Journal of Forestry Research and Management. Vol. 23 (2). 12-20; 2026, ISSN 0189-8418
www.jfrm.org.ng

PHYTOCHEMICAL PROFILE AND IN VITRO ANTIOXIDANT ACTIVITY OF METHANOL LEAF EXTRACT OF BLOOD LEAF (*Justicia secunda* VAHL)

EMMANUEL, I.B.[1*], OGUNBAMOWO, P. O.[1], EMMANUEL, J.O.[2], SOWUNMI, L.I.[1], OYEYINKA, B.O.[1],ONIYA, E.A.[1], and CHUKWUDEBE, E.P.[3]

[1]Biomedicinal Research Centre, Forestry Research Institute of Nigeria (FRIN), Ibadan
[2] Federal College of Forestry Mechanization Afaka, Kaduna, Nigeria
[3]Forest Research Station, Asaba, Nigeria
Corresponding author: Emmanuel, I.B., Tel: +234-813-169-4728, E-mail: eibusayo01@gmail.com; emmanuel.ib@frin.gov.ng

ABSTRACT

The increasing global burden of oxidative-stress-linked diseases has intensified interest in plant-derived antioxidants as safe and affordable therapeutic alternatives. *Justicia secunda* Vahl (Acanthaceae), locally known as blood leaf.It is a tropical medicinal herb with documented ethnomedicinal applications in the management of inflammation, hypertension, sickle cell disease, and diabetes. The present study characterized the phytochemical constituents and evaluated the *in-vitro* antioxidant capacity of the methanol leaf extract of *J. secunda*. Qualitative phytochemical screening was conducted using standard methods, while total phenolic content (TPC) and total flavonoid content (TFC) were estimated by Folin-Ciocalteu and aluminium chloride colorimetric assays, respectively. Antioxidant activity was assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method across five concentrations (10–200 µg/mL), with ascorbic acid as a reference standard. Qualitative screening revealed the presence of alkaloids, flavonoids, tannins, saponins, phenolics, and terpenoids, while steroids and glycosides were absent. TPC and TFC were 185.4 ± 3.2 and 112.7 ± 2.5 mg/g extract, respectively. DPPH scavenging activity was concentration-dependent, ranging from 28.3 ± 1.2% at 10 µg/mL to 85.2 ± 1.4% at 200 µg/mL; ascorbic acid exhibited comparably higher scavenging (42.1–94.6%) at the same concentrations. These findings confirm that *J. secunda* is a rich source of phenolic and flavonoid antioxidants and substantiate its ethnomedicinal use in oxidative stress-related conditions.

Keywords: antioxidant, DPPH, flavonoids, *Justicia secunda*, phenolics, phytochemistry

Introduction

Oxidative stress, arising from an imbalance between reactive oxygen species (ROS) production and the cellular antioxidant defence, plays a central role in the pathogenesis of numerous non-communicable diseases, including cancer, cardiovascular disorders, diabetes mellitus, and neurodegenerative conditions (Martemucci *et al.*, 2023). Free radicals, particularly superoxide anion, hydroxyl radical, and hydrogen peroxide, inflict cellular damage through lipid peroxidation, protein oxidation, and DNA strand breaks, ultimately compromising organ function and cellular viability (Bitwell *et al.*, 2023). Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been employed industrially; however, concerns about their potential hepatotoxicity and carcinogenicity have redirected attention towards naturally occurring plant antioxidants as safer alternatives (Chaachouay and Zidane, 2024).

Medicinal plants represent an inexhaustible repository of structurally diverse bioactive secondary metabolites, including

polyphenols, flavonoids, alkaloids, tannins, and terpenoids that confer antioxidant, anti-inflammatory, antimicrobial, and cytoprotective activities (Bitwell*et al.*, 2023). The phenolic class, in particular, scavenges free radicals through hydrogen and electron donation mechanisms, chelates pro-oxidant metal ions, and up-regulates endogenous antioxidant enzymes (Kruk*et al*., 2022). It is therefore scientifically rational to investigate the phenolic content and radical-scavenging potential of underexplored medicinal species.

