

Gynura procumbens Leaf Extract Nanoparticles and Anti-Müllerian Hormone (AMH) in a Polycystic Ovary Syndrome (PCOS) Rat Model: A Systematic Literature Review of Evidence Gaps and Research Opportunities

Potensi Nanopartikel Ekstrak Daun Sambung Nyawa (*Gynura procumbens*) terhadap Anti-Müllerian Hormone (AMH) pada Model Tikus Polycystic Ovary Syndrome (PCOS): Sebuah Tinjauan Literatur Sistematis tentang Celah Bukti dan Peluang Penelitian

Ricky Febri Maulana ^a, Evi Lusiana ^{b*}, Shaum Shiyan ^{c,d},

^a Master's Program in Biomedical Sciences, Faculty of Medicine, Universitas Sriwijaya, Palembang, South Sumatra, Indonesia.

^b Department of Pharmacology, Faculty of Medicine, Universitas Sriwijaya, South Sumatra, Indonesia.

^c Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Indralaya (OI) Sumatera Selatan, Indonesia.

^d Phytopharmaceutical Research Center (PRC), Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Indralaya (OI) Sumatera Selatan, Indonesia.

*Corresponding Author: mrs.evilusiana@fk.unsri.ac.id

Abstract

Polycystic ovary syndrome (PCOS) is a complex endocrine-metabolic disorder. It involves hyperandrogenism, ovulatory dysfunction, insulin resistance, low-grade inflammation, oxidative stress, and altered ovarian morphology. Anti-Müllerian hormone (AMH) is a key biomarker. It is primarily produced by granulosa cells of preantral and small antral follicles. AMH is typically elevated in PCOS. *Gynura procumbens* (Lour.) Merr. exhibits antioxidant, anti-inflammatory, and metabolic activities relevant to PCOS pathophysiology. Nanoparticle technology may enhance the dispersibility, stability, and delivery of its active compounds. This review synthesizes 2021–2026 scientific evidence on *G. procumbens* pharmacology, nanoformulation, herbal nanoparticle efficacy in PCOS models, and the role of AMH as a PCOS biomarker/mediator. It also identifies research gaps regarding *G. procumbens* nanoparticle effects on AMH. The review followed PRISMA 2020 methodology. Searches were conducted in PubMed/MEDLINE, Scopus, ScienceDirect, Google Scholar, and additional academic sources. The search was limited to 2021–2026. A layered search strategy mapped four domains: *G. procumbens*, nanoformulation, PCOS, and AMH. *G. procumbens* contains polyphenols with antioxidant and anti-inflammatory activity. In vitro studies show modulation of COX-2, NF-κB, PI3K/Akt, and MAPK. Nanoformulation studies (chitosan nanoparticles, self-microemulsifying systems, nanoliposomes) demonstrated improved delivery and biological effects in animal models. In PCOS rat models, curcumin, naringenin, and other plant-based nanoparticles improved hormonal, metabolic, histological, or ovulatory outcomes. Recent studies reinforce the causal role of AMH/AMHR2 in the PCOS phenotype. However, no primary study has directly tested the combination of *G. procumbens* nanoparticles and AMH outcome in PCOS rat models. Convergent evidence provides a reasonable biological basis for experimental research. The relationship remains a hypothesis, not proof of efficacy.

Keywords: *Gynura procumbens*; nanoparticles; Anti-Müllerian hormone; polycystic ovary syndrome; rat model

Abstrak

Polycystic ovary syndrome (PCOS) merupakan gangguan endokrin-metabolik kompleks. Gangguan ini melibatkan hiperandrogenisme, gangguan ovulasi, resistensi insulin, inflamasi derajat rendah, stres oksidatif, dan perubahan morfologi ovarium. Anti-Müllerian hormone (AMH) merupakan biomarker penting. Hormon ini terutama diproduksi oleh sel granulosa folikel preantral dan antral kecil. AMH cenderung meningkat pada PCOS. *Gynura procumbens* (Lour.) Merr. atau sambung nyawa mempunyai aktivitas antioksidan, antiinflamasi, dan metabolik yang relevan dengan mekanisme PCOS. Teknologi nanopartikel berpotensi meningkatkan dispersibilitas, stabilitas, dan penghantaran senyawa aktif ekstrak. Review ini disusun untuk menilai dan mensintesis bukti ilmiah 2021–2026 mengenai aktivitas farmakologis *G. procumbens*, penggunaan nanoformulasi *G. procumbens*, efektivitas nanopartikel berbasis herbal pada model PCOS, dan peran AMH sebagai biomarker/mediator PCOS. Review ini juga mengidentifikasi celah penelitian mengenai nanopartikel ekstrak *G. procumbens* terhadap AMH. Review ini mengikuti metodologi PRISMA 2020. Penelusuran dilakukan pada PubMed/MEDLINE, Scopus, ScienceDirect, Google Scholar, dan sumber akademik tambahan. Literatur utama dibatasi pada 2021–2026. Hasil penelusuran menunjukkan bahwa *G. procumbens* mengandung polifenol dan mempunyai aktivitas antioksidan serta antiinflamasi. Studi in vitro menunjukkan pengaruh terhadap COX-2, NF-κB, PI3K/Akt, dan MAPK. Bukti nanoformulasi pada *G. procumbens* menunjukkan bahwa nanopartikel kitosan, sistem self-microemulsifying, dan nanoliposom dapat meningkatkan karakteristik penghantaran dan efek biologis ekstrak pada model hewan. Pada model PCOS, nanopartikel kurkumin, naringenin, dan bahan alam lain menunjukkan perbaikan hormonal, metabolik, histologis, atau ovulasi. Studi terbaru juga memperkuat peran kausal AMH/AMHR2 pada fenotipe PCOS. Namun demikian, belum ditemukan studi primer yang secara langsung menguji kombinasi *G. procumbens*, nanopartikel, model tikus PCOS, dan AMH sebagai outcome utama. Konvergensi bukti dari ketiga domain memberikan dasar biologis yang masuk akal untuk penelitian eksperimental. Hubungan tersebut masih berupa hipotesis dan tidak boleh dianggap sebagai bukti efikasi langsung.

Kata Kunci: *Gynura procumbens*; sambung nyawa; nanopartikel; Anti-Müllerian hormone; polycystic ovary syndrome



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Introduction

Polycystic ovary syndrome (PCOS) is a complex endocrine-metabolic disorder with reproductive and metabolic manifestations, including hyperandrogenism, ovulatory dysfunction, and altered ovarian morphology [1]. Because no single animal model fully replicates the human PCOS spectrum, experimental models must be selected according to the research question; induction protocols such as dehydroepiandrosterone combined with a high-fat diet illustrate this principle [1,14]. Within this pathophysiological framework, anti-Müllerian hormone (AMH)—a glycoprotein of the transforming growth factor- β family produced mainly by granulosa cells of preantral and small antral follicles—has gained attention as both a biomarker and a potential mediator of disordered folliculogenesis [15,16]. In PCOS, the expanded cohort of small follicles and the altered intra-ovarian hormonal milieu are frequently accompanied by elevated circulating AMH [15,16], reinforcing its value as an experimental endpoint.

