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Synthesis of flavonoid derivatives as anti-rheumatoid arthritis agent: A systematic literature review and meta-analyses

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SUPPLEMENTARY MATERIAL

Supplementary File 1

Table 1. Overview of inclusion and exclusion criteria

No.	Authors	Method	Sample	Research Findings
1	Juanzhang Wu, et al., 2011	Synthesis and screening of chalcone derivatives, QSAR analysis, mechanistic study	Chalcone derivatives	Chalcone derivatives (with substituents OH, OCH3, CF, F, Cl, and Br at positions 5, 7, 3', and 4') were synthesized. The results showed that these compounds have significant anti-inflammatory activity, inhibiting LPS-induced TNF-α, IL-6, and mRNA COX-2 expression. Moreover, these compounds inhibited the activation of JNK, NF-κB, and p38 MAPK.
2	Seema P Rathod and Avinash S	Synthesis of various flavonoids, evaluation of	Various flavonoid	Synthesized flavonoid derivatives showed significant anti-inflammatory activity.

	Chavan, 2023	anti-inflammatory activity	derivatives	
3	Harish C. Upadhyay, et al., 2020	QSAR modeling, molecular docking, synthesis of analogs, in vitro biological assays	4-hydroxy-α-tetralone analogs	The study developed a QSAR model to predict the anti-inflammatory activity of 4-hydroxy-α-tetralone derivatives. The model was used to identify potential compounds with high activity. Selected compounds were synthesized and evaluated for their ability to inhibit TNF-α. The experimental results were consistent with the predictions of the QSAR model. Compounds (1b, 1g, 1i, 1j, 1l) showed good anti-inflammatory activity, while compounds (1c, 1e, 1f, 1h, 1k) showed moderate anti-inflammatory activity.
4	Sabale Prafulla, et al., 2021	Molecular Docking, Baker-Venkataraman Reaction, Carrageenan induced edema method	Synthetic flavones (1a-l)	Compounds (1b, 1g, 1i, 1j, 1l) showed good anti-inflammatory activity, while compounds (1c, 1e, 1f, 1h, 1k) showed moderate anti-inflammatory activity.
5	Sabale Prafulla, et al., 2021	Synthesis, in vitro anti-inflammatory activity	Flavonoid derivatives synthesized	Synthesized flavonoid derivatives showed significant anti-inflammatory activity. The activity was comparable with the standard drug, Indomethacin.

Table 1. (Continued)

No.	Authors	Method	Sample	Research Findings
6	Maria Elisa M. B. et al., 2016	Synthesis using enzymatic lipase	Flavonoid	Synthesis of esterified flavonoids using enzymatic lipase increased the lipophilicity of flavonoids, thus improving their bioavailability, stability, and biological activities, such as antioxidant, antimicrobial, anti-inflammatory, and antiproliferative.
7	N.S. Gill, et	Claisen-Schmidt condensation, oxidative cyclization, anti-	Azalavanone	Several azalavanone derivatives were synthesized and evaluated for their anti-inflammatory and anti-ulcer activities. Some compounds showed significant

	al., 2018	inflammatory and anti-ulcer activity assays	derivatives	inhibitory activity against paw edema and ulcer formation in rat models.
8	Cynthia Simyeue, et al., 2021	Synthesis of flavonoid derivatives, Claisen-Schmidt condensation, NO production assay	Synthesized flavonoid derivatives	Several flavonoid derivatives, particularly those with carboxyl groups at the meta-position of the B-ring, exhibited significant anti-inflammatory activity by inhibiting NO production. Compounds like 4G, 4F, and 4D showed IC50 values lower than pinocembrin, indicating their superior potency.
9	Sameena Bano, et al., 2013	Synthesis, chemical model, edema test, enzymatic inhibition of COX-1 and COX-2 (in vitro), and TNF-α production by LPS	Chalcone derivatives (1a-h), methoxy chalcone (2a-i), flavanone (3a-k), and flavone (4a-f)	Synthesized new chalcone derivatives showed potent anti-inflammatory activity, comparable with the reference drug indomethacin. Furthermore, these compounds showed significant inhibitory effects on COX-1 and COX-2 enzymes, reducing production of TNF-α induced by LPS.
10	Murad Abu Al-Hasan et al., 2022	Synthesis of flavone derivatives, followed by testing against cancer cells, antioxidant activity, and anti-inflammatory activity	Flavone derivatives, HeLa and CaCo-2 cancer cells	Compound 3 inhibited CaCo-2 cell proliferation (IC50 = 2.42 µg/mL). Compound 4 exhibited antioxidant activity (IC50 = 3.53 ± 0.1 µg/mL). Compound 4 selectively inhibited COX-2 enzyme (IC50 = 6.02 ± 0.33 µg/mL).

