Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 16 May 2026
Revised 30 June 2026
Accepted 18 July 2026

Natural Resources for Human Health 2026, Vol. 6 (8s): 629–648

KEYWORDS:
ABTS, total flavonoid content, DPPH, total phenolic content, Phyllanthus emblica, Curcuma longa.

Comparative Antioxidant and GC–MS Profiling of Phyllanthus emblica L. and Curcuma longa L.: Implications for Microplastic-Induced Hepatotoxicity

B Gyani Priyanka Patnaik[1], R. Sanjeevi[2]*, Dushyant Singh Chauhan[3], J. Anuradha[4]

[1]PhD Research Scholar, Nims Institute of Allied Medical Science and Technology, Nims University Rajasthan, Jaipur, Rajasthan, India – 303121.
[2]Associate Professor, Nims Institute of Allied Medical Science and Technology, Nims University Rajasthan, Jaipur, Rajasthan, India – 303121.
[3]Professor, Nims Institute of Allied Medical Science and Technology, Nims University Rajasthan, Jaipur, Rajasthan, India – 303121.
[4]Assistant Professor, Nims Institute of Allied Medical Science and Technology, Nims University Rajasthan, Jaipur, Rajasthan, India – 303121.
*Correspondence Author: r.sanjeevi@nimsuniversity.org

ABSTRACT: Phyllanthus emblica and Curcuma longa are two medicinal herbs commonly used with their biological properties linked to phenolic, flavonoid and volatile constituents. In this study, the total flavonoid content (TFC), total phenolic content (TPC), ABTS and DPPH radical-scavenging activity and gas chromatography-mass spectrometry (GC-MS) profile of the authenticated powdered materials were compared. Each spectrophotometric assay was repeated three times, and the percentage inhibition was computed directly from the absorbance. When the 50% response was bracketed, IC50 values were computed by local linear interpolation. The data of the GC-MS were processed in the Agilent MassHunter and compared with the NIST 23 mass-spectral library. P. emblica exhibited a TPC of 43.12 ± 0.24 mg gallic acid equivalents/100 mg, compared with 2.69 ± 0.23 mg/100 mg for C. longa. TFC values were 23.87 ± 0.37 and 22.86 ± 0.36 mg quercetin equivalents/100 mg, respectively. At 25 µg/mL, DPPH inhibition reached 81.43 ± 0.08% for P. emblica and 31.66 ± 0.12% for C. longa. The DPPH IC50 value of P. emblica was 12.86 ± 0.01 µg/mL While C. longa exhibited weaker inhibition compared to P. emblica, with the DPPH activity by 50% even at the tested concentration. ABTS IC50 values were 11.13 ± 0.04 and 31.46 ± 0.31 µg/mL, respectively. The TFC responses and the C. longa TPC response were below the lowest standards and should be interpreted with caution. In case of P. emblica, 37 integrated peaks and 45 library annotations were obtained and for C. longa, 23 integrated peaks and 31 library annotations were obtained from GC-MS analysis. The tentative identification of representative compounds in P. emblica and C. longa were alpha-pinene, dill ether and 2-caren-4-ol, and furfural, 1,2,3-benzenetriol, trans-cinnamic acid and ar-turmerone, respectively. Retention indices and authentic standards were not available and therefore compound assignments were tentative. In Summary, the present study compares the phytochemical composition, antioxidant activity, and GC–MS profiles of Phyllanthus emblica and Curcuma longa. P. emblica exhibited markedly higher phenolic content and radical-scavenging activity, while C. longa showed distinct volatile constituents. These

findings provide a chemical basis for evaluating their complementary roles in mitigating oxidative stress and hepatotoxicity associated with microplastic exposure.

1. INTRODUCTION

The presence of microplastic contamination is a new health issue as ingested particles can get to the liver. Experimental studies have demonstrated liver accumulation, response to oxidative stress and metabolic biomarkers (Deng et al., 2017; Li et al., 2025; Forutan et al., 2026). Polypropylene microplastics have also been found to disrupt the lipid-droplet homeostasis and lipid metabolism in mouse liver, suggesting a potential hepatotoxic risk (Liu et al., 2023). There is still limited evidence in humans (Lu et al., 2026).

The oxidative stress, inflammation and lipid dysregulation provide the rationale for using antioxidant-rich botanicals as potential ameliorative agents. *Phyllanthus emblica* fruit extract has been found to be beneficial for the antioxidant status of liver in experimental liver disease (Huang et al., 2017). Curcumin (*Curcuma longa)* has been shown to reduce oxidative stress and inflammation liver damage in rats (Reyes-Gordillo et al., 2007). They should be directly validated for their effectiveness against hepatotoxicity due to polypropylene microplastic.

These two botanicals might offer complementary protection that is pertinent to liver injury caused by pollutants. The antioxidant and anti-inflammatory activity of *P. emblica* may be attributed to the phenolic rich matrix, which may help in direct scavenging of radicals and limit lipid peroxidation, while the curcuminoid and turmerone related constituents of *C. longa* may help in the antioxidant and anti-inflammatory activity (Huang et al., 2017; Reyes-Gordillo et al., 2007; Singh et al., 2010). Together, these activities may aid in alleviating oxidative stress, inflammatory signaling and lipid-metabolic disruption induced by polypropylene microplastics and other environmental pollutants. The present work is however a characterization study of analytical nature and the direct hepatoprotection of both plants was not tested and needs to be confirmed in suitable biological models.

Thus, chemical and antioxidant characterization is essential as a preliminary step. None of the in vitro assays is a complete reflection of antioxidant capacity since antioxidants vary in polarity and reaction kinetics (Huang et al., 2005). TPC and TFC should be used in conjunction with DPPH and ABTS (Prior et al., 2005). Phenolics like ellagic acid and gallic acid are good sources of *P. emblica* (Liu et al., 2008; Yang et al., 2026). *C. longa* is rich in curcuminoids and volatile sesquiterpenoids like ar-turmerone (Singh et al., 2010). The main application of conventional GC-MS is to characterize volatile and semi-volatile compounds (Fahmy et al., 2023; Tripathi et al., 2022).

Hence, the aim of this work was to perform a comparative phytochemical and antioxidant evaluation of *P. emblica* and *C. longa* powders through TPC, TFC, DPPH, ABTS and GC-MS and to investigate if the comparative phytochemical and antioxidant profile of these powders would provide a rational basis for subsequent evaluation as complementary protective agents against hepatotoxicity and associated oxidative injuries caused by polypropylene microplastic associated environmental pollutants. The novelty of the study is the combination of botanical authentication, calibration-domain evaluation, concentration-response antioxidant analysis, and library-assisted GC-MS profiling for both species in a single comparative workflow, explicitly connected to a microplastic-hepatotoxicity research framework. The results can be used for botanical standardization, selection of candidate extracts and marker compounds, prioritization of formulations and doses, and design of future cellular and animal toxicology studies.

2. MATERIALS AND METHODS

2.1 Study design and analytical workflow

The use of an experimental comparative design to assess the performance of authenticated materials of *P. emblica* and *C. longa* under a common analytical workflow. Samples E and C were coded respectively. All assays (TPC, TFC, DPPH and ABTS) were repeated three times and the GC-MS profiling was performed using the same acquisition and data-processing platform for both species. The spectrophotometric absorbance values were kept for the calculation of concentration, percentage inhibition, IC50 values, coefficient of variation and normalized response area. Table 1 shows the procedures and analytical endpoints.

Table 1. Summary of experimental procedures and analytical endpoints.

Analytical component	Procedure	Analytical endpoint / quality measure
Botanical material	*P. emblica* and *C. longa* were collected from local market of Chandwaji region (Jaipur), and authenticated by the Department of Botany, University of Rajasthan (RUBL 21776 and RUBL 21777).	Authenticated powdered materials coded E and C
Qualitative screening	Initial color, precipitate, phase-separation, and foam/emulsion reactions were photographed immediately after development.	Visual documentation of reaction endpoints
Total flavonoid content	Aluminum chloride colorimetry using quercetin standards (0.2-1.0 mg/mL); absorbance at 510 nm; triplicate analysis.	mg QE/100 mg; calibration range and precision
Total phenolic content	Folin-Ciocalteu colorimetry using gallic acid standards (0.02-0.10 mg/mL); absorbance at 760 nm; triplicate analysis.	mg GAE/100 mg; calibration range and precision
DPPH assay	Samples at 5-25 µg/mL were reacted with 0.1 mM DPPH for 1 h and measured at 517 nm.	Inhibition (%), IC_{50}, and normalized AUC
ABTS assay	ABTS radical cation was generated with ammonium persulfate; samples at 10-50 µg/mL were incubated for 10 min and measured at 734 nm.	Inhibition (%), IC_{50}, and normalized AUC
GC-MS profiling	A 1 µL aliquot was analyzed on an Agilent triple-quadrupole GC-MS system; data were processed in MassHunter/Chrom Decon and searched against NIST23.L.	Peak count, relative area, library score, and tentative annotation
Data analysis	Triplicate values were summarized as mean ± SD and CV. IC50 was interpolated when bracketed; normalized AUC was calculated by the trapezoidal rule.	Descriptive comparison without inferential testing

2.2 Botanical materials, collection, and authentication

The plant materials of *Phyllanthus emblica L.* and *Curcuma longa L.* were collected from NIMS University campus and Chandwaji region of Jaipur, Rajasthan, India respectively. Botanical identification was done in the Jaipur region. The authentication numbers of *P. emblica* and *C. longa* were RUBL 21776 and RUBL 21777 respectively. Analytical assays were done with powdered authenticated material.