Justicia secunda Vahl (Acanthaceae), commonly called blood leaf or scarlet justicia, is a perennial herbaceous plant native to tropical America and now widely naturalised across West Africa, including Nigeria, Ghana, and Cameroon (Ajuru *et al.*, 2017). The plant is characterized by dark reddish-purple leaves, showy purple tubular flowers, and a strong preference for humid soils near water bodies (Bako *et al.*, 2024). Ethnomedicinally, preparations from *J. secunda* leaves are used across West Africa to manageanaemia, haemorrhage, hypertension, malaria, sickle cell disease, rheumatism, and skin infections (Ololade *et al.*, 2024). The plant has gained particular attention for its anti-sickling properties, anti-inflammatory, antidiabetic, anxiolytic, and antioxidant activities, as documented in experimental studies (Onoja *et al.*, 2017; Anyasor*et al.*, 2019; Emmanuel *et al*., 2024).

Chemical characterisation studies have identified a diverse array of bioactive compounds in *J. secunda*, including pyrrolidine alkaloids (secundallerone B and C), hydroxycinnamic acids (2-caffeoyloxy-4-hydroxy-glutaric acid), flavonoids, kaempferitrin, anthocyanins, and various phenolic acids (Świątek*et al.*, 2023). Various extracts, including methanol, which preferentially solubilises polar bioactives, has been identified as particularly rich in phenolic compounds with measurable free radical scavenging capacity (Corrêa *et al*., 2012; Osioma and Hamilton-Amachree, 2017; Bako *et al.*, 2024). Despite existing pharmacological reports, quantitative data on phenolic and flavonoid content, along with comparative DPPH radical-scavenging profiles, remain incompletely documented, particularly regarding the concentration-response relationship. This study, therefore, characterised the phytochemical composition and evaluated the concentration-dependent antioxidant activity of the methanol leaf extract of *J. secunda*, providing a scientific basis for its ethnomedicinal applications.

Materials and Methods

Collection and Identification of Plant Material

The fresh healthy leaves of *Justicia secunda* Vahl (Acanthaceae) were collected from the staff quarters of Obafemi Awolowo University Ile-Ife, Osun state. It was identified and authenticated at the Department of Forest Herbarium, Forestry Research Institute of Nigeria, Jericho, Ibadan, Oyo State, Nigeria, with voucher specimen number FHI112604.

The collected plant material was rinsed with distilled water to remove surface contaminants and shade-dried at ambient temperature (25-30°C) for about 3 weeks, when the leaves were crisp and ready to be pulverised. The dried material was subsequently pulverised to a fine homogeneous powder using a mechanical grinder and stored in airtight containers before extraction.

Journal of Forestry Research and Management. Vol. 23 (2). 12-20; 2026, ISSN 0189-8418
www.jfrm.org.ng



Figure 1: fresh healthy leaves of *Justicia secunda* Vahl (Acanthaceae)

Preparation of Methanol Extract

Cold maceration was performed by soaking into 70% aqueous methanol ata ratio of 1:10 (w/v) for 3 days. The extract was filtered using a Whitman filter paper and then evaporated in the Rotary evaporator to dryness to obtain a solid extract with a yield of 23.3g. The crude extract was dried in a desiccator and stored at 4°C until required for analysis.

Qualitative Phytochemical Screening

Qualitative phytochemical analysis of the extract was performed using standard chemical methods described by Harborne (1973) and Edeoga *et al.* (2005) for the detection of alkaloids (Dragendorff's and Mayer's reagents), flavonoids (Shinoda test), tannins (ferric chloride test), saponins (foam test), phenolics (ferric chloride test), terpenoids (Salkowski test), steroids (Liebermann-Burchard test), and glycosides (Keller-Killiani test).

