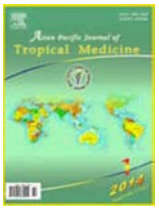
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm



Document heading doi: 10.1016/S1995-7645(14)60289-3

Bioprospecting medicinal plants for antioxidant components

Ahmed Yacouba Coulibaly[1,2*], Rokiah Hashim[2], Shaida Fariza Sulaiman[3], Othman Sulaiman[2], Lily Zuin Ping Ang[2], Kheng Leong Ooi[3]

[1]*Laboratory of Applied Biochimistry and Chemistry (LABIOCA), UFR/SVT, University of Ouagadougou, 09 BP 848 Ouagadougou 09, Burkina Faso*

[2]*Division of Bioresource, Paper and Coatings Technology, School of Industrial Technology, Universiti Sains Malaysia, Penang, Malaysia*

[3]*School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia*

ARTICLE INFO

Article history:
Received 26 Mar 2014
Received in revised form 19 May 2014
Accepted 11 Jun 2014
Available online 10 Sep 2014

Keywords:
Antioxidant
Polyphenols
Chemical identification
UPLC analysis
Medicinal plants

ABSTRACT

Objective: To evaluate antioxidant activities of seven medicinal plant species and their fractions, and to identify their phenolic compounds.
Methods: Two extractions were processed and further fractionated by column chromatography to evaluate the concentration that inhibit 50% of 2,2′-azinobis (3-ethylbenzothiazoline-6-suslfonic acid, 1,1-diphenyl-2-picryl-hydrazyl radicals, and their ferric reducing antioxidant power. The identification of the fractions of phenolic compounds was done by ultra performance liquid chromatography.
Results: The aqueous-acetone extracts of *Feretia apodanthera* and *Ozoroa insignis* exhibited the highest antioxidant potentials comparable to those of the standard quercetin. Their subsequently silica gel column fractionation showed three most active fractions from which the major constituents quercetin, myricetin, kampferol, rutin and isoquercetin were identified.
Conclusions: These plant species have potent antioxidant profiles and polyphenol compounds that may help to manage with radical related disease and aging.

1. Introduction

Free radical formation is associated with the normal natural metabolism of aerobic cells that occur during the respiratory process. These reactive oxygen species (ROS) can be produced from both endogenous and exogenous sources.

Under normal circumstances, the generated ROS are detoxified by the antioxidant systems of the body to maintain the balance between ROS production and their removal. Due to ROS overproduction and/or inadequate antioxidant defense, this equilibrium is hampered leading to ROS upsurge and oxidative stress. Then, ROS readily attack and induce oxidative damage to various biomolecules, including proteins, lipids, lipoproteins and DNA[1]. This oxidative damage is implicated in several chronic human diseases and aging such as diabetes mellitus, cataract, rheumatoid, cancer, atherosclerosis, arthritis and neurodegenerative diseases[2,3].

Antioxidants are substances that are able to counter free radical, and they may help to suppress the imbalance that occurs during oxidative stress. They play a key role in protection of plants from pollution damage, disease prevention in both plants and animals, and are very important for the body defense system[4].

Natural products of plant origin have been proposed as a potential source of natural antioxidants with strong activity[5]. In the nature, polyphenols are among the most widespread class of metabolites with ubiquitous distribution and they are able to act as antioxidants in a number of ways. Several plant species have been screened for their antioxidant component, but there is still a lack of such information about a number of medicinal plant species. The current study investigated the antioxidant properties of tropical plants belonging to the families Euphorbiaceae, Rubiaceae, Anacardiaceae, Scrophulariaceae and Poaceae.

Fadogia agrestis (*F. agrestis*) is a woody herb or shrub that grows in the savannah ecosystem of Africa[6]. Its aphrodisiac, antiplasmodial and diuretic potentials have been reported[6-8], and two monoterpene glycosides were isolated from its leaves[9]. Ethnopharmacological surveys

*Corresponding author: Ahmed Y. Coulibaly, Division of Bioresource, School of Industrial Technology, Universiti Sains Malaysia, 11800 Penang, Malaysia.
Tel: +601126461217
E-mail: coulahmede@yahoo.fr
Foundation Project: Supported by the World Academy of Sciences (TWAS) and Universiti Sains Malaysia (USM) (Fellowship Reference Number: 3240255139).

indicated that *Euphorbia balsamifera* (*E. balsamifera*) is used to treat chronic wound, and *Feretia apodanthera* (*F. apodanthera*) for infective wounds[10]. The stem charcoal of *Ozoroa insignis* (*O. insignis*) is traditionally used as a milk preservative by pastoralists in West Africa[11]. The antiradical activities of *O. insignis*[11], *Scoparia dulcis* (*S. dulcis*) and *Lepidagathis anobrya* (*L. anobrya*) were reported[12,13].

