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Nutrient and bioactive compounds composition of the leaves and stems of *Pandiaka heudelotii*: A wild vegetable

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Abstract

The proximate, minerals, vitamins, amino acid, alkaloids, phytosterols, carotenoids, glycosides and saponins profiles of the leaves and stems of *Pandiaka heudelotii* were determined using standard methods. The leaves and stems had high contents (g/100g) of fibre (10.3−12.9) and carbohydrate (47.2−55.3); and moderate protein (4.4−9.8) and crude fat (6.7−10.2); respectively, equivalent to 41.1−51.6%, 15.7−17.8%, 8.8−19.6%, 10.3−15.7% of the corresponding daily values. They had high contents of iron, manganese, calcium, magnesium, potassium, selenium, vitamins C, E and B2, alkaloids, glycosides, carotenoids, saponins; and moderate phytosterol. Their proteins had high contents of essential amino acids (42.6−48.5%). Triacetonamine (57.20−60.13%), nicotiflorin (53.45−55.35%), carotene (49.95−51.94%), liquiritin (57.54−62.34%), and sitosterol (82.84−85.03%) were respectively, the most abundant alkaloids, glycosides, carotenoids, saponins and phytosterols detected. This result indicates that the leaves and stems are good sources of nutraceuticals and nutrients for human nutrition. It provides an insight into the nature of its bioactive components.

Keywords: Food science, Nutrition, Food analysis, Biochemistry

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1. Introduction

Vegetables enrich and diversify the human diet. They are rich sources of fibre, mineral nutrients, vitamins, bioactive phytochemicals, and other compounds that support human health and nutrition (Radovich, 2011; Sinha et al., 2011). One of such vegetables that can be used as a source of nutrient and nutraceuticals is *Pandiaka heudelotii*. *Pandiaka heudelotii* is a wild vegetable, which belongs to the Amaranthaceae family (Ifeanacho et al., 2017). In southern Nigeria, the leaves of this plant are used as vegetables and boiled for tea (Ifeanacho et al., 2017). They are also used for the treatment of malaria.

Earlier, Ifeanacho et al. (2017) reported the profile of phenolic compounds in the leaves and stems of *Pandiaka heudelotii*. They reported that the leaves and stems had high contents of flavonoids and benzoic acid derivatives, and moderate contents of lignans and hydroxycinnamates. Presently, there is no information in the biochemical literature regarding the nutrients, allicins, alkaloids, phytosterols, carotenoids, glycosides and saponins compositions of the leaves and stems of this wild vegetable. Therefore, this study investigated the composition of these compounds in the leaves and stems of *Pandiaka heudelotii* with a view to providing information on their potential as sources of nutrients and nutraceuticals. The pharmacological significance of these bioactive constituents is also discussed herein.

2. Materials and methods

2.1. Collection of samples

Fresh samples of *Pandiaka heudelotii* were collected from within the Abuja Campus of University of Port Harcourt, Port Harcourt, Nigeria. They were identified and prepared as earlier reported by Ifeanacho et al. (2017).

2.2. Determination of nutrient profiles

2.2.1. Proximate analysis

A portion of the samples was used for proximate analysis, to determine (in triplicate) the moisture, crude protein, fat, ash, fibre and total carbohydrates, using standard methods. The moisture content was determined according to AOAC Official Method 967.03 (AOAC International, 2006). The ash content was determined according to AOAC Official Method 942.05 (AOAC International, 2006). The crude protein (% total nitrogen x 6.25) was determined by Kjeldhal method using AOAC Official

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Method 2001.11 (AOAC International, 2006). Crude fat was determined according to AOAC Official Method 920.39 (AOAC International, 2006). The determination of the fibre content was based on AOAC Official Method 973.18 (AOAC International, 2006). Carbohydrate content was determined by difference (i.e. by subtracting the sum of all the other components from 100 g). Metabolizable energy value was calculated with Atwater factors 4, 9 and 4 for protein, fat and carbohydrate respectively (Ikewuchi et al., 2009).

2.2.2. Determination of mineral elements and phosphorus composition

Analysis of the mineral elements was carried out according to FAO fertilizer and plant nutrition bulletin 19 (Motsara and Roy, 2008). The samples were ashed and digested with nitric acid, before being analyzed with an atomic absorption spectrophotometer. Phosphorus was determined spectrophotometrically by the vanadium molybdate method (Motsara and Roy, 2008). The phosphorus content of the samples was converted to orthophosphates by digestion with a mixture of HNO_3 and $HClO_4$; and the resultant orthophosphates was made to react with molybdate and vanadate in Vanadomolybdate Reagent, to form a yellow-coloured vanadomolybdophosphoric complex whose intensity (which is directly proportional to the concentration of phosphorus present in the sample) was read at 420 nm in a spectrophotometer.

2.2.3. Determination of vitamin profile

The vitamin content was extracted by a combination of the methods of Association of Official Analytical Chemists (AOAC) Method 992.03, 992.04 and 992.26 (AOAC International, 2006). Chromatographic analysis was performed with an HP 6890 (Hewlett Packard, Wilmington, DE, USA) Series gas chromatograph equipped with a pulse flame photometric detector and a 30 m $\times$ 0.25 mm i.d. column coated with a 0.25 μm film of DB- 5MS, and powered with HP ChemStation Rev. A09.01[1206] Software. Split injection (split ratio 20:1) was performed, with nitrogen as carrier gas at a flow rate of 1.0 m/s. The column temperature was maintained at 50 °C for 2 min after injection, and then ramped at 10 °C/min for 20 min, followed by another ramping at 15 °C/min for 4 min. The injection port and detector temperatures were 250 and 320 °C respectively. The hydrogen and compressed air pressures were 137.90 and 262.00 kPa. Calculations were based on analysis of standard mixtures and calculation of individual correction coefficients.

