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Nutritive value, mineral composition, phytochemical constituents, and antioxidant properties of freshly collected *Justicia secunda* Vahl leaves from two locations in Oyo State, Nigeria

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A B S T R A C T

Justicia secunda Vahl is an important medicinal plant in African ethnomedicine, where it is traditionally used in the management of anaemia, sickle cell disease, and inflammatory conditions. Its bioactive potential has been linked to its phytochemical composition. Because the phytochemical, nutritional, and antioxidant profiles of medicinal plants can vary with geographical location and prevailing environmental conditions, this study compared the proximate, mineral, phytochemical, and antioxidant properties of freshly collected *J. secunda* leaves from Iseyin and Oyo Town, Oyo State, Nigeria, designated JSVI and JSVE, respectively. The samples were authenticated, air-dried, and analysed using standard Association of Official Analytical Chemists (AOAC) methods and validated *in vitro* assays. Proximate analysis showed similar protein contents in JSVI ($18.76 \pm 0.04\%$) and JSVE ($18.17 \pm 0.04\%$). JSVE had higher moisture ($8.91 \pm 0.12\%$), ash ($4.06 \pm 0.08\%$), and crude fibre ($19.08 \pm 0.54\%$) contents, whereas JSVI had higher carbohydrate ($51.81 \pm 0.78\%$) and gross energy (292.81 kcal/100 g) contents. Both samples had comparable qualitative phytochemical profiles and tested positive for alkaloids, tannins, saponins, steroids, phlobatannins, flavonoids, and cardiac glycosides. Quantitatively, JSVI contained more phenolics (196.97 ± 3.03 mg/100 g), flavonoids (64.89 ± 4.68 mg/100 g), saponins (2.28 ± 0.44 mg/100 g), and cardiac glycosides (46.35 ± 3.34 mg/100 g) than JSVE, whereas JSVE had marginally more tannins. Mineral analysis showed higher potassium, sodium, iron, zinc, manganese, and copper concentrations in JSVI, while JSVE had higher magnesium and phosphorus concentrations; both samples supplied only a small fraction of the cited adult dietary reference values for most minerals. JSVI also showed greater 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity, ferric-reducing antioxidant power (FRAP), and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical-scavenging activity than JSVE, consistent with its higher phenolic and flavonoid contents. These findings indicate that geographical and environmental variation measurably influences the nutritive and phytochemical composition of *J. secunda* and that the Iseyin sample may possess a comparatively stronger antioxidant profile than the Oyo Town sample. Further *in vivo* and toxicological studies are recommended to substantiate therapeutic inferences drawn from these *in vitro* observations.

Keywords: *Justicia secunda*, Phytochemical screening, Mineral composition, Antioxidant activity, Geographical variation

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1. INTRODUCTION

Green plants remain among the most fundamental resources for human survival and well-being, providing nutrition, remedies

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for minor health disorders, functional additives that enhance and preserve food, and protection against disease. Because plant-derived metabolites can help buffer the body against redox imbalance and other oxidative-stress-related conditions, medicinal plants continue to attract considerable global research interest [1, 2].

Among these plants, *Justicia secunda* Vahl (family Acanthaceae) is a flowering species originally native to South America but now naturalised and widely cultivated across tropical and subtropical Africa, where the genus comprises several recognised species [3]. In many parts of Nigeria, the plant is commonly grown as a hedge around residential compounds and is valued for its vigorous growth and protective vegetative structure. It is known locally as "ogwu obara" among the Igbo of eastern Nigeria and as "Ewe Eje" (blood-tonic leaves) among the Yoruba of southwestern Nigeria, names that reflect its long-standing ethnomedicinal reputation.

J. secunda is traditionally used in the management of several ailments, most notably conditions associated with sickle cell disease. The leaves are commonly boiled or soaked in water to produce a deep-purple decoction consumed as a tonic or herbal tea [4]. Several other species in the genus are similarly used across the region to manage a range of health disorders [3] and are valued for their antioxidant and cardioprotective properties [4]. Ethnomedicinal applications also include wound healing and the treatment of anaemia and abdominal pain [5]; in south-eastern Nigeria, the Congo, and southern Côte d'Ivoire, the leaf decoction is used specifically for anaemia management.

