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

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Abbreviations: PN: Picralima Nitida; QU: Quassia Undulata; SA: Senecio Abyssimicus; TT: Tetrapleura Tetraptera; UP: Uraria Picta

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.

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.

Introduction

About 80% of Nigerian population reside in rural area [1] and the non-availability of modern healthcare facilities in these rural communities in Nigeria has made people to depend on medicinal plants in treating a lot of degenerative diseases since ancient time. The efficacy of these plants lies on the numerous bioactive compounds present in them.

Tetrapleura tetraptera (Schum. & Thonn.) Taub (TT) fruit, locally called aridan in South Western Nigeria belongs to the Mimosaceae family, with 4 edged sides. The fruit is common on the fringe of the West African rainforest belt [2]. It has different ways of preparation; It can be crushed into pieces, soak in water and taken orally or cooked, the ground powder can be

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used as a spice, and cooked as soup. *T. tetraptera* 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-cholinesterase 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 subtropical 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 abeere 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 without an insight into the bioactive constituents of the herbs. This study, therefore, sought to characterize the bioactive constituents of the aqueous extract from the herbs using HPLC-DAD technique.

Materials and methods

Chemicals and reagents

All chemicals were of analytical grade and water was glass distilled. Acetonitrile, methanol, formic acid, phosphoric acid, acetic acid, gallic acid, caffeic acid, ellagic acid, *p*-coumaric acid and chlorogenic acid were purchased from Merck (Darmstadt, Germany). Catechin, rutin, quercetin, luteolin and apigenin were acquired from Sigma Chemical Co. (St. Louis, MO, USA). High performance liquid chromatography (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A Integrator, SPD-M20A diode array detector and LC solution 1.22 SP1 software.

Sample collection and preparation

Picralima nitida, *Quassia undulata*, *Senecio abyssimicus*, *Tetrapleura tetraptera* and *Uraria picta* were obtained from the Botanical garden, Adekunle Ajasin University Akungba Akoko date. Authentication of the plants was carried out in the Department of Biology, Federal University of Technology, Akure, Nigeria by A. A. Shorungbe and voucher specimens were deposited in the Federal University of Technology herbarium. The samples were washed under running water, air-dried and then ground into powder.

Extraction of aqueous extract

This was carried out according to the method described by Odubanjo et al [7]. Briefly, 10 g of each sample was soaked in 200 ml of distilled water and placed in an orbital shaker for 48 hr for absolute extraction. The mixture was then filtered through a What man No. 1 filter paper and the filtrate centrifuged at 805 × g for 10 minutes. The clear supernatant 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 & discusion

The studied plants are good source of phenolic compounds. Plant materials have been employed in the treatment/manage-

ment of cognitive dysfunction over the years, most especially in African countries. Plants are very effective in boosting the immune system, healing the allergies, raising and renewing the body vitality [12]. There is a strong evidence that intake of dietary phenolics have positive correlation with cognitive performance [13]. There is a growing body of evidence to suggest that flavonoids and flavonoid-rich foods may be capable of counteracting neuronal injury, thereby delaying the progression of disease pathologies [14].