2. Material and methods

2.1. Plant material

Aerial parts (stems and leaves) of seven herbaceous plant species *E. balsamifera* Ait. (Euphorbiaceae), *F. apodanthera* Del. (Rubiaceae), *L. anobrya* Nees (Acanthaceae), *F. agrestis* Schweinf. ex Hiern. (Rubiaceae), *Sporobolus pyramidalis* P. Beauv. (Poaceae) (*S. pyramidalis*), *S. dulcis* L. (Scrophulariaceae) and *O. insignis* Del. (Anacardiaceae) were freshly-collected at Gampela (25 km east of Ouagadougou, Burkina Faso) on June 2013. Taxonomic identification was verified by Prof. Jeanne F. Millogo (Laboratoire de Biologie et Ecologie Vegetales, University of Ouagadougou, Burkina Faso) and a voucher specimen was deposited for each plant species under the following numbers: *E. balsamifera* (EB_aca 001), *F. apodanthera* (FA_rca 001), *L. anobrya* (LA_nca 001), *F. agrestis* (FA_sca 001), *S. pyramidalis* (SP_pca 001), *S. dulcis* (SD_ca 002) and *O. insignis* (OI_dca 001). Each plant species was separately air-dried in the laboratory and then reduced into powder for future use.

2.2. Chemicals

All chemicals used in this study are of analytical grade. Acetone, methanol and hexane were purchased from Fisher. Folin-Ciocalteu reagent, 2,2′-azinobis(3-ethylbenzothiazoline-6-suslfonic acid) (ABTS), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), sodium carbonate, ferric chloride hexahydrate, iron(II), 2,4,6-Tris(2pyridyl)-S-traizine (TPTZ), potassium persulfate ($K_2S_2O_8$) and aluminium trichloride were obtained from Sigma.

2.3. Extraction

From each of the seven plant species, 25 g of powdered plant material was separately boiled for 45 min to make a decoction, which was air-dried at 50 °C to give a dried extract (the water-extract). Another 25 g of powdered plant material from each plant species was separately soaked in 250 mL of a mixture of acetone/water (70:30 v/v) for 48 h and after filtration, the solvent was evaporated under reduced pressure (132 hPa, 40 °C) and then air-dried at 50 °C to give a dried extract (the aqueous-acetone extract). Both of the extraction processes were repeated for three times.

2.4. Fractionation

The aqueous-acetone extracts of *F. apodanthera* and *O. insignis*, which showed the strongest antioxidant activities, were chromatographed on a silica gel column (60-120 mesh) and successively eluted with increasing polarity gradients of a mixture (60 mL) of hexane/acetone/water (5:2:0.2, 5:3:0.2, 5:4:0.2, 5:5:0.2, 5:6:0.2, 5:6:0.4, 5:6:0.6, 5:6:0.8, 5:6:1, 5:6:1.2 v/v) and then isocratic elution with acetone/water (7:3 v/v).

Thirty-seven and thirty-three initial fractions [(20±2) mL] were respectively collected from *F. apodanthera* and *O. insignis*. Then, each of these fractions were separated on silica gel GF254 thin layer chromatography developed with eluted hexane/acetone/water (5:6:0.4 v/v) and identified by UV light at 254 nm and 365 nm. After merger of the same reference frontal (R_f) values, 9 fractions were finally obtained from *F. apodanthera* (F1 to F9), and 10 fractions from *O. insignis* (O1 to O10).

2.5. Total polyphenol content

The total phenolic content of each extract was determined by the Folin-Ciocalteu method as described previously[12]. The diluted aqueous solution of each extract (0.5 mL, 1 mg/mL) was mixed with Folin-Ciocalteu reagent (0.2 mol/L, 2.5 mL). This mixture was incubated at room temperature for 5 min and then sodium carbonate solution (2 mL, 75 g/L in distilled water) was added. After 2 h of incubation, the absorbencies were measured at 760 nm against water blank. A standard calibration curve was plotted using gallic acid (0-200 mg/L). The results were expressed as mg of gallic acid equivalents (GAE) per 100 mg of dried extract (mg GAE/100 mg). All tests were run in triplicates.

2.6. Total flavonoids content

The total flavonoids were estimated as described previously[12]. A diluted methanolic solution of each extract (2 mL, 1 mg/mL) was mixed with a solution of aluminium trichloride (2 mL, 2% in methanol). The absorbance was read at 415 nm after 10 min against a blank (2 mL extract, 2 mL methanol). Quercetin was used as reference compound to produce the standard curve, and the results are expressed as mg quercetin equivalent (QE) per 100 mg of dried extract (mg QE/100 mg). Each assay was repeated three times.

2.7. β-carotene content

β-carotene was determined as described previously[12]. Dried extract (100 mg) was vigorously shaken with 10 mL of acetone-hexane mixture (4:6) for 1 min and filtered through Whatman filter paper. The absorbance of the filtrate was then measured at 453, 505, and 663 nm. All tests were run in triplicates. Content of β-carotene was calculated according to the following equation:

β-carotene (mg/100 mL)= $0.216\ A_{663} - 0.304\ A_{505} + 0.452\ A_{453}$.

2.8. DPPH assay

Radical-scavenging activities of the plant extracts, fractions and quercetin were determined using DPPH radical on a 96-well microplate reader[14]. A solution of DPPH radical (0.3 mmol/L in methanol) was prepared and then 150 μL of this solution was mixed with 50 μL of 2-fold serial dilutions of sample solution (1.96-4 000.00 μg/mL for crude extracts and 0.78-100.00 μg/mL for fractions and quercetin). The mixture was incubated for 30 min in darkness at room temperature. Scavenging capacity was measured on a spectrophotometer by monitoring the decrease in absorbance at 515 nm using a Multiskan EX microplate reader, Thermo Fisher Scientific. A blank containing methanol and sample solution was prepared for background subtraction. A control (150 μL of DPPH, 50 μL of methanol) was also run. All tests were run in triplicates. The percentage of DPPH scavenging effect was calculated using the following equation:

DPPH scavenging effect (%)=($A_{control}$-A_{sample})×100/$A_{control}$

Where, $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of the sample test.

