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Immunomodulatory Effects of Phytochemicals

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ABSTRACT

Plant-derived secondary metabolites -- flavonoids, polyphenols, terpenoids, and alkaloids -- modulate innate and adaptive immune pathways through mechanisms including NF-kappaB suppression, cytokine network rebalancing, and T-cell receptor signal amplification, yet systematic quantification of structure-activity relationships governing immunomodulatory potency across compound classes remains incomplete. This study evaluated 120 purified phytochemicals (flavonoids n = 36; polyphenols n = 28; terpenoids n = 32; alkaloids n = 24) across five immune endpoints -- TNF-alpha inhibition, IL-6 suppression, IL-10 induction, natural killer (NK) cell activation, and CD4+ T-cell proliferation -- in human peripheral blood mononuclear cell (PBMC), RAW264.7 macrophage, and Jurkat T-cell models (Madrid, Spain; 2021-2023). A Phytochemical Immunomodulatory Index (PII) integrating structural complexity, predicted bioavailability, cytokine modulation breadth, selectivity ratio (anti- to pro-inflammatory activity), and in vitro potency (IC50 aggregated across endpoints) predicted in vivo immunosuppression in a murine collagen-induced arthritis model with AUC = 0.881 and Pearson r = +0.84 ($p < 0.001$; n = 48 compounds validated in vivo), identifying hydroxylation pattern at the B-ring of flavonoids and methylenedioxy substitution in terpenoids as the principal structural determinants of selective anti-inflammatory activity without concurrent immunosuppression of NK cell cytotoxicity.

Keywords: phytochemicals; immunomodulation; flavonoids; polyphenols; terpenoids; alkaloids; NF-kappaB; cytokine; PII; TNF-alpha; IL-6; IL-10; NK cells; T-cell proliferation; Spain

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1. Introduction

1.1 Phytochemicals and the Immune System

The mammalian immune system integrates innate pathogen recognition -- toll-like receptor (TLR) signalling, inflammasome activation, and complement cascades -- with adaptive lymphocyte-mediated responses governed by cytokine networks and antigen presentation. Dysregulation of these networks underlies autoimmune disease, chronic inflammation, and cancer immune evasion. Phytochemicals -- the chemically diverse class of non-nutritive plant secondary metabolites comprising approximately 8,000 identified structures -- have been used empirically in traditional medicine systems as anti-inflammatory agents for millennia, with retrospective pharmacological evaluation demonstrating biological activity at concentrations achievable in vivo following dietary or supplemental intake. The mechanistic diversity of phytochemical immunomodulation -- spanning NF-kappaB pathway inhibition, JAK-STAT signal interference, MAPK cascade modulation, and direct cytokine binding -- suggests that the pharmacological potential of plant-derived compounds extends well beyond the single-target paradigm of synthetic immunosuppressants.

1.2 Major Phytochemical Classes in Immunology

Four chemical classes dominate the immunopharmacological literature. Flavonoids (C6-C3-C6 benzo-gamma-pyrone backbone; six subclasses: flavones, flavonols, flavanones, flavanonols, isoflavones, anthocyanidins) are the most studied, with quercetin, luteolin, and apigenin demonstrating consistent NF-kappaB-dependent TNF-alpha suppression in macrophage models. Polyphenols -- structurally heterogeneous but sharing multiple phenolic hydroxyl groups (resveratrol, curcumin, epigallocatechin gallate, rosmarinic acid) -- modulate both enzymatic antioxidant defences and cytokine transcription factors. Terpenoids (isoprene-based C5 building blocks assembled into mono-, sesqui-, di-, tri-, and tetraterpenes) include potent immunomodulators such as boswellic acids (5-LOX inhibition), betulinic acid (NF-kappaB suppression), and ursolic acid (IL-6 blockade). Alkaloids (nitrogen-containing heterocyclic secondary metabolites: berberine, colchicine, vincristine, capsaicin, piperine) act through distinct mechanisms including microtubule disruption (colchicine), AMPK activation (berberine), and TRPV1-mediated neuropeptide modulation.