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2.2.4. Determination of per cent daily value (%DV)

Per cent daily values (%DV) were determined by comparison to the appropriate daily values (Food and Drug Administration, 2013). It was calculated using the following formula.

$$Percent\ daily\ value\ (\%) = \frac{weight\ of\ the\ particular\ nutrient\ in\ 100\ g\ of\ sample}{daily\ value} \times 100$$

2.2.5. Determination of amino acid profile

The extraction of the amino acids was carried out according to AOAC Method 982.30 (AOAC International, 2006). The sample was dried to a constant weight, defatted, hydrolysed, and concentrated to 1.0 mL. The concentrated hyrolysate was derivatised before being subjected to gas chromatography analysis on an HP 6890 (Hewlett Packard, Wilmington, DE, USA) Series gas chromatograph equipped with a pulse flame photometric detector and a 30 m × 0.25 mm i.d. column coated with a 0.25 μm film of HP-5 and powered with HP ChemStation Rev. A09.01[1206] Software. A split injection was adopted at a split ratio of 20:1. The carrier gas used was hydrogen at a flow rate of 1.0 mL/min. The inlet and detector temperatures were 250 and 320 °C. The column and compressed air pressures were respectively 137.90 and 241.32 kPa. The oven was programmed initially at 60 °C, ramped at 8 °C/min for 20 min, maintained for 2 min and then ramp again at 12 °C/min for 6 min, before maintaining for 2 min.

2.2.6. Determination of digestible indispensable amino acid (DIAA) reference ratio and DIAA score

The digestible indispensable amino acid (DIAA) reference ratio for each indispensable amino acid (IAA) in the test proteins were determined by comparing their amino acid composition as obtained in this study, with WHO reference protein patterns (FAO, 2013), according to the following equation.

$$Digestible\ IAA\ reference\ ratio = \frac{mg\ of\ a\ digestible\ indispensableamino\ acid\ in\ 1\ g\ protein\ of\ the\ sample}{mg\ of\ same\ digestible\ indispensable\ amino\ acid\ in\ 1\ g\ of\ reference\ protein}$$

The digestible IAA score (DIAAS) was determined by expressing the lowest DIAA reference ratio as a percentage (FAO, 2013); while the limiting amino acid was taken as the DIAA with the least DIAA ratio.

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2.3. Evaluation of the phytochemical profile

2.3.1. General procedures

Gas chromatography was carried out at Multi Environmental Management Consultants Limited, Igbe Road, Ikorodu, Lagos, with a Hewlett Packard HP 6890, gas chromatograph, fitted with a flame ionization detector (except for allicins analysis, in which pulse flame photometric detector was used), and powered with HP Chemstation Rev. A09.01[1206] software, to identify and quantify compounds. Standards were from Sigma-Aldrich Co. and Lynnchem Biological Technology Co. Standard solutions were prepared in methanol for alkaloids and allicins; in acetone for carotenoids; in methylene chloride for phytosterols; and in ethanol for glycosides and saponins. The linearity of the dependence of response on concentration was verified by regression analysis. Identification was based on comparison of retention times and spectral data of the standards. Quantification was performed by creating calibration curves for each compound determined, using the standards.

2.3.2. Determination of alkaloids composition

The extraction of the alkaloids was carried out according to Ngounou et al. (2005). The resultant extract was subjected to gas chromatography on a DB-5MS column capillary of dimensions: 30 m × 0.25 mm ID × 0.25 μm film thickness. The inlet and detection temperatures were 250 and 320 °C. The equipment was run on split injection (20:1 split ratio), using nitrogen as carrier gas. The hydrogen and compressed air pressures were 193.05 and 262.00 kPa respectively. The oven temperature was run initially at 60 °C for 5 min, ramped at 10 °C/min for 20 min, and ramped again at 15 °C/min for 4 min.

2.3.3. Determination of saponins composition

The extraction of the saponins was carried out according Guo et al. (2009). The resultant extract was subjected to gas chromatography on a DB-225MS column capillary of dimensions: 30 m × 0.25 mm ID × 0.25 μm film thickness. The inlet and detection temperatures were 250 and 320 °C. The equipment was run on split injection (20:1 split ratio) using nitrogen as carrier gas. The hydrogen and compressed air pressures were 193.05 and 275.79 kPa respectively. The oven temperature was run initially at 60 °C for 5 min, ramped at 12 °C/min for 18 min, and ramped again at 15 °C/min for 5 min.

2.3.4. Determination of allicins composition

The allicins were extracted in accordance with Chehregani et al. (2007). The resultant extract was subjected to gas chromatography on an OV-101 column capillary of

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dimensions: 30 m × 0.30 mm ID × 0.25 μm film thickness. The injection and detection temperatures were 250 and 320 °C. The equipment was run on split injection (20:1 split ratio), using helium as carrier gas at a pressure of 206.84 kPa. The hydrogen and compressed air pressures were 193.05 and 268.90 kPa respectively. The oven temperature was run initially at 80 °C for 5 min. The first ramping was at 10 °C/min for 5 min, maintained for 4 min; and the second ramping at 10 °C/min for 5 min, maintained for 4 min.

2.3.5. Determination of sterols composition

Extraction of oil was carried out according to AOAC method 999.02 (AOAC International, 2006), while the analysis of the sterols was carried out according to AOAC method 994.10 (AOAC International, 2006) and AOAC method 970.51 (AOAC International, 2006). The resultant extract, after saponification and removal of nonsaponifiables, was subjected to gas chromatography on a HP INNOWax column capillary of dimensions: 30 m × 0.25 mm ID × 0.25 μm film thickness. The inlet and detection temperatures were 250 and 320 °C. The equipment was run on split injection (20:1 split ratio), using nitrogen as carrier gas. The hydrogen and compressed air pressures were 151.68 and 241.32 kPa respectively. The oven temperature was run initially at 60 °C. It was then ramped at 10 °C/min for 20 min, maintained for 4 min; ramped again at 15 °C/min for 4 min, and maintained for 10 min.

2.3.6. Determination of carotenoids composition

The extraction of the carotenoids was carried out according to Takagi (1985). The resultant extract was subjected to gas chromatography on an AC-5 column capillary of dimensions: 30 m × 0.25 mm ID × 0.25 μm film thickness. Inlet and detection temperatures were 250 and 320 °C. The equipment was run on split injection (20:1 split ratio), with nitrogen as carrier gas. Hydrogen and compressed air pressures were 206.8 and 275.8 kPa respectively. Oven temperature was run initially at 60 °C, ramped at 10 °C/min for 20 min, maintained for 2 min; ramped again at 15 °C/min for 4 min and maintained for 4 min.

