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Anti-oxidant and Antimicrobial Flavonoid Glycosides from *Alstonia boonei* De Wild Leaves

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Authors' contributions

This work was carried out in collaboration between both authors. Author NNO designed the work, carried it out and wrote the first draft of the manuscript while author COBO supervised the work, proof read the manuscript and assisted in the analyses. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: *Alstonia boonei* De Wild (Apocynaceae) leaves are used traditionally in several parts of West Africa (including Nigeria) and Asia for the treatment of various ailments such as rheumatic and muscular pains as well as hypertension and malaria. The aim of this study is to isolate the phytoconstituents responsible for its anti-oxidant and anti-inflammatory activities.
Methodology: The leaves of *Alstonia boonei* were extracted in methanol and the methanol extracted subjected to series of chromatographic separation for the isolation of 8 flavonoid glycosides. The chemical structures of these compounds were elucidated by a combination of UV, HPLC-MS, 1 D and 2 D NMR spectroscopy. The antioxidant activity of the isolated flavonoid glycosides was determined by DPPH free radical scavenging model while the antimicrobial activity was determined by Agar well diffusion technique.
Results: The flavonoid glycosides were elucidated as Rutin **(1)**, Quercetin robinobioside **(2)**, Kaempferol-3-O-rutinoside **(3)**, Kaempferol-3-O-robinobioside **(4)** and compounds **5, 7** and **8** which are glycosides of quercetin and compound **8** which is a flavonoid of kaempferol. Compounds **1, 2, 5, 7** and **8** which are all glycosides of quercetin showed good antioxidant activities (IC_{50} <66 µg/mL)

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on DPPH free radical scavenging model. Compounds **3, 4** and **6** which are derivatives of kaempferol, however, showed poor activity (IC_{50} >200 µg/mL). Of all the compounds, only **7** and **8** were active against *Escherichia coli* with IZD (inhibition zone diameter) values of 17 and 13 mm at 62.5 µg/mL respectively.

Conclusion: The profound antioxidant activity of the isolated quercetin derivatives may explain the ethnomedicinal use of the plant extracts in the management of inflammatory diseases and other disorders associated with oxidative stress.

Keywords: Alstonia boonei De Wild; flavonoid glycosides; anti-microbial; anti-oxidant.

1. INTRODUCTION

Oxidation is an essential part of life and metabolism being required for the production of energy in the form of ATP. This process, however often leads to the generation of free radicals. The existence of physiopathological situations (cigarette smoke, air pollutants, UV radiation, high poly unsaturated fatty acid diet, inflammation, ischemia/reperfusion etc) in which Reactive Oxygen Species (ROS) are produced in excess and at the wrong time and place, makes dietary antioxidants needful for diminishing the cumulative effects of oxidative damage over the life span [1,2]. Antioxidant substances block the action of these free radicals which have been implicated in the pathogenesis of many diseases. Currently there are several synthetic antioxidants in use, however, an ever increasing preference for natural remedies due to their tendencies for low toxicity has led to a continuous quest for such.

Among the well established plant derived antioxidants are vitamins C, E, A, and carotenoids. Plant polyphenols, present in several plants also serve as antioxidants [3]. *Alstonia boonei* De Wild (Apocynaecae) is a medicinal plant that is widely used across Africa for various ailments. Its leaves and latex are used topically to reduce swellings and for the treatment of rheumatic pains, muscular pains and hypertension [4]. *A. boonei* is widely distributed in some parts of Asia and the tropical Africa for instance Senegal (Ti Keung), Guinea (Ekouk, Kanja), Ivory Coast (Ouguie, Sinduru), Ghana(Osen-nuri, Onyame-dua), Nigeria (Égbú-Ọ̀rà, Awun), Cameroon (Bókùkà), Sudan, Uganda (Myna, Mujwe). It is also referred to as cheesewood, stoolwood, patternwood.

Independent studies [4-7] have confirmed the indigenous medicinal usefulness of *A. boonei* for the treatment of malaria in south-western part of Nigeria. Other pharmacological activities of *A. boonei* include: antihelminthic [8], hypotensive [4,9], aphrodisiac and antidiabetic [10] properties. This wide range of medicinal activity of *A. boonei* is probably due to its antioxidant activity. Our preliminary study on the plant showed that it is rich in polyphenolics, which have been implicated as natural antioxidant. In the present study, we report the isolation and structure elucidation of the antioxidant flavonoid glycosides from the leaves of *A. boonei*. The result of the investigation of their antimicrobial properties are also reported.