2.9. ABTS assay

The ABTS assay was carried out using a method based on the original and classic method previously developed with slight modifications[15]. The ABTS radical was prepared by reacting equal volumes of 1.1 mg/mL aqueous ABTS and 0.68 mg/mL aqueous potassium persulfate ($K_2S_2O_8$), and then storing in the dark for 6 h at room temperature. Then the absorbance of $ABTS^{\cdot+}$ solution was adjusted to 0.7±0.02 by a dilution in distilled water and 200 μL of this diluted solution were added to 2-fold serial dilutions of sample solution (50 μL, 0.49-1000.00 μg/mL for crude extracts and 0.78-100.00 μg/mL for fractions and quercetin). These solutions were gently mixed and incubated in darkness for 30 min at room temperature. Then the absorbencies of the resulting solutions were measured at 734 nm on a microplate reader. A blank (200 μL of distilled water, 50 μL of sample) and a control (200 μL of ABTS solution, 50 μL of distilled water) were simultaneously performed. Quercetin was used as standard. All assays were run in triplicates. The scavenging ability of ABTS radical by the sample was calculated with comparison to the control as following:

ABTS scavenging effect (%)=($A_{control}$-A_{sample})×100/$A_{control}$

Where, $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of the sample test.

2.10. FRAP assay

The ferric reducing antioxidant power (FRAP) assay was conducted according to the previous method with slight modifications[16]. FRAP solution is a mixture of acetate buffer (0.3 mol/L, pH 3.6), ferric chloride hexahydrate (0.02 mol/L in distilled water) and 2,4,6-Tris(2pyridyl)-S-traizine (0.01 mol/L in 0.04 mol/L HCl) with proportion 10:1:1, respectively. A 2-fold serial dilution of samples was prepared (0.49-1000.00 μg/mL for crude extracts and 0.78-100.00 μg/mL for fractions and quercetin) and an aliquot (50 μL) was added to 150 μL of FRAP solution. After 30 min of incubation at a temperature of 37 °C, absorbance of reaction mixture was measured at 593 nm using Multiskan EX microplate reader, Thermo Fisher Scientific. A water blank without FRAP solution was also run. Simultaneously, a control was prepared by mixing distilled water (50 μL) with a new FRAP solution in which ferric chloride was replaced by iron (II) solution (150 μL, 0.020 mol/L). Quercetin was used as a standard. All tests were run in triplicates.

2.11. UPLC identification of polyphenolic compounds

The identification of polyphenolic compounds from column fractions of *F. apodanthera* and *O. insignis* was performed using an Acquity ultraperformance liquid chromatography (UPLC) system (Waters, Milford, MA, USA) equipped with a reverse-phase Acquity UPLC BEH C-18 column, 1.7 μm (100 mm×2.1 mm inner diameter) and a photodiode array detector. The mobile phase consisted of solvent A (1% acetic acid) and solvent B (methanol/acetic acid/water; 18:1:1). Each sample was separated using a gradient mode that was initially set at an A:B ratio of 85:15 and then linearly increased to 65:35 at 1.3 min, 30:70 at 3 min, 5:95 at 4 min and 85:15 at 5.3 min until 6.3 min (378 seconds). The detector was set at 320 nm for identification of caffeic acid and at 370 nm for flavonoids (quercetin, isoquercitrin, luteolin, myricetin, rutin and kampferol) with a flow rate of 0.20 mL/min and an injection volume of 1.0 μL for standards, 6.0 μL for non-hydrolysed fractions and 12.0 μL for hydrolysed fractions. Phenolic compounds in the extracts were identified by comparison of their retention times and UV spectra (detected by the photodiode array detector) with those of authentic standards.

2.12. Statistical analysis

All the reactions were performed in triplicate and data are presented as mean±SD. Data were analyzed by One-way analysis of variance followed by the Tukey multiple-comparison test with XLSTAT 2013.4.08. A *P*-value less than 0.05 was used as the criterion for statistical significance.

3. Results

3.1. Antioxidant components

The yield and phytochemical content of crude extracts from the seven plants species are presented in Table 1. The yield of extraction ranged from (10.54±1.89)% to (22.04±0.62)% for water-extracts (decoction) and from (10.24±0.39)% to (34.90

±8.57)% for aqueous-acetone extracts; the highest yield was obtained from *E. balsamifera* and *F. apodanthera* aqueous-acetone extracts.

The total phenolic contents as determined by Folin-Ciocalteu method were expressed in mg GAE/100 mg. These values ranged from (3.45±0.05) to (12.81±0.14) mg GAE/100 mg for water extracts and from (5.77±0.04) to (29.32±0.66) mg GAE/100 mg for aqueous-acetone extracts (Table 1). Along the seven plant species, the richest in total phenolic compounds are *F. apodanthera* and *O. insignis* and the poorest is *S. pyramidalis*. For each plant species, the amounts of total phenolic, flavonoids and β-carotene are higher in aqueous-acetone extracts than in water-extracts. Therefore, flavonoids as a part of polyphenols and β-carotene were in lower amounts in comparison to total phenolic. Along the seven plant species, flavonoid contents are expressed in mg QE/100 mg, and ranged from (0.65±0.01) to (5.61±0.12) mg QE/100 mg, and β-carotene contents from (0.060±0.001) to (0.640±0.005) mg/g (Table 1).

