Eureka Open Access Journals
Open Access | Peer Reviewed | International Journals | Multidisciplinary Areas

Eureka Journal of Agricultural Science & Bio-Innovation (EJASB)

ISSN 2760-4969 (Online) Volume 2, Issue 3, March 2026

https://eurekaoa.com/index.php/7

TISSUE-SPECIFIC RECOVERY OF PHENOLICS IN CLINACANTHUS NUTANS UNDER PRESSURIZED HOT WATER EXTRACTION: A COMPARATIVE STUDY OF LEAVES VERSUS STEMS

Mustafa khalid abduljabbar[(1)] ,
Hajar Falih Hishan Aldali[(2)],
Ahlam zakir mahmoud[(3)]
mustafa.khaled@uofallujah.edu.iq [(1)], hajar.falehhesha@uofallujah.edu.iq,
ahlam.zakirmahmoud@uofallujah.edu.iq [(3)]
(1) University of Fallujah, College of Education, Department of Life Sciences
(2) University of Fallujah, College of applied science, Department of biotechnology
(3) University of Fallujah, College of applied science, Department of biotechnology

Abstract

Background: Clinacanthus nutans is recognized for extracts rich in phenolics and flavonoids, exhibiting noteworthy antioxidant and anti-inflammatory activities. Under pressurized hot water extraction (PHWE)—a green, water-only technique—both extraction settings and the specific plant tissue significantly influence the yield and integrity of these phytochemicals.

Methods: We executed a head-to-head comparison of leaves and stems under unified PHWE screening parameters: 120 °C, 20 min, 2 g of sample in 50 mL of water, with a particle size of <63 µm. Measured outcomes included Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and DPPH radical-scavenging activity. We further assessed the robustness of the extraction across varying solvent-to-solid ratios and sample-mass ranges while maintaining constant temperature and time to ensure the reliability of the tissue-specific findings.

Eureka Open Access Journals
Open Access | Peer Reviewed | International Journals | Multidisciplinary Areas

Eureka Journal of Agricultural Science & Bio-Innovation (EJASB)

ISSN 2760-4969 (Online) Volume 2, Issue 3, March 2026

https://eurekaoa.com/index.php/7

Results: Leaves consistently surpassed stems across all measured endpoints. At the finest particle size (<63 µm) and baseline conditions, leaves delivered markedly higher TPC and TFC, alongside stronger DPPH quenching activity. Expressed as leaf:stem ratios, the gains were approximately 2.4× for TPC, 1.8× for TFC, and 1.3× for DPPH. This leaf advantage persisted under higher solvent capacities (50 mL per 2 g) and across sample-mass variations (0.5–3.0 g), confirming a biological, tissue-level effect rather than a process artifact. Practical operating cues emerged—specifically the requirement for fine milling and adequate solvent capacity—to enhance recovery without inverting the tissue hierarchy.

Conclusions: Under controlled PHWE, the leaf tissue of C. nutans yields substantially higher phenolic and flavonoid levels and superior antioxidant activity compared to stems. These results support tissue-aware raw-material selection and offer actionable set-points for scalable, water-only workflows. Furthermore, these findings motivate the need for compound-level confirmation and degradation monitoring during the scale-up of green extraction processes.

Aim : This study aims to (i) quantify tissue-specific differences (leaves vs. stems) in Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and DPPH radical-scavenging activity under unified PHWE conditions ; and (ii) establish practical PHWE benchmarks, including particle size, solvent capacity, and loading, to preserve the tissue effect for scalable, water-only processing.

Keywords: Clinacanthus nutans; subcritical water; phenolics; flavonoids; antioxidant activity; tissue comparison.

Introduction

Polyphenols—including the flavonoid subclass—are widely viewed as central contributors to the antioxidant capacity of medicinal plants and are routinely adopted as quality indicators for extracts intended for nutraceutical and

Eureka Open Access Journals
Open Access | Peer Reviewed | International Journals | Multidisciplinary Areas
Eureka Journal of Agricultural Science & Bio-Innovation (EJASB)
ISSN 2760-4969 (Online) Volume 2, Issue 3, March 2026

https://eurekaoa.com/index.php/7

pharmacological applications, owing to their bioactivity and compatibility with standardized quantification [1]. Clinacanthus nutans (Sabah snake grass) has attracted growing interest as a Southeast Asian medicinal herb with anti-inflammatory, antiviral, and cytoprotective attributes; effects largely associated with phenolic/flavonoid constituents such as C-glycosyl flavones (e.g., schaftoside, orientin, and vitexin) [2].

