Available online at www.ijpsdr.com

International Journal of Pharmaceutical Sciences and Drug Research 2014; 6(4): 323-328

Research Article

ISSN: 0975-248X
CODEN (USA): IJPSPP

Antioxidant Activity of Flavonoids Isolated From the Fruits of *Xylopia parviflora* (A. Rich.) Benth

AT Tchinda[1*], GA Agbor[1], DE Tsala[2], AJG Yaya[1], EN Nga[3], E Talla[4], J-N Wauters[5], M Frédérich[5]

[1]*Center for Research on Medicinal Plants and Traditional Medicine, Institute of Medical Research and Medicinal Plants Studies (IMPM), P.O. Box 6163, Yaoundé, Cameroon*
[2]*Department of Biological Sciences, Faculty of Sciences, University of Maroua, P.O. Box 46 Maroua, Cameroon*
[3]*Department of Galenic Pharmacy and Pharmaceutical Legislation, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, P.O. Box 1364, Yaoundé, Cameroon*
[4]*Department of Chemistry, Faculty of Sciences, University of Ngaoundéré, P.O. Box 454, Ngaoundéré, Cameroon*
[5]*Laboratory of Pharmacognosy, Department of Pharmacy, CIRM, University of Liège, B36, 4000 Liège, Belgium*

ABSTRACT

The present study evaluates the antioxidant activity of the different flavonoids of the fruits of *Xylopia parviflora* used in Cameroon as spice in common traditional dishes. The fruits were successively extracted with hexane and methanol. The isolation of flavonoids was guided by the DPPH-TLC technique. The methanol crude extract and isolated compounds were screened for antioxidant and free radical scavenging activities using DPPH radical-scavenging, β-carotene/linoleic acid and ferric reducing antioxidant power (FRAP) assays. The total phenolic content of the methanol crude extract was determined by Folin–Ciocalteu method. The DPPH-TLC technique led to the isolation of (+)-catechin (**1**), kaempferol 3-*O*-arabinofuranoside (**2**) and quercetin 3-*O*-arabinofuranoside (**3**) identified by NMR and mass spectra analysis. In the colorimetric DPPH test, compound **1** had the same activity (EC_{50} 8.1µg/ml) as butylated hydroxytoluene (BHT) used as standard while compound **3** and the methanol crude extract were less active (EC_{50} 17.2µg/ml). Compound **2** was completely inactive. The total phenolic content of the fruit extracts was 113.03 mg gallic acid equivalents per g of extract. In the carotene bleaching test at the highest concentration of 100µg/mL, the order of inhibition of β-carotene discoloration was BHT > crude extract > quercetin 3-*O*-arabinofuranoside (**3**) > catechin (**1**) > kaempferol 3-*O*- arabinofuranoside (**2**). In the reducing power assay, compound **3** was more active at concentrations 40-100µg/mL. The HPLC analysis of the methanol crude extract revealed the presence of compounds **1**-**3** and unidentified phenolic compounds. The antioxidant activity of the methanol extract is probably due to the presence of compounds **1** and **3**.

Keywords: *Xylopia parviflora*, fruits, isolation, flavonoids, antioxidant activity.

***Corresponding author: Dr. AT Tchinda,**
Center for Research on Medicinal Plants and Traditional Medicine, Institute of Medical Research and Medicinal Plants Studies (IMPM), P.O. Box 6163, Yaoundé, Cameroon; **Tel.:** +/237/76925929**;**
E-mail: alembertt2002@yahoo.fr
Received: 16 September, 2014; **Accepted:** 18 September, 2014

