Investigation of the profile of phenolic compounds in the leaves and stems of *Pandiaka heudelotii* using gas chromatography coupled with flame ionization detector

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Abstract

The profile of phenolic compounds in the leaves and stems of *Pandiaka heudelotii* was investigated using gas chromatography coupled with flame ionization detector. The leaves and stems had high flavonoids and benzoic acid derivatives content, and moderate levels of lignans and hydroxycinnamates. Twenty-eight known flavonoids were detected, which consisted mainly of kaempferol (41.93% in leaves and 47.97% in stems), (+)-catechin (17.12% in leaves and 16.11% in stems), quercetin (13.83% in leaves and 9.39% in stems), luteolin (7.34% in leaves and 7.71% in stems), and artemisin (6.53% in leaves and 4.83% in stems). Of the six known hydroxycinnamates detected, chlorogenic acid (80.79% in leaves and 87.56% in stems) and caffeic acid (18.98% in leaves and 12.30% in stems) were the most abundant, while arctigenin (77.81% in leaves and 83.40% in stems) and retusin (13.82% in leaves and 10.59% in stems) were the most abundant of the nine known lignans detected. Twelve known benzoic acid derivatives were detected, consisting mainly of ellagic acid (65.44% in leaves and 72.89% in stems), p-hydroxybenzoic acid (25.10% in leaves and 18.95% in stems), and vanillic acid (8.80% in leaves and 7.30% in stems). The rich phytochemical profile of the leaves and stems is an indication of their ability to serve as sources of nutraceuticals.

KEYWORDS

flavonoids, hydroxycinnamates, lignans, *Pandiaka heudelotii*, phenolic acids

1 | INTRODUCTION

The wide use of phytonutrients reflects a fact that nutrition science has advanced beyond the treatment of deficiency syndromes to the reduction of disease risk. Food is no longer evaluated only in terms of macronutrient and micronutrient levels, but their contents of some biologically active compounds are becoming more important (Zhao, 2007). In addition to providing macro- and micro-nutrients, vegetables are rich sources of bioactive phytochemicals, and other compounds that support human health and nutrition (Radovich, 2011; Sinha, Hui, Evranuz, Siddiq, & Ahmed, 2011). *Pandiaka heudelotii* (family: Amaranthaceae) is a wild vegetable commonly consumed in southern Nigeria. It is used for the preparation of soup, and boiled for tea. Despite the use of this plant as both food and medicine, we found no information in the biochemical literature regarding its phytochemical and phenolic compounds composition. Therefore, this study investigated the phenolic compounds composition of the leaves and stems of *Pandiaka heudelotii* with a view to providing information on their potential as sources of nutraceuticals.
2 | MATERIALS AND METHODS

2.1 | Collection of samples

Fresh samples of *Pandiola heudeletii* were collected from within the Abuja Campus of University of Port Harcourt, Port Harcourt, Nigeria. They were duly identified at the Herbarium of the Department of Plant Science and Biotechnology, University of Port Harcourt. The leaves were collected and rid of dirt, and used for the analysis.

2.2 | General procedures

Gas chromatography was carried out at Multi Environmental Management Consultants Limited, Igbiri Road, Ikorodu, Lagos, with a Hewlett Packard HP 6890, gas chromatograph, fitted with a flame ionization detector (FID), 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 flavonoids and benzoic acid derivatives, acetone for lignans, and ethanol for hydroxycinnamates. 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 establishing calibration curves for each compound determined, using the standards.

