Quantification of polyphenols present in some herbs used for neurological disorder management in Nigeria by high-performance liquid chromatography (HPLC-DAD)

Objective: This work aims at providing information on the polyphenols present in five different herbs traditionally used in Nigeria for the management of neurological disorders. The Yoruba folkloric system of medicine in Nigeria gives a whole lot of effective treatments for disorders associated with the central nervous system, such as memory loss, anxiety, aging, etc. Aridan (*Tetrapleura tetraptera* Schum. & Thonn. Taub), Oriji (*Quassia undulata* Guill. & Perr. D. Dietr), Abeere (*Picralima nitida* Stapf Th. & H.Dur), Amunimuye (*Senecio abyssimicus* Sch. Bip), and Alupayida (*Uraria picta* Jacq. DC) are herbs used traditionally in the treatment of mentally derailed individuals in Nigeria.

Methods: Fresh samples of the herbs were obtained from the botanical garden, Adekunle Ajasin University, Akungba – Akoko. The samples were air dried, grounded into fine powder and the aqueous extract prepared (1:20 w/v) was used for the HPLC analysis.

Results: The HPLC characterization of the herbs revealed the presences of essential polyphenols such as, caffeic acid, chlorogenic acid, gallic acid, rutin, *p*-coumeric acid, quercetin, ellagic acid, catechin, apigenin and luteolin.

Conclusion: The Presence of these essential polyphenols in the herbs might be a justification for their used in folklore in the management/treatment of some neurological disorders.

Keywords: HPLC analysis; Polyphenol; Herbs; Neurological disorder

Abstract

**Objective:** This work aims at providing information on the polyphenols present in five different herbs traditionally used in Nigeria for the management of neurological disorders. The Yoruba folkloric system of medicine in Nigeria gives a whole lot of effective treatments for disorders associated with the central nervous system, such as memory loss, anxiety, aging, etc. Aridan (*Tetrapleura tetraptera* Schum. & Thonn. Taub), Oriji (*Quassia undulata* Guill. & Perr. D. Dietr), Abeere (*Picralima nitida* Stapf Th. & H.Dur), Amunimuye (*Senecio abyssimicus* Sch. Bip), and Alupayida (*Uraria picta* Jacq. DC) are herbs used traditionally in the treatment of mentally derailed individuals in Nigeria.

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Cite this article: Odubanjo VO. Quantification of polyphenols present in some herbs used for neurological disorder management in Nigeria by high-performance liquid chromatography (HPLC-DAD). J Plant Biol Crop Res. 2019; 2(1): 1013.
used as a spice, and cooked as soup. *T. tetraperta* is commonly used due to its high medicinal and aromatic values. Its used for several purposes ranging from culinary, healing, and therapeutic to cosmetology. Researchers also reveal that this plant has anti-cholesterolesterase activity [3], anti-inflammatory, hypotensive, neuromuscular, cardiovascular, anti-ulcerative, molluscicidal and anti-microbial properties [4].

*Quassia undulata* (Guill. & Perr.) D. Dietr (QU) is a plant of the Simaroubaceae family. It is a perennial shrub distributed mostly on open grassland and woodland in tropical and sub-tropical Africa, Asia, Australia and America. It is locally called oriji (Yoruba). The leaves can be consumed as soup. The leaf is used as memory enhancer and has anti-aging properties [2,3]. It also has antibacterial and antifungi properties [5].

*Picralima nitida* Stapf Th. & H.Dur (PN seed) belongs to the Apocynaceae family, mostly found in the West tropical Africa. It is called abere in the South Western Nigeria. The seeds are extensively used in place of quinine for treatment of fevers in Nigeria and Ghana [6]. The powdered seeds are also used in Nigeria for pneumonia and other chest congestion [6]. Studies have also proved the seed to be a good memory enhancer consumed orally by soaking in water [2].

*Senecio abyssinicus* Sch. Bip (SA) is an annual herb belonging to the Compositae family which is about 50 cm in height, found in open places, lowlands and mountains in North and South Nigeria. It. The plant is locally called amunimuye (Yoruba). Nigerians considered the plant to be a stomachic and blood-purifier. The bruised leaves are applied topically to painful areas of rheumatism, and with bruises and cuts [6].

*Uraria picta* (Jacq.) DC (UP) is a Papilionaceae (http://www.efloraofgandhinagar.in/plant-families/papilionaceae) family herb widely found in the Tropics through tropical Africa, India and China. It is locally called alupayida (Yoruba). The powdered leaves are used against gonorrhea and for memory enhancement in Nigeria [2]. Odubanjo et al. [7] have also established that the aqueous extract of *Uraria picta* exhibits antioxidant and anticholinesterase ability. The most commonly used methods to analyze flavonoids is the High performance liquid chromatography with diode array (HPLC-DAD) [8]. All these herbs are used in treating mental health problems in folklore in Nigeria. What man No. 1 filter paper and the filtrate centrifuged at 805 × g for 10 minutes. The clear supernatant was obtained was freeze dried in a lyophilizer. The dried powder was stored in a small capped plastic container (labeled) at 4°C until required. This was later reconstituted in water for the quantification analysis.

