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Anthocyanin Profile and Antioxidant Activity of Edible Leaves of *Dissotis brazzae* Cogn (Melastomataceae)

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Abstract: Anthocyanins are colored bioactive phytochemicals in the class of flavonoids with high potential as dietary antioxidants. Demand for anthocyanins in functional food and nutraceutical industries has continued to increase over the past decades. However, sources of anthocyanins for commercial exploitation are currently limited. Tropical Africa and Uganda in particular, harbors many plant species with potential to serve as new sources of anthocyanins. In this study, the anthocyanin profile and antioxidant activity of edible leaves of *Dissotis brazzae*, a plant of tropical African origin, were investigated. Two known anthocyanins, (1) cyanidin 3-*O*-β-glucopyranoside (1.28±0.44 mg/g fr. wt.) and (2) cyanidin 3-*O*-α-rhamnopyranoside (1.89±0.42 mg/g fr. wt.), were isolated from *D. brazzae* purple leaves by a combination of chromatographic techniques. Their structures were elucidated mainly by homo- and heteronuclear NMR spectroscopy and online HPLC/UV-Vis spectroscopy. The isolated anthocyanins showed appreciable level of antioxidant activity against DPPH, with 1 (IC50=9.2±0.2 µg/ml) showing slightly higher antioxidant potency than 2 (IC50=14.0±0.6 µg/ml). However, quercetin (IC50=4.5±0.4 µg/ml) used as a reference showed a higher antioxidant activity than 1 and 2. The total anthocyanin content of *D. brazzae* leaves (317 mg/100g) was within the range (80 – 680 mg/100g) of anthocyanins content in most dietary sources. This is the first report of fully characterised anthocyanins from the genus *Dissotis*, which may be of taxonomic importance. A new dietary source of the rare cyanidin 3-*O*-α-rhamnopyranoside has been identified.

Keywords: *Dissotis brazzae*, Anthocyanins, Nutraceutical, Colorant, Dietary Antioxidant, Functional Food

1. Introduction

Day by day anthocyanins are increasingly becoming popular phytochemicals because of their safety and potential applications in functional food, nutraceutical, cosmetic, pharmaceutical and flower industries [1-3]. Anthocyanins are a class of flavonoids responsible for most of the red to blue colors exhibited by the various plant parts. They exist in a diversity of structures which impart characteristic properties including color hue, intensity, bioactivity and metabolism pathway [4]. The biological activity of anthocyanins may be attributed to their antioxidant potency which is also structure dependent [5]. Distribution of anthocyanins in plants varies with genotype, cultivar, geographical location, agricultural practices and climatic conditions [6, 7]. Therefore, in order to utilise plant sources of anthocyanins as food colorant, functional food as well as nutraceutical, chemical characterisation of the anthocyanin extracts has been recommended [8]. Currently, plants are the main source of anthocyanins for commercial application. However, the available plant sources have so far failed to meet the increasing demand for commercial diversity of anthocyanins.

More so, advances in technology such as cell culture and genetic engineering in anthocyanin production have also at the moment failed to produce commercial amounts of anthocyanins [9-11]. This therefore calls for a search for more plant sources of anthocyanins.

Many plants of African origin have not been adequately investigated with regard to anthocyanins. In our effort to unearth new sources of anthocyanins for food colorant, functional food and nutraceutical application from tropical African plants, *Dissotis brazzae* was investigated. *D. brazzae* is a native plant of the tropical region of Africa ranging from Guinea Republic to Angola, Democratic Republic of Congo, Uganda, Kenya and Tanzania [12]. It belongs to Melastomataceae family and the genus Dissotis is comprised of 140 species [13]. It is regarded as an important wild plant due to its medicinal and food value. In Western Uganda, the leaves are consumed as snacks by mainly herdsmen and children. In Democratic Republic of Congo, the plant is used as a medicinal herb against sickle cell [14]. The Hayas of the Kagera region in Tanzania use the plant to manage syphilis and cerebral malaria [15]. The leaf decoction has an anesthetic effect and is drunk to induce sleep and as an anthelmintic [16]. There are also reports of antimicrobial activity and antiplasmodial activity of *D. brazza*e leaves [15].