Beyond ethnomedicinal use, a growing body of *in vitro*, cell-based, and animal evidence supports several of these traditional claims, although these categories provide different levels of evidence. *In vitro* and cell-based studies have reported antidiabetic and antiarthritic activity [6], anti-inflammatory and antinociceptive effects [7], antibacterial and antisickling potential [8, 9], and protective activity against oxidative-stress-related processes [10, 11]. Animal studies have similarly demonstrated antiplasmodial activity [12], antianaemic and haemopoietic effects reported to exceed those of ferrous sulphate and vitamin B_{12} [4, 13], and ameliorative effects on phenylhydrazine-induced haematological toxicity in rats co-administered Astymin [14].

Reports on the safety of *J. secunda* extracts, however, are not fully consistent. Ref. [15] reported adverse cardiac and renal effects following the administration of an ethanolic leaf extract to Wistar rats, whereas Ref. [16] reported no cytotoxicity for crude, dichloromethane, ethyl acetate, and methanol leaf extracts (antiplasmodial IC_{50} from 5.74 to 100.26 μgmL^{-1}), alongside a reduction in induced haematological abnormalities. This discrepancy may reflect differences in extraction solvent, dose, and duration of exposure and underscores the need for standardised toxicological evaluation before firm therapeutic recommendations are made.

The antioxidant and nutritional properties of medicinal plants are also known to vary with plant material, geographical location, and prevailing environmental conditions [17–20]. Comparisons within a single species indicate that such differences can translate into measurable variation in bioactivity: leaves of *J. secunda* show greater pharmacognostic potential than stems [21], and root phytochemical and proximate contents differ between

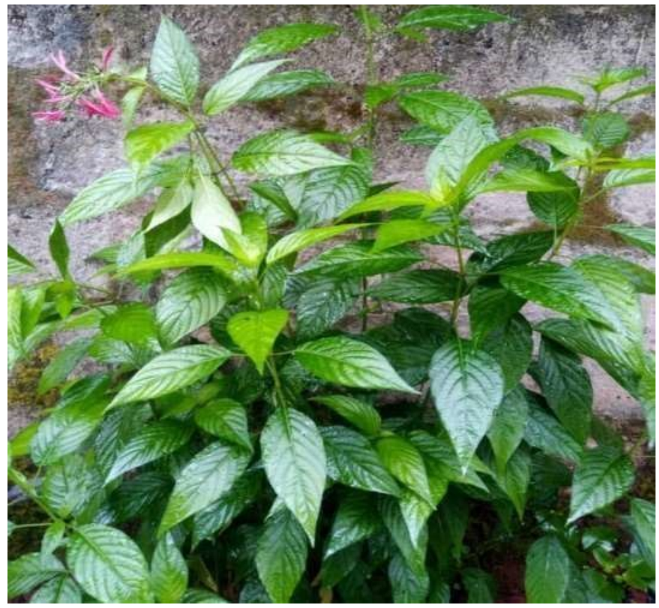

Figure 1. *Justicia secunda* **Vahl leaves. Source: field photograph.**

J. carnea and *J. secunda* [22]. Solvent strength also affects antioxidant yield; 0.1% HCl-acidified methanolic extracts of the same species have been investigated [23]. Despite this evidence, no study has directly compared the proximate, mineral, phytochemical, and antioxidant profiles of *J. secunda* harvested from two distinct locations within the same agroecological state. This omission limits understanding of how local environmental variability shapes the nutritional and pharmacological value of this widely used plant.

This study therefore evaluated and compared the proximate composition, mineral content, phytochemical constituents, and *in vitro* antioxidant potential of *J. secunda* leaves harvested from Iseyin and Oyo Town in Oyo State, Nigeria, to determine whether, and to what extent, geographical variation influences these properties.

2. MATERIALS AND METHODS

2.1. SAMPLE COLLECTION AND TREATMENT

Fresh, healthy leaves of *Justicia secunda* Vahl 1 were collected in November 2024 from home gardens in Iseyin (sample designated JSVI) and Oyo Town (sample designated JSVE), Oyo State, Nigeria. Plant identity was authenticated by Mr. Esimekhuai, D.P.O., at the Herbarium of the Department of Botany, University of Ibadan, Nigeria (voucher number UIH-23492). The leaves were separated from the stems and roots, washed with distilled water to remove surface debris, air-dried at room temperature, and pulverised using an electric blender. The resulting powder was passed through a 60-mesh Wiley mill to obtain a uniform particle size. The fine powder was stored in labelled polythene bags at 4 °C until analysis. No reference plant material was included as the study focused on comparing samples from the two collection sites.