All sample tubes and analytical records were kept under the codes E and C, and the solutions used for the assay were made up to the concentrations specified for each procedure and measurements were made for the two species under similar experimental conditions.

2.3 Qualitative phytochemical screening

Qualitative phytochemical reactions were carried out as a preliminary screening. Photographic documentation of the color development, precipitate formation, phase separation and foam- or emulsion-like endpoints. These observations were not recorded numerically, but were utilized as supporting visual evidence.

2.4 Total flavonoid content

TFC was assessed by aluminium chloride colorimetric method (Zhishen et al., 1999). The sample aliquot of 0.2 mL was diluted to 4.0 mL with distilled water and 0.30 mL of 10% sodium nitrite was added. 0.30 mL of 10% aluminium chloride and 2.0 mL of 1% sodium hydroxide were added after 5 min. The solution was then homogenized and the absorbance measured at 510nm using a reagent blank. Quercetin standards of 0.2-1.0 mg/mL generated the calibration equation $y = 2.783x - 0.0934$ ($R^2 = 0.9892$). The results were presented as mg QE/100 mg.

2.5 Total phenolic content

The Folin-Ciocalteu method (Singleton & Rossi, 1965) was selected to define the TPC. The standards of gallic acid (0.02-0.10 mg/mL) were mixed with ten-fold diluted Folin-Ciocalteu reagent (5 mL) and 7.5% sodium carbonate (4 mL). The absorbance was measured at 760 nm. The calibration equation obtained was y = 2.46x + 0.0158 (R2 = 0.9947) and the findings were detected as mg gallic acid equivalents (GAE)/100 mg.

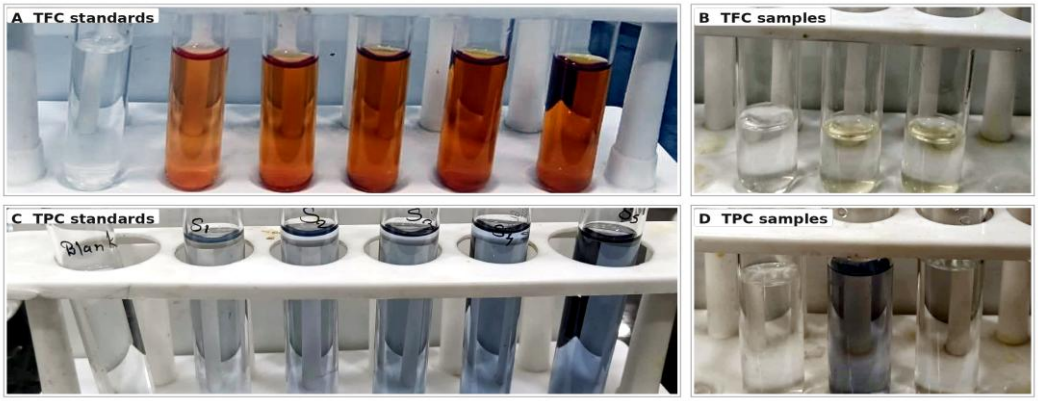

Figure 1. Photographic documentation of total flavonoid content standards and samples (A, B) and total phenolic content standards and samples (C, D).

2.6 DPPH radical-scavenging assay

DPPH radical-scavenging activity was carried out according to the technique of Brand-Williams et al. (1995). The sample solutions (5, 10, 15, 20 and 25 µg/mL) were mixed with 3 mL of 0.1 mM DPPH in methanol mixed thoroughly and incubated for 1 h at room temperature. The absorbance was calculated at 517 nm with methanol as blank. The assay is assessed according to the reduction of the stable DPPH radical (Blois, 1958). The %age inhibition was determined according to this formula: 100 × (Acontrol - Asample)/Acontrol. Control absorbances were 0.827, 0.828, and 0.828.

2.7 ABTS radical-cation-scavenging assay

The ABTS activity was calculated as described by Re et al. (1999). The ABTS solution (7 mM) was mixed with the ammonium persulfate (2.45 mM) and enabled to incubate in the dark for 16 h. The radical solution was then diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. The sample solutions of 10, 20, 30, 40 and 50 µg/mL were mixed with 3 mL of ABTS radical solution, diluted to 5 mL with ethanol and measured after 10 minutes at 734 nm. The percentage inhibition was resulted as 100 × (Acontrol - Asample)/Acontrol with the absorbance of the control being 0.745.

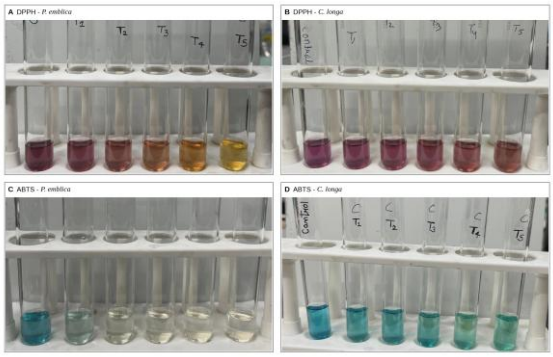

Figure 2. DPPH reactions for *P. emblica* and *C. longa* (A, B) and ABTS reactions for *P. emblica* and *C. longa* (C, D) across the tested concentration series.

2.8 GC-MS acquisition and library searching

GC-MS analysis was carried out with an Agilent triple-quadrupole GC-MS system with 1 µL injection. Data were collected and analyzed in Agilent MassHunter, chromatographic deconvolution was done in Chrom Decon, and mass spectra were searched in NIST23.L library. Both botanical samples were analyzed using the same analytical approach.

Library matches were considered as tentative annotations. To summarize the spectral agreement, match scores were grouped descriptively as ≥70, 60-69.9, 50-59.9, and <50. The categories were only used for comparative assessment and were not considered as formal identification levels. Transparent documentation of chemical analysis is stressed in minimum reporting standards (Sumner et al., 2007). Further evidence is needed such as retention indices, authentic standards, MS/MS or NMR, to confirm small-molecule identification (Schymanski et al., 2014). It is recommended that system-suitability and quality-control observations be selected to monitor analytical stability (Broadhurst et al., 2018).

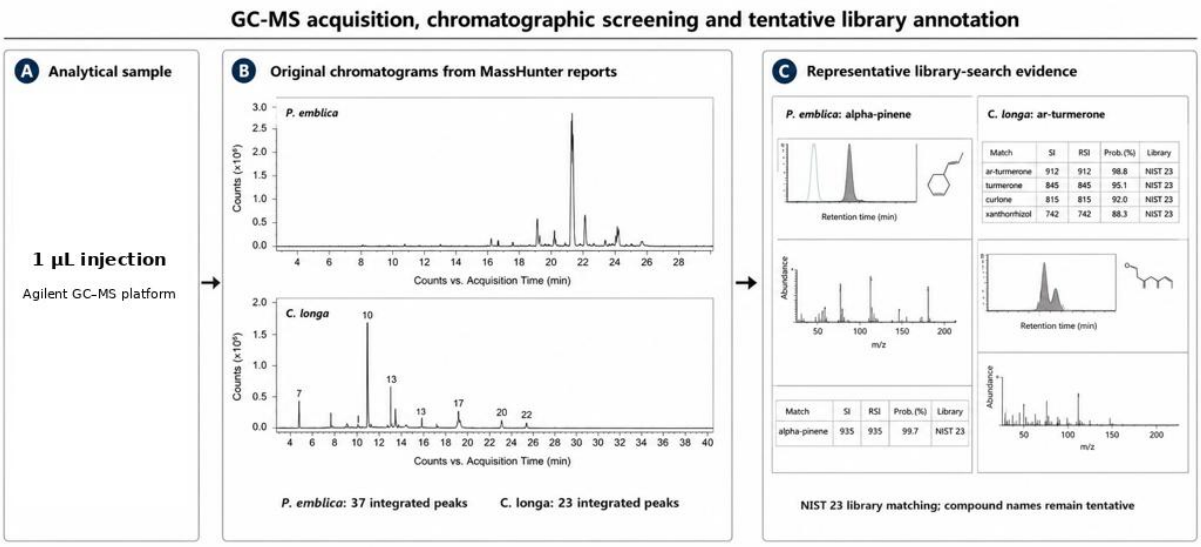

Figure 3. GC-MS analytical workflow showing sample injection, representative chromatograms, spectral library searching, and tentative compound annotation.

2.9 Data processing and statistical analysis

The absorbance and GC-MS summary data were placed in a structured worksheet and tested for internal consistency. The findings are expressed as the mean value ± sample SD of three analytical measurements. CVs were used to characterize the precision of measurements. Since the replicates were repeated measurements of the same botanical material, no inferential hypothesis testing was done.

If the 50% inhibition was between two adjacent concentrations, IC50 was determined by local linear interpolation. The 50% endpoint was not observed for *C. longa* in the DPPH assay between 5-25 µg/mL; the primary result is reported as IC50 >25 µg/mL, with the linear extrapolation shown as a secondary estimate. Integrated response was presented as the normalized area under the concentration-response curve (AUC) using the trapezoidal rule and evaluated as a %age of the maximum possible response area within the range of the tested concentrations.