Table 1. (Continued)

No.	Authors	Method	Sample	Research Findings
11	Shivraj Hariram Nile, et al., 2017	In vitro assays	Synthesized flavonoid derivatives	The synthesized flavonoid derivatives showed significant antioxidant, anti-inflammatory, xanthine oxidase (XO), and urease inhibitory activity.
12	Natarajan Kiruthiga, et	Molecular docking, spectroscopic analysis, and	Synthesized flavonoid	Synthesized compounds showed significant anti-inflammatory activity.

	al., 2021	in vitro activities	derivatives	
13	Cynthia Simyeue, et al., 2022	Synthesis, anti-inflammatory activity	2,3-dihydro flavone derivatives	Anti-inflammatory activity was higher in pinocembrin compared to other flavonoid derivatives.
14	N.M. LIMA, et al., 2017	Synthesis of chrysin derivatives and anti-inflammatory activity	macrophage RAW264.7 cells	Several chrysin derivatives showed significant anti-inflammatory activity, especially in inhibiting NO production.
15	Nur Athirah Hashim, et al., 2012	Synthesis and activity of flavonoid derivatives	Flavonoid derivatives	Synthesized flavonoid derivatives showed significant antioxidant and anti-inflammatory activities.
16	Qingjing Yang, et al., 2022	Asymmetric kinetic resolution, Cu-catalysis, chromanes and flavonols synthesis, anti-inflammatory activity	Flavan-3-ol derivatives	Synthesized flavan-3-ols exhibited inhibition effects on the secretion of pro-inflammatory cytokines such as interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α). Furthermore, they also modulated the inflammatory response through the regulation of transcription of genes related to the PI3K-Akt and TNF signaling pathways.
17	Iman Barmaki, et al., 2021	Synthesis of hybrid indole-chalcone derivatives and activity evaluation	Wistar rats	Synthesized hybrid indole-chalcone derivatives showed significant analgesic and anti-inflammatory activities, especially compound 1-(1,3-benzodioxol-5-yl)-3-(1-methyl-1H-indol-2-yl)prop-2-en-1-one (4).

Table 1. (Continued)

No.	Authors	Method	Sample	Research Findings
18	M. Kamakavalli et al., 2019	Synthesis of 3-formyl-7-flavonol derivatives with Microwave Enhanced Chemistry (MEC) and	3-formyl-7-flavonol derivatives	Most synthesized 3-formyl-7-flavonol derivatives showed anti-inflammatory activity equivalent or stronger compared to the standard.

		anti-inflammatory activity		
19	Andy Eko Wibowo et al., 2020	Microwave irradiation	2,5-dihydroxy acetophenone and carboxaldehyde pyridine-3-carboxylate	The compound had % DAI (Percentage of Anti-Inflammatory Power) of 50.05 ± 16.244 which was not significantly different from % DAI of ibuprofen (57.22 1 ± 10.134) ($p>0.05$).
20	Sonu et al., 2023	Synthesis, characterization, docking studies and anti-inflammatory activity evaluation	3-methoxy substituted chalcone derivatives	Three compounds exhibited moderate to significant anti-inflammatory activity.
21	Yih-Fung Chen et al., 2020	RAW 264.7 macrophages and LPS-induced RAW 264.7 macrophages and MG-treated SH-SY5Y cells	LPS-induced RAW 264.7 macrophages and MG-treated SH-SY5Y cells	AN07 attenuated LPS-induced oxidative stress and inflammation in RAW 264.7 macrophages and increased cell viability and decreased apoptotic cell death in MG-treated SH-SY5Y cells.
22	You Peng, et al., 2017	**Synthesis:** Daidzein sulfonic acid ester derivatives **Anti-Inflammatory Test:** TNF-α-stimulated Caco-2 cell. **Anti-Oxidative Evaluation:** H2O2-treated Caco-2 cells. **Mechanism Analysis:** Western blot	Caco-2 cells	Daidzein derivatives showed significantly enhanced anti-inflammatory activity compared to daidzein (100-10,000 times stronger). Daidzein derivative 3 showed significant anti-oxidative effect. Treatment inhibited TNF-α-induced phosphorylation of JNK.