INTRODUCTION

Reactive oxygen species, such as superoxide anion, hydroxyl radical and peroxyl radicals, are highly reactive and are produced from the reduction of molecular oxygen. [1] Damages mediated by reactive oxygen species include the disruption of membrane

fluidity, protein denaturation, lipid peroxidation, DNA denaturation and alteration of platelet functions. [2] These have generally been considered to be linked with many chronic diseases such as cancers, inflammation, aging and atherosclerosis. An antioxidant, which can quench reactive oxygen species, has the potentials of preventing the oxidation of other molecules and may therefore have health-benefit effects in the prevention of degenerative diseases. [3] There is a growing interest in antioxidants research because of their high capacity in scavenging free radicals [4] with particular focus in natural antioxidants found in medicinal and dietary plants. [5] These natural antioxidants not only protect dietary lipids from oxidation, but may also provide health benefits associated with preventing damages to biological molecules. [6-7] Flavonoids are the major antioxidants in dietary constituents. [8] As part of our efforts to find antioxidants from edible herbs, we have evaluated the antioxidant potential of the fruits of *Xylopia parviflora* (A. Rich.) Benth which is used as condiment in several traditional dishes in the Western Region of Cameroon. [9] Previous studies carried out on the stem bark resulted in the isolation of isoquinoline alkaloids. [10] The present study reports on the antioxidant activity of flavonoids isolated from methanolic extract of *X. parviflora* and represent the first significant phytochemical study of the fruits of *X. parviflora*.

MATERIAL AND METHODS

General experimental procedures

The TOF-ESI mass spectra were recorded on a Synapt HDMS (Waters) spectrophotometer with internal calibration in nano ESI-positif mode. NMR spectra were recorded in MeOH-d_4 on a Bruker 500 MHz NMR spectrometer, with TMS as internal reference. Column chromatography (CC) was carried out on silica gel (70-230 mesh, Merck). TLC was performed on Merck precoated silica gel 60 F_{254} aluminium foil and spots were detected using 5% sulfuric acid in ethanol and 0.5% DPPH solution in methanol as spraying reagents. The absorbance in the experiments was read on an Advance spectrophotometer (model V-110).

Plant material

The fruits of *Xylopia parviflora* were purchased at the Mfoundi market, a local market in Yaoundé (Cameroon) and the identity confirmed by M. Nana Victor, National Herbarium Yaoundé, where a voucher specimen (Number 42351HNC) is deposited.

Extraction and isolation of compounds

The dried fruits of *X. parviflora* (3 Kgs) were grounded in a mill and extracted with hexane (6 L) to give 226 g of oil. The residue after hexane extraction was dried at room temperature and further extracted with methanol (6 L). The filtrate was concentrated to give a brown extract (340 g). One hundred grams of the methanol extract was chromatographed over silica gel eluted with mixtures of hexane-ethyl acetate (50:50, 30:70), pure ethyl acetate and ethyl acetate-methanol (95:5, 90:10 and 80:20). Fraction 5 obtained with the mixture hexane-ethyl acetate (30:70) was separated through a silica gel chromatography column (CC) eluted with the same solvent mixture to give compound **1** (70 mg). Fractions 12-15 obtained with ethyl acetate-MeOH (95:5) were combined and passed through a silica gel CC eluting with a gradient of hexane-ethyl acetate (70:30 to 0:100). Fractions 9-11 collected from the latter column yielded compound **2** (120 mg) whilst compound **3** (170 mg) was obtained from fraction 14.

Antioxidant assay

Reagents

2, 2-Diphenyl-1-picrylhydrazyl (DPPH), BHT, ferrozine, phosphate buffer (pH 6.6), potassium ferricyanide [$K_3Fe(CN)_6$] and methanol were obtained from Sigma (St. Louis, MO, USA). Ferric chloride, β-carotene, trichloroacetic acid and Folin–Ciocalteu's phenol reagent were obtained from Fluka.

Determination of total phenolic content

The total phenolic content of the methanolic extract was determined using Folin-Ciocalteu reagent as previously described by Singleton *et al*. [11] Gallic acid in methanol was used as standard and absorption read at 765 nm at 20°C after 30 min (standard) and 2 hours (extract) of incubation. All analyses were performed in triplicate. The total phenolic content of the extract was presented as gallic acid equivalent.

C= c. V/m where C is the total content of phenolic compounds (in mg/g of plant extract or GAE); c is the concentration of gallic acid established from the calibration curve in mg/mL; v is the volume of extract in mL; m is the weight of pure plant methanol extract in g.