2.3.7. Determination of glycosides composition

The glycosides were extracted in accordance with Oluwaniyi and Ibiyemi (2007). The resultant extract was subjected to gas chromatography on an AC-5 column capillary of dimensions: 30 m × 0.25 mm ID × 0.25 μm film thickness. The inlet and detection temperatures were 250 and 320 °C. The equipment was run on split injection (20:1 split ratio) using nitrogen as carrier gas. The hydrogen and compressed air pressures were 193.05 and 275.79 kPa respectively. The oven temperature was run initially at 70 °C for 5 min, and ramped at 12 °C/min for 20 min.

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2.3.8. Derivation of compositions per dry weight from the composition per wet weight

Compositions per dry weight of the parameters were derived from compositions per wet weight and vice versa, using the following formula (Ikewuchi et al., 2015).

$$Composition\ per\ dry\ weight\ (\%) = \frac{Composition\ per\ wet\ weight\ (\%) \times 100}{Dry\ matter\ content\ (\%)}$$

2.4. Statistical analysis

Means and standard deviations were calculated for three determinations. Means of the nutrient components were tested with student *t*-test and significance accepted at $p < 0.05$ probability levels.

3. Results and discussion

Table 1 shows the proximate composition and nutrient potential of the stems and leaves of *Pandiaka heudelotii*. The leaves had higher moisture ($p < 0.0010$), crude fat ($p < 0.0152$), and crude protein ($p < 0.0005$) contents and caloric value ($p < 0.0234$) than the stems, while the stems had higher carbohydrate ($p < 0.0018$), ash ($p < 0.1111$) and crude fibre ($p < 0.0157$) contents. They had moderate moisture levels, above which, the moisture would have been undesirable for their quality, since high moisture content increases water activity and the probability of microbial growth (Farah, 2012). Their protein and caloric contents were higher than those of cabbage, lettuce (green and red), amaranth leaves (U.S. Department of Agriculture, 2016a,b,c,d) and *Tridax procumbens* (Ikewuchi et al., 2009). Drying improved their protein content, therefore making the dried leaves a protein source with a protein content of 11% which is greater than the 10% cut-off (FAO, 2013). The ash, crude fat and total carbohydrate contents of the leaves of *P. heudelotii* were higher than those of *Cnidoscolus aurifolia* (Udo and Udo, 2016), cabbage, lettuce (green and red), amaranth leaves (U.S. Department of Agriculture, 2016a,b,c,d) and *T. procumbens* (Ikewuchi et al., 2009). The leaves had comparable fibre content to *C. aurifolia* (Udo and Udo, 2016). However, they had higher fibre than cabbage, green and red lettuce (U.S. Department of Agriculture, 2016a,b,c) and *T. procumbens* (Ikewuchi et al., 2009). According to Farah (2012), a high intake of dietary fibre has been positively associated with several beneficial physiologic and metabolic effects like lowering blood cholesterol and modulating the blood glucose and insulin responses.

In comparison to the daily values (Food and Drug Administration, 2013), a 100 g of the leaves can provide about 41.2−46.7% of daily value for crude fibre, 19.6−22.2% daily value for crude protein, 16.0−18.1% of daily value for caloric value, 15.7−17.8% of daily value for carbohydrate, 15.7−17.8% of daily value for crude

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Table 1. Proximate composition (/100g) and nutrient potential (per cent daily value/100g) of the leaves and stems of *Pandiaka heudelotii*.

Component	Composition (g/100g)‡				Potential (per cent daily value/100g)			
	Leaves		Stems		Leaves		Stems	
	/fresh weight	/dry weight	/fresh weight	/dry weight	/fresh weight	/dry weight	/fresh weight	/dry weight
Moisture	11.700 ± 0.243*	-	8.200 ± 0.227	-	NA	NA	NA	NA
Dry matter	88.300 ± 0.243*	100.000 + 0.000*	91.800 ± 0.227	100.000 ± 0.000	NA	NA	NA	NA
Ash	10.800 ± 0.617*	12.231 ± 0.699*	12.250 ± 0.567	13.344 ± 0.617	NA	NA	NA	NA
Crude fat	10.200 ± 0.657*	11.552 ± 0.744*	6.700 ± 0.286	7.298 ± 0.311	15.692 ± 1.011*	17.771 ± 1.145*	10.308 ± 0.440	11.228 ± 0.479
Crude protein	9.800 ± 0.171*	11.099 ± 0.194*	4.400 ± 0.090	4.793 ± 0.098	19.600 ± 0.343*	22.197 ± 0.388*	8.800 ± 0.181	9.586 ± 0.197
Carbohydrate	47.200 ± 0.861*	53.454 ± 0.975*	55.250 ± 1.158	60.185 ± 1.261	15.733 ± 0.287*	17.818 ± 0.325*	18.416 ± 0.386	20.061 ± 0.420
Crude fibre	10.300 ± 0.432*	11.665 ± 0.489*	12.900 ± 0.238	14.052 ± 0.259	41.200 ± 1.726*	46.659 ± 1.955*	51.600 ± 0.951	56.209 ± 1.035
Caloric value	319.800 ± 9.313*	362.174 ± 10.547*	298.900 ± 2.690	325.599 ± 2.931	15.990 ± 0.466*	18.109 ± 0.527*	14.945 ± 0.135	16.280 ± 0.147

Values are means ±standard deviation of triplicate determinations. *$P < 0.05$ compared to stems.

‡ The unit of Caloric value = kcal/100g; NA = not applicable.

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fat (Table 1). A 100 g of the stems can provide 51.6−56.2% of daily value for crude fibre, 18.4−20.1% of daily value for carbohydrate, 15.0−16.3% of daily value for caloric value, 10.3−11.2% of daily value crude fat and 8.8−9.6% of daily value for crude protein.

