Natural Sciences Engineering and Technology Journal
Vol. 6, No. 1, 2026, pp. 34-50 · e-ISSN 2807-2820 *journal homepage: nasetjournal.com*

Systematic Review / Meta-analysis

Green energy-assisted versus conventional solvent extraction for the recovery of phenolic antioxidants from marine macroalgae: A meta-analysis

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ARTICLE INFO

Article history:
Received: July 1, 2026
Revised: July 15, 2026
Accepted: August 2, 2026
Available Online: August 26, 2026
Published: August 28, 2026
Keywords:
Green chemistry
Macroalgae
Phenolic antioxidants
Ultrasound-assisted extraction
Sustainable biorefinery
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DOI: https://doi.org/10.37275/nasetjournal.v6i1.77

ABSTRACT

Background: Marine macroalgae are a renewable source of phenolic antioxidants. Energy-assisted extraction may replace prolonged solvent-intensive maceration, but its effect on recovery had not been quantitatively pooled.

Methods: PubMed and Europe PMC were searched. Of 493 de-duplicated records, 35 full texts were assessed. Eligible reports compared energy-assisted and conventional extraction using paired arms from one homogenised macroalgal biomass. Hedges' g was pooled using random-effects models with cluster-robust inference; the ratio of means was a secondary measure. Risk of bias used laboratory analogues of five Cochrane domains.

Results: Thirteen studies yielded 35 comparisons across 22 species and 93 estimates; ultrasound contributed 56 estimates, brown seaweeds 73, red seaweeds 20, and green seaweeds none. Energy-assisted extraction increased phenolic antioxidant recovery (cluster-robust Hedges' g = 8.62, 95% CI 2.98 to 14.26, p = 0.0063; ratio of means 1.385, 95% CI 1.149 to 1.671, a 38.5% gain) in a median 10 versus 960 minutes. The advantage persisted in 12 duration-matched comparisons. Heterogeneity was high (I^2 = 87.8%); the prediction interval -2.26 to 16.16 included zero. No moderator survived robust testing. Supercritical carbon dioxide recovered 51.8% less than conventional extraction.

Conclusion: Energy-assisted extraction recovered about 39% more phenolic antioxidant activity about 21 times faster. Direction was robust, but magnitude and certainty remained uncertain; a green label did not guarantee superior recovery.

Both authors have reviewed and approved the final version of the manuscript.

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1. Introduction

Marine macroalgae accumulate a structurally distinctive family of phenolic secondary metabolites, and interest in recovering them has grown steadily as the pharmaceutical, food and cosmetic industries have sought renewable alternatives to synthetic antioxidants.[1,2] Brown seaweeds elaborate phlorotannins, oligomers of phloroglucinol that have no terrestrial counterpart, while red and green seaweeds accumulate bromophenols, mycosporine-like amino acids and simpler phenolic acids.[3,4] Extracts of these organisms have shown antioxidant, photoprotective, anti-inflammatory, cholinesterase-inhibitory and antiproliferative activity in laboratory models,[5-8] and their incorporation into food and cosmetic matrices has been demonstrated at pilot scale.[9] The commercial obstacle has rarely been whether useful molecules are present; it has been how efficiently, how cleanly and how quickly they can be liberated from a tough, highly hydrated and polysaccharide-rich cell wall.[10]

Conventional recovery relies on prolonged solid-liquid contact. Biomass is macerated, stirred or refluxed in an organic solvent for periods measured in hours or days, and the process is limited by passive diffusion across an intact cell wall in which phenolics are extensively bound to alginate, fucoidan and cellulose.[10,11] The approach consumes large solvent volumes, occupies equipment for long periods and exposes thermolabile phenolics to extended heating. The alternative is to stop waiting for diffusion and to apply energy that disrupts the cell wall directly. Throughout this article that alternative is termed **energy-assisted extraction**, a single term used consistently for the whole family of techniques; the word green is reserved for discussion of environmental credentials, because one of the findings reported below is that the two are not synonymous. Acoustic cavitation in ultrasound-assisted extraction generates microjets that perforate cell walls; microwave irradiation heats intracellular water volumetrically until the cell ruptures; carbohydrases in enzyme-assisted extraction hydrolyse the wall matrix under mild aqueous conditions; and pressurised liquid, subcritical water and supercritical fluid systems manipulate solvent polarity and diffusivity under elevated temperature and pressure.[12-14]

Evidence that these technologies outperform maceration has accumulated in adjacent fields. In terrestrial matrices energy-assisted extraction has repeatedly increased polyphenol recovery from fruit pomace, leaves, seeds and agro-industrial residues,[15-19] and comparative work on distillery stillage and peach by-products has placed ultrasound, microwave and conventional extraction side by side within a single design.[20,21] Deep eutectic solvents combined with ultrasound have extended the same logic to solvent selection,[22,23] and response-surface optimisation of green protocols is now routine.[24-26] Macroalgal biomass,

however, differs from terrestrial biomass in wall chemistry, water content and mineral load, so results cannot simply be transferred.

Within phycology the primary literature is substantial but fragmented. Individual laboratories report single-species comparisons in incompatible units, some expressing phenolic content per gram of dry biomass and others per gram of recovered extract, and antioxidant capacity is variously expressed as percentage inhibition, half-maximal inhibitory concentration or Trolox equivalents.[27-30] Published meta-analyses involving algae and antioxidants have addressed crop biostimulation, methane mitigation in ruminants, blood pressure and anthropometric outcomes in humans; none has pooled the effect of the extraction process itself. The field's working belief that green extraction is superior has therefore rested on narrative synthesis rather than on a quantitative estimate, and neither the magnitude of the advantage, the consistency across algal lineages, nor the possibility that the advantage is an artefact of mismatched process conditions has been tested.

This gap has practical consequences that reach beyond process engineering. Because the extraction platform determines which phenolics are liberated and in what proportion, it determines the material that any subsequent pharmacological assay actually receives; the process question therefore precedes the pharmacological one rather than following it, and inconsistencies in the reported potency of macroalgal extracts may in part be inconsistencies of extraction.[6,30] The consequences are sharpest in tropical archipelagic economies, where red seaweeds are cultivated at scale and where the choice of extraction platform determines both capital outlay and solvent cost.[31,32,33] A process engineer deciding whether to install an ultrasonic reactor needs to know how much additional phenolic material is recovered, not merely that a difference has been reported somewhere.

The novelty of this study lies in being the first quantitative synthesis to pool the effect of the extraction process itself, rather than the effect of algal extracts, on the recovery of phenolic antioxidants from marine macroalgal biomass; in restricting the evidence base to paired arms drawn from a single homogenised biomass within a single laboratory, which removes between-species and between-laboratory confounding at source; in reporting the standardised mean difference alongside the ratio of means, so that a measure inflated by analytical-replicate dispersions is accompanied by one that is not; and in subjecting every moderator claim to cluster-robust inference, which revealed that several apparently strong process-level effects did not survive correct treatment of the dependence between estimates arising from the same study. **The aim of this study was to** estimate the pooled effect of green extraction relative to conventional solvent extraction on total phenolic content, phlorotannin content and antioxidant capacity in marine macroalgae, to quantify the accompanying reduction in processing time, and to determine whether the advantage depended on algal lineage, extraction technology or the degree to which the two arms were matched for solvent, temperature and duration.

2. Methods

2.1. Reporting and registration

The review was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.[34] The review was not prospectively registered.

2.2. Search strategy

Two bibliographic databases were searched: PubMed, through the National Center for Biotechnology Information E-utilities interface, and Europe PMC. Three search arms were executed. The initial arm interrogated PubMed and returned 84 records. A verification arm re-interrogated PubMed with a broadened technology vocabulary and returned 60 unique records. A third arm was added against Europe PMC after the PubMed-only strategy was found to under-retrieve, and returned 396 records; that arm recovered the two methodologically strongest reports in the final pool, and its addition is reported here rather than concealed.

The Boolean string, given in the syntax of the final Europe PMC arm, was:

("ultrasound-assisted extraction" OR "ultrasonic extraction" OR "microwave-assisted extraction" OR "enzyme-assisted extraction" OR "supercritical" OR "subcritical water" OR "pressurised liquid extraction" OR "pressurized liquid extraction" OR "deep eutectic solvent" OR "green extraction") AND (seaweed OR macroalga OR macroalgae OR Rhodophyta OR Phaeophyceae OR Chlorophyta OR "marine alga") AND ("total phenolic" OR "phenolic content" OR polyphenol OR phlorotannin OR antioxidant OR DPPH OR ABTS OR FRAP) AND (conventional OR maceration OR Soxhlet OR "solid-liquid extraction" OR stirring OR reflux OR comparison OR compared) NOT (review[Publication Type])

Reference lists of the retrieved reports were inspected for additional eligible studies. No language restriction was applied. Searching was performed by a single reviewer, which is stated as a limitation rather than presented as a dual-reviewer process.

2.3. Eligibility criteria

Reports were eligible when all of the following held. The biomass was a marine macroalga of the Rhodophyta, Phaeophyceae or Chlorophyta; all microalgae and all freshwater and terrestrial biomass were excluded. At least one green or energy-assisted extraction technique was applied. A comparator arm was run on the same homogenised biomass within the same report, so that the contrast was internal rather than across laboratories. At least one of total phenolic content, phlorotannin content, DPPH radical-scavenging, ABTS radical-scavenging, ferric-reducing antioxidant power or gravimetric extract yield was reported. Both arms were reported as a mean with a dispersion measure, so that a standardised mean difference could be computed. Reviews, meta-analyses, conference abstracts, theses and book chapters were excluded.

The protocol specification of randomised controlled trials or cohort studies could not be applied, because neither design exists in extraction science: there are no participants to randomise and no cohort to follow. The design that occupies the equivalent evidential position is the controlled comparative laboratory experiment, in which a single homogenised biomass is divided between two or more extraction methods under otherwise identical conditions and replicate determinations are reported as mean and standard deviation. This design constrains confounding more tightly than a cohort, because biomass, laboratory, analyst, instrument and assay are held constant across arms. Reviews were excluded as specified.