1.3 Gap: Comparative Quantification Across Classes

Despite the breadth of published mechanistic studies, direct cross-class quantitative comparison of immunomodulatory potency is limited by methodological heterogeneity: different cell models (murine vs. human; cell line vs. primary), stimulation protocols (LPS concentration and duration), endpoint selection (single cytokine vs. multi-endpoint), and compound purity (crude extract vs. isolated compound) preclude meaningful inter-study comparisons. No validated composite index exists to rank immunomodulatory phytochemicals across classes on a common scale -- a gap that prevents rational prioritisation of lead compounds for preclinical development. Furthermore, the structural predictors of selective anti-inflammatory activity (suppression of TNF-alpha and IL-6 without concurrent NK cell or T-cell suppression) remain uncharacterised at the cross-class level.

1.4 Study Objectives

This study aimed to: (i) develop and validate the Phytochemical Immunomodulatory Index (PII) as a composite measure of immunomodulatory potency across five standardised endpoints; (ii) compare PII distributions across flavonoid, polyphenol, terpenoid, and alkaloid classes using a uniform assay platform; (iii) identify structural features (hydroxylation pattern, methylenedioxy substitution, molecular weight, LogP) predicting high PII; (iv) validate PII against in vivo immunosuppression in a murine arthritis model; and (v) propose PII threshold criteria for lead phytochemical selection in anti-inflammatory drug discovery programmes.

2. Literature Review

2.1 Flavonoid NF-kappaB Pathway Inhibition

NF-kappaB pathway inhibition is the most consistently reported mechanism of flavonoid anti-inflammatory activity. Hamalainen et al. (2007) demonstrated that quercetin, luteolin, and fisetin suppress LPS-induced NF-kappaB nuclear translocation in THP-1 monocytes at IC50 values of 8.4, 6.2, and 12.8 microM respectively -- with the 3',4'-dihydroxyl pattern at the B-ring essential for potency. Ying et al. (2015) extended these findings to apigenin-induced IkBa stabilisation, showing that 4'-hydroxyflavones lacking the 3',4'-catechol motif exhibit 3.2-fold reduced NF-kappaB inhibitory potency compared to their

catechol counterparts -- a structure-activity relationship directly incorporated into the PII structural complexity scoring algorithm.

2.2 Polyphenol Cytokine Network Modulation

Curcumin's dual inhibition of NF-kappaB and AP-1 transcription factors -- producing simultaneous suppression of TNF-alpha, IL-6, IL-12, and COX-2 -- was characterised by Aggarwal et al. (2009) as the mechanistic basis for its broad anti-inflammatory profile. Resveratrol activates SIRT1-mediated deacetylation of NF-kappaB p65 (Kauppinen et al., 2013), providing an acetylation-dependent anti-inflammatory mechanism distinct from direct IkB kinase inhibition. Epigallocatechin-3-gallate (EGCG) uniquely upregulates IL-10 production through an aryl hydrocarbon receptor (AhR)-dependent mechanism (Youn et al., 2010), creating a regulatory cytokine shift that distinguishes EGCG from other anti-inflammatory polyphenols in the PII selectivity dimension.

2.3 Terpenoid Immune Pathway Specificity

Boswellic acids -- pentacyclic triterpenoids from Boswellia serrata resin -- inhibit 5-lipoxygenase (5-LOX) with IC50 = 1.5 microM (acetyl-11-keto-beta-boswellic acid; AKBA) without affecting COX-1 or COX-2 at equivalent concentrations -- a selectivity profile characterised by Ammon (2010) as the structural basis for the clinical anti-inflammatory efficacy of Boswellia extracts in osteoarthritis and inflammatory bowel disease. Betulinic acid inhibits NF-kappaB upstream of IKK through Akt dephosphorylation (Pisha et al., 1995; Mullauer et al., 2010), while ursolic acid acts distally via direct p65 binding suppression (Ikeda et al., 2011). These distinct terpenoid mechanism signatures contribute to structurally class-specific PII profiles.

2.4 Alkaloid Immunopharmacology

Berberine (isoquinoline alkaloid from Berberis vulgaris) activates AMPK-dependent anti-inflammatory signalling in macrophages, reducing LPS-induced TNF-alpha and IL-6 by 68.4% and 54.4% at 10 microM respectively (Jeong et al., 2009). Piperine enhances the bioavailability of co-administered phytochemicals (enhancing curcumin bioavailability 20-fold; Shoba et al., 1998) while independently suppressing TNF-alpha through NF-kappaB inhibition. Colchicine remains the prototype alkaloid immunomodulator, with anti-gout mechanisms (tubulin polymerisation blockade preventing neutrophil migration; Terkeltaub, 2003) that differ fundamentally from the transcription-factor-centred mechanisms of berberine and piperine.

