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Bioactive proanthocyanidins from the root bark of *Cassia abbreviata*

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ABSTRACT

Cassia abbreviata is an important medicinal plant used in the treatment of various infectious diseases. The ethnomedical efficacy of extracts of this plant species is attributed to its phytochemical constituents most of which are phenolics and anthraquinones. The aim of this study was to isolate and elucidate bioactive phenolic compounds from the root bark of this species. Consequently, two novel trimmeric proanthocyanidins; 3,7,4'-trihydroxyflavan-(4β→8)-3,5,7,4'-tetrahydroxyflavan-(3'→6)-3,5,7,2',4'-pentahydroxyflavan (cassinidin A) and 3,7,2',4'-tetrahydroxyflavan-(4α→8)-3,5,7,4'-tetrahydroxyflavan-(4α→6)-3,5,7,2',4'-pentahydroxy-flavan (cassinidin B) were isolated from the root bark of *Cassia abbreviata*. The chemical structures were determined using NMR, MS and HRMS spectroscopic data. The cassinidin A and B showed higher to moderate antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Candida mycoderma*.
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Keywords: Medicinal plant, *Cassia abbreviata*, Caesalpinioideae, root bark, Cassinidin A, Cassinidin B, antimicrobial.

INTRODUCTION

Cassia abbreviata Oliv. (Caesalpinioideae) is a widely used medicinal plant in East and South African countries. It is a medium to large tree reaching 6 -7 m high. It grows in low to medium grassland, along rivers, on hillsides and often associated with termite moulds. It is used in the treatment of various diseases including bilharzias, skin diseases, cough, pneumonia, fever, gonorrhea, abdominal pains, headaches and snakebites. It is also used in the treatment of water fever and heart diseases (Barakanye 1998; Erasto, 2003). The Bushmen in Botswana and South Africa use decoction of this species for dysentery, diarrhea, severe abdominal pain and toothache (Watt and Brandwijk, 1962; Palgrave, 1977). In Bukoba and Morogoro Tanzania the root barks of *C. abbreviata* are used in the treatment of oral and vaginal candidiasis particularly in HIV/AIDS patients (Hamza et al., 2006; Runyoro et al., 2006). The pharmacological studies have indicated that stem bark extracts and some chemical compounds of this plant species have laxative and antimicrobial properties (Shiv, 1997; Barakanye, 1998).

The chemistry of *C. abbreviata* has been a subject of various studies; this is due to its efficacy in the ethnomedical treatment of

various diseases. Different classes of compounds have been reported from various parts of this plant species including flavonoids, anthraquinones, and triterpenes (Dehmlow et al., 1997; Barakanye, 1998; Erasto, 2003). *Cassia* species are known to have polyphenols particularly those made up of flavonoid units. For instance dimmeric flavonoids also known as proanthocyanidins have been reported from *Cassia abbreviata* (Malan et al., 1996; Rao et al., 1999). The polyphenolic nature of proanthocyanidins and their structural diversity makes them potential targets for the discovery of new antimicrobial agents. In an attempt to search for more bioactive polyphenols, this paper presents two novel trimmeric bioactive proanthocyanidins, cassinidin A and B isolated from the root bark of *C. abbreviata*. Their chemical structures were elucidated on the basis of NMR and MS spectroscopic data and by comparison with the spectral data of the known proanthocyanidins available in literatures.

MATERIALS AND METHODS
General experimental procedures

Melting points were measured on a Stuart Scientific (SMP1) melting point apparatus; UV spectra were obtained from a Shimadzu UV-2101PC spectrophotometer. The NMR spectra for 1D {1H (300 MHz), 13C (75.5 MHz), DEPT} and the 2D (COSY, HMQC, HMBC) spectra were acquired on a Bruker Avance DPX 300 spectrometer and referenced to residual solvent signal. HMBC experiments were run using the standard pulse sequence while the EI-MS spectra were measured on a Finnigan MAT SSQ 7000 single quadrupole instrument. The HRESIMS was performed on a micromass Q-TOF ultima mass spectrometer. Column chromatographs were conducted using silica gel 60F particles size 0.04-0.063 mm (Merck) and sephadex LH-20 (Sigma) stationary phase. Preparative TLC plates were prepared using silica gel 60 PF$_{254+366}$ for preparative thin layer chromatography (Merck) while analytical TLC were conducted on a TLC silica gel 60-F$_{254}$ precoated alumina sheets (Merck). Visualization of developed TLC plates were

achieved using UV (254 and 366 nm) and vanillin-sulphuric acid spray.

Plant materials

The root bark of *Cassia abbreviata* was collected from Mapoka village in the North East District of Botswana in August 2001. The plant was identified by Dr. Turton and the identity further confirmed by comparison with a voucher specimen (960) by Sykes, which is deposited at the University of Botswana Herbarium.