2.3 | Determination of benzoic acid derivatives composition

Benzoic acid derivatives were extracted by the two-stage process described by Andary et al. (2013). The concentrated extract (2.0 ml) was transferred to a 5.0 ml glass vial. It was then saturated with sodium chloride salt before adding 250.0 μl of ethyl acetate to it. The mixture was agitated manually for 10 min at room temperature and later centrifuged for 15 min at 2500 rpm, before removing the organic phase to a 1-ml vial. The extraction was repeated twice and the organic phases were combined. Aliquot of 50.0 μl of N,O-bis (trimethylsilyl) trifluoroacetamide was added, and the mixture was manually agitated for 2 min at room temperature for derivatization. The column type was a capillary HP-1 with the dimension (30 m × 0.25 mm × 0.25 μm film thickness). The inlet and detector temperatures were 250°C and 320°C, respectively. Split injection was adopted with a split ratio of 20:1. Nitrogen was used as the carrier gas. The hydrogen and compressed air pressures were 193.1 kPa and 220.6 kPa. The oven was programmed as follows: initial temperature at 60°C for 5 min, ramping at 15°C/min for 15 min, and attained temperature maintained for 1 min, followed by a second ramping at 10°C/min for 4 min.

2.4 | Determination of the flavonoids composition

The flavonoids extraction was carried out according to the method of Millogo-Kone et al. (2009). One gram of the dried ethanol and aqueous extracts were weighed into 100 ml of distilled water in a 250-ml conical flask and boiled for 10 min. To this was added 100 ml of boiling methanol/water (70:30, v/v) mixture. The mixture was allowed to macerate for about 2 hr, and then filtered with Whatman filter paper No.1. The filtrate was concentrated to 5 ml for gas chromatographic analysis. The column was a capillary HP INNOWax (30 m × 0.25 mm × 0.25 μm film thickness). The inlet and detection temperatures were 250 and 320°C. Split injection was adopted with a split ratio of 20:1. Nitrogen was used as the carrier gas. The hydrogen and compressed air pressures were 151.7 kPa and 241.3 kPa. The oven was programmed as follows: initial temperature of 50°C, first ramping at 8°C/min for 20 min, attained temperature maintained for 4 min, followed by a second ramping at 12°C/min for 4 min, and maintaining attained temperature for 4 min.

2.5 | Determination of hydroxycinnamates composition

The hydroxycinnamates extract was prepared as described by Ortan, Popescu, Gaita, Dinu-Pîrvu, and Câmpeanu (2009), and subjected to gas chromatographic analysis. The column was HP-5 (30 m × 0.32 mm × 0.25 μm film thickness). The samples were introduced via an all-glass injector working in the split mode, with nitrogen as the carrier gas, at a flow rate of 1 ml/min. The injection and detector temperatures were 260°C and 300°C, respectively. The oven temperature was programmed at the start of the run from 170°C to 250°C at 5°C/min.

2.6 | Determination of the lignans composition

The lignan extract was prepared as reported by Chapman, Knoy, Kingscher, Brown, and Niemann (2006), and subjected to gas chromatography. The column was ZP-5 (30 m × 0.32 mm × 0.25 μm film thickness), detected at 300 nm. One microliter of sample was injected. The conditions for the GC were initial oven temperature of 40°C, injector 250°C, transfer line 280°C, a solvent delay of 2 min; and ramped temperature at 10°C/min to a final temperature of 230°C, which was held for 1 min.

2.7 | Determination of tannins composition

The tannin extract was prepared as reported by Luther (1992), and subjected to gas chromatographic analysis. The column was a capillary HP-5 (30 m × 0.25 mm × 0.25 μm film thickness). The inlet and detection temperatures were 250 and 320°C. Split injection was adopted with a split ratio of 20:1. Nitrogen was used as the carrier gas. The hydrogen and compressed air pressures were 173.1 kPa and 275.8 kPa. The oven was programmed to an initial temperature at 120°C, before ramping at 10°C/min for 20 min.

3 | RESULTS

The leaves (5885.5 mg/kg dry weight) and stems (3644.0 mg/kg dry weight) had high total flavonoid contents (Table 1). Twenty-eight
known flavonoids were detected, which consisted of kaempferol (41.9% in leaves and 48.0% in stems), (+)-catechin (17.1% in leaves and 16.1% in stems), quercetin (13.8% in leaves and 9.4% in stems), luteolin (7.3% in leaves and 7.7% in stems), artemetin (6.5% in leaves and 4.8% in stems), naringin (6.1% in leaves and 4.7% in stems), apigenin (4.0% in leaves and 4.7% in stems), hesperidin (3.1% in leaves and 2.4% in stems) all comprising above 99%. The remainder (less than 1%) consisted ofisorhamnetin, naringenin, myricetin, (−)-epicatechin, daidzein, genistein, biochanin, silymarin, daidzin, gallocatechin, butein, robinetin, tangeretin, baicalein, resveratrol, baicalin, nobiletin, (−)-epicatechin-3-gallate, (−)-epigallocatechin, and (−)-epigallocatechin-3-gallate.