3. RESULTS

3.1 Calibration performance and analytical range

Both the quercetin and gallic acid calibration curves exhibited good linearities with R2 of 0.9892 and 0.9947, respectively. The absorbances of the TFC samples (0.015-0.025) were lower than the lowest absorbance of the quercetin standard (0.426). The absorbances of the *C. longa* TPCs (0.021-0.022) were also below the lowest gallic-acid-standard absorbance (0.072), while the absorbances of the *P. emblica* TPCs were within the calibration response range. Hence, the TFC values of both the samples were

interpreted with caution and the TPC value of C. longa with caution. Figures 4 and 5 show the calibration-domain assessment, and Table 2 shows the analytical ranges.

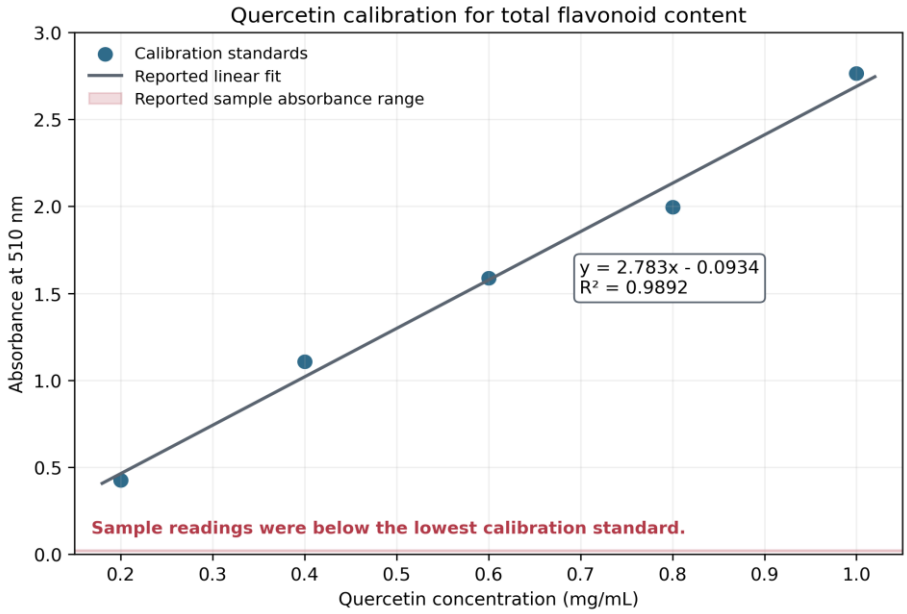

Figure 4. Quercetin calibration curve for the total flavonoid assay. The shaded band indicates the sample absorbance range, which was below the lowest standard.

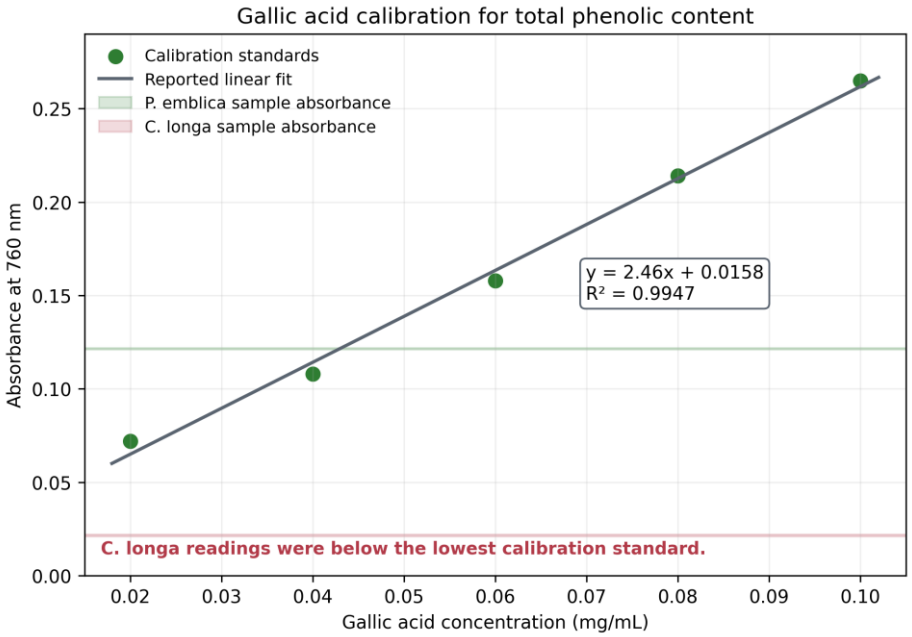

Figure 5. Gallic acid calibration curve for the total phenolic assay. The *P. emblica* absorbance was within the standard response range, whereas the *C. longa* response was below it.

Natural Resources for Human Health
eISSN: 2583-1194

Table 2. Calibration characteristics and analytical-range assessment.

Assay	Calibration range	Regression / R^2	Sample absorbance	Interpretation
TFC	0.2-1.0 mg/mL quercetin	y = 2.783x - 0.0934; R^2 = 0.9892	E: 0.021-0.025; C: 0.015-0.019	Both samples below the lowest standard response; values interpreted cautiously
TPC	0.02-0.10 mg/mL gallic acid	y = 2.46x + 0.0158; R^2 = 0.9947	E: 0.121-0.122; C: 0.021-0.022	*P. emblica* within the response range; *C. longa* below the lowest standard response
DPPH	5-25 µg/mL sample	Raw absorbance available	Monotonic decrease for both samples	Suitable for descriptive response analysis; *C. longa* did not bracket 50%
ABTS	10-50 µg/mL sample	Raw absorbance available	Monotonic decrease for both samples	Suitable for interpolation across the tested concentration range

3.2 Total phenolic and flavonoid contents

TPC was 43.12 ± 0.24 mg GAE/100 mg in *P. emblica* and 2.69 ± 0.23 mg GAE/100 mg in C. longa, corresponding to a 16.05-fold difference and an absolute difference of 40.44 mg GAE/100 mg. Their CVs were 0.55% and 8.60% respectively. Total flavonoid content was 23.87 ± 0.37 mg QE/100 mg in *P. emblica* and 22.86 ± 0.36 mg QE/100 mg in *C. longa*, giving a 1.04-fold ratio. The two phytochemical indices are compared in figure 6 and replicate summaries are provided in table 3.

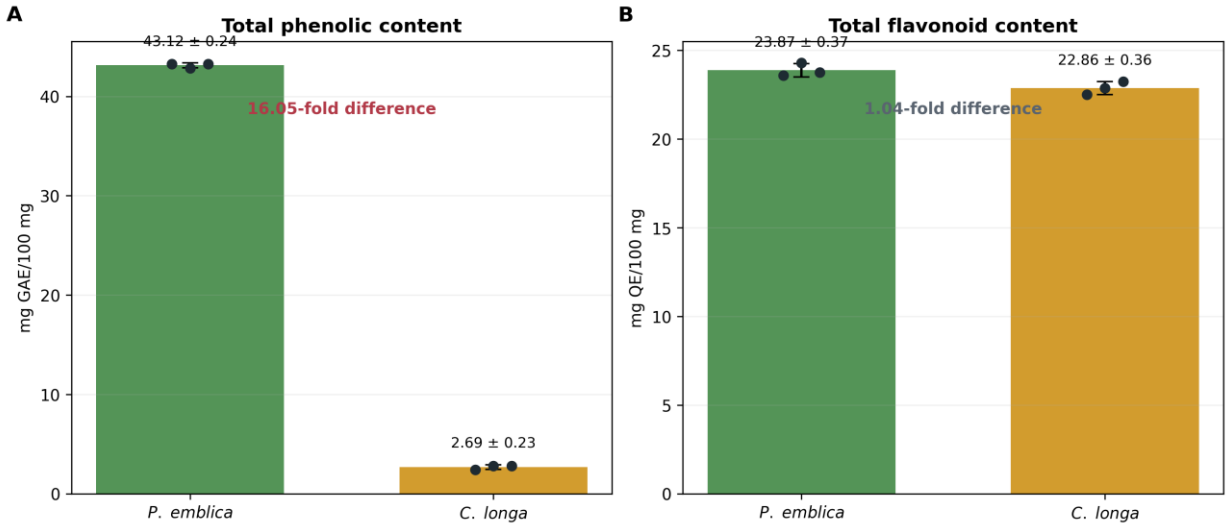

Figure 6. TPC (A) and TFC (B). Bars show mean ± SD, and points show the three analytical measurements.

Table 3. Total phenolic and flavonoid contents.

Measurement	P. emblica, mean ± SD	CV (%)	C. longa, mean ± SD	CV (%)	E/C ratio
TPC (mg GAE/100 mg)	43.12 ± 0.24	0.55	2.69 ± 0.23	8.60	16.05
TFC (mg QE/100 mg)	23.87 ± 0.37	1.55	22.86 ± 0.36	1.57	1.04

3.3 DPPH radical-scavenging activity

The DPPH response for *P. emblica* was found to be steeply increasing with concentration from 20.30 ± 0.12% at 5 µg/mL to 81.43 ± 0.08% at 25 µg/mL. Its locally interpolated IC50 was 12.86 ± 0.01 µg/mL. However, C. longa rose only from 15.59 ± 0.11% to 31.66 ± 0.12% across the same range and was not able to reach the 50% endpoint. The linear extrapolation was 45.82 ± 0.18 µg/mL, which is model dependent and not recommended to be used in lieu of testing at higher concentrations. The concentration-response curves and the in-range IC50 status are displayed in figure 7 and the calculated inhibitions at each concentration are displayed in table 4.