Extraction and isolation of compounds

The root barks were chopped into small pieces and air dried for two weeks. Thereafter the dried root barks were milled to fine powder using Thomas-Wiley Laboratory Mill Model 4. The powdered materials (1.4 kg) were extracted in 100% Methanol, then filtered and concentrated in *vacuo* to give 130 g of a brown extract. Thereafter the extract was dissolved in distilled water and separately partitioned against chloroform, ethyl acetate and *n*-butanol respectively. After TLC analysis, the chloroform and ethyl acetate extracts were combined and loaded on a silica gel PF 60 column chromatography and eluted with 100% CHCl$_3$ with increasing polarity to CHCl$_3$/EtOAc (6:4, 4:6, 3:7) and finally eluted with CHCl$_3$/EtOAc/MeOH (2:6:2) to give 26 fractions each with 150 ml of an eluate. After TLC analysis, the fractions with similar spots were combined to give three major fractions namely; Ca 1/6, 7/15 and 16/26. After TLC analysis, a combined fraction Ca 16/26 contained compounds of interest hence was subjected on further chromatographic separation. The fraction was concentrated in *vacuo* to remove the solvent system used in the elution of the column. Thereafter, a dried fraction was re-dissolved in CHCl$_3$/MeOH (1:1) and loaded on a sephadex LH-20 column eluting with CHCl$_3$/MeOH (1:1) to give ten sub-fractions of 50 ml each. Out of 50, only three sub-fractions 4, 5 and 6 contained two conspicuous and well resolved spots and therefore combined to give sub-fraction C. Sub-fraction C was loaded on preparative

TLC plates and developed four times in *n*-hexane/acetone/EtOAc (4:4:2) to afford 13.5 mg of Cassinidn A (**1**) and 10.4 mg of cassinidin B (**2**).

Antibacterial and antifungal assays

A TLC agar overlay bioautography method as described by Rahalison et al. (1991) and later adopted by Bojase et al. (2002) and Erasto et al. (2004) was used to screen compounds against one Gram negative and two Gram positive bacteria species. The same method was also used to screen compounds for antifungal activity against one yeast fungi species. The organisms used in the assays were *Escherichia coli* (NCTC 09001), *Bacillus subtilis* (NCTC 3610), *Staphylococcus aureus* (NCTC 8532) and a yeast *Candida mycoderma* (NCTC 10716).

RESULTS
Identification of cassinidin A (1)

Compound **1** was isolated as a brown solid with mp 198-200° C. The EI-MS spectrum showed a base peak at *m/z* 529 [M-289]$^+$ (100), and strong peaks at *m/z* 253 [M-565]$^+$ (95), 273 [M-545]$^+$ (58) and 289 [M-529]$^+$ (30). A weak molecular ion peak appeared at m/z 818 (15%) consistent with the molecular formula $C_{45}H_{38}O_{15}$. Additionally, the positive HRESIMS of compound 1 showed a pseudo-molecular ion peak at *m/z* 819.7742 [M + H]$^+$ which is consistent with the molecular formula $C_{45}H_{38}O_{15}$.

The ^{1}H and COSY NMR spectrum showed three spin systems of eleven aliphatic protons. The first system comprised of protons at δ 4.68 (1H, *br s*), 4.50 (1H, *t, J* = 9.5 Hz) and δ 4.71 (1H, *br s*) corresponding to the methine – methine – methine structure. On the basis of HMQC and HMBC correlations, the protons were assigned to H-2 (δ$_c$ 83.3), H-3 (δ$_c$ 70.8) and H-4 (δ$_c$ 41.2) of ring C in the upper flavan unit respectively (Table 1). The second spin system comprised of protons resonating at δ 4.89 (1H, *br s*), 4.12 (1H, *br s*) and prochiral protons resonating at δ 2.83 (1H, *dd, J* = 4.5, 16.7 Hz) and 2.79 (1H, *dd, J* = 4.4, 16.8 Hz). Based on HMQC and HMBC correlations the protons corresponded to a

methine – methine – methylene structure of ring C of a flavan unit and were assigned to H-2 (δ$_c$ 78.4), H-3 (δ$_c$ 66.3) and H-4 (δ$_c$ 32.1) of ring C in the middle flavan unit respectively. The third spin system comprised of protons at δ 5.09 (1H, *br s*), δ 4.27 (1H, *br s*) and two prochiral protons at δ 2.99 (1H, *dd, J* = 4.5, 16.7 Hz) and δ 2.88 (1H, *dd, J* = 3.2, 16.8 Hz), also describing a methine – methine – methylene structure of ring C of a flavan residue. Based on HMQC and HMBC correlations, the protons were assigned to H-2 (δ$_c$ 79.3), H-3 (δ$_c$ 66.2) and H-4 (δ$_c$ 28.8) of ring C in the lower flavan unit respectively. The three spin systems described above are the ^{1}H NMR signals of three different C rings of flavan units. This was further confirmed by the ^{13}C NMR spectrum which showed 45 signals indicating three C6-C3-C6 systems of flavonoid units (Table 1). These information together with the MS data indicated that compound **1** was a trimeric flavonoid made up of three flavan monomers.