Building on this biomarker role, recent animal studies have strengthened the case for AMH as a causal contributor to the PCOS phenotype. High AMH exposure during early life can induce a PCOS-like phenotype in mice, whereas antibodies against AMHR2 can prevent or partially rescue this phenotype [11]. In a spontaneous PCOS rat model, AMH inhibition restored ovulation and normalized androgen levels [12]. Parallel work has linked AMH signalling to granulosa cell proliferation and apoptosis via SMAD4 in DHEA-induced PCOS rats [16]. Importantly, PCOS pathogenesis is not confined to the reproductive axis: insulin resistance, oxidative stress, low-grade inflammation, and altered lipid metabolism interact with hyperandrogenism and ovarian dysfunction [1,13]. This multi-target nature of the disease makes pharmacological approaches that simultaneously address redox balance, inflammation, and metabolic disturbance particularly relevant.

Given this multi-target pathophysiology, plant-derived compounds with concurrent antioxidant, anti-inflammatory, and metabolic effects are of particular interest. In this context, *Gynura procumbens* (Lour.) Merr., known in Southeast Asia as sambung nyawa, has been investigated for antioxidant, anti-inflammatory, and metabolic activities that align with several PCOS-related pathways. HPLC-MS/MS analyses have identified diverse polyphenols and flavonoids in the plant that contribute to its antioxidant and anti-inflammatory profile [3]. Mechanistically, *G. procumbens* extract reduces inflammatory mediators such as nitric oxide (NO), tumour necrosis factor- α (TNF- α), and prostaglandin E2 (PGE2), and down-regulates iNOS and COX-2 expression through modulation of AP-1, NF- κ B, PI3K/Akt, and MAPK signalling in macrophage models [4]. Although these data derive primarily from non-ovarian cellular systems, the implicated pathways are shared with ovarian inflammation and metabolic stress in PCOS.

Beyond inflammation, the metabolic potential of *G. procumbens* is supported by a 2025 review of four in vitro and twelve in vivo studies reporting effects on glucose and lipid profiles, while also highlighting extract heterogeneity, limited standardization, sparse pharmacokinetic data, and a paucity of clinical trials [13]. These limitations are especially pertinent because insulin resistance is a core component of PCOS. A further practical challenge is the poor solubility, stability, and bioavailability of many plant-derived actives. Nanotechnology has been explored to address these delivery constraints: ethanol extract of *G. procumbens* combined with chitosan nanoparticles produced hypoglycaemic effects in diabetic rats [5]; nanoliposomal formulations (approximately 112 nm) improved dispersibility and antioxidant markers in a hepatorenal injury model [6]; and a self-microemulsifying drug delivery system enhanced delivery characteristics of the leaf extract [7].

Nanoparticle approaches have likewise been applied directly in PCOS models. Curcumin-encapsulated self-assembled nanoparticles improved hormonal, metabolic, ovarian morphological, and fertility parameters in PCOS rats [8], and curcumin (with or without zinc) reduced AMH in letrozole-induced PCOS rats [18]. Polymer-based naringenin nanoparticles normalized hormonal and ovarian disturbances in letrozole-induced Wistar rats [9], while Fagonia indica-coated chitosan nanoparticles improved LH, testosterone, ovulation, and histology in a PCOS Wistar model [10]. Collectively, these findings raise a focused research question: can the antioxidant, anti-inflammatory, and metabolic actions of *G. procumbens* be enhanced by nanoformulation sufficiently to modify the ovarian environment and, ultimately, AMH in PCOS? As of the literature search conducted for this review, no primary study has directly tested this four-component combination (*G. procumbens* nanoparticles, validated PCOS rat model, and AMH as primary outcome). Accordingly, this systematic literature review does not claim proven efficacy; rather, it maps available evidence, articulates the biological rationale, and defines the knowledge gap that can be addressed in subsequent experimental (thesis) work.

Taken together, the evidence streams summarized above provide a coherent scientific rationale for investigating nanoformulated *G. procumbens* in a PCOS rat model with AMH as a primary endpoint. The present review systematically maps supporting evidence across pharmacology, nanoformulation, nano-PCOS interventions, and AMH biology; clarifies remaining knowledge gaps; and outlines design considerations for subsequent experimental research. By adhering to transparent PRISMA 2020 reporting standards and emphasizing methodological rigor, this synthesis aims to contribute both to the academic literature and to the practical planning of thesis-level research in biomedical sciences.

Method

This section synthesizes evidence across four interrelated domains relevant to the research question: pharmacology of *G. procumbens*, nanoformulation of *G. procumbens* extracts, nanoparticle interventions in PCOS animal models, and the role of AMH as both biomarker and mediator in PCOS. The synthesis is narrative rather than meta-analytic due to heterogeneity in study designs, interventions, dosing, and outcomes. Findings are organized to make transparent the convergence of biological plausibility and the absence of direct primary evidence for the four-component combination central to the thesis hypothesis.

Methods (PRISMA 2020)

This systematic literature review was conducted as part of a thesis project and was not prospectively registered in PROSPERO or any other systematic review registry. This is common for thesis-level systematic reviews; the absence of prospective registration is explicitly stated here for transparency in accordance with PRISMA 2020 item 24a.

Table 1. PICO-based research question

Component	Operational Definition	Relevance
Population	Rat/mouse PCOS model or relevant biological model	Assess reproductive and metabolic phenotype
Intervention	<i>G. procumbens</i> leaf extract nanoparticles or relevant herbal nanoformulation	Assess nano-delivery effects
Comparison	Normal control, PCOS control, non-nano extract, or comparator therapy	Determine relative intervention effects
Primary Outcome	AMH serum/ovary	Primary thesis outcome
Secondary Outcomes	LH, FSH, testosterone, insulin, HOMA-IR, MDA, SOD, GSH, histology, estrous cycle	Explain systemic effects and mechanisms

Table 2. Literature search strategy

Domain	Key Search Terms
Plant	"G. procumbens" OR "sambung nyawa"
Nano	nanoparticle OR nanoformulation OR nanocarrier OR nanoliposomal OR nanoemulsion OR chitosan nanoparticle
PCOS	"polycystic ovary syndrome" OR PCOS OR "polycystic ovarian syndrome"
AMH	"anti-Müllerian hormone" OR AMH OR AMHR2
Model	rat OR rats OR mice OR animal model OR letrozole OR DHEA