Quantitative Determination of Total Phenolic Content (TPC)

TPC was determined by the Folin-Ciocalteu colorimetric method as described by Singleton and Rossi (1965) with modifications. A 0.5 mL aliquot of the extract at an appropriate dilution was mixed with 2.5 mL of a 1:10 diluted Folin-Ciocalteu reagent and allowed to stand for 5 minutes, after which 2.0 mL of 7.5% sodium carbonate solution was added. The mixture was incubated in the dark at room temperature for 30 minutes, and absorbance was measured at 760 nm against a blank. TPC was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract) from a gallic acid calibration curve:($y = 0.024x - 0.014$, $R^2 = 0.996$), and the results were calculated using the equation:

$$C = \frac{c\,x\,V}{m} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where: C = TPC (mgGAE/g), c = the concentration established from the gallic acid calibration curve (µg/mL), V = volume of extract solution in millilitre, m = weight of dried extract in grams.

Quantitative Determination of Total Flavonoid Content (TFC)

TFC was measured using the aluminium chloride colorimetric assay following Zhishen *et al.* (1999). One millilitre of extract solution was mixed with 4 mL of distilled water, followed by the sequential addition of 0.3 mL of 5% sodium nitrite, 0.3 mL of 10% aluminium chloride (after 5 minutes), and 2 mL of 1 M sodium hydroxide (after a further 6 minutes). The volume was made up to 10 mL with distilled water, and absorbance was read at 510 nm. Results were expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract)based on the quercetin

calibration curve ($y = 0.011x + 0.132$, $R^2 = 0.97$), and the results were calculated using the equation:

$$C = \frac{c \times V}{m} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Where: C = TFC (mgQE/g), c = the concentration established from the quercetin calibration curve (µg/mL), V = the volume of extract solution in millilitre, m = the weight of the dried extract in gram.

DPPH Free Radical Scavenging Assay

The DPPH radical scavenging assay was performed according to the method of Blois (1958) as described by Onoja *et al.* (2017). A stock solution of DPPH (0.5 mM in methanol) was freshly prepared. Various concentrations (10, 25, 50, 100, and 200 µg/mL) of the extract and ascorbic acid (positive control) were each mixed with 1 mL of 0.5 mM DPPH solution. Mixtures were incubated in the dark at room temperature for 30 minutes, and absorbance was read at 517 nm using a UV-Vis spectrophotometer. Methanol (1 mL) mixed with DPPH served as the negative control. Percentage inhibition was calculated as:

$$\%\ Inhibitin = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

where $A_{control}$ is the Absorbance of the negative control, and A_{sample} is the Absorbance of the test sample. All assays were conducted in triplicate, and results were expressed as mean ± SD.

Data Analysis

Data are presented as mean ± standard deviation (SD) of three replicates (n = 3). Comparisons between the extract and ascorbic acid at each concentration were evaluated using IC_{50}, which is determined as the inhibitory concentration at which 50% of the DPPH free radicals have been scavenged by either the extract or standard ascorbic acid.

Results

Qualitative Phytochemical Composition

The qualitative phytochemical screening of the methanol leaf extract of *J. secunda* revealed the presence of six major classes of secondary metabolites: alkaloids, flavonoids, tannins, saponins, phenolics, and terpenoids. Steroids and glycosides were absent from the extract (Table 1). The positive detection of flavonoids and phenolics is particularly noteworthy given their well-established roles in free radical neutralisation and antioxidant defence.

Table 1: Qualitative phytochemical composition of the methanol leaf extract of *Justicia secunda*

Phytochemical	Result
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Phenolics	+
Terpenoids	+
Steroids	−
Glycosides	−

Key: + = Present; − = Absent

Journal of Forestry Research and Management. Vol. 23 (2). 12-20; 2026, ISSN 0189-8418
www.jfrm.org.ng

Quantitative Phytochemical Content

The quantitative analysis of the extract revealed a high TPC of 185.4 ± 3.2 mg GAE/g extract and a TFC of 112.7 ± 2.5 mg QE/g extract (Table 2). The TPC was approximately 1.64-fold higher than the TFC, suggesting that phenolic acids and condensed tannins contribute substantially to the overall phenolic pool beyond the flavonoid fraction alone.