2. MATERIALS AND METHODS

2.1 General Experimental Procedures

The optical rotation values were measured with a Perkin–Elmer-241 MC polarimeter. NMR spectra (^{1}H, ^{13}C, DEPT, HMQC and HMBC) were recorded with Bruker ARX 500 NMR spectrometers. MS (ESI) data were obtained with Finnigan LCQ Deca mass spectrometer. Analytical HPLC was carried out with a Dionex P580 HPLC system coupled to a photodiode array detector (UVD340S) and routine detection was at 235, 254, 280 and 354 nm. The separation column (125 X 4 mm, length X internal diameter) was prefilled with Eurospher-10 C18 (Knauer, Berlin, Germany). A linear gradient of nanopure water (adjusted to pH 2 by addition of formic acid) and methanol was used as eluent. Semipreparative HPLC was performed with Merck Hitachi L-7100 coupled to a UV detector (L-7400). A gradient of HPLC grade methanol and nanopure water was used in each case of separation. Vacuum Liquid Chromatography (VLC) was carried out on silica gel (230-400 mesh, Merk), Sephadex LH-20 was used for CC while TLC was performed on silica gel G_{254} coated plates (Merk) using the following solvent systems: DCM-MeOH (9:1 and 4:1).

2.2 Plant Material

The leaves of *A. boonei* De Wild were collected in Nsukka, Enugu State, Nigeria and identified by

a taxonomist Mr. Alfred Ozioko of the Bioresearch and Conservation Program (BDCP), Nsukka, Nigeria. Voucher specimens were deposited in the herbarium section of the BDCP under the herbarium number INTERCEDD/024. The plant material was air dried for 10 days and pulverized.

2.3 Extraction and Isolation

The extraction, fractionation and isolation procedures adopted in this work was based on previously reported and optimized procedures for isolation flavonoid glycosides [11,12]. Briefly, about 500 g of the dried and pulverized leaves were extracted for 48 h by cold maceration in 5 L methanol with continuous stirring using a magnetic stirrer. The combined extracts was dried at 40℃ under reduced pressure to obtain the methanol extract (ME). The methanol extract (20 g) was dispersed in 200 mL of distilled water and defatted by partitioning with n-hexane (500 mL X 3), The defatted extract was thereafter partitioned with ethyl acetate (500 mL X 3) to obtain ethyl acetate fraction (EF). About 5 g of ethyl acetate fraction was subjected to VLC (silica gel 500 g, sinterred funnel 5 L) eluting with 750 or 500 mL each of hexane:ethyl acetate (90:10, 80:20, 70:30, 60;40, 50:50, 40:60, 30:70, 20:80, 0:100) and dichloromethane:MeOH (90:10, 80:20, 60:40, 40:60, 20:80, 0:100). This resulted in 15 pooled fractions EF1 to EF16. The fraction eluted with dichloromethane:MeOH 80:20 (EF11) was separated on a Sephadex LH-20 column (3 X 60 cm) eluted with dichloromethane: MeOH (1:1) to afford 7 pooled fractions EF11A to EF11G. Similarly the fraction eluted with dichloromethane:MeOH 70:30 (EF12) was separated on a Sephadex LH-20 column (3 X 60 cm) eluted with 100% MeOH to afford 20 pooled fractions EF10A to EF10T. The Sephadex fraction EF11E was purified by semipreparative HPLC to obtain compounds **3** (25 mg), **4** (7 mg) and **8** (4 mg). EF12J was similarly purified to obtain compounds **1** (30 mg) and **6** (5 mg) while the purification of EF12M, EF12N and EF12O gave compounds **5** (4 mg), **7** (4 mg), and **2** (8 mg) respectively.

2.4 Antioxidant and Antimicrobial Activities

The antioxidant activity of the isolated compounds was assessed by the DPPH free radical-scavenging activity of the isolated using ascorbic acid as a standard as previously reported [13]. The compounds were also screened for their antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Pseudomona aerigunosa* and *Candida albicans* using agar well diffusion technique [14].

