

Original Research Article

HPLC-PDA-ESI-MS identification of polyphenolic phytochemicals in different market classes of common beans (*Phaseolus vulgaris* L.)

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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.

ABSTRACT

Aim: The aim of the study was to screen the market classes of common beans popularly grown in Zambia for their polyphenolic phytochemical profiles.

Methodology: Three market classes of common bean (red, grey mottled and brown) were investigated for their polyphenolic phytochemical profiles using the High Performance Liquid Chromatography-Photo Diode Array-Electrospray Ionization-Mass Spectrometry (HPLC-PDA-ESI-MS).

Results: Various phenolic compounds were identified in the Zambian market classes of common beans. Quinic acid, a syringic acid derivative, ferulic acid derivatives, medioresinol, *p*-coumaric acid, catechin, gallic acid and ferulic acid were identified in all the market classes investigated. However, the isomers of ferulic acid derivatives observed were 4 in red, 3 in brown and 1 in grey

mottled beans. Epicatechin was only identified in the red and grey mottled beans. A compound with the molecular ion at m/z 567, tentatively identified as a flavonone derivative was only observed in red beans. Catechin glucoside was only identified in grey mottled and brown beans. Compounds tentatively identified as kaempferol glucoside and carnosol were only observed in brown beans and not in the other market classes.

Conclusion: The study has shown that the market classes of common beans investigated contain various polyphenolic compounds that may be useful as nutraceuticals. The study assumes that the diversity in the phenolic phytochemical profile is an excellent opportunity for genetic improvement in the nutraceutical attributes of these common bean market classes by crop breeders.

Key words: Common beans, phenolics, phytochemicals, nutraceuticals

1. INTRODUCTION

Common beans (*Phaseolus vulgaris* L.) occupy an important position in human nutrition and have great potential to deal with health problems associated with non-communicable diseases if appropriately positioned in the diet. It is also an important income generating crop for smallholder farmers in Sub-Saharan Africa and other developing countries in the world. Common beans are a staple food crop for many regions of the world where they are eaten in large quantities on daily basis as a rich source of protein, resistant starch, and dietary fibre [1]. They provide an economical source of proteins, especially among the low income group who cannot afford the more expensive meat proteins. Legume-based foods are also one of the best non-meat sources of iron, providing 23 – 30% of daily recommended levels [2].

Common beans also contain phytonutrients that are believed to play protective functions in the body. Consumption of common bean has been associated with reduced risk of some diseases such as coronary heart disease and cancer [3]. The physiological effects of common bean may be due to the presence of abundant phytochemicals including polyphenols, which possess both anticarcinogenic and antioxidant properties [3]. Basic qualitative screening for common beans has shown that alkaloids, anthraquinone, catechitannins, flavonoids, tannins, glucosides, polyphenols, saponins, steroids and terpenoids are present [4]. Phytochemical screening showed

the presence of some bioactive components such as alkaloids, anthocyanin, carbohydrate, catechin, fibers, flavonoids, phasine, phytic acid, quercetin, saponins, steroids, tannins and terpenoids and trypsin inhibitor [4]. Analysis using HPLC-MS, identified compounds such as anthocyanins, flavanol monomers, and heterogeneous flavanol oligomers up to hexamers [4]. The presence of catechin, delphinidin, cyaniding, and phenolic acids such as gallic, vanillic, caffeic, coumaric and ferrulic in the seed coat of common beans have been reported [5]. Phenolic compounds such as antocyanins, quercetin glycosides and protoanthocyanidins (condensed tannins), were isolated and identified in dark red kidney beans [6]. Kaempferol and quercetin have been reported as the main flavonoids in different market classes of Mexican beans [7].

