ACTA ABP BIOCHIMICA POLONICA

Vol. 63, No 2/2016
353–358
http://dx.doi.org/10.18388/abp.2015_1178

Regular paper

Isolation and antioxidant activity of flavonoids from *Holarrhena floribunda* (G.don) leaves

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Bioactive polyphenolics are ubiquitously present in plants and may play an important role in the prevention and management of certain human diseases. Three known flavonoids viz Kaemperol-3-*O*-rutinoside (1), quercetin-3-*O*-glucoside (2) and kaemperol-3-*O*-glucoside (3) and inseparable mixture (1:1) of quercetin-3-*O*-glucose/galactose (4) were isolated, and identified for the first time from *Holarrhena floribunda*. The antioxidant capacity using the ORAC, FRAP and TEAC assays and inhibition of lipid peroxidation were measured for isolated flavonoids. The result showed that compounds 2 and 4 showed significantly increased ORAC, TEAC, and FRAP activities with low pro-oxidant potential as well as improved lipid peroxidation inhibition levels when compared to compounds 1 and 3. The most active compounds were found to be flavonoids with a quercetin basic structure. These results imply that the isolated flavonoid glycosides are responsible for the antioxidant activity of the plant leaves and it forms the scientific basis for its traditional usage.

Key words: polyphenolics, plants, *Holarrhena floribunda*, antioxidant, flavonoids

Received: 17 August, 2015; revised: 13 January, 2016; accepted: 11 February, 2016; available on-line: 12 May, 2016

INTRODUCTION

Flavonoids are low molecular weight, structurally related compounds with basic features of the 2-phenylbenzo-γ-prone nucleus consisting of two benzene rings linked through a heterocyclic pyran ring (Cushnie & Lamb, 2005). The protective health benefits of natural flavonoids and its broad pharmacological activity cannot be overemphasized and has led to increased interest among scientists with bias in functional foods (Kahkonen *et al*., 1999). Flavonoids and other phenolic compounds with antioxidant activities are reputed to play a preventive role in the development of oxidative stress-related diseases like certain cancers (Kahkonen *et al*., 1999). Reactive oxygen species (ROS) produced during normal metabolism or induced by exogenous factors, have been implicated in the aetiology of several human diseases (Tapas *et al*., 2008). ROS is known to contribute to cellular aging, mutagenesis, carcinogenesis and coronary heart diseases (Sastre *et al*., 2000, Takabe *et al*., 2001, Kawanishi *et al*., 2001). The scavenging of ROS, metal chelating ability or induction of antioxidant enzymes by the flavonoids are essential in preventing impending damage to important cellular components such as DNA, proteins and lipids (Suganya *et al*., 2007). Oxidation of lipids for example, leads to lipid peroxidation, which is a free-radical mediated propagation of oxidative insult to the polyunsaturated fatty acid component of cell membranes (Heim *et al*., 2002). The potential of the health benefit of these compounds for the prevention and therapeutic uses has led to the investigation and identification of a wide range of bioactive principles, that include mainly flavonoids and phenolic compounds in the plants (vegetables, fruits, leaves, seeds, cereal, roots, spices and herbs) (Suganya *et al*., 2007). The protective effects of flavonoids are attributed to their ability to transfer electrons, chelate metals, activate antioxidant enzymes, reduce alpha-tocopherol radicals and inhibit oxidases (Heim *et al*., 2002). Anti-inflammatory, anti-diarrhoeal, anti-ulcer, anti-viral, anti-allergic and vasodilatory actions have also been attributed to these phytochemicals (Proestos & Komaitis, 2006). Previously, the methanolic extract and sub-fractions from the leaves of *Holarrhena floribunda* was reported to exhibit strong antioxidant activity (Badmus *et al*., 2010, Badmus *et al*., 2013). The present work sought to isolate and characterize the compounds responsible for the antioxidant activities.

PLANT MATERIAL

Holarrhena floribunda leaves were collected in Igbajo, Osun state, Nigeria during the raining season in July, 2011. It was identified and authenticated by the duo of Chukwuma, E.C and Ugbogu O.A at the Federal Research Institute of Nigeria (FRIN). Herbarium number FHI 109764 was issued at the Institute.

