 µM) showed significant α-glucosidase inhibitory activity, while compounds **1**-**4** were not active against the enzyme. The *ortho*-dihydroxyl substituents and the high number of hydroxyl groups in the structure of

quercetin (**5**) and piceatannol (**8**) are responsible for their stronger enzyme inhibitory activity over kaempferol (**6**) and dihydrokaempferol (**7**) [13]. Flavonoids such as quercetin (**5**) and stilbene such as piceatannol (**8**), with high number of hydroxyl groups are potentially useful in the treatment of diabetes [16,17].

The prevalence use of the root of *C. sieberiana* by traditional herbalist in sub-Saharan Africa, for the treatment of oxidative stress attributed diseases such as diabetes, can be associated to the presence of phenolic compounds, more especially the flavonoids and stilbene present in the root of *C. sieberiana*.

Table 3. Free radical scavenging (DPPH and $ABTS^{+}$) and α-glucosidase inhibitory activities of compounds from root of *C. sieberiana*

	IC_{50} (mM)		IC_{50} (µM)
Compound	DPPH radical	$ABTS^{+}$ radical	α-glucosidase
1	NA	NA	NA
2	NA	NA	NA
3	NA	NA	NA
4	NA	NA	NA
5	1.58 ± 0.1	1.30 ± 0.4	5.73 ± 3.6
6	7.75 ± 0.3	18.5 ± 1.3	65.0 ± 5.6
7	82.92 ± 0.1	87.34 ± 1.6	92.0 ± 7.4
8	3.96 ± 0.2	3.28 ± 0.2	7.37 ± 2.9
AA	2.44 ± 0.3	-	-
BHT	8.93 ± 0.2	-	-
BHA	10.84 ± 0.4	-	-
Trolox	-	8.25 ± 0.2	-
Quercetin	1.33 ± 0.3	0.81 ± 0.1	9.20 ± 5.4
Acarbose	-	-	14.12 ± 1.5

Values are means ± SD of three determinations. $P < 0.05$ compared to the control

Acknowledgments

The authors would like to thank the Ministry of Higher Education (MOHE) for financial support under the FRGS grant Vote 4F635 and the Faculty of Science, Universiti Teknologi Malaysia for the research facilities. One of the authors is also grateful to Tertiary Education Trust Fund-Nigeria (TETFund) for doctoral study of Saidu Jibril.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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