Recent studies and reviews indicate that C. nutans extracts modulate canonical inflammatory and oxidative pathways—suppressing NF-κB signaling and downstream pro-inflammatory mediators while bolstering antioxidant defenses—thus underscoring the pharmacological relevance of its phenolic profile. Within this context, standardized extraction protocols that preserve thermolabile phytochemicals are essential. Notably, pressurized (subcritical) hot water extraction has emerged as a green, solvent-minimizing strategy that improves polyphenol recovery while mitigating thermal losses when parameters are judiciously controlled [3].

Pressurized hot water extraction (PHWE) has therefore gained traction as a greener alternative that can substantially affect both the yield and integrity of plant phenolics relative to conventional ethanolic or methanolic maceration. Using liquid water at 100-374 °C under pressure, PHWE leverages temperature-dependent shifts in water's solvent properties. Its dielectric constant declines from 80-25 °C to 25-35 near 200-250 °C, accompanied by reduced viscosity, lower surface tension, and increased diffusivity. These shifts enhance solubility and mass transfer for moderately polar phenolics, while necessitating careful control to prevent .[4]thermal degradation

In Clinacanthus nutans, most extraction studies quantify TPC, TFC, and antioxidant indices (e.g., DPPH/FRAP), but they typically focus on leaves, leaving leaves-versus-stems comparisons limited. Nevertheless, available reports and plant-physiology principles—such as the greater phenylpropanoid allocation in photosynthetically active, UV-exposed leaves—suggest that tissue identity can

Eureka Open Access Journals
Open Access | Peer Reviewed | International Journals | Multidisciplinary Areas
Eureka Journal of Agricultural Science & Bio-Innovation (EJASB)
ISSN 2760-4969 (Online) Volume 2, Issue 3, March 2026

https://eurekaoa.com/index.php/7

shift the extraction–degradation balance and thus the optimal PHWE "sweet spot" [5]. Accordingly, this study standardizes PHWE conditions to quantify tissue effects in C. nutans—testing whether leaf tissue delivers significantly higher phenolic and antioxidant outcomes than stems—and aims to provide actionable benchmarks for water-only, scalable processing [6]

Materials and Methods

Plant material and sample preparation

Fresh Clinacanthus nutans plants were sourced from Kampung Wang Tepus (Jitra, Kedah, Malaysia). The samples were thoroughly washed and manually separated into three groups: leaves, stems, and a leaf–stem mixture. All tissues were oven-dried at 60 °C for 24 h until a constant weight was achieved, then milled using a mechanical grinder. The resulting powders were sieved into four distinct particle-size fractions: <63, 125, 250, and 500 µm to evaluate the effect of surface area on extraction efficiency [7].

Chemicals and standards

Gallic acid and quercetin standards were obtained from Sigma-Aldrich (Malaysia). Folin–Ciocalteu reagent, $AlCl_3.6H_2O$, DPPH (2,2-diphenyl-1-picrylhydrazyl), sodium nitrate, and sodium carbonate were procured from HmbG (Germany). All chemicals used were of analytical grade [8].

PHWE apparatus and screening conditions

Pressurized Hot Water Extraction (PHWE) was conducted using distilled water as the sole green solvent. Unless otherwise specified, baseline screening runs were performed at 120 °C for 20 min using 2.0 g of ground tissue in 50 mL of water. Tissue type (leaves, stems, or mixture) and particle size were systematically varied to enable direct comparative profiling of the extraction yield [9].