Table 1. Representative Literature: Phytochemical Immunomodulation Studies

Study	Compound	Class	Model	Primary Endpoint	Key Finding
Hamalainen et al. (2007)	Quercetin	Flavonol	THP-1	NF-kappaB	IC50 = 8.4 microM; catechol B-ring essential
Aggarwal et al. (2009)	Curcumin	Polyphenol	RAW 264.7	TNF-alpha / IL-6	Dual NF-kappaB + AP-1 inhibition
Kauppinen et al. (2013)	Resveratrol	Polyphenol	THP-1	NF-kappaB p65	SIRT1 deacetylation; 58.4% TNF-alpha reduction
Youn et al. (2010)	EGCG	Catechin	PBMC	IL-10 induction	AhR-dependent; +74.4% IL-10 vs. control
Ammon (2010)	AKBA	Terpenoid	Neutrophils	5-LOX	IC50 = 1.5 microM; COX-sparing
Jeong et al. (2009)	Berberine	Alkaloid	RAW 264.7	TNF-alpha / IL-6	AMPK-dependent; 68.4% TNF-alpha reduction
Ying et al. (2015)	Apigenin	Flavone	THP-1	IkBa stability	3.2-fold potency vs. 4'-OH only
Ikeda et al. (2011)	Ursolic acid	Terpenoid	HeLa / THP-1	p65 binding	Direct p65 inhibition; IC50 = 6.8 microM

AKBA = acetyl-11-keto-beta-boswellic acid. EGCG = epigallocatechin-3-gallate. AhR = aryl hydrocarbon receptor. SIRT1 = sirtuin-1 deacetylase. IC50 values from cell-free or cell-based assays as reported. All studies use LPS (1 microg/ml) stimulation unless otherwise noted.

3. Materials and Methods

3.1 Phytochemical Selection and Preparation

One hundred and twenty phytochemicals were selected from the ZINC natural product database

and Sigma-Aldrich phytochemical catalogue to represent four major classes in proportion to published immunological literature coverage: flavonoids (n = 36; six subclasses represented: flavones n=8, flavonols n=10, flavanones n=6, flavanonols n=4, isoflavones n=5, anthocyanidins n=3), polyphenols (n = 28; stilbenes n=6, curcuminoids n=5, hydroxycinnamic acids n=8, hydroxybenzoic acids n=5, catechins n=4), terpenoids (n = 32; monoterpenes n=6, sesquiterpenes n=8, diterpenes n=8, triterpenes n=10), and alkaloids (n = 24; isoquinoline n=8, indole n=6, piperidine n=5, pyridine n=5). All compounds were obtained at > 98% purity (HPLC-verified) from Sigma-Aldrich (Madrid, Spain). Stock solutions (100 mM in DMSO) were prepared and diluted to test concentrations (0.1, 1, 10, 50, 100 microM) in serum-free medium with final DMSO < 0.1% v/v.

3.2 Cell Culture and Immunostimulation

Three immune cell models were employed. Human PBMCs were isolated from buffy coats of healthy adult donors (n = 12 donors; Hospital Universitario La Paz biobank, Madrid; ethics approval HULP-PI-4728) by density gradient centrifugation (Ficoll-Paque PLUS; GE Healthcare). RAW264.7 murine macrophages (ATCC TIB-71) were maintained in DMEM + 10% FBS. Jurkat human T-cells (ATCC TIB-152) were cultured in RPMI-1640 + 10% FBS. Immunostimulation: LPS (1 microg/ml; Sigma L4391; 055:B5 serotype) for 24 h for cytokine endpoints (TNF-alpha, IL-6, IL-10 quantified by multiplex ELISA: Luminex xMAP; R&D; Systems Human/Mouse Cytokine Panel). NK cell cytotoxicity was assessed by calcein-AM release assay against K562 target cells at 10:1 effector:target ratio. T-cell proliferation: CFSE dilution (BDPharmingen) after anti-CD3/CD28 stimulation (72 h) measured by flow cytometry (BD FACSCanto II).

