British Journal of Applied Science & Technology
6(1): 77-85, 2015, Article no.BJAST.2015.068
ISSN: 2231-0843

SCIENCEDOMAIN *international*
www.sciencedomain.org

Idenfication of Nutraceutical Phenolic Compounds in Bambara Groundnuts (*Vigna subterranea* L. Verdc) by HPLC-PDA-ESI-MS

V. Nyau[1*], S. Prakash[2], J. Rodrigues[3] and J. Farrant[3]

[1]Department of Food Science and Technology, University of Zambia, Lusaka, Zambia.
[2]Department of Chemistry, University of Zambia, Lusaka, Zambia.
[3]Department of Molecular and Cell Biology, University of Cape Town, 7701 Rondebosch, South Africa.

Authors' contributions

This work was carried out in collaboration between all authors. Authors VN and SP designed the study, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors JR, JF and VN managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJAST/2015/14780
Editor(s):
(1) Teresa De Pilli, Department of Science of Agriculture of Food of Environnement (SAFE), University of Foggia, Italy.
Reviewers:
(1) Anonymous, Nigeria.
(2) Slobodan Jankovic, University of Kragujevac, Serbia.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=764&id=5&aid=7216

Original Research Article

Received 21st October 2014
Accepted 10th November 2014
Published 15th December 2014

ABSTRACT

Aim: The aim of the study was to explore the nutraceutical potential of bambara groundnuts (*Vigna subterranea* L. Verdc) commonly grown in Zambia based on the phenolic phytochemical profiles.
Methodology: Two market classes of bambara groundnuts (red and brown) commonly grown in Zambia were screened in raw dry form. The study employed the High Performance Liquid Chromatography-Photo Diode Array-Electrospray Ionization-Mass Spectrometry (HPLC-PDA-ESI-MS) to screen for phenolic phytochemical profiles of the 70% methanol extracts from bambara groundnuts
Results: HPLC-PDA-ESI-MS- based identification revealed the presence of various phenolic compounds, mainly phenolic acids and flavonoids. In both the red and brown bambara groundnuts, the following phenolic compounds were tentatively identified: Quinic acid, (E) GC-hexoside, catechin glucoside, catechin, epicatechin, medioresinol, *p*-coumaric acid, salicylic acid, caffeic acid

**Corresponding author: E-mail: vincentnyau@yahoo.co.uk;*

derivative and catechin dimer. The red bambara groundnuts revealed the following phenolic compounds that were absent in the brown: myricetin hexoside, quercetin-3-O-rutinoside and quercetin-3-O-glucoside.

Conclusion: The nuts contain various polyphenolic compounds, mainly from the class of flavonoids. It is suggested that consumption of bambara groundnuts could possibly offer some health benefits since they contain phytochemical constituents that have been reported to possess protective functions. These data indicate that bambara groundnuts studied have the potential for use as nutraceuticals.

Keywords: Bambara groundnuts; phenolics; phytochemicals; nutraceuticals; Vigna subterranea L. Verdc.

1. INTRODUCTION

Phenolic compounds are the most widely distributed secondary metabolites, ubiquitously present in the plant kingdom [1]. They have highly diverse chemical structures and more than 500 polyphenols have been described in common foods and beverages [2]. The main classes of phenolic compounds are phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), flavonoids (flavanols, flavones, flavanones, isoflavones and anthocyanins), chalcones, aurones (hispidol), hydroxycoumarins, lignans, hydroxystilbenes and polyflavans (proanthocyanidins and prodeoxyanthocyanidins) [3,4].

Flavonoids constitute the largest class of phenolic compounds with more than 3000 structures, possessing in common a flavylium unit (C6-C3-C6) [5].

Phenolic acids includes hydroxybenzoic acids (gallic and ellagic acids being major ones) and hydroxycinnamic acids (most common being coumaric, caffeic and ferulic acid). Added to this is quinic acid, a conjugate of caffeic acid and chlorogenic acid [6]. Tannins are polyphenolic compounds that constitute hydrolysable and non-hydrolysable tannins. They are complex polyphenols that can be degraded to sugars and phenolic acids by both enzymatic and non-enzymatic hydrolytic processes [7].