Rapid evaluation of radical scavenging activity by DPPH method

The TLC-DPPH guided isolation of compounds from the fruits extract of *X. parviflora* was determined as previously described [12] with a slight modification. Briefly, the solutions of the crude extract (10 mg/ml), fractions (10 mg/ml) and isolated compounds (1 mg/ml) were spotted on silica gel F_{254} aluminium sheets and developed in ethyl acetate or dichoromethane-methanol (9:1). The silica gel sheets were sprayed with 0.5% solution of 1, 1-diphenyl-2-picrylhydrazyl in MeOH. Any spot that bleached the purple colour background of DPPH reagent within three minutes was taken as positive results. Only fractions showing positive results were considered for further purification.

Quantitative DDPH assay

The hydrogen atoms or electron-donating ability of the test compounds and BHT was determined from the bleaching of purple-colored methanol solution of DPPH. This spectrophotometric assay uses the stable radical DPPH as a reagent. Experiments were carried out as described previously. [13] Briefly, 0.5 mM DPPH solution in methanol was prepared, and then 1 ml of this solution was mixed with 3 ml of the sample solution in ethanol. Various concentrations of extracts

were prepared. BHT was used as a positive control at 100µg/ml. After incubation for 30 min in the dark, the absorbance was measured at 517 nm. Decreasing the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity. This activity is given as percent DPPH radical scavenging, which is calculated with the equation: % DPPH radical scavenging = [(control absorbance- sample absorbance)/control absorbance] x 100. Control contained 1 ml of DPPH solution and 3 ml of methanol. The measurements of DPPH radical scavenging activity were carried out in triplicates, and values are an average of three replicates.

Determination of the antioxidant activity using β-carotene bleaching test

The antioxidant activity of the methanol extract and compounds **1-3** was evaluated by β-carotene–linoleic acid model system. [14] Briefly, 0.5 mg of β-carotene in 1 mL of chloroform was added to 25µL of linoleic acid and 200 mg of Tween 40 emulsifier mixture. After chloroform was evaporated under vacuum, distilled water saturated with oxygen was added by vigorous shaking up to 200 mL. Four millilitres of this mixture were transferred into different test tubes containing different concentrations of the sample. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. The emulsion system was incubated for 2 h at 50°C. A blank, devoid of β-carotene, was prepared for background subtraction. BHT was used as standard; test was carried out in triplicates. The antioxidant activity (AA) was calculated in terms of percent inhibition relative to absorbance of the control, using following equation:

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

Reducing power

The reducing power of the methanol extract and compounds **1-3** was determined using the method described previously. [15] A serial dilution of the extract and compounds was performed (20, 40, 60, 80 and 100µg/mL) in 0.2 M phosphate buffer pH 6.6 containing 1% ferrocyanate. The mixture was incubated at 50°C for 20 min. 10 % trichloroacetic acid (TCA, 2.5 mL) was added to a portion of this mixture (5 mL) and centrifuged at 3.000 g for 10 minutes. The supernatant was separated and mixed with distilled water (2.5 mL) containing 1% ferric chloride (0.5 mL). The absorbance of the mixture was measured at 700 nm. BHT was used as a positive control and prepared as described for the extract. The intensity in absorbance was the measurement of antioxidant activity. The reducing power values were an average of three replicates.

HPLC identification of flavonoids from the fruits of *X. parviflora*

An HP Agilent HPLC system comprised of a quaternary pump Agilent 1100 (G1311A), a Diode Array Detector HP 1100 (G1315A), an autosampler Agilent 1100 (G1313A), a degasser Agilent 1100 (G1379A), a solvent module and a HP Chemstation and computer control was used for HPLC analysis. A Hypersil ODS column (250 mm × 4.6 mm i.d.), 5µ from Thermo Electron Corporation was employed, at 30°C. Separations were done in gradient mode, using acetonitrile: water at a flow rate of 1 mL/min with an injection volume of 10µL; UV detection was at 280 and 350 nm. The crude extract and pure compounds were dissolved in methanol at concentrations of 10mg/ml and 1 mg/ml respectively and filtered through a Chromafil filter PET-45/25 (polyester, 0.45µm) from Macherey-Nagel GmbH & Co. The mobile phase consisted of solvent A (0.05% trifluoroacetic acid) and solvent B (CH_3CN) with the following gradient: 0-15 min, 3% B in A; 15-45min, 40% B in A, 45-55 min, 60% B in A, 55-70 min, 100% A in B.