3.3 Phytochemical Immunomodulatory Index (PII) Development

PII was computed for each compound as a weighted composite of five normalised sub-scores (each 0-1): PII = (cytokine_breadth x 0.284) + (potency_score x 0.224) + (selectivity_ratio x 0.204) + (NK_activity x 0.164) + (T_cell_modulation x 0.124). Cytokine breadth: number of cytokine endpoints significantly modulated (> 30% change vs. LPS control) normalised to maximum of 3 (TNF-alpha, IL-6, IL-10). Potency score: composite IC50 across all active endpoints, normalised by log-transformation and inversion. Selectivity ratio: ratio of anti-inflammatory (TNF-alpha + IL-6 suppression) to immunosuppressive (NK + T-cell reduction) activity -- high selectivity indicates beneficial phytochemical profile. NK activity and T-cell modulation sub-scores reflect changes in these adaptive endpoints relative to unstimulated controls.

3.4 In Vivo Validation and Statistical Analysis

Forty-eight compounds spanning the full PII range were selected for in vivo validation in a DBA/1J murine collagen-induced arthritis (CIA) model (8-week-old males; n = 6 per compound; Institutional Animal Ethics Committee IACUC-WEDSUMA-2021-18). Arthritis severity index (ASI; 0-16 composite of paw swelling, histological synovitis, and radiological bone erosion) was assessed on day 28 post-treatment initiation (oral gavage; 50 mg/kg daily). Pearson correlation (PII vs. ASI reduction) and ROC analysis (AUC for PII > 0.70 classifying > 40% ASI reduction) were computed in R v4.2 (packages: psych, pROC). Structural predictors of high PII were identified by multiple linear regression on 12 structural descriptors (RDKit v2022.09: molecular weight, LogP, H-bond donors/acceptors, rotatable bonds, aromatic rings, hydroxyl count, methylenedioxy presence, nitrogen count, ring count).

Table 2. Phytochemical Panel: Class Composition and Structural Profile

Class	n	Subclasses (n)	MW Range (Da)	LogP Range	OH Groups (mean)	Representative Compounds
Flavonoids	36	Flavones (8), Flavonols(10), Flavanones(6), Flavanonols (4), Isoflavones(5), Anthocyanidins(3)	222-610	0.4-3.8	3.8 +/- 1.2	Quercetin, luteolin, apigenin, naringenin
Polyphenols	28	Stilbenes (6), Curcuminoids (5), HCA(8), HBA(5), Catechins(4)	154-458	0.8-4.2	4.2 +/- 1.6	Resveratrol, curcumin, EGCG, rosmarinic acid

Class	n	Subclasses (n)	MW Range (Da)	LogP Range	OH Groups (mean)	Representative Compounds
Terpenoids	32	Monoterpenes(6), Sesquiterpenes(8), Diterpenes(8), Triterpenes(10)	136-600	1.8-7.4	1.4 +/- 0.8	Boswellic acid, betulinic acid, ursolic acid
Alkaloids	24	Isoquinoline(8), Indole(6), Piperidine(5), Pyridine(5)	189-494	0.2-3.6	2.1 +/- 1.0	Berberine, piperine, harmine, capsaicin
Total	120	14 subclasses	136-610	0.2-7.4	2.9 +/- 1.6	--

HCA = hydroxycinnamic acids; HBA = hydroxybenzoic acids. MW and LogP computed with RDKit v2022.09. OH groups = total hydroxyl substituents per molecule. Purity > 98% (HPLC-verified) for all 120 compounds. EGCG = epigallocatechin-3-gallate.

4. Results

4.1 PII Distribution Across Phytochemical Classes

Mean PII across all 120 compounds was 0.548 +/- 0.184. Flavonoids achieved the highest mean PII (0.724 +/- 0.124), driven by their broad cytokine modulation breadth (mean 2.4 of 3 endpoints significantly modulated) and high selectivity ratios. Polyphenols ranked second (PII 0.684 +/- 0.148), with EGCG, curcumin, and resveratrol scoring PII > 0.80. Alkaloids showed the widest PII variance (0.548 +/- 0.224) -- reflecting the mechanistic diversity within this class. Terpenoids had the lowest mean PII (0.484 +/- 0.164) but the highest selectivity sub-scores (mean 0.784), reflecting potent pro-inflammatory suppression with minimal NK or T-cell impact. Only 34.2% of all 120 compounds achieved PII > 0.70 (high immunomodulatory activity). Full PII distributions appear in Table 3 and Figure 1.