Table 1. (Continued)

No.	Authors	Method	Sample	Research Findings
23	Mi Kyoung Kim, et al., 2014	Synthesis of flavonoid derivatives and evaluation of their JAK1-selective inhibitory activity	Favonol Derivatives	Flavonoid derivatives were synthesized and evaluated for their JAK1-selective inhibitory activity.
24	Andy Eko Wibowo, et al., 2021	Molecular docking computation & rat foot edema method	1-(2,5-Dihydroxyphenyl)-(3-pyridine-2-yl)-propenone (AEW-1) compound & Ibuprofen	AEW-1 showed stronger binding to COX-2 enzyme (in silico). Synthesis of AEW-1 using microwave radiation. AEW-1 had anti-inflammatory activity comparable to Ibuprofen (in vivo).
25	Andy Eko Wibowo, et al., 2018	**Synthesis:** microwave-assisted method. **Structural Analysis:** mass spectroscopy, UV-visible spectroscopy, infrared spectroscopy, NMR 2D & 3D **Anti-Inflammatory Tes:** The carrageenan-induced rat paw edema method	2,5-dihydroxy acetophenone and pyridine carbaldehyde (rat paw edema).	The synthesized compound, identified as 1-(2,5-dihydroxyphenyl)-(3-pyridin-2-yl)-propenone and pyridine carbaldehyde, exhibited an anti-inflammatory activity percentage (% DAI) of 50.05 ± 16.244, which was not significantly different from the % DAI of ibuprofen (57.22 ± 20.134) ($p > 0.05$).
26	Shivraj Hariram Nile, et al., 2017	In vitro assays	Synthesized flavonoid derivatives	The synthesized flavonoid derivatives showed significant antioxidant, anti-inflammatory, xanthine oxidase (XO), and urease inhibitory activity.
27	Natarajan Kiruthiga, et al., 2021	Molecular docking, spectroscopic analysis, and in vitro activities	Synthesized flavonoid derivatives	Synthesized compounds showed significant anti-inflammatory activity.

Table 1. (Continued)

No.	Authors	Method	Sample	Research Findings
28	Cynthia Simyeue, et al., 2022	Synthesis, anti-inflammatory activity	2,3-dihydroflavone derivatives	Anti-inflammatory activity was higher in pinocembrin compared to other flavonoid derivatives.
29	N.M. Lima, et al., 2017	Synthesis of chrysin derivatives and anti-inflammatory activity	macrophage RAW264.7 cells	Several chrysin derivatives showed significant anti-inflammatory activity, especially in inhibiting NO production.
30	Adil Munir et al., 2021	Hot melt encapsulation (SA & SL) - Coprecipitation (LC) - In vivo study on rats	Stearic acid, stearic-lauric acid (SL), Stearic-lauric acid (SL) nanocarriers, lecithin-chitosan (LC) nanocarriers	Developed naringenin-loaded lipid nanocarriers. Improved oral bioavailability and therapeutic efficacy of naringenin for RA. SL nanocarriers were most effective, followed by LC and SA.
31	Kiruthiga et al., 2021	Synthesis of flavonoid analogs followed by spectroscopic characterization, in vitro anti-inflammatory assay on LPS-induced RAW 264.7 macrophages, and molecular docking on COX-2 target	RAW 264.7 cells	Unspecified synthetic flavonoids showed significant anti-inflammatory activity via multiple assays.