2.2. PROXIMATE AND MINERAL ANALYSES

Moisture, crude protein, crude fat, ash, crude fibre, and carbohydrate contents were determined in triplicate ($n = 3$) using standard AOAC methods [24]. Crude protein was estimated using the micro-Kjeldahl method ($N \times 6.25$) [25]; ash and crude fibre were determined by incineration and gravimetric analysis [26, 27]; crude fat was determined by Soxhlet solvent extraction [28]; and carbohydrate content was obtained by difference [29]. Gross energy was calculated from the proximate values using Atwater conversion factors of 4 kcal/g for protein and carbohydrate and 9 kcal/g for fat.

Mineral content (K, Na, Ca, P, Fe, Zn, Mn, Mg, Cu, and Se) was determined after acid digestion of the dried powdered samples. A Buck Scientific Model 210 VGP atomic absorption spectrophotometer was used for Fe, Zn, Mn, Mg, Cu, and Se, while a flame photometer (Model FP902) was used for K, Na, and Ca. Phosphorus was determined colorimetrically using the vanadomolybdate method. Reagent blanks and spiked recovery samples were analysed alongside the test samples for quality control, and all readings were calibrated against certified standard solutions.

2.3. QUALITATIVE PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening was performed to detect the presence or absence of major secondary-metabolite classes following Ref. [30]. Each 15.0-g portion of powdered sample was extracted in 100 mL of ethanol to yield a crude extract of 50 mg/mL; the resulting residue was stored in a desiccator before analysis. Alkaloids, tannins, saponins, steroids, and phlobatannins were screened using standard colour and precipitation tests [31]; flavonoids were screened using the methods of Refs. [32, 33]; and phenolic compounds and cardiac glycosides were screened following Refs. [34] and [35], respectively.

2.4. QUANTITATIVE PHYTOCHEMICAL AND ANTIOXIDANT ANALYSES

Total phenolic content, total flavonoid content, phytate, oxalate, saponin, and cardiac glycoside contents were quantified in triplicate using established spectrophotometric procedures [36, 37]. DPPH and ABTS radical-scavenging activities and FRAP were determined following Ref. [38] and the International Union of Pure and Applied Chemistry (IUPAC)-recommended protocols of Ref. [39]. Results were expressed as percentage inhibition and EC_{50} (mg/mL), with ascorbic acid (vitamin C) used as the reference standard.

2.5. STATISTICAL ANALYSIS

All determinations were performed in triplicate ($n = 3$), and the results are expressed as mean ± standard deviation (SD). Data for JSVI and JSVE were compared using Student's independent-samples t-test, with differences considered statistically significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. PROXIMATE COMPOSITION

The moisture contents of JSVI (8.38 ± 0.20%) and JSVE (8.91 ± 0.12%) were comparable (Table 1). These values were similar to those reported for *Ficus capensis* leaves [40] but considerably lower than the 68.40–89.40% reported for *Mangifera indica* and *Chenopodium ambrosioides* [41]. The relatively low moisture content of the air-dried *J. secunda* samples suggests good storage stability because reduced moisture limits microbial growth [42]. Statistical analysis showed no significant difference in moisture content between the samples ($p = 0.067$).

Crude protein content was high in both samples (JSVI: 18.76%; JSVE: 18.17%), exceeding values reported for *Senna singueana*, *Nymphaea lotus*, *Cochlospermum planchonii*, and *Acacia nilotica* [43] and for *F. capensis* leaves [44]. This finding suggests that *J. secunda* could serve as a supplementary protein source [45]. The difference in protein content between JSVI and JSVE was statistically significant ($p = 0.031$).

Crude fat content was low in both samples (JSVI: 1.17%; JSVE: 0.91%) relative to values reported for other medicinal plants [41, 46]. The low values may be nutritionally favourable in contexts where excessive dietary fat is a concern [47]. The difference in fat content between the samples was statistically significant ($p = 0.042$). Crude fibre content was substantial (JSVI: 16.35%; JSVE: 19.08%); the JSVE value exceeded the 10.9–18.4% range reported across several plants by Ref. [48], in which *Tribulus terrestris* recorded the highest value (18.4%). High dietary-fibre intake is generally associated with improved digestive health and a reduced risk of coronary heart disease [49]. The fibre-content difference between JSVI and JSVE was also statistically significant ($p = 0.028$).