RESULTS AND DISCUSSION

Isolation of compounds

The fruits of *X. parviflora* were successively extracted with hexane and methanol. Both extracts were subjected to chromatography separations using a series of techniques including open silica gel and medium pressure column chromatography. The isolation process was guided by DPPH-TLC screening. The hexane fraction did not show any purple spot upon spraying with a solution of 0.2% DPPH in methanol whilst the methanol fraction showed three spots. The methanol extract was fractionated using a silica gel chromatography column. The fractions collected were pooled according to their TLC profile after spraying with a methanolic solution of 2% DPPH. Only fractions showing yellow spots upon purple coloration were considered for isolation. Three flavonoids namely (+)-catechin **1** [16], kaempferol 3-*O*-arabinofuranoside (juglanin) **2**, quercetin 3-*O*-arabinofuranoside (avicularin) **3** [17] were isolated. Their structures were determined by interpretation of their NMR and MS data.

Total phenolic content of the extract

Total phenolic content of the crude methanol extract of *X. parviflora* was 113.03 (mgGAE)/g of extract (mg of gallic acid per g of sample). This result indicated the presence of a good amount of phenolic compounds in the extract. The three flavonoids isolated in the methanol extract are all phenolic compounds characterized by to the presence of hydroxyl groups attached to aromatics rings. However, it is possible that some more polar phenolic compounds such as tannins were present in the extract and were not isolated.

DPPH radical scavenging activity

DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extract. DPPH is a stable, nitrogen-centered free radical which produces violet color in ethanol solution. It is reduced to a yellow colored product, 2, 2-Diphenyl-1-picrylhydrazyl, with the addition of the fractions in a concentration-dependent manner. [17] The crude methanol extract was spotted on a TLC plate and sprayed with a 0.5% methanolic solution of DPPH.

Three yellow spots appeared and corresponded to three flavonoids which were isolated by the DPPH-TLC guided fractionation of the methanol extract. In the quantitative DPPH experiment, all the tested extract and pure compounds except compound **2** exhibited free radical scavenging activity with respectively EC50 values of 8.1µg/ml for compound **1**, 17.2µg/ml for compound **3** and 17.2µg/ml for the crude extract. The activity of compounds **1** and **3** and crude extract was comparable to that of BHT used as standard (Figure 1). It took only one to two minutes for these compounds to completely bleach the DPPH purple background. According to kinetic studies of aryloxy (Ar-O.) radical formation and decomposition reactions, the antioxidant activity of a flavonoid is closely related to its chemical structure. Three structural requirements are important for high antioxidant activity of a flavonoid : (i) the *ortho*-dihydroxy (catechol) structure in the B-ring, imparting a greater stability to the formed aryloxy radicals as a result of flavonoid oxidation, possibly through H-bonding and electron delocalization (ii) the 2, 3-double bond, in conjugation with the 4-oxo function, enhancing electron-transfer and radical scavenging actions through electron-delocalization; (iii) the presence of both 3- and 5-OH groups, enabling the formation of stable quinonic structures upon flavonoid oxidation. A typical flavonoid which meets the above three criteria is quercetin, showing the highest antioxidant capacity. [18] Compounds **1** and **3** possess a catechol moiety with hydroxyl groups at C-3′ and C-4′. This structural future plays an important role to their observed radical scavenging activity. In contrary, compound **2** has only one hydroxyl on ring B and is inactive. Studies have shown that the suppression of a hydroxyl group as for instance from quercetin to kaempferol reduces the activity. [17] This explains the great activity difference between compounds **2** and **3**. It has been shown that the glycosylation of 3-OH of flavonoids reduce the radical scavenging activity. [19] The results obtained for compounds **1** and **3** are comparable to those obtained previously in the same experiment. [20]