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_R = 11.25 min; peak 1), chlorogenic acid (t_R = 19.83 min; peak 2), caffeic acid (t_R = 26.47 min; peak 3), *p*-coumaric acid (t_R = 33.08 min; peak 4), rutin (t_R = 43.56 min; peak 5) and quercetin (t_R = 54.91 min; peak 6) (Figure 1 and Table 1). *Quassia undulata* extract has gallic acid (retention time - t_R = 11.79 min; peak *a*), catechin (t_R = 15.86 min; peak *b*), ellagic acid (t_R = 30.17 min; peak *c*), *p*-coumaric acid (t_R = 35.94 min; peak *d*) and rutin (t_R = 42.39 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_R = 11.95 min, peak 1), chlorogenic acid (t_R = 21.63 min, peak 2), caffeic acid (t_R = 25.41 min, peak 3), ellagic acid (t_R = 33.17 min, peak 4), quercetin (t_R = 49.98 min, peak 5) and apigenin (t_R = 64.87 min, peak 6). *Senecio abyssimicus* extract (Figure 4) contains other minor compounds in addition to Gallic acid (retention time-t_R = 10.85 min, peak *a*), chlorogenic acid (t_R = 19.76 min, peak *b*), caffeic acid (t_R = 24.39 min, peak *c*), ellagic acid (t_R = 28.75 min, peak *d*) and quercetin (t_R = 39.85 min, peak *e*). While, *Uraria picta* extract revealed the presence of gallic acid (retention time - t_R = 11.39 min; peak 1), chlorogenic acid (t_R = 20.45 min; peak 2), caffeic acid (t_R = 25.06 min; peak 3), rutin (t_R = 44.13 min; peak 4), quercetin (t_R = 50.09 min; peak 5) and luteolin (t_R = 55.87 min; peak 6) (Figure 5 and Table 1).

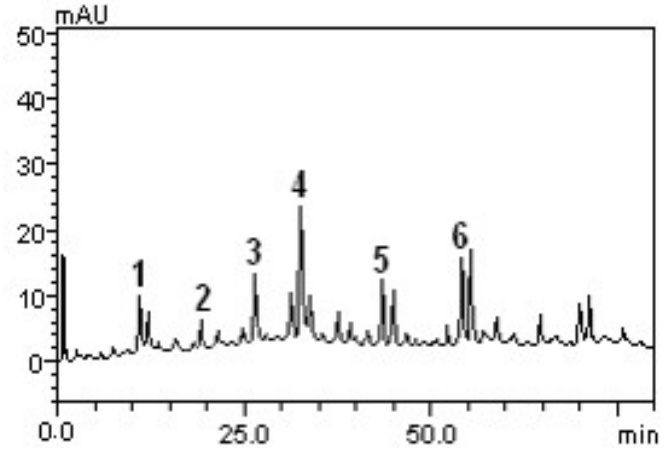

Figure 1: Representative reverse-phase HPLC analysis of *Tetrapleura tetraptera* extract using standard and spectral analysis, where peaks 1, 2, 3, 4, 5 and 6 were identified as gallic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, rutin and quercetin, respectively.

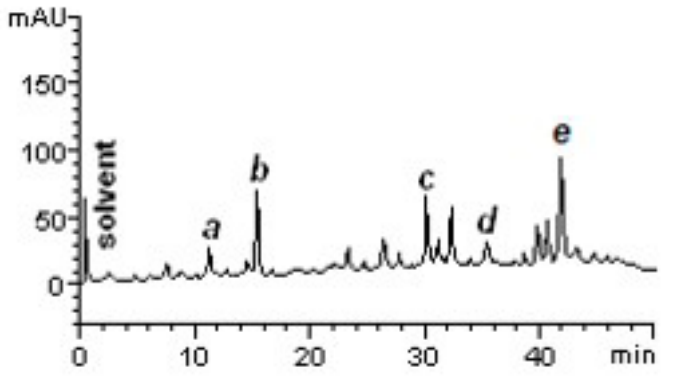

Figure 2: Representative reverse-phase HPLC analysis of *Quassia undulata* extract using standard and spectral analysis, where peaks *a*, *b*, *c*, *d* and *e* were identified as gallic acid, catechin, ellagic acid, *p*-coumaric acid and rutin respectively.

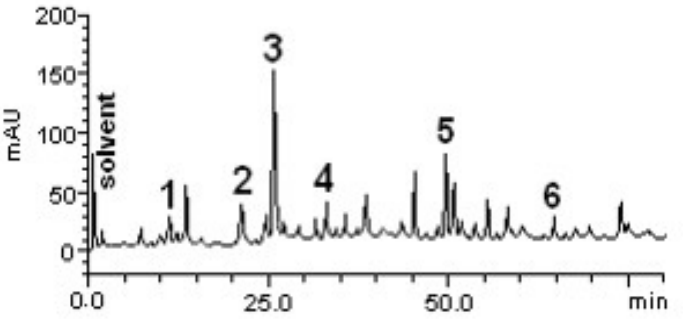

Figure 3: Representative high performance liquid chromatography profile of *Picralima nitida*. Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), ellagic acid (peak 4), quercetin (peak 5) and apigenin (peak 6).