Ash content, an indicator of total mineral matter, was 3.53% in JSVI and 4.06% in JSVE, comparable to values reported for *Zingiber officinale* and *Ocimum gratissimum* [50]. Higher ash values reported for other plants [46, 48] suggest that those species have a comparatively richer total mineral profile than *J. secunda*. Carbohydrate content (JSVI: 51.81%; JSVE: 48.86%) was consistent with levels reported for other medicinal plants [51] and indicates meaningful energy-supplying potential. Gross-energy values (JSVI: 292.81 kcal/100 g; JSVE: 276.31 kcal/100 g) reflect the combined contributions of protein, fat, and carbohydrate to each sample.

Overall, both samples showed a broadly favourable nutritional profile, with low fat and ash contents, moderate carbohydrate content, and comparatively high protein and fibre contents. JSVE had modestly higher moisture, ash, and fibre contents, whereas JSVI had modestly higher protein, fat, carbohydrate, and gross-energy values.

3.2. MINERAL COMPOSITION

Potassium, sodium, and magnesium contribute to osmotic balance, nerve transmission, and muscle function [52, 53]. As shown in Table 2, the K (38.20 and 11.10 mg/100 g), Na (9.55 and 2.78 mg/100 g), and Mg (4.63 and 6.67 mg/100 g) concentrations in JSVI and JSVE, respectively, were considerably below the adult dietary reference values cited by the authors (approximately 200 mg/day K, 500 mg/day Na, and 320–420 mg/day Mg) [54]. They were also below ranges reported for other plants (K: 37.71–51.22 mg/100 g; Na: 27.35–37.50 mg/100 g; Mg: 49.10–66.49 mg/100 g). Calcium content (JSVI: 62.55 mg/100 g; JSVE: 61.32 mg/100 g) fell within the range reported for *Strophanthus hispidus* and *Terminalia avicennoides*, while phosphorus concentrations were very low (0.173–0.214 mg/100 g). Relative to the cited adult reference values of 800–1200 mg/day for Ca and 700 mg/day for P, *J. secunda* would contribute minimally to daily intake of these minerals, which are nevertheless important for bone and tooth formation, blood clot-

Table 1. Proximate composition of JSVI and JSVE.

Sample	Protein (g/100 g)	Moisture (g/100 g)	Lipid (g/100 g)	Ash (g/100 g)	Fibre (g/100 g)	CHO (g/100 g)	Gross energy (kcal/100 g)
JSVI	18.76 ± 0.04	8.38 ± 0.20	1.17 ± 0.14	3.53 ± 0.08	16.35 ± 0.48	51.81 ± 0.78	292.81
JSVE	18.17 ± 0.04	8.91 ± 0.12	0.91 ± 0.05	4.06 ± 0.08	19.08 ± 0.54	48.86 ± 0.47	276.31

Table 2. Mineral composition of JSVI and JSVE.

Mineral (mg/100 g)	JSVI	JSVE
K	38.20 ± 0.10	11.10 ± 0.10
Na	9.550 ± 0.025	2.780 ± 0.025
Ca	62.55 ± 0.05	61.32 ± 0.07
P	0.173 ± 0.009	0.214 ± 0.007
Fe	0.213 ± 0.003	0.200 ± 0.003
Zn	0.081 ± 0.001	0.054 ± 0.001
Mn	0.065 ± 0.001	0.056 ± 0.001
Mg	4.627 ± 0.006	6.667 ± 0.006
Cu	0.068 ± 0.001	0.045 ± 0.001
Se	BDL	BDL

BDL: below detection limit.

Table 3. Qualitative phytochemical screening of JSVI and JSVE.

Phytochemical class	JSVI	JSVE
Alkaloids	+	+
Tannins	+	+
Saponins	+	+
Steroids	+	+
Phlobatannins	+	+
Flavonoids	+	+
Cardiac glycosides	+	+
Phytochemical extractable	100	100

+: detected.

Table 4. Quantitative phytochemical constituents of JSVI and JSVE.