Table 3. Inclusion and exclusion criteria

Criteria	Inclusion	Exclusion
Year	2021–2026	<2021
Type	Primary articles, relevant reviews, animal/in vitro mechanistic studies	Editorials, comments, abstracts without data, grey literature (preprints, theses, unpublished reports)
Topic	G. procumbens, nanoformulation, PCOS, AMH, or mechanistic relationship	Not relevant to the research question
Language	Indonesian/English	Other languages without adequate translation
Data	Methodology/outcome extractable	Insufficient data
Duplication	One final-version article	Duplicate

Grey literature, including preprints, theses, conference abstracts or proceedings without full methodological and outcome data, and unpublished reports, was excluded a priori. This decision prioritised peer-reviewed primary studies and reviews that provided complete, extractable data for qualitative synthesis and risk-of-bias appraisal. Consequently, formal quality assessment of grey literature was not undertaken. The study selection process followed the PRISMA 2020 flow. Title and abstract screening and full-text eligibility assessment were performed by two reviewers (R.F.M. and E.L.). Disagreements were resolved by discussion until consensus was reached; a third arbitrator was not required. Data extraction was performed using a pre-defined matrix (author, year, design/model, intervention, nanoparticle characteristics, primary and secondary outcomes, and notes) and cross-checked by both reviewers. A total of 343 records were identified from the databases and additional sources (PubMed/MEDLINE, Scopus, ScienceDirect, Google Scholar, and SciSpace).

After removal of 56 duplicates, 287 records remained for title and abstract screening. Of these, 224 records were excluded because they did not address any of the four domains of interest (G. procumbens pharmacology, nanoformulation of G. procumbens, nanoparticle interventions in PCOS models, or AMH as biomarker/mediator in PCOS), leaving 63 full-text articles for eligibility assessment. Forty-five full-text articles were excluded. The reasons fell into four categories. First, 22 articles were not relevant to the four-domain research question or only tangentially related (for example, studies on other *Gynura* species, general herbal PCOS reviews without nanoparticle or AMH focus, or nanoformulations of unrelated plants without PCOS outcomes).

Second, 12 articles had insufficient extractable outcome or methodological data (for example, conference abstracts without full methods or results, incomplete nanoparticle characterization, or absence of measurable hormonal or ovarian endpoints). Third, 7 articles were the wrong study type (editorials, commentaries, letters without original data, or non-research articles). Fourth, 4 articles fell outside the predefined publication window (before January 2021) after full-text verification, or were duplicate publications of the same study. This resulted in 18 studies included in the qualitative synthesis. These figures are the final verified counts from the original database export logs and screening records conducted for this review (PubMed/MEDLINE, Scopus, ScienceDirect, Google Scholar, and SciSpace; January 2021 – August 2026). The definitive PRISMA 2020 flow diagram is presented in Figure 1. Representative full-text articles excluded at the eligibility stage, with specific reasons, are listed in Supplementary Table S3 (PRISMA 2020 item 16b); the full screening log is retained by the authors.

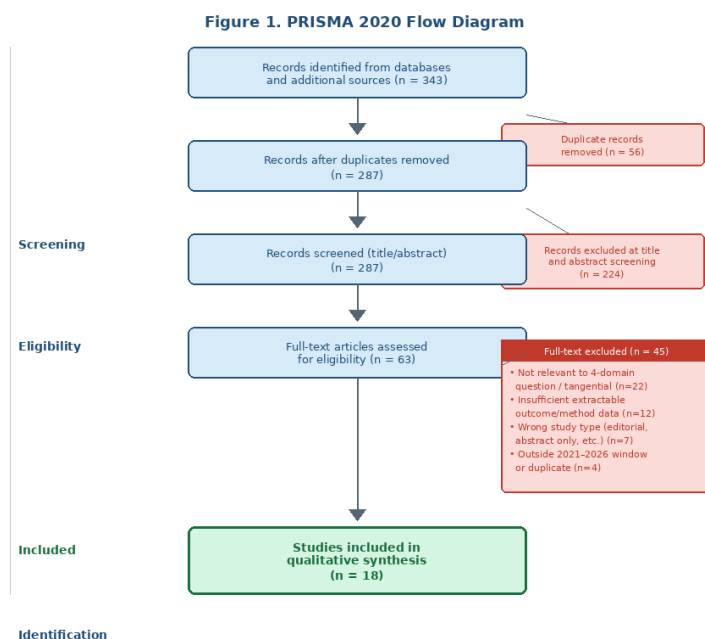


Figure 1. PRISMA 2020 flow diagram

Figure 1. PRISMA 2020 flow diagram of the study selection process. Records were identified from PubMed/MEDLINE, Scopus, ScienceDirect, Google Scholar, and SciSpace (January 2021 – August 2026). Numbers are final verified counts reconciled against original database export logs. Detailed reasons for full-text exclusion (n = 45) are listed in the eligibility stage of the diagram and described in the Methods text. Eighteen studies were included in the qualitative synthesis.

PRISMA 2020 stage	
Records identified from databases and additional sources	343
Duplicate records removed	56
Records after duplicates removed	287
Records excluded at title and abstract screening	224
Full-text articles assessed for eligibility	63
Full-text articles excluded	45
– Not relevant to four-domain question / only tangential (other species, unrelated nano, no AMH/PCOS focus)	22
– Insufficient extractable outcome or methodological data (abstracts only, incomplete NP characterisation)	12
– Wrong study type (editorial, commentary, letter, or non-research article)	7
– Outside 2021–2026 window after full-text check, or duplicate publication	4
Studies included in qualitative synthesis	18

Detailed reasons for full-text exclusion are described in the text above and summarised in Figure 1. The complete screening log and data-extraction matrix for the 18 included studies are provided in the Supplementary Materials.

Results

The 18 included studies were published between 2021 and 2025. They originated predominantly from Asia (including Indonesia, Malaysia, Bangladesh, China, India and Iraq) for the G. procumbens and nano-phytochemical work, with additional European and North-American contributions for the AMH mechanistic studies. Study designs encompassed in vitro mechanistic assays (e.g., macrophage and granulosa-cell models), in vivo animal experiments (primarily Wistar or Sprague-Dawley rats and mice), and narrative or systematic reviews. Where PCOS was induced, the principal methods were letrozole, dehydroepiandrosterone (DHEA) with or without a high-fat diet, bisphenol A, and spontaneous models. Animal sample sizes were typically modest (commonly 6–12 animals per group). Interventions ranged from crude or standardised leaf extracts and their nanoformulations (chitosan nanoparticles, nanoliposomes, self-microemulsifying systems) to single-phytochemical nanoparticles (curcumin, naringenin) and AMH/AMHR2-targeted antibodies. Primary

outcomes varied by domain and included antioxidant/anti-inflammatory markers, glucose and lipid parameters, reproductive hormones (including AMH), ovarian histology, and ovulatory or fertility endpoints. Full details of author, year, country/region, study design, animal model, PCOS induction method, intervention, dose, treatment duration, and main outcomes for each of the 18 records are provided in the data-extraction matrix (Supplementary Table S1).