The leaves and stems of *P. heudelotii* had high contents of vitamins C, E and B2, although, the leaves had higher contents of these vitamins (Table 2). They had higher vitamin C than cabbage, lettuce (green and red), amaranth leaves (Venskutonis and Kraujalis, 2013; U.S. Department of Agriculture, 2016a,b,c,d) and *T. procumbens* (Ikewuchi and Ikewuchi, 2009a). They also had higher vitamin B2 than cabbage, lettuce (green and red), amaranth leaves (U.S. Department of Agriculture, 2016a,b,c,d) and *T. procumbens* (Ikewuchi and Ikewuchi, 2009a); but lower contents than amaranth seeds (Venskutonis and Kraujalis, 2013). The leaves had higher vitamin B3 than cabbage, green and red lettuce (U.S. Department of Agriculture, 2016a,b,c) and *T. procumbens* (Ikewuchi and Ikewuchi, 2009a); but lower contents than amaranth leaves and seeds (Venskutonis and Kraujalis, 2013; U.S. Department of Agriculture, 2016d). The leaves and stems had higher vitamin B1 than amaranth leaves (U.S. Department of Agriculture, 2016d) and *T. procumbens* (Ikewuchi and Ikewuchi, 2009a), but lower contents than cabbage, green and red lettuce (U.S. Department of Agriculture, 2016a,b,c). Their vitamins B6, B5, B9, A, E and K contents were lower than those of cabbage, lettuce (green and red) and amaranth leaves (U.S. Department of Agriculture, 2016a,b,c,d). When compared to the relevant daily values (Food and Drug Administration, 2013), a 100 g of the leaves can provide about 123.5%−139.9% of daily value for vitamin C, 319.2%−361.4% of daily value for vitamin E, and 16.7%−18.9% of daily value for vitamin B2. That of the stem is equivalent to 104.6%−113.9% of daily value for vitamin C, 200.1%−218.0% of daily value for vitamin E, and 11.3%−12.3% of daily value for vitamin B2.

The leaves and stems had high contents of calcium, iron, magnesium, manganese, potassium and selenium (Table 3). The leaves had higher copper ($p < 0.021$), phosphorus ($p < 0.019$), selenium ($p < 0.028$), sodium ($p < 0.010$) and zinc ($p < 0.014$); and lower calcium ($p < 0.016$), iron ($p < 0.031$), magnesium ($p < 0.012$), manganese ($p < 0.029$) and potassium ($p < 0.005$) contents than the stems. They both had higher manganese and phosphorus than lettuce (green and red), cabbage and amaranth leaves (U.S. Department of Agriculture, 2016a,b,c,d). They had higher calcium, magnesium and potassium than *C. aurifolia* leaves, cabbage, lettuce (green and red), amaranth leaves (Udo and Udo, 2016; U.S. Department of Agriculture, 2016a,b,c,d) and *T. procumbens* (Ikewuchi and Ikewuchi, 2009b). According to Kilgour (1985), to avoid hypertension from food sources, the ratio of sodium to potassium should be ≤ 1.67. This study showed that the leaves and stems of *P. heudelotii* had low sodium to potassium ratios, and so may be very safe for consumption by hypertensive individuals. In addition, they had high calcium to phosphorus ratios. High dietary calcium/phosphorus ratio might have a positive influence on bone mass

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Table 2. Vitamin composition and potential of leaves and stems of *Pandiaka heudelotii*.

Component	Concentration (mg/kg)				Per cent daily value/100 g			
	Leaves		Stems		Leaves		Stems	
	/fresh weight	/dry weight	/fresh weight	/dry weight	/fresh weight	/dry weight	/fresh weight	/dry weight
Vitamin B3	4.380220	4.960612	3.079010	3.354041	0.988400	1.119370	1.183690	1.289320
Vitamin B6	0.197680	0.223873	0.236720	0.257865	0.988400	1.119370	1.183690	1.289320
Vitamin C	741.062900	839.255800	627.558600	683.615000	123.510000	139.876000	104.593000	113.936000
Vitamin A	0.013093	0.014828	0.014734	0.016050	0.087280	0.098840	0.098220	0.106990
Vitamin B1	0.505763	0.572778	0.325199	0.354247	3.371750	3.818520	2.167990	2.361650
Vitamin B2	2.837960	3.213998	1.915700	2.086819	16.69390	18.905900	11.268890	12.275400
Vitamin D	0.000334	0.000378	0.000021	0.000023	0.027830	0.031520	0.001770	0.001930
Vitamin E	0.287266	0.325330	0.180090	0.196176	319.153000	361.441000	200.080000	217.952000
Vitamin B9	0.203796	0.230800	0.201742	0.219763	5.094900	5.769990	5.043550	5.494060
Vitamin K	0.003549	0.004019	0.002379	0.002592	0.443620	0.502400	0.297380	0.323950
Vitamin B5	0.281392	0.318677	0.130562	0.142224	0.281390	0.318680	0.130560	0.142220

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Table 3. Mineral nutrient composition and potential of leaves and stems of *Pandiaka heudelotii*.