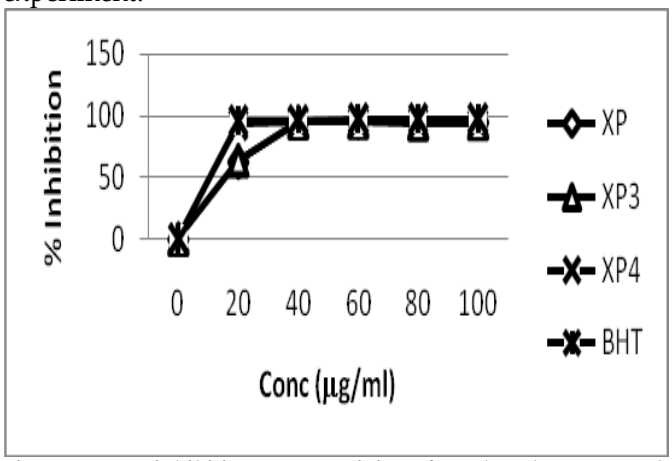


Fig. 1: DPPH inhibition assay activity of methanol extract and compounds 1-3.
XP: methanol extract; XP3: quercetin 3-*O*-arabinofuranoside (**3**); XP4: catechin (**1**)

Antioxidant activity using β-carotene bleaching test

The β-carotene bleaching assay is a commonly used model to analyze the antioxidant activity of the plant extracts because β-carotene is extremely sensitive to free radical mediated oxidation of linoleic acid. β-carotene in this model system undergoes rapid discoloration in the absence of an antioxidant. This is because of the coupled oxidation of β-carotene and linoleic acid, which generates free radicals. The linoleic acid free radical formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups attacks the highly unsaturated β-carotene molecules. As a result, β-carotene will be oxidized and broken down in part; subsequently the system loses its chromophore and characteristic orange colour, which can be monitored spectrophotometrically. [21] All the tested compounds and methanol extract of *X. parviflora* inhibited β-carotene oxidation, suggesting that the antioxidant activity could be related to high level of phenolic compounds in the extract and free hydroxyl groups in the compounds. In this study, at the highest concentration of 100µg/mL, the order of inhibition of β-carotene discoloration was BHT > crude extract > quercetin 3-*O*-arabinofuranoside (**3**) > catechin (**1**) > kaempferol 3-*O*- arabinofuranoside (**2**). Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. Especially, linoleic acid and arachidonic acid is the target of lipid peroxidation. Free radical chain reaction is widely accepted as a common mechanism of lipid peroxidation and it is generally thought that the inhibition of lipid peroxidation by antioxidants may be due to their free radical-scavenging activities. Radical scavengers may directly react and quench peroxide radicals to terminate the peroxidation chain reaction and improve the quality and stability of food products. [22] The fruits extracts of *X. parviflora* may therefore possess a lipid peroxidation inhibitory activity in the human body.

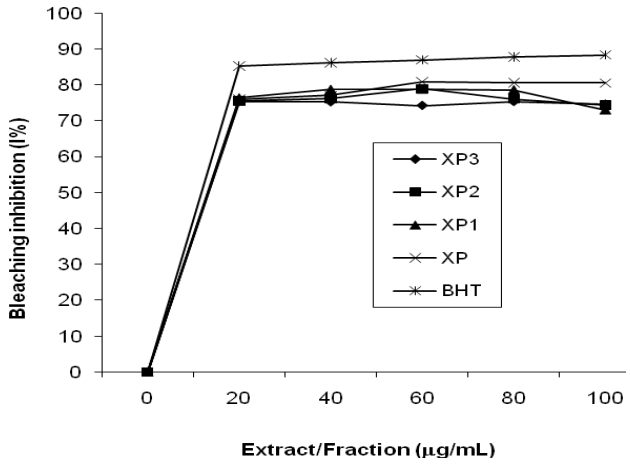


Fig. 2: β-Carotene bleaching activity of methanol extract and compounds 1-3.