The identified evidence indicates that direct literature on the combination of *G. procumbens*-nanoparticles-PCOS-AMH remains limited. Most evidence can be mapped to three domains: (i) studies on antioxidant, anti-inflammatory, and metabolic activities of *G. procumbens*; (ii) development of nano-delivery systems for *G. procumbens* extract; and (iii) studies of plant/phytochemical nanoparticles in PCOS models and studies specifically evaluating AMH. Table 4 summarizes the evidence domains and their strength relative to the thesis question.

The synthesis employed a structured narrative approach organised around the four predefined evidence domains (pharmacology of *G. procumbens*, nanoformulation of *G. procumbens*, nanoparticle interventions in PCOS models, and AMH biology in PCOS). Because of marked heterogeneity in study designs, interventions, dosing regimens, and outcome measures, quantitative meta-analysis or formal vote-counting was not feasible or appropriate. Instead, findings within and across domains were integrated thematically by examining consistency of direction of effect, biological plausibility, and relevance to the four-component research question. Convergence was judged present when independent streams of evidence (mechanistic, formulation, and intervention) pointed in the same direction toward a testable hypothesis, even in the absence of a single study that tested all four components simultaneously. To make the appraisal transparent and reproducible, the strength of evidence for the thesis question in Table 4 (and the related synthesis in Table 9) was rated using predefined internal criteria adapted to the predominantly preclinical, heterogeneous literature: (1) Directness — whether the evidence addresses the thesis combination (*G. procumbens* + nano + PCOS + AMH) or only a related domain. (2) Study design hierarchy — in vivo animal models with defined PCOS induction ranked higher than in vitro or non-reproductive models; reviews were used for context but not for strength of primary effect. (3) Consistency — agreement of findings across independent studies within the same domain. (4) Outcome relevance — whether AMH, reproductive hormones, ovarian histology, or closely related metabolic/oxidative markers were measured. (5) Reporting quality — adequacy of extract or nanoparticle characterization, model validation, and statistical description. Ratings were assigned as follows. Strong = multiple in vivo studies with consistent, outcome-relevant findings and acceptable reporting; Moderate = supportive mechanistic or feasibility evidence that is relevant but indirect (e.g., different model or endpoint), or limited number of studies; Moderate-strong = in vivo nano-PCOS evidence that is consistent but does not use *G. procumbens*; Not yet found = no primary study of the four-component combination. A formal GRADE assessment was not applied because GRADE is designed primarily for clinical bodies of evidence; the criteria above serve as a transparent, domain-adapted alternative for this narrative preclinical synthesis. Table 4 summarizes the evidence domains and their strength relative to the thesis question.

Table 4. Characteristics of evidence domains

Domain	Example Evidence	Strength for Thesis Question
Pharmacology of <i>G. procumbens</i>	Polyphenols; antioxidant; anti-inflammatory; antidiabetic	Moderate; mechanistically relevant
Nanoformulation of <i>G. procumbens</i>	Chitosan NP; nanoliposome; SMEDDS	Moderate; supports feasibility
Nanoparticles in PCOS	Curcumin, naringenin, Fagonia indica	Moderate-strong for nano-PCOS concept
AMH in PCOS	Animal models and AMH/AMHR2 interventions	Strong for outcome relevance
Direct combination	<i>G. procumbens</i> + nano + PCOS + AMH	Not yet found

Pharmacological Evidence of *G. procumbens*

Kim et al. provided evidence that polyphenols of *G. procumbens* can be chemically analyzed and linked to antioxidant and anti-inflammatory activities [3]. This is important for standardization of active substances in thesis research because extract composition can determine biological response. Cao et al. showed that *G. procumbens* extract affects inflammatory mediators and signaling pathways, including reductions in NO,

TNF- α , and PGE2 and modulation of NF- κ B, PI3K/Akt, and MAPK in RAW264.7 macrophages [4]. However, the RAW264.7 model is a macrophage cell line, so results cannot be directly extrapolated to ovary or AMH. The 2025 antidiabetic review indicated potential effects on glucose and lipid profiles but emphasized methodological heterogeneity and limited pharmacokinetic data [13], reinforcing that thesis work should document extraction methods, standardization, and nanoformulation characterization in detail.

Table 5. Pharmacological evidence of *G. procumbens*

Author/Year	Model/Method	Key Findings	Relevance
Kim et al., 2021 [3]	HPLC-MS, DPPH, COX-2, RAW264.7	Polyphenols identified; antioxidant/anti-inflammatory activity	Mechanistic basis
Cao et al., 2022 [4]	RAW264.7, <i>G. procumbens</i> extract	↓ NO, TNF- α , PGE2; NF- κ B, PI3K/Akt, MAPK modulation	Inflammation relevance
Mohd Nor et al., 2025 [13]	Review of 4 in vitro + 12 in vivo	Antidiabetic potential; heterogeneous evidence	Insulin resistance relevance

Nanoformulation Evidence of *G. procumbens*

Badsha et al. tested a combination of ethanol extract of *G. procumbens* and chitosan nanoparticles in diabetic rats, demonstrating that a nano-system can be combined with plant extract and producing hypoglycemic effects [5]. Bhowmik et al. developed a nanoliposomal formulation with an average size of approximately 112 ± 2.6 nm and reported improved dispersibility; in a hepatorenal injury model, the formulation increased SOD and GSH and reduced oxidative stress markers [6]. Although not a PCOS model, these oxidative parameters are conceptually relevant. Shill et al. used a self-microemulsifying system to improve the delivery characteristics of the extract, supporting that nano-approaches for *G. procumbens* have been tested in animal models [7]. Table 6 summarizes the nanoformulation evidence.