Table 1

Phytochemical content of water and aqueous-aceton extracts.

Plant species	Extracts	Yield (%)	Phenolic (mg/100mg)	Flavonoid (mg/100mg)	β-carotene (mg/g)
E. balsamifera	Water	22.04±0.62^{c}	6.06±0.14^{a,b}	2.37±0.33^{b}	0.110±0.001^{a,b}
	H_2O-aceton	34.90±8.57^{d}	10.69±0.16^{b}	4.53±0.15^{c}	0.640±0.005^{e}
F. agrestis	Water	17.20±4.58^{b}	9.53±0.11^{b}	1.82±0.20^{a,b}	0.150±0.040^{b}
	H_2O-aceton	13.86±1.10^{a,b}	10.61±0.21^{b}	5.05±0.21^{d}	0.560±0.003^{d}
F. apodanthera	Water	16.44±4.41^{b}	12.81±0.14^{b}	1.34±0.04^{a,b}	0.220±0.030^{b,c}
	H_2O-aceton	28.06±4.04^{c,d}	28.36±0.57^{c}	3.58±0.19^{b,c}	0.570±0.001^{d}
L. anobrya	Water	10.54±1.89^{a}	3.83±0.10^{a}	1.13±0.15^{a,b}	0.100±0.005^{a,b}
	H_2O-aceton	13.56±4.69^{a,b}	7.39±0.11^{a,b}	1.68±0.22^{a,b}	0.160±0.001^{b}
O. insignis	Water	13.48±1.01^{a,b}	12.39±0.22^{b}	2.29±0.08^{b}	0.160±0.001^{b}
	H_2O-aceton	18.58±5.28^{b}	29.32±0.66^{c}	5.61±0.12^{d}	0.490±0.010^{c}
S. dulcis	Water	12.06±0.59^{a}	5.03±0.12^{a}	1.43±0.02^{a,b}	0.060±0.001^{a}
	H_2O-aceton	12.36±2.77^{a}	8.82±0.17^{a,b}	5.54±0.17^{d}	0.580±0.002^{d}
S. pyramidalis	Water	14.36±2.48^{a,b}	3.45±0.05^{a}	0.65±0.01^{a}	0.130±0.010^{b}
	H_2O-aceton	10.24±0.39^{a}	5.77±0.04^{a}	3.33±0.13^{b,c}	0.590±0.001^{d}

Values within each column with different superscripted letters differ significantly (P<0.05).

3.2. Antioxidant activity

The primary antioxidant capacity of the plant extracts and fractions was evaluated through inhibition of DPPH and ABTS radicals and also the ferric reducing antioxidant power (FRAP). The antioxidant activity of water and aqueous-acetone extracts are showed in Table 2 as final concentration in the test solution that reduce 50% of radical or iron (III) ion (IC_{50}).

The DPPH radical scavenging activity ranged from (6.52±1.35) μg/mL to (402.31±7.21) μg/mL while ABTS cation radical inhibition ranged from (5.96±0.24) μg/mL to (90.16±4.69) μg/mL. The ferric reducing antioxidant power start from (32.13±0.92) μg/mL to above the limit concentration tested (250 μg/mL). Along each plant, the antioxidant activity in aqueous-acetone extract was superior or equal to that of water extract. For both DPPH, ABTS and FRAP assays, *O. insignis* and *F. apodanthera* aqueous-acetone extracts exhibited the best antioxidant activities with lowest values of IC_{50}. Crude extract of aqueous-acetone extract of *O. insignis* inhibited DPPH radical as well as quercetin, the standard flavonoid compound. These two extracts also got the highest amount of polyphenol compounds.

Table 2

Antioxidant activity of water and aqueous-aceton extracts.

Plant species	Extracts	IC_{50} (μg/mL)		
		DPPH	ABTS	FRAP
E. balsamifera	Water	74.82±2.25^{b,c}	43.81±3.60^{d}	157.21±12.24^{c,d}
	H_2O-aceton	56.21±4.39^{b}	17.53±2.18^{c}	116.34±5.26^{c}
F. agrestis	Water	61.20±1.90^{b}	23.45±3.67^{c}	118.02±3.22^{c}
	H_2O-aceton	52.43±1.20^{b}	24.70±1.32^{c}	126.31±4.56^{c}
F. apodanthera	Water	27.84±3.20^{a,b}	21.14±2.59^{c}	121.33±6.38^{c}
	H_2O-aceton	11.90±2.67^{a}	5.96±0.24^{a,b}	51.02±2.74^{b}
L. anobrya	Water	402.31±7.21^{e}	78.97±3.76^{e}	>250^{e}
	H_2O-aceton	166.18±8.56^{c}	44.85±1.57^{d}	239.07±13.24^{d}
O. insignis	Water	12.17±1.34^{a}	15.98±0.28^{c}	57.25±4.87^{b}
	H_2O-aceton	6.52±1.35^{a}	6.07±0.29^{a,b}	32.13±0.92^{a,b}
S. dulcis	Water	157.40±4.67^{c}	61.27±7.43^{e}	>250^{e}
	H_2O-aceton	142.54±6.21^{c}	64.01±5.26^{e}	>250^{e}
S. pyramidalis	Water	263.87±6.22^{d}	90.16±4.69^{f}	>250^{e}
	H_2O-aceton	224.63±11.53^{d}	81.24±3.68^{f}	>250^{e}
Quercetin		7.80±0.67^{a}	1.8±0.32^{a}	8.62±1.07^{a}

The concentrations that inhibit 50% (IC_{50}) of DPPH and ABTS radicals and iron (FRAP) are expressed in μg/mL as final concentration in the test solution. Values within each column with different superscripted letters differ significantly (P<0.05).