Plant-derived phenolic compounds are important as nutraceutical constituents in our diet. They have antioxidant properties and may protect against major clinical conditions such as heart disease and cancer in which reactive oxygen species (i.e., superoxide anion, hydroxyl radicals and peroxy radicals) are involved [8,9]. Plant phenolics represent one of the major groups of compounds acting as primary antioxidants or free radical terminators [10,11].

This study was undertaken to explore the nutraceutical phenolic profiles of the bambara groundnut (*Vignea subterranean* L. verdc) market classes grown in Zambia. Whilst the nutritional value of bambara groundnuts is well known, their potential as nutraceuticals has not been exploited. Bambara groundnuts remain uncharacterised in many aspects and have not been the subject of sustained research [12]. Since under-utilised crops are grown for subsistence and contribute to the food security of many of the world's poorest people, attempts to improve them rarely attract interest from international agencies or commercial sponsors [13]. Studies on the nutraceutical properties of bambara groundnuts are essential to establish their potential for use in the functional foods and nutraceutical industry. Additionally, this information is necessary for strategies aimed at promoting consumption of bambara groundnuts, which are slowly drifting into the category of "neglected species" or "forgotten crops" of Africa.

2. MATERIALS AND METHODS

2.1 Sample Collection

Popularly cultivated market classes of bambara groundnuts (red and brown) were procured directly from the farmers in the Eastern region of Zambia immediately after harvest. The two market classes were botanically identified as *Vigna subterranea* L. Verdc by the National Plant Genetic Resources Centre Gene bank at Mount Makulu, Lusaka, Zambia. In order to make the samples representative, an attempt was made to collect the seeds of each market class from 15 farmers in the area with not less than 0.5 kg per farmer. Both market classes are landraces that have become well adapted to the local climate and soils, and indigenous knowledge about the germplasm is well preserved in the communities.

2.2 Preparation of the Extracts

Raw dry bambara groundnuts and common beans were ground into powder of the same consistency using a coffee grinder (Braun, Mexico). The 70% methanol extracts were obtained using Ultrasound-Assisted Extraction (UAE) from the seed flour [14]. Approximately 15 g of seed powder in 150 ml of 70% methanol was sonicated for 30 minutes at 25°C using the Eumax UD500SH 40 kHz ultrasonic bath. After extraction, the mixture was centrifuged at a speed of 10,000 rpm for 15 minute in Beckman Coulter JE centrifuge. The resulting supernatant first concentrated to 30 ml by evaporation under reduced pressure in a rotary evaporator (Buchi R-210 model, Switzerland) to remove methanol. The extract was then frozen at -80°C and freeze dried to obtain a powdered methanolic extract using the Telstar LyoQuest -85 freeze dryer. The freeze dried extracts were stored at -4°C until further analysis.

2.3 HPLC –DAD-ESI-MS Instrumentation and Chromatographic Conditions

The freeze dried 70% methanolic extracts of bambara groundnuts were analysed using a Waters ZMD 4000 system that was equipped with a Waters 2690 HPLC, Waters 996 photodiode array, ZMD mass spectrophotometer, 717 Plus autosampler, and a quaternary pump (Waters Corp, Milford, MA, USA). Separations were carried out on a 300x3.9 mm, 4 µm reversed phase Nova-Pak C18 (Waters) column that was maintained at 40°C. The photodiode array detector (PDA) was linked directly to a sprayer needle where ions were generated by electrospray ionisation (ESI) in a negative mode. The mobile phase A consisted of 5% (v/v) acetonitrile/water, containing 0.1% (v/v) formic acid and mobile phase B consisted of 100% acetonitrile containing 0.1% (v/v) formic acid. The sample was injected at a volume of 25 µl. The elution profile consisted of a stepwise linear gradient from 0% to 28% solvent B for 22 minutes with a flowrate of 0.3 ml/min. The PDA dectector was set to a scanning range of 200 to 700 nm and the UV-Vis absorption spectra were recorded online during the HPLC analysis. Phenolic acids and flavonols were detected at 280 and 360 nm respectively. Continuous mass spectra data were recorded on a full scan negative ionisation mode for a mass range of m/z 85 to 1000. The capillary voltage was set at 2.5 kV, the cone at 20 V and the extractor at 5 V. Nitrogen gas was used for nebulising and drying at different fragmentation voltages. Data acquisition was controlled using MassLynx 4.1 (Micromass, Waters Corp., Beverly, MA, USA).