2.4. Data extraction

For each report the following were transcribed exactly as printed, without rounding, recomputation or unit conversion: bibliographic identifiers; species and algal phylum; geographic origin of the biomass; the green technique and its

process conditions, comprising power, frequency, duration, temperature, solvent identity and concentration, and solid-to-solvent ratio; the comparator method and its corresponding conditions; the number of analytical replicates; and, for each outcome, the mean and dispersion in each arm together with the unit as printed and the source table. Values absent from a report were recorded as not reported; values that were present but ambiguous, internally inconsistent or of uncertain unit were flagged for adjudication and their handling recorded. Where a report nominated an optimal green condition, that condition was extracted; where several arms existed, each was retained as a separate comparison.

Two data-handling conventions were fixed in advance. Five estimates were expressed as half-maximal inhibitory concentrations, for which a lower value denotes greater activity; their sign was reversed before pooling so that a positive effect always favoured energy-assisted extraction, and the forest plot legend restates this convention. One comparator concentration was censored rather than missing, because the macerated extract reached only 38.02% scavenging at the highest concentration tested; it was excluded rather than imputed. The direction of that censoring is not neutral. The comparator failed to reach the threshold, so the excluded observation would have favoured the energy-assisted arm, and its exclusion therefore biases the pooled estimate downward rather than upward. One study never stated a replicate count anywhere in its text; three replicates were assumed for its three estimates, the assumption is recorded here, and a sensitivity analysis excluding that study entirely is reported. All numeric values were subsequently cross-checked against the source reports by script.

2.5. Risk of bias assessment

The protocol specified the Cochrane risk-of-bias tool for randomised trials. That instrument appraises randomised trials of interventions in human participants, and its domains, which concern randomisation, deviation from intended interventions, missing outcome data, measurement of the outcome and selection of the reported result, have no referent where there is no allocation sequence, no participant and no attrition. The five domains were therefore mapped one-to-one onto their structural analogues for the controlled comparative laboratory experiment: allocation of biomass to arms; matching of process conditions between arms; completeness of replicate and dispersion data; measurement of the outcome; and selection of the reported result. Each domain was rated low, some concerns or high, and an overall judgement assigned by the standard algorithm in which the least favourable domain governs.

2.6. Statistical analysis

Effect sizes were expressed as Hedges' g, the standardised mean difference with the small-sample correction. The standardised form was necessary because phenolic content is reported on incompatible denominators across laboratories. Estimates were pooled under a random-effects model with the DerSimonian-Laird between-study variance estimator, as specified in the protocol.

Because most reports contributed several estimates measured on the same extracts, the assumption of independence required by that model was violated, and the resulting confidence interval is anti-conservative. Inference was therefore drawn from cluster-robust variance estimation with the study as the clustering unit, and a three-level random-effects model fitted by restricted maximum likelihood was used to partition heterogeneity between studies and between comparisons within studies. The ratio of means was computed as a secondary measure because the dispersions in this literature are analytical replicate standard deviations describing the repeatability of a spectrophotometric assay rather than biological variability; dividing by such dispersions inflates the standardised mean difference to values with no counterpart in clinical research. Both measures are reported throughout.

Heterogeneity was quantified by the I^2 statistic, the between-study variance and a 95% prediction interval. Subgroup analyses were pre-specified for outcome domain, algal phylum, green technique and the degree of matching between arms for solvent, temperature and duration, and were tested by cluster-robust moderator tests. Meta-regression examined whether the logarithm of the ratio of arm durations predicted effect size. Sensitivity was examined across 28 scenarios, comprising leave-one-study-out removal, alternative variance estimators, alternative treatment of unlabelled dispersions, restriction to single outcome domains, restriction to independent estimates, and exclusion of extreme values. Small-study effects were assessed by regression-based and rank-correlation tests and by trim-and-fill; because the sampling variance of the standardised mean difference is itself a function of the effect size, an additional regression test was performed on the ratio of means, which carries no such artefact. Certainty of evidence was appraised across five domains. Analyses were performed in R with the metafor and clubSandwich packages and in Python 3.

Two features of the inferential strategy require explicit statement. First, five outcome domains were analysed and no adjustment for multiplicity was applied. The domains are strongly correlated measurements performed on the same extracts rather than independent hypotheses, so a Bonferroni-type correction would be unduly conservative, but the consequence is that the domain-level analyses are exploratory and their nominal p values should not be read as confirmatory. Only the composite estimate, which is a single pre-specified comparison, is treated as inferential. Second, cluster-robust standard errors are computed from the empirical dispersion of the within-cluster residuals and therefore do not depend on the between-study variance estimator; the cluster-robust interval is consequently unchanged when that estimator is varied, which is why it is identical across those sensitivity scenarios.

3. Results

3.1. Study selection

The three search arms returned 540 records in total. After 47 duplicates were removed, 493 records were screened on title and abstract and 455 were excluded, most commonly because the biomass was not a macroalga, because the report was a review or narrative account, because the extraction target was a polysaccharide, protein or pigment rather than a phenolic fraction, or because no head-to-head extraction contrast existed. Thirty-eight reports were sought for retrieval and three could not be obtained because they had no PubMed Central deposit and their publisher full texts were paywalled. Thirty-five reports were assessed at full text and 22 were excluded. The selection process is set out in Figure 1.

The dominant reason for exclusion at full text is itself a finding. Eighteen of the 22 excluded reports optimised a green technique against itself, typically by response-surface methodology, and never ran a conventional comparator at all. Laboratories in this field have largely treated the superiority of green extraction as settled background rather than as a proposition to be tested. Three further reports were excluded because the head-to-head values carried no dispersion

measure, and one because the phenolic outcome was reported only as a pooled range across all arms. Thirteen studies were included. The ceiling of 13 is a property of the literature rather than of the search.

The three reports that could not be retrieved would have addressed precisely the gaps that most constrain this synthesis. One concerns a red seaweed compared by ultrasound against infusion, a lineage represented here by only two studies; one concerns four brown seaweeds compared by microwave against conventional extraction, which would have roughly doubled the microwave evidence; and one concerns a species already represented, which would have supplied an independent second estimate for it. Anyone extending this work should begin by obtaining them.

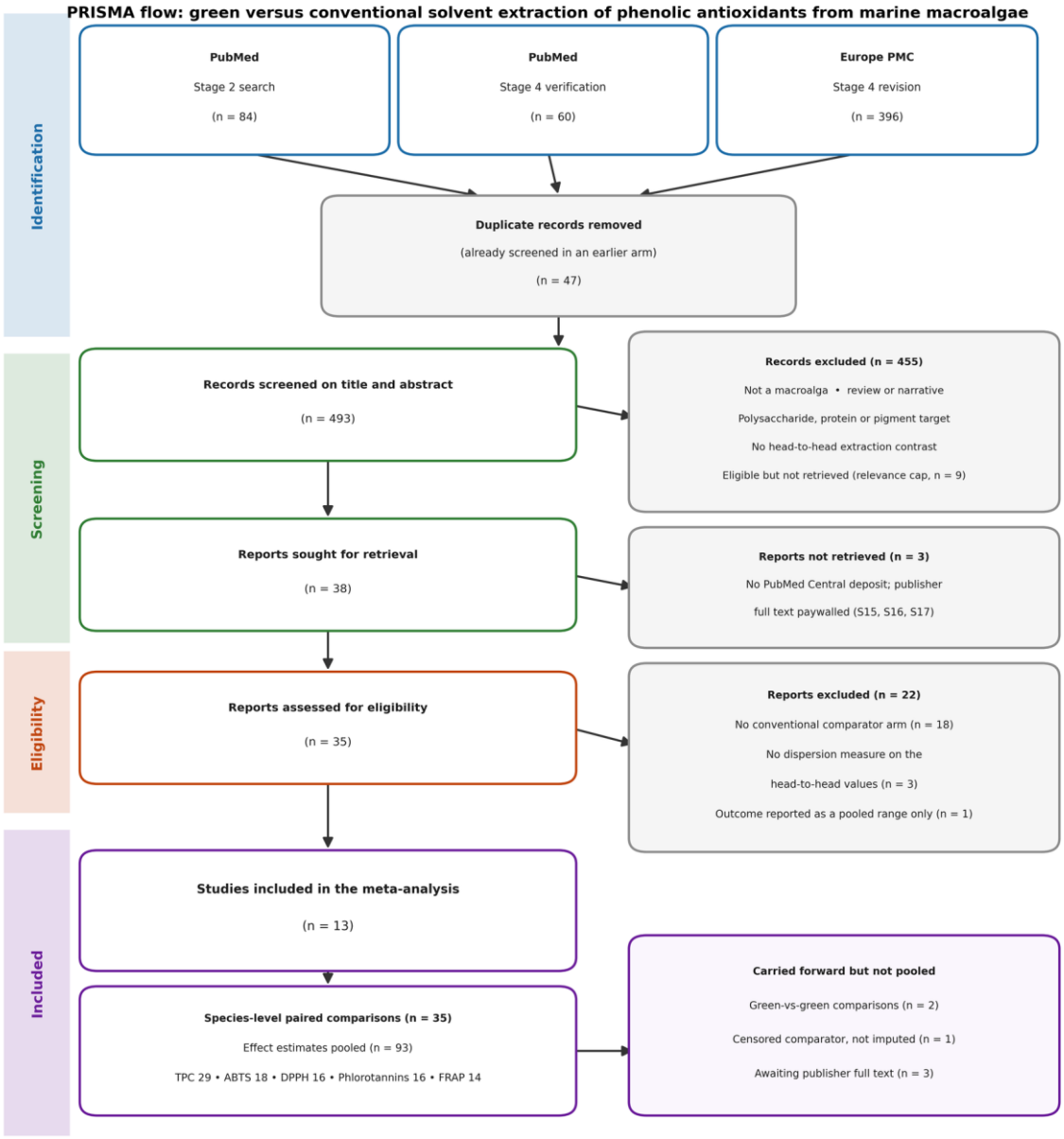

Figure 1. Study selection across the three search arms. The Europe PMC arm was added after the initial database-specific strategy was found to under-retrieve, and recovered the two methodologically strongest reports in the pool.