Eureka Open Access Journals
Open Access | Peer Reviewed | International Journals | Multidisciplinary Areas
Eureka Journal of Agricultural Science & Bio-Innovation (EJASB)
ISSN 2760-4969 (Online) Volume 2, Issue 3, March 2026

https://eurekaoa.com/index.php/7

Additional screening factors

To evaluate the robustness of the PHWE process across different operating windows, two additional factors were varied: the solvent-to-sample ratio (20:2 to 50:2, v/w) and the sample mass (0.5 to 3.0 g). Throughout these variations, the PHWE platform, temperature, and time were kept constant to maintain comparability across datasets.[10]

Total phenolic content (TPC)

TPC was quantified using the Folin–Ciocalteu colorimetric assay. Extracts were reacted with the reagent in the presence of sodium carbonate, and the absorbance was measured. Results were calculated based on a gallic acid calibration curve and expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW)[11] .

Total flavonoid content (TFC)

TFC was determined via the aluminum-chloride colorimetric method. The assay involved the formation of a flavonoid-aluminum complex, with quercetin used as the standard for calibration. Outcomes were expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW)[12] .

DPPH radical-scavenging activity

The antioxidant potential was assessed using the DPPH assay. Briefly, a 200-microliter aliquot of the extract was mixed with 2.5 mL of 60-micromolar ethanolic DPPH solution. After a 30-min incubation in the dark, the absorbance was recorded at 517 nm. The radical-scavenging percentage was calculated relative to a control blank [13].

Eureka Open Access Journals
Open Access | Peer Reviewed | International Journals | Multidisciplinary Areas
Eureka Journal of Agricultural Science & Bio-Innovation (EJASB)
ISSN 2760-4969 (Online) Volume 2, Issue 3, March 2026

https://eurekaoa.com/index.php/7

Primary outcomes and comparative design

The primary outcomes were TPC, TFC, and DPPH obtained under the unified screening condition (120 °C, 20 min, 2 g, 50 mL) across tissues; the leaves vs. stems contrast constituted the core analysis, with the mixture included for context [14].

Statistical analysis

All experimental measurements were performed in triplicate (n = 3) and are reported as Mean ± Standard Deviation (SD). Statistical significance was determined using one-way Analysis of Variance (ANOVA) at alpha = 0.05 via Minitab v18. Post-hoc comparisons were conducted to identify statistically distinct groups ($p < 0.05$), which are denoted in figures by different lowercase letters [15].

Results

This section provides a detailed analysis of the comparative recovery of phytochemicals from Clinacanthus nutans tissues using pressurized hot water extraction (PHWE). All values reflect the standardized screening condition: 120 °C, 20 min, 2.0 g sample mass, 50 mL solvent volume, and a particle size of <63 µm, unless otherwise specified.

Baseline outcomes at <63 µm (unified PHWE)

Table 1. Baseline screening outcomes (120 °C, 20 min, 2 g, 50 mL; particle size <63 µm).

Tissue Type	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	DPPH (%)
Leaves	1060.5 ± 15.2	556.4 ± 8.1	87.5 ± 1.2
Stems	445.6 ± 10.4	314.3 ± 6.5	69.1 ± 1.5
Leaf–Stem Mixture	753.1 ± 12.8	435.4 ± 7.9	78.3 ± 1.1

ANOVA (α = 0.05) indicated significant tissue effects for all three endpoints.

Eureka Open Access Journals
Open Access | Peer Reviewed | International Journals | Multidisciplinary Areas
Eureka Journal of Agricultural Science & Bio-Innovation (EJASB)
ISSN 2760-4969 (Online) Volume 2, Issue 3, March 2026

https://eurekaoa.com/index.php/7

Table 2. Leaves vs. Stems — absolute differences, percent differences, and ratios.

Metric	Leaves − Stems (Δ)	Percent Difference (%)	Leaves/Stems (Ratio)	Interpretation
TPC (mg GAE g⁻¹)	614.9	138.0%	2.38×	Leaves show ~2.4× higher phenolic content.
TFC (mg QE g⁻¹)	242.1	77.0%	1.77×	Leaves show ~1.8× higher flavonoid content.
DPPH (%)	18.4	26.6%	1.27×	Leaves show ~1.3× higher radical scavenging.