The ^{1}H and COSY NMR spectrum showed an AA'BB' spin system made up of protons at δ 7.10 (2H, *br d, J* = 8.5 Hz) and 6.72 (2H, *br s*). A proton at δ 7.10 showed HMBC correlation with carbons resonating at δ 83.3, 131.3, 157.4 ascribed to C-2, C-1', C-3' and C-4' of ring B in the upper flavan unit while a proton at δ 6.72 correlated with carbons at δ 129.5, 131.3, 157.4 and 114.9 ascribed to C-1', C-2', C-4' and C-5' of ring B in the upper flavan unit. Consequently the AA'BB' protons were assigned to H-2' (δ$_c$ 129.5), H-6' (δ$_c$ 129.5) and H-3' (δ$_c$, 114.9) H-5' (δ$_c$ 114.9) of ring B in the upper flavan unit respectively. A broad singlet proton observed at δ 4.71 correlated with carbons at δ 70.8, 129.1 and δ 118.4 ascribed to C-3 of ring C, C-5 and C-10 of ring A of the upper flavan unit respectively. The proton showed another key HMBC correlation with a carbon resonating at δ 106.9 which was identified as C-8 of the middle flavan unit. Consequently this proton was assigned to H-4 (δ$_c$ 41.2) of ring C in the upper flavan monomer (Table 1).

The correlation of H-4 (δ$_c$ 41.2) proton with the C-8 (δ 106.9) of the middle flavan

residue indicated that an inter-flavan linkage between the upper and middle flavan units involved C-4 (U) and C-8 (M) carbons (i.e. 4→8). This was further confirmed by the carbon C-4 (U) resonating up field at δ 41.2 which is a characteristic signal of a C-4 bearing flavan substituent. Furthermore, a singlet signal at δ 4.71 ascribed to H-4 (U) and a carbon shift of C-2 (U) are characteristic signals for the *β*-linkage of two flavan units (Fletcher et al., 1977; Czochanska et al., 1980; Foo, 1986; Ghanzi et al., 1999). This observation confirmed further that ring C of the upper flavan residue had a 2,3-*trans* and 3,4-*trans* configuration and thus the upper and middle flavan units were 4*β*→8 linked.

The ^{1}H and COSY NMR spectrum showed two ABD spin systems, the first had protons resonating at δ 6.69 (1H, *br s*), 6.32 (1H, *dd, J* = 2.4, 8.3 Hz) and δ 6.39 (1H, *d, J* = 2.4 Hz). Based on HMQC and HMBC correlations, the protons were assigned to H-5 (δ_c 129.1), H-6 (δ_c 108.5) and H-8 (δ_c 102.8) of ring A in the upper flavan unit respectively. The second ABD spin system comprised of protons at δ 6.78 (1H, *br s*), 6.84 (1H, *d, J* = 8.6 Hz) and δ 7.35 (1H, *br d, J* = 8.6Hz). Based on HMQC and HMBC correlations, the protons were assigned to H-6' (δ_c 129.7), H-2' (δ_c 129.8) and H-5' (δ_c 115.1) of ring B in the middle flavan unit respectively (Table 1). The proton H-2' showed an important HMBC correlation with a carbon resonating at δ 118.1 identified as C-6 of ring A in the lower flavan unit, and this indicated that an inter-flavan linkage between the middle and lower flavan units involved C-3' (M) and C-6 (L) (i.e. 3'→6).

The ^{1}H and COSY NMR spectra showed another ABD spin system which comprised of protons resonating at δ 7.46 (1H, *d, J* = 8.4 Hz), 6.80 (1H, *br d, J* = 8.4 Hz) and δ 6.69 (1H, *br s*). Through HMQC and HMBC correlations the protons were assigned to H-6', H-3' (δ_c 115) and H-5' (δ_c 115) of ring C in the lower flavan unit respectively. In all three flavan units (monomers) the coupling constant between H-2 and H-3 were obscured by the broadening effect caused by restricted rotation around the inter-flavan bond and also steric interactions in the vicinity of the biaryl bond. However the chemical shifts of the C-2 and C-3 of ring C in the ^{13}C NMR spectrum could indicate whether the system was 2, 3-*trans* or 2, 3-*cis* structure. In this case, when C-2 resonates within δ 78 - 79.5 and C-3 between δ 65 - 66.6 chemical shifts, the structure is 2, 3-*cis* and when C-2 resonates at δ 80 and above, the orientation is 2, 3-*trans* structure (Czochanska et al., 1980; Porter, 1994; Coetzee et al., 2000). Compound 1 was thus identified as a trimmeric proanthocyanidin 3,7,4'-trihydroxyflavan-(4*β*→8)-3,5,7,4'-tetrahydroxyflavan-(3'→6)-3,5,7,2',4'-pentahydroxyflavan (cassinidin A).