**Quantification of compounds present in the samples by HPLC-DAD**

For analysis of the samples extract, 10 mg/mL of each extract was injected into a Phenomenex C_{18} column (4.6 mm x 250 mm) packed with 5μm diameter particles; the mobile phases were: water containing 1% formic acid, water: Acetic acid (98:2, v/v), 0.5% (v/v) aqueous formic acid, water containing 1% phosphoric acid (A) and acetonitrile, methanol, 1% (v/v) acetic acid in acetonitrile (B), and the gradient was as follows: 13% B up to 10 min and then changed to obtain 20, 30, 50, 60, 70, 80 and 10% B in 20, 30, 40, 50, 60, 70 and 80 min, respectively, according to Brito et al [9], with slight modifications. The flow rate was 0.6 ml/min and the injection volume was 40 μL. Each sample and mobile phase were filtered through 0.45 μm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. The wavelengths used ranged from 250 nm to 366 nm. Stock solutions of standard references were prepared in the HPLC mobile phase at a concentration range of 0.025 - 0.300 mg/mL. Chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 600 nm). All chromatography operations were carried out at ambient temperature and in three replicates.

**Limit of detection (LOD) and limit of quantification (LOQ)**

Limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves, as defined by Boligon et al. [10] LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve.

**Statistical analysis**

Differences between groups of HPLC were assessed by One-way analysis of variance model and Tukey’s test. The level of significance for the analyses was set to p < 0.05. These analyses were performed using the free software R version 3.1.1. [11].

**Results & discussion**

The studied plants are good source of phenolic compounds. Plant materials have been employed in the treatment/manage-
Flavonoids are low molecular weight secondary metabolic compounds, generally found in the cell vacuoles of green plants [15]. Studies have shown that flavonoids have anti-inflammatory, antiviral, antibacterial, vasodilatory, anticancer, anti-ischemic and neuro protective properties [16]. Historically, the biological actions of flavonoids on the brain were attributed to their ability to exert antioxidant actions [17], through their ability to scavenge reactive species or through their possible influences on intracellular redox status [18].

HPLC fingerprinting composition of the *Tetrapleura tetraptera* extract revealed the presence of gallic acid (retention time \( t_1 = 11.25 \text{ min} \); peak 1), chlorogenic acid \(( t_2 = 19.83 \text{ min} \); peak 2), caffeic acid \(( t_3 = 26.47 \text{ min} \); peak 3), \( p \)-coumaric acid \(( t_4 = 33.08 \text{ min} \); peak 4), rutin \(( t_5 = 43.56 \text{ min} \); peak 5) and quercetin \(( t_6 = 54.91 \text{ min} \); peak 6) (Figure 1 and Table 1). *Quassia undulata* extract has gallic acid (retention time \( t_1 = 11.79 \text{ min} \); peak a), catechin \(( t_2 = 15.86 \text{ min} \); peak b), ellagic acid \(( t_3 = 30.17 \text{ min} \); peak c), \( p \)-coumaric acid \(( t_4 = 35.94 \text{ min} \); peak d) and rutin \(( t_5 = 42.39 \text{ min} \); peak e) (Figure 2 and Table 1). HPLC analysis of *Picralima nitida* extract is shown in Figure 3 and table 1. The sample contains other minor compounds in addition to gallic acid (retention time \( t_1 = 11.95 \text{ min} \); peak 1), chlorogenic acid \(( t_2 = 21.63 \text{ min} \); peak 2), caffeic acid \(( t_3 = 25.41 \text{ min} \); peak 3), ellagic acid \(( t_4 = 33.17 \text{ min} \); peak 4), quercetin \(( t_5 = 49.98 \text{ min} \); peak 5) and apigenin \(( t_6 = 64.87 \text{ min} \); peak 6). *Senecio abyssimicus* extract (Figure 4) contains other minor compounds in addition to Gallic acid (retention time \( t_1 = 10.85 \text{ min} \); peak a), chlorogenic acid \(( t_2 = 19.76 \text{ min} \); peak b), caffeic acid \(( t_3 = 24.39 \text{ min} \); peak c), ellagic acid \(( t_4 = 28.75 \text{ min} \); peak d) and quercetin \(( t_5 = 39.85 \text{ min} \); peak e). While, *Uraria picta* extract revealed the presence of gallic acid (retention time \( t_1 = 11.39 \text{ min} \); peak 1), chlorogenic acid \(( t_2 = 20.45 \text{ min} \); peak 2), caffeic acid \(( t_3 = 25.06 \text{ min} \); peak 3), rutin \(( t_4 = 44.13 \text{ min} \); peak 4), quercetin \(( t_5 = 50.09 \text{ min} \); peak 5) and luteolin \(( t_6 = 55.87 \text{ min} \); peak 6) (Figure 5 and Table 1).
TT contains the highest amount of gallic acid and P-Coumaric acid. Catechin was found only in QU. Also with QU having the highest amount of rutin and ellagic acid. PN has the highest amount of caffeic acid and quercetin with a considerable amount of apigenin. While, SA and UP have the least amount of chlorogenic acid and quercetin. Reports have shown that caffeic acid and quercetin are capable of affecting several aspects of memory and learning, notably rapid and slow memory acquisition [19, 20], short-term working memory [21], long-term reference memory [22], reversal learning and memory retention/retrieval [20]. Studies have also shown that ellagic acid has neuroprotective ability, such as diminishing the diabetic neuropathy, protecting cerebral ischemic damage, improving cognitive impairment caused by 6-hydroxy dopamine and preventing cognitive and hippocampal long-term potentiation deficits induced by traumatic brain injury [23, 24].