Table 2: Quantitative phytochemical content of the methanol leaf extract of *Justicia secunda*

Parameter	Value (mg/g extract)
Total Phenolic Content (TPC)	185.4 ± 3.2 mg GAE/g
Total Flavonoid Content (TFC)	112.7 ± 2.5 mg QE/g

Values are mean ± SD (n = 3)

DPPH radical scavenging activity

The methanol leaf extract of *J. secunda* demonstrated a clear concentration-dependent increase in DPPH radical scavenging activity, ranging from 28.3 ± 1.2% at 10 µg/mL to 85.2 ± 1.4% at 200 µg/mL (Figure 2). Ascorbic acid, the reference antioxidant, exhibited consistently higher scavenging percentages (42.1 ± 0.9% to 94.6 ± 0.8%) at corresponding concentrations, reflecting its superior hydrogen-donating capacity. The IC_{50} value estimated by interpolation from the regression dose-response curve for the extract was approximately 47.2 µg/mL, compared to approximately 20.8 µg/mL for ascorbic acid, indicating that the extract possesses moderate but biologically meaningful antioxidant potency, though lower than the standard ascorbic acid.

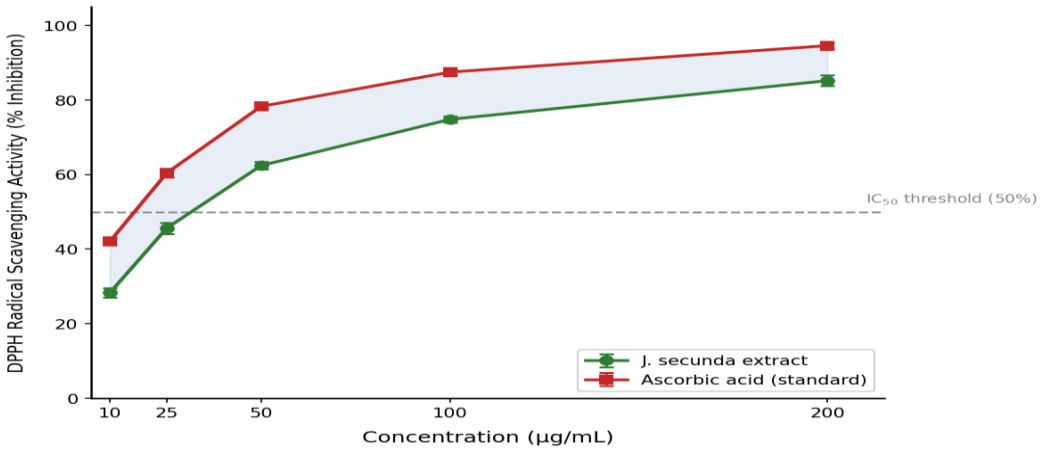


Figure 2: Concentration-dependent DPPH radical scavenging activity of the methanol leaf extract of *Justicia secunda* and ascorbic acid. Values are mean ± SD (n = 3).

Discussion

The detection of alkaloids, flavonoids, tannins, saponins, phenolics, and terpenoids in the methanol leaf extract of *J. secunda* is consistent with previous phytochemical screenings of the plant (Onoja *et al.*, 2017; Osioma and Hamilton-Amachree, 2017;Ogunbamowo*et al.*, 2020; Okafor, 2023). The absence of steroids and glycosides in the present extract may reflect solvent polarity effects, as methanol preferentially dissolves polar phytochemicals while excluding non-polar

steroidal terpenoids that are better recovered in hexane or dichloromethane fractions (Świątek*et al.*, 2023; Bako *et al.*, 2024). The detected phytochemicals possess well-documented health-promoting properties: alkaloids exhibit analgesic, antimicrobial and anti-inflammatory activities; flavonoids are potent antioxidants and enzyme modulators; tannins demonstrate astringent, antibacterial, and antiviral properties; saponins display hypocholesterolaemic and immunostimulatory effects; phenolics act as primary free radical scavengers; and terpenoids contribute to anti-inflammatory and cytotoxic bioactivities (Harborne, 1973; Chaachouay and Zidane, 2024).