3.3. UPLC identification of antioxidant components

Aqueous-acetone extracts of *O. insignis* and *F. apodanthera* were then chromatographed on a silicagel column and the antioxidant activity of the obtained fractions was evaluated (Table 3). Two fractions of *F. apodanthera* (F4 and F5) and the O7 fraction of *O. insignis* showed the best antioxidant activities, but not significantly different from those of their respective crude extracts for DPPH and ABTS radical inhibitions; therefore, their reducing activities are significantly better in comparison to their respective crude extracts.

Table 3

Antioxidant activity of fractions from *F. apodanthera* and *O. insignis*.

Fractions	Yield (%)	IC_{50} (μg/mL)		
		DPPH	ABTS	FRAP
F1	1.46^{c}	>25^{d}	>20^{d}	>25^{c}
F2	0.38^{b}	>25^{d}	>20^{d}	>25^{c}
F3	1.58^{c}	>25^{d}	>20^{d}	>25^{c}
F4	2.02^{c}	11.62±0.32^{a}	5.63±0.87^{a}	23.62±2.54^{b}
F5	23.02^{d}	10.57±1.05^{a}	4.58±0.41^{a}	18.85±1.36^{a}
F6	0.43^{b}	>25^{d}	>20^{d}	>25^{c}
F7	39.65^{e}	19.83±2.10^{c}	9.07±1.44^{b}	>25^{c}
F8	17.67^{d}	>25^{d}	18.41±2.17^{c}	>25^{c}
F9	5.48^{c}	>25^{d}	>20^{d}	>25^{c}
O1	5.71^{c}	>25^{d}	>20^{d}	>25^{c}
O2	0.11^{b}	>25^{d}	>20^{d}	>25^{c}
O3	0.61^{b}	>25^{d}	>20^{d}	>25^{c}
O4	0.32^{b}	>25^{d}	>20^{d}	>25^{c}
O5	0.65^{b}	>25^{d}	10.60±0.89^{b}	>25^{c}
O6	0.54^{b}	>25^{d}	9.88±0.08^{b}	23.33±2.07^{b}
O7	43.87^{e}	15.21±1.35^{b}	6.71±0.76^{a}	22.47±1.41^{b}
O8	0.08^{a}	>25^{d}	17.43±1.63^{c}	>25^{c}
O9	17.65^{d}	>25^{d}	17.98±1.19^{c}	>25^{c}
O10	4.00^{c}	>25^{d}	>20^{d}	>25^{c}

The concentrations that inhibit 50% (IC_{50}) of DPPH and ABTS radicals and iron ion III (FRAP) are expressed in μg/mL as final concentration in the test solution. F1 to F9 from *F. apodanthera* and O1 to O10 from *O. insignis* are fractions obtained by column chromatography. Values within each column with different superscripted letters differ significantly (P<0.05).