Table 6. Nanoformulation evidence of *G. procumbens*

Author/Year	System	Model	Key Findings	Implication
Badsha et al., 2024 [5]	Ethanol extract + chitosan NP	Diabetic rat	Combination formulation showed hypoglycemic effect	Nano feasibility
Shill et al., 2025 [7]	SMEDDS leaf extract	Animal organ injury	Improved dispersion and protective characteristics	Drug delivery
Bhowmik et al., 2025 [6]	Nanoliposome	Hepatorenal injury rat	~112 nm; ↑ dispersibility; ↑ SOD/GSH; protective effect	Feasibility and characterization

Nanoparticle Evidence in PCOS Models

Raja et al. developed curcumin-encapsulated self-assembled nanoparticles and demonstrated improvements in hormonal, ovarian, and fertility parameters in PCOS rats [8]. Dashputre et al. reported that naringenin nanoparticles normalized hormonal and ovarian disturbances in letrozole-induced PCOS Wistar rats [9]. Abd-Alqader et al. evaluated curcumin (standard and supplement) with or without zinc in letrozole-induced PCOS rats and showed decreased AMH in the curcumin group [18]. Zia et al. demonstrated that *Fagonia indica*-coated chitosan nanoparticles improved LH, testosterone, ovulation, and histological parameters in PCOS Wistar rats [10]. These findings support the feasibility of plant-based nanoformulations in PCOS but do not constitute evidence for *G. procumbens*. Table 7 summarizes this evidence.

Table 7. Nanoparticle/nanomedicine evidence in PCOS models

Author/Year	Material/System	Model	Outcomes	Findings
Raja et al., 2021 [8]	Curcumin self-assembled NP	PCOS rat	Hormone, ovary, fertility	Improved reproductive phenotype
Dashputre et al., 2023 [9]	Naringenin NP	Letrozole PCOS Wistar rat	Hormone, ovarian function	Normalized hormonal/ovarian disturbances
Abd-Alqader et al., 2024 [18]	Curcumin $\pm$ zinc	Letrozole PCOS rat	Glucose, insulin, HOMA-IR, AMH	AMH decreased in curcumin group
Zia et al., 2025 [10]	<i>Fagonia indica</i> chitosan NP	PCOS Wistar rat	LH, testosterone, ovulation, histology	Improved hormonal profile/ovulation

Evidence of AMH in PCOS Models

The study by Abd-Alqader et al. is highly relevant to the thesis because it measured AMH in a letrozole-induced PCOS rat model and demonstrated that curcumin, in both standard and supplement forms, decreased AMH [18]. However, the intervention was not *G. procumbens* nanoparticles. The 2025 study on blocking antibodies against AMH in spontaneous PCOS rats provided important translational evidence that AMH can be a therapeutic target [12]. The high-AMH mouse model study by Cotellessa et al. demonstrated that blocking AMHR2 prevents or ameliorates PCOS-like phenotype [11]. The SMAD4 study in DHEA-induced PCOS rats showed increased AMH/SMAD4 and that AMH affects granulosa cell proliferation and apoptosis [16]. The BPA-induced PCOS model also reported increased AMH alongside hormonal and metabolic changes [15]. These findings suggest that AMH changes in the thesis should be interpreted alongside ovarian and other hormonal parameters rather than as a single outcome. Table 8 summarizes the AMH evidence.

Table 8. Evidence regarding AMH in PCOS models

Author/Year	Model	AMH-related Findings	Significance
Stener-Victorin et al., 2022 [1]	Animal model review	Discusses models and limitations	Basis for model selection
Abd-Alqader et al., 2024 [18]	Letrozole PCOS rat	AMH increased; curcumin decreased AMH	Intervention outcome evidence
SMAD4 study, 2024 [16]	DHEA PCOS rat	AMH/SMAD4 increased; AMH affects granulosa proliferation/apoptosis	Mechanism
BPA rat model, 2025 [15]	BPA-induced PCOS	AMH increased alongside hormonal/metabolic changes	Model and biomarker
Racine et al., 2025 [12]	Spontaneous PCOS rat	Blocking AMH restored ovulation and androgens	Causal evidence
Cotellessa et al., 2025 [11]	High-AMH mouse model	Blocking AMHR2 prevents/ameliorates PCOS-like phenotype	Causal evidence

Synthesis Matrix and Quality Assessment

Table 9 synthesizes the converging evidence and its relevance to the thesis question. It applies the same predefined strength criteria used for Table 4. These criteria are: directness to the four-component combination; study design hierarchy (in vivo PCOS models ranked above in vitro or non-reproductive models); consistency across independent studies; relevance of measured outcomes (especially AMH and related reproductive or metabolic markers); and adequacy of reporting (extract or nanoparticle characterization, model validation, and statistics). The matrix indicates that each evidence stream supports the conceptual framework. However, none directly tests the combination of *G. procumbens* nanoparticles, a PCOS rat model, and AMH as primary outcome.

Table 9. Synthesis matrix of evidence and relevance to thesis

Evidence	Result	Link to Thesis	Limitation
Polyphenols GP [3]	Antioxidant/anti-inflammatory	Supports mechanism	In vitro
GP anti-inflammatory [4]	↓ NO/TNF- α /PGE2; PI3K/Akt-MAPK	Inflammation relevance	Not ovary
GP antidiabetic [13]	Glucose/lipid effects	Insulin resistance relevance	Heterogeneity
GP + chitosan NP [5]	Hypoglycemic effect	Supports feasibility	Not PCOS
GP nanoliposome [6]	~112 nm; ↑ dispersibility; ↑ SOD/GSH	Supports delivery	Not PCOS
Curcumin NP [8]	Improved PCOS phenotype	Supports nano-PCOS concept	Not GP
Naringenin NP [9]	Improved hormone/ovary	Supports nano-PCOS concept	Not GP
Curcumin + zinc [18]	↓ AMH in letrozole PCOS	Supports AMH outcome	Not GP/nano
AMH blockade [12]	Ovulation/androgen improved	Supports AMH target	Not herbal
AMHR2 blockade [11]	PCOS-like phenotype improved	Supports causality	Mouse model

Risk of bias for included in vivo animal studies was assessed using SYRCLE's Risk of Bias tool (Hooijmans et al., 2014). This tool adapts the Cochrane framework to animal intervention studies across ten domains: sequence generation, baseline characteristics, allocation concealment, random housing, blinding of caregivers and outcome assessors, random outcome assessment, incomplete outcome data, selective reporting, and other bias. Judgments (Low / Unclear / High) for the principal animal studies are presented in Supplementary Table S4. In vitro studies and methodological reviews were not scored with SYRCLE. Their

limitations are summarized narratively in Table 10. Predefined domains include extract characterization; animal randomization and allocation concealment; blinding of outcome assessment (particularly histology); validity of the PCOS induction model; whether AMH was a primary or secondary outcome; completeness of nanoformulation characterization; and sample size and statistical reporting. Extract characterization is partially reported across studies. Randomization is inconsistently documented. Blinding of histological evaluation is often unclear. These patterns align with the frequent Unclear judgments on SYRCLE randomization and blinding domains. The strongest evidence for the thesis question remains conceptual. Separate evidence exists for *G. procumbens* pharmacology, *G. procumbens* nanoformulation, nanoparticles in PCOS, and AMH as a target. However, no direct evidence unifies all components.