This study was undertaken to screen the market classes of common beans commonly grown in Zambia for their phenolic phytochemical profiles using High Performance Liquid Chromatography-Photo Diode Array-Electrospray Ionization-Mass Spectrometry (HPLC-PDA-ESIMS). Published reports on the phenolic phytochemicals of market classes of Zambian common beans are limited. Germplasm screening for traits of interest is an important first step to genetic improvement and it is imperative to evaluate locally available germplasm to have a baseline of information on the nutritional traits [8]

2. MATERIALS AND METHODS

2.1. Sample collection

Three market classes of common beans (red, grey mottled and brown) commonly grown in Zambia were obtained directly from the farmers immediately after harvest. A description of their characteristics is shown in Table 1. In order to make the samples representative, an attempt was made to collect the seeds of each market class from 15 farmers in each growing area with not less than 0.5 kg per farmer.

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95 **Table 1. Market classes of common beans investigated**

Market class	Local variety name	*Descriptive characteristics	Appearance
Red beans	<i>Lundazi</i>	• Non-climber • 78-80 days to maturity • Potential yield: 1500 kg/ha	
Brown beans	<i>Chambeshi</i>	• Non-climber • 78-80 days to maturity • Potential yield: 1500 kg/ha	
Grey mottled beans	<i>Kabulangeti</i>	• Climber • 80-100 days to maturity • Potential yield: 1000 kg/ha	

96 *Information about the general attributes such as days to maturation, growth pattern and
 97 potential yields was obtained through informal interviews with the local people.

98 **2.2.Preparation of the extracts**

99 Raw dry common beans were ground into powder of the same consistency using a coffee grinder
 100 (Braun, Mexico). The 70% methanol extracts were obtained using Ultrasound-Assisted

Extraction (UAE) from the seed flour [9]. 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 using Beckman Coulter JE centrifuge. The resulting supernatant was 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 extract powder of common beans 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 300 x 3.9 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 flow rate of 0.3 ml/min. The PDA detector 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). The gradient solvent system used for the analysis of phenolic compounds is summarised in Table 2.

Table 2. Gradient solvent system for analysis of phenolic compounds by HPLC -PDA –ESI-MS

Time (minutes)	Composition of the mobile phase (%)	
	*Mobile phase	*Mobile phase
	(A)	(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.

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 Gülçin procedure with slight modifications [10]. One hundred mg of the freeze dried 70% methanolic extract was dissolved in 5 ml of ethanol-water (50:50 v/v). One hundred 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 DISCUSSIONS

3.1. Phenolic compounds identified by HPLC-PDA-ESI-MS in red beans

Thirteen phenolic compounds were identified by HPLC-PDA-ESI-MS in red beans. The results of the identified compounds are presented in Table 3, and the numbered peaks in the chromatogram (Figure 1). A compound with a negatively charged $[M - H]^-$ ion at m/z 191 (peak 1), with a fragment ion at m/z 127 was identified as quinic acid. Peak 2 was identified as gallic acid with $[M - H]^-$ at m/z 169 that fragmented to yield a MS/MS spectrum at m/z 125, an indicator of trihydroxy phenol moiety [11]. Furthermore, co-chromatography of the authentic standard verified the identification of this peak as gallic acid.

Peaks 3, 6, 7 and 8 were identified as isomers of ferulic acid derivatives. These compounds were eluted at different retention times but showed similar deprotonated molecules $[M - H]^-$ at m/z 385 that fragmented to yield MS/MS spectra with ions at m/z 193, indicative of ferulic acid fragment. Four isomers of ferulic acid derivatives have been reported previously in common beans [12].

Peak 4 showed a deprotonated molecule $[M - H]^-$ at m/z 259 and its MS/MS fragmentation yielded fragment ions at m/z (241, 223, 197), indicating the presence of a syringic acid fragment (a phenolic acid with a deprotonated molecule at m/z 197, and with a molecular weight of 198). The fragmentation pattern is characterised by the loss of two water moieties (18 amu, each subsequently) and a hydrocarbon moiety (26 amu), which probably could be the acetylene (C_2H_2). This compound was tentatively identified as a syringic acid derivative.