3,7,4'-trihydroxyflavan-(4*β*→8)-3,5,7,4'-tetrahydroxyflavan-(3'→6)-3,5,7,4',5'-pentahydroxyflavan (cassinidin A) 2: Brown solid, mp 198-200° C, [α] $_D$ –55.2° (MeOH *c* = 0.005) UV λ_{max} (MeOH) nm (logε$_{max}$) 280 (4.16), +NaOMe 281 (4.17), +AlCl$_3$ 281 (4.08) +AlCl$_3$/HCl 276 (4.10), 210 (4.70), +NaOAc 278 (4.15), 222 (4.12), +NaOAc/H$_3$BO$_3$ 278 (4.15), 224 (4.80). IR (KBr) ν$_{max}$ (cm^{-1}) 3421, 2924, 1617, 1517, ^{1}H and ^{13}C NMR in methanol-d$_4$ (see Table 1) EI-MS, *m/z* 529 [M-289]+ (100), 253 (60), 818 (15), [MF C$_{45}$H$_{38}$O$_{15}$; Mwt 818]. HRESIMS: *m/z* 818.7742 [M + H]$^+$ calculated for C$_{45}$H$_{38}$O$_{15}$.

Identification of cassiadin B (2)

Compound 2 was isolated as a brown solid with mp 202-204° C. The EI-MS spectrum showed a base peak at *m/z* 273 [M – 561]$^+$ (100), and other strong peaks at 255 [273 – H$_2$O] (85), 529 [M – 305]$^+$ (50). A weak molecular ion peak appeared at *m/z* 834 (15%) which was consistent with a molecular formula C$_{45}$H$_{38}$O$_{16}$. Furthermore, the positive HRESIMS of compound **2** showed a pseudo-molecular ion peak at *m/z* 835.7736 [M + H]$^+$, consistent with the molecular formula C$_{45}$H$_{38}$O$_{16}$.

The ^{1}H and COSY NMR spectrum showed ten aliphatic protons corresponding to three spin systems. The first system comprised of protons at δ 5.09 (1H, *br s*), 4.40 (1H, *br s*) and δ 4.65 (1H, *br d, J* = 4.3 Hz), which

indicated a methine – methine – methine structure, typical of ring C in the flavan system. The second spin system had protons at δ 4.62 (1H, *br s*), 4.44 (1H, *t, J* = 9.2 Hz) and δ 4.73 (1H, *br d, J* = 6.3 Hz), also made up a methine – methine – methine structure, corresponding to ring C of a flavan system (Table 1). The third spin system consisted of protons at δ 4.85 (1H, *br s*), 4.42 (1H, *br s*) and two prochiral protons resonating at δ 2.80 (1H, *dd, J* = 4.3, 16.1 Hz) and 2.89 (1H, *dd, J* = 4.5, 16.1 Hz), corresponding to the methine – methine – methylene structure. The three spin systems are typical characteristic signals of three C rings of three different flavan units. Additionally, the ^{13}C NMR spectrum showed 45 signals which corresponds to three different C6-C3-C6 systems of flavonoids (Table 1). These information together with the MS data indicated that compound 2 was a trimeric flavonoid made up of three flavan monomers.

The ^{1}H and COSY NMR spectrum showed further two ABD spin systems. The first one comprised of protons resonating at δ 7.41 (1H, *d, J* = 8.4 Hz), 6.78 (1H, *dd, J* = 2.7, 8.3 Hz) and δ 6.66 (1H, *br s*). The protons at δ 7.41 and δ 6.66 showed key HMBC correlation with carbons at δ 130.8, 156.4, 157, 127.8 and δ 79.2 ascribed to C-1', C-2', C-4', C-5' and C-2 of ring B and C in the upper flavan unit respectively. The two protons together with that at δ 6.78 were assigned to H-6' (δ$_c$ 128.3), H-3' (δ$_c$ 114.8) and H-5' (δ$_c$ 127.8) of ring B of the upper flavan monomer respectively. A broad doublet signal at δ 4.65 ascribed to H-4 of ring C in the upper flavan unit showed a key HMBC correlation with a carbon at δ 106.8 which was identified as C-8 of ring A in the middle flavan unit. This correlation indicated that an inter-flavan bond involved C-4 of the upper and C-8 of the middle flavan units (i.e. 4→8 linkage). The second ABD spin system comprised of protons resonating at δ 6.65 (1H, *d, J* = 8.4 Hz), δ 6.27 (1H, *dd, J* = 2.4, 8.2 Hz) and δ 6.30 (1H, *d, J* = 2.2 Hz). Based on HMQC and HMBC correlations, they were assigned to H-5 (δ$_c$ 129.1), H-6 (δ$_c$ 108.4) and

H-8 (δ$_c$ 102) of ring A in the upper flavan residue respectively.