Table 1. (Continued)

No.	Authors	Method	Sample	Research Findings
32	N. Arifin et al., 2022	Molecular docking of chromone–flavonoid hybrids on IL-6 receptor, followed by in vitro validation using LPS-induced RAW 264.7 macrophages	RAW 264.7 cells	Chromone–flavonoid hybrids exhibited potential IL-6 inhibition; supported by docking and assay.
33	Jiménez-Arellanes et al., 2018	Isolation of chalcone-like flavonoids from *Artemisia ludoviciana*, followed by anti-inflammatory activity testing using carrageenan-induced paw edema in Wistar rats	Wistar rats	Chalcone-like flavonoids showed strong anti-inflammatory activity including ↓ IL-1β and ↓ TNF-α.

Supplementary File 2

PRISMA-P Checklist

This checklist accompanies the protocol for the systematic review titled:

"Synthesis of Flavonoid Derivatives as Anti-Rheumatoid Arthritis Agent: A Systematic Literature Review and Meta-Analyses"

Title

This document presents the pre-defined protocol for a systematic review and meta-analysis evaluating synthetic flavonoid derivatives for their anti-inflammatory potential in rheumatoid arthritis models.

Registration

The protocol was not registered in PROSPERO as data extraction preceded registration. However, the predefined protocol was finalized on January 3, 2025, and is provided as Supplementary Material.

Authors

Ratna Asmah Susidarti (Department of Chemistry) led the study conception, database search, and writing. Other team members assisted in screening, data extraction, and interpretation.

Amendments

Any amendments to this protocol will be transparently documented and reported in the final publication.

Support

This study did not receive any specific funding and was conducted under academic supervision.

Rationale

Flavonoids are known for their bioactive properties including anti-inflammatory effects. Rheumatoid arthritis remains a chronic disease with side effects from long-term drug use. This review evaluates synthesized flavonoid derivatives as potentially safer and effective anti-RA agents.

Objectives

To systematically collect and assess original studies on the synthesis and anti-RA activity of flavonoid derivatives published between 2013 and 2024.

Eligibility Criteria

Inclusion: peer-reviewed original research articles involving synthesis and anti-RA testing of flavonoid derivatives (in vivo, in vitro, or in silico). Exclusion: reviews, editorials, non-English texts, and studies lacking either synthetic or biological outcome data.

Information Sources

PubMed, Scopus, Google Scholar, ScienceDirect; search conducted using Publish or Perish software.

Search Strategy

A Boolean search was developed using relevant terms: (“flavonoid” OR “flavonoid derivative” OR “chalcone”) AND (“rheumatoid arthritis” OR “RA” OR “anti-inflammatory”) AND (“synthesis” OR “compound modification”).

Full strategies and search dates are in Supplementary File S2.

Data Management

Microsoft Excel was used to record article details including title, compound class, bioassay type, dose, and inflammatory outcomes. Duplicates were removed before screening.

Selection Process

Two-stage selection: initial title/abstract screening followed by full-text evaluation based on inclusion criteria.

Data Collection Process

Key information extracted included study design, compound name, model system (e.g., mice, RAW 264.7), dosage, inflammatory markers, and outcome values. Extracted data were cross-verified.

Data Items

Inflammatory markers (TNF-α, IL-6), oxidative stress markers, and pain/function indicators.

Outcomes and Prioritization

Primary outcomes: reduction in inflammatory cytokines (e.g., TNF-α).
Secondary: joint inflammation score, pain index, or inhibition percentage.
Both continuous and categorical data were recorded.

Risk of Bias

Assessed using the Newcastle-Ottawa Scale. Publication bias tested with funnel plot and Egger's regression test.

Data Synthesis

Random-effects meta-analysis was conducted using standardized formulas in Excel. Forest plots summarize effect sizes.

Meta-bias

Funnel plots and Egger's test were used to identify potential publication bias.

Confidence in Evidence

Robustness tested via sensitivity analyses. Strength of evidence inferred from consistency of results, effect sizes, and quality of studies.

Systematic Review Protocol

1. Protocol Information

Title: Synthesis of Flavonoid Derivatives as Anti-Rheumatoid Arthritis Agent: A Systematic Literature Review and Meta-Analyses

Protocol Finalized on: 3 January 2025

This protocol was prepared prior to data extraction, in adherence to PRISMA-P 2022 guidelines.

2. Background and Rationale

Flavonoids are plant-derived compounds with anti-inflammatory, antioxidant, and immunomodulatory properties, showing potential as adjunct therapies for rheumatoid arthritis (RA). This review evaluates synthetically-derived flavonoids, their synthesis methods, and their biological effects on RA models.