Table 10. Narrative quality and risk-of-bias assessment

Domain	Strength	Main Limitation
Extract characterization	Some studies use chemical analysis	Standardization not uniform across studies
Animal randomization	Reported in some studies	Not consistent
Blinding	Often unclear	May increase histology assessment bias
PCOS model	Diverse and defined	No single model replicates all human PCOS
AMH outcome	ELISA available in some studies	Not always primary outcome
Nanoformulation	Some studies report particle size	Zeta potential/EE/release not always complete
Statistics	Generally compare groups	Small sample sizes in animal studies

Discussion

Main Synthesis.

The SLR results indicate biological convergence but no direct efficacy evidence. *G. procumbens* exhibits antioxidant, anti-inflammatory, and metabolic activities; nano-technology can improve delivery characteristics; phytochemical nanoparticles can improve PCOS phenotypes; and AMH can act as a mediator/target [3–13]. This convergence supports the hypothesis that an ovarian environment undergoing oxidative stress and inflammation may be influenced by *G. procumbens* intervention. However, AMH reduction should not be assumed to result solely from antioxidant effects. AMH is influenced by small follicle count, granulosa cell function, androgens, and the TGF- β /SMAD pathway, so research must link AMH to ovarian histology and reproductive hormones.

Beyond the direct pharmacological and nanoformulation evidence, the broader literature on herbal interventions in PCOS provides additional context for interpreting the potential of *G. procumbens*. Multiple plant extracts rich in polyphenols and flavonoids have been shown to modulate ovarian steroidogenesis, improve insulin sensitivity, and reduce oxidative stress markers in rodent PCOS models. The mechanistic overlap between these interventions and the known activities of *G. procumbens* strengthens the biological plausibility of the proposed hypothesis, even in the absence of direct experimental data linking *G. procumbens* nanoparticles to AMH outcomes. Nevertheless, extrapolation must remain cautious because phytochemical profiles differ substantially among plant species, and dose-response relationships established for one extract cannot be assumed for another.

Oxidative Stress and AMH.

Oxidative stress can affect granulosa cell function and follicle quality. The antioxidant activity of *G. procumbens* provides a basis for testing whether redox improvement is associated with ovarian environment improvement. However, the relationship between ROS reduction and AMH reduction must be empirically demonstrated. MDA and SOD parameters can be used as secondary outcomes to test this hypothesis.

Granulosa cells are particularly sensitive to redox imbalance because of their high metabolic activity during folliculogenesis. Elevated reactive oxygen species can disrupt mitochondrial function, impair steroidogenic enzyme activity, and alter expression of genes involved in follicle survival and atresia. In PCOS, chronic oxidative stress coexists with hyperandrogenism and insulin resistance, creating a self-reinforcing cycle that may sustain the elevated AMH phenotype. Antioxidant interventions that successfully lower ovarian or systemic markers of oxidative damage have, in some studies, been associated with partial

normalization of follicle distribution and hormone profiles. Whether such changes reliably translate into measurable reductions in circulating AMH remains an empirical question that the proposed thesis research is positioned to address.

Inflammation and Ovarian Function.

Chronic low-grade inflammation is frequently associated with PCOS. Evidence from *G. procumbens* in macrophages shows modulation of NF- κ B and MAPK, but these pathways have not been directly tested in PCOS ovaries after nanoformulated *G. procumbens* administration. Cytokine or inflammatory pathway marker measurements can strengthen interpretation.

The ovarian microenvironment in PCOS is characterized by elevated local production of inflammatory mediators, including interleukin-6, tumor necrosis factor- α , and various chemokines. These cytokines can influence granulosa cell proliferation, steroidogenesis, and responsiveness to gonadotropins. Because *G. procumbens* has been shown to down-regulate NF- κ B-dependent transcription in macrophage models, it is plausible that similar anti-inflammatory effects could occur in ovarian tissue if adequate concentrations of active metabolites reach the ovary; however, this remains to be demonstrated experimentally. Nanoparticle delivery systems have the potential to improve solubility and protect labile polyphenols from gastrointestinal degradation, but any advantage in ovarian exposure requires empirical verification. Confirmation of such effects would require measurement of both systemic and ovarian inflammatory markers in future experimental designs.

Insulin Resistance and AMH.

Insulin resistance can worsen hyperandrogenism through ovarian-adrenal-liver interactions. The antidiabetic evidence for *G. procumbens* indicates potential effects on glucose and lipids [13]. However, whether HOMA-IR improvement by nanoformulated *G. procumbens* is accompanied by AMH changes is unknown. This relationship can be tested by correlating AMH with insulin/HOMA-IR.

The relationship between insulin resistance and AMH is bidirectional and complex. Hyperinsulinemia can stimulate ovarian androgen production, which in turn may increase the cohort of small antral follicles that secrete AMH. Conversely, elevated AMH may contribute to follicular arrest and thereby perpetuate the metabolic and reproductive phenotype of PCOS. Interventions that improve insulin sensitivity, such as lifestyle modification or pharmacological agents targeting AMPK or PI3K/Akt pathways, have been associated with reductions in AMH in some clinical and preclinical studies. Given that *G. procumbens* possesses documented antidiabetic activity, concurrent assessment of HOMA-IR and AMH in a nanoformulation study would allow evaluation of whether metabolic improvement mediates or merely accompanies any observed change in AMH.

Why Nanoparticles May Be Superior.

Evidence on *G. procumbens* indicates that nanoformulation can improve dispersibility and biological effects in non-PCOS models [5–7]. Improved formulation characteristics (e.g., particle size, dispersibility) may facilitate better delivery of active constituents, but any claim of increased tissue exposure or bioavailability remains theoretical until supported by pharmacokinetic data or at least comprehensive formulation characterisation. In the thesis, cautious phrasing such as “may improve delivery characteristics” or “has the potential to enhance dispersibility” should be preferred when bioavailability has not been directly measured.

From a pharmaceutical perspective, the choice of nanoparticle system is critical. Chitosan-based nanoparticles offer mucoadhesive properties and relatively simple preparation, while nanoliposomes can encapsulate both hydrophilic and lipophilic constituents of a complex plant extract. Self-microemulsifying systems are designed to form fine emulsions in the gastrointestinal lumen and thereby have the potential to improve oral delivery characteristics; however, actual bioavailability gains require experimental verification. Each system has distinct advantages and limitations with respect to particle size distribution, zeta potential, encapsulation efficiency, release kinetics, and stability under physiological conditions. For a thesis investigating *G. procumbens* nanoparticles, rigorous physicochemical characterization prior to in vivo testing is essential to ensure reproducibility and to allow meaningful comparison with existing nano-PCOS literature.