Phytochemical (mg/100 g)	JSVI	JSVE
Phenolics	196.97 ± 3.03	190.91 ± 6.06
Flavonoids	64.887 ± 4.68	40.447 ± 2.04
Phytate	2.283 ± 0.11	1.21 ± 0.15
Saponins	2.277 ± 0.44	1.74 ± 0.33
Oxalate	93.31 ± 4.53	90.27 ± 0.76
Tannins	59.05 ± 5.02	61.907 ± 2.18
Cardiac glycosides	46.35 ± 3.34	28.89 ± 1.46

ting, muscle contraction, and acid–base balance [55–57]. Statistically significant differences were reported for K ($p < 0.001$), Na ($p < 0.001$), Mg ($p = 0.003$), and Cu ($p = 0.011$).

Iron content was very low in both samples (JSVI: 0.213 mg/100 g; JSVE: 0.200 mg/100 g), below the cited adult reference value of 8.4–11.7 mg/day and far below values reported for *S. hispidus* (15.04–18.67 mg/100 g) and *T. avicennoides* (16.52–21.50 mg/100 g). Thus, *J. secunda* does not represent a significant dietary iron source, despite iron's essential roles in oxygen transport, immune function, and central nervous system activity [58]. Copper concentrations (0.068 and 0.045 mg/100 g) were below the cited adult reference value of 0.9–1.0 mg/day. Zinc (0.081 and 0.054 mg/100 g) and manganese (0.065 and 0.056 mg/100 g) were also far below the cited adult reference values of 8–11 mg/day for Zn and 2–5 mg/day for Mn and were substantially lower than values reported for *S. hispidus* and *T. avicennoides*.

In summary, *J. secunda* was not a significant source of most essential macro- and micronutrients relative to the cited adult dietary reference values and several comparator plants. The comparatively higher concentrations of several trace elements in JSVI suggest location-dependent mineral uptake.

3.3. QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL CONSTITUENTS

Both JSVI and JSVE tested positive for alkaloids, tannins, saponins, steroids, phlobatannins, flavonoids, and cardiac glycosides, indicating that the samples shared a comparable qualitative phytochemical profile (Table 3). Their differences were therefore quantitative rather than qualitative.

Phenolic content was slightly higher in JSVI (196.97 mg/100 g) than in JSVE (190.91 mg/100 g). Phenolics are established antioxidants [4, 59], and higher total phenolic content has been linked to stronger antioxidant activity [37], consistent with the antioxidant results in Section 3.4. The difference between the samples was statistically significant ($p = 0.038$). Flavonoid content was considerably higher in JSVI (64.89 mg/100 g) than in JSVE (40.45 mg/100 g). Flavonoids are similarly associated with antioxidant and anti-inflammatory activity [4, 48], and the magnitude of the JSVI–JSVE difference was comparable to variability reported among plant extracts [60]. This difference was also statistically significant ($p = 0.006$).

Saponin content was higher in JSVI (2.28 mg/100 g) than in JSVE (1.74 mg/100 g). Saponins have been associated with adjuvant and antitumour properties [4], and both values were close to the 1.257–1.573 mg/100 g range reported for other plant samples [60]. Tannin content was marginally higher in JSVE (61.91 mg/100 g) than in JSVI (59.05 mg/100 g); tannins are polyphenolic compounds noted for astringent, antioxidant, and antimicrobial activity [56]. Cardiac glycoside content was higher in JSVI (46.35 mg/100 g) than in JSVE (28.89 mg/100 g). This difference was statistically significant ($p = 0.004$), although pharmacological potential should be confirmed through dose–response and safety studies rather than inferred from concentration alone.

JSVI also contained more phytate (2.28 ± 0.11 mg/100 g) and oxalate (93.31 ± 4.53 mg/100 g) than JSVE (phytate: 1.21 ± 0.15 mg/100 g; oxalate: 90.27 ± 0.76 mg/100 g). The authors considered both antinutrient levels to be within the generally accepted range for food-grade plant material (below 10 g/100 g) and therefore unlikely to pose a significant nutritional risk at typical consumption levels. Statistically significant differences were reported for phytate ($p = 0.015$) and oxalate ($p = 0.042$).

Table 5. Antioxidant activity of JSVI and JSVE measured using DPPH, FRAP, and ABTS assays.

Sample	DPPH inhibition (%)	DPPH EC_{50} (mg/mL)	FRAP (μg/mL)	FRAP EC_{50} (mg/mL)	ABTS inhibition (%)	ABTS EC_{50} (mg/mL)
JSVI	40.67 ± 1.35	0.190 ± 0.007	245.78 ± 9.72	0.61 ± 0.03	49.91 ± 3.60	1.19 ± 0.09
JSVE	26.38 ± 1.24	0.292 ± 0.013	197.56 ± 11.74	0.76 ± 0.05	31.11 ± 1.57	1.91 ± 0.09

The reported ascorbic-acid reference EC_{50} was 0.154 mg/mL.