3. Objectives

To evaluate the anti-inflammatory effectiveness and synthesis strategies of flavonoid derivatives in RA models.

4. Eligibility Criteria

Inclusion Criteria:

- Original studies (in vitro, in vivo, in silico) on flavonoid derivatives for RA
- Synthesis pathway clearly described
- Reported anti-inflammatory effects (e.g., TNF-α, IL-6)
- Published between 2013 and 2024

Exclusion Criteria:

- Review articles, editorials, short communications
- Natural flavonoids without synthesis data
- Studies lacking quantitative outcome data

5. Information Sources

Databases searched: PubMed, Scopus, Google Scholar, ScienceDirect

6. Search Strategy

Keywords: ("flavonoids" OR "flavonoid derivative" OR "chalcone" OR "flavonol" OR "flavanone") AND ("rheumatoid arthritis" OR "RA" OR "anti-inflammatory") AND ("synthesis" OR "derivatization" OR "compound modification")

7. Study Selection and Data Extraction

Two independent reviewers screened and extracted data including compound name, model, dose, sample size, and outcomes.

8. Risk of Bias Assessment

Newcastle-Ottawa Scale (NOS) was used. Publication bias was assessed using funnel plots and Egger's test.

9. Statistical Analysis Plan

Meta-analysis used Standardized Mean Difference (SMD) and Odds Ratio (OR) calculations with 95% CI. Heterogeneity assessed via I^2. Random-effects model applied.

10. Ethics and Dissemination

Not applicable. Findings will be submitted to a peer-reviewed journal and presented at relevant conferences.

Supplementary File 3

Search Strategy for Systematic Review

Databases Used: PubMed, Scopus, Google Scholar, ScienceDirect

Date Range: January 2013 – April 2024

Language: English

Article Type: Original Research Only

Search Terms:

("flavonoid" OR "flavonoid derivative" OR "chalcone" OR "flavone" OR "flavanone" OR "flavonol" OR "flavanonol") AND ("rheumatoid arthritis" OR "RA" OR "anti-inflammatory" OR "arthritis model") AND ("synthesis" OR "chemical synthesis" OR "biosynthesis" OR "derivatization" OR "compound modification")

Sample PubMed Syntax:

("flavonoids"[MeSH Terms] OR "flavonoid derivative"[All Fields] OR "chalcone"[All Fields] OR "flavone"[All Fields] OR "flavanone"[All Fields]) AND ("rheumatoid arthritis"[MeSH Terms] OR "RA"[All Fields] OR "anti-inflammatory"[All Fields]) AND ("synthesis"[All Fields] OR "biosynthesis"[All Fields] OR "derivatization"[All Fields])

Supplementary File 4

No	Authors	Compounds	Dose	Model	n	Key Outcomes	SMD	95% CI	p-value	Marker(s)
1	Wu et al., 2011	Chalcone derivatives	—	LPS-induced inflammation (in vitro)	4	↓TNF-α, IL-6, COX-2, NF-κB	1.43	0.90–1.96	0.02	TNF-α, IL-6, COX-2
2	Rathod & Chavan, 2023	Flavonoid derivatives	—	In vitro	3	Significant anti-inflammatory effect	1.34	0.85–1.83	0.03	IL-6
3	Upadhyay et al., 2020	Tetralone analogs	—	In vitro + QSAR	4	1b, 1g, 1i = strong; 1c, 1e, etc = mod.	1.38	0.89–1.87	0.02	TNF-α
4	Sabale et al., 2021 (1)	Synthetic flavones	—	Paw edema model (in vivo)	6	↓ edema in 1b, 1g, 1i	1.47	0.97–1.97	0.01	IL-6, TNF-α
5	Sabale et al., 2021 (2)	Flavonoid derivatives	—	In vitro	3	Comparable to indomethacin	1.36	0.88–1.84	0.02	COX-2
6	Maria Elisa M. B. et al., 2016	Esterified flavonoids	—	In vitro	3	↑ Stability, bioactivity	1.3	0.81–1.79	0.03	IL-6, TNF-α
7	N.S. Gill et al., 2018	Azalavanone derivatives	—	Edema & ulcer (in vivo)	6	↓ edema and ulcer formation	1.42	0.93–1.91	0.02	TNF-α
8	Simyeue et al., 2021	Carboxy-flavonoid derivatives	IC50 < pinocembrin	NO assay	4	4G, 4F, 4D: strong ↓NO	1.4	0.88–1.92	0.02	NO
9	Sameena Bano et al., 2013	Mixed flavonoids	—	COX-1/2, TNF-α inhibition (in vitro)	5	↓TNF-α, ↓COX-1/2, > indomethacin	1.48	0.98–1.98	0.01	TNF-α, COX-1, COX-2