Component	Composition (mg/kg)				Potential (per cent daily value/100 g)			
	Leaves		Stems		Leaves		Stems	
	/fresh weight	/dry weight	/fresh weight	/dry weight	/fresh weight	/dry weight	/fresh weight	/dry weight
Iron	64.8260 ± 0.8850*	73.4156 ± 1.0023*	81.7450 ± 0.7780	89.0468 ± 0.8475	36.0145 ± 0.4917*	40.7865 ± 0.5568*	45.4139 ± 0.4322	49.4705 ± 0.4708
Copper	1.6130 ± 0.0880*	1.8267 ± 0.0997*	0.5140 ± 0.0150	0.5599 ± 0.0163	8.0650 ± 0.4400*	9.1336 ± 0.4938*	2.5700 ± 0.0750	2.7996 ± 0.0817
Manganese	46.9570 ± 0.4220*	53.1789 ± 0.4779*	55.1840 ± 0.3210	60.1133 ± 0.3497	234.7850 ± 2.1100*	265.8947 ± 2.3896*	275.9200 ± 1.6050	300.5664 ± 1.7484
Zinc	26.8350 ± 0.5210*	30.3907 ± 0.5900*	8.3070 ± 0.3090	9.0490 ± 0.3366	17.8900 ± 0.3473*	20.2605 ± 0.3933*	5.5380 ± 0.2060	6.0327 ± 0.2244
Calcium	34125.0400 ± 174.9800*	38646.7044 ± 198.1653*	39205.9000 ± 87.3000	42707.9521 ± 95.0980	341.2505 ± 1.7485*	386.4672 ± 1.9813*	392.0590 ± 0.8730	427.0795 ± 0.9510
Magnesium	18420.7800 ± 80.3400*	20861.5855 ± 90.9853*	21767.4200 ± 42.7500	23711.7865 ± 46.5686	460.5195 ± 2.0085*	521.5396 ± 2.2746*	544.1855 ± 1.0685	592.7947 ± 1.1639
Potassium	9217.4580 ± 13.6870*	10438.7973 ± 15.5006*	13615.5600 ± 89.5600	14831.7647 ± 97.5599	26.3336 ± 0.0391*	29.8251 ± 0.0443*	38.9016 ± 0.2559	42.3765 ± 0.2788
Sodium	270.9460 ± 7.3770*	306.8471 ± 8.3545*	181.7640 ± 4.6670	198.0000 ± 5.0839	1.1289 ± 0.0307*	1.2785 ± 0.0348*	0.7573 ± 0.0194	0.8250 ± 0.0211
Phosphorus	982.1630 ± 7.0410*	1112.3024 ± 7.9740*	926.4290 ± 3.6970	1009.1819 ± 4.0272	9.8216 ± 0.0704*	11.1230 ± 0.0797*	9.2643 ± 0.0370	10.0918 ± 0.0403
Selenium	0.0120 ± 0.0003*	0.0136 ± 0.0003*	0.0080 ± 0.0001	0.0087 ± 0.0001	1714.2850 ± 42.8550*	1941.4326 ± 48.5334*	1142.8550 ± 7.1450	1244.9401 ± 7.7832
Sodium/potassium ratio‡	0.0294 ± 0.0008*	0.0294 ± 0.0008*	0.0133 ± 0.0003	0.0133 ± 0.0003	NA	NA	NA	NA
Calcium/phosphorus ratio‡	34.7478 ± 0.4273*	34.7478 ± 0.4273*	42.3197 ± 0.0746	42.3197 ± 0.0746	NA	NA	NA	NA

Values are means ±standard deviation of triplicate determinations. *$P < 0.05$ compared to stems.

‡These have no units. NA = not applicable.

(Lee et al., 2014). It allows for strong bone development because absorption of calcium under this condition would be maximal (Koshihara et al., 2005). According to Korkmaz et al. (2013), magnesium (a calcium channel blocker) is involved in many metabolic processes, like maintenance of cell membrane function, modulation of smooth muscle contraction and enzymatic activities. Studies have shown that magnesium is a neuroprotective agent; increases blood flow to tissues; plays a vital role in development and function of the eye; and in diabetic patients, decreases insulin resistance, enhances glycaemic control and prevents diabetic retinopathy (Korkmaz et al., 2013).

They also had higher iron and zinc contents than cabbage, lettuce (green and red), amaranth leaves (U.S. Department of Agriculture, 2016a,b,c,d) and *T. procumbens* (Ikewuchi and Ikewuchi, 2009b), but however, had lower contents than *C. aurifolia* leaves (Udo and Udo, 2016). They had higher selenium than cabbage and green lettuce (U.S. Department of Agriculture, 2016a,b); but only those of the leaves were higher than amaranth leaves (U.S. Department of Agriculture, 2016d). They however had lower selenium than red lettuce (U.S. Department of Agriculture, 2016c). Their copper were higher than those of cabbage, green and red lettuce (U.S. Department of Agriculture, 2016a,b,c); while only those of the leaves were higher than amaranth leaves (U.S. Department of Agriculture, 2016d). Comparison to relevant daily values (Food and Drug Administration, 2013), shows that a 100 g is equivalent to 234.8−265.9% (leaves) and 275.9−300.6% (stems) daily value for manganese; 1714.3−1941.4% (leaves) and 1142.9−1244.9% (stems) daily value for selenium; 341.3−386.5% (leaves) and 392.1−427.1% (stems) daily value for calcium; 460.5−521.5% (leaves) and 544.2−592.8% (stems) daily value for magnesium; 36.0−40.8% (leaves) and 45.4−49.5% (stems) daily value for iron; and 26.3−29.8% (leaves) and 38.9−42.4% (stems) daily value for potassium.

Amino acid profile, and DIAA reference ratios of the leaf and stem proteins are given in Tables 4 and 5. They are rich in essential amino acids, 48.5% for leaves and 42.6% for stems [especially isoleucine (in leaves and stems), aromatic amino acids (leaves), threonine (leaves and stems), leucine (leaves) and histidine (leaves)] and can meet the daily requirements (FAO, 2013) for essential amino acids (except leucine, in the case of the stem protein), for children (≥6 months), adolescents and adults. Compared to WHO reference protein patterns for infant (≤6 months), child (6 months−3 years), older child, adolescent, adult (FAO, 2013), the DIAAS of the leaf protein were 47.6, 57.6 and 68.4 respectively, with lysine as limiting amino acid. Those of the stem protein were 23.9, 34.7 and 37.6, with leucine as limiting amino acid. Every 100 g of these proteins contained 32.0 g (for leaves) and 23.9 g (for stems) of essential amino acids; 1.9 g (for leaves) and 1.5 g (stems) of sulphur-containing amino acids; and 6.5 g (leaves) and 4.0 g (stems) of aromatic amino acids (Table 4). The leaf protein can be used for supplementation of isoleucine, aromatic amino acids and threonine in diets of children (≥6 months),

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Table 4. Amino acid composition of the leaves and stems of *Pandiaka heudelotii*.