3.2. Characteristics of the included studies

The 13 included studies contributed 35 species-level paired comparisons across 22 macroalgal species and yielded 93 effect estimates. Their characteristics are summarised in Table 1. Brown seaweeds dominated the evidence base, supplying 73 of the 93 estimates across 11 studies, while red seaweeds supplied 20 estimates from only two studies.[35,36] No study of a green seaweed met the eligibility criteria. Biomass originated from Ireland, Portugal, Spain, Norway, Australia, Turkey, Iran, China, Vietnam and Indonesia, so the pool was geographically broad even though it was taxonomically narrow.

Ultrasound-assisted extraction was by far the most frequently studied technology, contributing 56 estimates from nine studies, as detailed in the green-arm column of Table 1. Microwave-assisted and pressurised-liquid extraction contributed eight estimates each, while hydrothermal-assisted, high-pressure-assisted, combined

ultrasound-microwave, supercritical-fluid and mechanical homogenisation arms contributed four estimates each and enzyme-assisted extraction a single estimate. Outcome coverage was likewise uneven, as the outcomes column of Table 1 shows: total phenolic content was reported in 29 estimates, ABTS in 18, DPPH and phlorotannins in 16 each, and ferric-reducing antioxidant power in 14.

Table 1. Characteristics of the 13 included controlled comparative laboratory experiments.

Study	Species (phylum)	Origin	Green arm	Conventional comparator	Outcomes	Estimates	Risk of bias
Goksen 2023[35]	*Laurencia papillosa* (R)	Turkey	UAE, probe, 15 min	Stirring, 50 °C, 24 h	TPC, DPPH, ABTS, FRAP	4	Some concerns
Lee 2024[37]	*Cystophora* sp., *Durvillaea potatorum*, *Sargassum fallax*, *Ecklonia radiata*, *Phyllospora comosa* (P)	Australia	UAE, 8 min	Shaking, 4 °C, 16 h	TPC, DPPH, ABTS, FRAP	20	High
Sonno 2026[36]	*Asparagopsis armata*, *A. taxiformis* (R)	Australia	UAE, 3 min	Shaking, 210 rpm, 24 h	TPC, DPPH, ABTS, FRAP	16	Some concerns
He 2026[38]	*Sargassum fusiforme* (P)	China	UAE, 28 kHz, 320 W, 75 min	Water bath, identical solvent, temperature and time	TPC, phlorotannins	2	Some concerns
Taherkhani 2024[39]	*Nizimuddinia zanardini* (P)	Iran	MAE 300 W, 15 min; UAE 37 kHz, 120 min	Maceration, 30 °C, 24 h	TPC, phlorotannins	4	High
Paulo 2025[40]	*Rugulopteryx okamurae* (P)	Portugal	UAE 40 kHz; mechanical homogenisation	Overnight agitation, 20 °C	TPC, DPPH, ABTS, FRAP	5	High
Ummat 2020[41]	*Fucus vesiculosus*, *Laminaria digitata* (P)	Ireland	UAE, 35 kHz, 30 min	No-ultrasound control; shaking water bath, 4 h	TPC, phlorotannins, DPPH, FRAP	4	Some concerns
da Silva 2025[42]	*Saccharina latissima* (P)	Spain	MAE 80 °C, 8 min; PLE 80 °C, 30 min	Conventional reflux, 6 h	TPC, phlorotannins, DPPH, ABTS	4	High
Amarante 2020[43]	*Fucus vesiculosus* (P)	Portugal	MAE, 400 W, 75 °C, 5 min	Solid-liquid, 70% acetone, 3 h	Phlorotannins, ABTS	2	High
Sunarwidhi 2022[44]	*Sargassum polycystum*, *S. cristaefolium*, *Turbinaria ornata* (P)	Indonesia	UAE, 30 kHz, 90 min	Cold maceration, 72 h	ABTS	3	High
Nguyen 2024[45]	*Padina gymnospora* (P)	Vietnam	EAE, Alcalase, 60.5 °C, 1.95 h	70% ethanol, 10 °C, 16 h	DPPH	1	High
Garcia-Vaquero 2021[46]	*Fucus vesiculosus*, *Pelvetia canaliculata* (P)	Ireland	UAE, MAE, UMAE, HAE, HPAE; all 10 min	Magnetic-stirring maceration, 10 min	TPC, phlorotannins	20	Low
Baek 2025[47]	*Sargassum serratifolium* (P)	Republic of Korea	PLE 100 °C, 70 min; SFE 45 °C, 90 min	Methanol maceration, 24 h	TPC, DPPH, ABTS, FRAP	8	High

Note. R, Rhodophyta; P, Phaeophyceae. UAE, ultrasound-assisted extraction; MAE, microwave-assisted extraction; UMAE, combined ultrasound-microwave-assisted extraction; HAE, hydrothermal-assisted extraction; HPAE, high-pressure-assisted extraction; PLE, pressurised liquid extraction; SFE, supercritical fluid extraction; EAE, enzyme-assisted extraction; TPC, total phenolic content. Nomenclatural correction: the source report for Lee 2024 prints its material as Cytospora sp. throughout. Cytospora is a genus of terrestrial ascomycete fungi and cannot be the material studied; the intended brown-algal genus Cystophora is reported here instead. The correction is recorded rather than made silently, and no value from that report has been altered.

3.3. Risk of bias

Of the 13 studies, one was judged at low risk of bias on every domain,[46] four raised some concerns and eight were judged at high overall risk. The domain-level judgements are displayed in Figure 2. Allocation of biomass to arms was rated low in every study, because a single homogenised biomass was invariably divided between arms.

The dominant defect lay in the matching of process conditions, shown as domain D2 in Figure 2. Energy-assisted arms were routinely tuned by response-surface or algorithmic optimisation while the conventional arm was implemented as a single fixed protocol taken from earlier work, so that the contrast confounded the technology with the effort invested in optimising it. In several studies the two arms used different solvent systems altogether: one compared a 57% ethanolic microwave arm against a 70% acetone conventional arm,[43] and another compared an aqueous enzymatic arm against a 70% ethanolic comparator.[45] A further study applied a 30-minute sonication assist to its own conventional arm.[47] Completeness of dispersion data, domain D3 in the same figure, was the second recurrent problem: two studies never labelled their dispersions as standard deviations or standard errors,[39,42] one never stated a replicate count,[44] and one reported its optimised microwave arm with no dispersion at all.[42] The per-study judgements behind these statements are detailed in Figure 2, and the study-by-study risk ratings are also listed in the final column of Table 1. This pattern of optimisation asymmetry inflates the apparent advantage and is the single most important limitation of the synthesis.

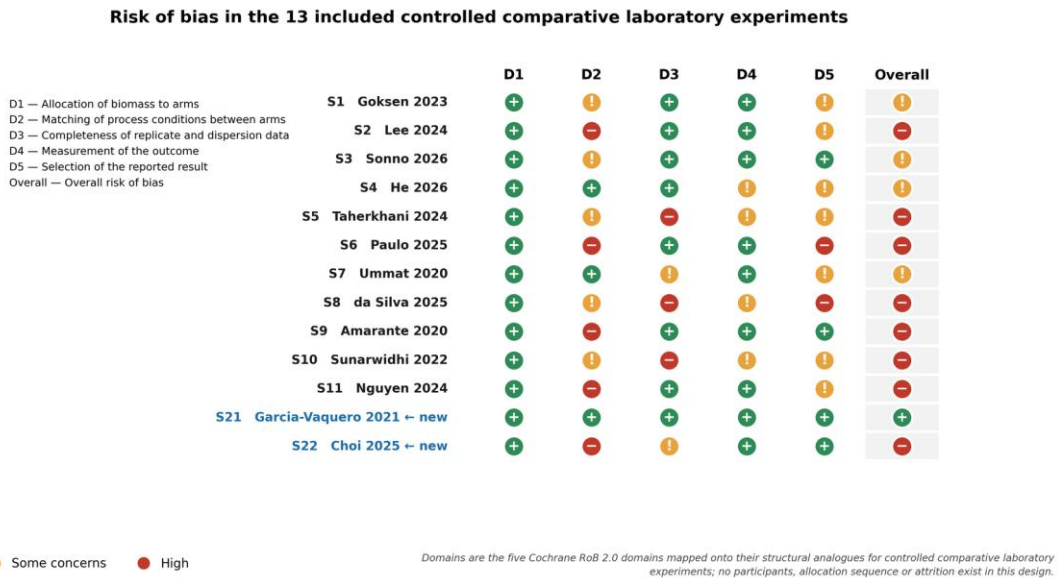

Figure 2. Risk of bias across the five Cochrane domains mapped onto their structural analogues for controlled comparative laboratory experiments. D1, allocation of biomass to arms; D2, matching of process conditions; D3, completeness of replicate and dispersion data; D4, measurement of the outcome; D5, selection of the reported result.

3.4. Pooled effect of green extraction

Across all 93 estimates the random-effects pooled standardised mean difference was 6.95 (95% CI 5.73 to 8.17) with the protocol-specified DerSimonian-Laird estimator. That interval assumes independence between estimates and is anti-conservative; it is retained for transparency but is not the basis of any claim made here. The primary inferential result is the cluster-robust estimate, in which the study is the clustering unit: Hedges' g = 8.62 (95% CI 2.98 to 14.26, p = 0.0063). The three-level model returned the same point estimate with a closely comparable interval (95% CI 3.55 to 13.69, p = 0.0009). On the interpretable scale, energy-assisted extraction recovered 38.5% more than conventional extraction (ratio of means 1.385, 95% CI 1.149 to 1.671, p = 0.0027). These results, and the corresponding estimates for each outcome domain, are presented in Table 2, and the individual estimates are displayed in Figure 3.