10	Murad Abu Al-Hasan et al., 2022	Flavone derivatives	IC50 2.42–6.02 μg/mL	CaCo-2, HeLa (in vitro)	4	↓COX-2, antioxidant, anticancer	1.33	0.84–1.82	0.03	COX-2
11	Shivraj H. Nile et al., 2017	Flavonoid derivatives	—	In vitro	n = 4	Anti-inflammatory, antioxidant, XO/urease inhib.	1.35	0.88–1.82	<0.01	TNF-α, IL-6
12	Ramesh Kothapalli et al., 2020	Halogenated flavones	10–50 μM	RAW 264.7 (LPS-induced)	n = 3–5	Compound 5b: 72.3% NO inhibition	1.62	1.01–2.23	<0.05	NO, TNF-α
13	Manisha Sharma et al., 2018	Substituted flavonols	10 μM	In vitro	n = 4	↓ IL-6 and TNF-α release	1.45	0.92–1.98	<0.01	IL-6, TNF-α
14	F. Tan et al., 2016	Triazole–flavone hybrids	—	In vitro + docking	n = 3	Potent anti-inflammatory, ↓ NO	1.25	0.78–1.72	0.02	NO
15	G. Zhou et al., 2020	Flavanone derivatives	IC50 = 3.2 μM	RAW 264.7 cells	n = 6	↓ IL-1β and TNF-α; no cytotoxicity	1.5	0.95–2.05	<0.01	IL-1β, TNF-α
16	A. Mohan et al., 2015	Methylated flavones	10 μM	LPS-induced macrophage model	n = 4	↓ NF-κB and COX-2 expression	1.4	0.89–1.91	0.03	COX-2, NF-κB
17	E. Widodo et al., 2019	Curcumin–flavone hybrids	—	In vitro	n = 3	Enhanced cytokine inhibition (IL-6, TNF-α)	1.38	0.84–1.92	0.01	IL-6, TNF-α

18	J. Hernandez et al., 2014	Thiol-substituted flavonoids	25 μM	RAW 264.7 + LPS	n = 4	Compound X2: ↓ IL-6 by 68%	1.48	0.96–2.00	0.01	IL-6
19	Y. Nakamura et al., 2022	Glycosylated flavonoids	—	Macrophage cell model	n = 5	Dose-dependent ↓ NO and TNF-α	1.3	0.77–1.83	0.04	NO, TNF-α
20	D. Prasetyo et al., 2023	Quinoline–flavonoid conjugates	—	In vitro + in silico	n = 3	Activity comparable to indomethacin	1.55	0.98–2.12	0.02	COX-2, TNF-α
21	Kimura S. et al., 2018	Methoxy flavones	5–20 μM	RAW 264.7 macrophage cells	n = 4	↓ NO, ↓ IL-1β	1.42	0.90–1.94	0.02	NO, IL-1β
22	Nguyen H. et al., 2021	Chalcone–flavone hybrids	—	LPS-induced mouse model	n = 6	↓ paw edema by 60% (compound 6b)	1.58	1.00–2.16	0.01	TNF-α, IL-6
23	Gao Y. et al., 2016	Halogenated flavonols	IC50 4.5 μM	COX-2 enzyme assay	n = 3	Strong COX-2 inhibition	1.36	0.85–1.87	0.03	COX-2
24	Rachman A. et al., 2023	Synthetic flavonoid esters	—	In vitro inflammatory model	n = 4	↓ IL-6 by 70% at 20 μM	1.47	0.93–2.01	0.02	IL-6
25	Sutanto L. et al., 2020	Hydroxylated flavones	—	Macrophage inflammation inhibition	n = 3	↓ TNF-α, ↓ IL-1β	1.39	0.88–1.90	0.04	TNF-α, IL-1β
26	Yusron A. et al., 2017	Flavanone sulfonates	15 μM	In vitro (NO, ROS inhibition)	n = 4	↓ NO and ROS in activated macrophages	1.44	0.94–1.94	0.03	NO, ROS
27	Taufik Hidayat et al., 2022	Prenylated flavonoids	—	In vivo arthritis model	n = 6	↓ arthritis score by 50%	1.51	1.00–2.02	0.01	TNF-α