Peaks 5 and 9 were isomers that eluted at 6.08 and 8.12 minutes, respectively. These two compounds showed similar deprotonated molecules $[M - H]^-$ at m/z 289 that fragmented to yield MS/MS spectra with ions at 245. Co-chromatography with authentic standards verified peak 5 as catechin and peak 9 as epicatechin. Peak 10 revealed a negatively charged $[M - H]^-$ ion at m/z 387 with MS/MS fragmentation at m/z 207. The compound was identified as medioresinol, a

phenolic lignin. A compound in lamiaceae with similar fragmentation pattern that was tentatively identified previously as medioresinol [13]

Peaks 11 and 12 revealed negatively charged ions $[M - H]^-$ at m/z 163 and 193, and HPLC retention times at 8.92 and 11.1 minutes, respectively. Co-chromatography with authentic standards verified the identification of peak 11 as *p*-coumaric acid and peak 12 as *t*-ferulic acid. These compounds have been reported previously in common beans [12]

Peak 13 showed a deprotonated molecule $[M - H]^-$ ion at m/z 567 and its MS/MS fragmentation yielded a fragment ion at m/z 341 with absorption maxima at 217 and 287 nm, suggesting the presence of a flavanone skeleton [14]. The fragmentation observed for this deprotonated molecule has been previously described in the literature for a flavanone derivative [15].

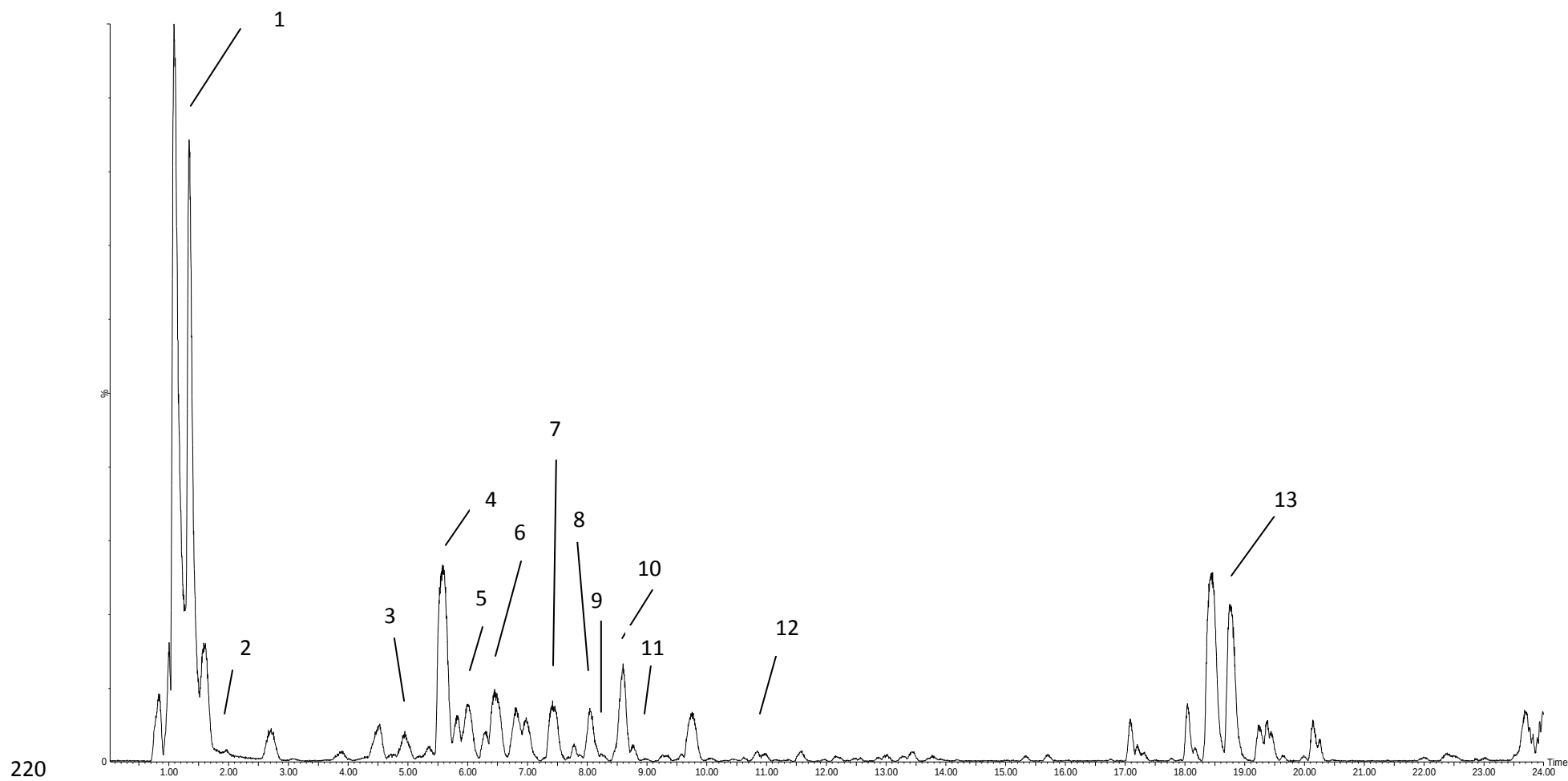
212 **Table 3. HPLC-PDA-ESI-MS -based identification of phenolics in red beans**