The total benzoic acid derivatives’ contents of the leaves and stems of Pandiaka heudelotii were 3384.1 mg/kg dw and 1975.4 mg/kg dw, respectively (Table 2). Twelve known compounds were detected including ellagic acid (65.4% in leaves and 72.9% in stems), p-hydroxybenzoic acid (25.1% in leaves and 19.0% in stems), vanillic acid (8.8% in leaves and 7.3% in stems), and rosmarinic acid (0.6% in leaves and 0.8% in stems); comprising above 99%. The remainder consisted of syringic acid, ferulic acid, sinapinic acid, o-coumaric acid, piperic acid, gentisic acid, protocatechuic acid, and cinnamic acid.

They had moderate total (448.4 mg/kg dw in leaves and 334.3 mg/kg dw in stems) hydroxycinnamates’ contents (Table 3). Six known hydroxycinnamates were detected, including chlorogenic acid (80.87% in leaves and 87.6% in stems), caffeic acid (19.0% in leaves and 12.3% in stems), and chiorotic acid (0.1% in leaves and 0.1% in stems). The remaining less than 0.2% consisted of p-coumaric acid, p-coumarin, and scopoletin. Tannic acid was the only compound detected in the tannins fraction.

The total lignans contents of the leaves and stems were 496.6 mg/kg dw and 914.6 mg/kg dw, respectively (Table 4). Nine known lignans

### Table 1: Isolated and detected flavonoids in the leaves and stems of Pandiaka heudelotii

| Compounds       | Composition (mg/kg) | Leaves | Stems |
|-----------------|--------------------|--------|-------|
|                 | Retention time (min) | Fresh weight | Dry weight | Retention time (min) | Fresh weight | Dry weight |
| (+)-Catechin    | 13.696             | 890    | 1000  | 13.698             | 540    | 590        |
| Apigenin        | 14.509             | 210    | 240   | 14.509             | 160    | 170        |
| Resveratrol     | 15.105             | 0.00027| 0.00031| 15.103             | 0.00015| 0.00016    |
| Genistein       | 15.537             | 0.00071| 0.00080| 15.536             | 0.00037| 0.00040    |
| Daidzein        | 15.805             | 0.00072| 0.00082| 15.805             | 0.00036| 0.00039    |
| Daidzin         | 16.254             | 0.00052| 0.00059| 16.254             | 0.00027| 0.00030    |
| Butein          | 16.539             | 0.00038| 0.00043| 16.542             | 0.00020| 0.00022    |
| Naringenin      | 16.926             | 0.014  | 0.016 | 16.925             | 0.0072 | 0.0078     |
| Biochanin       | 17.205             | 0.00067| 0.00076| 17.206             | 0.00035| 0.00039    |
| Luteolin        | 17.451             | 380    | 430   | 17.451             | 260    | 280        |
| Kaempferol      | 17.711             | 2200   | 2500  | 17.706             | 1600   | 1700       |
| (−)-Epicatechin | 18.784             | 0.0014 | 0.0016 | 18.782             | 69     | 75         |
| (−)-Epigallocatechin | 20.677 | 0.00062| 0.00071| 20.450             | 0.00017| 0.00019    |
| Quercetin       | 21.094             | 720    | 810   | 21.209             | 310    | 340        |
| Gallocatechin   | 22.577             | 0.00044| 0.00050| 22.578             | 0.00010| 0.00011    |
| (−)-Epicatechin-3-gallate | 22.858 | 0.00015| 0.00017| 22.856             | 0.000073| 0.000079   |
| (−)-Epigallocatechin-3-gallate | 23.575 | 0.00005| 0.00006| 23.573             | 0.000024| 0.000026   |
| Isorhamnetin    | 24.037             | 0.27   | 0.30  | 24.038             | 0.12   | 0.13       |
| Robinetin       | 24.154             | 0.00033| 0.00037| 24.153             | 0.00016| 0.00018    |
| Myricetin       | 24.714             | 0.0014 | 0.0016 | 24.787             | 0.00031| 0.00033    |
| Baicalin        | 25.564             | 0.00032| 0.00036| 25.557             | 0.00016| 0.00018    |
| Nobiletin       | 26.177             | 0.00016| 0.00019| 26.177             | 0.00008| 0.00009    |
| Baicalin        | 26.381             | 0.00027| 0.00030| 26.381             | 0.00014| 0.00015    |
| Tangeretin      | 26.529             | 0.00032| 0.00036| 26.530             | 0.00015| 0.00017    |
| Artemetin       | 26.741             | 340    | 380   | 26.742             | 160    | 180        |
| Silymarin       | 27.085             | 0.00063| 0.00071| 27.084             | 0.00033| 0.00036    |
| Naringin        | 27.490             | 320    | 360   | 27.489             | 160    | 170        |
| Hesperidin      | 28.321             | 160    | 180   | 28.315             | 81     | 89         |
| Total flavonoids content | 5200 | 5900 | 3300 | 3600 |
were detected, consisting of arctigenin (77.8% in leaves and 83.4% in stems), retusin (13.8% in leaves and 10.6% in stems), dehydroabi-
etic acid (8.1% in leaves and 5.8% in stems), and sakuranin (0.2% in leaves and 0.1% in stems). The remaining less than 0.2% consisted of epieudesmin, galgravin, apigenin-4’,7-dimethyl ether, 2-allyl-5-ethox-
y-4-methoxyphenol, and (9E, 12E, 15E)-9,12,15-octadecatrien-1-ol.