In spite of the seemingly popular ethnomedical and food utilisation of *D. brazzae*, its phytochemistry remains largely unknown. Generally, information on the phytochemical composition of the genus *Dissotis* is lacking. Out of the 140 species, only *Dissotis rotundifolia* and *Dissotis erecta* have been partly investigated for their phytochemical composition. The presence of alkaloids, tannins, saponins, flavonoids, anthraquinone, anthocyanin, terpenoids, phenols and cardiac glycosides in the leaves of *D. rotundifolia* has been reported [17, 18]. In addition, kaempferol-3-O-β-D-glucopyranose and kaempferol from *D. erecta* and apigenin-8-C-β-D-glucopyranoside and luteolin-8-C-β-D-glucopyranose from *D. rotundifolia* were identified by [19]. However, to the best of our knowledge the characterisation of anthocyanins from the Dissotis genus has not been reported before. In this paper, we report the anthocyanin profile and antioxidant activity of the mature purple leaves of *D. brazzae*.

2. Materials and Methods

2.1. Isolation of Anthocyanins

Fresh purple leaves of *D. brazzae* were collected from the bushes in Kyeizooba Sub-county, Bushenyi district, Uganda (approximate coordinates: 00°36'46.2"S, 30°15'35.7"E) in June, 2014. The plant was identified at the Herbarium of Botany Department, Makerere University where a voucher specimen (Adaku No. 03) was deposited. The fresh leaves (536 g) were extracted with 2L of methanol containing 0.5% (v/v) trifluoroacetic acid (TFA) (Merck, Darmstadt, Germany) in a deep freezer at 4°C for 20 hours. The filtered extract was concentrated under reduced pressure at 28°C in order to remove the methanol. The concentrate was defatted by partition against ethlyacetate (x2) and afterwards concentrated under reduced pressure to remove traces of the solvent. The unwanted polar compounds were removed by applying the extract to an Amberlite XAD-7 column (70 x 5 cm, Sigma-Aldrich, Steinheim, Germany). The anthocyanins adsorbed on the column were washed with water and eluted with methanol containing 0.5% TFA. The anthocyanins in the concentrated eluate from Amberlite column were separated on Sephadex LH-20 column (10 x 80 cm, Amersham Biosciences, Uppsala, Sweden) using H_2O/MeOH/TFA (79.5:20:0.5, v/v/v) solvent with a flow rate of 5 mL/min. The Sephadex LH-20 fractions with similar qualitative anthocyanin contents (revealed by analytical HPLC) were combined and further purified by preparative reverse-phase HPLC to yield two compounds (1 and 2).

2.2. Preparative HPLC

The preparative HPLC system consisted of a Gilson 321 pump and an UltiMate 3000 variable wave length UV detector (Dionex Corporation, Sunnyvale, CA, US), equipped with a 25 x 2.2 cm (10 μm) Econosphere C18 column (Grace, Deerfield, IL) and solvents (A) water (0.5% TFA, v/v) and (B) acetonitrile (0.5% TFA, v/v). The elution profile consisted of initial conditions with 90% A and 10% B followed by a linear gradient elution for the next 10 min to 14% B, isocratic elution (10-14 min), and the subsequent linear gradient conditions: 14-18 min (to 16% B), 18-22 min (to 18% B), 22-26 min (to 23% B), 26-31 min (to 28% B), and 31-32 min (to 40% B), with isocratic elution at 32-40 min (to 40% B) and a final linear gradient elution at 43-46 min (to 10% B). The flow rate was 15 mL/min, and aliquots of 250 μL were injected manually. Prior to injection, all samples were filtered through a 0.45 μm Millipore membrane filter.