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	1.99	170	169	125	Gallic acid
3	4.95	386	385	193	Ferulic acid derivatives
4	5.57	260	259	241,233,197	Syringic acid derivative
5	6.08	290	289	245	Catechin
6	6.45	386	385	193	Ferulic acid derivates
7	7.42	386	385	193	Ferulic acid derivatives
8	8.04	386	385	193	Ferulic acid derivatives
9	8.12	290	289	245	Epicatechin
10	8.62	388	387	207	Medioresinol
11	8.92	164	163	119	<i>p</i> -coumaric acid
12	11.1	194	193	149, 134	<i>t</i> -ferulic acid
13	19.38	568	567	342, 341, 330	Flavanone derivative

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Figure 1. HPLC-PDA-ESI-MS chromatogram of 70% methanol extract of red beans.

3.2. Phenolic compounds identified by HPLC-PDA-ESI-MS in grey mottled beans

The chromatogram of methanolic extract of grey mottled beans is presented in Figure 2. Eleven phenolic compounds were identified and are summarised in Table 4. The following phenolic compounds that were identified in the red beans were also found in the grey mottled one: quinic acid (peak 1), gallic acid (peak 2), a syringic acid derivative (peak 4), catechin (peak 5), ferulic acid derivatives (peak 6), epicatechin (peak 7), medioresinol (peak 8), *p*- coumaric acid (peak 9) and *t*-ferulic acid (peak 10). For the peaks in the brackets, refer to the chromatogram (Figure 2). Only one ferulic acid derivative was reported in grey mottled beans compared to the four that were detected in red beans.

Peak 11 with the retention time of 12.37 minutes was identified as salicylic acid based on the co-chromatography with the authentic standard. Peak 3 showed a deprotonated molecule $[M - H]^-$ ion with m/z at 451 that fragmented to yield a fragment ion at m/z 289 indicating the presence of catechin residual and a loss of a hexose moiety (162 amu). This compound was tentatively identified as catechin glucoside and has been reported previously in pinto beans [16]