The three-level decomposition, reported in the third row of Table 2, attributed 50.3% of the variance to differences between studies and 46.8% to differences between species-level comparisons within studies, so roughly half of the variability was biological and species-specific rather than a matter of some laboratories being systematically more favourable than others.

The domain-level results require careful reading, and they are exploratory rather than confirmatory: five correlated domains were analysed and no multiplicity adjustment was applied, so a nominal p value of 0.0144 would not survive even a permissive correction across five tests. Once the correlation between outcomes measured on the same extract was respected, only DPPH radical-scavenging reached nominal significance (g = 16.84, 95% CI 4.58 to 29.11, p = 0.0144). Total phenolic content was marginal (g = 5.74, 95% CI 0.13 to 11.34, p = 0.0459; the corresponding DerSimonian-Laird estimate for this domain was 5.61 with an interval of 3.75 to 7.47). The phlorotannin, ABTS and ferric-reducing pools had cluster-robust intervals that included the null. The composite remained significant because pooling across domains draws on all 13 studies rather than on the six to ten contributing to any single domain. On the ratio-of-means scale every domain favoured energy-assisted extraction, from a 23.0% gain for ABTS to a 54.9% gain for DPPH.

Table 2. Pooled effect of energy-assisted extraction relative to conventional solvent extraction on phenolic and antioxidant outcomes. The cluster-robust row is the inferential basis of this article.

Analysis	Estimates	Studies	Hedges' g (95% CI)	p	Ratio of means (95% CI)	Gain, % (95% CI)	I^2 (%)
Protocol-specified estimate, DerSimonian-Laird (retained for transparency; interval anti-conservative)	93	13	6.95 (5.73 to 8.17)	<0.0001	1.385 (1.149 to 1.671)	38.5 (14.9 to 67.1)	87.8
PRIMARY INFERENTIAL RESULT: cluster-robust variance estimation	93	13	**8.62 (2.98 to 14.26)**	**0.0063**	**1.385 (1.149 to 1.671)**	**38.5 (14.9 to 67.1)**	—
Three-level random-effects model, restricted maximum likelihood	93	13	8.62 (3.55 to 13.69)	0.0009	—	—	97.1
Total phenolic content (exploratory)	29	10	5.74 (0.13 to 11.34)	0.0459	1.313	31.3	86.6
Phlorotannins (exploratory)	16	6	11.70 (−0.37 to 23.77)	0.0528	1.511	51.1	86.5
DPPH radical-scavenging (exploratory)	16	8	16.84 (4.58 to 29.11)	0.0144	1.549	54.9	90.2
ABTS radical-scavenging (exploratory)	18	8	13.00 (−0.62 to 26.62)	0.0585	1.230	23.0	89.7
Ferric-reducing antioxidant power (exploratory)	14	6	6.84 (−1.33 to 15.00)	0.0797	1.479	47.9	86.4

Note. All intervals are cluster-robust except the DerSimonian-Laird row. The 95% prediction interval for the pooled effect ran from −2.26 to 16.16. Domain rows are exploratory: five correlated domains were analysed without adjustment for multiplicity, and their p values are not confirmatory. The I^2 value of 97.1% for the three-level model is the sum of its between-study (50.3%) and between-comparison (46.8%) components; the corresponding total between-study variance in the analysis output is 102.78 and is a variance, not a percentage. Gravimetric extract yield is a different construct and is reported separately in Table 5; it does not contribute to the pooled estimate.

3.5. Subgroup analyses and process meta-regression

No moderator reached significance under cluster-robust testing. Algal phylum, which had appeared to be a strong moderator when estimates were treated as independent, returned a between-group test of Q = 1.19 (p = 0.425) once clustering was respected; brown seaweeds gave a pooled g of 10.08 against 1.24 for red seaweeds, but the red-seaweed stratum rested on two studies and its interval was uninformative. Matching between arms likewise failed to reach significance (Q = 0.48, p = 0.775), as did solvent matching (Q = 1.63, p = 0.292). The subgroup results are presented in Table 3.

The red-seaweed stratum requires individual description, because its pooled interval of −63.18 to 72.32 is not merely imprecise but uninformative, and it should not be read as a weak estimate of a real quantity. That stratum comprises exactly two studies which disagree qualitatively rather than quantitatively. One reported a large and internally consistent advantage across all four outcomes in a single species, with total phenolic content rising from 169.21 to 212.03 and ferric-reducing capacity from 20.50 to 72.47 in the printed units.[35] The other examined two species at two developmental stages and reported 16 estimates that were close to null throughout, several of which favoured the conventional arm; its authors stated explicitly that they observed no universal advantage.[36] The width of the Rhodophyta interval therefore reflects genuine disagreement between two reports, not merely a small number of observations, and no statement about red seaweeds can be supported from it.

These null moderator tests reflect limited power rather than demonstrated equivalence, and the descriptive pattern set out in Table 3 remains informative. The stratum in which the energy input was the only difference between arms, all other conditions being identical, produced the largest effect (g = 12.74) and by far the lowest heterogeneity (I^2 = 64.6% against 87.8% overall), and on the ratio-of-means scale a 90.2% gain. That stratum, however, comprised only three studies, and three clusters cannot support a significant robust test. Conversely, the stratum in which the comparator itself received a sonication assist showed no advantage at all (g = −0.06; ratio of means 0.886), which is precisely what would be expected if the effect is real and attributable to acoustic energy. Both strata are given in the comparator-matching block of Table 3.

One technology stood apart in the opposite direction. As the final row of Table 3 records, supercritical carbon dioxide recovered significantly less phenolic material than conventional extraction (g = −9.19, 95% CI −14.59 to −3.79; ratio of means 0.482, a 51.8% reduction). The label green therefore did not guarantee superior recovery, and this counter-example is reported rather than suppressed.

Meta-regression addressed the most obvious sceptical explanation for the pooled result, namely that green arms might appear superior only because the two arms were run for different lengths of time. Across the pool the conventional arm ran between 1 and 480 times longer than the green arm. The logarithm of that duration ratio did not predict effect size, either for the standardised mean difference (β = −1.09, 95% CI −5.00 to 2.81, p = 0.4805) or for the ratio of means (β = −0.02, 95% CI −0.16 to 0.12, p = 0.7170), and it remained non-significant in a multivariable model additionally containing solvent matching and phylum (p = 0.9385). In 12 of the 35 comparisons the two arms ran for exactly the same duration, and the advantage was still present among them.

Table 3. Subgroup analyses by algal phylum, degree of matching between arms and green extraction technology.

Moderator	Level	Estimates	Studies	Hedges' g (95% CI)	Ratio of means	I^2 (%)	Q between (p)
Algal phylum	Phaeophyceae	73	11	10.08 (3.90 to 16.73)	1.412	87.3	1.19 (0.425)
	Rhodophyta	20	2	1.24 (−63.18 to 72.32)	1.279	79.7	
Comparator matching	Isolated variable	20	3	12.74 (−5.16 to 40.16)	1.902	64.6	0.48 (0.775)
	Same solvent	62	9	6.12 (1.34 to 17.02)	1.409	87.1	
	Different solvent	3	2	1.25 (−8.41 to 10.90)	1.028	91.3	
	Partially sonicated comparator	8	1	−0.06 (−5.75 to 5.63)	0.886	91.4	
Solvent matching	Same	82	10	7.95 (3.23 to 16.86)	1.518	87.3	1.63 (0.292)
	Different	11	3	0.09 (−15.09 to 20.15)	0.976	90.4	
Green technology	Ultrasound-assisted	56	9	6.36 (−2.46 to 19.64)	1.431	87.5	—
	Pressurised liquid	8	2	9.07 (5.09 to 13.05)	1.598	81.5	
	Microwave-assisted	8	3	6.27 (−59.45 to 103.24)	1.194	88.8	
	Ultrasound-microwave	4	1	14.36 (9.27 to 19.45)	1.364	26.9	
	Hydrothermal-assisted	4	1	13.77 (9.72 to 17.83)	1.366	0.0	
	High-pressure-assisted	4	1	9.57 (5.88 to 13.26)	1.250	35.1	
	Mechanical homogenisation	4	1	5.89 (1.19 to 10.60)	1.583	80.4	
	Supercritical carbon dioxide	4	1	**−9.19 (−14.59 to −3.79)**	**0.482**	73.7	

Note. Intervals are cluster-robust. Between-group tests were not computed for green technology because several levels were represented by a single study. Isolated variable denotes comparisons in which solvent, temperature, duration and solid-to-solvent ratio were identical and the energy input was the only difference.