2.4 Preparation of the Samples for HPLC - DAD-ESI-MS Analysis

Preparation of the test solution for HPLC-DAD-ESI-MS was done according to the procedure by Gülçin et al. [15] with slight modifications. One hundred mg of the freeze dried 70% methanolic extract was dissolved in 5 ml of ethanol-water (50:50 v/v). 100 µl of the prepared extract was transferred into a 5 ml volumetric flask and diluted to the volume with ethanol-water (50:50). From the final solution, an aliquot of 1.5 ml was transferred into a capped autosampler vial and 25 µl of the sample was injected into the HPLC-DAD-ESI-MS system. Identification of phenolic compounds was accomplished using UV spectra and ESI-MS spectral data and by comparison with published data reported in the literature. Authentic standards were also used where available by comparing their chromatograms with those of the samples. The available standards were *t*-ferrulic acid, gallic acid, salicylic acid, *p*-coumaric acid, epicatechin and catechin.

3. RESULTS AND DISCUSSION

3.1 Phenolic Compounds Identified by HPLC-PDA-ESI-MS in Brown Bambara Groundnuts

Ten phenolic compound were identified based on co-chromatography with available authentic standards and mass spectra obtained in the negative mode by using their fragmentation pattern and data from published literature [16,17]. The gradient solvent system used for the analysis of phenolic compounds is summarised in Table 1. The chromatogram of the 70% methanol extract of brown bambara groundnuts is presented in Fig. 1 and the identified compounds are summarised in Table 2. The mass spectrum in full scan negative ionisation mode showed the deprotonated molecule $[M - H]^-$ of quinic acid at m/z 191 (peak 1) that fragmented to yield MS/MS spectrum with an ion at m/z 127, characteristic of quinic acid [18].

Peak 2 was provisionally identified as (E) GC-hexoside, a flavan-3-ol monomer with $[M - H]^-$ ion at m/z 467, a double molecular ion $[2M - H]^-$ at m/z 935, and a fragment ion at m/z 305 indicative of the hexosyl residual. These fragmentation patterns are in agreement with the previously published data, where this phenolic compound

was detected in bayberry [19]. The presence of flavanol compounds in free and conjugated forms has been reported as one of the principal phenolics in common legumes [20].

Peak 3 was tentatively identified as catechin glucoside with $[M - H]^-$ at m/z 451 that fragmented to yield a MS/MS spectrum with an ion at m/z 289 indicative of the catechin fragment and a loss of the hexose moiety (162 amu). This compound has been identified previously in pinto beans with similar fragmentation pattern [21].

Peak 4 had a deprotonaed molecular ion $[M - H]^-$ with m/z 205. MS/MS fragmentation yielded fragments at m/z (179, 143, 129) indicating the presence of caffeic acid. The fragmentation pattern is characterised by the loss of a hydrocarbon moiety (amu 26). This molecule was be tentatively assigned as caffeic acid derivative.

Peak 5 showed a deprotonated molecule $[M - H]^-$ of catechin at m/z 289, a fragment at m/z 245, and HPLC retention time of 6.08 min. Co-chromatography of authentic standard verified the identification of this peak as catechin.

Peak 6 revealed a negatively charged $[M - H]^-$ ion at m/z 289, MS/MS spectrum with m/z at 245, and HPLC retention time of 8.12 min . The spectra and the UV data gave a suggestive indication that compounds represented in peaks 5 and 6 are isomers. Co-chromatography of authentic standard verified the identification of peak 6 as epicatechin.

Peak 7 had $[M - H]^-$ion at m/z 387 with MS/MS fragmentation at m/z 207 was identified at medioresinol, a phenolic lignin. The compound has been identified previously in lamiaceae species with similar fragmentation pattern [22].

Peaks 8 and 9 revealed negatively charged ions $[M- H]^-$ at m/z 163 and 193, and HPLC retention times at 8.92 and 11.1 respectively. Co-chromatography with authentic standards verified the identification of peak 8 as *p*-coumaric acid and peak 9 as *t*-ferulic acid.

Peaks 10 revealed negatively charged ions $[M-H]^-$ at m/z 137, and HPLC retention time at 12.37 min. Co-chromatography with authentic standard verified peak 10 as salicylic acid.