3. RESULTS AND DISCUSSION

Investigation of the ethyl acetate fraction of the methanol extract of the leaves of *A. boonei* De Wild led to the isolation and structure elucidation of eight known flavonoid glycosides, **1**-**8** (Fig. 1).

Compound **1**, was isolated as a yellow amorphous solid with an optical rotation of +88.00±1.8 The LC-ESI-MS indicated a strong peak at *m/z* 611 in the positive mode and 609 in the negative mode indicating a molar mass of 610 g/mol. The LC-ESI-MS also showed fragment peaks at *m/z* 465.0 $(M-147)^+$ corresponding to a loss of desoxyhexose and 303.3 $[M-147-163]^+$ a subsequent loss of a hexose suggesting that compound **1** is a diglycoside. The fragment 303.3 is also diagnostic of quercetin aglycone and this was supported by the observed UV maxima (λ_{max}) of 256.0 and 356.0 nm. ^{1}H-NMR spectrum of compound **1** revealed five aromatic proton peaks which included an ABX aromatic spin system at δ_H 6.87(d, *J*=8.4, 1H) 7.63 (dd, *J*=2.1, 8.4, 1H) 7.67 (d, *J*=2.1, 1H) assigned to H-5', H-6' and H-2' respectively of the of Ring 'B' of the quercetin/morin nucleus. A-HMBC correlation between H-2' and C-2 as well as C-4' show a 3',4'-dihydroxy system of quercetin, not 2'4' system of morin. The other two aromatic protons at δ_H 6.20 (d, *J*=2.0, 1H) and 6.39 (d, *J*=2.0, 1H) are assignable to the meta coupled H-6 and H-8, respectively of the 'Ring A' of quercetin nucleus.

Two anomeric proton signals at δ_H 5.12 ppm (d *J*=7.6 Hz, 1 H) and 4.52 ppm (br s, 1 H) were observed thus confirming the proposed diglycoside nature of compound **1**. The anomeric protons are assignable to a β-D-glucosyl unit and an α-L-rhamnose respectively. Anomeric protons of β-linked glucopyranosides exhibit H-1/H-2 coupling constants of 7-8 Hz. This is readily distinguishable from the α-linked glucopyranoside with 3-4 Hz coupling [15]. Anomeric protons belonging to α-L-rhamnose have coupling constant values of 1-2 Hz and sometimes appear as a broad singlet. ^{1}H-NMR spectrum of compound **1** also showed a rhamnosyl methyl doublet signal at δ_H 1.12 (d, *J*=6.2, 3H) having COSY correlations with H-5''' and HMBC correlations with C-5''' as well as a long range correlation with H-4''' [16]. The *β*-glucose moiety was evident from the ^{1}H NMR

resonances of oxymethine protons, together with a pair of diastropic oxymethylene protons at δ_H 3.81 and 3.28 ppm. The remaining sugar protons resonate in the range of 3.2-3.5 ppm. The observed HMBC correlation of the anomeric proton of the rhamnosyl unit (δ_H 4.52) to C-6'' of the glucosyl unit confirms suggested a 1→6 interglycosidic linkage in compound **1.** This was further confirmed by a downfield shift of the C-6 signal. The attachment of the glucosyl moiety at C-3 of the quercetin nucleus was confirmed by the HMBC correlation of the anomeric proton (δ_H 5.11) of the glucosyl moiety to C-3 (δ_C 135.92) of the quercetin nucleus to C-3 of the quercetin. This was also confirmed by the downfield shift of the signals of C-2 and C-3 (δ_C 159.47 and 135.92, respectively) [17]. Each of the remaining carbons were accounted for using C-13 NMR as well as DEPT-135. Compound **1** was thus identified as **Rutin** {Quercetin-3-O-[α-L-rhamnopyranosyl(1→6) β-D-glucopyranoside]}.

Compound **2** was isolated as a yellow amorphous solid with optical rotation of +38.17±0.8 and UV absorption maxima peaks of 256.0 and 354.0 nm, typical of the quercetin flavonoids. A molecular mass of 610 g/mol was deduced on the basis of its pseudomolecular ion peaks at *m/z* 611.1 $[M+1]^+$ in the positive mode and *m/z* 609.4 $[M-1]^-$ in the negative mode of its ESIMS. Compound **2** showed very similar UV, MS and NMR data as compound **1.** The major differences, however, lie in the NMR signals of the hexose unit. A slight upfield shift of the hexose anomeric proton in compound **2** (δ_H 5.07) compared to that in compound **1** (δ_H 5.11) was observed revealing that **2** is a galactoside as opposed to **1** which is a glucoside [18].