Preparation of methanolic leaf extract of *Holarrhena floribunda*. The leaves of *Holarrhena floribunda* were dried at room temperature. The dried leaves weighing 1.748 kg were extracted with 5.25 L methanol under stirring for 48 h (X 2). The filtrate was evaporated till dryness to give 380.70 g representing 21.78% yield (dry weight).The part of the dried total extract filtrate (50 g) was loaded into silica gel column eluted with a gradient

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Abbreviations: ABTS, 2,2′azabis-(3-ethyl benzothiozoline-6-sulfonate); AAPH, 2,2′-azobis (2-aminopropane); DCM, dichloromethane; EGCG, Epigallocatechin gallate; FRAP, ferric reducing antioxidant power; MEOH, methanol; ORAC, oxygen radical absorption capacity; ROS, reactive oxygen species; TE, Trolox equivalent; TEAC, Trolox equivalent antioxidant capacity

mixture of ethyl acetate and methanol. The ratio of ethyl acetate: methanol used was 9:1, 8:2, 6:4 and 4:6, respectively. A total of 51 eluents (500 ml each) was collected and pooled together according to their TLC profile to 18 main fractions.

Sub-fractions 13 and 14 from the main total extract were shown to contain flavonoids as indicated by TLC and selected for further purification. Both fractions (13 & 14) were subjected to silica gel column eluted with dichloromethane:methanol (DCM: MEOH) mixture 1% to 15%. The sub-fractions obtained were purified on Sephadex column using 100% ethanol as mobile phase and finally high performance liquid chromatography (HPLC) using different ratios of acetonitrile and water to yield three pure compounds (**1–3**) and inseparable mixture (1:1) (**4**).

Oxygen radical absorption capacity (ORAC). ORAC generates both qualitative and quantitative measures which present fast and slow acting antioxidant activity of a test compound (DeLange & Glazer, 1989). Briefly, 12 µl of test sample (0.1, 1, 10 and 100 µg/ml) was combined with 138 µl of the fluorescein working solution. The reaction was started by the addition of 50 µl reactive species in 96-well plate. The absorbance was measured with a Fluoroskan spectrum plate reader with excitation/emission wavelengths set at 485/530 nm at 37°C against a reagent blank prepared with phosphate buffer. The method measures the antioxidant scavenging capacity of thermal decomposition generated by (1) peroxyl radical of 2,2'-azobis (2-aminopropane) dihydrochloride (AAPH; $ORAC_{ROO}$. assay), (2) hydroxyl radical ($ORAC_{OH}$. assay), generated by H_2O_2-Cu^{2+} (H_2O_2, 0.3%; Cu^{2+} [as $CuSO_4$], 18 µM), or (3) Cu^{2+} [as $CuSO_4$], 18 µM as a transition metal oxidant at 37°C. ORAC values were expressed as micromole of Trolox equivalents (TE) per milligram dry weight (µM TE/mg DW) of test sample, except when Cu^{2+} (without H_2O_2) was used as an oxidant in the assay. In the presence of Cu^{2+} without H_2O_2, test samples acted as prooxidants rather than antioxidants in the ORAC assay. The copper-initiated prooxidant activity was calculated using $[(Area_{Blank} - Area_{Sample})/Area_{Blank}] \times 100$ and expressed as prooxidant units; one unit equals the prooxidant activity that reduces the area under the fluorescein decay curve by 1% in the ORAC assay (Cao *et al*., 1997).

Ferric reducing antioxidant power (FRAP). FRAP measures a single electron transfer reaction from the antioxidant molecule to the oxidant. The change in absorbance value of either oxidant or antioxidant is a measure of reducing capability of the antioxidant (Ou *et al*., 2002).

A working FRAP reagent was prepared in accordance with previously described method (Benzie and Strain, 1999). The Samples (100 µl; [0.1, 1, 10, 100 µg/ml]) were prepared in triplicate followed by the addition of 3 ml freshly prepared FRAP reagent. The reaction mixture was incubated at 37°C for 4 min and absorbance read at 593 nm against the blank prepared with distilled water. L-Ascorbic acid was used as standard and the results were expressed as micromole ascorbic acid equivalent per milligram dry weight (µM AAE/mg DW) of the test samples.