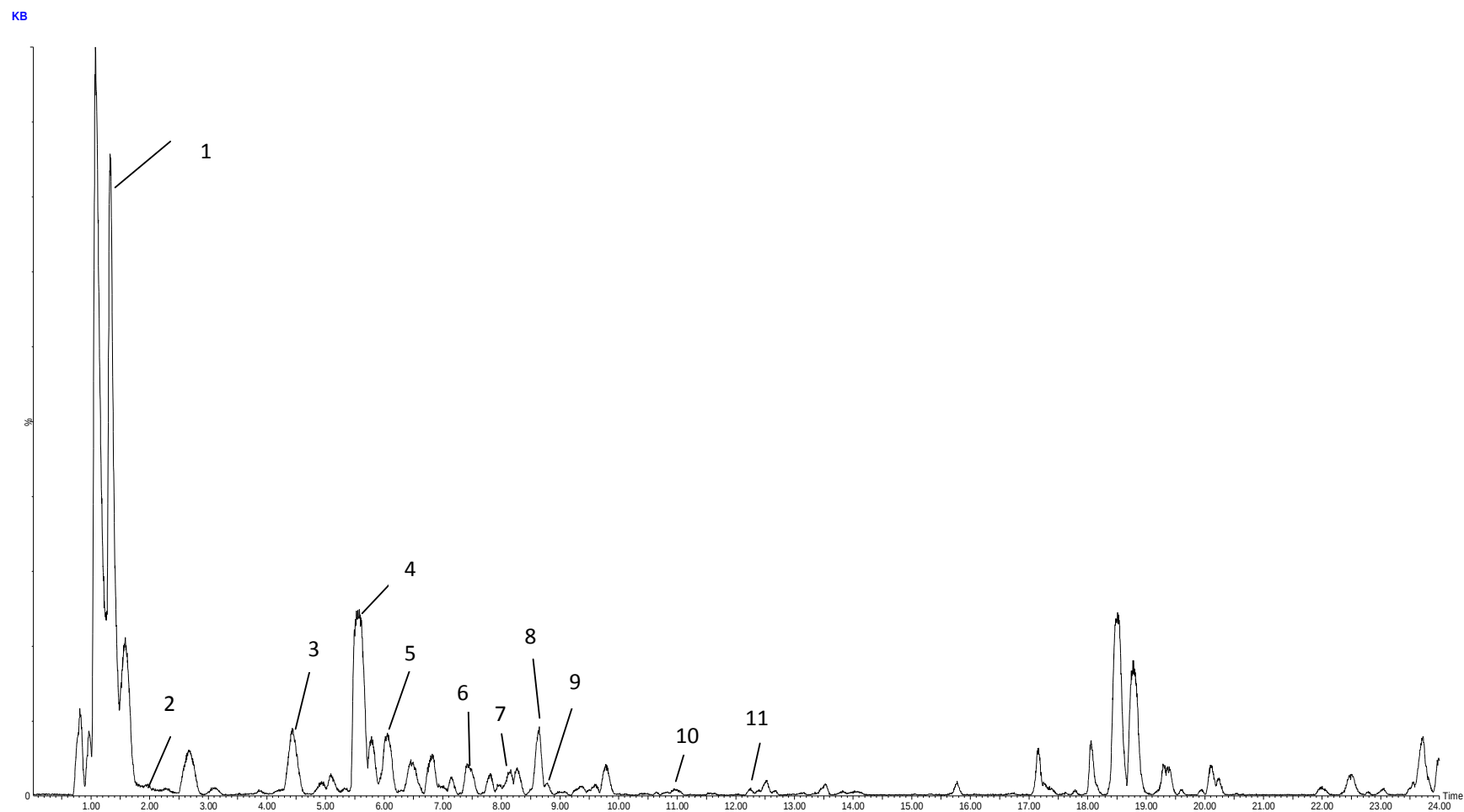
A flavone derivative with $[M - H]^-$ on at m/z 567 that was observed in red beans was not detected in grey mottled beans. Similarly, catechin glucoside observed in grey mottled beans was not detected in red beans.

250 **Table 4. HPLC-PDA-ESI-MS -based identification of phenolics in grey mottled beans**

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	1.98	170	169	125	Gallic acid
3	4.50	452	451	289	Catechin glucoside
4	5.57	260	259	241,233,197	Syringic acid derivative
5	6.08	290	289	245	Catechin
6	7.42	386	385	193	Ferulic acid derivates
7	8.12	290	289	245	Epicatechin
8	8.62	388	387	207	Medioresinol
9	8.92	164	163	119	<i>p</i> -coumaric acid
10	11.1	194	193	149, 134	<i>t</i> -ferulic acid
11	12.37	138	137		Salicylic acid

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Figure 2. HPLC-PDA-ESI-MS chromatogram of 70% methanol extract of grey mottled beans.