2.3. Analytical HPLC

Analytical HPLC was performed using Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a HP 1050 diode array detector (Hewlett-Packard), an ODS-Hypersil column (20 x 0.5 cm, i.d., 5 μm; Supelco, Bellefonte, PA, USA), two solvents; A (water/0.5% TFA; v/v) and B (acetonitrile/0.5% TFA; v/v). The elution profile consisted of initial conditions with 90% A and 10% B followed by a linear gradient elution for the next 10 min to 14% B, isocratic elution (10-14 min), and then subsequent linear gradient conditions; 14-18 min (to 16% B), 18-22 min (to 18% B), 22-26 min (to 23% B), 26-31 min (to 28% B), and31-32 min (to 40% B), isocratic elution 32-40 min (40% B), and final linear gradient elution 43-46 min (to 10%B). The flow rate was 1.0 mL/min, and aliquots of 15 μL were injected with an Agilent 1100 Series microautosampler. Prior to injection, all samples were filtered through a 0.45 μm Millipore membrane filter. The UV-Vis absorption spectra were recorded online during HPLC analysis over the wavelength range of 240-600 nm in steps of 2 nm.

2.4. NMR Spectroscopy

The NMR spectra were obtained at 600.13 MHz (^{1}H) and

150.92 MHz (^{13}C) on a Bruker Biospin Ultrashield Plus AV-600 MHz instrument equipped with a TCI ^{1}H-^{13}C/^{15}N CryoProbe head (Bruker BioSpin, Zürich, Switzerland). All spectra were recorded at 298 K and the deuteriomethyl ^{13}C signal and the residual ^{1}H signal of the solvent (CF_3COOD-CD_3OD; 5:95, v/v) were used as secondary references (δ 49.0 and 3.40 from TMS, for ^{1}H and ^{13}C, respectively) [20]. The NMR experiments ^{1}H, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC, ^{1}H-^{1}H DQF-COSY and ^{1}H-^{1}H TOCSY were recorded.

2.5. Quantitative Determination of Anthocyanins

HPLC method was used to determine the amounts of the individual anthocyanins as described by [21]. Briefly, fresh purple leaves of *D. brazzea* (10 g) was extracted for 15 hours in 20 mL of methanol containing 0.5% TFA (v/v) in a refrigerator. The extraction was repeated twice to exhaust the pigments and the combined extracts were transferred into a volumetric flask to determine the total volume. The quantitative amounts of anthocyanins 1 and 2 were determined from a HPLC calibration curve based on pure cyanidin 3-glucoside (>95%, determined by HPLC-DAD/NMR standardization) isolated from wild Norwegian blackberries, without taking into account the variation of molar absorption coefficients for individual pigments. Five replicates of the sample were analysed. The results were presented as milligrams of cyanidin 3-glucoside equivalents per 100 g of fresh weight.

2.6. Antioxidant Assay

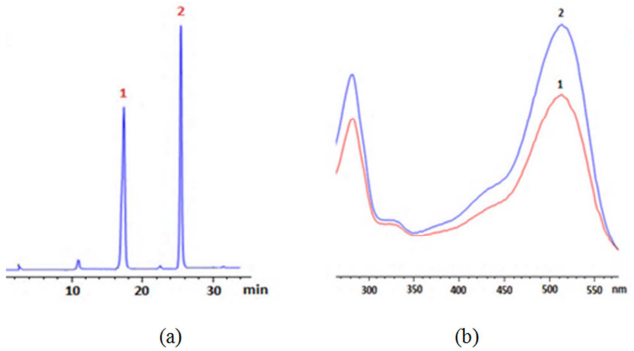
The free radical scavenging activity of the extract was determined according to the method described by [22]. Briefly, solutions of 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) in methanol (4 mg/ml) and the test compound in DMSO (10 mg/ml) were prepared. UV absorbance at 517 nm (A_{517}=1.0) for an aliquot of the DPPH solution (2.95 ml) was measured. The aliquot was mixed with the test compound solution (0.05 ml) and the UV absorbance at 517 nm was measured for 5 min. The procedure was repeated for the test compound serial dilutions of 5, 2.5, 1.3, 0.7 mg/ml. Quercetin was used as a positive control. All aliquots were analyzed in triplicate and results given as averages±standard deviation.