Trolox equivalent antioxidant capacity (TEAC). The TEAC assay estimates inhibition of radical cation production by the antioxidant in the sample. The concentration of antioxidant in the sample is inversely proportional to the absorbance of the radical cation produced by 2,2'-azo-bis-(3-ethyl benzothiozoline-6-sulfonate) (ABTS) (Gupta *et al*., 2009). TEAC assay is a useful assay for tracking down unknown antioxidants in a complex mixture (Arts *et al*., 2004). The assay was evaluated in accordance with previously described method (Re *et al*., 1999). Briefly, 25 µl of test sample (0.1, 1, 10, 100 µg/ml) was added to 300 µl of ABTS (1 ml of ABTS in 20 ml ethanol) in triplicate. The reaction mixture was incubated for 30 min. Absorbance of the reaction was measured with a Multiskan spectrum plate reader at a wavelength of 734 nm at 25°C against the blank prepared with ethanol. Results were expressed as micromole Trolox equivalents per milligram dry weight (µM TE/mgDW) of test samples.

Inhibition of Fe(II)-induced microsomal lipid peroxidation. Inhibition of Fe(II)-induced microsomal lipid peroxidation was determined by the method described by Snijman and co-workers (Snijman *et al*., 2009). Liver microsomes were prepared from male Fischer 344 rats. Microsomes were purified from the liver S9 fraction using a Sepharose 2B column as previously described (Gelderblom *et al*., 1984). The absorbance was read at 532 nm. The percentage inhibition of sample relative to control was calculated by the equation below;

$$[(A_{control} - A_{sample})/A_{control}] \times 100.$$

Where $A_{control}$ and A_{sample} refer to the absorbance of reacting mixture without the sample and the absorbance in the presence of sample respectively.

Statistical analyses. Data are expressed as means ± S.D. of experiments performed in triplicate. The values were analyzed by Two-Way ANOVA, followed by Tukey's multiple comparison tests using GraphPad Prism software version 6 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com). A *P* value of less than 0.05 was considered significant.

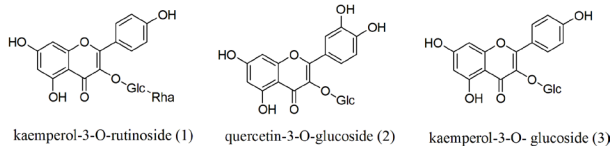
RESULTS

Isolation and characterization of flavonoids from *Holarrhena floribunda* leaves

TLC screening of different fractions from the methanolic leaf extract of *Holarrhena floribunda*, showed that sub-fraction 13 and 14 were rich in flavonoids, which were submitted for chromatographic purification using the combination of silica gel and sephadex column and HPLC. Compound **1** yielded 17.6 mg while **2**, **3** and **4** (inseparable mixture (1:1) of quercetin-3-O-glucose/galactose) gave 12.3, 17.3 and 14.72 mg, respectively (Fig. 1). The compounds were fully identified based on 1D and 2D NMR spectra. The data were compared with previously published study (Gudej, 2003; Sikorska & Matlawska, 2000; Nowak & Wolbis, 2002). These flavonoids were further subjected to antioxidant capacity test, including ORAC, FRAP, TEAC and lipid peroxidation inhibition.

Evaluation of oxygen radical absorbance capacity

The antioxidant evaluation of flavonoid-rich extracts using the ORAC assay in the presence of AAPH, Cu^{2+}-



kaemperol-3-O-rutinoside (1) quercetin-3-O-glucoside (2) kaemperol-3-O- glucoside (3)

Figure 1. The structure of flavonoids isolated from the *Holarrhena floribunda* leaves.

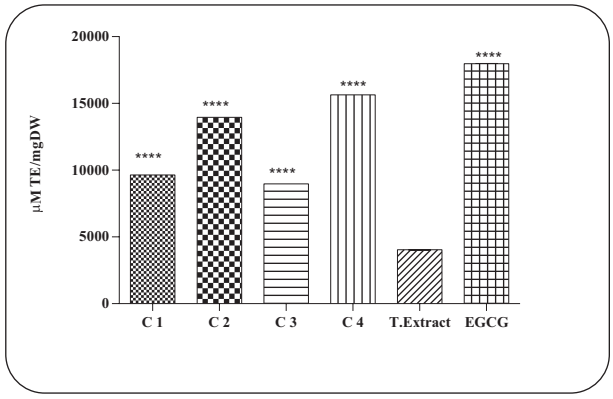


Figure 2. The ORAC values of isolated flavonoids, total extract and the standard (EGCG) using AAPH as an oxidant.
Data are expressed as mean ±S.D. µM TE/mgDW. ****P<0.0001 when compared to T.Extract (Total extract). Note that the standard deviation is very low and as such is not showing in the figure.