Peak 11 had $[M - H]^-$ ion at m/z 529 with MS/MS fragmentation at m/z 368, 367 and 179 (indicating the presence of caffeic acid fragment). Compounds with similar fragmentation characteristics have been reported previously in plants [18]. Based on the fragmentation pattern and published data, this compound was tentatively identified as a caffeic acid derivative.

Peak 12 revealed a negatively charged ion ions $[M- H]^-$ at m/z at 582 with MS/MS fragmentation at 245, 205 and 289 (indicative of the presence of catechin fragment). The compound was provisionally assigned to catechin dimer.

Table 1. Gradient solvent system for analysis of phenolic compounds by HPLC -PDA –ESI-MS

Time (minutes)	Composition of the mobile phase (%)	
	*Mobile phase (A)	*Mobile phase (B)
1	100	0
22	72	28
22.50	60	40
23	0	100
24.50	0	100
25	100	0
26	100	0

** Mobile phase A consisted of 5% (v/v) acetonitrile/water, containing 0.1% (v/v) formic; mobile phase B consisted of 100% acetonitrile containing 0.1% (v/v) formic acid*

3.2 Phenolic Compounds Identified by HPLC-PDA-ESI-MS in Red Bambara Groundnuts

The chromatogram of the 70% methanol extract of red bambara groundnuts is presented in Fig. 2. Fifteen phenolic compounds were provisionally identified and are summarised in Table 3. The following phenolic compounds that were identified in the brown market class of bambara groundnuts were also found in the red one: Quinic acid (peak 1), (E) GC –hexoside (peak 2), catechin glucoside (peak 3), caffeic acid derivative (peak 4), catechin (peak 5), epicatechin (peak 6), medioresinol (peak 7), *p*-coumaric acid (peak 8), *t*-ferulic acid (peak 11) salicylic acid (peak 13), caffeic acid derivative (peak 14) and catechin dimer (peak 15).

The red bambara groundnuts contained the following phenolic compounds that were not detected in the brown market class:

Peak 9 revealed a negatively charged [M – H]- ion at m/z 479 and MS/MS spectrum with m/z at 317, corresponding to a loss of 162 amu: hexose moiety, probably a galactoside or glucoside

leaving the myricetin fragment. This compound was tentatively identified as myricetin hexoside. This compound has been identified in other legumes with similar fragmentation pattern [23]

Peak 10 revealed a negatively charged ions [M – H]- at m/z 609 with MS/MS fragmentation at 301 (indicative of the presence of quercetin fragment). Tentatively, this compound is a quercetin derivative. A compound in common beans with similar fragmentation pattern has been identified as quercetin-3-O-rutinoside by confirmation with a standard previously [23]. In this research, the standard was not available to do the confirmation. Based on the literature data and the fragmentation pattern, this compound can provisionally be identified as quercetin-3-0-rutinoside.

Peak 12 had a negatively charged [M – H]- ion at m/z 463. MS/MS fragmentation yielded a fragment with m/z 301 (quercetin), after losing a hexose moiety (amu 162). This compound was identified as quercetin-3-O-glucoside. The presence of this flavonoid in legumes has been reported previously by Lin et al. [23]. A compound with similar fragmentation pattern has been reported previously in strawberries [24].

Table 2. HPLC-PDA-ESI-MS -based identification of phenolics in brown bambara groundnuts

Peak	t_R(min)	Molecular weight	[M-H]- (m/z)	[M-H]- fragments (m/z)	Tentative identification
1	1.36	192	191	127	Quinic acid
2	4.31	468	467	935, 467	(E)GC-hexoside
3	4.58	452	451	289	Catechin glucoside
4	5.27	206	205	179,143,129	Caffeic acid derivative
5	6.08	290	289	245	Catechin
6	8.12	290	289	245	Epicatechin
7	8.62	388	387	207.1	Medioresinol
8	8.92	164	163	119	*p*-coumaric acid
9	11.1	193	194	149,134	*t*-ferulic acid
10	12.37	138	137		Salicylic acid
11	15.7	530	529	368, 367, 179	Caffeic acid derivative
12	16.71	583	582	289, 245, 205	Catechin dimer