3. Results and Discussion

3.1. Structure Determination

3.1.1. HPLC and On-line UV-Visible Analysis

The HPLC profile (Figure 1a) of *D. brazzea* purple leaves extract detected at 520±20 nm revealed two major anthocyanins (1&2). The On-line UV-Visible spectra (Figure 1b) showed that both anthocyanins absorb at the same wavelength in the visible region ($\lambda_{Vis\text{-}max}$ at 516 nm), which points to an aglycone with two oxygen-functions on the B-ring (cyanidin or peonidin) [23]. From table 1, the $A_{440}/A_{Vis\text{-}max}$ of 31.8% for 1 and 32.3% for 2 were in accordance with 3-glycosyl substitution and the $A_{UV\text{-}max}/A_{Vis\text{-}max}$ of 0.54 for each pigment indicated one sugar moiety [24]. The absence of local λ_{max} around 310-330 nm in the UV region of the spectra showed absence of aromatic acylation [25]. Thus, compounds 1 and 2 were deemed to be 3-monoglycosides of cyanidin or peonidin.



(a) (b)

Figure 1. *(a) HPLC profile for anthocyanins 1 and 2 (b) On-line UV-Visible spectra for anthocyanins 1 and 2.*

Table 1. *UV-Vis data for anthocyanins 1 and 2.*

Compound	$\lambda_{Vis\ max}$	$\lambda_{UV\ max}$	$A_{440}/A_{vis\ max}$	$A_{UV\text{-}max}/A_{Vis\text{-}max}$
Anthocyanin 1	516	280	31.8	0.54
Anthocyanin 2	516	280	32.3	0.54

3.1.2. NMR Spectroscopy Analysis

The 1D ^{1}H NMR spectrum of compound 1 showed six signals in the aromatic region. The signals at δ 8.14 (d, J=2.3 Hz, H-2′), δ 8.35 (dd, J=2.3, 8.7 Hz, H-6′) and δ 7.11 (d, J=8.7 Hz, H-5′) arose from AMX-system of the anthocyanidin B-ring, while the signals at δ 9.12 (d, J=0.8 Hz, H-4), δ 6.75 (d, J=1.9 Hz, H-6) and δ 6.98 (dd, J=0.8, 1.9 Hz, H-8) belonged to the A- and C-rings (Table 2). The chemical shifts for the corresponding carbons of aglycone of compound 1 were assigned from the HSQC spectrum. The chemical shifts for quaternary carbon atoms of the aglycone were assigned from the HMBC spectrum using the procedure reported by [26]. The assignment of the chemical shifts were in conformity with a cyanidin aglycone. Peonidin was ruled out because there was no absorption corresponding to the OCH_3 group.

The 1D ^{1}H NMR spectrum of compound 2 showed similar signals in the aromatic region; δ 8.01 (d, J=2.3 Hz, H-2′), δ 8.11 (dd, J=2.3, 8.6 Hz, H-6′) and δ 7.14 (d, J=8.6 Hz, H-5′) for AMX-system of the anthocyanidin B-ring and δ 9.06 (d, J=0.8 Hz, H-4), δ 6.76 (d, J=1.9 Hz, H-6) and δ 6.98 (dd, J=0.8, 1.9 Hz, H-8) for A- and C-rings (Table 3), also in accordance with cyanidin aglycone.