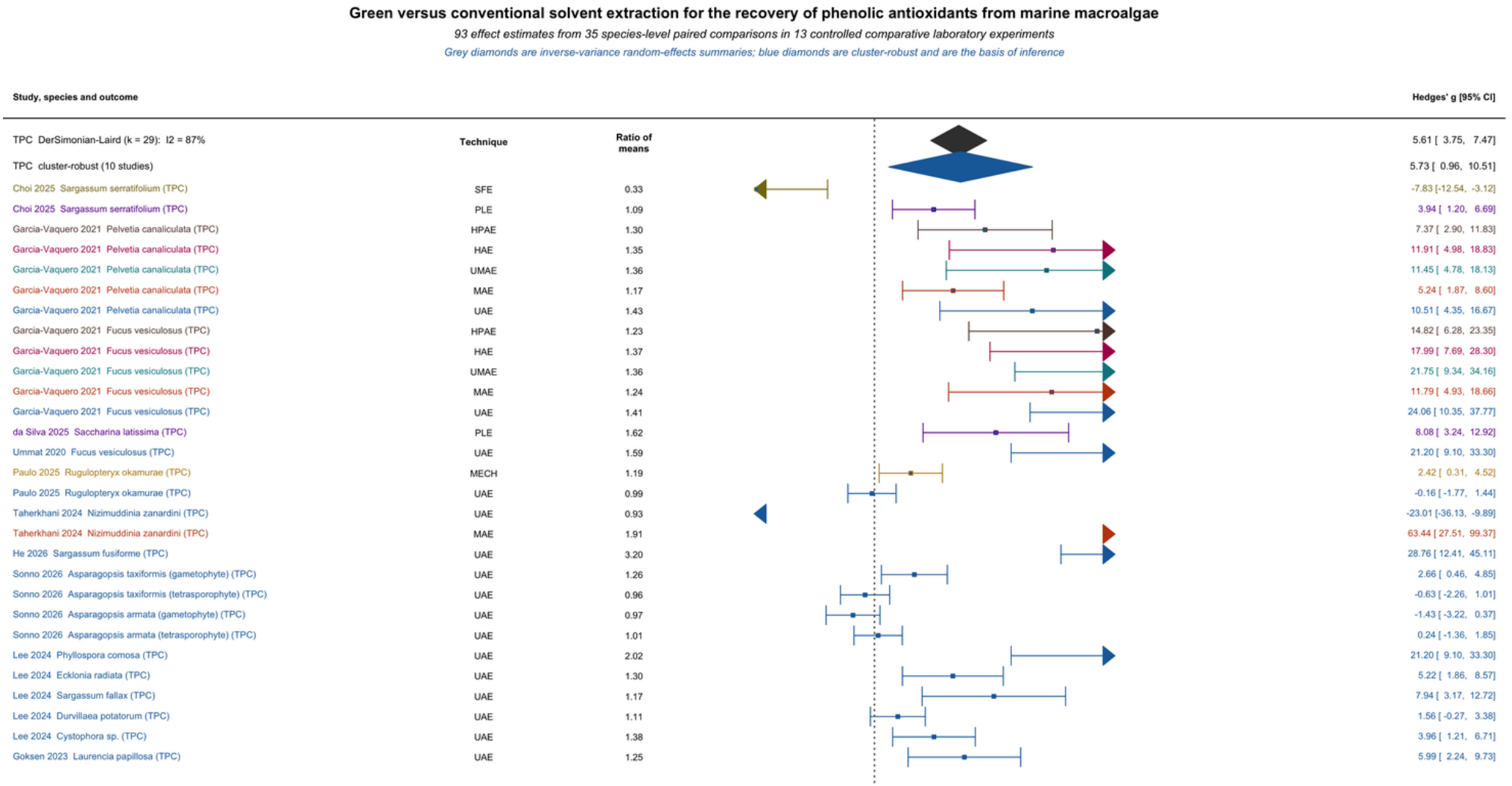

Figure 3 (part 1 of 5). Forest plot of all 93 effect estimates grouped by outcome domain and colour-coded by extraction technology. Individual estimates are not intended to be read one by one; the diamonds carry the message. Grey diamonds are inverse-variance summaries and blue diamonds are cluster-robust, the latter forming the basis of inference. Positive values favour energy-assisted extraction. Five estimates expressed as half-maximal inhibitory concentrations have had their signs reversed, so that a positive value denotes greater antioxidant activity in every panel.

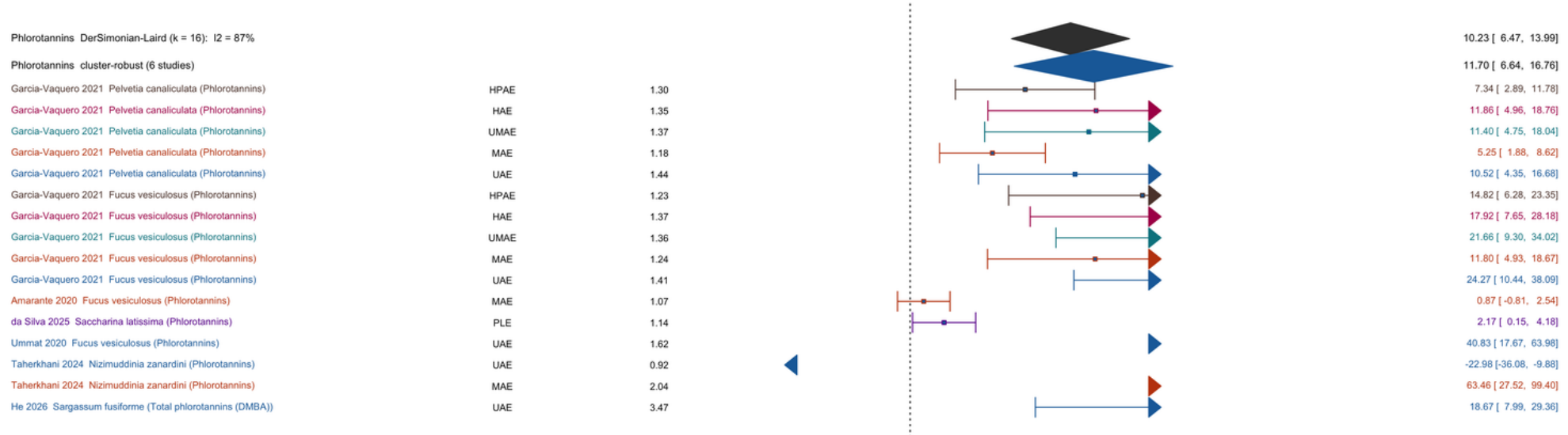

Figure 3 (continued, part 2 of 5). Phlorotannins.

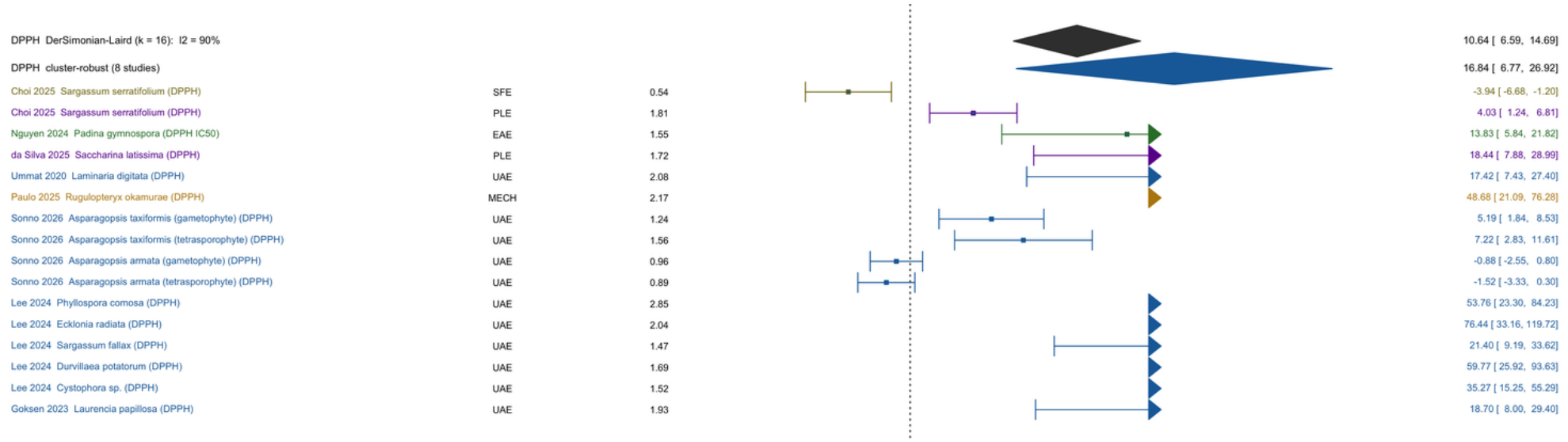

Figure 3 (continued, part 3 of 5). DPPH.

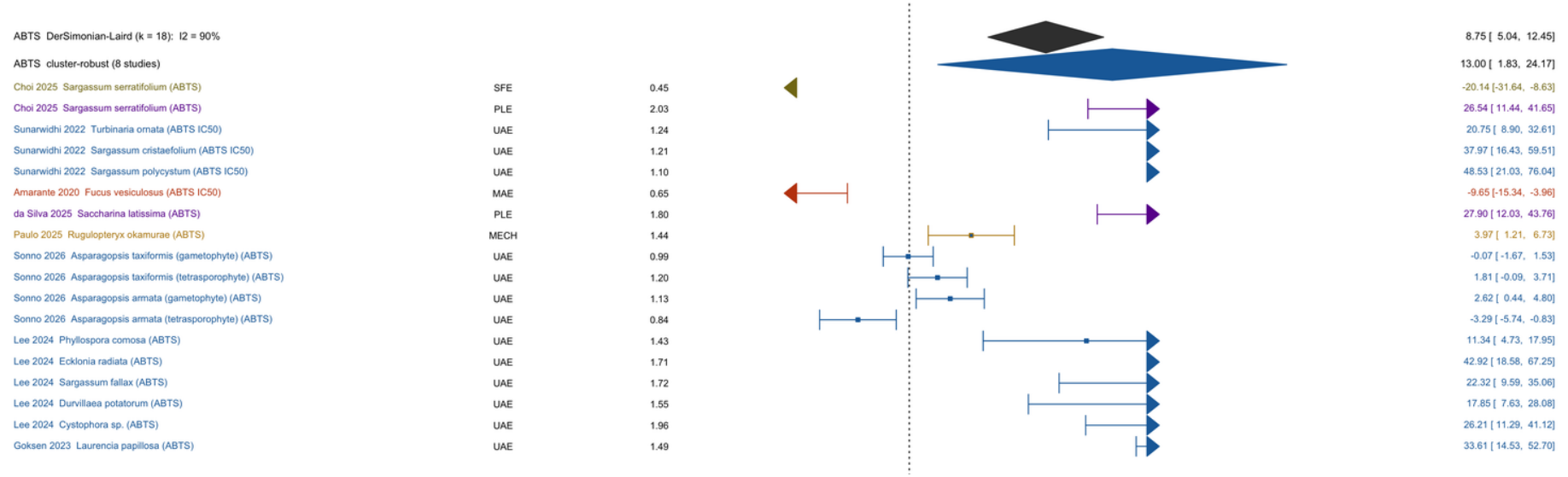

Figure 3 (continued, part 4 of 5). ABTS.