4 | DISCUSSION

This study showed that Pandiaka leaves have higher artemetin con-
tents than *Artemisia annua* (Weathers & Towler, 2012). Artemetin has

### TABLE 2 Isolated and detected benzoic acid derivatives in the leaves and stems of *Pandiaka heudelotii*

| Compounds       | Composition (mg/kg) | Leaves Retention time (min) | Fresh weight | Dry weight | Stems Retention time (min) | Fresh weight | Dry weight |
|-----------------|---------------------|-----------------------------|--------------|------------|-----------------------------|--------------|------------|
| Cinnamic acid   | 9.325               | 0.0025                      | 0.0029       |            | 9.008                       | 0.00064      | 0.00069    |
| Gentisic acid   | 10.848              | 0.0044                      | 0.0050       |            | 10.844                      | 0.00072      | 0.00079    |
| Protocatechelic acid | 12.357                | 0.0021                      | 0.0024       |            | 12.367                      | 0.00017      | 0.00018    |
| Vanillic acid   | 14.918              | 260                         | 300          |            | 14.917                      | 130          | 140        |
| o-Coumaric acid | 15.812              | 0.082                       | 0.093        |            | 15.815                      | 0.030        | 0.033      |
| p-Hydroxybenzoic acid | 16.037                | 750                         | 850          |            | 16.038                      | 340          | 370        |
| Ferulic acid    | 18.493              | 0.21                        | 0.24         |            | 18.498                      | 0.14         | 0.15       |
| Syringic acid   | 19.688              | 1.1                         | 1.2          |            | 19.626                      | 0.42         | 0.46       |
| Piperic acid    | 20.467              | 0.0064                      | 0.0073       |            | 20.473                      | 0.0049       | 0.0053     |
| Sinapinic acid  | 21.327              | 0.092                       | 0.10         |            | 21.329                      | 0.090        | 0.098      |
| Ellagic acid    | 22.602              | 1900                        | 2200         |            | 22.606                      | 1300         | 1400       |
| Rosmarinic acid | 23.232              | 18                          | 21           |            | 23.235                      | 15           | 16         |
| Total phenolic acids content |            | 3000                        | 3400         |            |                             |              |            |