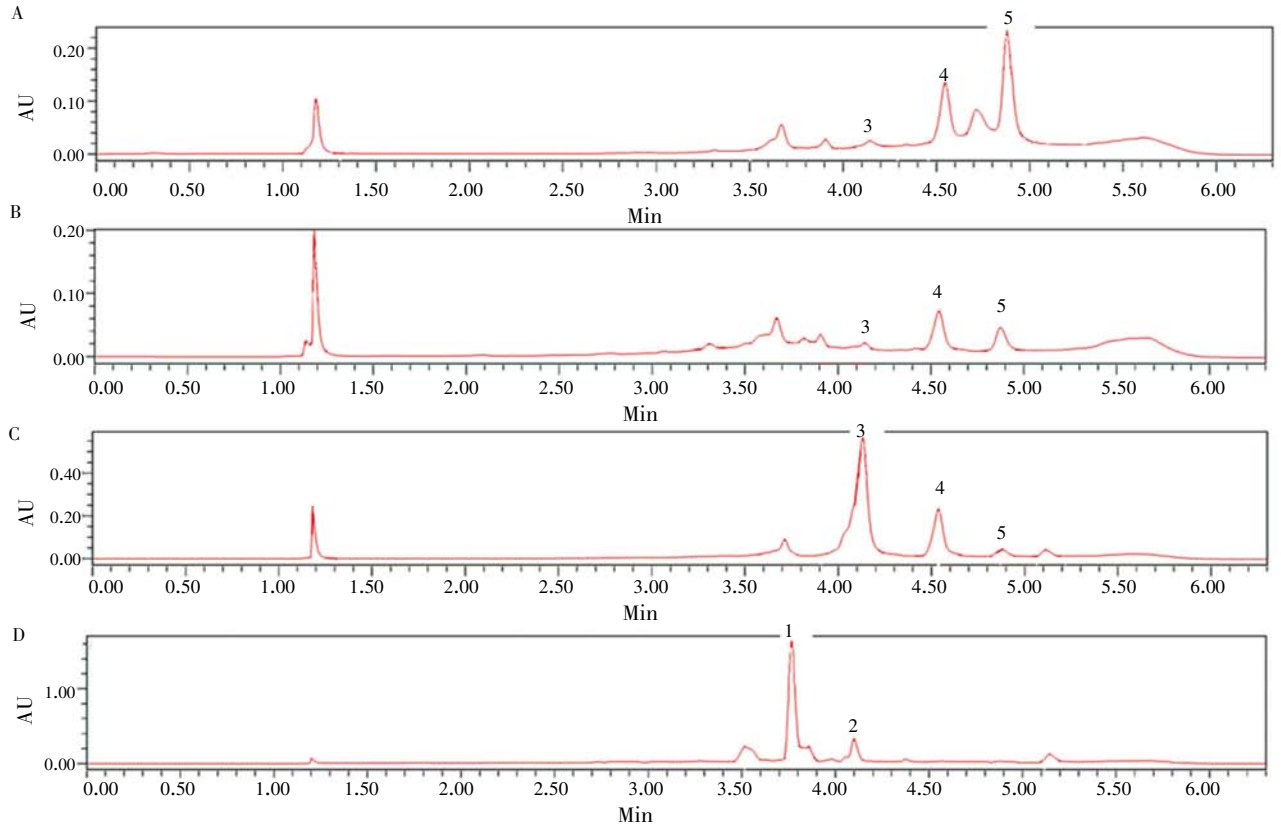


Figure 1. UPLC chromatograms of column fractionations of *F. apodanthera* and *O. insignis*.

A: Hydrolysed fraction F4; B: Hyrolysed fraction F5; C: Hydrolysed fraction O7; D: Non hyrolysed fraction O7; 1: Rutin; 2: Isoquercetin; 3: Myricetin; 4: Quercetin; 5: Kampferol.

Further studies were then undertaken to identify polyphenolic compounds in these fractions (F4, F5 and O7) of *F. apodanthera* and *O. insignis* with greatest antioxidant activity. Three flavonols (quercetin, kampferol and myricetin) were identified in the hydrolysed fractions F4, F5 and O7 of *F. apodanthera* and *O. insignis* (Figure 1). From the non-hydrolyzed fractions, myricetin and quercetin were found in F4 (data not shown), isoquercetin in F5 (data not shown), rutin and isoquercetin in O7 (Figure 1). Kampferol was the most abundant compound in the hydrolyzed fraction F4. Myricetin was the most abundant compound identified in the hydrolyzed fraction O7 and rutin in the non-hydrolyzed fraction O7.

4. Discussion

The different crude extracts from the seven plant species exhibited significant amounts of phenolics compounds and mainly *F. apodanthera* and *O. insignis*. Previous studies indicated higher amount of total phenolic content in *L. anobrya* and *S. dulcis*[12,13]. This could be due to the difference in the extraction process since 70% aqueous acetone was used in this study.

The antioxidative assays of the extracts were tested *in vitro* as DPPH radical scavenging activity, the cation ABTS radical inhibition and the reducing antioxidant power. The crude aqueous-acetone extracts of *F. apodanthera* and *O. insignis* with highest amount of polyphenols also got best antioxidant activities. The potent DPPH radical scavenging activity of a water extract from the stem charcoal of *O. insignis* was previously reported[11].

Indeed, several previous studies demonstrated strong relationship between total phenolic content and antioxidant activity[17,18]. Phenolic compounds include flavonoids and non-flavonoids such as phenolic acid, phenolic diterpenes and volatile oils. As a part of phenolic compounds, flavonoids are in a lesser extent along the different plant species but they might have a greater contribution to phenolic antioxidative action, since they are well known antioxidants[19]. The antioxidant activity of the phenolics compounds was also related not necessarily to their content, but to their hydrogen donating free radical scavengers and their chemical structure[19,20]. Phenolic and aromatic compounds can act as antioxidants by donating hydrogen to free radical and become themselves a radical, which will be stabilized by the resonance delocalization of the electron within the aromatic ring and formation of quinone structures. Then, free radical scavenging ability increases as the number of phenolic hydroxyl groups increases[21]. Phenolic compounds antioxidant activity was also related to their structure; phenolic acids for example generally act as antioxidants by trapping free radicals while flavonoids can scavenge free radicals and chelate

metals as well[22].

Moreover, the antioxidant activity of these crude extracts can be justified by their content in β-carotene, a carotenoid pigment acting mainly as provitamin A in animals. These amounts are significant since some are higher than other amounts found in plant species known for their richness in β-carotene as *Cucurbita moschata* Duch.[23]. Despite its lower amount in the extracts in comparison to polyphenols, β-carotene may contribute to their antioxidant activity. This carotenoid is well-known for its ability to quench the singlet oxygen and other radicals and is also highly reactive and energized molecule[24,25].

The antioxidant activities of the most potent fractions (F4, F5 and O7) from *F. apodanthera* and *O. insignis* showed similar DPPH and ABTS radicals scavenging ability with their respective crude extracts but stronger reducing activity. This can be explained by the fact that beside the DPPH and ABTS methods which are based on a hydrogen donating ability, the FRAP mechanism is a total electron transfer rather than their hydrogen donating ability; the ferric reducing activity in FRAP assay measures the ability of antioxidant compounds in the sample to reduce the Fe (III)/ferric cyanide complex to form ferrous iron by donating an electron.