The ^{1}H NMR spectrum showed an AA'BB' spin system made up of protons resonating at δ 7.05 (2H, *br d, J* = 8.4 Hz) and 6.68 (2H, *dd, J* = 2.5, 8.2 Hz). The proton at δ 7.05 had HMBC correlations with carbons at δ 130.3, 114.7, 157.4, 129.4 and δ 83.2 ascribed to C-1', C-3', C-4', C-6' and C-2 of ring B and C in the middle flavan unit. The proton at δ 6.68 showed HMBC correlation with C-1', C-2' (δ 129.4), C-4' and C-5' (δ 114.7) of ring B in the middle flavan unit. These protons were assigned to H-2' (δ$_c$ 129.4), H-6' (δ$_c$ 129.4) and H-3' (δ$_c$ 114.7), and H-5' (δ$_c$ 114.7) of ring B in the middle flavan unit respectively (Table 1).

Two important aliphatic protons identified earlier on as H-3 (δ$_c$ 70.8) and H-4 (δ$_c$ 40.8) showed key HMBC correlations with a carbon at δ 106.7 ascribed to C-6 of ring A in the lower flavan unit. This correlation indicated that an inter-flavan bond between the middle (M) and lower (L) flavan units is between carbon C-4 (M) and C-6 (L) (i.e. 4→6 linkage). Furthermore the ^{1}H and COSY NMR spectrum showed another ABD spin system with protons resonating at δ 7.32 (1H, *d, J* = 8.4 Hz), 6.80 (1H, *br d, J* = 8.2 Hz) and δ 6.65 (1H, *br s*). Through HMQC and HMBC correlations, the protons were assigned to H-6' (δ$_c$ 129.6), H-5' (δ$_c$ 114.9) and H-3' (δ$_c$ 114.8) of ring B in the lower (L) flavan unit respectively.

The coupling constants between H-2 and H-3 were obscured by the broadening effect caused by restricted rotation around the inter-flavan bond. It has been suggested that 4→8 connection causes more crowded structure than the 4→6 connection for the inter-flavan bond (Fletcher et al., 1977; Porter, 1994; Coetzee et al., 2000). However the chemical shifts of C-2 in the ^{13}C NMR spectrum can indicate whether the stereochemistry is 2,3-*trans* structure or 2,3-*cis* structure (Czochanska et al., 1980; Coetzee et al., 2000). The 2,3-cis-3,4-cis configuration of the trimmer is unusual because the general observation in both

synthetic and natural oligometric proanthocyanidins is that the C-4 substituent is usually *trans* to the C-3 hydroxy group (Foo, 1986), which is not the case for compound **2**. Thus the stereochemistry of protons H-2, H-3 and H-4 of ring C in the upper (U) flavan unit were suggestive of a 4α→8 linkage of the upper and middle flavan units. Furthermore the 2,3-trans-3,4 trans stereochemistry of ring C in the middle flavan unit, together with the correlations described earlier on confirmed the 4α→6 linkage of the middle and lower flavan units. On the basis of spectral data described above compound **2** was identified as a trimmeric proanthocyanidin 3,7,2',4'-tetrahydroxyflavan-(4α→8)-3,5,7,4'-tetrahydroxy-flavan-(4α→6)-3,5,7,2',4'-pentahydroxyflavan (cassinidin B).

3,7,2',4'-tetrahydroxyflavan-(4α→8)-3,5,7,4'-tetrahydroxyflavan-(4α→6)-3,5,7,2',4'-pentahydroxyflavan (cassinidin B) 2: Brown solid, mp 202-204° C, [α] $_D$ –47.0 (MeOH, *c* = 0.005) UV λ_{max} (MeOH) nm (logε$_{max}$) 284 (4.18), NaOMe 282 (4.19), AlCl$_3$ 281 (4.00), AlCl$_3$/HCl 278 (4.12), 210 (4.80), NaOAc 278 (4.15), NaOAc/H$_3$BO$_3$ 278 (4.18). IR (KBr) v_{max} (cm^{-1}) 3421, 2921, 1618, and 1518. ^{1}H and ^{13}C NMR in methanol-d$_4$ (see Table 1). EI-MS, *m/z* 273 [M-561]$^+$ (100), 255 [273-H$_2$O]$^+$ (30), 834 (15), [MF C$_{45}$H$_{38}$O$_{16}$; Mwt 834]. HRESIMS: *m/z* 835.7736 [M + H]$^+$ calculated for C$_{45}$H$_{38}$O$_{16}$.