Table 1. NMR spectra data of Rutin (1) and Quercetin robinobioside (2)

Position	Compound 1			Compound 2
	δ_H (*J* in *Hz*)	δ_C (*J* in *Hz*)	HMBC	δ_H (*J* in *Hz*)
2	-	159.5		-
3	-	135.9		-
4	-	179.5		-
5	-	163.1		-
6	6.20 (d, *J*=2.0)	100.2	5, 7, 8, 10	6.20 (s)
7	-	166.4		-
8	6.39 (d, *J*=2.0)	95.1	6, 7, 9, 10	6.40 (s)
9	-	158.8		-
10	-	105.7		-
1'	-	123.3		-
2'	7.67 (d, *J*=2.1)	117.2	2, 1', 3', 4'	7.87(s)
3'	-	149.8		-
4'	-	146.0		-
5'	6.87 (d, *J*=8.4)	116.2	1'	6.87 (d, *J*=8.5)
6'	7.63 (dd, *J*=2.1,8.4)	123.7	2,1'	7.60 (d, *J*=8.5)
1''	5.11 (d, *J*=7.6)	104.9	2''	5.07 (d, *J*=7.8)
2''	3.4			.84 (d, *J*=9.5)
3''	3.4			3.54 (d, *J*=9.9)
4''	3.26			3.81 (s)
5''	3.32			3.64 (d, *J*=6.3)
6''	3.81 (d, *J*=10.9) 6''A 3.28 (dd, *J*= 8.9) 6''B	68.7	1'''	3.75 (dd,*J*=5.8) 6''A 3.41 m 6''B
1'''	4.52 (s,1H)	104.9	2''',6''	4.53 (br s)
2'''	2.64 (s)			3.58 (br s)
3'''	3.54 (dd, *J*=3.4, 9.5)			3.50 (dd,*J*=3.4, 9.9)
4'''	3.28 (dd, *J*=4.2, 8.9)			3.27 m
5'''	3.4			3.54 (d, *J*=9.9)
6'''	1.12 (d, *J*=6.2, 3H)	18.3	5''', 4'''	1.19 (d, *J*=6.2)

NMR was measured at 500 MHz (^1H) and 125 MHz (^{13}C) (CD_3OD)

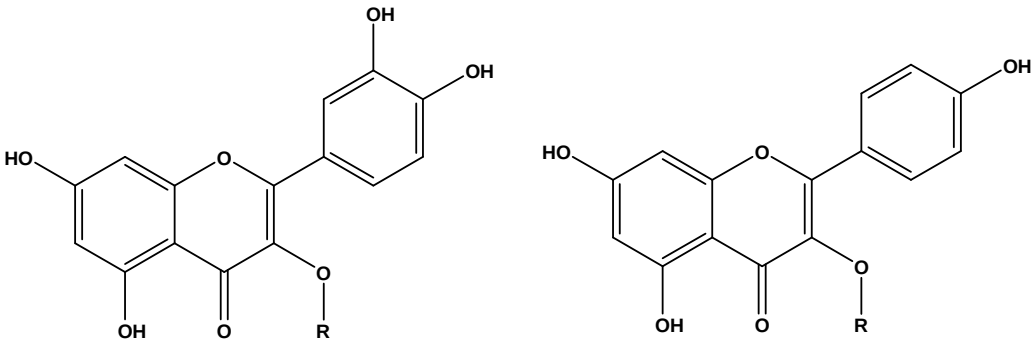
Table 2. NMR spectra data of Kaempferol-3-O-rutinoside (3) and Kaempferol-3-O-robinobioside (4)