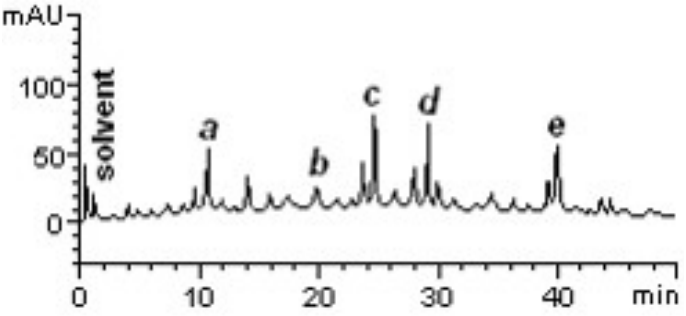

Figure 4: Representative high performance liquid chromatography profile of *Senecio abyssimicus*. Gallic acid (peak *a*), chlorogenic acid (peak *b*), caffeic acid (peak *c*), ellagic acid (peak *d*) and quercetin (peak *e*).

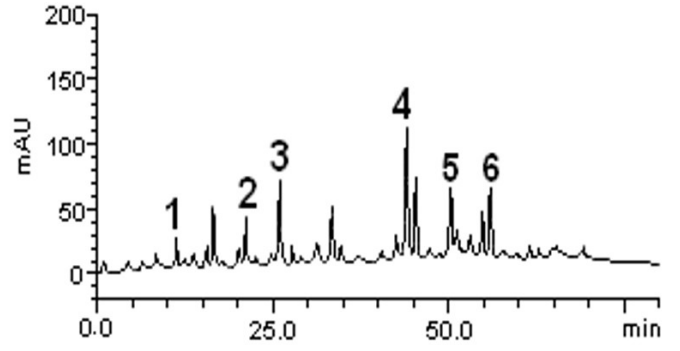

Figure 5: Representative reverse-phase HPLC analysis of *Uraria picta* extract using standard and spectral analysis, where peaks 1, 2, 3, 4, 5 and 6 were identified as gallic acid, chlorogenic acid, caffeic acid, rutin, quercetin and luteolin respectively

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 cholorogenic 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 shows 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*

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

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 undulate*, *Picralima nitida*, *Senecio abyssimicus* and *Uraria picta* (µg/mL) By High-Performance Liquid Chromatography (HPLC-DAD)

	T. Tetraptera		*Q. Undulata*		*P. nitida*		*S. abyssimicus*		*U. picta*	
Compounds	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ
Gallic acid	0.015	0.040	0.017	0.056	0.015	0.049	0.016	0.053	0.009	0.031
Catechin	-	-	0.028	0.091	-	-	-	-	-	-
Chlorogenic acid	0.027	0.090	-	-	0.009	0.028	0.023	0.076	0.017	0.054
Caffeic acid	0.008	0.026	-	-	0.026	0.085	0.009	0.028	0.024	0.079
Ellagic acid	-	-	0.009	0.030	0.011	0.037	0.011	0.037	-	-
P-Coumaric acid	0.011	0.037	0.013	0.042	-	-	-	-	-	-
Rutin	0.019	0.063	0.022	0.073	-	-	-	-	0.028	0.092
Quercetin	0.023	0.075	-	-	0.023	0.076	0.025	0.084	0.013	0.043
Apigenin	-	-	-	-	0.013	0.045	-	-	-	-
Luteolin	-	-	-	-	-	-	-	-	0.023	0.076

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.

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