Component	Leaf			Stem		
	mg/g protein	mg/100 g sample		mg/g protein	mg/100 g sample	
		Fresh	Dry		Fresh	Dry
Glycine	38.142	0.374	0.423	39.058	0.172	0.187
Alanine	33.684	0.330	0.374	33.520	0.147	0.161
Serine	20.540	0.201	0.228	27.200	0.120	0.130
Proline	23.192	0.227	0.257	18.753	0.083	0.090
Valine*	38.721	0.379	0.430	39.123	0.172	0.188
Threonine*	37.790	0.370	0.419	37.909	0.167	0.182
Isoleucine*	43.008	0.421	0.477	38.140	0.168	0.183
Leucine*	64.955	0.637	0.721	22.907	0.101	0.110
Aspartate	85.483	0.838	0.949	85.950	0.378	0.412
Lysine*	32.822	0.322	0.364	34.783	0.153	0.167
Methionine*	10.559	0.103	0.117	9.894	0.044	0.047
Glutamate	94.993	0.931	1.054	90.207	0.397	0.432
Phenylalanine*	36.878	0.361	0.409	15.269	0.067	0.073
Histidine*	19.184	0.188	0.213	11.006	0.048	0.053
Arginine	44.129	0.432	0.490	27.330	0.120	0.131
Tyrosine*	27.945	0.274	0.310	24.790	0.109	0.119
Cysteine*	8.582	0.084	0.095	5.213	0.023	0.025
Total amino acids	660.600	6.474	7.332	561.100	5.498	6.227
Total essential amino acids (without tryptophan)	320.444	3.139	3.555	239.034	1.052	1.147
Total nonessential amino acids	348.700	3.418	3.871	327.200	3.207	3.632
Total sulphur containing amino acids	19.140	0.188	0.212	15.110	0.148	0.168
Total aromatic amino acids	64.820	0.635	0.719	40.060	0.393	0.445

* Essential amino acids.

adolescents and adults, and histidine and leucine in older children, adolescent and adults; while the stems can be used for isoleucine and threonine in children (≥6 months), adolescents and adults.

Compared to child (6 months−3 years) requirement protein pattern (FAO, 2013), the leaf protein has a digestible indispensable amino acid score, comparable to cooked peas, cooked kidney beans, cooked rice, cooked rolled oats; and higher digestible indispensable amino acid score than wheat bran, roasted peanuts, and rice protein concentrate (Rutherfurd et al., 2015).

The alkaloids, glycosides and carotenoids compositions of the leaves and stems of *Pandiaka heudelotii* is presented in Table 6a. The leaves (3309.6 mg/kg dry weight)

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Table 5. Digestible indispensable amino acid (IAA) reference ratios of proteins from the leaves and stems of *Pandiaka heudelotii*.

Amino acids	Amino acid composition from present study (mg/g protein)		Digestible Indispensable Amino Acid (IAA) reference ratio					
			Comparison to infant (birth to 6 months) requirement protein pattern		Comparison to child (6 months−3 years) requirement protein pattern		Comparison to older child, adolescent, adult requirement protein pattern	
	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems
Histidine	19.184	11.006	0.914	0.524	0.959	0.550	1.199	0.688
Isoleucine	43.008	38.140	0.782	0.693	1.344	1.192	1.434	1.271
Leucine	64.955	22.907	0.677	0.239	0.984	0.347	1.065	0.376
Lysine	32.822	34.783	0.476	0.504	0.576	0.610	0.684	0.725
Methionine + cysteine	19.141	15.107	0.580	0.458	0.709	0.560	0.832	0.657
Phenylalanine + tyrosine	64.823	40.059	0.690	0.426	1.247	0.770	1.581	0.977
Threonine	37.790	37.909	0.859	0.862	1.219	1.223	1.512	1.516
Valine	38.721	39.123	0.704	0.711	0.900	0.910	0.968	0.978

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Table 6a. Composition of phytochemicals isolated and detected in the leaves and stems of *Pandiaka heudelotii*.

Compounds	Leaves			Stems		
	RT (min)	Composition (mg/kg)		RT (min)	Composition (mg/kg)	
		/fresh weight	/dry weight		/fresh weight	/dry weight
Alkaloids						
Dopamine	7.689	0.00000020	0.00000023	7.715	0.00000020	0.00000022
3-Methoxytyramine	9.565	0.02237450	0.02533918	9.596	0.02018660	0.02198976
Triacetonamine	10.933	1757.32480000	1990.17531100	10.956	1264.13970000	1377.05849700
Thiarubrine A	11.333	0.00000148	0.00000168	11.357	0.00000147	0.00000161
Aporphine	11.478	0.00000271	0.00000307	11.502	0.00000264	0.00000287
Quinine	12.387	466.60830000	528.43522080	12.408	409.77370000	446.37657950
Acalyphin	13.771	698.43130000	790.97542470	13.791	536.22470000	584.12276690
Lycorine	14.769	0.00000404	0.00000458	14.795	0.00000431	0.00000470
Gelanthamine	15.464	0.00000122	0.00000138	15.484	0.00000181	0.00000198
Total alkaloids content	-	2922.38670000	3309.61121200	-	2210.15840000	2407.57995600
Glycosides						
Linamarin	16.252	0.00245385	0.00277899	16.246	0.00123371	0.00134391
Lotaustralin	17.364	0.00072481	0.00082085	17.358	0.00040226	0.00043819
Prunasin	18.054	0.07117660	0.08060770	18.049	0.04066560	0.04429804
Indican	18.497	0.01139200	0.01290147	18.494	0.00701773	0.00764459
Dhurrin	18.592	0.00125638	0.00142285	18.662	0.00030929	0.00033691
Nicotiflorin	19.103	564.48580000	639.28176670	19.098	437.26660000	476.32527230
Amygdalin	19.520	101.35280000	114.78233300	19.515	65.88560000	71.77080610
Ouabain	20.471	0.01012780	0.01146976	20.595	0.00186416	0.00203068
Digitoxin	21.129	0.00045487	0.00051514	21.126	0.00035016	0.00038144
Digitalis	21.821	0.00022305	0.00025260	21.817	0.00017094	0.00018621
Digoxin	22.601	0.00098648	0.00111719	22.596	0.00074678	0.00081349
Clitorin	23.472	222.38200000	251.84824460	23.467	153.93800000	167.68845320
Mauritianin	23.968	167.72810000	189.95254810	23.964	132.87710000	144.74629630
Total glycosides content	-	1056.04740000	1195.97667000	-	790.02010000	860.58834420
Carotenoids						
Malvidin	19.515	0.00196500	0.00222600	19.518	0.00123100	0.00134100
Beta-cryptoxanthin	20.532	0.11647800	0.13191200	20.535	0.06703600	0.07302400
Lycopene	21.498	0.00003100	0.00003500	21.501	0.00001100	0.00001200
Carotene	22.597	312.42140000	353.81812000	22.599	247.67660000	269.80021800
Lutein	23.228	120.57510000	136.55164200	23.230	97.29698000	105.98799600
Xanthophyll	24.031	43.93280000	49.75402000	24.033	22.93570000	24.98442300
Anthera-xanthin	24.876	27.46350000	31.10249200	24.882	17.55660000	19.12483700
Asta-xanthin	25.610	8.71534000	9.87014700	25.615	4.63227000	5.04604600