Two important performance characteristics in method validation are, the limit of detection (LOD) and limit of quantification (LOQ). LOD and LOQ are terms used to describe the smallest concentration of an analyte that can be reliably measured by an analytical procedure [25]. The result of the limit of detection (LOD) and limit of quantification (LOQ) of the samples is presented in table 2. The LOD was in the range of 0.009-0.028 μg/mL for the polyphenols present in the samples.

Table 1: High-Performance Liquid Chromatography (HPLC-DAD) Composition of Aqueous Extract of *Tetrapleura tetraptera*, *Quassia undulata*, *Picralima nitida*, *Senecio abyssimicus* and *Uraria picta*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>T. tetraptera</th>
<th>Q. undulata</th>
<th>P. nitida</th>
<th>S. abyssimicus</th>
<th>U. picta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>1.85 ± 0.01^c</td>
<td>0.76 ± 0.01^b</td>
<td>0.59 ± 0.03^a</td>
<td>1.62 ± 0.02^c</td>
<td>0.41 ± 0.01^a</td>
</tr>
<tr>
<td>Catechin</td>
<td>-</td>
<td>3.05 ± 0.03</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>0.73 ± 0.01^c</td>
<td>-</td>
<td>1.27 ± 0.016^d</td>
<td>0.59 ± 0.01^a</td>
<td>0.92 ± 0.01^c</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>1.97 ± 0.04^c</td>
<td>-</td>
<td>5.83 ± 0.04^c</td>
<td>2.17 ± 0.01^b</td>
<td>1.83 ± 0.04^c</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>-</td>
<td>2.49 ± 0.02^c</td>
<td>0.61 ± 0.029^a</td>
<td>2.13 ± 0.03^b</td>
<td>-</td>
</tr>
<tr>
<td>P-Coumaric acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rutin</td>
<td>1.95 ± 0.03^a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Quercetin</td>
<td>2.24 ± 0.02^a</td>
<td>-</td>
<td>2.94 ± 0.01^c</td>
<td>1.65 ± 0.04^a</td>
<td>1.65 ± 0.03^c</td>
</tr>
<tr>
<td>Apigenin</td>
<td>-</td>
<td>-</td>
<td>0.45 ± 0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Luteolin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.67 ± 0.02</td>
<td>-</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard deviations (SD) of three replications. Averages followed by different letters on a row differ by Tukey test at p < 0.05.

Table 2: Limit of Detection (LOD) and Limit of Quantification (LOQ) Values of *Tetrapleura tetraptera*, *Quassia undulata*, *Picralima nitida*, *Senecio abyssimicus* and *Uraria picta* (μg/mL) By High-Performance Liquid Chromatography (HPLC-DAD)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>T. Tetraptera</th>
<th>Q. Undulata</th>
<th>P. nitida</th>
<th>S. abyssimicus</th>
<th>U. picta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>0.015</td>
<td>0.040</td>
<td>0.017</td>
<td>0.056</td>
<td>0.015</td>
</tr>
<tr>
<td>Catechin</td>
<td>-</td>
<td>0.028</td>
<td>0.091</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>0.027</td>
<td>0.090</td>
<td>-</td>
<td>0.009</td>
<td>0.028</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>0.008</td>
<td>0.026</td>
<td>-</td>
<td>0.026</td>
<td>0.085</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>-</td>
<td>0.009</td>
<td>0.030</td>
<td>0.011</td>
<td>0.037</td>
</tr>
<tr>
<td>P-Coumaric acid</td>
<td>0.011</td>
<td>0.037</td>
<td>0.013</td>
<td>0.042</td>
<td>-</td>
</tr>
<tr>
<td>Rutin</td>
<td>0.019</td>
<td>0.063</td>
<td>0.022</td>
<td>0.073</td>
<td>-</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.023</td>
<td>0.075</td>
<td>-</td>
<td>0.023</td>
<td>0.076</td>
</tr>
<tr>
<td>Apigenin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.013</td>
<td>0.045</td>
</tr>
<tr>
<td>Luteolin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.023</td>
<td>0.076</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard deviations (SD) of three replications.
Conclusion/recommendation

The Presence of essential polyphenols in the herbs might be the reason why they are used in folklore in the management of some neurological disorders but, further scientific investigations need to be carried out to fully clarify the efficacy of these herbs and to determine their effective safe dose.

Acknowledgment

I acknowledge the support of Adekunle Ajasin University for the release of Year 2014 NEEDS Assessment Fund (AAUA/VC/APU/047) to complete this work.

References