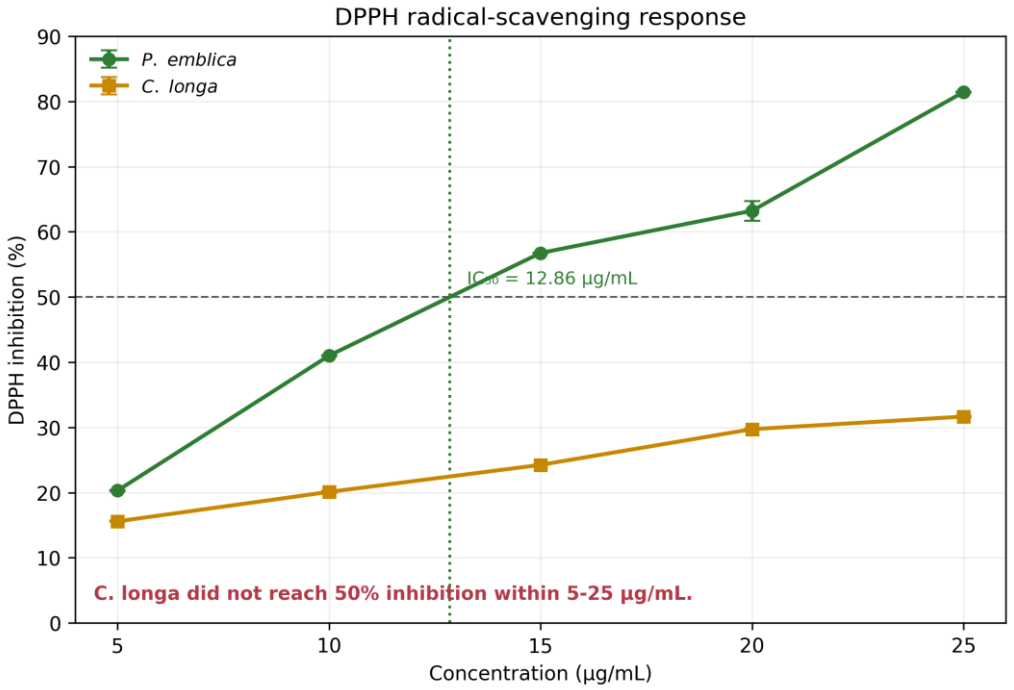

Figure 7. DPPH radical-scavenging responses calculated from measured absorbance. Data are mean ± SD of three analytical measurements. The *C. longa* curve did not bracket 50% inhibition.

Table 4. DPPH inhibition values calculated from absorbance.

Concentration (µg/mL)	P. emblica (%)	C. longa (%)	Difference (percentage points)
5	20.30 ± 0.12	15.59 ± 0.11	4.71
10	41.00 ± 0.06	20.10 ± 0.17	20.90

Concentration (µg/mL)	P. emblica (%)	C. longa (%)	Difference (percentage points)
15	56.75 ± 0.03	24.24 ± 0.05	32.50
20	63.23 ± 1.51	29.72 ± 0.10	33.51
25	81.43 ± 0.08	31.66 ± 0.12	49.78

3.4 ABTS radical-cation-scavenging activity

P. emblica produced 46.89 ± 0.15% ABTS inhibition at 10 µg/mL and 98.97 ± 0.08% at 50 µg/mL. C. longa increased from 5.73 ± 0.21% to 90.65 ± 0.08%. The corresponding IC50 values were 11.13 ± 0.04 µg/mL and 31.46 ± 0.31 µg/mL, respectively. The IC50 of *P. emblica* was 2.83-fold lower, which shows its higher ABTS-scavenging potency. The concentration-response curves and interpolated IC50 values are shown in figure 8 and ABTS inhibition values are shown in table 5.

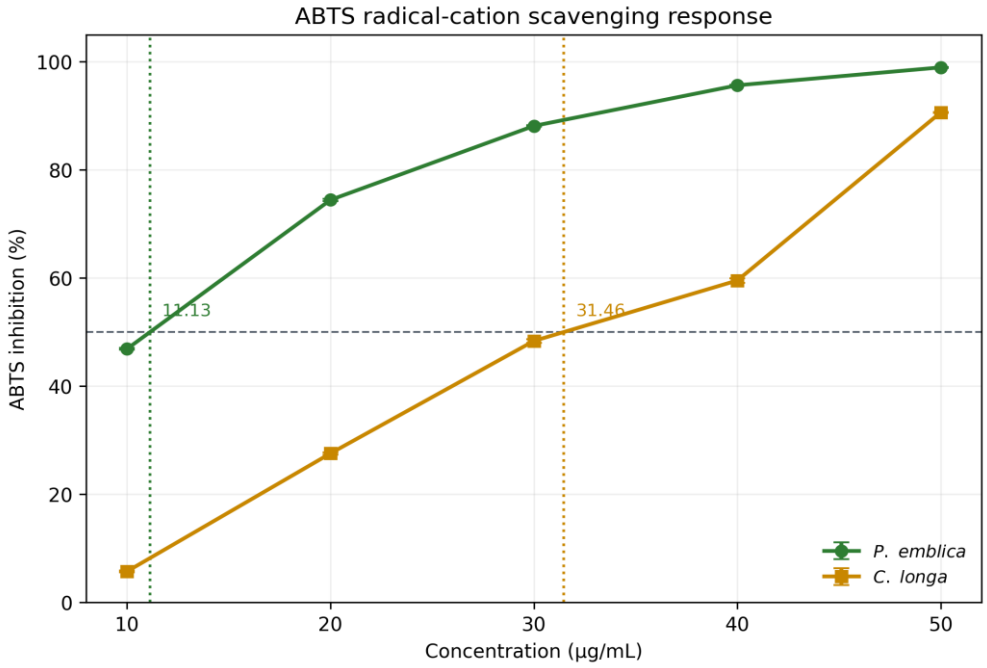

Figure 8. ABTS radical-cation-scavenging responses calculated from measured absorbance. Vertical dotted lines indicate locally interpolated IC50 values.

Table 5. ABTS inhibition values calculated from absorbance.

Concentration (µg/mL)	P. emblica (%)	C. longa (%)	Difference (percentage points)
10	46.89 ± 0.15	5.73 ± 0.21	41.16
20	74.45 ± 0.21	27.56 ± 0.21	46.89
30	88.14 ± 0.15	48.37 ± 0.34	39.78

Natural Resources for Human Health
eISSN: 2583-1194

Concentration (µg/mL)	P. emblica (%)	C. longa (%)	Difference (percentage points)
40	95.66 ± 0.08	59.55 ± 0.41	36.11
50	98.97 ± 0.08	90.65 ± 0.08	8.32

3.5 Integrated antioxidant performance

P. emblica exhibited the superior integrated response throughout the entire tested range. Normalized AUC was 52.96 ± 0.38% for *P. emblica* and 24.42 ± 0.08% for C. longa in the DPPH assay, and 82.80 ± 0.09% versus 45.92 ± 0.27% in the ABTS assay. DPPH inhibition was about 2.57-fold different at the highest concentration tested, while the ABTS responses were converging with both samples reaching high inhibition at 50 µg/mL. Integrated potency and response metrics are compared in Figure 9, the underlying absorbance profiles are defined in Figure 10 and the endpoints are described in Table 6.

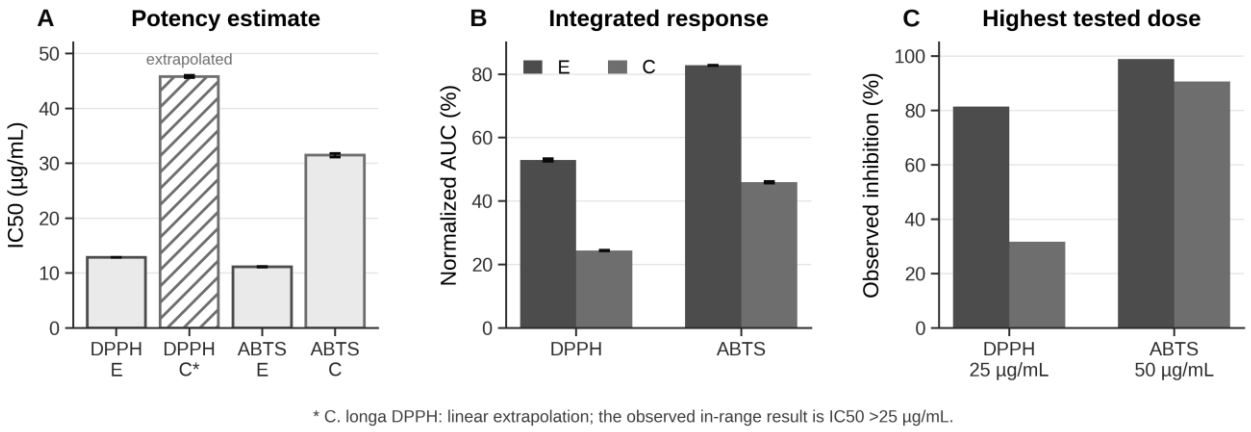

Figure 9. Integrated antioxidant metrics: IC50 estimates (A), normalized concentration-response AUC (B), and observed inhibition at the highest tested concentration (C). The hatched *C. longa* DPPH bar is a linear extrapolation, not an observed IC50.