Reduction power assay

Reduction power using the Prussian blue assay (Ferrocyanate method) is an electron transfer assay

which is based on ferric–to–ferrous reduction in the presence of a Fe (II)-stabilizing ligand. [19] Increasing absorbance with increasing concentrations at 700 nm indicates an increase in reductive ability. At the concentration of 40-100µg/ml, compound **1** was more active than the methanol extract, compounds **2** and **3** and BHT (standard). At 40 g/mL, the order of activity was catechin > quercetin 3-*O*-arabinofuranoside > BHT > methanol extract. At the dose of 100µg/mL the reduction power order was respectively catechin > methanol extract > quercetin 3-*O*-arabinofuranoside > BHT. Compound **2** was inactive.

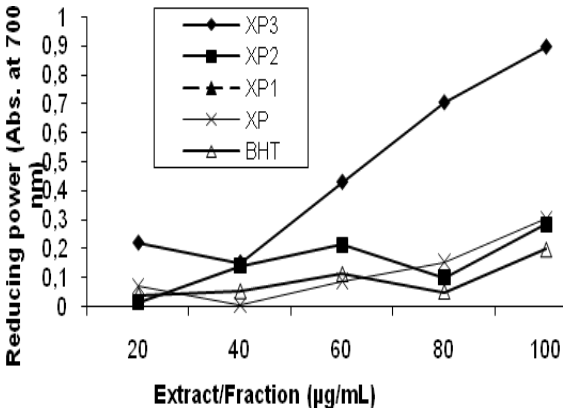
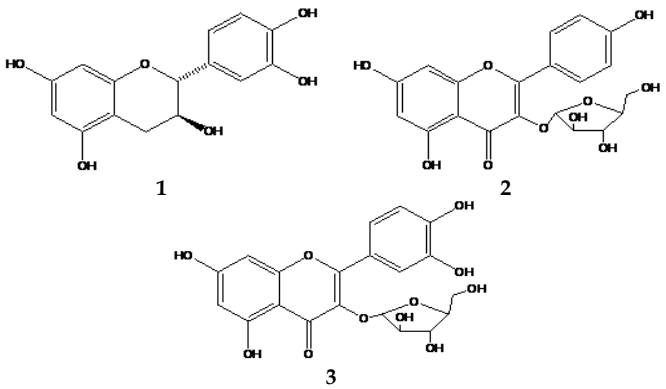


Fig. 3: Reducing power activity of methanol extract and compounds 1-3.

HPLC identification of compounds 1-3

The HPLC fingerprint of the methanol extract at 350 nm showed intense peaks at Rt 14.2, 18.6, 19.2, 23.2, 31.1 and 34.0 min. The methanol extract was injected in the same conditions as compounds **1-3** isolated in the course of this work. The peaks corresponding to compounds **1-3** at Rt 21.7, 34.0 and 31.1 min respectively were clearly identified in the HPLC chromatogram of the methanol extract (Figure 4). The peak corresponding to compound **1** is less intense in the chromatogram recorded at 350 nm though it absorbs very well at 280 nm. Other important peaks at 14.2, 18.6 and 19.2 min could not be identified. They may correspond to phenolic acids. [23-24] These results confirm the presence of compounds **1-3** in the fruits of *X. parviflora*.



HPLC chromatogram of isolated flavonoids (**1-3**)

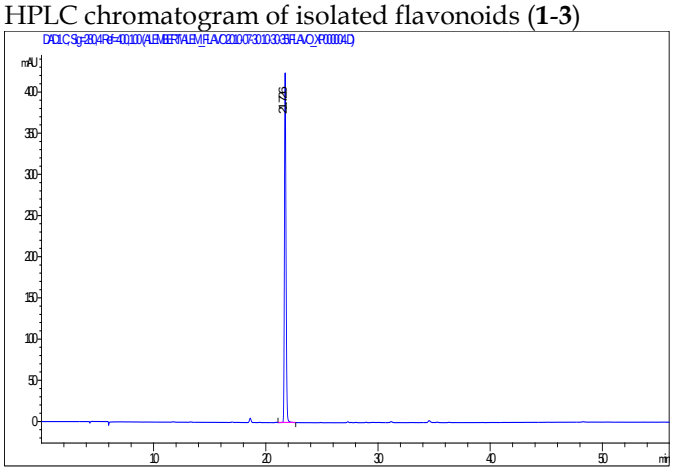


Fig. 4: HPLC chromatogram of compound 1 (280 nm); Column: Hypersil C_{18} ODS, 250 × 4.6 mm, i.d., 5µM.