### TABLE 3 Isolated and detected hydroxycinnamates and tannins in the leaves and stems of *Pandiaka heudelotii*

| Compounds       | Composition (mg/kg) | Leaves Retention time (min) | Fresh weight | Dry weight | Stems Retention time (min) | Fresh weight | Dry weight |
|-----------------|---------------------|-----------------------------|--------------|------------|-----------------------------|--------------|------------|
| Hydroxycinnamates |                     |                             |              |            |                             |              |            |
| p-Coumarin      | 7.790               | 0.083                       | 0.094        |            | 7.793                       | 0.061        | 0.066      |
| p-Coumaric acid | 11.522              | 0.34                        | 0.38         |            | 11.528                      | 0.15         | 0.17       |
| Caffeic acid    | 14.400              | 75                          | 85           |            | 14.397                      | 38           | 41         |
| Scopoletin      | 16.335              | 0.11                        | 0.12         |            | 16.365                      | 0.030        | 0.033      |
| Chlorogenic acid| 19.030              | 320                         | 360          |            | 19.016                      | 270          | 290        |
| Chicoric acid   | 20.349              | 0.40                        | 0.45         |            | 20.341                      | 0.18         | 0.20       |
| Total hydroxycinnamates content |            | 390                        | 450          |            |                             | 310          | 330        |
| Tannins         |                     |                             |              |            |                             |              |            |
| Tannic acid     | 19.518              | 50                          | 57           |            | 19.518                      | 51           | 56         |
| Total tannins content |            | 50                        | 57           |            |                             | 51           | 56         |
When compared to blueberry, the leaves and stems had higher quercetin (73 mg/kg, US Highbush Blueberry Council, 2005; 17–24 mg/kg, Hakkinen, Karenlampi, Heinonen, Mykkänen, & Torronen, 1999), while the stems had higher epicatechin (11.1 mg/kg, US Highbush Blueberry Council, 2005) contents. Pandiaka leaves and stems also had higher apigenin, catechin, hesperidin, luteolin, kaempferol, and quercetin contents than lettuce, onions, and carrots (Harney et al., 2006). The leaves and stems were also rich in arctigenin, retusin, ellagic, p-hydroxybenzoic, vanillic, chlorogenic, caffeic, tannic, and dehydroabietic acids. This means that they can serve as sources of these bioactive compounds.

Studies have shown that kaempferol, apigenin, quercetin, catechin, and luteolin have antioxidant, anti diabetic, hypolipidemic, hypotensive, antibacterial, anti-inflammatory, and anticancer properties (Dillard & German, 2000; Sutherland, Rahman, & Appleton, 2006; Lahkanpal & Rai, 2007; Calderón-Montaño, Burgos-Morón, Pérez-Guerrero, & López-Lázaro, 2011; Ren et al., 2016). Other properties of kaempferol include analgesic, antiallergic, anti protozoal, antiviral, antifungal, neuroprotective, cardio-protective, and hepatoprotective properties (Calderón-Montaño et al., 2011; Dillard & German, 2000); while those of apigenin are diuretic, hepatoprotective, and cardio-protective properties (Dillard & German, 2000; Panda & Kar, 2007; Ren et al., 2016). In addition to the above, catechin also have antiviral, antiallergic, antiobesity, antiplaquelet, anti ulcer, chemo-preventive, neuroprotective, cardio-protective, antispasmodic, bronchodilator, and vasodilator properties (Dillard & German, 2000; Ghayur, Khan, & Gilani, 2007; Sutherland et al., 2006); while quercet in has antiallergic, antiarthritic, anticataractogenic, antiviral, cardio-protective, gastro-protective, and hepatoprotective activities (Dillard & German, 2000; Lahkanpal & Rai, 2007). Luteolin also has antiallergic, antiandrogenic, antiestrogenic, neuroprotective, and radio-protective activities (Dillard & German, 2000; López-Lázaro, 2009).