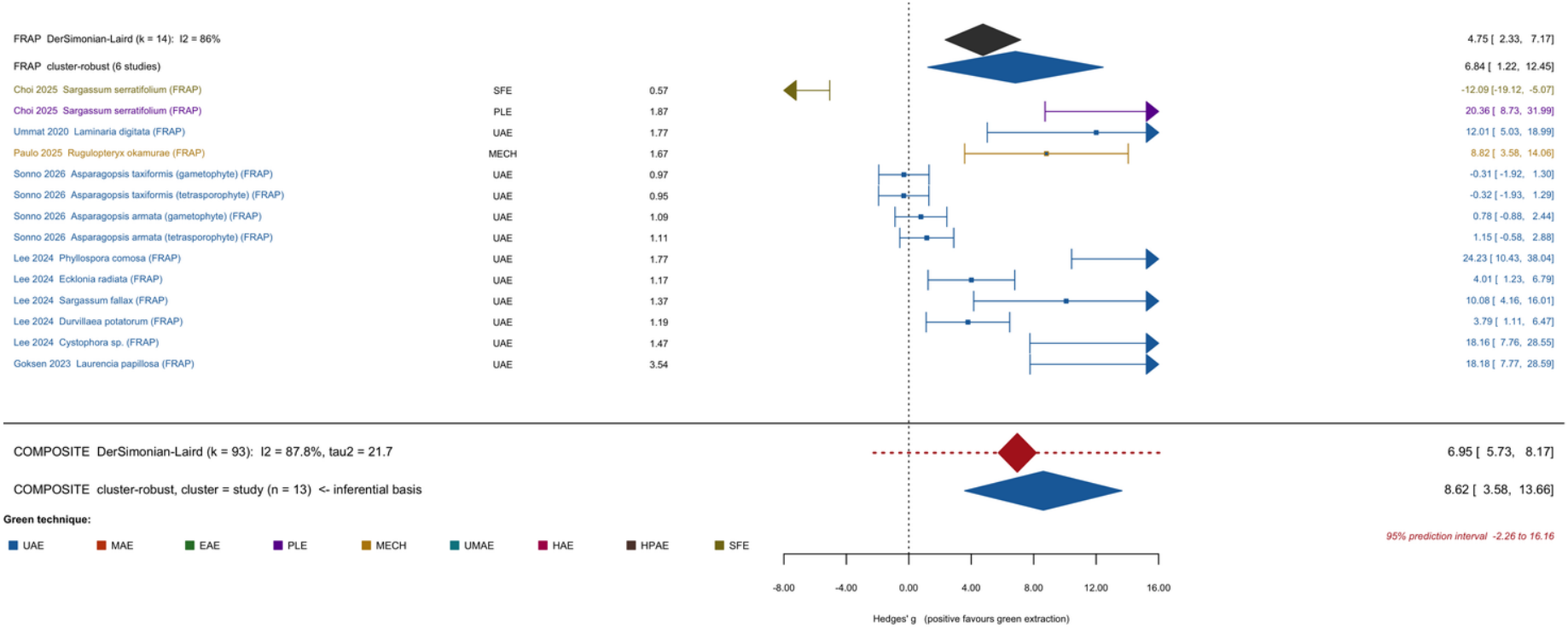

Figure 3 (continued, part 5 of 5). FRAP and composite pooled effect estimate.

3.6. Sensitivity analysis

Twenty-eight sensitivity scenarios were examined and are summarised in Table 4. The pooled estimate ranged from g = 1.87 to g = 12.74 and never reversed direction. Removing any one of the 13 studies left the cluster-robust interval entirely above zero, so no single study drove the result. Substituting restricted maximum likelihood or the Paule-Mandel estimator for the between-study variance raised the point estimate rather than lowering it. Treating the two unlabelled dispersions as standard errors rather than standard deviations changed the pooled estimate trivially, from 6.95 to 6.75.

The most informative scenario, highlighted in bold in Table 4, excluded all estimates with an absolute standardised mean difference above 10, which guards against inflation by very small analytical replicate dispersions. This reduced the pooled effect to g = 1.87 (95% CI 0.20 to 4.63) and the ratio of means to 1.085, an 8.5% gain. The direction was preserved and the interval still excluded the null, but the magnitude collapsed, which is the clearest available demonstration that the standardised mean difference is inflated in this literature and that the ratio of means is the more trustworthy expression of magnitude.

Restricting the analysis to one estimate per comparison, which enforces full independence, gave g = 9.02 (95% CI 3.39 to 17.50). Excluding the two studies added at the revision stage reproduced the earlier evidence base and gave g = 5.86 (95% CI 2.46 to 17.07), so those additions strengthened rather than created the finding. Only two scenarios produced an interval crossing the null, and both did so because too few clusters remained for robust inference.

Table 4. Selected sensitivity analyses. Twenty-eight scenarios were examined in total.

Scenario	Estimates	Studies	Hedges' g (95% CI)	Ratio of means	Direction preserved
Base case	93	13	6.95 (2.98 to 14.26)	1.385	—
Most influential single-study removal (Sonno 2026)	77	12	10.36 (4.31 to 16.07)	1.420	Yes
Least favourable single-study removal (Garcia-Vaquero 2021)	73	12	5.26 (1.98 to 15.36)	1.383	Yes
Unlabelled dispersions treated as standard errors	93	13	6.75 (3.35 to 14.21)	1.385	Yes
Excluding the study with no stated replicate count	90	12	6.63 (2.19 to 11.90)	1.407	Yes
Excluding mechanical homogenisation arms	89	13	7.03 (2.98 to 14.56)	1.357	Yes
Excluding the partially sonicated comparator	85	12	7.60 (3.57 to 15.40)	1.448	Yes
Total phenolic content only (DerSimonian-Laird point estimate)	29	10	5.61 (0.13 to 11.34)	1.313	Yes
Isolated-variable comparators only	20	3	12.74 (−5.16 to 40.16)	1.902	Yes
Excluding inverse-scaled concentration outcomes	88	11	6.72 (1.64 to 11.83)	1.432	Yes
Excluding estimates with |g| > 10	39	8	**1.87 (0.20 to 4.63)**	**1.085**	Yes
Excluding the two studies added at revision	65	11	5.86 (2.46 to 17.07)	1.453	Yes
One estimate per comparison	35	13	9.02 (3.39 to 17.50)	1.418	Yes
Restricted maximum likelihood estimator	93	13	10.12 (2.98 to 14.26)	1.385	Yes
Paule-Mandel estimator	93	13	10.87 (2.98 to 14.26)	1.385	Yes

Note. Point estimates are the random-effects estimates for each scenario and intervals are cluster-robust. Because cluster-robust standard errors are computed from the empirical dispersion of within-cluster residuals, they do not depend on the between-study variance estimator; the final three rows therefore share the base-case interval by construction rather than by coincidence, while their point estimates differ. The row restricted to total phenolic content carries the DerSimonian-Laird point estimate of 5.61 against the cluster-robust interval; the cluster-robust point estimate for that domain, 5.74, is given in Table 2.

3.7. Gravimetric extract yield

Gravimetric extract yield was pooled separately throughout, because it measures the mass of material recovered rather than the concentration of phenolics within it, and a process that recovers more mineral matter and polysaccharide will register a higher yield without delivering more antioxidant activity. Fifteen estimates from four studies were available. The inverse-variance estimate was large (g = 26.68), but once clustering was respected the effect was not significant (g = 16.99, 95% CI −14.67 to 48.66, p = 0.1816), and the ratio of means gave a 41.3% gain whose interval crossed unity (1.413, 95% CI 0.877 to 2.276, p = 0.1023). Yield should therefore be regarded as suggestive only, and it is set out separately in Table 5.

Table 5. Gravimetric extract yield, pooled separately from the phenolic and antioxidant outcomes.

Analysis	Estimates	Studies	Estimate (95% CI)	p	I^2 (%)	Status
Hedges' g, inverse-variance	15	4	26.68 (17.21 to 36.15)	<0.0001	90.6	Anti-conservative
Hedges' g, cluster-robust	15	4	**16.99 (−14.67 to 48.66)**	**0.1816**	—	**Inferential; not significant**
Ratio of means, cluster-robust	15	4	1.413 (0.877 to 2.276)	0.1023	99.9	41.3% gain; interval crosses unity

Note. Yield does not contribute to the pooled phenolic estimate reported in Table 2 and should not be averaged with it.

3.8. Processing time

Processing time was the most robust practical finding and depended on no modelling assumption. Across the 35 comparisons the median duration of the energy-assisted arm was 10 minutes against 960 minutes for the conventional arm, a median 20.6-fold reduction with an interquartile range from 1-fold to 108-fold. Energy-assisted arms ran for between 3 and 120 minutes and conventional arms for between 10 and 4320 minutes. In 12 of the 35 comparisons the two arms ran for exactly the same length of time, and the

advantage was still present among them; this is the most direct evidence in the article that the benefit is attributable to the energy input rather than to contact time, because it requires no model at all.

Because processing time is only one axis on which a process is judged to be green, the solvent and matching characteristics of the two arms were also tabulated descriptively, and are set out in Table 6. Solvent identity and concentration were identical between arms in 31 of the 35 comparisons, so the pooled estimate is predominantly a comparison of energy input rather than of solvent chemistry. Ten comparisons were fully isolated-variable contrasts, in which solvent, temperature, duration and phase ratio were all matched. Two comparisons used water alone as the energy-assisted solvent against an organic comparator, and 14 energy-assisted arms used an organic solvent against 5 conventional arms that did so, which reflects the frequent use of aqueous or dilute-ethanolic media in the energy-assisted literature. These are descriptive counts of the extracted dataset and no effect estimate is attached to them.

Table 6. Process and solvent characteristics of the 35 paired comparisons, reported descriptively.