Comparison with Phytochemical Nanoparticles in PCOS.

Curcumin and naringenin studies show that nanoparticles can affect multiple PCOS pathways [8,9]. This supports the feasibility of the approach but does not prove that *G. procumbens* will produce similar

effects. Differences in compound composition, dose, PCOS model, and nanoparticle system may yield different responses.

It is also important to note that the PCOS models employed across the nano-phytochemical studies are heterogeneous. Letrozole induction produces a hyperandrogenic phenotype with disrupted estrous cyclicity, whereas DHEA combined with high-fat diet may better recapitulate the metabolic component of human PCOS. Spontaneous models and high-AMH transgenic or exogenous-AMH models offer complementary insights into the causal role of AMH itself. The choice of model for future *G. procumbens* nanoparticle research should therefore be guided by the primary research question: if the focus is on AMH as both biomarker and mediator, a model in which AMH is demonstrably elevated and functionally relevant is preferable.

Research Gap and Novelty.

Table 11 summarizes the research gap and novelty. Current knowledge covers *G. procumbens* pharmacology (antioxidant, anti-inflammatory, and metabolic activities), *G. procumbens* nanoformulation (chitosan nanoparticles, nanoliposomes, and SMEDDS), and plant-based nanoparticles in PCOS models (curcumin, naringenin, *Fagonia indica*). The critical gap is the absence of any primary study that simultaneously tests a *G. procumbens* nanoformulation in a validated PCOS animal model with AMH as a measured—let alone primary—outcome. Several factors help explain why this combination has not yet been investigated. First, research on *G. procumbens* has historically concentrated on metabolic and anti-inflammatory endpoints in non-reproductive models (diabetes, hepatorenal injury), so reproductive endocrinology outcomes such as AMH have remained outside the dominant research agenda for this plant.

Second, nanoformulation work on *G. procumbens* is recent (2024–2025) and has focused on delivery characterization and organ-protection models rather than ovarian or hormonal endpoints. Third, the PCOS nanomedicine literature has preferentially used well-characterized single phytochemicals (curcumin, naringenin) rather than complex leaf extracts, partly because single compounds are easier to standardize, dose, and attribute mechanistically—whereas whole-extract nanoparticles raise additional challenges of batch consistency, marker-compound quantification, and regulatory clarity. Fourth, only in 2025 did causal evidence for AMH/AMHR2 in PCOS phenotypes become prominent in high-impact experimental work, so the incentive to designate AMH as a primary endpoint in herbal nano-PCOS studies is itself very recent. These converging timelines—late maturation of *G. procumbens* nanoformulation, fragmented domain-specific research agendas, and the recent elevation of AMH as a causal target—account for the absence of direct primary data and define a clear, timely research opportunity rather than a mere oversight.

Table 11. Research gap and novelty

Aspect	Current Knowledge	Gap	Thesis Novelty
Plant	GP antioxidant/anti-inflammatory/metabolic	Not specifically tested on PCOS-AMH	Test GP in PCOS-AMH
Nano-GP	Chitosan NP, nanoliposome, SMEDDS exist	Not in PCOS	Nano-GP in PCOS model
Nano-PCOS	Curcumin/naringenin/ <i>Fagonia</i> exist	Does not use GP	GP as candidate
AMH	AMH elevated and targetable	Not tested with nano-GP	AMH as primary outcome
Mechanism	Inflammatory/metabolic pathways known separately	Not integrated	Multi-target framework

The novelty of the proposed research therefore rests on three interlocking contributions. First, it integrates an under-studied medicinal plant with a nanoformulation strategy inside a PCOS model, rather than testing either component in isolation. Second, it elevates AMH from an optional secondary hormone to the primary outcome within a multi-parameter framework that still captures androgens, gonadotropins, insulin resistance, oxidative stress, and ovarian histology. Third, it explicitly addresses the biological implication of that choice. AMH is produced by the same granulosa-cell cohort that expands in PCOS. Recent work shows that manipulating AMH/AMHR2 can prevent or reverse PCOS-like phenotypes. A change in AMH is therefore more than a correlative marker: it is a readout of whether the intervention has altered the core follicular compartment that drives the syndrome. In contrast, LH, FSH, or testosterone, while clinically important, can fluctuate with stress, cycle stage, and extra-ovarian factors, and do not directly quantify the small-follicle pool. Insulin or HOMA-IR capture the metabolic axis but do not report on ovarian follicle dynamics. By prioritizing AMH, the experimental design gains a mechanistically anchored primary endpoint that links antioxidant, anti-inflammatory, and metabolic actions of nanoformulated *G. procumbens* to the

ovarian phenotype itself. Most existing nano-PCOS studies either omit AMH or treat it as secondary; making it primary, while fully characterizing extract composition and nanoparticle properties, would generate data of direct relevance to both the mechanistic understanding of PCOS and the translational evaluation of herbal nanomedicines.

Implications for Thesis Research Design

A concrete example of a thesis-ready experimental design is as follows. Induce PCOS in adult female Wistar rats with daily oral letrozole (1 mg/kg body weight) for 21 consecutive days; confirm induction by persistent diestrus on vaginal cytology, elevated serum testosterone, and polycystic ovarian morphology on histology. After confirmation, randomise animals into five groups (n = 8–10 per group, determined by a priori power analysis targeting a 30–40 % reduction in serum AMH based on effect sizes reported for curcumin nanoparticles [18]): (1) normal control (vehicle only), (2) PCOS control (vehicle), (3) PCOS + non-nano *G. procumbens* leaf extract (e.g., 200 mg/kg), (4) PCOS + low-dose nano-*G. procumbens* (e.g., 50–100 mg/kg of the nanoformulation), and (5) PCOS + high-dose nano-*G. procumbens* (e.g., 200 mg/kg of the nanoformulation). Treat daily by oral gavage for 28 days. Collect blood for serum AMH (primary outcome) at baseline (after induction), mid-treatment (day 14), and end of treatment (day 28); measure secondary outcomes (testosterone, LH, FSH, insulin, HOMA-IR, MDA, SOD) at the same time points and perform ovarian histology and estrous-cycle monitoring at termination. Characterise the nanoformulation before in-vivo use: particle size and polydispersity index by dynamic light scattering, zeta potential, encapsulation/loading efficiency, in-vitro release under simulated gastrointestinal conditions, and short-term stability. Quantify at least one marker polyphenol in the parent extract by HPLC for batch consistency. Include a non-nano extract arm to isolate the contribution of nanoformulation. If resources permit, assess ovarian mRNA or protein levels of AMH, AMHR2, FSHR, CYP19A1 and selected inflammatory or metabolic mediators (NF- κ B, AMPK, PI3K/Akt). Obtain institutional ethical approval and adhere to the 3Rs principles. Report all methodological details (extraction solvent and conditions, nanoparticle fabrication protocol, exact induction and treatment regimens, assay methods and kit manufacturers) to maximise reproducibility.