The TPC of 185.4 mg GAE/g extract recorded in this study is considerably higher than values reported by Onoja *et al.* (2017) using the same solvent system, and falls within the upper range of phenolic content reported for other African Acanthaceae species. Świątek *et al.* (2023) reported TPC values varying substantially across extraction solvents for *J. secunda*, with methanolic and infused extracts displaying the richest phenolic profiles, corroborating the present findings. High TPC values are ecologically significant because phenolics are primary antioxidant compounds in plants, protecting cellular biomolecules against ROS-induced oxidative damage (Martemucci *et al.*, 2023). A positive and statistically meaningful correlation between TPC and DPPH scavenging activity is well-established across diverse plant taxa (Limsuwan *et al.*, 2025), and the high TPC observed here is consistent with the substantial DPPH inhibition recorded in this study.

The TFC (112.7 mg QE/g) of the extract is comparably high and aligns with the report by Ajuru*et al.* (2017), who noted that flavonoids had the highest yield among all phytochemical classes analysed in *J. secunda*. Flavonoids are the largest class of plant phenolics, contributing to antioxidant activity through multiple mechanisms: direct radical scavenging, metal chelation, inhibition of pro-oxidant enzymes, and regeneration of other antioxidants such as vitamin C (Ololade *et al.*, 2024). The flavonoid content in the present extract exceeds values reported for several medicinal plants routinely cited as antioxidant-rich, including *Meyna spinosa* (58.50 mg QE/g), *Bergenia ciliata* (17.44 mg QE/g), and *Aloe vera* (14.68 mg QE/g) methanol extracts (Bajalan *et al., 2016;* Mehmood*et al.*, 2022), highlighting the significant flavonoid endowment of *J. secunda*.

The concentration-dependent DPPH scavenging activity observed in this study is consistent with the established free radical neutralization mechanism of plant polyphenols, which donate hydrogen atoms or single electrons to the stable DPPH radical, converting the purple-coloured radical to the yellow diphenylpicrylhydrazine form (Blois, 1958). The estimated IC_{50} of approximately 47.2 µg/mL for *J. secunda* extract compares favourably with values reported by Onoja *et al.* (2017) for the same plant under a slightly different concentration range, and is lower than IC_{50} values reported for several other tropical medicinal plant methanol extracts, including *Achillea millefolium* (IC_{50} = 72.3 µg/mL) and *Aloe vera* (IC_{50} = 77.9 µg/mL) (Mehmood*et al.*, 2022). The IC_{50} reported here is also notably lower than the DPPH IC_{50} of 50.0 µg/mL reported by Ololade *et al.* (2024) for the Galvinoxyl and DPPH assays of *J. secunda*, reflecting consistency across independent studies.

That the ascorbic acid standard achieved a higher percentage inhibition at every concentration tested is physiologically expected given its well-characterised, direct hydrogen-donating antioxidant mechanism and small molecular weight, which

facilitates rapid radical quenching kinetics (Martemucci *et al.*, 2023). Nonetheless, the difference in scavenging capacity between the extract and ascorbic acid narrowed progressively at higher concentrations, with the extract reaching 85.2% inhibition at 200 µg/mL compared to 94.6% for ascorbic acid. This convergence at higher concentrations is characteristic of polyphenol-rich plant extracts and is attributable to the synergistic contributions of multiple antioxidant compounds within the extract matrix (Świątek *et al.*, 2023; Bako *et al.*, 2024). The close relationship between TPC, TFC, and DPPH inhibitory activity recorded in this study further substantiates the role of phenolic and flavonoid compounds as the principal antioxidant agents in *J. secunda* leaf extract.

The pharmacological significance of these findings is multidimensional. First, the high phenolic and flavonoid load of *J. secunda*places the plant as a potential natural source of dietary antioxidants for functional food applications (Bako, 2024). Also, the antioxidant capacity of the extract provides mechanistic support for the ethnomedicinal use of the plant in conditions driven by oxidative stress, including sickle cell disease, hypertension, and inflammatory disorders (Anyasor *et al.*, 2019; Ololade *et al.*, 2024). Furthermore, the concentration-dependent scavenging profile allows for dose-optimisation strategies relevant to further in vivo and clinical validation studies. It is however important to note that the DPPH assay, while widely employed, measures only hydrogen-atom transfer and single-electron transfer mechanisms and may underestimate the total antioxidant capacity; complementary assays such as ABTS, FRAP, and CUPRAC are recommended in future studies to comprehensively characterise the antioxidant potential of *J. secunda* extracts (Świątek *et al.*, 2023).