H_2O_2 and Cu^{2+} showed that the isolated flavonoids and total extract have remarkable ORAC activities against the oxidants AAPH and Cu^{2+}-H_2O_2 as shown in Figs. 2 and 3 below. However, Compounds **2** and **4** were significantly (P< 0.05) higher compared with compounds **1**, **3** and the total extract in the AAPH and Cu^{2+}-H_2O_2 systems. Figure 4 shows the percentage pro-oxidant ability of the compounds in the presence of Cu^{2+}. The result (Fig. 4) showed that none of the isolated compounds and the total extract acted as a pro-oxidant in the presence of the Cu^{2+}.

Evaluation of ferric reducing antioxidant power

The ability of compounds to reduce Fe^{3+} to Fe^{2+} was evaluated using the established FRAP method. Figure 5 below shows the ability of each compound and the total extract to reduce Fe^{3+} to Fe^{2+} calculated as micromole ascorbic acid equivalent per milligram dry weight (µmol AAE/mgDW). The present investigation showed that compounds **1** and **3** have significantly lower FRAP activity (P<0.0001) when compared with compounds **2**, **4** and the methanolic extract with high activity (1561.37, 1527.63 and 850.06 µmol AAE/mgDW respectively). The standard (EGCG; 4754 µmol AAE/mgDW) was significantly higher than the isolated compounds and the total extract.

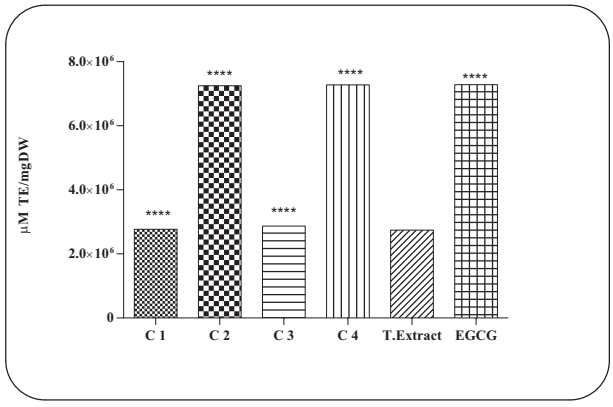


Figure 3. The ORAC values of isolated flavonoids, total extract and the standard (EGCG).
Cu^{2+}-H_2O_2 was used to generate OH radical as an oxidant. Data are expressed as mean ±S.D. µM TE/mgDW. ****P<0.0001 when compared to the T.Extract (total extract).

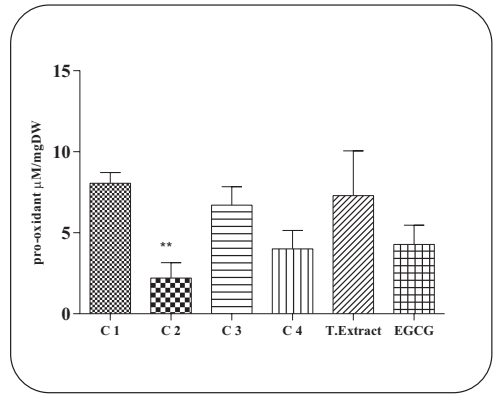


Figure 4. The percentage of pro-oxidant induction in relation to untreated control in the presence of Cu^{2+} evaluated using ORAC assay.
Data are expressed as mean ±S.D. pro-oxidant µM/mgDW. **P<0.05 when compared to the T.Extract (total extract).

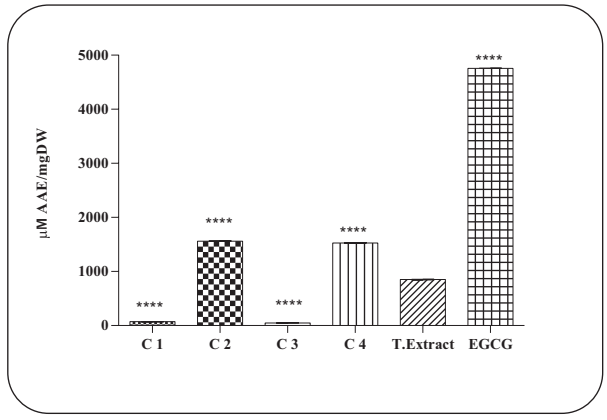


Figure 5. Ferric reducing antioxidant power assay (FRAP) of isolated compounds, total extract and the standard (EGCG).
Data are expressed as mean ±S.D. µM AAE/mgDW. ****P<0.0001 when compared to the T.Extract (total extract).