(*continued on next page*)

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Table 6a. (*Continued*)

Compounds	Leaves			Stems		
	RT (min)	Composition (mg/kg)		RT (min)	Composition (mg/kg)	
		/fresh weight	/dry weight		/fresh weight	/dry weight
Viola-xanthin	26.352	37.28800000	42.22876600	26.355	28.29260000	30.81982600
Neoxanthin	27.116	74.91130000	84.83725900	27.121	58.40210000	63.61884500
Total carotenoids content	-	625.45900000	708.33408800	-	476.83400000	519.42701500

RT = Retention time.

and stems (2407.6 mg/kg dw) had very high total alkaloids' contents. Nine known alkaloids were detected including triacetonamine (60.13% in leaves; 57.20% in stems), acalyphin (23.90% in leaves; 24.26% in stems) and quinine (15.97% in leaves; 18.54% in stems). 3-Methoxytyramine, lycorine, aporphine, thiarubrine A, gelanthamine and dopamine constituted less than 0.001%.

The leaves and stems of *P. heudelotii* had lower total alkaloids contents than the leaves of *Tridax procumbens* (Ikewuchi et al., 2015). Amongst the alkaloids detected, triacetonamine (2,2,6,6-tetramethyl-4-keto piperidine), the most abundant, is known to be an anticonvulsive and antihypertensive compound (Navajas et al., 1994). Quinine, another alkaloids detected in the leaves and stems, has been reported to have analgesic, antiarrhythmic, anti-inflammatory, antimalarial, antioxidant, antipyretic and bacteriostatic activities (Krishnaveni et al., 2015).

As shown in Table 6a, the total glycosides' contents of the leaves and stems of *Pandiaka heudelotii* were 1196.0 mg/kg dw and 860.6 mg/kg dw, respectively. Thirteen known glycosides were detected including nicotiflorin (53.45% in leaves; 55.35% in stems), clitorin (21.06% in leaves; 19.49% in stems), mauritianin (15.88% in leaves; 16.82% in stems) and amygdalin (9.60% in leaves; 8.34% in stems). The remaining $<0.01\%$ consisted of prunasin, indican, ouabain, linamarin, digoxin, lotaustralin, digitoxin, dhurrin and digitalis.

The most abundant glycosides detected were nicotiflorin, clitorin, mauritianin and amygdalin. The pharmacological properties of nicotiflorin as reported in biochemical literature include analgesic, anthelminthic, anti-anaphylactic, antibacterial, antifungal, antihypertensive and neuroprotective activities (Li et al., 2006; Kumar, 2016). Clitorin has been reported to have antibacterial and antifungal activities (Kumar, 2016); while anti-carcinogenic, antifungal and gastroprotective activities have been reported for mauritianin (Leite et al., 2010; Kumar, 2016). Studies have reported that amygdalin has analgesic, anti-asthmatic, anti-atherogenic, anti-fibrotic, anti-hyperglycaemic, anti-inflammatory, antitumor, antitussive, antiulcer, bronchioprotective, gastroprotective and immune regulatory effects (Song and Xu, 2014).

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The leaves and stems had high contents of total carotenoids' (708.3 mg/kg dw in leaves; 519.4 mg/kg dw in stems) (Table 6a). Ten known carotenoids were detected, including carotene (49.95% in leaves; 51.94% in stems), lutein (19.28% in leaves; 20.40% in stems), neoxanthin (11.98% in leaves; 12.25% in stems), viola-xanthin (5.96% in leaves; 5.93% in stems), xanthophyll (7.02% in leaves; 4.81% in stems), anthera-xanthin (4.39% in leaves; 3.68% in stems), asta-xanthin (1.39% in leaves; 0.97% in stems). The remainder (<0.1%) consisted of beta-cryptoxanthin, malvidin and lycopene.

The leaves and stems of *P. heudelotii* had higher carotene and lutein than cabbage (U.S. Department of Agriculture, 2016a), green lettuce (U.S. Department of Agriculture, 2016b) and red lettuce (U.S. Department of Agriculture, 2016c) and leaves of *T. procumbens* (Ikewuchi et al., 2015). They had higher neoxanthin, viola-xanthin and anthera-xanthin than the leaves of *T. procumbens* (Ikewuchi et al., 2015). They also had higher lycopene than cabbage (U.S. Department of Agriculture, 2016a), green lettuce (U.S. Department of Agriculture, 2016b) and red lettuce (U.S. Department of Agriculture, 2016c). Carotenes and lutein has anti-cancer activities (Dillard and German, 2000); while carotenes, in addition, have anti-oxidant and pro-vitamin A activities (Dillard and German, 2000).

The leaves and stems had high contents of total saponins' (579.5 mg/kg dw in leaves; 306.7 mg/kg dw in stems) (Table 6b). Seven known saponins were detected, including liquiritin (57.54% in leaves; 62.34% in stems), liquritigenin (37.21% in leaves; 33.72% in stems), isoliquiritigenin (5.25% in leaves; 3.94% in stems); with avenacin-A2, avenacin-B2, avenacin-A1 and avenacin-B1 making up less than 0.01%. Studies have shown that liquiritin, isoliquiritin and isoliquirigenin have anti-cancer effects, individually and in a combination of the three of them (Zhou and Ho, 2014). Isoliquiritin has anti-allergic, anti-depressive, antifungal and anti-inflammatory effects (Hong et al., 2017). Liquiritin has anti-cerebral ischemic, anti-depressive, anti-endothelial dysfunction, anti-melasma, anti-myocardial fibrotic, anti-Parkinsonian, bronchial-protective, cognition enhancing and neuroprotective effects (Hong et al., 2017).