Conclusion

The methanol leaf extract of *Justicia secunda* Vahl possesses a rich array of bioactive phytochemicals, particularly phenolics and flavonoids, and demonstrates substantial concentration-dependent DPPH radical scavenging activity. TPC and TFC values reported in this study represent among the highest values documented for this species under methanol extraction, and the estimated IC_{50} of approximately 47.2 µg/mL indicates biologically relevant antioxidant potency. These results provide a strong phytochemical and pharmacological rationale for the traditional use of the plant in oxidative stress-associated diseases. Further investigations are recommended to isolate and structurally characterise the individual antioxidant constituents, assess in vivo antioxidant efficacy, and evaluate additional mechanisms of radical scavenging through ABTS, FRAP, and metal chelation assays.

References

Ajuru, M. G., Williams, L. F. and Ajuru, G. (2017). Qualitative and Quantitative Phytochemical Screening of Some Plants Used in Ethnomedicine in the Niger Delta Region of Nigeria. *Journal of Food and Nutrition Sciences*. 5(5): 1-7. doi: 10.11648/j.jfns.20170505.16.

Anyasor, G. N., Okanlawon, A. A. and Ogunbiyi, B. (2019). Evaluation of Anti-Inflammatory Activity of *Justicia secunda* Vahl Leaf Extract Using In-vitro and In-vivo Inflammation Models.*ClinicalPhytoscience*. 5(49): 1–3.doi.org/10.1186/s40816-019-0137-8

Bajalan, I., Mohammadi, M., Alaei, M., andPirbalouti, A. G. (2016). Total Phenolic and Flavonoid Contents and Antioxidant Activity of Extracts from Different Populations of Lavandin.*Industrial Crops and Products*, 87: 255-260.

https://doi.org/10.1016/j.indcrop.2016.04.059

Bako, B., Danladi, A. H., Gambo, B. G. and Shinggu, J. P. (2024). A Comprehensive Review of Solvent-Induced Variability in Antioxidant Profiling of Plants Extract: *Justicia secunda*. *Progress in Chemial and Biochemical Research*, 7(1): 1–21. doi: 10.48309/pcbr.2024.417487.1292.

Bako, B. (2024). Applications of *Justicia secunda* Extracts in Functional Foods and Natural Products: A Review. *Advanced Journal of Chemistry B*, 6: 1–10.doi.org/10.48309/ajcb.2024.412568.1192

Bitwell, C., Sen, I. S., Luke, C. and Kakoma, M. K. (2023). A Review of Modern and Conventional Extraction Techniques and Their Applications for Extracting Phytochemicals from Plants. *Scientific African*, e01585. https://doi.org/10.1016/j.sciaf.2023.e01585

Blois, M. S. (1958). Antioxidant Determinations by the Use of a Stable Free Radical. *Nature*, 181(4617): 1199-1200. https://doi.org/10.1038/1811199a0

Chaachouay, N. and Zidane, L. (2024). Plant-Derived Natural Products: A Source for Drug Discovery and Development. *Drugs and Drug Candidates*, 3(1): 184–207. https://doi.org/10.3390/ddc3010011

Corrêa, G. M. and Alcântara, A. F. C. (2012). Chemical Constituents and Biological Activities of Species of Justicia – A Review. *Revista Brasileira de Farmacognosia*, 22(1): 220–238. https://doi.org/10.1590/S0102-695X2011005000196

Edeoga, H. O., Okwu, D. E. and Mbaebie, B. O. (2005). Phytochemical Constituents of Some Nigerian Medicinal Plants. *African Journal of Biotechnology*, 4(7): 685–688.

Emmanuel, I.B., Alabi, B., Sotunde, O., Olowoparija, S., Obaro, M., & Badejo, A., Ologe, O., Oguntoye, I., Hammed, O., and Iwalewa, O. (2024). Evaluation of Anxiolytic Effects of *Justicia secunda* Methanol Leaf Extract and Its Chemical Constituents in Mice. *Basic and Clinical Neuroscience*, 15(5), 683-702.https://doi.org/10.32598/bcn.2024.6335.1

Harborne, J. B. (1973). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, London.