Limitations of the SLR

Several limitations of this systematic literature review should be acknowledged. Direct primary literature on the four-component combination (*G. procumbens* nanoparticles, PCOS rat model, and AMH as primary outcome) is absent, so the synthesis necessarily relies on convergent but indirect evidence from separate domains. PCOS animal models are heterogeneous and do not fully replicate the human phenotype; some *G. procumbens* evidence derives from in vitro or non-reproductive models, limiting direct extrapolation to ovarian AMH. Heterogeneity in extract type, solvent, dose, nano-system, and treatment duration precluded quantitative meta-analysis. Title and abstract screening and data extraction were performed by two reviewers with consensus resolution of disagreements, as described in the Methods. The review was not prospectively registered in PROSPERO; this is common for thesis-level systematic reviews and is stated transparently in accordance with PRISMA 2020 item 24a. The PRISMA flow numbers reported herein (343 records identified 18 studies included) reflect the final verified counts reconciled against the original database export logs and screening records.

The temporal scope of the review was restricted to January 2021 through August 2026. This window was chosen deliberately to capture the most recent five-year developments in three rapidly evolving areas — nanoformulation of *G. procumbens* (largely published from 2024 onward), plant-based nanoparticles in PCOS models, and causal experimental work on AMH/AMHR2 (notably 2025) — while remaining manageable for a thesis-level synthesis. The implication is that earlier foundational studies establishing the basic pharmacological profile of *G. procumbens* or the first applications of nanotechnology to herbal extracts may be under-represented; readers seeking a longer historical perspective should consult the broader reviews cited herein (e.g., the antidiabetic review of *G. procumbens*). Restricting the search in this way may therefore bias the synthesis toward newer nano-delivery and AMH-targeted findings and away from older but still relevant phytochemical or formulation science. Language restriction to English and Indonesian may similarly introduce selection bias by excluding relevant publications in other languages (for example, Chinese or Malay literature on *Gynura* species).

Although major biomedical databases index predominantly English-language literature and the practical impact is expected to be modest, the possibility of missed non-English primary data cannot be ruled out. Publication bias is another concern. Studies reporting positive effects of herbal extracts or nanoparticles

on hormonal or metabolic outcomes are more likely to be published than null or negative findings. Because this review is narrative and draws heavily on preclinical literature—where negative results are less frequently reported—the apparent convergence of supportive evidence across domains may overestimate the true probability of a beneficial effect of nanoformulated *G. procumbens* on AMH. The absence of direct primary data for the four-component combination itself is a form of evidence gap rather than publication bias, but it underscores that the biological rationale advanced here remains a hypothesis to be tested, not a conclusion of efficacy. Future updates of this review should re-run searches without a narrow year filter where feasible, consider additional languages if resources allow, and explicitly search grey literature and trial registries to mitigate selective reporting.

In addition, the translational pathway from preclinical nanoformulation studies to clinical application remains long and requires careful consideration of safety, scale-up, and regulatory requirements. Acute and subchronic toxicity studies of both the extract and the final nanoparticle formulation are prerequisites for any future clinical investigation. Moreover, the complex composition of plant extracts raises questions about batch-to-batch consistency and the identification of marker compounds that can serve as quality control standards. Addressing these pharmaceutical and regulatory dimensions early in the research process will increase the likelihood that positive preclinical findings can be advanced toward meaningful clinical evaluation.

Conclusions and Future Directions

G. procumbens exhibits antioxidant, anti-inflammatory, and metabolic activities that are relevant to several pathophysiological components of PCOS. Nanoformulation of *G. procumbens* has been developed and tested in non-PCOS animal models, with evidence of improved dispersion and biological effects in several systems. Phytochemical and plant-based nanoparticles in PCOS models can improve hormonal, metabolic, ovarian, and ovulatory parameters. AMH holds an important position as both a biomarker and a probable mediator of PCOS, with recent evidence supporting the possibility of targeting the AMH/AMHR2 pathway. However, no primary study has directly tested *G. procumbens* leaf extract nanoparticles against AMH in a PCOS rat model. Therefore, the hypothesis that nanoformulated *G. procumbens* reduces AMH remains a rational research hypothesis. It is not a conclusion of efficacy.

Importantly, the findings of this systematic literature review must not be interpreted as evidence of clinical efficacy. No clinical trials of *G. procumbens* nanoparticles for PCOS or AMH-related outcomes were identified. The convergent preclinical rationale summarized here does not justify therapeutic claims, prescribing recommendations, or use of *G. procumbens* nanoformulations in clinical practice. Any practical recommendation would be premature and potentially misleading until controlled experimental and, eventually, clinical studies have demonstrated safety, dose–response relationships, and meaningful effects on validated endpoints including AMH.

Further experimental research is therefore essential before any practical application can be considered. Future studies should prioritise the following specific steps: (1) phytochemical characterisation and standardisation of *G. procumbens* leaf extract, including quantification of marker polyphenols by HPLC and batch-to-batch consistency data; (2) systematic optimisation and full physicochemical characterisation of the nanoparticle formulation (particle size, polydispersity index, zeta potential, encapsulation/loading efficiency, in-vitro release, and stability); (3) well-controlled in-vivo testing in a validated PCOS rat model (preferably letrozole- or DHEA-induced) that demonstrably elevates AMH; (4) designation of serum (and, where feasible, ovarian) AMH as the primary outcome, measured at multiple time points, with a non-nano extract comparator arm to isolate the contribution of nanoformulation; and (5) mechanistic analyses focused on the AMH/AMHR2/SMAD4 pathway together with secondary reproductive, metabolic, oxidative-stress, and histological endpoints. Acute and subchronic toxicity data for both the standardised extract and the final nanoparticle formulation are prerequisites for any later translational steps. Only after such preclinical evidence is available would exploratory clinical investigation be scientifically and ethically warranted.

Collectively, the evidence reviewed here supports the scientific merit of testing nanoformulated *G. procumbens* in a carefully designed PCOS rat study focused on AMH. The absence of direct prior data is an opportunity to generate novel findings that integrate pharmacology, nanotechnology, and reproductive endocrinology. Until those experiments are completed and independently replicated, the relationship between *G. procumbens* nanoparticles and AMH in PCOS should be treated strictly as a hypothesis, not as a basis for clinical or consumer-facing claims.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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