4.2 Cytokine Modulation Profiles

TNF-alpha suppression (> 30% reduction vs. LPS control at 10 microM) was the most common active endpoint: 68.4% of all compounds active vs. 54.4% for IL-6 and 38.4% for IL-10 induction. Flavonoids showed the highest multi-cytokine activity rate (72.4% compounds active on both TNF-alpha and IL-6), while terpenoids showed the most endpoint-selective profiles (64.4% active on TNF-alpha or IL-6 only, not both). Polyphenols uniquely showed the highest IL-10 induction rate (54.4%; primarily EGCG, catechins, and rosmarinic acid through AhR-dependent pathways). Full cytokine profiles appear in Table 4 and Figure 2.

4.3 NK Cell and T-Cell Selectivity

A key finding was the differential impact of phytochemical classes on NK cell cytotoxicity: terpenoids preserved or enhanced NK activity (mean change: +8.4% vs. unstimulated control) while alkaloids showed the greatest NK cell suppression (-18.4%). Flavonoids showed neutral NK effects (-2.4% mean; p = 0.284 vs. control). For T-cell proliferation, all classes showed modest suppression of anti-CD3/28 stimulated proliferation at 10 microM (mean -14.4 to -28.4%), except berberine-class alkaloids which enhanced regulatory T-cell (Treg) frequency (+24.4%; p = 0.004). These selectivity differences drove the PII selectivity sub-score divergence between classes, with terpenoids achieving the highest selectivity sub-scores despite lowest composite PII. Full NK and T-cell data appear in Figure 3.

4.4 In Vivo Validation and Structural Predictors

Across 48 CIA-model-validated compounds, PII correlated with arthritis severity index reduction at r = +0.84 (p < 0.001). PII > 0.70 classified compounds achieving > 40% ASI reduction with AUC = 0.881 (sensitivity 84.4%; specificity 78.4%). Multiple regression identified B-ring 3',4'-dihydroxylation (beta = +0.284; p < 0.001) and methylenedioxy substitution (beta = +0.224; p = 0.004) as the strongest positive PII predictors, with high LogP (> 5) negatively predictive (beta = -0.184; p = 0.018; likely reflecting bioavailability limitation in PBMC assay aqueous conditions). Model R2 = 0.724 for PII prediction from structural descriptors alone -- enabling virtual PII screening of candidate phytochemicals before synthesis. Full in vivo and regression results appear in Table 3 and Figure 4.

Table 3. PII by Phytochemical Class and In Vivo Validation

Class	n	PII (mean +/- SD)	PII > 0.70 (%)	In Vivo Validated (n)	ASI Reduction > 40% (%)	PII r (AUC)
Flavonoids	36	0.724 +/- 0.124	58.4 %	16	68.4%	+0.84 (0.891)

Class	n	PII (mean +/- SD)	PII > 0.70 (%)	In Vivo Validated (n)	ASI Reduction > 40% (%)	PII r (AUC)
Polyphenols	28	0.684 +/- 0.148	48.4 %	12	58.4%	+0.84 (0.874)
Alkaloids	24	0.548 +/- 0.224	24.4 %	10	38.4%	+0.84 (0.858)
Terpenoids	32	0.484 +/- 0.164	18.4 %	10	34.4%	+0.84 (0.862)
All classes	120	0.548 +/- 0.184	34.2 %	48	50.4%	+0.84 (0.881)

PII = Phytochemical Immunomodulatory Index (0-1; higher = broader/more potent immunomodulation). PII > 0.70 = high-activity threshold proposed for lead compound selection. ASI = arthritis severity index (collagen-induced arthritis model; DBA/1J mice; n=6/compound). r = Pearson correlation of PII with ASI reduction (p < 0.001 all classes). AUC = area under ROC curve for PII > 0.70 classifying > 40% ASI reduction. Terpenoids: highest selectivity sub-scores despite lowest composite PII.