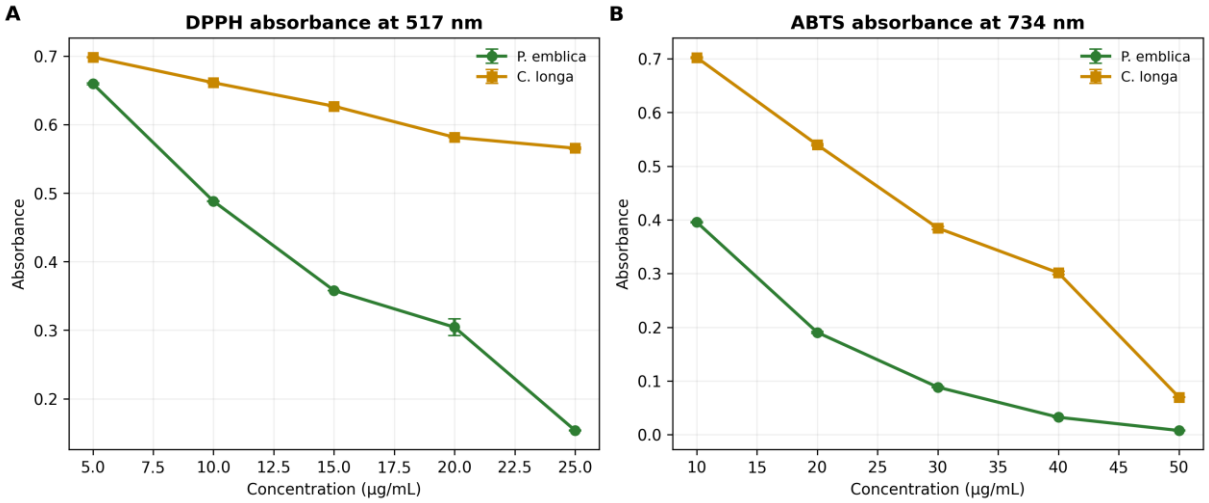

Figure 10. Raw absorbance profiles for DPPH (A) and ABTS (B). The lower absorbance of *P. emblica* across the concentration series is consistent with stronger radical decolourization.

Natural Resources for Human Health
eISSN: 2583-1194

Table 6. Integrated antioxidant metrics.

Metric	P. emblica	C. longa	Comparative interpretation
DPPH IC50 (µg/mL)	12.86 ± 0.01	>25 observed; 45.82 ± 0.18 extrapolated	C did not reach the endpoint in-range
DPPH normalized AUC (%)	52.96 ± 0.38	24.42 ± 0.08	2.17-fold higher for E
DPPH inhibition at 25 µg/mL (%)	81.43 ± 0.08	31.66 ± 0.12	49.78 percentage-point difference
ABTS IC50 (µg/mL)	11.13 ± 0.04	31.46 ± 0.31	2.83-fold lower for E
ABTS normalized AUC (%)	82.80 ± 0.09	45.92 ± 0.27	1.80-fold higher for E
ABTS inhibition at 50 µg/mL (%)	98.97 ± 0.08	90.65 ± 0.08	Both high at the top dose

3.6 GC-MS chromatographic features

GC-MS analysis of *P. emblica* yielded 37 integrated chromatographic peaks and 45 library-annotated features among 54 detected features (83.3% annotation yield). The main chromatographic region occurred between approximately 15.6 and 20.0 min. *C. longa* yielded 23 integrated peaks and 31 library-annotated features among 46 detected features (67.4%), with prominent signals near 3.7, 7.4, 12.0, 14.1-14.8, 17.9, and 22.2 min. MassHunter area values were treated as relative intensities rather than compositional percentages because the listed values did not sum to 100. Figure 11 presents the chromatograms, and Figure 12 shows the dominant signals after within-sample normalization.

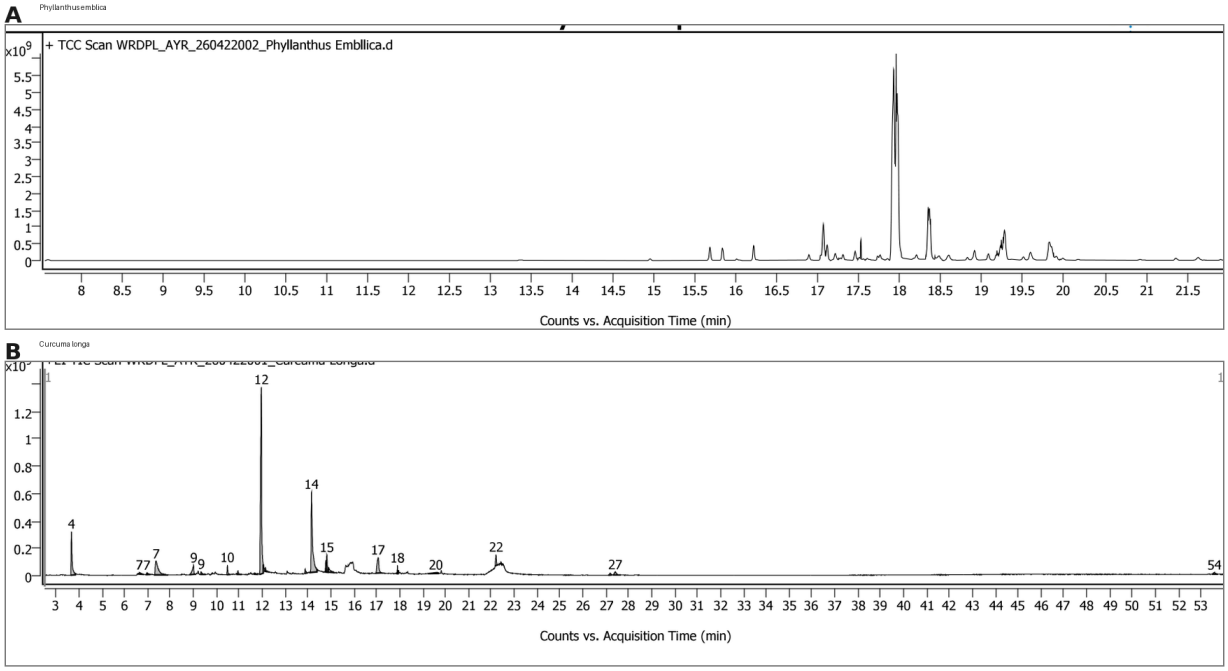

Figure 11. Representative total ion chromatograms for *P. emblica* (A) and *C. longa* (B) obtained using Agilent MassHunter.

Natural Resources for Human Health
eISSN: 2583-1194

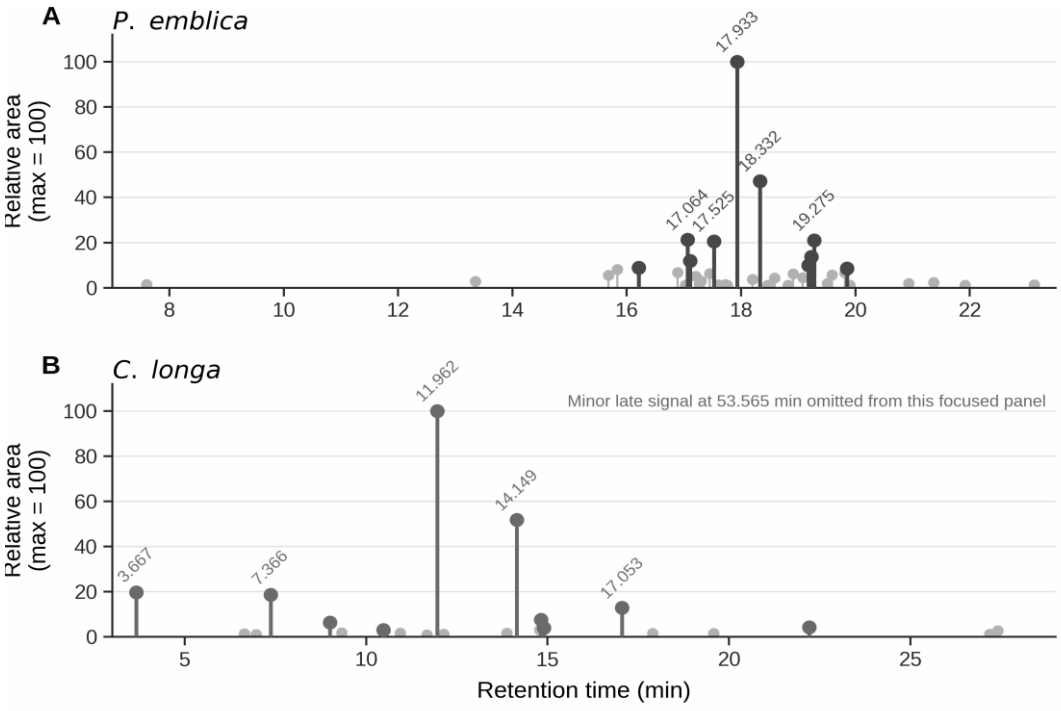

Figure 12. Dominant GC-MS signals plotted from relative area values. The largest peak within each sample was normalized to 100; values do not represent percentage composition.

3.7 GC-MS library-match confidence

The mean match score for *P. emblica* annotations was 58.44 ± 9.03 while that for *C. longa* annotations was 50.75 ± 8.68. For *P. emblica*, 5 annotations scored ≥70, 16 scored 60-69.9, 14 scored 50-59.9, and 10 scored <50. The numbers for C. longa were 1, 5, 8 and 17, respectively. Hence, 53.3% of the library assignments of *P. emblica* and 80.6% of *C. longa* library assignments scored below 60, highlighting the screening level nature of the output. The score distributions and annotation yields are shown in Figure 13 and the feature counts and match-score categories are summarized in Table 7.