The TOCSY spectrum of compound 1 revealed one sugar unit. The protons of the sugar unit were assigned from cross-peaks in the DQF-COSY spectrum supported by cross-peaks in the HSQC spectrum [26]. Starting from the anomeric proton at δ 5.38 the observed cross-peak at δ 5.38/3.76 permitted the assignment of δ 3.76 to 2-H″. A sequential walk through approach was afterwards used to assign the coupled protons, 3-H″, δ 3.63 (3.76/3.63), 4-H″, δ 3.53 (3.63/3.53), 5-H″, δ 3.65 (3.53/3.65), 6B-H″, δ 3.80 (3.65/3.80) and 6A-H″, δ 4.00 (3.65/3.80/4.00) (Table 2). The corresponding sugar carbons were assigned from their cross-peaks in the HSQC spectrum. The range of chemical shift values from δ 62.4 to δ 78.7 of the non-anomeric carbons

was indicative of a pyranose hexose [27]. The chemical shifts and coupling constants were in conformity with a β-glucopyranoside. A cross-peak at δ 5.38/145.5 (H-1"/C-3) in the HMBC spectrum confirmed the linkage of the glucopyranose moiety to the 3- position of the aglycone while the coupling constant of the anomeric proton at δ 5.38 (d, J=7.8 Hz, 1-H″) confirmed the β-configuration. Therefore, compound 1 was identified as cyanidin-3-*O*-β-glucopyranoside (Figure 2 (a))

From the TOCSY spectrum of compound 2, only one sugar unit was also revealed and it was observed that one of the proton signals of this sugar unit was at high-field, δ 1.37 (d, J=6.1 Hz). This signal is typically associated with a rhamnose moiety [28]. The chemical shifts and the ^{1}H-^{1}H coupling constants for the sugar moiety were established from the DQF-COSY and the HSQC spectra (Table 3), confirming the rhamnopyranosyl group. A cross-peak at δ 5.84/145.7 (1-H″/C-3) in the HMBC spectrum confirmed the linkage of the rhamnopyranosyl group to the 3- position of the aglycone while the coupling constant of the anomeric proton at δ 5.84 (d, J=1.7 Hz, 1-H″) confirmed the α-configuration. Therefore, compound 2 was identified as cyanidin-3-*O*-α-rhamnopyranoside (Figure 2 (b)).

Table 2. *Proton and Carbon-13 NMR spectral data for anthocyanin* ***1*** *(cyanidin-3-O-β-glucopyranoside).*

	^{1}H δ(ppm), multiplicity, *J* (HZ)	^{13}C δ(ppm)
Aglycone		
2		164.3
3		145.5
4	9.02, *d* 0.9	136.9
5		159.0
6	6.75, *d* 1.9	103.3
7		170.3
8	6.98, *dd* 0.8, 1.9	95.1
9		157.6
10		113.2
1'		121.1
2'	8.14, *d* 2.3	118.4
3'		147.3
4'		155.7
5'	7.11, *d* 8.7	117.4
6'	8.32, *dd* 2.3, 8.7	128.2
3-O-β- glucopyranosyl		
1"	5.38, *d* 7.8	103.7
2"	3.76, *dd* 7.8, 9.3	74.8
3"	3.68, *t* 9.3	78.2
4"	3.57, *dd* 9.1, 9.7	71.1
5"	3.65, *ddd* 2.3, 6.0, 9.7	78.7
6A"	4.00, *dd* 2.2, 12.1	62.4
6B"	3.80, *dd* 6.0, 12.1	62.4

s=singlet, d=doublet, t=triplet, dd=double doublet, ddd=double double doublet

Table 3. *Proton and Carbon-13 NMR spectral data for anthocyanin 2 (cyanidin-3-O-α-rhamnopyranoside).*