Trolox equivalent antioxidant capacity (TEAC)

Trolox equivalent antioxidant capacity of compounds and the total extract of *Holarrhena floribunda* are presented in Fig. 6. The TEAC assay involves evaluation of the quenching potential of antioxidants in the presence of

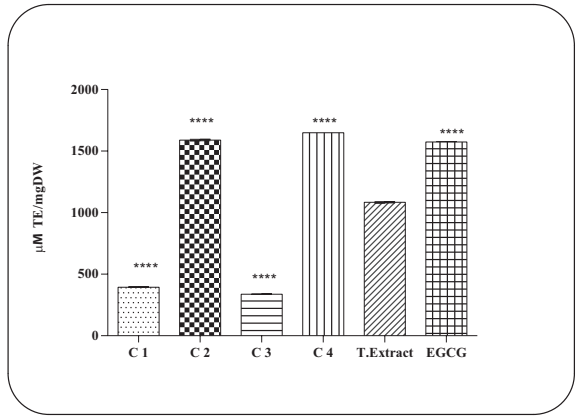


Figure 6. Trolox equivalent capacity (TEAC) of the isolated flavonoids and the total extract in µmole trolox equivalent per gram dry weight.
Data are expressed as mean ±.S.D. µM TE/mgDW. ****P<0.0001 when compared to the T.Extract (total extract).

long-lived radical cation chromophore 2,2' azinobis-(3-ethylbenzothiazoline-6-sulphonate) $ABTS^{\cdot+}$. The result of the TEAC assay follows the same manner as observed in the FRAP assay above. Compound **2** shows (1649.4 μmol TE/mgDW)>compound **4** (1589.9 μmol TE/mgDW)>EGCG (1573.5 μmol TE/mgDW)> Total extract (1084.4 μmol TE/mgDW)>compound **1** (394.8 μmol TE/mgDW)>compound **3** (337.5 μmol TE/mgDW). The results showed that the TEAC values for compounds **2**, **4**, Total extract and EGCG were significantly ($P<0.0001$) higher when compared with compounds **1** and **3**.

Inhibition of Fe-induced lipid peroxidation in rat liver microsome

The effects of the compounds and the total extract on the induced-lipid peroxidation were evaluated in S9 rat liver fraction. The compounds **2**, **4** and the extract showed dose dependent inhibition of lipid peroxidation, while dose dependent inhibitions were not observed in compounds **1** and **3**. The IC_{50} values of the results were calculated using the Prism 6 statistical software. The IC_{50} values (Table 1) showed that compound **2** has a 10.4 μg/ml while compounds **4** and the extract have 9.8 and 7.2 μg/ml, respectively and IC_{50} values for compounds **1** and **3** could not be determined because they do not show dose dependent inhibition of lipid peroxidation. There is, however, no significant difference in the IC_{50} values among the compound **2**, **4** and the extract.

DISCUSSION

Dietary antioxidants with potential for therapeutic and prevention use have been a major focus of research in recent years. Isolation and characterization of bioactive compounds from plants with health-maintaining and disease-preventing properties have led to the isolation and identification of an array of compounds among which include flavonoids (Suganya *et al.*, 2007). The present work isolated flavonoids by subjecting the methanolic

Table 1. Inhibition of Fe-induced lipid peroxidation by isolated compounds and the total extract in rat liver microsome.