Position	Compound 3			Compound 4
	δ_H (*J* in *Hz*)	δ_C (*J* in *Hz*)	HMBC	δ_H (*J* in *Hz*)
2	-	159.6		
3	-	135.6		
4	-	179.5		
5	-	163.2		
6	6.20 (d, *J*=2.1)	100.4	5, 7, 8, 10	6.18 (*J*=1.19)
7	-	166.8		
8	6.39 (d, *J*=2.0)	95.5	6, 7, 9, 10	6.36 (br s)
9	-	158.8		
10	-	105.7		
1'	-	123.04		
2'	8.06 (d, *J*=8.9)	132.5	6', 1'	8.09 (d, *J*=8.8)
3'	6.89 (d, *J*=8.9)	116.3	5'	6.88 (d, *J*=8.8)
4'	-	161.7		-
5'	6.89 (d, *J*=8.9)	116.3	3'	6.88 (d, *J*=8.8)
6'	8.06 (d, *J*=8.9)	132.5	1'	8.09 (d, *J*=8.8)
1''	5.12 (d, *J*=7.4)	105.1		5.01 (d, *J*=7.8)
2''	3.44	78.3	1'', 3''	3.77 (d, *J*=7.3)
3''				3.54 (d, *J*=3.4)
4''				
5''				3.61 (s)
6''	3.81 (d, *J*=10.6) 6''A	68.7	1'''	3.73 (dd, *J*=10.1) 6''A
	3.39 (d, *J*=6.7) 6''B			3.39 (s)
1'''	4.52 (br s)	102.8	2''', 3''', 6''	4.52 (s)
2'''	3.64 (br s)	72.2		3.59 (s)
3'''		72.5	1''', 2'''	3.52 (d, *J*=6.0)
4'''	3.27 m	74.0		3.27 (s)
5'''	3.44 m	69.9	4'''	3.52 (d, *J*=6.0)
6'''	1.13 (d, *J*=6.2)	18.1	5''', 4'''	1.19 (d, *J*=6.2)

NMR was measured at 500 MHz (^1H) and 125 MHz (^{13}C) (CD_3OD)

The galactosyl unit was further confirmed by the equatorial conformation of the H-4 (broad singlet signal) which appeared much deshielded at δ_H 3.81 (brs) as compared to the axial and much shielded (δ_H 3.26 dd *J*=4.2, 8.9) H-4 of the glucose unit [18, 19]. More so, there is a slight downfield shift (Δ≈0.07) of the methyl protons of the rhamnosyl unit of compound **2** (δ_H 1.19) compared to that of compound **1** (δ_H 1.12). Compound **2** was therefore identified as **Quercetin-3-O-robinobioside** {quercetin-3-O-[α-L-rhamnopyranosyl(1→6) β-D-galactopyranoside]}.

Compound **3** was obtained as a yellow powdery solid with optical rotation of -34.17±1.8. The molar mass was deduced as 594 g/mol based on the observed ESIMS peaks at *m/z* 595.0 $[M+1]^+$ and 617.3 $[M+Na]^+$ in the positive mode and at *m/z* 593.2 $[M-1]^-$ in the negative mode. The ESIMS also showed fragments due to loss of one desoxyhexose (a rhamnose) *m/z* 449.1 [M+1-146] and a subsequent loss of hexose (glucose/galactose) *m/z* 287.3 [M+1-146-162]. The latter fragment ion at *m/z* 287.3 as well as the observed UV maxima at 265.5 nm and 348.7 nm confirms the compound to be a kaempferol derivative [16,17,19]. Compound **3** was also shown to be a diglycoside from the ESIMS data and presence of two anomeric proton signals at δ_H 5.12(d, *J*=7.4, 1H) and 4.52 (s, 1 H). The NMR data of compound **3** is similar to that of compound **1**. The major difference is the absence of ABX system observed in ring B of 1. This was replaced by an AA'BB' aromatic signals at δ_H 8.06 (d, *J*=8.9) and δ_H 6.89 (d, *J*=8.9 Hz), each integrating to two protons and assigned to H-2'/H-6' and H-3'/H-5', which further confirmed the presence of kaempferol nucleus. Compound **3** was therefore identified as **Kaempferol-3-O-rutinoside** {kaempferol-3-O-[α-L-rhamnopyranosyl(1→6) β-D-glucopyranoside]}.