Characteristic	Value
Duration of the energy-assisted arm	3 to 120 minutes (median 10)
Duration of the conventional arm	10 to 4320 minutes (median 960)
Median fold reduction in processing time	20.6-fold (interquartile range 1 to 108)
Comparisons in which both arms ran for identical durations	12 of 35
Comparisons with identical solvent identity and concentration in both arms	31 of 35
Comparisons in which solvent differed between arms	4 of 35
Fully isolated-variable comparisons (solvent, temperature, duration and phase ratio all matched)	10 of 35
Comparisons with a conventional arm that itself received a sonication assist	2 of 35
Energy-assisted arms using water or an aqueous system alone	2 of 35
Energy-assisted arms using an organic or hydro-organic solvent	14 of 35
Conventional arms using an organic or hydro-organic solvent	5 of 35
Comparisons in which arm temperatures were matched	10 of 35 (9 differed, 16 not reported)

Note. Counts describe the extracted dataset and carry no pooled estimate. Solvent volume per unit of biomass could not be tabulated for every comparison because solid-to-solvent ratio was not reported in all reports, which is itself a reporting deficiency of the primary literature.

3.9. Small-study effects and publication bias

Small-study effects were present, and the tests that establish this are set out in Table 7. The regression test applied to all 93 estimates was highly significant ($t = 11.15$, $p < 0.0001$), but that test is not interpretable on its own, because the sampling variance of a standardised mean difference is a function of the effect size and a pool containing large effects is forced into an asymmetric funnel; that artefact is visible as the distortion in panel A of Figure 4. Two artefact-free tests were therefore applied. The regression test on the 13 study-level means was significant ($t = 2.80$, $p = 0.0172$), as was the regression test on the ratio of means, which carries no such artefact ($t = 4.42$, $p < 0.0001$); these correspond to panels B and C of Figure 4 respectively. Trim-and-fill imputed 27 estimates, 29% of the pool, and reduced the effect from 6.95 to 4.42 (95% CI 3.17 to 5.68); because that method performs poorly under heterogeneity of the magnitude observed here, the adjusted value is offered as an illustrative lower bound rather than as a corrected estimate, as noted beneath Table 7.

A genuine reporting bias is highly plausible on mechanistic grounds. Eighteen of the 22 reports excluded at full text never ran a conventional comparator, which suggests that laboratories finding no advantage may simply not frame their work as a comparison, so that null contrasts are absent from the literature by design rather than by suppression.

Table 7. Tests for small-study effects and publication bias.

Test	Units entering the test	Statistic	p or interval	Interpretation
Regression test, all estimates	93 estimates	t = 11.15	<0.0001	Confounded by the dependence of the sampling variance on the effect size
Regression test, study-level means	13 studies	t = 2.80	0.0172	Aggregated to one estimate per study; artefact removed
Regression test, ratio of means	**93 estimates**	**t = 4.42**	**<0.0001**	**Free of the variance artefact; the decisive test**
Rank-correlation test	93 estimates	0.822	<0.0001	Subject to the same mechanical caveat as the first row
Trim-and-fill, estimates imputed	93 estimates	27	—	29% of the pool imputed; a very large adjustment
Trim-and-fill, adjusted effect	93 estimates	g = 4.42	3.17 to 5.68	Illustrative bound, not a corrected value

Note. Regression and rank-correlation tests are conventionally regarded as uninformative with fewer than ten units; the study-level test sits only marginally above that threshold and should be read with corresponding caution. Trim-and-fill imputed 27 estimates, or 29% of the pool, and the method is known to perform poorly when heterogeneity is as high as it is here; its adjusted value is therefore presented as an illustrative lower bound rather than as a corrected estimate.

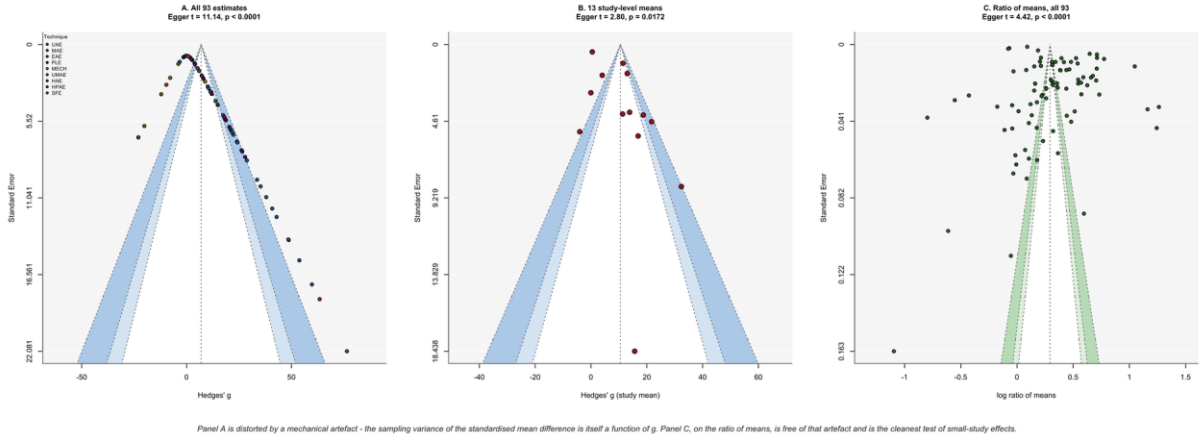

Figure 4. Contour-enhanced funnel plots. A, all estimates, distorted by the dependence of the sampling variance on the effect size; B, study-level means; C, the ratio of means, which is free of that artefact and provides the cleanest test of asymmetry.

3.10. Certainty of evidence

No outcome exceeded low certainty, as detailed in Table 8, and the phlorotannin outcome was rated very low because its cluster-robust interval included the null in addition to the concerns applying to every outcome. Every outcome was downgraded for risk of bias, driven by the optimisation asymmetry visible in Figure 2, and for inconsistency, driven by the I^2 values above 80% reported in Table 2. Reporting bias was suspected throughout, on the evidence set out in Table 7. The direction of effect may therefore be regarded as well supported and the magnitude as uncertain.

Table 8. Certainty of evidence across five appraisal domains.

Outcome	Studies	Risk of bias	Inconsistency	Indirectness	Imprecision	Reporting bias	Certainty
Total phenolic content	10	Serious	Serious	Not serious	Not serious	Suspected	Low
Phlorotannins	6	Serious	Serious	Not serious	Serious	Suspected	**Very low**
DPPH radical-scavenging	8	Serious	Serious	Not serious	Not serious	Suspected	Low
ABTS radical-scavenging	8	Serious	Serious	Not serious	Not serious	Suspected	Low
Ferric-reducing antioxidant power	6	Serious	Serious	Not serious	Not serious	Suspected	Low
Gravimetric extract yield	4	Serious	Not serious	Not serious	Not serious	Suspected	Low

4. Discussion

4.1. Principal findings

This synthesis established that green extraction recovered more phenolic antioxidant activity from marine macroalgal biomass than conventional solvent extraction, and that it did so a great deal faster. The pooled advantage was 38.5% on the ratio-of-means scale, with a cluster-robust confidence interval on the ratio of means of 1.149 to 1.671, that is a gain of between 14.9% and 67.1%, and the median green protocol achieved that advantage in 10 minutes against 16 hours. The direction of effect proved unusually durable: it survived all 28 sensitivity scenarios, the removal of any single study, three different between-study variance estimators, the enforcement of full independence between estimates, and the exclusion of the extreme estimates most likely to be inflated by small analytical dispersions.

The magnitude, by contrast, was not durable, and the honest interpretation of this work is that direction and magnitude carry very different evidential weight. Excluding estimates with an absolute standardised mean difference above 10 reduced the pooled gain from 38.5% to 8.5%, and trim-and-fill adjustment reduced the standardised effect from 6.95 to 4.42. Both operations preserved the sign while collapsing the size. A reader should therefore take from this synthesis that green extraction helps, that it helps quickly, and that the precise size of the benefit for any given species and technology remains an open question.

An equally important finding was negative. Under correct treatment of the dependence between estimates measured on the same extract, no moderator survived testing. The apparent superiority of brown seaweeds over red, and the apparent importance of matching between arms, both dissolved once clustering was respected. These were not weak findings that became weaker; they were artefacts of treating 93 correlated estimates as 93 independent observations. This has a methodological implication for the wider field, in which multiple outcomes measured on a single extract are routinely analysed as though they were independent replicates.

4.2. Comparison with previous syntheses

No previous meta-analysis has pooled the effect of extraction technology on phenolic recovery from macroalgae, so no direct comparison is available. The published quantitative syntheses involving algae and antioxidant outcomes have addressed entirely different questions, examining seaweed-extract biostimulants and crop yield, salinity tolerance in cereals, methane mitigation and performance in ruminants, and blood pressure, anthropometric indices and metabolic status in humans. The present work therefore occupies genuinely unoccupied ground, and its pooled estimate provides the first quantitative benchmark against which future process studies can be positioned.

Indirect comparison with primary work in adjacent matrices is nonetheless instructive. Head-to-head studies in terrestrial biomass have reported ultrasound- and microwave-assisted extraction outperforming conventional methods for polyphenol recovery from distillery stillage and peach by-products,[20,21] and optimisation studies in fruit

pomace, leaves and seeds have consistently recorded gains of comparable order to those found here.[15,16,18,19] The convergence between two literatures with different wall chemistries strengthens the mechanistic case that the advantage arises from cell-wall disruption rather than from the peculiarities of any single matrix. Studies combining green solvents with ultrasound have reported further gains again,[22,23] which is consistent with the pressurised-liquid arms in the present pool performing well.[42,47]

The single most instructive discordant result came from within the pool itself. The supercritical carbon dioxide arm recovered roughly half as much as its conventional comparator.[47] This is mechanistically unsurprising, because supercritical carbon dioxide is a non-polar solvent poorly suited to polar phenolics unless substantially modified, and it is a useful corrective to the assumption that any technology bearing the green label will improve recovery. Selection of the platform must be governed by the polarity of the target compounds, not by the environmental credentials of the solvent.