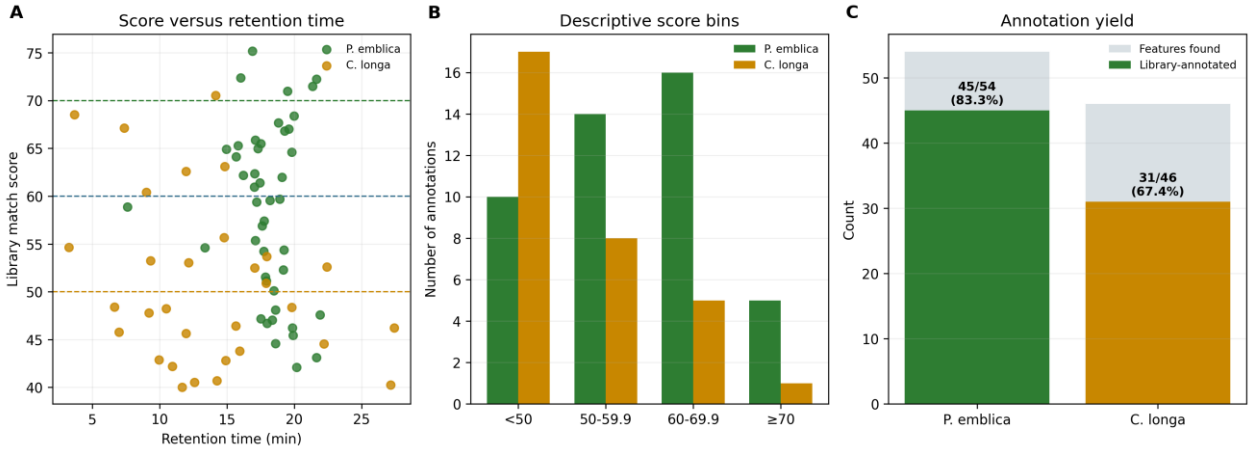

Figure 13. GC-MS library-match assessment: score versus retention time (A), descriptive match-score categories (B), and annotation yield (C).

Table 7. GC-MS feature and match-score summary.

Indicator	P. emblica	C. longa
Integrated chromatographic peaks	37	23
Features found by software	54	46
Library-annotated features	45	31
Annotation yield	83.3%	67.4%
Mean match score	58.44 ± 9.03	50.75 ± 8.68
Score ≥70	5 (11.1%)	1 (3.2%)
Score 60-69.9	16 (35.6%)	5 (16.1%)
Score 50-59.9	14 (31.1%)	8 (25.8%)
Score <50	10 (22.2%)	17 (54.8%)

3.8 Representative tentative annotations

Among the highest-scoring biologically plausible annotations in *P. emblica,* terpene-related structures were the most prevalent, such as 1,3,5-cycloheptatriene, 3,7,7-trimethyl-; spiro[2.4]heptane, 1,5-dimethyl-6-methylene-; dill ether; and 2-caren-4-ol. Alpha-pinene was tentatively annotated at RT 15.832 min with a score of 65.29. The plausible annotations with higher scores in C. longa were 1,2,3-benzenetriol, furfural, 2H-pyran-2,6(3H)-dione, trans-cinnamic acid, 3-furancarboxylic acid, a trihydroxybenzoic-acid annotation, and ar-turmerone. The match score between ar and turmerone was 50.90 and this was interpreted with caution. The highest-scoring plausible annotations are compared in figure 14 and selected matches for each species are listed in tables 8 and 9.

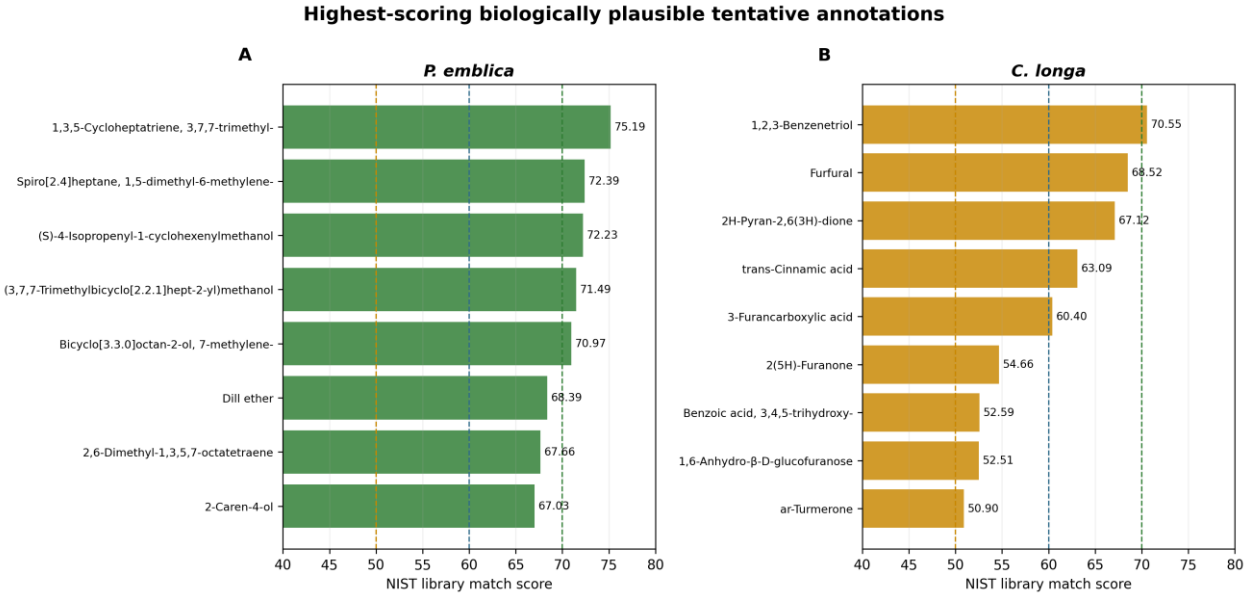

Figure 14. Highest-scoring biologically plausible tentative GC-MS annotations. Obvious background or artefact candidates were excluded from this display but retained in the audit.

Table 8. Selected tentative annotations for *P. emblica*.

Tentative annotation	RT (min)	Formula	Library score	Interpretive note
1,3,5-Cycloheptatriene, 3,7,7-trimethyl-	16.889	C10H14	75.19	Highest score; tentative terpene-related match
Spiro[2.4]heptane, 1,5-dimethyl-6-methylene-	16.011	C10H16	72.39	Higher-score terpene-related match
(S)-4-Isopropenyl-1-cyclohexenylmethanol	21.640	C10H16O	72.23	Oxygenated terpene-like match
(3,7,7-Trimethylbicyclo[2.2.1]hept-2-yl)methanol	21.364	C11H20O	71.49	Oxygenated bicyclic match
Bicyclo[3.3.0]octan-2-ol, 7-methylene-	19.503	C9H14O	70.97	Tentative oxygenated bicyclic constituent
Dill ether	19.984	C10H16O	68.39	Plausible oxygenated monoterpenoid
2,6-Dimethyl-1,3,5,7-octatetraene	18.822	C10H14	67.66	Unsaturated hydrocarbon match
2-Caren-4-ol	19.593	C10H16O	67.03	Plausible oxygenated monoterpenoid
alpha-Pinene	15.832	C10H16	65.29	Representative monoterpene; unconfirmed
2-Methoxy-4-vinylphenol	13.354	C9H10O2	54.63	Phenolic volatile; low-score match

Table 9. Selected tentative annotations for *C. longa*.

Tentative annotation	RT (min)	Formula	Library score	Interpretive note
1,2,3-Benzenetriol	14.148	C6H6O3	70.55	Highest score; phenolic match
Furfural	3.665	C5H4O2	68.52	Plausible furan; may reflect processing or thermal chemistry
2H-Pyran-2,6(3H)-dione	7.366	C5H4O3	67.12	Oxygenated heterocycle
trans-Cinnamic acid	14.823	C9H8O2	63.09	Phenylpropanoid-related match

Natural Resources for Human Health
eISSN: 2583-1194

Tentative annotation	RT (min)	Formula	Library score	Interpretive note
3-Furancarboxylic acid	9.002	C5H4O3	60.40	Furan-derived acid
2(5H)-Furanone	3.245	C4H4O2	54.66	Furanone match
Benzoic acid, 3,4,5-trihydroxy-	22.428	C7H6O5	52.59	Provisional gallic-acid-like match; confirmation required
1,6-Anhydro-beta-D-glucofuranose	17.064	C6H10O5	52.51	Possible thermal carbohydrate product
ar-Turmerone	17.901	C15H20O	50.90	Chemotaxonomically plausible but low-score
4-Decanol	19.815	C10H22O	48.36	Very-low-score aliphatic match

3.9 Integrated evidence and photographic documentation

Integration of the quantitative and chromatographic endpoints showed that the largest between-species difference occurred in TPC, followed by DPPH response and normalized AUC. TFC values were similar, while ABTS inhibition converged at the highest concentration. The assay photographs documented concentration-dependent decolorization in the DPPH and ABTS series and distinct color development in the TPC and TFC reactions. Figure 115 summarizes the relative evidence and analytical quality checkpoints, and Figure 15 (B) presents the assay photographs.