	^{1}H δ(ppm), multiplicity, *J* (HZ)	^{13}C δ(ppm)
Aglycone		
2		164.4
3		145.7
4	9.06, *d* 0.8	136.6
5		158.6
6	6.76, *d* 1.9	103.4
7		170.3
8	6.98, *dd* 0.8, 1.9	95.1
9		157.8
10		113.3
1'		121.2
2'	8.01, *d* 2.3	118.1
3'		147.6
4'		155.5
5'	7.14, *d* 8.6	117.4
6'	8.11, *dd* 2.3, 8.6	127.3
3-O-α- rhamnopyranosyl		
1"	5.84, *d* 1.7	102.5
2"	4.31, *dd* 1.8, 3.4	71.6
3"	4.01, *dd* 3.4, 9.3	72.3
4"	3.65, *t* 9.4	73.3
5"	3.72, *dd* 6.2, 9.5	72.2
6A"	1.37, *d* 6.1	17.9
6B"		

s=singlet, d=doublet, t=triplet, dd=double doublet, ddd=double double doublet

Of the two anthocyanins isolated from *D. brazzae* leaves, cyanidin-3-glucoside is the most common anthocyanin found in plants, with 50% distribution in fruits and vegetables [29, 1]. Previously, this pigment was reported to occur in Melastomataceae family in the flowers of *Melastoma sanguineurn Sims* and *Oritrephes grandijlora Ridl* [30]. Cyanidin-3-glucoside may be regarded as an important phytochemical due to its beneficial effects on human health. Studies have revealed that cyanidin-3-glucoside exhibits chemopreventive and chemotherapeutic activity in cultured JB6 cells, suggesting that this pigment may function as a prospective anticancer agent [31].

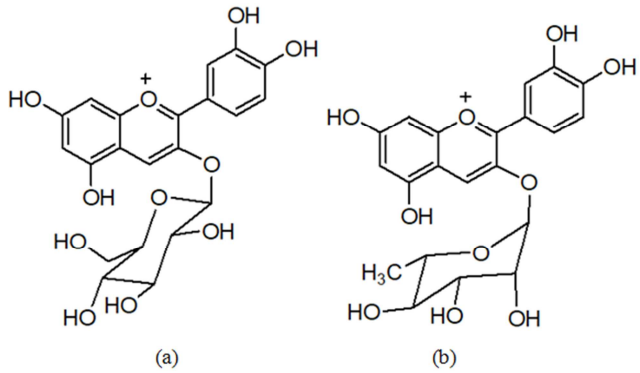


Figure 2. *(a) Structure of cyanidin-3-O-β-glucopyranoside (b) Structure of cyanidin-3-O-α-rhamnopyranoside.*

On the contrary, the occurrence of cyanidin 3-rhamnoside is quite rare and we have not found any report of its occurrence in Melastomataceae family. It has so far been isolated from few plants including taro (*Colocasia esculenta* (L.)) corms [32], phosphorous-deficient leaves of maize (*Zeamays* Var. Ganga-5) [33], flowers of *Salvia splendes* Var. Amethyst [34], acerola (*Malpighia emarginata* DC) fruits [35] and buckwheat (*Fagopyrum esculentum* Moench) flowers [36]. This distribution clearly shows that cyanidin 3-rhamnoside has not been previously identified in the leaves that are produced

under natural conditions. The distribution also shows that the pigment is not readily available in the human diet, appearing only as a minor component in acerola fruits. Therefore, to the best of our knowledge this is the first time cyanidin 3-rhamnoside is being reported as a major component of human diet. In addition, the occurrence of rhamnose as a monosaccharide moiety in anthocyanins is less common. It mainly occurs as a rutinose disaccharide moiety and other polysaccharides. Owing to the limited availability, the functional properties of cyanidin 3-rhamnoside have not been extensively studied. However, [35] reported that cyanidin 3-rhamnoside isolated from acerola exhibited strong antioxidant activity and strongly inhibited advanced glycation end products (AGES) which are associated with diabetes mellitus. In another study on effects of anthocyanidins and anthocyanins on drug metabolizing cytochromes P450 in primary human hepatocytes and human liver microsomes, cyanidin 3-rhamnoside together with other anthocyanins did not induce human CYP2A6, CYP2B6, CYP2C9, and CYP3A4. Unfortunately, the anthocyanins inhibited the marker activities of CYP enzymes to a significant extent, with cyanidin 3-rhamnoside exerting the strongest inhibitory effect [37]. However, the low levels of ingestion of anthocyanins as food or dietary supplement will seldom cause an induction or inhibition of CYP.