Furthermore, five flavonoids (quercetin, isoquercitrin, myricetin, rutin and kampferol) were identified as major components from these fractions (F4, F5 and O7) that may justify their potent antioxidant activities. The antioxidant activity of flavonoids was related to the presence of multiple hydroxyl groups and the ortho-3,4-dihydroxy group in their structure[22,26]. For example, the glycosylation of isoquercetrin at the 3-position of the C-ring decrease its antioxidant activity in comparison to the non-glycosidic form that is quercetin[16]. Glycosylation of flavonoids at the 3-OH group usually decreases the antioxidative activity due to the reduction of the number of phenolic group. In the quercetin structure, the antioxidant functional groups such as o-dihydroxy groups at the B-ring and a 2,3-double bond in conjugation with a 4-oxo function are essential structural criteria that strengthen its activities[27]. This potent activity of quercetin in inhibiting both metal and non-metal induced oxidative damage is partially ascribed to its free 3-OH substituent, which is thought to increase the stability of the flavonoid radical.

Due to the presence of both aglycones and glycosides in these fractions of *F. apodanthera* and *O. insignis*, their antioxidant activity can be attributed to synergistic effect of the different flavonoids that they contain. Therefore, along these three fractions, O7 significantly exhibited lower DPPH radical scavenging activity [IC_{50}= (15.21±1.35) μg/mL] that can be explained by its low hydrogen donating capability. Indeed, the glycosylation of rutin, the most abundant compound in this fraction reduce the number of phenolic group along with the hydrogen donating capability and the scavenging activity. Therefore, previous studies indicated that the flavonols (quercetin, myricetin and kampferol) identified in the three hydrolyzed fractions of both *F. apodanthera* and *O. insignis* are potent radical scavengers in both ABTS and DPPH models[28,29], and this can justify the strong activity of the three fractions and particularly F4 and F5 that got the best activities.

The fraction F5 got significantly higher ferric reducing value with the lowest IC_{50} [(18.85±1.36) μg/mL] in FRAP assay suggesting a potent action of quercetin, the most important flavonol identified in this fraction and a synergistic action with myricetin and kampferol also present.

However, the reducing activity of polyphenols in the FRAP model have been suggested to be involved in prooxidant behavior since the resulting iron (II) ion is implicated in hydroxyl radicals production and hydroperoxide decomposition that occurs in the Fenton reaction. Nevertheless, structural advantages to radical stability that increase antioxidant activity may modulate adverse oxidative effects of flavonoids. Moreover, the intracellular cytoplasmic environment is known to be quite reducing and iron may mostly be present in reduced form *in vivo*[30]. Polyphenols antioxidant potential was also related to their iron binding ability and that might be a supplement to the strong intracellular antioxidant systems that implies antioxidant enzymes and metal chelators. Both quercetin and rutin are highly effective chelators of transition metals[31], and their presence in the F4, F5 and O7 fractions of *F. apodanthera* and *O. insignis* may contribute to the observed antioxidant activities of these fractions.

Singlet oxygen and free radicals are involved in several damages to macromolecules and membrane constituents in the body. The antioxidative potential of the selected plants can help to manage free radical related diseases and aging. The aqueous-acetone extracts of *F. apodanthera* and *O. insignis* and their fractions are particularly potent sources of antioxidant compounds.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

We are grateful to The World Academy of Sciences (TWAS) and Universiti Sains Malaysia (USM) for providing the postdoctoral fellowship (Fellowship Reference Number: 3240255139), which allowed this work to be undertaken.

References

[1] Mahantesh SP, Gangawane AK, Patil CS. Free radicals, antioxidants, diseases and phytomedicines in human health:

future perspects. *World Res J Med Aromatic Plants* 2012; **1**(1): 6-10.

[2] Pong K. Oxidative stress in neurodegenerative diseases: therapeutic implications for superoxide dismutase mimetics. *Expert Opin Biol Ther* 2003; **3**: 127-139.

[3] Niki E, Yoshida Y, Saito Y, Noguchi N. Lipid peroxidation: mechanisms, inhibition, and biological effects. *Biochem Biophys Res Commun* 2005; **338**: 668-676.

[4] Ou B, Huang D, Hampsch-Woodil M, Flanagan JA, Deemer EK. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. *J Agric Food Chem* 2002; **50**(11): 3122-3128.

[5] Liu J, Henkel T. Traditional Chinese medicine (TCM): are polyphenols and saponins the key ingredients triggering biological activities? *Curr Med Chem* 2002; **9**(15): 1483-1485.

[6] Yakubu MT, Akanji MA, Oladiji AT. Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem in male albino rats. *Asian J Androl* 2005; **7**(4): 399-404.

[7] Sanon S, Ollivier E, Azas N, Mahiou V, Gasquet M, Ouattara CT, et al. Ethnobotanical survey and *in vitro* antiplasmodial activity of plant used in traditional medicine in Burkina Faso. *J Ethnopharmacol* 2003; **86**(3): 143-147.

[8] Adjanohoun EJ, Ahyi MRA, Ake Assi L, Akpagana K, Chibon P, EI-Hadji A, et al. [*Contribution to ethnobotanic and floristic studies in Togo*]. Paris, France: Agency of cultural and technical cooperation; 1986, p. 308-309. French.