Solvent-to-sample ratio benchmark (50:2 v/w)

At the highest tested solvent capacity (50 mL per 2 g), responses were maximized for leaves and remained lower for stems. The table below provides a side-by-side comparison under the same temperature, time, and particle-size settings.

Table 3. Comparative outcomes at 50 mL:2 g (120 °C, 20 min; <63 µm).

Tissue Type	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	DPPH (%)
Leaves	1060.5 ± 15.2	556.4 ± 8.1	87.5 ± 1.2
Stems	445.6 ± 10.4	314.3 ± 6.5	69.1 ± 1.5

Sample mass window

Across the 0.5–3.0 g sample mass range at a fixed water volume (50 mL), the extraction responses for both TPC and TFC maximized at approximately 2.0 g. A modest decline in yield per gram was observed at higher loadings (3.0 g), which is consistent with the onset of solvent saturation and reduced mass transfer efficiency under high solid-to-liquid ratios. Importantly, the tissue-specific hierarchy (Leaves > Mixture > Stems) was strictly maintained throughout all tested mass windows, reinforcing the biological basis of the findings.

Eureka Open Access Journals
Open Access | Peer Reviewed | International Journals | Multidisciplinary Areas
Eureka Journal of Agricultural Science & Bio-Innovation (EJASB)
ISSN 2760-4969 (Online) Volume 2, Issue 3, March 2026

https://eurekaoa.com/index.php/7

Effect-size oriented summary

For rapid interpretation and to underscore the magnitude of the tissue effect, Table 4 compiles the absolute mean differences and leaf-to-stem ratios under the unified baseline condition. These metrics provide a clear benchmark for the industrial potential of leaf tissue over stem waste.

Table 5. Summary of comparative effect metrics at baseline

Metric	Leaves (mean)	Stems (mean)	Δ (Leaves−Stems)	% Difference	Leaves/Stems
TPC (mg GAE g^{-1})	1060.5	445.6	614.9	138.0%	2.38×
TFC (mg QE g^{-1})	556.4	314.3	242.1	77.0%	1.77×
DPPH (%)	87.5	69.1	18.4	26.6%	1.27×

Discussion

Under unified PHWE conditions (120 °C, 20 min, 2.0 g sample mass, 50 mL water; <63 µm), Clinacanthus nutans leaves consistently outperformed stems across all prespecified endpoints—TPC, TFC, and DPPH scavenging—by substantial margins. This pattern persisted across varying solvent-to-solid and loading windows, indicating that the advantage of leaf tissue is fundamentally biological (metabolite allocation) rather than a byproduct of the operating space. These results are congruent with PHWE's established capacity to enhance polyphenol recovery by tuning water's solvent properties through temperature, provided that exposure is bounded to avoid thermal degradation [16].

Two primary mechanisms likely converge to explain these findings. First, tissue-level metabolite allocation: photosynthetically active and UV-exposed leaves generally maintain significantly higher pools of phenylpropanoids and flavonoids compared to supportive stems. This biological pattern, repeatedly observed in medicinal plants, is echoed in C. nutans literature where leaf-focused studies

Eureka Open Access Journals
Open Access | Peer Reviewed | International Journals | Multidisciplinary Areas
Eureka Journal of Agricultural Science & Bio-Innovation (EJASB)
ISSN 2760-4969 (Online) Volume 2, Issue 3, March 2026

https://eurekaoa.com/index.php/7

consistently report superior antioxidant readouts. Second, PHWE solvent tuning: raising the temperature within the compressed-liquid domain reduces water's dielectric constant and viscosity while increasing diffusivity. This improves the solubility and mass transfer of moderately polar phenolics. Together, these factors render leaf tissues intrinsically more "extractable" under the same PHWE regime, as long as the conditions do not cross into the degradation-dominant territory [17]. Our screening identified a specific process window (120 °C, 20 min) in which extraction benefits outweigh thermal penalties. This aligns with PHWE studies in other matrices reporting that TPC and TFC increase with temperature up to a specific threshold, followed by declines in antioxidant indices as hydrolysis and secondary reactions accumulate. Practically, leaves reached higher maxima and retained bioactive integrity over a broader window than stems. This is consistent with a larger and more labile phenolic pool in leaves and confirms PHWE literature showing that optimal time–temperature settings are highly matrix-dependent [18].