3.2. Antioxidant Activity of the Anthocyanins

The IC50 (the concentration of sample that reduced the initial DPPH concentration by 50%) values for *D. brazzae* anthocyanins (See Table 4) suggest appreciable radical scavenging activity against DPPH, which is consistent with other reports [38, 39]. Generally, the electron deficient structure of anthocyanins earns them natural antioxidant activity which is modulated by their structure. Among the anthocyanins, cyanidin and cyanidin-glycosides have been more widely studied for their antioxidant activity mainly because of their greater distribution in plants. Various *in vitro* and *in vivo* studies have reported remarkable antioxidant activity of cyanidin and cyanidin glycosides. In fact, in many instances cyanidin and cyanidin 3-glucoside exhibited higher antioxidant activity than Trolox (Vitamin E analogue) [38, 40] and comparable or higher activity than tert-butylhydroquinone and butylated hydroxytoluene [41, 42] which are renown antioxidants. Cyanidin 3-glucoside also showed a significant antioxidant capacity in the protection of human low density lipoprotein (LDL) oxidation, which was superior to both ascorbic acid and resveratrol [43]. According to [44] cyanidin and cyanidin 3-glucoside demonstrated higher antioxidant efficacy than α-tocopherol in rat liver microsomes but similar activity as α-tocopherol in liposomes and rabbit erythrocyte. More importantly, the antioxidant activity of cyanidin and cyanidin 3-glucoside reduced cytotoxicity and apoptosis without causing toxic side effects on the cell [45, 46]. However, in this study the remarkable antioxidant activity demonstrated by *D. brazzae* anthocyanins was lower than the antioxidant activity of quercetin.

3.3. Quantification of the Anthocyanins

The total anthocyanin content of *D. brazzae* leaves was 317 mg/100g fresh weight (Table 4). Since leaves are scarce as dietary sources of anthocyanins, most studies on anthocyanins in leaves tend to focus on the qualitative rather than the quantitative content. Therefore, information regarding the quantitative content of anthocyanins in leaves is generally scanty. Many dietary sources contain variable quantitative amounts of anthocyanins due to several factors including stage of maturity, period of harvest, genotype, cultural practices, etc. However, the total anthocyanin content in *D. brazzae* leaves was within the range (80 – 680 mg/100g) of anthocyanins content in some edible fruits [47] and within the range (181.2-716.4 mg/100g) of anthocyanin content in grape varieties [48] which serve as a major commercial source for anthocyanins. This shows that the quantity of anthocyanins in *D. brazzae* leaves is appreciable.

4. Conclusion

Anthocyanins have been identified in Dissotis genus for the first time. The revelation of these anthocyanins may be of paramount chemotaxonomic importance. A new source for the uncommon cyanidin 3-rhamnoside has been identified, which may enhance comparative studies of anthocyanins. The appreciable quantity and remarkable antioxidant potency of *D. brazzae* anthocyanins suggest that this plant may serve as a useful source of anthocyanins for dietary antioxidants and nutraceutical and functional food development.

Table 4. *Quantitative amounts of anthocyanins 1 and 2 and IC50 for anthocyanins 1 and 2 and Quercetin.*

Compound	Amount (mg/100g fr. wt)	IC50±S. D. (µg/ml)
Anthocyanin 1	128±0.44	9.2±0.2
Anthocyanin 2	189±0.42	14.0±0. 6
Anthocyanins 1 & 2	317±0.43	12.6±0.4
Quercetin		4.5±0.4

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