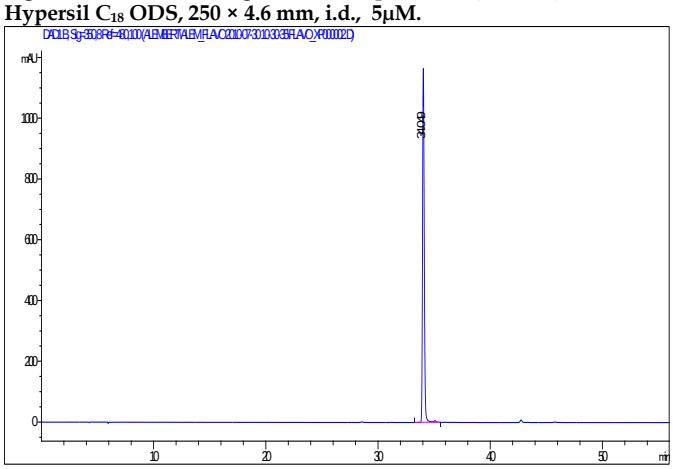


Fig. 5: HPLC chromatogram of compound 2 (350 nm); Column: Hypersil C_{18} ODS, 250 × 4.6 mm, i.d., 5µM.

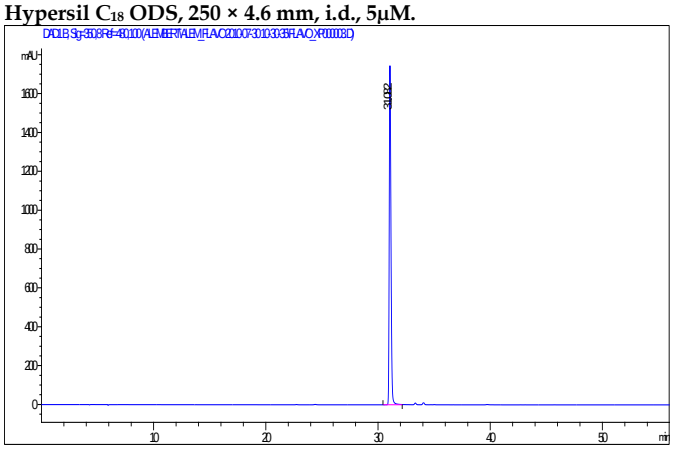


Fig. 6: HPLC chromatogram of compound 3 (350 nm); Column: Hypersil C_{18} ODS, 250 × 4.6 mm, i.d., 5µM.

The result of the present study showed that the fruits of *X. parviflora* contained three flavonoids (+)-catechin (**1**), kaempferol 3-*O*-arabinofuranoside (**2**) and quercetin 3-*O*-arabinofuranoside (**3**) and probably other phenolic compounds. All these constituents may contribute to the antioxidant activity of this plant material. Definitely, the fruits of *X. parviflora*, used as spice, constitute an important source of antioxidant substances with potential health benefits.

ACKNOWLEDGEMENTS

This work was supported by TWAS (grant Nº 07-145 RG/CHE/AF/AC) and F.R.S.-FNRS (grant N°

T.0190.13). We are grateful to the University of Liege for the award of a postdoctoral fellowship to A.T.T. through the 'Subside federal de la recherche' scheme and also to M. Gabriel Mazzucchelli, Giga mass center, University of Liege for the recording of mass spectra.