Vanillic and 4-hydroxybenzoic acids have antifungal, antimutagenic, anti sickling, estrogenic, and antimicrobial activities (Khadem & Marles, 2010; Oksana, Marian, Mahendra, & Bo, 2012). In addition, vanillic acid is a flavoring, anthelmintic, hepatoprotective, immunomodulating, and anti-inflammatory agent (Khadem & Marles, 2010; Oksana et al., 2012). Ellagic acid is reported to have antioxidant, anti- malarial, anti-inflammatory, antileukocyte, anti diabetic, antiatherogenic, anti wrinkle, antidepressant, neuroprotective, antiapoptotic, anticancer, antiproliferative, and chemo-preventive activities (Dhingra & Chhillar, 2012; Özkaya et al., 2013).

Chlorogenic acid reduces the risk of cardiovascular disease, and exhibits many biological properties such as antibacterial, antiviral, anti-inflammatory, antioxidant, anticancer, antiobesity, hypolipidemic, hepatoprotective, immunostimulatory, hypoglycemic, and anti hypertensive activities (Cho et al., 2010; Farah, 2012; Lafay, Morand, Manach, Besson, & Scalbert, 2006; Li, Habasi, Xie, & Aisa, 2014; Meng, Cao, Feng, & Hu, 2013; Zhao, Wang, Ballebre, Luo, & Zhang, 2011). Caffeic acid increases collagen production, in addition to having antiaging, antioxidant, antimicrobial, antiatherosclerotic, anti diabetic, antitumor, anti inflammatory, and photo-protective properties (Dhingra & Chhillar, 2012; Gugliucci, Bastos, Schulze, & Souza, 2009; Magnani, Isaac, Correa, & Salgado, 2014; Oksana et al., 2012). Studies have shown that arctigenin has antioxidant, antitumor, anti-inflammatory, antiviral, analgesic, neuroprotective, and memory-enhancing activities (Chakraborty & Borah, 2013; Du et al., 2016; Lu et al., 2015; Park, Hong, Moon, Kim, & Kim, 2011; Srivastava & Shukla, 2015; Zhu et al., 2013). Retusin is an antiemetic, antitumor, and psychoactive agent; and teas containing it are used as anti-inflammatory agents, analgesics, purgative, laxative, and cathartic (Chakrapani et al., 2013; Chapman et al., 2006).

From the foregoing, it can be seen that the leaves and stems of Pandiaka heudelotii contain a variety of biologically active phytochemicals. The beneficial roles of these bioactive phytochemical constituents can be harnessed in the diet, making them important tools for nutritional therapy. This, therefore, emphasizes the potential of the leaves as a candidate for use as functional food.

### TABLE 4  Isolated and detected lignans in the leaves and stems of Pandiaka heudelotii

| Compounds | Composition (mg/kg) | Leaves | | | Stems | | |
|---|---|---|---|---|---|---|---|
| | Retention time (min) | Fresh weight | Dry weight | Retention time (min) | Fresh weight | Dry weight |
| 2- Allyl-5-ethoxy-4-methoxyphenol | 11.449 | 0.0034 | 0.0039 | 11.298 | 0.0066 | 0.00720 |
| (9E, 12E, 15E)-9,12,15-Octadecatrien-1-ol | 14.143 | 0.0021 | 0.00024 | 14.130 | 0.00010 | 0.00011 |
| Apigenin-4′,7-dimethyl ether | 16.372 | 0.048 | 0.054 | 16.429 | 0.049 | 0.053 |
| Dehydroabietic acid | 18.133 | 35 | 40 | 18.551 | 49 | 53 |
| Retusin | 19.675 | 0.09 | 0.098 | 20.008 | 89 | 97 |
| Galgravin | 20.463 | 0.13 | 0.15 | 20.320 | 0.090 | 0.098 |
| Arctigenin | 21.131 | 340 | 39 | 21.393 | 700 | 760 |
| Epieudesmin | 22.234 | 0.24 | 0.27 | 22.251 | 32 | 0.35 |
| Sakuranin | 23.968 | 0.78 | 0.89 | 23.963 | 1.2 | 1.3 |
| Total lignans content | 440 | 50 | | 840 | 910 |
CONFLICT OF INTEREST

None declared.

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