3.3. Phenolic compounds identified by HPLC-PDA-ESI-MS in brown beans

Phenolic compounds identified by HPLC-PDA-ESI-MS in brown beans are presented in Table 5 and the numbered peaks in the chromatogram (Figure 3). Quinic acid, gallic acid, a syringic acid derivative, catechin, ferulic acid derivatives, medioresinol, *p*-coumaric acid and *t*-ferulic acid detected in both the red and the grey mottled beans were also observed in the brown beans. Three isomers of ferulic acid derivatives were observed as opposed to one detected in grey mottled and four in red beans. Epicatechin, observed in both the red and grey mottled, was not detected in the brown beans. However, catechin glucoside and salicylic acid which were absent in the red beans but present in the grey mottled were detected in the brown beans. A flavanone derivative observed in red beans was not detected in brown beans.

Peak 14 revealed a negatively charged ion $[M - H]^-$ at m/z 447 with MS/MS fragment ion at m/z 285 indicating the presence of kaempferol fragment and a loss of hexose moiety (162 amu). Based on the similar fragmentation pattern reported previously [17], this compound was identified as kaempferol glucoside. The presence of kaempferol glucoside in common beans has been reported previously [12].

Peak 15 had $[M - H]^-$ ion at m/z 329 with MS/MS fragmentation yielding a major fragment ion at m/z 285, indicating the loss of carbon dioxide (44 amu). The fragmentation pattern observed for this ion after MS/MS experiment has been previously described in literature for carnosol [13]. Carnosol, a phenolic antioxidant has been reported previously in rosemary extracts [18, 13]. To the authors' knowledge, this is the first time carnosol has been reported to be present in common beans.

Kaempferol glucoside and carnosol detected in this market class were not observed in the red and grey mottled beans.

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Table 5. HPLC-PDA-ESI-MS -based identification of phenolics in brown beans

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	1.98	170	169	125	Gallic acid
3	4.50	452	451	289	Catechin glucoside
4	5.57	260	259	241,233,197	Syringic acid derivative
5	6.08	290	289	245	Catechin
6	6.43	386	385	193	Ferulic acid derivates
7	7.42	386	385	193	Ferulic acid derivates
8	8.10	386	385	193	Ferulic acid derivates
9	8.62	388	387	207	Medioresinol
10	8.92	164	163	119	<i>p</i> -coumaric acid
11	9.79	388	387	207	Medioresinol
12	11.1	194	193	149, 134	<i>t</i> -ferulic acid
13	12.37	138	137		Salicylic acid
14	13.13	148	447	287	Kaempferol glucoside
15	23.55	330	329	285	Carnosol