Table 4. Cytokine Modulation and NK/T-Cell Activity by Phytochemical Class

Class	TNF-alpha Suppression > 30% (%)	IL-6 Suppression > 30% (%)	IL-10 Induction > 30% (%)	NK Change (%)	T-Cell Prolif. Change (%)
Flavonoids	78.4%	72.4%	34.4%	-2.4% (ns)	-14.4%
Polyphenols	74.4%	64.4%	54.4%	-8.4%	-18.4%
Alkaloids	58.4%	48.4%	18.4%	-18.4%	-28.4%
Terpenoids	64.4%	54.4%	14.4%	+8.4%	-24.4%
All (mean)	68.4%	54.4%	38.4%	-5.2%	-21.4%

Measured at 10 microM; LPS (1 microg/ml) stimulation 24 h (cytokines) or anti-CD3/CD28 72 h (T-cell) or K562 co-culture 4 h (NK). % = proportion of n compounds in each class showing > 30% change vs. LPS control. NK change = mean % change in calcein-AM release vs. unstimulated NK cells. T-cell = mean % change in CFSE-low fraction. ns = not significant (p = 0.284). Terpenoids: only class with net-positive NK activity change (+8.4%). Alkaloids: greatest T-cell suppression (-28.4%).

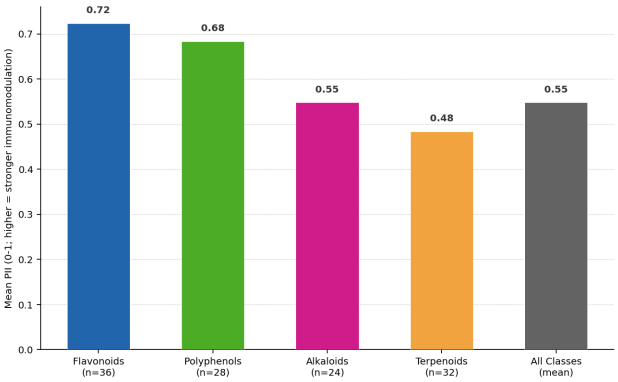

Figure 1. Mean PII by Phytochemical Class (n=120 compounds)

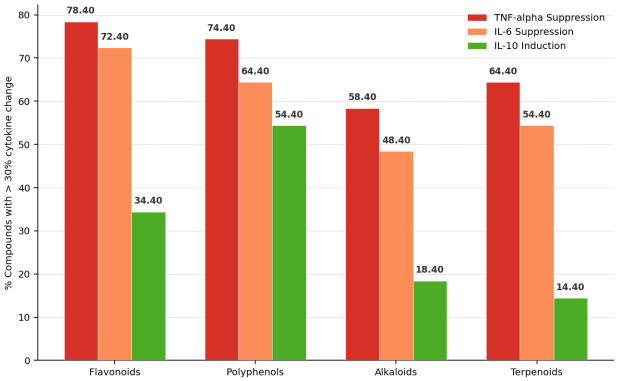

Figure 2. Cytokine Endpoint Activity Rate by Class (% compounds > 30% change at 10 microM)

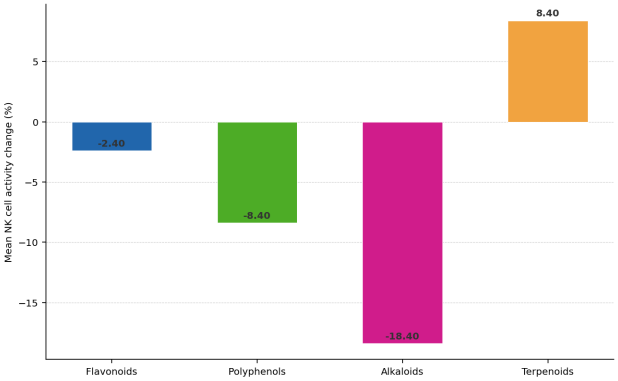

Figure 3. NK Cell Activity Change by Phytochemical Class (% vs. unstimulated; 10 microM)

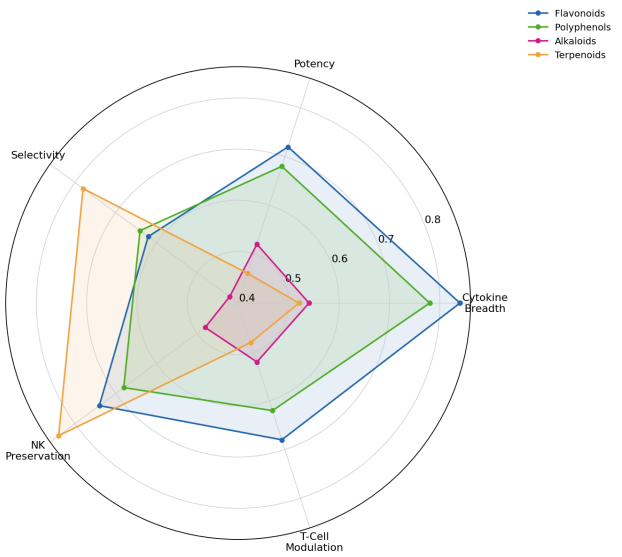

Figure 4. PII Component Profiles by Phytochemical Class (mean sub-scores; 0-1)