Compound	Concentration (μg/ml)	Inhibition (%)	IC_{50} (μg/ml)	Regression equation
1	0.67	4.50±0.37	N.D.	Y=0.4353*X + 3.093
	6.67	6.00±1.76		R^2=0.8370
	13.40	7.55±1.86		
	16.70	9.27±2.19		
	26.70	16.06±0.98		
2	0.67	11.59±1.62	10.4	Y=2.202*X + 13.13
	6.67	20.44±9.98		R^2=0.8193
	13.40	55.52±0.81		
	16.70	55.37±1.27		
	26.70	63.94±6.45		
3	0.67	0.43±0.7	N.D.	Y = 0.4822*X – 0.8752
	6.67	0.44±0.4		R^2 =0.6700
	13.40	4.58±2.14		
	16.70	9.75±1.11		
	26.70	11.34±0.55		
4	0.67	8.58±0.64	9.8	Y=2.333*X + 14.05
	6.67	31.11±2.74		R^2=0.9074
	13.40	50.67±0.81		
	16.70	61.21 ±1.27		
	26.70	68.34±0.47		
Extract	0.67	2.14±1.70	7.2	Y=2.742*X + 3.333
	6.67	17.33±5.77		R^2=0.9369
	13.40	50.13±1.68		
	16.70	52.20±0.84		
	26.70	70.70±0.82		
Quercetin	0.27	5.31±1.73	2.95	Y=3.909*X + 11.80
	0.53	26.00±1.70		R^2=0.8739
	1.13	23.53±1.73		
	2.26	51.23±0.83		
	4.53	65.28±0.99		

N.D. = Not detected

leaf fractions of *Holarrhena floribunda* to different chromatography techniques. The flavonoids were isolated, purified using HPLC, identified with NMR spectroscopy and data compared with that of the literature. Three pure compounds and an inseparable mixture of two compounds were isolated and identified. The compounds showed a dull spot under UV lights, which indicate the nature of flavonoids.

However, further to the isolation of flavonoids from the leaves, isolated flavonoids were subjected to antioxidant evaluation using ORAC, FRAP, TEAC and the *in vitro* inhibition of lipid peroxidation. The ORAC assay takes into account both inhibition time and the degree of inhibition into a single assay by considering area under the curve (Cao *et al*., 1995). In this study, in addition to the commonly used AAPH as an oxidant, Cu^{2+}-H_2O_2 and Cu^{2+} alone were used as a source of oxidant. Cu^{2+}-H_2O_2 is used to mimic the *in vivo* generation of oxidant as H_2O_2 and transition metal are available *in vivo* and are frequently used *in vitro* to induce oxidative damage to proteins and nucleic acids (Cao *et al*., 1997; Parthasarathy *et al*., 1989; Sato *et al*., 1992). In the presence of a transition metal like Cu^{2+} alone, it is believed that flavonoids act as a pro-oxidant (Cao *et al*., 1997). The Cu^{2+} was used in this study to evaluate pro-oxidative potential of the isolated flavonoids. Evaluation of the antioxidant capacity of the isolated compounds using the ORAC peroxyl radical ($ORAC_{ROO\cdot}$) assay showed a high degree of antioxidant capacity for all the isolated compounds. However, compounds **2** and **4** showed significantly higher activities compared with the compounds **1** and **3**. The $ORAC_{OH\cdot}$ activity of these compounds also followed a similar pattern to what is obtained in $ORAC_{ROO\cdot}$ activities. The $ORAC_{OH\cdot}$ activities of the flavonoids were several folds higher than what was obtained for $ORAC_{ROO\cdot}$. This could be that the flavonoids isolated were more specific to and aimed at OH· radical protection. Chen *et al*. in their structural-activity relationships concluded that phenolic hydroxyls in flavonoids, hydroxyl groups in A and B rings (*ortho*-dihydroxyl groups in A and B rings), are important to the hydroxyl radical scavenging activity of flavonoids (Chen *et al*., 2002). Inversely however, the pro-oxidant activities using Cu^{2+} in the ORAC assay showed that compounds **1** and **3** were more prone to behave like prooxidants in the presence of the metal than the compounds **2** and **4**. This is not unexpected because the previous reports have correlated the number of flavonoids' OH group substitution to its ORAC activity (Rice-Evans *et al*., 1996). The observed results can be related to the basic structure of the flavonoids as compounds **2** and **4** have quercetin as their basic structure, while **1** and **3** have kaempferol. Cao *et al*. have demonstrated that $ORAC_{ROO\cdot}$ activity of quercetin is higher than that of kaempferol (Cao *et al*., 1997). The pro-oxidant activity can also be related to the number of substituted OH groups in the flavonoids as it showed in this study that the lower the number of OH groups the higher the pro-oxidation capacity in the presence of Cu^{2+}.