Antimicrobial activity of cassinidin A (1) and B (2)

Cassinidin A and B exhibited moderate to high antimicrobial activity against both Gram-positive and Gram negative bacteria. Cassinidin A was more active than cassinidin B, which had moderate to weak activities. Cassinidin A had MIC values of 0.6, 0.6 and 0.1 µM which were ten times higher than those of cassinidin B (Table 2). The difference of activities between the two compounds could be attributed to their chemical structures in which the nature of an inter-flavan bond between three flavan monomers and the stereochemistry derived thereof may have strong influence to the activities of the two compounds.
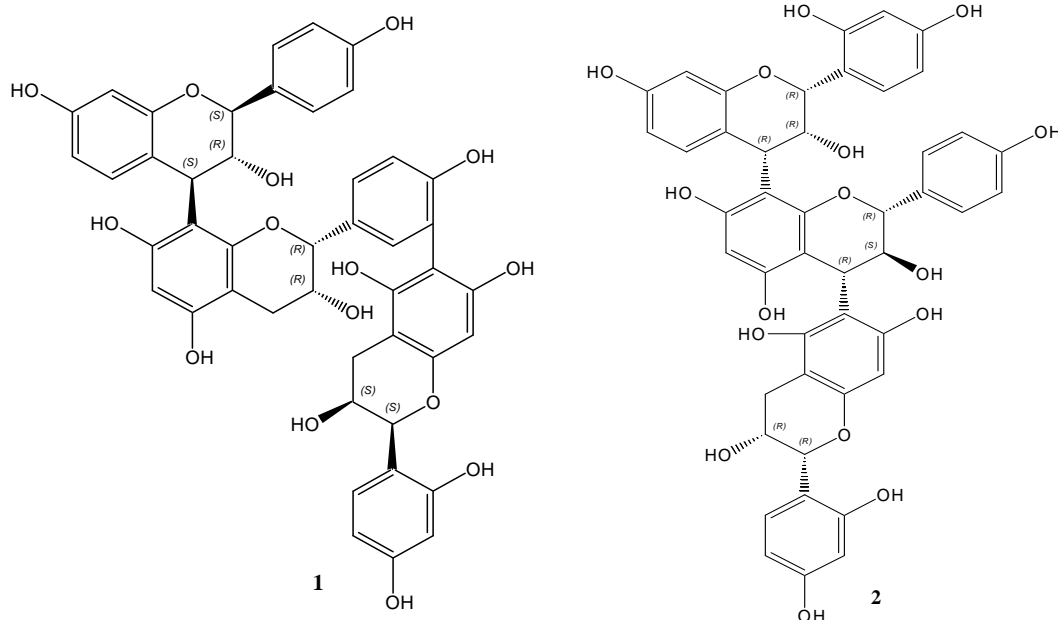

Figure 1: The chemical structures of cassinidin A (**1**) and B (**2**) isolated from the root bark of *Cassia abbreviata.*

Table 1: ^{1}H and ^{13}C NMR data for cassinidin A and B in methanol - d$_4$ isolated from the root bark of *Cassia abbreviata*

Monomers	Position	Cassinidin A		Cassinidin B	
		δ_H (J_H)	δ_C	δ_H (J_H)	δ_C
	2	4.68 1H, *br s*	83.3 (*d*)	5.09 1H, *br s*	79.2 (*d*)
	3	4.50 1H, *t*, (9.5 Hz)	70.8 (*d*)	4.40 1H, *br s*	66.3 (*d*)
	4	4.71 *α*H, *br s*	41.2 (*d*)	4.65 *β*H, *br d* (4.3Hz)	41 (*d*)
	5	6.69 1H, *br s*	129.1 (*d*)	6.65 1H, *br d* (8.4 Hz)	129.1 (*d*)
Upper flavan Unit (U)	6	6.32 1H, *dd*, (2.4, 8.3 Hz)	108.5 (*d*)	6.27 1H, *dd* (2.4, 8.2 Hz)	108.4 (*d*)
	7		156.2 (*s*)		155.8 (*s*)
	8	6.36 1H, *d*, (2.4 Hz)	102.8 (*d*)	6.30 1H, *d*, (2.2 Hz)	102.4 (*d*)
	9		155.8 (*s*)		155.7 (*s*)
	10		118.4 (*s*)		118.4 (*s*)
	1'		131.3 (*s*)		130.8 (*s*)
	2'	7.10 1H, *br d*, (8.5 Hz)	129.5 (*d*)		156.4 (*s*)
	3'	6.72 1H, *br s*	114.9 (*d*)	6.66 1H, *br s*	114.8 (*d*)
	4'		157.4 (*s*)		157 (*s*)
	5'	6.72 1H, *br s*	114.9 (*d*)	6.78 1H, *dd* (2.7, 8.3 Hz)	127.8 (*d*)
	6'	7.10 1H, *br d*, (8.5 Hz)	129.5 (*d*)	7.41 1H, *d* (8.4 Hz)	128.3 (*d*)
	2	4.89 1H, *br s*	78.4 (*d*)	4.62 1H, *br s*	83.2 (*d*)
	3	4.12 1H, *br s*	66.3 (*d*)	4.44 1H, *t* (9.2 Hz)	70.8 (*d*)
Middle flavan Unit (M)	4	2.83 *α*H, *dd*, (4.5, 16.7 Hz) 2.79 *β*H, *dd*, (4.4, 16.8 Hz)	32.1 (t)	4.73 *β*H, *br d*,6.3 Hz)	40.8 (*d*)
	5		155.3 (*s*)		155.3 (*s*)
	6	6.07 1H, *br s*	96.9 (*d*)	6.03 1H, *s*	96.3 (*d*)
	7		155.4 (*s*)		156.2 (*s*)
	8		106.9 (*s*)		106.8 (*s*)
	9		154.3 (*s*)		154.2 (*s*)
	10		98.8 (*s*)		118.2