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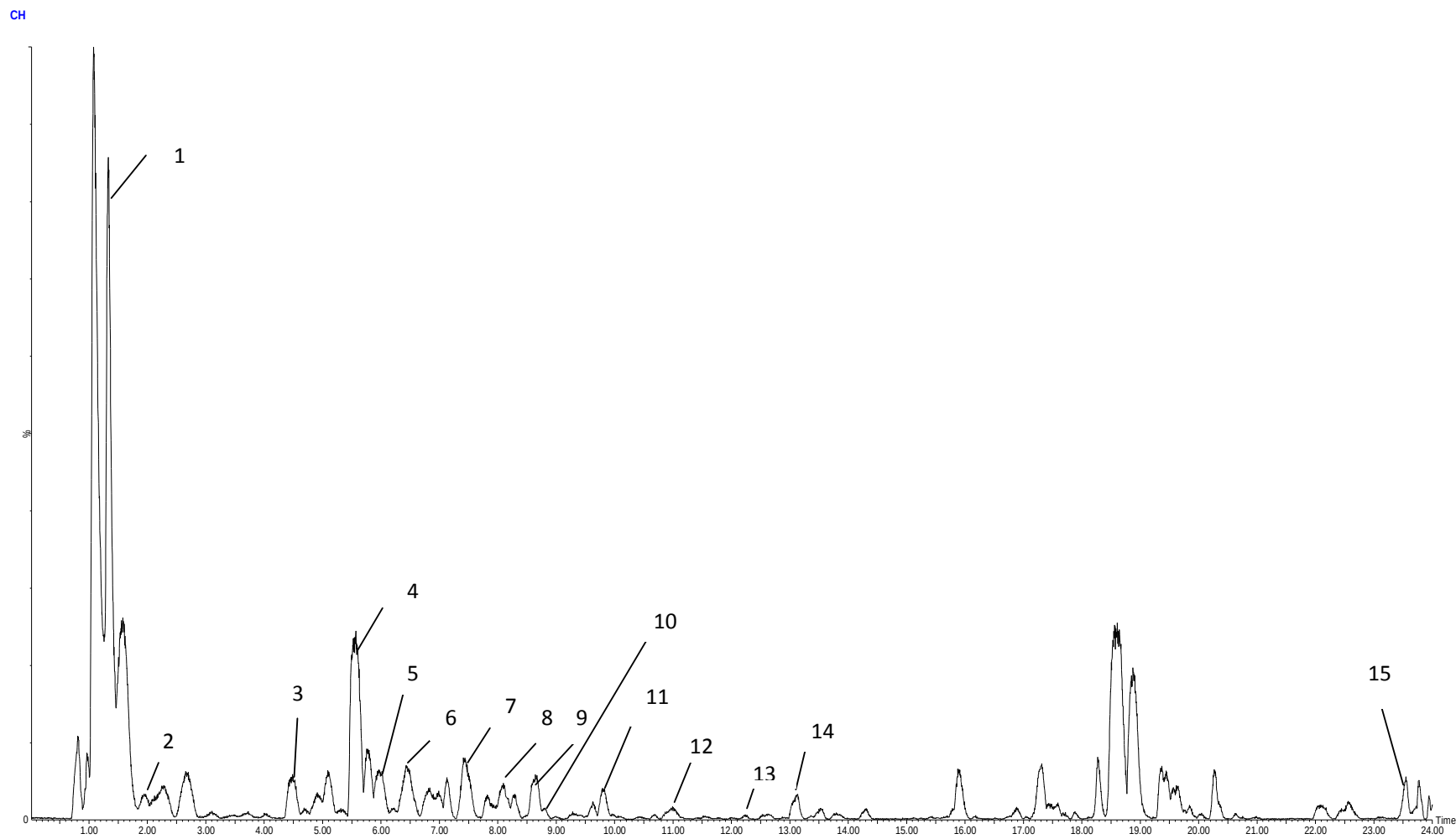


Figure 3 HPLC-PDA-ESI-MS chromatogram of 70% methanol extract of brown beans.

The use of HPLC-PDA-ESI-MS led to the identification of various phenolic compounds in the
Zambian market classes of common beans. Quinic acid, a syringic acid derivative, ferulic acid
derivatives, medioresinol, *p*-coumaric acid, catechin, gallic acid and ferulic acid were identified
in all the market classes investigated. However, the isomers of ferulic acid derivatives observed
were 4 in red, 3 in brown and 1 in grey mottled beans. Epicatechin was only identified in the red
and grey mottled beans. A compound with the molecular ion at m/z 567, tentatively identified as
a flavonone derivative was only observed in red beans. Catechin glucoside was only identified in
grey mottled and brown beans. Compounds tentatively identified as kaempferol glucoside and
carnosol were only observed in brown beans and not in the other market classes.

Though some phenolic compounds were common in all the market classes of common beans,
there was variation in the types of other phenolic compounds identified. It is possible to discover
variation in almost every conceivable trait, including agronomical and nutritional qualities. And
if a trait cannot be found in the crop itself, it can often be found in a wild relative of the crop; a
plant that has similar species that have not been farmed or used in agriculture, but exist in the
wild [19]. Diversity in the phenolic phytochemical profile offers great opportunities for the
improvement of common beans by plant breeders.

4. CONCLUSION

The study has shown that the market classes of common beans investigated contain various
polyphenolic compounds that may be useful as nutraceutical targets. Some identified phenolic
compounds are common in all the market classes while others are variety associated. The study
assumes that the diversity in the phenolic phytochemical profile is an excellent opportunity for
genetic improvement in the nutraceutical attributes of these common bean market classes by crop
breeders.

COMPETING INTERESTS

We declared that no competing interests exist.

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