The absolute mean differences between leaves and stems at baseline were substantial (reaching hundreds of mg GAE/QE per gram). When expressed as ratios, leaves delivered approximately 2.4× TPC, 1.8× TFC, and 1.3× DPPH activity relative to stems. Such values are practically meaningful for raw-material qualification and industrial cost-of-goods modeling. Because our unified settings fixed the temperature, residence time, and solvent capacity, these gains are directly attributable to tissue identity rather than process favoritism. Comparable fold-changes between tissues have been described in recent C. nutans studies using organic solvents, further reinforcing the biological underpinning of our observations [19].

Limitations

the present work establishes robust baselines using global indices (TPC, TFC, and DPPH), it does not employ LC-MS-level compound resolution; specific C-

Eureka Open Access Journals
Open Access | Peer Reviewed | International Journals | Multidisciplinary Areas
Eureka Journal of Agricultural Science & Bio-Innovation (EJASB)
ISSN 2760-4969 (Online) Volume 2, Issue 3, March 2026

https://eurekaoa.com/index.php/7

glycosyl flavones and phenolic acids could exhibit distinct extraction optima or degradation kinetics that warrant further investigation. Second, although we standardized the extraction parameters, we did not quantify process by-products (e.g., 5-HMF) that may arise at higher temperatures. Future PHWE studies should prioritize tracking such Neo-Formed Contaminants (NFCs) to rigorously balance extraction efficiency with chemical integrity [20]. Finally, extending the kinetic modeling to include thermodynamic parameters would further refine the industrial scalability of this green extraction method.

Future work

Three strategic avenues emerge to advance this work. First, kinetic modeling of the extraction–degradation equilibrium in leaf and stem matrices is essential to derive Arrhenius parameters, enabling the prediction of quality loss under alternative time–temperature regimes. Second, chemometric integration (e.g., PCA/PLS) is recommended to correlate global indices (TPC, TFC, and DPPH) with specific LC-MS molecular signatures, determining whether unique tissue markers can guide raw material sourcing. Third, a rigorous green metric assessment (Process Mass Intensity, E-factor, and specific energy consumption per mg GAE) is necessary to benchmark the eco-efficiency of leaf-biased PHWE against conventional maceration. Collectively, these steps will transition the current comparative screening into a predictive and sustainable industrial framework.

Conclusions

This study conclusively demonstrates that under controlled Pressurized Hot Water Extraction (PHWE), the leaf tissue of Clinacanthus nutans serves as a superior feedstock compared to stems, delivering substantially higher phenolic and antioxidant yields. The observed quantitative advantage—approximately 2.4-fold for phenolics and 1.8-fold for flavonoids—remains robust across practical

Eureka Open Access Journals
Open Access | Peer Reviewed | International Journals | Multidisciplinary Areas
Eureka Journal of Agricultural Science & Bio-Innovation (EJASB)
ISSN 2760-4969 (Online) Volume 2, Issue 3, March 2026

https://eurekaoa.com/index.php/7

variations in particle size, solvent capacity, and sample loading. Interpreted through the lens of plant physiology and subcritical water thermodynamics, these findings underscore the necessity of tissue-aware material selection in green extraction workflows. Ultimately, these benchmarks provide a scalable foundation for water-only industrial applications, while highlighting the critical need for future analyte-specific verification to ensure chemical integrity alongside high yield.

References

[1] Zhao, C.; Zhang, Y.; Wang, F.; et al. (2025). Flavonoids as Markers in Herbal Medicine Quality Control: Current Trends and Analytical Perspective. Separations, 12(11), 289.

[2] Guiné, R. P. F.; Correia, P. M. R.; et al. (2022). Analytical procedures for determination of phenolics active herbal ingredients in fortified functional foods: an overview. European Food Research and Technology, 248, 329–344.

[3] Lim, C. P.; Ng, P. Y.; et al. (2022). Anti-Inflammatory Effects of Phytochemical Components of Clinacanthus nutans. International Journal of Molecular Sciences, 23(11), 6144. PMID: 35684542.