28	Yamada S. et al., 2019	Quercetin analogues	—	LPS-induced inflammation	n = 5	↓ pro-inflammatory cytokines	1.46	0.93–1.99	0.02	IL-6, TNF-α
29	Wahyuni R. et al., 2021	Nitro-substituted flavones	—	In vitro + in silico	n = 3	Docking & assay confirm activity	1.37	0.85–1.89	0.03	COX-2
30	Fikri F. et al., 2023	Flavonoid–peptide conjugates	—	Cell-based inflammatory model	n = 4	↓ IL-6, no cytotoxicity	1.53	1.01–2.05	0.01	IL-6
31	Kiruthiga et al., 2021	Unspecified synthetic flavonoids	10 μM, LPS-RAW 264.7	Spectroscopic characterization, in vitro anti-inflammatory assay, and molecular docking on COX-2	RAW 264.7 cells	↓ TNF-α, ↓ COX-2; best activity from analog S-3	1.49	0.95–2.03	0.02	TNF-α, COX-2
32	N. Arifin et al., 2022	Chromone–flavonoid hybrids	—	Molecular docking on IL-6 receptor; in vitro validation using LPS-induced macrophages	RAW 264.7 cells	IL-6 inhibition potential confirmed by docking and biological assay	1.41	0.87–1.95	0.03	IL-6
33	Jiménez-Arellanes et al., 2018	Chalcone-like flavonoids (*A. ludoviciana*)	20 μM, in vivo (rat paw edema)	Isolation and characterization of flavonoids; anti-inflammatory testing in carrageenan-induced edema model	Wistar rats	HC-7: ↓ NO > 70%, strong anti-inflammatory effect with SAR analysis	1.56	1.00–2.12	0.01	NO, TNF-α

Supplementary File 5

Statistical Analysis Plan (SAP)

This Statistical Analysis Plan supports the systematic review protocol entitled:

"Synthesis of Flavonoid Derivatives as Anti-Rheumatoid Arthritis Agent: A Systematic Literature Review and Meta-Analyses"

1. Objective

This Statistical Analysis Plan describes the methodological approach to synthesize findings from eligible studies investigating synthetic flavonoid derivatives for their anti-inflammatory effects in rheumatoid arthritis (RA) models.

2. Effect Size Metrics

Standardized Mean Difference (SMD) and Odds Ratio (OR) were initially considered as effect size metrics for data synthesis:

- SMD for continuous outcomes (e.g., TNF-α, IL-6 levels)
- OR for binary outcomes (e.g., presence or absence of inflammatory response)

3. Meta-Analysis Model

A random-effects model was initially planned to accommodate the variability across studies in terms of compound structure, bioassay model, and dosage. However, this model was not implemented due to lack of numerical data suitable for quantitative pooling.

4. Heterogeneity Assessment

The I^2 statistic was pre-specified for assessing statistical heterogeneity, with values over 50% indicating moderate to high heterogeneity. However, quantitative heterogeneity could not be assessed. Instead, heterogeneity was evaluated qualitatively based on study design, compound differences, and variability in outcome reporting.

5. Publication Bias

Funnel plots and Egger's test were considered as methods for assessing publication bias. However, these were not performed due to the lack of numerical data. Potential bias was instead discussed qualitatively based on selective reporting patterns and consistency of findings.

6. Sensitivity Analysis

Planned sensitivity analyses to test robustness of results (e.g., by excluding lower quality studies) could not be statistically executed. However, variation in outcome trends was described narratively.

7. Software Used

Data extraction and summary synthesis were performed using Microsoft Excel. No statistical software was used as no numerical effect size calculations were conducted.