1. **R** = Rhamnopyranosyl (1→6) Glucopyranoside

2. R = Rhamnopyranosyl (1→6) Galactopyranoside

5. R = Rhamnopyranosyl (1→4) Glucoopyranoside

7. R = Rhamnopyranosyl (1→2) Glucopyranoside

8. R = Rhamnopyranosyl (1→2) Galactopyranoside

3. R = Rhamnopyranosyl (1→6) Glucopyranoside

4. R = Rhamnopyranosyl (1→ 6) Galactopyranoside

6. R = Rhamnopyranosyl (1→4) Glucopyranoside

Fig. 1. The chemical structures of the isolated flavonoid glycosides

Table 3. ^{1}H NMR spectra data of compounds 5, 6, 7 and 8

Position	Compound 5	Compound 6	Compound 7	Compound 8
	δ_H (*J* in *Hz*)	δ_C (*J* in *Hz*)	HMBC	δ_H (*J* in *Hz*)
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
6	6.21 (s)	6.21 (*J*=2.0)	6.21 (s)	6.21 (*J*=2.0)
7	-	-	-	-
8	6.40 (s)	6.40 (*J*=2.0)	6.40 (s)	6.41 (*J*=2.0)
9	-	-	-	-
10	-	-	-	-
1'	-	-	-	-
2'	7.67 (s)	8.09 (*J*=8.8)	7.67	7.88 (d, 2.1)
3'	-	6.91 (*J*=8.8)	-	-
4'	-	-	-	-
5'	6.87 (d, *J*=8.4)	6.91 (*J*=8.8)	6.87	6.87 (d, 8.5)
6'	7.64 (d, *J*=8.4)	8.09 (*J*=8.8)	7.63	7.61 (d, 8.5)
1''	5.11 (d, *J*=7.6)	5.13 (*J*=7.3)	5.11(d, 7.7)	5.07 (d, 7.8)
2''	3.47	3.45	3.48	3.81
3''		-		3.42
4''	3.63	3.37	3.63	
5''		3.33	3.32	
6''	3.80 (d, *J*=10.9) 6"A	3.80 6"A	3.80 6"A	
	3.38 (d, *J*=10.5) 6"B	3.40 6"B	3.40 6"B	
1'''	4.52 (s)	4.54, s	4.52	4.53 (br s)
2'''	3.63 (s)		3.53	
3'''	3.54			
4'''	3.27 (d, *J*=6.3)			
5'''	3.42 (d, *J*=9.8)	3.50	3.48 (d, 7.7)	
6'''	1.12 (d, *J*=6.2)	1.15 *J*=6.2	1.13 (d, 6.2)	1.19 (d, 6.2)

NMR was measured at 500 MHz (^{1}H) and 125 MHz (^{13}C) (CD_3OD)

Compound **4** was isolated as a yellow powdery solid with optical rotation of -50.47±0.8. It showed identical UV, ESIMS and NMR data with compound **3**. Chemical shifts observed in its H-NMR, however revealed it to be a galactopyranoside as seen with compound **2**. Compound **4** was thus identified as **Kaempferol-3-O-robinobioside** {kaempferol-3-O-[α-L-rhamnopyranosyl(1→6) β-D-galactopyranoside]}.

Compound **5** was isolated as a yellow amorphous solid with optical rotation of -23.83±0.8. It showed identical mass and UV spectra with compound **1**. An observed downfield shift of H-5'' of compound **5** relative to compound **1**, indicates a substitution on its C-4'. Based on this, Compound **5** was identified as Quercetin-3-O-[α-L-rhamnopyranosyl(1→4) β-D-glucopyranoside].

Compound **6** appeared as a yellow amorphous solid with optical rotation -28.29±1. It showed identical UV and mass spectra as well as very similar NMR with Compound **3**. Similarly as observed in **5** a slight upfield shift of H-5'' indicatedd a substitution on C-4'' and compound **6** was therefore identified as Kaempferol-3-O-[α-L-rhamnopyranosyl (1→4) β-D-glucopyranoside].