The importance of substitutions such as OH-group, methoxy group and O-dihydroxy in the B-ring are related to the high antioxidant activity of the flavonoids using a FRAP assay (Firuzi *et al*., 2005). The results of the FRAP assay of this study show that OH group substitution in the compounds **2** and **4** are responsible for the significant antioxidant activity compared to compounds **1** and **3**. In the same vain, similar activities were shown by the isolated flavonoids when subjected to TEAC antioxidant assay. The result implies that the activities of these flavonoids remarkably correlate with the 3'-OH group on the B-ring of flavonoids basic structure which is the only different between quercetin and kaempferol flavonoids structure. The strong antioxidant activity of the extract, however, implies that the isolated compounds **2** and **4** are responsible for the observed antioxidant activity in the total extract although they are not complementary.

Endogenous and exogenous factors such as metabolism, chemicals and ionizing radiations are linked to the induction of free radicals in biological tissues (Iqbal *et al*., 2003). Iron is known to be involved in the generation of reactive oxygen species (ROS) *in vivo* which may attack lipids to form damaged products (Imlay & Linn, 1988; Aruoma *et al*., 1989). Lipid peroxidation is an important product of reaction between free radicals and lipids (D'Souza & Prabhu, 2006). The ability of an agent to protect lipids from oxidative damage by ROS can be related to its antioxidant ability.

Iron-induced lipid peroxidation is a reliable biological marker of cellular oxidative stress (Dargel, 1992). The result, showed that compounds **1** and **3** did not exhibit significant inhibition of lipid peroxidation, as the values did not reach up to 50% inhibition in the concentration used while compounds **2** , **4** and total extract showed non-significant IC_{50} values when compared to each other. The lipid peroxidation inhibitory activities of flavonoids are shown to be related to the number of hydroxyl group, substitution of the hydroxyl group, catechol moiety on the B-ring and double bonds between carbon 2 and 3 of the C-ring (Cholbi *et al*., 1991, Mora *et al*., 1990). The presence of a sugar moiety however affects the activity of the lipid peroxidation inhibition due to steric hindrance between the sugar and adjacent hydroxyl group likewise methoxy group (Cholbi *et al*., 1991). This can be applied to the results obtained in the present study when the inhibition of lipid peroxidation of the isolated glycosylated flavonoids was compared with the quercetin standard. The result showed that IC_{50} inhibition of peroxidation by the standard is about 3 folds lower than the isolated flavonoids. On the other hand, glycosylation of flavonoids increased the hydrophilicity and hence enhances bioavailability better than for the aglycone flavonoids (Kumar & Pandey, 2013). However, despite lower activity of glycosylated flavonoids compared to their aglycone, bioavailability will be a determining factor of their bioactivity *in vivo* (Thilakarathna & Rupasinghe, 2013). Enhancement of bioavailability will be an important factor in order to exert an eventual beneficial effect *in vivo* (Thilakarathna & Rupasinghe, 2013). In addition, the results obtained in this study is in agreement with the number of hydroxyl group substitution on the B-ring as compounds **2** and **4** with hydroxyl group in B-ring showed effective dose dependent lipid peroxidation compared with compounds **1** and **3**.

In summary, the antioxidant activity of flavonoids, which involve neutralization of free radicals initiating oxidative-cascade of reactions or termination of the free radical chain reaction due to hydrogen donating property, can be related to their structures (Cao *et al*., 1997; Suganya *et al*., 2007). Compounds **2** and **4** have an *ortho*-dihydroxyl in the B-ring of the flavonoid skeleton (catechol) which plays an essential role in the antioxidant activity of flavonoids (Bors *et al*., 1990) and is responsible for the observed effects in those compounds. The antioxidant activity of flavonoids is known to depend largely on the functional groups attached to the nuclear structure (Heim *et al*., 2002). It is noteworthy to observe that in the battery of antioxidant assays done in this study, no significant differences could be shown when compar-

ing compounds **2** and **4** and compounds **1** and **3**, which showed selective antioxidant activities only. This implies that the only common factor that is responsible for the observed differences in the antioxidant activities of the flavonoids in this study is *ortho*-dihydroxyl in the B-ring.

This work has lend scientific credence to the claim by the tradition that the leave of *Holarrhena floribunda* has medicinal values and also that the plant can be a source of natural antioxidant which is beneficial for maintaining the health status of humans.

Acknowledgements

The authors thank TETFund (Nigeria) for PhD travel scholarship granted to the first author. The work was sponsored, in part, by the National Research Foundation (NRF), South Africa.

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