Taken together, the quantitative data in Table 4 show that JSVI contained higher levels of phenolics, flavonoids, saponins, and cardiac glycosides than JSVE, while JSVE contained marginally more tannins. These location-linked differences, consistent with variability in phytochemical content reported for medicinal plants generally [60], suggest that environmental or soil conditions at the two sites influence secondary-metabolite biosynthesis.

3.4. ANTIOXIDANT ACTIVITY

In the DPPH assay (Table 5), JSVI showed greater percentage inhibition (40.67 ± 1.35%) than JSVE (26.38 ± 1.24%), supported by a lower DPPH EC_{50} for JSVI (0.190 ± 0.007 mg/mL) than for JSVE (0.292 ± 0.013 mg/mL). Extracts with an EC_{50} below 0.05 mg/mL are generally classified as very strong antioxidants [37]; by this criterion, neither sample was “very strong”. However, the EC_{50} of JSVI was closer to the reported ascorbic-acid reference value (0.154 mg/mL), indicating stronger DPPH radical-scavenging activity than JSVE. Differences in DPPH inhibition ($p = 0.003$) and DPPH EC_{50} ($p = 0.005$) were statistically significant.

The FRAP assay showed a greater reducing capacity for JSVI (245.78 ± 9.72 μg/mL) than for JSVE (197.56 ± 11.74 μg/mL), with a correspondingly lower FRAP EC_{50} for JSVI (0.61 ± 0.03 mg/mL) than for JSVE (0.76 ± 0.05 mg/mL). Both EC_{50} values were within the 0.2224–0.87257 mg/mL range reported for sesame-seed extracts [37], indicating broadly comparable ferric-reducing capacity to that reference plant. Differences in FRAP ($p = 0.008$) and FRAP EC_{50} ($p = 0.012$) were statistically significant.

In the ABTS assay, JSVI again showed greater inhibition (49.91 ± 3.60%) than JSVE (31.11 ± 1.57%), with a lower ABTS EC_{50} (JSVI: 1.19 ± 0.09 mg/mL; JSVE: 1.91 ± 0.09 mg/mL). Both EC_{50} values were markedly higher, and therefore weaker, than the 0.02491–0.14119 mg/mL range reported for sesame-seed extracts [37]. Thus, although both *J. secunda* samples exhibited ABTS radical-scavenging activity, this activity was comparatively modest against that reference plant. Differences in ABTS inhibition ($p = 0.002$) and ABTS EC_{50} ($p = 0.007$) were statistically significant.

Across all three assays, JSVI consistently outperformed JSVE, an outcome consistent with its higher phenolic and flavonoid contents. These results suggest that *J. secunda*, particularly the JSVI sample from Iseyin, has meaningful *in vitro* antioxidant potential; confirmation of clinical or therapeutic relevance, however, requires further *in vivo* evaluation.

4. CONCLUSION

Freshly collected *Justicia secunda* leaves from two locations in Oyo State, Nigeria, shared a broadly similar qualitative phytochemical profile but differed quantitatively in proximate composition, mineral content, phytochemical concentration, and *in vitro* antioxidant activity. The Iseyin sample (JSVI) had higher protein, fat, carbohydrate, and gross-energy values, as well as higher phenolic, flavonoid, saponin, and cardiac-glycoside contents and greater DPPH, FRAP, and ABTS antioxidant activity. The Oyo Town sample (JSVE) had higher moisture, ash, crude fibre, tannin, magnesium, and phosphorus contents. These findings support the view that geographical and environmental variation measurably influences the nutritive and phytochemical composition of *J. secunda* and, by extension, its potential functional and antioxidant value. Most essential minerals in both samples were well below the cited adult dietary reference values, indicating that *J. secunda* is better regarded as a source of bioactive phytochemicals than as a primary dietary mineral source.

4.1. RECOMMENDATIONS

Extensive *in vivo* and toxicological studies are needed to establish the safety, bioavailability, and potential health applications of *J. secunda* for human consumption.

DATA AVAILABILITY

The data supporting the findings are incorporated in the article. Raw data available on request.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no competing interests.

FUNDING

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