REFERENCES

1. Williams GM, Jeffrey AM. Oxidative DNA damage: Endogenous and chemically induced. Regul Toxicol Pharmacol. 2000; 32: 283–292.
2. Kinsella JE, Frankel E, German B, Kanner J. Possible mechanisms for the protective role of antioxidants in wine and plant foods. Food Technol. 1993; 47: 85–89.
3. Shahidi F. Natural antioxidants: Chemistry, health effects, and applications. Urbana, IL: AOCS Press. 1997.
4. Silva EM, Souza JNS, Rogez H, Rees JF, Larondelle Y. Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. Food Chem. 2007; 101: 1012–1018.
5. Silva BA, Ferreres F, Malva JO, Dias ACP. Phytochemical and antioxidant characterization of *Hypericum perforatum* alcoholic extracts. Food Chem. 2005; 90: 157–167.
6. Hu C, Kitts DD. Dendelion (*Taraxacum officinale*) flower extract suppresses both reactive oxygen species and nitric oxide and prevents lipid oxidation *in vitro*. Phytomedicine 2005; 12: 588–597.
7. Shahidi F, Wanasundara PKJPD. Phenolic antioxidants. Crit Rev Food Sci Nutr. 1992; 32: 67–103.
8. Pier-Giorgio P. Flavonoids as antioxidants. J Nat Prod. 2000; 63: 1035-1042.
9. Tchiégang C, Mbougueng PD. Composition chimique des épices utilisées dans la préparation du Nah-poh et du Nkui de l'ouest Cameroun. Tropicultura. 2005 ; 23: 193-200.
10. Nishiyama Y, Moriyasu M, Ichimaru M, Iwasa K, Kato A, Mathenge SG, Mutiso PBC, Juma FD. Secondary and tertiary isoquinoline alkaloids from *Xylopia parviflora*. Phytochemistry 2006; 67: 2671–2675.
11. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu Reagent. Meth Enzymol. 1999; 299: 152-178.
12. Cuendet M, Hostettmann K, Potterat O. Iridoid glycosides with free radical scavenging properties from *Fagrea blumei*. Helv Chim Acta. 1997; 80: 1144-1152.
13. Yaya AJG, Feumba RD, Talla E, Tchinda AT, Fredérich M, Oben J. Antioxidant activity of compounds isolated from the root woods of *Erythrina droogmansiana*. Int J Pharm Sci Drug Res. 2014; 6(2): 160-163.
14. Miller HEA. A simplified method for the evaluation of antioxidant. J Amer Chem Soc. 1971; 45: 91-98.
15. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Japan J Nutr. 1986; 44: 307-315.
16. Young HS, Park JC, Choi JS. Isolation of (+)-catechin from the roots of *Rosa rugosa*. Kor J Pharmacog. 1987; 18: 177-179.
17. Olszewska M, Wolbis M. Flavonoids from the flowers of *Prunus spinosa* L. Acta Pol Pharm. 2001, 58: 367-372.
18. Huang D, Ou B, Prior RL. The Chemistry behind antioxidant capacity assays. J Agric Food Chem. 2005; 53: 1841-1856.
19. Apak R, Güçlü K, Demirata B, Özyürek M, Çelik SE, Bektaşoğlu B, Berker KI, Özyurt D. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. Molecules 2007; 12: 1496-1547.
20. Ren-Bo A, Hyun-Chul K, Yu-Hua T, Youn-Chul K. Free radical scavenging and hepatoprotective constituents from the leaves of *Juglans sinensis*. Arch Pharm Res. 2005; 28: 529-533.
21. Abdille MdH, Singh RP, Jayaprakasha GK, Jena BS. Antioxidant activity of the extracts from Dillenia indica fruits. Food Chem. 2005; 90:891–896.
22. Sharififar F, Dehghn-Nudeh G., Mirtajaldini M. Major flavonoids with antioxidant activity from *Teucrium polium* L. Food Chem. 2009; 112: 885–888.
23. Shalini, Srivastava R. Antifungal activity screening and HPLC analysis of crude extract from *Tectona grandis*, shilajit, *Valeriana wallachi*. Electron J Environ Agric Food Chem. 2009; 8: 218-229.
24. Nuengchamnong N, Krittasilp K, Ingkaninan K. Rapid screening and identification of antioxidants in aqueous extracts of *Houttuynia cordata* using LC–ESI–MS coupled with DPPH assay. Food Chem. 2009; 117: 750–756.

Source of Support: Nil, Conflict of Interest: None declared.