				(*s*)
1'		130.5 (*s*)		130.3 (*s*)
2'	6.78 1H, *br s*	127.8 (*d*)	7.05 1H, *br d*, (8.4 Hz)	129.4 (*d*)
3'		131.3 (*s*)	6.68 1H, *dd*, (2.5, 8.2 Hz)	114.7 (*d*)
4'		157.5 (*s*)		157.4 (*s*)
5'	6.84 1H, *d*, (8.6 Hz)	115.1 (*d*)	6.68 1H, *dd*, (2.5, 8.2 Hz)	114.7 (*d*)
6'	7.35 1H, *br d*, (8.6 Hz)	129.7 (*d*)	7.05 1H, *br d*, (8.4 Hz)	129.4 (*d*)
2	5.09 1H, *br s*	78.6 (*d*)	4.85 1H, *br s*	78.3 (*d*)
3	4.27 1H, *br s*	65.6 (*d*)	4.42 1H, *br s*	66.2 (*d*)
4	2.99 αH, *dd*, (4.5, 16.7 Hz)	29.1 (*t*)	2.89 αH *dd*, (4.5, 16.1 Hz)	28.6 (*t*)
	2.88 βH, *dd*, (4.2, 16.8 Hz)		2.80 βH, *dd*, (4.3, 16.1 Hz)	
5		155.9 (*s*)		155.1 (*s*)
6		97.5 (*s*)		106.7 (*s*)
7		156.4 (*s*)		156.7 (*s*)
8	6.23 1H, *s*	105.6 (*d*)	6.17 1H, *s*	95.4 (*d*)
9		154.8 (*s*)		155.6 (*s*)
10		99.0 (*s*)		96.6 (*s*)
1'		130.1 (*s*)		131.3 (*s*)
2'		127.5 (*s*)		156.4 (*s*)
3'	6.69 1H, *br s*	115.2 (*d*)	6.65 1H, *br s*	114.8 (*d*)
4'		156.8 (*s*)		157.6 (*s*)
5'	6.80 1H, *br d*, (8.4 Hz)	115.2 (*d*)	6.80 1H, *br d*, (8.2 Hz)	114.8 (*d*)
6'	7.46 1H, *d*, (8.4 Hz)	127.5 (*d*)	7.32 1H, *d*, (8.4 Hz)	129.6 (*d*)

Lower flavan Unit (L) — (rows 2 through 6')

The assignment confirmed by DEPT135, HMQC and HMBC

Table 2: Antimicrobial activity of cassinidin A and B from the root bark of *C. abbreviata.*

Compounds/drug	Test organisms and minimum inhibitory concentration in µg/ml (and µM)			
	E. coli	*B. subtilis*	*S. aureus*	*C. mycoderma*
Cassinidin A (**1**)	1 (1.2)	0.5 (0.6)	0.5 (0.6)	0.1 (0.1)
Cassinidin B (**2**)	10 (12)	5 (6)	5 (6)	0.5 (0.6)
Chloramphenicol	0.001 (0.003)	0.0001 (0.0003)	0.0001 (0.0003)	-
Miconazole	-	-	-	0.0001 (0.0002)

DISCUSSION

The antimicrobial activity of crude extacts of *Cassia abbreviata* has been previously documented. The whole stem extract of this plant species was found to be active against various bacteria which include *Staphylococcus aureus*, *Shigella dysentrie*, and several *Bacilus* and *Proteus* species. The MIC values of the extract ranged from 500 - 700 µg/ml (Shiv, 1997). Therefore, the observed efficacy of cassinidin A and B, corroborates with the earlier reports on this plant species. The higher antibacterial activity of cassinidin A as compared to cassinidin B may be related to their differences in chemical structures. The two compounds differs in their inte-flavan linkages orientation and the stereochemistries derived thereof. Cassinidin A has a $4\beta{\rightarrow}8$ and $3'{\rightarrow}6$ inter-flavan bonds while cassinidin B has $4\alpha{\rightarrow}8$ and $4\alpha{\rightarrow}6$ bonds. It can therefore be suggested that the bond that involves two sp^2 aromatic carbons increased the activity of cassinidin A. It also seems that the stereochemistry around the $4{\rightarrow}8$ bond influenced the antibacterial activity of the triflavonoids as cassinidin A has a $4\beta{\rightarrow}8$ and cassinidin B has $4\alpha{\rightarrow}8$ link. In the antifungal assay, cassinidin A exhibited moderate activity against *Candida mycoderma* compared to cassinidin B (Table 2). Again, the influence of the inter-flavan bonds and the stereochemistry at the $4{\rightarrow}8$ inter-flavan bond as described above may have had a strong influence to the anticandida activities of the two compounds.