Table 3. HPLC-PDA-ESI-MS -based identification of phenolics in red bambara groundnuts

Peak	t_R(min)	Molecular weight	[M-H]- (m/z)	[M-H]- fragments (m/z)	Tentative identification
1	1.36	192	191	127	Quinic acid
2	4.31	468	467	935,467	(E)GC-hexoside
3	4.58	452	451	289	Catechin glucoside
4	5.27	206	205	178,143,129	Caffeic acid derivative
5	6.08	290	289	245	Catechin
6	8.12	290	289	245	Epicatechin
7	8.62	388	387	207.1	Medioresinol
8	8.92	164	163	119	*p*-coumaric acid
9	9.81	480	479	317	Myricetin hexoside
10	10.11	610	609	301	Quercetin-3-O-rutinoside
11	11.1	194	193	149, 134	*t*-ferulic acid
12	11.59	464	463	301	Quercetin-3-O-glucoside
13	12.37	138	137	-	Salicylic acid
14	15.7	530	529	368, 367, 179	Caffeic acid derivative
15	16.71	583	582	289, 245, 205	Catechin dimer

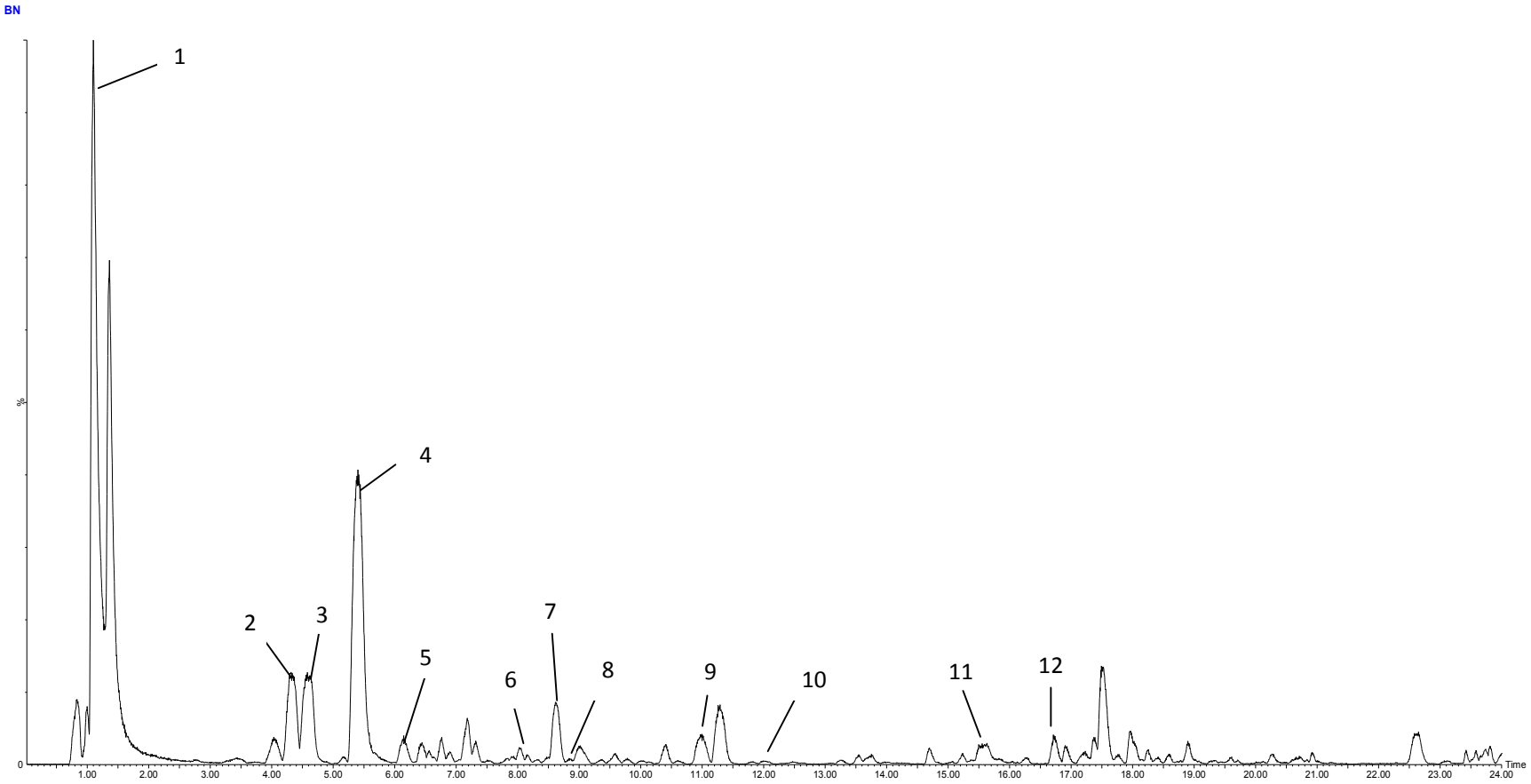

Fig. 1. HPLC-PDA-ESI-MS chromatogram of 70% methanol extract of brown bambara groundnuts

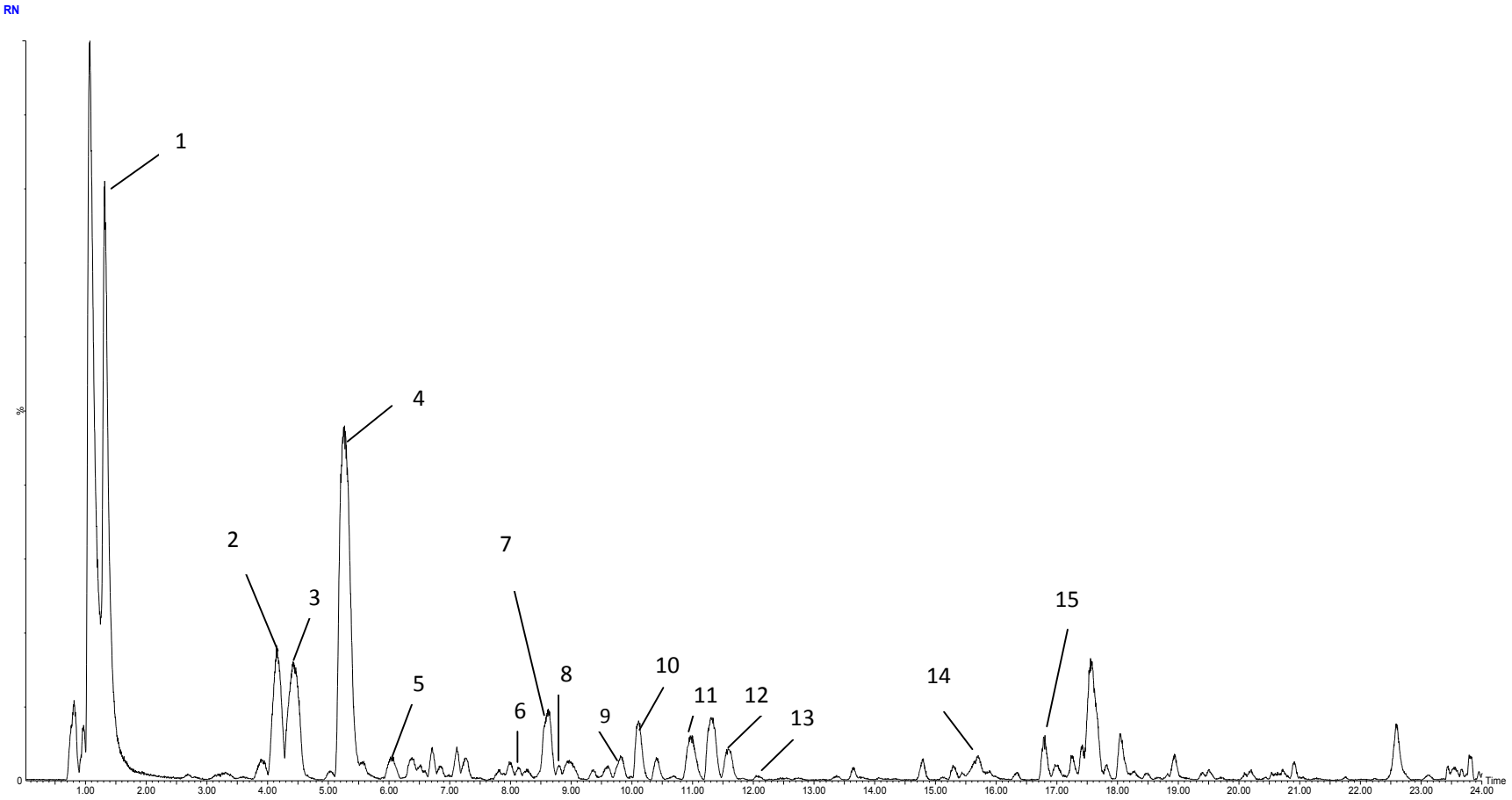

Fig. 2. HPLC-PDA-ESI-MS chromatogram of 70% methanol extract of red bambara groundnuts