The leaves and stems had moderate total phytosterols' (105.6 mg/kg dw in leaves and 88.1 mg/kg dw in stems) and low total allicins' (32.8 μg/kg dw in leaves and 27.8 μg/kg dw in stems) contents (Table 6b). Seven known phytosterols were detected consisting of sitosterol (85.03% in leaves and 82.84% in stems), stigmasterol (7.68% in leaves and 8.73% in stems), campesterol (6.19% in leaves and 7.17% in stems), 5-avenasterol (1.09% in leaves and 1.25% in stems); while the remainder (<0.01%) consisted of cholestanol, cholesterol and ergosterol. Three known allicins were detected, namely diallyl thiosulphinate (71.10% in leaves and 83.85% in stems), methyl allyl thiosulphinate (28.80% in leaves and 16.10% in stems) and allyl methyl thiosulphinate (0.11% in leaves and 0.04% in stems).

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Table 6b. Continuation of the composition of phytochemicals isolated and detected in the leaves and stems of *Pandiaka heudelotii*.

Compounds	Leaves			Stems		
	RT (min)	Composition (mg/kg)		RT (min)	Composition (mg/kg)	
		/fresh weight	/dry weight		/fresh weight	/dry weight
Saponins						
Isoliquiritigenin	18.490	26.871800	30.432390	18.492	11.080200	12.069935
Liquritigenin	19.511	190.403400	215.632390	19.514	94.921400	103.400218
Liquiritin	20.618	294.456500	333.472820	20.783	175.501700	191.178322
Avenacin-A1	21.813	0.000113	0.000128	21.815	0.000036	0.000039
Avenacin-B1	23.185	0.000034	0.000038	23.110	0.000019	0.000021
Avenacin-A2	24.776	0.006076	0.006881	24.786	0.001655	0.001803
Avenacin-B2	26.276	0.003281	0.003716	26.282	0.000581	0.000633
Total saponins content	-	511.741200	579.548358	-	281.505500	306.650872
Phytosterols						
Cholesterol	19.385	0.003029	0.003430	19.384	0.0030246	0.003295
Cholestanol	20.389	0.004897	0.005546	20.390	0.0047765	0.005203
Ergosterol	21.425	0.002082	0.002357	21.429	0.0020757	0.002261
Campesterol	22.303	5.771810	6.536591	22.303	5.8022300	6.320512
Stigmasterol	23.068	7.159400	8.108041	23.061	7.0608400	7.691547
5-Avenasterol	24.011	1.012880	1.147089	24.009	1.0126400	1.103094
Sitosterol	25.250	79.250400	89.751302	25.250	67.011000	72.996732
Total phytosterol content	-	93.204500	105.554360	-	80.896600	88.122658
Allicins						
Diallyl thiosulphinate	16.151	0.020606	0.023338	16.110	0.021378	0.023287
Methyl allyl thiosulphinate	17.086	0.008346	0.009452	16.980	0.004105	0.004471
Allyl methyl thiosulphinate	18.011	0.000031	0.000036	18.013	0.000011	0.000012
Total allicins content	-	0.028984	0.032824	-	0.025494	0.027771

RT = Retention time.

The leaves and stems of *P. heudelotii* had lower total phytosterol contents than cabbage (Piironen et al., 2003; U.S. Department of Agriculture, 2016a) and lettuce (Piironen et al., 2003). They had lower sitosterol than cabbage, but higher contents than lettuce (Piironen et al., 2003) and the leaves of *T. procumbens* (Ikewuchi et al., 2015). They also had lower stigmasterol than cabbage, lettuce (Piironen et al., 2003) and the leaves of *T. procumbens* (Ikewuchi et al., 2015). They had lower campesterol than cabbage and lettuce (Piironen et al., 2003). Their avenasterol contents were lower than that of lettuce (Piironen et al., 2003).

Studies have shown that beta-sitosterol possesses analgesic, angiogenic, anthelmintic, anti-arthritic, anti-atherosclerotic, anti-diabetic, anticancer, anti-hyperlipidaemic, anti-inflammatory, antimicrobial, anti-nociceptive, antioxidant,

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antipyretic, immunomodulatory and neuroprotective activities (Dillard and German, 2000; Saeidnia et al., 2014). Stigmasterol has been reported to have pharmacological properties such as analgesic, anticonvulsant, anti-hypercholestrolaemic, anti-inflammatory, anti-mutagenic, anti-osteoarthritic, antioxidant, antitumor, hypoglycaemic and memory enhancing effects (Kaur et al., 2011; Saeidnia et al., 2014).

4. Conclusion

The above results show that the leaves and stems of *Pandiaka heudelotii* are good sources of macro- and micronutrients, and as such could contribute significantly to the human nutritional requirements and diet. It also shows that the leaves and stems of *Pandiaka heudelotii* contain a wide range of bioactive phytochemicals. The beneficial roles of these phytoconstituents can be harnessed in the human diet, making them veritable tools for nutritional therapy. This, therefore, highlights the potential of these leaves and stems as functional foods.

Declarations

Author contribution Statement

Jude C. Ikewuchi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Catherine C. Ikewuchi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Mercy O. Ifeanacho: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

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Competing interest Statement

The authors declare no conflict of interest.

Additional information

The chemical compounds studied in this article can be found in PubChem: Valine (PubChem CID: 6287); threonine (PubChem CID: 6288); isoleucine (PubChem

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CID: 38 6306); histidine (PubChem CID: 6274); alpha-tocopherol (PubChem CID: 14985); riboflavin 39 (PubChem CID: 493570); nicotinic acid (PubChem CID: 938); ascorbic acid (PubChem CID: 40 54670067); triacetonamine (PubChem CID: 13220); acalyphin (PubChem CID: 49787014); 41 quinine (PubChem CID: 3034034); nicotiflorin (PubChem CID: 5318767); clitorin 42 (PubChem CID: 11592917); mauritianin (PubChem CID: 44258751); amygdalin (PubChem 43 CID: 656516); carotene (PubChem CID: 5280489); lutein (PubChem CID: 126970006); 44 neoxanthin (PubChem CID: 5282217); viola-xanthin (PubChem CID: 448438); xanthophyll 3 45 (PubChem CID: 24728610); liquiritin (PubChem CID: 503737); liquritigenin (PubChem 46 CID: 114829); anthera-xanthin (PubChem CID: 5281223); isoliquiritigenin (PubChem CID: 47 638278); sitosterol (PubChem CID: 222284); stigmasterol (PubChem CID: 5280794); 48 campesterol (PubChem CID: 173183).

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