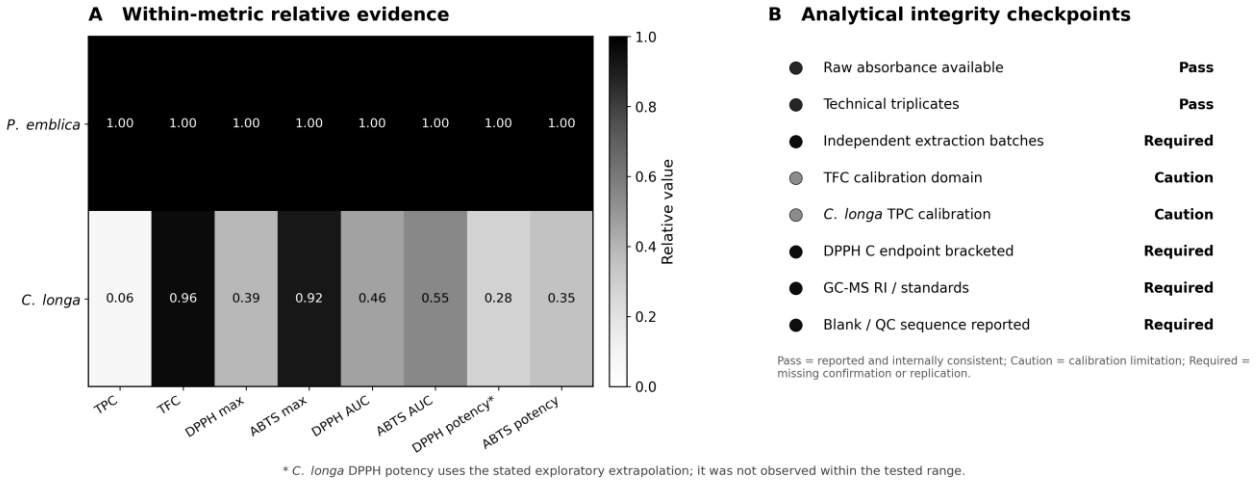

Figure 15. Relative evidence matrix (A) and analytical integrity checkpoints (B). Each matrix metric was normalized to the higher of the two sample values.

4. DISCUSSION

4.1 Comparative phenolic and antioxidant profile

The principal finding was the markedly stronger phenolic and radical-scavenging profile of *P. emblica*. The approximately 16-fold TPC difference was accompanied by a 2.17-fold higher DPPH normalized AUC and a 1.80-fold higher ABTS normalized AUC. This concordance is chemically plausible because hydrolyzable tannins and gallic- and ellagic-acid-related constituents

are prominent in *P. emblica* fruit (Yang & Liu, 2014). Phenolics isolated from the fruit have shown substantial radical-scavenging activity (Liu et al., 2008).

The similarity of the TFC values suggests that the contrast between species was not captured by the aluminum-chloride-reactive fraction alone. The assay does not respond equally to all flavonoid subclasses (Khoddami et al., 2013). The Folin-Ciocalteu method reflects reducing capacity and can respond to nonphenolic reductants (Prior et al., 2005). Extraction and matrix composition can further influence apparent phenolic values (Dai & Mumper, 2010). Consequently, TPC should be interpreted as a comparative reducing index rather than a definitive mass of structurally confirmed polyphenols. The calibration-domain findings also indicate that the TFC results and *C. longa* TPC value require cautious interpretation.

4.2 Assay-dependent differences between DPPH and ABTS

The same overall ranking was obtained for the DPPH and ABTS assays, but different response shapes were observed. In both the assays *P. emblica* exhibited 50% inhibition at the lowest concentration. *C. longa* failed to achieve 50% DPPH inhibition at 25 µg/mL and achieved >90% ABTS inhibition at 50 µg/mL. In organic media, steric accessibility and reaction kinetics can affect the DPPH reaction (Huang et al., 2005). ABTS is sensitive to a wider variety of electron donors, both hydrophilic and lipophilic (Re et al., 1999). It is therefore preferable to interpret either of these methods alone with complementary antioxidant assays (Apak et al., 2013).

Direct calculation of the IC50 from the concentration-response data is important as the estimate is dependent on whether the 50% endpoint is bracketed. If concentrations of adjacent areas are below and above 50%, it is appropriate to interpolate locally, but extrapolation beyond the range of the tested concentrations is less certain. Therefore, the *C. longa* DPPH result is mainly expressed as IC50 >25 µg/mL, and the extrapolated value is only included to show the concentration range.

4.3 Interpretation of the *C. longa* profile

The low TPC of *C. longa* does not mean that there are no biologically relevant turmeric constituents. Conventional GC-MS did not sufficiently reflect the presence of curcuminoids unless they were derivatized or validated in their preparation (Fahmy et al., 2023). The processing and extraction have significant impact on antioxidant activity and volatile composition of turmeric (Singh et al., 2010; Anuradha et al., 2015; Yun et al., 2025). The composition of essential oils is also different from one Curcuma material to another (L. Zhang et al., 2017). Thus, complementary HPLC-DAD or LC-MS analysis would be helpful to quantify curcumin, demethoxycurcumin and bisdemethoxycurcumin in addition to the volatile profile.

4.4 GC-MS annotation confidence and artefact control

GC-MS screening gave clear chemical fingerprints and did not allow for definitive compound identification. Most of the annotations were below 70% match and some of the hits were chemically unlikely such as tetrafluoromethane, krypton, and silane derivatives in the *C. longa* library output. These assignments may be due to background, column or septum bleed, solvent contamination, co-elution, low intensity peaks, or to nonspecific library matching and were not used in the biological interpretation.

The output of *P. emblica* also contained turmeric associated annotations such as dihydro-ar-turmerone and turmeronol A. They can be due to low specificity matching, unresolved co-elution, carry-over, cross-contamination, or unusual sample chemistry. Priority compounds would need to be confirmed by replicate injections, authentic standards and retention-index comparison. This tentative status is in line with the principles of minimum chemical-analysis reporting (Sumner et al., 2007). Explicit communication of identification confidence should be made (Schymanski et al., 2014). Analytical stability can be further ensured with routine blanks and pooled quality-control samples (Broadhurst et al., 2018).

4.5 Biological relevance

The significant radical-scavenging profile of *P. emblica* indicates its prioritization for further biological studies, and the unique *C. longa* volatile profile indicates complementary chemistry. In vitro antioxidant assays, however, do not show bioavailability, cellular protection or hepatoprotective efficacy. Any therapeutic interpretation should thus be validated in suitable cellular or animal models, with biochemical, oxidative-stress, inflammatory and histopathological endpoints.

4.6 Positive aspects and limitations

The advantages of this work are the application of complementary spectrophotometric assays, the retention of triplicate absorbance data, concentration-response analysis, integration with GC-MS profiling and documented botanical authentication. Limitations are the use of technical instead of independent extraction replicates, incomplete details of sample preparation and GC-MS acquisition, the sample absorbances were not in the stated calibration range for TFC and *C. longa* TPC, no reference antioxidant was used, and no retention indices or authentic standards were used to confirm GC-MS results. These factors affect absolute quantification and identification at the compound level, but do not affect the observed ranking of radical-scavenging activity. Table 10 provides a summary of the main analytical issues and how they have been addressed in the current study.

Analytical consideration	**Observation**	**Treatment in the present study / recommended follow-up**
TFC calibration range	Sample absorbances 0.015-0.025 versus lowest standard 0.426	Values interpreted cautiously; future measurements should use an appropriate sample concentration and validated range
C. longa TPC calibration range	Sample absorbances 0.021-0.022 versus lowest standard 0.072	Value interpreted cautiously; confirmation with lower dilution or an extended validated range is recommended
DPPH C value at 10 µg/mL	One tabulated percentage conflicted with the corresponding absorbance	The absorbance-derived mean value was used in the analysis
ABTS C value at 50 µg/mL	One tabulated percentage conflicted with the corresponding absorbance	The absorbance-derived mean value was used in the analysis
ABTS E IC50	The 50% response was bracketed between 10 and 20 µg/mL	Local interpolation was used to estimate IC50
DPPH C IC50	Fifty percent inhibition was not reached by 25 µg/mL	The observed result is reported as >25 µg/mL; extrapolation is secondary only
GC-MS compound identity	Retention indices and authentic standards were not available	All compound names are reported as tentative annotations
Replication	Three analytical measurements were obtained from one material per species	Results are summarized descriptively without inferential testing

Table 10. Analytical considerations and treatment of the corresponding data.

5. CONCLUSION AND FUTURE WORK

Under the experimental conditions used, the phenolics and in vitro radical-scavenging profile of authenticated *P. emblica* was markedly superior to that of *C. longa*. It had a TPC of about 16 times higher, and it had lower IC50 values and higher normalized AUC in both DPPH and ABTS assays. TFC values were comparable, but with limitations in the calibration domain, caution is advised in interpretation. GC-MS showed different volatile and semi-volatile fingerprints, and there were more integrated peaks in *P. emblica* with generally higher library-match scores. All compound names are tentative, as no retention-index or authentic-standard confirmation was done. The results, when combined, provide the basis for comparing the two botanicals for standardization and for future testing of their biological activity. Future perspectives are in silico model should be integrated to prioritize the tentatively identified phytochemicals from both plants against molecular targets associated with the oxidative stress, inflammation, lipid dysregulation, and hepatotoxicity caused by microplastic exposure. The most plausible compounds and pathways might be selected for further validation in cell and animal models using molecular docking, network pharmacology, molecular-dynamics simulation, and ADME/toxicity prediction.

Declarations

Ethics approval: Not applicable; the study involved only plant materials and in vitro chemical assays.