The majority of the phenolic compounds identified in bambara groundnuts were flavonoids. Various health benefits have been described previously for flavonoids. An inverse correlation between flavonoid intake and total plasma cholesterol concentrations has been shown [25]. Flavonoid intake has also been reported to have a protective effect against coronary heart disease [26,27]. Cocoa beans have historically been used as a treatment for diarrhoea [28]. The nature of the active ingredient, nor the exact mechanism of action was not known but the recent research attributes the antidiarrhoea effect to the flavonoids present in cocoa [28]. Flavonoids like quercetin have been shown to have anti-inflammatory properties and do so by inhibiting the cyclooxygenase pathway [29]. Quercetin has also been shown to inhibit the growth of helicobacter pylori bacteria in in-vitro studies [27]. Methyl-3-(+)-catechin interferes with the formation of histamine in gastric mucosa and hence produces the protective effect [30]. Most flavonoids have anti-viral effects against Herpes simplex virus, respiratory syncytial virus, parainfluenza virus, and adenovirus [29]. Flavonoids have also been shown to have free radical scavenging properties [31]. In a summary, one may speculate that bambara groundnuts may be useful as nutraceuticals due to various phenolic compounds from the flavonoids class that they contain.

4. CONCLUSION

The study has shown that bambara groundnuts contain various polyphenolic compounds, mainly from the class of flavonoids. Based on this, it is suggested that consumption of bambara groundnuts could possibly offer some health benefits since they contain phytochemical constituents that have been reported to possess protective functions. These findings have demonstrated that bambara groundnuts have the potential for use in the nutraceutical industry. Consumption of bambara groundnuts can be as good as other commonly consumed legumes and this is an opportunity for dietary and crop diversification on the part of the consumers and farmers respectively.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mamoudou HD, Gruppen H, Alfred S, TraoréAlphons GJ, Willem, JH. Phenolic compounds and related enzymes as determinants of sorghum for food use. Biotechnology and Molecular Biology. 2006;1(1):21-38.
2. Neveu V, Perez-Jime'nez J, Vos F, Crespy V, du Chaffaut L, Mennen L, et al. Phenol Explorer: An online comprehensive database on polyphenol contents in foods. Database; 2010. Article ID bap024. DOI:10.1093/database/bap024.
3. Chung T, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: A review. Crit. Rev. Food Sci. Nutr. 1998;38:421–464.
4. Krueger CG, Vestling MA, Reed JD. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of heteropolyflavan-3-ols and glucosylated heteropoly flavans in sorghum (*Sorghum bicolor* (L.) Moench. J. Agric. Food Chem. 2003;51:538–543.
5. Iacobucci GA, Sweeny JG. The chemistry of anthocyanins, anthocyanidins and related flavylium salts. Tetrahedron. 1983;39:3005-3015.
6. Goldberg G. Plants: Diet and health. British nutrition foundation. 1st ed. Oxford: Blackwell; 2003.
7. Wahle KW, Brown I, Rotondo D Heys SD. Plant phenolics in the prevention and treatment of Cancers. Adv. Exptl. Med. Biol. 2010;698:36–51.
8. Rhodes MJC, Price KR. Identification and analysis of plant phenolic antioxidants. Eur. J. Cancer prev. 1997;6:5188.
9. Duthie G, Crozier A. plant-derived phenolic antioxidants. Curr Opin Clin Nutr Metab Care. 2000;3(6):447-451.
10. Miliauskas G, Venskutonis P, van Beek T. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry. 2004;85:231-237.
11. Saura-Calixto F, Serrano J, Goñi I. Intake and bioaccessibility of total phenols in a whole diet. Food Chemistry. 2007;101:492-501.
12. Massawe FJ, Mwale SS, Azam-Ali SN, Roberts JA. Breeding in bambara groundnuts (*Vigna subterranea* (L.) Verdc.): Strategic considerations. Afr. J. Biotechnol. 2005;4(6):463–471.