Kruk, J., Aboul-Enein, B. H., Duchnik, E., &Marchlewicz, M. (2022). Antioxidative Properties of Phenolic Compounds and their Effect on Oxidative Stress Induced by Severe Physical Exercise. *The Journal of Physiological Sciences:JPS*,*72*(1): 19. https://doi.org/10.1186/s12576-022-00845-1

Limsuwan, S., Awaeloh, N., Na-Phatthalung, P., Kaewmanee, T. and Chusri, S. (2025). Exploring Antioxidant Properties of Standardized Extracts from Medicinal Plants Approved by the Thai FDA for Dietary Supplementation. *Nutrients,* 17(5): 898. https://doi.org/10.3390/nu17050898

Martemucci, G., Fracchiolla, G., Muraglia, M., Tardugno, R., Dibenedetto, R. S. and D'Alessandro, A. G. (2023). Metabolic Syndrome: A Narrative Review from the Oxidative Stress to the Management of Related Diseases. *Antioxidants*, 12(12): 2091. https://doi.org/10.3390/antiox12122091.

Mehmood, A., Javid, S., Khan, M.F., Ahmad, K.S., Mustafa, A. (2022). In vitro Total Phenolics, Total Flavonoids, Antioxidant and Antibacterial Activities of Selected Medicinal Plants using Different Solvent Systems. *BMC Chemistry*, 16: 64. https://doi.org/10.1186/s13065-022-00858-2

Ogunbamowo, P.O., Olaniyi, M.B., Awotedu, O.L., and Lawal, I.O. (2020).

Assessment of the Foliar Micromorphology, Phytochemical and Mineral Composition of *Justicia secunda*Valh leaves. *Anales de Bioloia*, 42: 183-192.

Okafor, A. C. (2023). Phytochemical Screening and Antioxidant Activity of *Justicia secunda* Stem. *FUAM Journal of Pure and Applied Science*, 5(1): 44–52.

Ololade, Z. S., Onifade, O. F., Olugboye, B. O., Alabi, T. E., Faleye, B. C. and Jimson, F. E. (2024). Secondary Metabolites, Anti-Diabetic, Antioxidant, Anti-Arthritic and Antimicrobial Potential of *Justicia secunda* for Health Benefits. *Journal of Clinical Microbiology and BiochemicalTechnolgy*, 10(1): 9-12. https://dx.doi.org/10.17352/jcmbt.000057

Onoja, S. O., Ezeja, M. I., Omeh, Y. N. and Onwukwe, B. C. (2017). Antioxidant, Anti-Inflammatory and Antinociceptive Activities of Methanolic Extract of *Justicia secunda* Vahl Leaf. *Alexandria Journal of Medicine*, 53(3): 207-213. https://doi.org/10.1016/j.ajme.2016.06.001

Osioma, E. and Hamilton-Amachree, A. (2017). Comparative Study on the Phytochemical and In Vitro Antioxidant Properties of Methanolic Leaf Extract of *Justicia secunda* Vahl. *Nigerian Journal of Science and Environment*, 15(1): 111–117.

Singleton, V. L. and Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, 16(3): 144–158.

Świątek, Ł., Sieniawska, E., Sinan, K. I., Zengin, G., Boguszewska, A., Hryć, B., Bene, K., Polz-Dacewicz, M., Dall'Acqua, S. (2023). Chemical Characterization of Different Extracts of *Justicia secunda* Vahl and Determination of Their Anti-Oxidant, Anti-Enzymatic, Anti-Viral, and Cytotoxic Properties. *Antioxidants (Basel)*, 12(2):509. https://doi.org/10.3390/antiox12020509

Zhishen, J., Mengcheng, T. and Jianming, W. (1999). The Determination of Flavonoid Contents in Mulberry and their Scavenging Effects on Superoxide Radicals. *Food Chemistry*, 64(4): 555–559.https://doi.org/10.1016/S0308-8146(98)00102-2