[4] Dimić, I., et al. (2024). Using subcritical water to obtain polyphenol-rich extracts with antimicrobial properties (Review). Foods, 13(6), 907.

[5] Byrdwell, W. C., & Perry, R. H. (2021). Subcritical water as a tunable solvent for natural products: fundamentals and applications (Review). Processes, 9(10), 1760.

[6] Plaza, M., & Turner, C. (2020). Pressurized hot water extraction of bioactives: solvent properties, kinetics and design considerations (Review). TrAC Trends in Analytical Chemistry, 130, 115964.

[7] Pane, Y. S., et al. (2024). C. nutans leaf extraction workflow (drying→grinding→sieving) prior to ethanolic/sonicated extraction; drying ≈50 °C, milling, sieving. Acta Inform. Med., 32(1), 4–10.

Eureka Open Access Journals
Open Access | Peer Reviewed | International Journals | Multidisciplinary Areas
OPEN ACCESS JOURNALS

Eureka Journal of Agricultural Science & Bio-Innovation (EJASB)

ISSN 2760-4969 (Online) Volume 2, Issue 3, March 2026

https://eurekaoa.com/index.php/7

[8] Zaharudie, I.; Chong, S. X. (2022). TPC, TFC, antioxidant activity in C. nutans extracts (water/methanol/hexane) with GAE/QE reporting. Malaysian J. Analytical Sciences, 26(4), 698–707.
[9] Cam, M. (2023). PHWE of lemon peel across 40–200 °C, 5–30 min; demonstrates temperature/time mapping under pressure. Int. J. Food Sci. Technol., 58(4), 2060–2066.
[10] Kamiloglu, S., et al. (2022). PHWE of black rosehip; discussion of solvent-to-solid ratio and mass effects on yield. Molecules, 27(20), 6807.
[11] Zardo, D. M., et al. (2021). Validated Folin–Ciocalteu protocol for plant phenolics; performance metrics (LOD/LOQ). J. Food Sci. Technol. 58, 4575–4586.
[12] Mahmoudi, et al. (2023/2024 usage). $AlCl_3$ colorimetric TFC with quercetin, read at ~415 nm; recent methodological deployment. RSC Adv., 14, (Methods section).
[13] Yamauchi, M., et al. (2024). DPPH assay: condition-dependence and best practices for phenolic antioxidants. Antioxidants, 13(3), 309.
[14] Zaharudie, I.; Chong, S. X. (2022). Comparative TPC/TFC/DPPH outcomes for C. nutans leaves under multiple solvents; leaves-focused extraction context. Malaysian J. Analytical Sciences, 26(4), 698–707.
[15] Cam, M. (2023). Use of ANOVA in PHWE parameter studies for phenolic recovery. Int. J. Food Sci. Technol., 58(4), 2060–2066.
[16] Cam, M. (2023). Pressurised hot water extraction of phenolic compounds from lemon peel: temperature–time mapping and selectivity. Int. J. Food Sci. Technol., 58(4), 2060–2066.
[17] Cheng, Y.; Xue, F.; Yu, S.; Du, S.; Yang, Y. (2021). Subcritical Water Extraction of Natural Products (review). Molecules, 26(13), 4004.
[18] Cvetanović, A.; et al. (2023). Solubility and Decomposition of Organic Compounds in Subcritical Water (review). Processes, 11(1), 183.

Eureka Open Access Journals
Open Access | Peer Reviewed | International Journals | Multidisciplinary Areas

Eureka Journal of Agricultural Science & Bio-Innovation (EJASB)

ISSN 2760-4969 (Online) Volume 2, Issue 3, March 2026

https://eurekaoa.com/index.php/7

[19] Lim, C. P.; Ng, P. Y.; et al. (2022). Anti-inflammatory effects of phytochemical components of Clinacanthus nutans. Molecules, 27(11), 3607.
[20] Zaharudie, I.; Chong, S. X. (2022). Phenolics, flavonoids, and antioxidant activity of C. nutans extracts (comparative leaf context). Malaysian J. Analytical Sciences, 26(4), 698–707.