13. FAO. A global mapping system for bambara groundnuts production. FAO Agricultural Information Management Series. Vialedelle Terme di Caracalla, 00100 Rome, Italy. 2001;1:48.
14. Dobiáš P, Pavlíková P, Adam M, Eisner A, Beňová B, Ventura K. Comparison of pressurised fluid and ultrasonic extraction methods for analysis of plant antioxidants and their antioxidant capacity. Cent. Eur. J. Chem. 2010;8:87-95.
15. Gülçin E, Bursal HM, Sehitoglu M, Gören AC. Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. Food Chem. Toxicol. 2010;48:2227-2238.
16. Wu XL, Prior LR. Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/ MS in common foods in the United States: Fruits and Berries. J. Agric. Food Chem. 2005;53:2589–2599.
17. Seeram PN, Lee R, Scheuller SH, Heber D. Identification of phenolic compounds in strawberries by liquid chromatography electrospray ionizationmass spectrescopy. Food Chemistry. 2006;97:1–11.
18. Gouveia S, Castilho PC. Characterisation of phenolic acid derivatives and flavonoids from different morphological parts of *Helichrysum obconicum* by a RP-HPLC–DAD–ESI-MS^n method. Food Chemistry. 2011;129:333–344.
19. Yang H, Ge Y, Sun Y, Liu D, Xingqian YEX, et al. Identification and characterisation of low-molecular-weight phenolic compounds in bayberry (*Myrica rubra* Sieb. et Zucc.) leaves by HPLC-DAD and HPLC-UV-ESIMS. Food Chemistry. 2011;128:1128–1135.
20. Dinelli G, Bonetti A, Minelli M, Marotti I, Catizone P, Mazzanti A. Content of flavonols in Italian beans (*Phaseolus vulgaris* L.) ecotypes. Food Chemistry. 2006;99:105-114.
21. Estrella I, Aguilera Y, Benitez V, Rosa M, Esteban MC. Bioactive phenolic compounds and functional properties of dehydrated beans flours. Food Research International. 2011;44:774–780.
22. Hassain M, Dilip K, Brunton N, Martin-Diana A, Barry-Ryan C. Characterization of phenolic composition in lamiaceae spices by LC-ESI-MS/MS. J. Agric. Food Chem. 2010;58(19):10576–10581.
23. Lin LZ, James M, Harnly-Matcial S, Pastor-Corrales, Devanand L. The Polyphenolic profiles of common beans (*Phaseolus vulgaris* L.). Food Chemistry. 2008;107: 399-410.
24. Kajdžanoska M, Gjamovski V, Stefova M. HPLC-DAD-ESI-MSn identification of phenolic compounds in cultivated straberries from Macedonia. Macedonian Journal of Chemistry and Chemiscal Engineering. 2010;29(2):181-194.
25. Patel JM. A Review of Potential Health Benefits of flavonoids. Lethbridge Undergraduate Research Journal. 2008;3:2.
26. Hertog MG, Kromhout D, Aravanis,C, et al. Flavonoid intake and long term risk of coronary heart disease and cancer in the seven countries study. Arch Intern Med.1995;155:381-386.
27. Middleton E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: Implications for Inflammation, Heart Disease, and Cancer. Pharmacol. Rev. 2000;52:673-751.
28. Schuier M, Sies H, Illek B, et al. Cocoa-related flavonoids inhibit cftr-mediated chloride transport across t84 human colon epithelia. J Nutr. 2005;135:2320-2325.
29. Nijveldt RJ, Nood E, Hoorn D, et al. Flavonoids: A review of probable mechanisms of action and potential applications. Am J Clin Nutr. 2001;74:418-425.
30. Farkas L, Gabor M, Wagner H, et al. Flavonoids and bioflavonoids. 1st ed. Amsterdam: Elsevier; 1981.
31. Pietta PG. Flavonoids as antioxidants. J Nat. Prod. 2000;63:1035-1042.

© 2015 Nyau et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=764&id=5&aid=7216