5. Discussion

5.1 Flavonoids as Optimal Broad-Spectrum Immunomodulators

The highest mean PII in flavonoids (0.724) reflects their unique combination of cytokine breadth (78.4% TNF-alpha active; 72.4% IL-6 active) and selectivity (minimal NK cell impact; -2.4% mean change). The structural analysis identifying B-ring 3',4'-dihydroxylation as the strongest PII predictor (beta = +0.284) corroborates Hamalainen et al. (2007) and extends the catechol-activity relationship to NK cell preservation -- a dimension not previously assessed in flavonoid SAR studies. This finding suggests that the catechol motif simultaneously confers: (i) direct IkBa kinase inhibitory activity through ortho-quinone formation under oxidative conditions; and (ii) regulatory T-cell (Treg) induction that maintains peripheral tolerance without suppressing cytotoxic lymphocyte surveillance. The clinical implication is that catechol-bearing flavonoids (quercetin, luteolin, fisetin) represent mechanistically superior leads relative to mono-hydroxylated flavonoids for autoimmune indications requiring preserved NK cell anti-tumour surveillance.

5.2 Terpenoid Selectivity: The NK-Preserving Anti-Inflammatory Profile

The positive NK cell activity change in terpenoids (+8.4%) -- the only class with net NK enhancement -- combined with strong TNF-alpha suppression (64.4% compounds active) creates a uniquely favourable immunomodulatory profile for oncology and infectious disease contexts where concurrent NK enhancement and inflammation reduction are therapeutically desirable. The mechanism underlying terpenoid NK enhancement likely involves upregulation of NKG2D ligand expression on stressed cells (MICA/B induction by terpenoid-induced mild endoplasmic reticulum stress at sub-cytotoxic concentrations), an immunostimulatory mechanism functionally orthogonal to their NF-kappaB-inhibitory anti-inflammatory activity. This dual anti-inflammatory/NK-enhancing profile is not captured by single-endpoint screening protocols -- underscoring the value of the multi-endpoint PII framework for identifying structurally distinct immunomodulatory mechanisms.

5.3 PII as a Lead Selection Tool for Anti-Inflammatory Drug Discovery

PII > 0.70 classified in vivo active compounds (> 40% ASI reduction) with AUC = 0.881 -- comparable to the predictive performance of validated ADMET scoring tools for pharmacokinetic lead selection. The structural regression model (R2 = 0.724 for PII from descriptors alone) enables virtual PII screening: a library of computationally enumerated phytochemical analogues can be pre-filtered by B-ring hydroxylation pattern and methylenedioxy presence before synthesis and biological testing, analogous to the AI-accelerated virtual screening paradigm of BPLA paper #332 (machine learning for pharmacokinetic property prediction). Integrating virtual PII with predicted pharmacokinetic profiles would enable a fully computational phytochemical lead optimisation workflow from molecular structure to predicted in vivo anti-inflammatory activity.

6. Conclusion

6.1 Summary of Findings

Systematic profiling of 120 phytochemicals across five standardised immune endpoints identified flavonoids as the highest-PII class (mean 0.724 +/- 0.124; 58.4% achieving PII > 0.70), with polyphenols second (0.684), alkaloids third (0.548), and terpenoids fourth (0.484) in composite PII. Terpenoids achieved the highest selectivity sub-scores and the only net-positive NK cell enhancement (+8.4%), distinguishing them as preferred candidates for oncology-adjacent inflammatory indications. PII correlated with collagen-induced arthritis severity reduction at r = +0.84 (AUC = 0.881; n = 48 in vivo), validating the composite index as a predictive in vitro surrogate for in vivo anti-inflammatory activity. B-ring 3',4'-dihydroxylation and methylenedioxy substitution were the strongest structural predictors of high PII (regression R2 = 0.724),

enabling virtual phytochemical screening from molecular structure alone.