[9] Anero R, Díaz-Lanza A, Ollivier E, Baghdikian B, Balansard G, Bernabé M. Monoterpene glycosides isolated from *Fadogia agrestis*. *Phytochemistry* 2008; **69**: 805-811.

[10] Inngjerdingen K, Nergård CS, Diallo D, Mounkoro PP, Paulsen BS. An ethnopharmacological survey of plants used for wound healing in Dogonland, Mali, West Africa. *J Ethnopharmacol* 2004; **92**: 233-244.

[11] Nyaberi MO, Onyango CA, Mathooko FM, Maina JM, Makobe M, Mwaura F. Bioactive fractions in the stem charcoal of *Ozoroa insignis* used by the pastoral communities in West Pokot to preserve milk. *J Appl Biosci* 2010; **26**: 1653-1658.

[12] Coulibaly AY, Kiendrebeogo M, Kehoe PG, Sombie PA, Lamien CE, Millogo JF, et al. Antioxidant and anti-inflammatory effects of *Scoparia dulcis* L. *J Med Food* 2011; **14**(12): 1576-1582.

[13] Sawadogo WR, Meda A, Lamien CE, Kiendrebeogo M, Guissou IP, Nacoulma OG. Phenolic content and antioxidant activity of six Acanthaceae from Burkina Faso. *J Biol Sci* 2006; **6**(2): 249-252.

[14] Sulaiman SF, Yusoff NAM, Eldeen IM, Seow EM, Sajak AAA, Supriatno, Ooi KL. Correlation between total phenolic and mineral contents with antioxidant activity of eight Malaysian bananas (*Musa* sp.). *J Food Compost Anal* 2011; **24**: 1-10.

[15] Li WJ, Cheng XL, Liu J, Lin RC, Wang GL, Du SS, et al. Phenolic compounds and antioxidant activities of *Liriope muscari*. *Molecules* 2012; **17**: 1797-1808.

[16] Firuzi O, Lacanna A, Petrucci R, Marrosu G, Saso L. Evaluation of the antioxidant activity of flavonoids by "ferric reducing antioxidant power" assay and cyclic voltammetry. *Biochim Biophys Acta* 2005; **1721**: 174-184.

[17] Iyawe HOT, Azih MC. Total phenolic contents and lipid peroxidation potentials of some tropical antimalarial plants. *Eur J Med Plants* 2011; **1**(2): 33-39.

[18] Granato D, Katayama FCU, Castro IA. Assessing the association between phenolic compounds and the antioxidant activity of Brazilian red wines using chemometrics. *LWT Food Sci Technol* 2010; **43**: 1542-1549.

[19] Dauqan EMA, Abdullah A, Sani HA. Natural antioxidants: lipid profile, lipid peroxidation, antioxidant enzymes of different vegetable oils. *Adv J Food Sci Technol* 2011; **3**(4): 308-316.

[20] Dorman HJD, Peltoketo A, Hiltunen R, Tikkanen MJ. Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. *Food Chem* 2003; **83**(2): 255-262.

[21] Eleazu CO, Amajor JU, Ikpeama AI, Awa E. Studies on the nutrient composition, antioxidant activities, functional properties and microbial load of the flours of 10 elite cassava (*Manihot esculenta*) varieties. *Asian J Clin Nutr* 2011; **3**(1): 33-39.

[22] Geldof N, Engeseth NJ. Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of *in vitro* lipoprotein oxidation in human serum samples. *J Agric Food Chem* 2002; **50**: 3050-3055.

[23] de Carvalho LMJ, Gomes PB, de Oliveira Godoy RL, Pacheco S, do Monte PHF, de Carvalho JLV, et al. Total carotenoid content, α-carotene and β-carotene, of landrace pumpkins (*Cucurbita moschata* Duch): a preliminary study. *Food Res Int* 2012; **47**: 337-340.

[24] Yurtcu E, Kasapoğlu E, Şahin FI. Protective effects of β-carotene and silymarin on human lymphocytes. *Turk J Biol* 2012; **36**: 47-52.

[25] Sarada SK, Dipti P, Anju B, Pauline T, Kain AK, Sairam M, et al. Antioxidant effect of beta-carotene on hypoxia induced oxidative stress in male albino rats. *J Ethnopharmacol* 2002; **79**: 149-153.

[26] MC Meena, V Patni. Isolation and identification of flavonoid "quercetin" from *Citrullus colocynthis* (Linn.) Schrad. *Asian J Exp Sci* 2008; **22**: 1137-1142.

[27] Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun T. Phenolics as potential antioxidant therapeutic agents: mechanism and actions. *Mutat Res* 2005; **579**: 200-213.

[28] Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med* 1996; **20**: 933-956.

[29] Hirano R, Sasamoto W, Matsumoto A, Itakura H, Igarashi O, Kondo K. Antioxidant ability of various flavonoids against DPPH radicals and LDL oxidation. *J Nutr Sci Vitaminol (Tokyo)* 2001; **47**: 357-362.

[30] Woodmansee AN, Imlay JA. Quantitation of intracellular free iron by electron paramagnetic resonance spectroscopy. *Methods Enzymol* 2002; **349**: 3-9.

[31] Ke Y, Ming Qian Z. Iron misregulation in the brain: a primary cause of neurodegenerative disorders. *Lancet Neurol* 2003; **2**: 246-253.