The antimicrobial activity of two polyphenols supports the ethnomedical use of *Cassia abbreviata* in the treatment of various microbial infections. These compounds together with other unidentified phytochemicals may be responsible for the efficacy of the roots of this plant species when used in the treatment of microbial infections. Identification of the two polyphenols, has further expanded the knowledge of chemical constituents of *Cassia abbreviata*. Investigation of a possible synergistic effect of the chemical constituents of the roots of this species, and further elucidation of more compounds is needed for the better understanding of the medicinal potential of *Cassia abbreviata*.

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REFERENCES

Barakanye BB. 1998. Secondary metabolites from the roots of *Cassia abbreviata*. MSc Dissertation, Department of Chemistry, University of Botswana.

Bojase G, Wanjala CCW, Gashe BA, Majinda RRT. 2002. Antimicrobial flavonoids from *Bolusanthus speciosus*. *Planta Medica,* **68**: 615 – 620

Coetzee J, Mciteka L, Malan F, Ferreira D. 2000. Structure and synthesis of the first procassinidin dimmers based on epicatechin and gallo- and epigallocatechin. *Phytochemistry*, **53**: 795–804.

Czochanska Z, Foo LY, Newman RH, Porter LJ. 1980. Polymeric proanthocyanidins.

Stereochemistry, structural units, and molecular weight. *J. Chem. Soc. Perkin Trans.,* **1**: 2278 – 2285.

Dehmlow EV, Ree TV, Guntenhoner M. 1997. 2,4 - *trans* - 7,4 – dihydroxy - 4-methoxyflavan from *Cassia abbreviata. Phytochemistry*, **49**: 1805 – 1806.

Erasto P. 2003. Phytochemical analyses and antimicrobial studies on *Bolusanthus speciosus* and *Cassia abbreviata*. MPhil Thesis, Department of Chemistry, University of Botswana.

Erasto P, Bojase-moleta G, Majinda RRT. 2004. Antimicrobial and antioxidant flavonoids from the root wood of *Bolusanthus speciosus. Phytochemistry*, **65**: 875–880.

Fletcher CA, Porter JL, Haslam E, Gupta KR. 1977. Plant proanthocyanidins. Part 3; Conformational and configurational studies of natural proanthocyanidins. *J. Chem. Soc.,* **1**: 1628 – 1637.

Foo LY. 1986. A novel pyrogallal A-ring proanthocyanidins dimmers from *Acacia melanoxylon*. *J. Chem. Soc. Chem. Comm*., 236 -237.

Ghanzi H, Norio N, Meselhy RM, Masao H. 1999. Phenolics from *Maytenus senegalensis*. *Phytochemistry*, **50**: 689-694.

Hamza OJM, van den Bout-van den Beukel CJP, Matee MIN, Moshi MJ, Mikx FHM, Semani HO, Mbwambo ZH, Van der ven AJAM. 2006. Antifungal activity of some Tanzanian plants used traditionally for the treatment of fungal infection. *J. Ethnopharmacol*., **108**: 124 - 132.

Malan E, Swinny E, Ferreira D, Steynberg P. 1996. Structure and synthesis of proguibourtinidins from *Cassia abbreviata*. *Phytochemistry*, **41**: 1209 – 1213.

Palgrave KC. 1977. *Trees of Southern Africa*. Struik: Cape Town; 287.

Porter LJ. 1994. Advances in research since 1986. In *The Flavonoids,* Harbone JB (Ed). Chapman and Hall, University Press: Cambridge, London; 23, 90-93.

Rahalison L, Hamburger M, Hostettman K. 1991. A bioautographic agar overlay method for the detection of antifungal compounds from higher plants. *Phytochem. Analysis,* **2**: 119 - 203.

Rao KVD, Jayaprakasam B, Gunasekar D. 1999. Flavanol glycoside from *Cassia hirsute. J. Nat. Prod*., **62**: 305 – 306.

Runyoro DKB, Ngassapa OD, Matee MIN, Joseph CC, Moshi MJ. 2006. Medicinal plants used by Tanzanian traditional healers in the management of Candida infections. *J. Ethnopharmacol.,* **106**: 158 – 165.

Shiv P. 1997. Anti-bacterial and anti-diarrhea studies on Zambian medicinal plants. Abstract and Programme. Seventh NAPRECA Symposium on Natural Products, Dar es Salaam, p. 32.

Watt JM, Brandwijk MGB. 1962. Medicinal and poisonous plants of southern and eastern Africa. Livingstone Ltd: Edinburgh.