Compound **7** ($[\alpha]_D^{20}$ = -18.17±1) was isolated as a major compound in an isomeric mixture with **8** ($[\alpha]_D^{20}$ = +48.00±0.3). They have identical UV MS and NMR spectra data as compound **1**. A downfield shift of proton 3'' (δ_H=3.63) in NMR data of compounds **7**/**8** supported substitution at position C-2. These shifts are analogous to those reported for flavonol rhamnosyl (I→2) [15]. Compound **7** and **8** are very closely related as can easily be observed in their proton and COSY spectra. Compound **7** is a glucoside as evident in the upfield shift in its rhamnosyl protons (δ_H=1.13), downfield shift of the anomeric proton of the directly attached hexose etc as discussed earlier, while compound 8 is a galactoside. Compound **7** is therefore confirmed to be Quercetin-3-O-[α-L-rhamnopyranosyl(1→2) β-D-glucopyranoside] while Compound **8,** also isolated as a major compound in another isomeric mixture with Compound **7** was confirmed to be Quercetin-3-O-[α-L-rhamnopyranosyl(1→2) β-D-galactopyranoside].

All the compounds were screened for antioxidant and antimicrobial activities. Rutin **(1)**, Quercetin -3-O-robinobioside **(2)**, Quercetin-3-O- [α-L-rhamnopyranosyl (1→4) β-D-glucopyranoside] **(5)**, Quercetin-3-O-[α-L-rhamnopyranosyl (1→2) β-D-glucopyranoside] (**7)** and Quercetin-3-O-[α-L-rhamnopyranosyl(1→2) β-D-galactopyranoside] **(8)** which are derivatives of quercetin, showed a dose-dependent antioxidant activity on DPPH free radical scavenging model with IC_{50} values of 52, 48, 36, 66 and 56 µg/mL respectively. The three kaempferol derivatives Kaempferol -3-O-rutinoside **(3)**, Kaempferol-3-O-robinobioside **(4)** and Kaempferol-3-O-[α-L-rhamnopyranosyl (1→4) β-D-glucopyranoside] **(6)** did not show any antioxidant activity (IC_{50}>100 µg/mL). This observation is consistent with an earlier report which showed that an ortho-dihydroxyl functional group at the B-ring of flavonoids is highly effective for scavenging free radicals [20]. Previous reports also showed that rutin, robinobioside and other quercetin derivatives exhibited profound anti-oxidant activity in DPPH free radical scavenging and other *in vitro* models [21-23]. Compound **5** showed better antioxidant activity than the standard drug, Vitamin C (IC_{50}= 49 µg/mL) The antioxidant activity of compounds **1**, **2**, **5**, **7**, **8** may be attributed to their proton donating ability [24].

Compounds **7** and **8** were active against *Escherichia coli* with IZD (inhibition zone diameter) values of 17 and 13 mm at 62.5 µg/mL respectively; and MIC (minimum inhibitory concentration) values of 1.77 µg/mL and 1.92 µg/mL respectively. Flavonoids have also been shown in previous studies to exhibit profound antimicrobial activity [25]. The profound

Table 4. Antioxidant assay of the compounds using the DPPH free radical scavenging model

Conc µg/ml	Percent inhibition (%) of the compounds and IC_{50} (µg/mL) values								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Vitamin C**
100	62.14	64.85	-	-	62.68	-	61.69	62.5	66.85
80	62.32	62.50	-	-	64.13	-	62.31	61.78	66.30
40	44.75	47.30	-	-	53.53	-	27.71	42.03	45.11
20	18.21	24.46	-	-	37.51	-	12.5	26.63	14.67
10	11.41	7.07	-	-	24.19	-	3.44	14.95	7.61
(IC_{50})	(52.0)	(48.0)	-	-	(36.0)	-	(66)	(56.0)	(49.0)

antioxidant activity of some of the isolated quercetin derivatives may explain the ethnomedicinal use of the plant extracts in the management of inflammatory disorders.

4. CONCLUSION

Flavonoids are reputed to have anti inflammatory, anti-allergic, antitumor, antimicrobial and antioxidant effects. The profound antioxidant activity of the isolated quercetin derivatives may thus, explain the ethnomedicinal use of *Astonia boonei* leaf extracts in the management of inflammatory diseases and other disorders associated with oxidative stress. The antioxidant activity (and to some extent, antimicrobial activity) of the compounds isolated appear to depend on their molecular structure. This study is therefore, a typical case where nature provides unique and complex molecules for structure activity relationship studies. These group of compounds can also be useful in the investigation of the effect of differences in glycosyl substitution patterns on chemical shifts of the ring carbons of flavonoid nuclei.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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