4.3. Heterogeneity

Heterogeneity was very high by any conventional standard, and the prediction interval included zero. Three sources were identifiable. The first was biological. The three-level decomposition attributed 46.8% of the variance to differences between species-level comparisons within the same study, meaning that two species processed in the same laboratory on the same day by the same operator responded differently. This is consistent with the known variation in cell-wall architecture and phenolic composition between macroalgal taxa and with reported seasonal and developmental variation within a single species.[27,28,29,36]

The practical consequence for a producer is direct. Because half the variance arises between species processed within the same laboratory, an optimised protocol cannot be transferred from one species to another with any expectation of reproducing the same gain, and species-specific process development remains necessary even after a technology has been selected. The prediction interval of −2.26 to 16.16 makes the same point in inferential terms: the pooled estimate describes an average across heterogeneous pairings and does not guarantee a benefit for any particular new one.

The second source was methodological, arising from the optimisation asymmetry documented in the risk-of-bias appraisal. Where a green arm had been tuned across dozens of design points and the conventional arm had not, the measured contrast reflected optimisation effort as well as technology. The stratum in which the energy input was the only difference between arms showed markedly lower heterogeneity, at 64.6% against 87.8% overall, which supports this interpretation.

The third source was analytical. Antioxidant capacity was expressed in at least six different ways across the pool, and phenolic content on at least three different denominators. Standardisation absorbs the unit differences but not the fact that DPPH, ABTS and ferric-reducing assays interrogate different chemistry, which is reflected in their differing pooled gains and is consistent with reports that assay choice materially affects the apparent antioxidant ranking of seaweed extracts.[11,48]

4.4. Limitations

Six limitations require explicit statement, and they are set out here in the order of the weight we place on them. The first and most serious is optimisation asymmetry between the arms. In eight of the 13 studies the energy-assisted arm was optimised, often across dozens of response-surface design points, while the conventional arm was implemented as a single fixed protocol inherited from earlier work, and in three studies the two arms used different solvent systems altogether. The pooled estimate therefore reflects the combined effect of the technology and of the effort invested in tuning it, and it is biased upward by an amount that cannot be recovered from the published record. The second concerns the primary effect measure itself. The dispersions reported in this literature are analytical replicate standard deviations describing the repeatability of a spectrophotometric assay rather than biological variability, and dividing by them produced individual estimates exceeding 70, values that have no counterpart in the clinical framework from which the standardised mean difference is borrowed. Reporting the ratio of means throughout mitigates this, and the sensitivity analysis excluding extreme estimates quantifies it, but the measure specified in the protocol remains inflated.

The third limitation is that small-study effects appear to be real rather than artefactual. The regression test on the ratio of means, which carries none of the mechanical asymmetry that afflicts the same test applied to standardised mean differences, was clearly significant, and 18 of the 22 reports excluded at full text had never run a conventional comparator at all. Trim-and-fill adjustment reduced the pooled estimate by roughly a third. Fourth, the evidence base is severely imbalanced taxonomically: red seaweeds contributed 20 estimates from two studies that disagree with one another, and green seaweeds contributed none. Because the practical interest motivating this work concerns red algae of tropical waters, the near-absence of Rhodophyta is a substantial constraint, and no confident statement about red seaweeds can be supported. Fifth, heterogeneity was very high and the prediction interval spanned zero, so the pooled estimate describes an average across a heterogeneous set of species and technology pairings and must not be read as a forecast for any particular new one.

The sixth limitation is procedural rather than substantive, and concerns the conduct of the review itself. Screening and data extraction were performed by a single reviewer rather than independently in duplicate; three eligible reports could not be obtained because their publisher full texts were inaccessible; and the evidence base was capped at 13 studies by the scarcity of conventional comparator arms in the primary literature rather than by any narrowness in the search. Each of these constraints is stated here rather than presented as a fully executed dual-reviewer systematic review.

4.5. Implications for biotechnology and green chemistry

For process biotechnology the practical case rests less on the effect size than on the time. Achieving equal or better recovery in 10 minutes rather than 16 hours changes the economics of a macroalgal biorefinery: reactor occupancy falls by an order of magnitude, throughput per unit of capital rises correspondingly, and the solvent inventory held at temperature for prolonged periods shrinks. These are the quantities that determine whether a valorisation route is viable, and they are robust to every modelling assumption examined here.

For green chemistry the finding that shorter, lower-solvent processes did not sacrifice recovery is the substantive result. The conventional expectation of a trade-off between environmental performance and yield was not observed; in the isolated-variable stratum, where energy input was the only difference between arms, recovery rose by 90.2%. The corollary is that solvent-reduction strategies and enzyme-assisted aqueous routes deserve closer examination,[45,14] since

the single study using water alone as the green solvent achieved a clear advantage over a 70% ethanolic comparator.

For pharmaceutical and nutraceutical development the implication is that extraction protocol must be reported and controlled as rigorously as species identity. Two laboratories working on the same alga with different platforms may recover substantially different phenolic profiles, which complicates the comparison of bioactivity results across studies and may partly explain inconsistencies in the reported potency of macroalgal extracts.[6,4,49,30]

For tropical archipelagic producers the priority indicated by this synthesis is the generation of Rhodophyta evidence. Red seaweeds are cultivated at very large scale in Indonesian waters and are already used in food, pharmaceutical and wound-care applications,[31,32,33,8] yet the extraction literature is dominated by cold-water brown seaweeds. Well-matched comparative experiments on cultivated red species, in which the green and conventional arms share solvent, temperature, duration and solid-to-solvent ratio, would address the largest single gap this review identified.

4.6. What the evidence does and does not support

Because the analyses above yield a mixture of robust and fragile conclusions, Table 9 states compactly which claims the evidence supports and which it does not. The distinction matters for anyone using this synthesis as a basis for a process decision, and the unsupported column is as important as the supported one.

Table 9. Claims supported and not supported by this synthesis.

Supported by the evidence	Not supported, and should not be claimed
Energy-assisted extraction recovers more phenolic antioxidant activity than conventional solvent extraction; the direction survived all 28 sensitivity scenarios and the removal of any single study.	That the advantage holds for every outcome. Only the composite reached inferential significance; the domain analyses were exploratory and three of five intervals included the null.
The advantage is obtained with a median 20.6-fold reduction in processing time, and persists in the 12 comparisons in which both arms ran for identical durations.	That any one technology outperforms another. No between-technology comparison survived cluster-robust testing.
The advantage is present where energy input is the only difference between arms, so it is not merely an artefact of protocol mismatch.	That brown seaweeds benefit more than red. The phylum test was not significant and the red-seaweed interval is uninformative.
Solvent identity was matched between arms in 31 of 35 comparisons, so the estimate reflects energy input rather than solvent chemistry.	That every green technology helps. Supercritical carbon dioxide recovered 51.8% less than its conventional comparator.
The direction is stable across three between-study variance estimators and under full enforcement of independence.	The exact magnitude. Small-study effects are real, the standardised measure is inflated by analytical dispersions, and optimisation asymmetry biases upward.
Extraction platform should be reported and controlled as rigorously as species identity in any bioactivity study.	Anything confident about red or green seaweeds. Rhodophyta rest on two disagreeing studies and Chlorophyta on none.

4.7. Recommendations for future comparative studies

Four design changes would materially improve the evidence base. Arms should be matched for solvent, temperature, duration and solid-to-solvent ratio so that the energy input is the only variable, as in the one study judged at low risk of bias throughout.[46] Both arms should receive equivalent optimisation effort, or the conventional arm should be optimised and the green arm run at a single condition, so that the direction of any residual asymmetry is at least conservative. Dispersions should be labelled explicitly as standard deviations or standard errors, and the number of independent extractions distinguished from the number of analytical replicates. Finally, absolute values should be reported for both arms in a table rather than only in a figure, and in a stated unit with an unambiguous denominator; four otherwise eligible reports were lost from this synthesis for want of a printed dispersion or a numeric table.

5. Conclusion

This meta-analysis pooled 93 effect estimates from 35 paired comparisons across 22 macroalgal species in 13 controlled comparative laboratory experiments. Energy-assisted extraction recovered more phenolic antioxidant activity than conventional solvent extraction: cluster-robust Hedges' g = 8.62 (95% CI 2.98 to 14.26, p = 0.0063), a 38.5% gain on the ratio-of-means scale (95% CI 1.149 to 1.671), obtained in a median of 10 minutes against 960. The advantage persisted in the 12 comparisons whose arms ran for identical durations, so it did not depend on contact time.

The direction was robust, surviving all 28 sensitivity scenarios, the removal of any single study, three variance estimators and the enforcement of full independence. The magnitude was not: excluding estimates with an absolute standardised mean difference above 10 reduced the gain to 8.5%, and adjustment for demonstrated small-study effects reduced the standardised effect to 4.42. Certainty did not exceed low for any outcome, domain analyses were exploratory, and the 95% prediction interval of −2.26 to 16.16 included zero, so no individual new pairing of species and technology is assured of benefit.

Three qualifications must accompany any use of these results. No moderator survived cluster-robust testing. The green label did not guarantee superior recovery, supercritical carbon dioxide having recovered 51.8% less than its comparator. And the evidence was taxonomically lopsided, with 73 of 93 estimates from brown seaweeds, 20 from two disagreeing red-seaweed studies and none from green seaweeds. The defensible practical conclusion is that energy-assisted extraction offers a large reduction in processing time without loss of phenolic recovery, and probably with a gain. Well-matched experiments on cultivated red seaweeds are the priority.

6. Declarations

Statement on the Use of Artificial Intelligence

None declared. The authors retain full responsibility for reviewing, verifying, and approving the final content, and no artificial-intelligence tool is listed as an author.

Author Contribution Statement

ZVN: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, and Writing - original draft. HC: Supervision, Validation, and Writing - review and editing. Both authors approved the final manuscript.

Funding Statement

The authors declare that this research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest / Competing Interests

The authors declare that they have no competing interests.

Data Availability Statement

All data analysed in this review were obtained from published studies cited in the article; no new participant-level data were generated.

Ethics Approval and Consent to Participate

Not applicable. This study is a meta-analysis of previously published data and did not involve new human participants, animals, or identifiable personal information.

Consent for Publication

Not applicable.

Review Registration

This review was not prospectively registered.

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