Consent for publication: Not applicable.

Competing interests: The author has no competing interests.

Funding: The work was carried out using institutional research facilities.

Author contributions: B Gyani Priyanka Patnaik, R.Sanjeevi, Dushyant Singh Chauhan and J.Anuradha, all the authors have equally contributed in Conceptualization, investigation, methodology, formal analysis, visualization, data curation, writing - original draft, and writing - review and editing.

Data availability: All data generated or analysed during this study are included in this article. Additional raw spectrophotometric and chromatographic files are available from the corresponding author on reasonable request.

Acknowledgements: The author acknowledges the Department of Botany, University of Rajasthan, Jaipur, for botanical authentication of the plant materials and NIMS University for institutional support.

REFERENCES

[1] Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, *6*(4), 42. https://doi.org/10.3390/plants6040042

[2] Apak, R., Gorinstein, S., Böhm, V., Schaich, K. M., Özyürek, M., & Güçlü, K. (2013). Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC Technical Report). *Pure and Applied Chemistry*, *85*(5), 957-998. https://doi.org/10.1351/PAC-REP-12-07-15

[3] Anuradha, J., Abbasi, T., & Abbasi, S. A. (2015). An eco-friendly method of synthesizing gold nanoparticles using an otherwise worthless weed *Pistia stratiotes* L. **Journal of Advanced Research, 6**(5), 711–720.

[4] Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*, 1199-1200. https://doi.org/10.1038/1811199a0

[5] Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

[6] Broadhurst, D., Goodacre, R., Reinke, S. N., Kuligowski, J., Wilson, I. D., Lewis, M. R., & Dunn, W. B. (2018). Guidelines and considerations for the use of system suitability and quality control samples in mass spectrometry assays applied in untargeted clinical metabolomic studies. *Metabolomics*, *14*(6), 72. https://doi.org/10.1007/s11306-018-1367-3

[7] Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, *15*(10), 7313-7352. https://doi.org/10.3390/molecules15107313

[8] Deng, Y., Zhang, Y., Lemos, B., & Ren, H. (2017). Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. *Scientific Reports*, *7*, 46687. https://doi.org/10.1038/srep46687

[9] Fahmy, N. M., Fayez, S., Uba, A. I., Shariati, M. A., Aljohani, A. S. M., El-Ashmawy, I. M., Batiha, G. E.-S., Eldahshan, O. A., Singab, A. N., & Zengin, G. (2023). Comparative GC-MS analysis of fresh and dried *Curcuma* essential oils with insights into their antioxidant and enzyme inhibitory activities. *Plants*, *12*(9), 1785. https://doi.org/10.3390/plants12091785

[10] Forutan, G., Farbood, Y., Lamoochi, Z., & Nardast, S. (2026). Chronic microplastic exposure dose-dependently induces liver failure via oxidative stress, inflammation, and apoptosis in rats. *Journal of Applied Toxicology*, *46*(5), 1687-1696. https://doi.org/10.1002/jat.70002

[11] Huang, C.-Z., Tung, Y.-T., Hsia, S.-M., Wu, C.-H., & Yen, G.-C. (2017). The hepatoprotective effect of *Phyllanthus emblica* L. fruit on high fat diet-induced non-alcoholic fatty liver disease (NAFLD) in SD rats. *Food & Function*, *8*(2), 842-850. https://doi.org/10.1039/C6FO01585A

[12] Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, *53*(6), 1841-1856. https://doi.org/10.1021/jf030723c

[13] Khoddami, A., Wilkes, M. A., & Roberts, T. H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules*, *18*(2), 2328-2375. https://doi.org/10.3390/molecules18022328

[14] Li, T., Yao, Z., Huang, Y., Li, R., Zhang, L., Chen, R., & Gu, W. (2025). Chronic waterborne exposure to polystyrene microplastics induces Kupffer cell polarization imbalance and hepatic lipid accumulation. *The FASEB Journal*, *39*(17), e70980. https://doi.org/10.1096/fj.202500910RR

[15] Liu, M., Mu, J., Wang, M., Hu, C., Ji, J., Wen, C., & Zhang, D. (2023). Impacts of polypropylene microplastics on lipid profiles of mouse liver uncovered by lipidomics analysis and Raman spectroscopy. *Journal of Hazardous Materials*, *458*, 131918. https://doi.org/10.1016/j.jhazmat.2023.131918

[16] Liu, X., Cui, C., Zhao, M., Wang, J., Luo, W., Yang, B., & Jiang, Y. (2008). Identification of phenolics in the fruit of emblica (*Phyllanthus emblica* L.) and their antioxidant activities. Food Chemistry, 109(4), 909-915. https://doi.org/10.1016/j.foodchem.2008.01.071

[17] Lu, Y., Tu, N., Zhang, H., Li, B., Qi, G., Chen, S., Pang, Y., & Chen, L. (2026). Association between microplastic exposure and hepatic dysfunction in humans: A cross-sectional study of occupationally exposed individuals. *Environment & Health*. Advance online publication. https://doi.org/10.1021/envhealth.5c00716

[18] Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, *53*(10), 4290-4302. https://doi.org/10.1021/jf0502698

[19] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9-10), 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

[20] Reyes-Gordillo, K., Segovia, J., Shibayama, M., Vergara, P., Moreno, M. G., & Muriel, P. (2007). Curcumin protects against acute liver damage in the rat by inhibiting NF-kappaB, proinflammatory cytokines production and oxidative stress. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1770*(6), 989-996. https://doi.org/10.1016/j.bbagen.2007.02.004

[21] Schymanski, E. L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H. P., & Hollender, J. (2014). Identifying small molecules via high resolution mass spectrometry: Communicating confidence. *Environmental Science & Technology*, *48*(4), 2097-2098. https://doi.org/10.1021/es5002105

[22] Singh, G., Kapoor, I. P. S., Singh, P., de Heluani, C. S., de Lampasona, M. P., & Catalan, C. A. N. (2010). Comparative study of chemical composition and antioxidant activity of fresh and dry rhizomes of turmeric (*Curcuma longa* Linn.). Food and Chemical Toxicology, 48(4), 1026-1031. https://doi.org/10.1016/j.fct.2010.01.015

[23] Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*(3), 144-158.

[24] Sumner, L. W., Amberg, A., Barrett, D., Beale, M. H., Beger, R., Daykin, C. A., Fan, T. W.-M., Fiehn, O., Goodacre, R., Griffin, J. L., Hankemeier, T., Hardy, N., Harnly, J., Higashi, R., Kopka, J., Lane, A. N., Lindon, J. C., Marriott, P., Nicholls, A. W., . . . Viant, M. R. (2007). Proposed minimum reporting standards for chemical analysis: Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics*, *3*(3), 211-221. https://doi.org/10.1007/s11306-007-0082-2

[25] Tripathi, S., Sanjeevi, R., Anuradha, J., Chauhan, D. S., & Rathoure, A. K. (2022). *Nano-bioremediation: Nanotechnology and bioremediation*. In **Research Anthology on Emerging Techniques in Environmental Remediation** (pp. 135–149). IGI Global.

[26] Yang, B., Kortesniemi, M., Liu, P., Karonen, M., & Salminen, J.-P. (2012). Analysis of hydrolyzable tannins and other phenolic compounds in emblic leafflower (*Phyllanthus emblica* L.) fruits by HPLC-ESI-MS. Journal of Agricultural and Food Chemistry, 60(35), 8672-8683. https://doi.org/10.1021/jf302925v

[27] Yang, B., & Liu, P. (2014). Composition and biological activities of hydrolyzable tannins of fruits of *Phyllanthus emblica*. Journal of Agricultural and Food Chemistry, 62(3), 529-541. https://doi.org/10.1021/jf404703k

[28] Yang, Y., Li, J., Li, X., Huang, Q., Jiang, W., Zhang, W., Jiang, Y., & Tang, Y. (2026). UHPLC-MS/MS and GNPS elucidate gallic acid transformation and accumulation mechanism in *Phyllanthus emblica* L. during hot air drying. *Journal of Agricultural and Food Chemistry*, *74*(9), 8012-8026. https://doi.org/10.1021/acs.jafc.5c13849

[29] Yun, C.-I., Kim, M.-A., Jung, S. K., Kim, Y.-J., & Lee, J. (2025). Thermal processing effects on phytochemical profiles, antioxidant activity, and anti-inflammatory potential of extracts from dried rhizomes of *Curcuma longa* L. evaluated by UHPLC-MS/MS and in vitro assays. *Food Chemistry*, *496*, 146756. https://doi.org/10.1016/j.foodchem.2025.146756

[30] Zhang, J., Miao, D., Zhu, W.-F., Xu, J., Liu, W.-Y., Kitdamrongtham, W., Manosroi, J., Abe, M., Akihisa, T., & Feng, F. (2017). Biological activities of phenolics from the fruits of *Phyllanthus emblica* L. (Euphorbiaceae). Chemistry & Biodiversity, 14(12), e1700404. https://doi.org/10.1002/cbdv.201700404

[31] Zhang, L., Yang, Z., Chen, D., Huang, Z., Li, Y., Lan, X., Su, P., Pan, W., Zhou, W., Zheng, X., & Du, Z. (2017). Variation on composition and bioactivity of essential oils of four common *Curcuma* herbs. *Chemistry & Biodiversity*, *14*(11), e1700280. https://doi.org/10.1002/cbdv.201700280

[32] Zhishen, J., Mengcheng, T., & Jianming, W. (1999). 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