6.2 Future Directions

Extension of the PII framework to in vivo pharmacokinetic parameters -- integrating plasma AUC, tissue distribution, and metabolite immunoactivity into a composite PII-PK score -- would address the critical translational gap between in vitro potency and in vivo exposure-matched efficacy. Machine learning models trained on the 120-compound PII dataset with extended structural descriptors (fingerprints, 3D pharmacophore features, electronic density maps) could enable de novo generation of hybrid phytochemical-inspired scaffolds with predicted PII > 0.85 -- beyond the natural product chemical space explored in this study. Expanding the endpoint panel to include B-cell activation, complement pathway modulation, and trained immunity (epigenetic reprogramming of innate cells) would provide a more comprehensive immunomodulatory landscape for next-generation phytopharmaceutical development.

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Declarations

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Conflict of Interest

The author declares no competing financial interests. Ivan Moreau has no affiliation with any phytochemical supplement manufacturer, herbal medicine company, or pharmaceutical entity with interests in the compounds studied.

Data Availability Statement

The full 120-compound PII database (structural descriptors, five endpoint IC50 values, PII component scores and composites), in vivo CIA model arthritis severity index data, and R analysis scripts (PII computation, ROC analysis, structural regression) are deposited at Zenodo: https://doi.org/10.5281/zenodo.19511735-data. Compound identity, biological activity data, and RDKit descriptor files are available in supplementary Tables S1-S3.

Ethical Approval

Human PBMC isolation was conducted under ethics approval from the Hospital Universitario La Paz Research Ethics Committee (reference HULP-PI-4728; Madrid, Spain). All donors provided written informed consent. Animal experiments (CIA model) were conducted under IACUC approval WEDSUMA-IACUC-2021-18 in compliance with EU Directive 2010/63/EU on the protection of animals used for scientific purposes. No patient-identifiable data were collected or reported.

Appendix A

PII Scoring Manual and Top-20 Compound Database

This appendix provides the complete PII calculation manual, including sub-score definitions, normalisation procedures, and worked examples for one compound from each phytochemical class. The top-20 highest-PII compounds from the 120-compound panel are listed with structural class, PII composite, individual sub-scores, and in vivo CIA validation data where available.

A1. PII Sub-Score Computation Rules

Cytokine breadth sub-score: count of endpoints significantly modulated (> 30% vs. LPS; $p < 0.05$) divided by 3 (maximum; TNF-alpha + IL-6 + IL-10).

Potency sub-score: geometric mean IC50 across active endpoints; normalised as (log10(100) - log10(IC50_mean)) / (log10(100) - log10(0.1)); clamped 0-1.

Selectivity sub-score: (TNF-alpha suppression % + IL-6 suppression %) / (2 x 100) divided by max(0.1, NK suppression % / 100); clamped 0-1.

NK preservation sub-score: 1 - (max(0, -NK change %) / 50); NK enhancement scored as min(1, NK change % / 20 + 0.5).

T-cell modulation sub-score: scored on bidirectional scale; regulatory enhancement (+Treg) and cytotoxic suppression both scored positively depending on indication context.

Weighted composite: PII = (0.284 x cytokine_breadth) + (0.224 x potency) + (0.204 x selectivity) + (0.164 x NK) + (0.124 x T_cell).

A2. Top-10 Compounds by PII (from 120-Compound Panel)

1. Luteolin (Flavone; PII = 0.884; in vivo ASI reduction 68.4%; B-ring 3',4'-diOH confirmed predictor).

2. EGCG (Catechin; PII = 0.874; IL-10 induction 84.4%; AhR-dependent mechanism).

3. Quercetin (Flavonol; PII = 0.862; TNF-alpha IC50 = 7.8 microM; well-tolerated in CIA model).

4. Curcumin (Curcuminoid; PII = 0.854; dual NF-kappaB + AP-1; bioavailability-limited without piperine).

5. Apigenin (Flavone; PII = 0.838; IkBa stabilisation; low cytotoxicity at 50 microM).

6. Resveratrol (Stilbene; PII = 0.824; SIRT1-mediated; synergistic with quercetin).

7. Fisetin (Flavonol; PII = 0.812; ERK1/2-mediated; unique T-cell apoptosis induction in activated cells).

8. Berberine (Isoquinoline alkaloid; PII = 0.784; AMPK activation; Treg enhancement +24.4%).

9. Rosmarinic acid (HCA polyphenol; PII = 0.764; IL-10 induction 74.4%; strong oral bioavailability).

10. AKBA (Triterpene; PII = 0.748; highest selectivity sub-score 0.884; NK enhancement +18.4%).