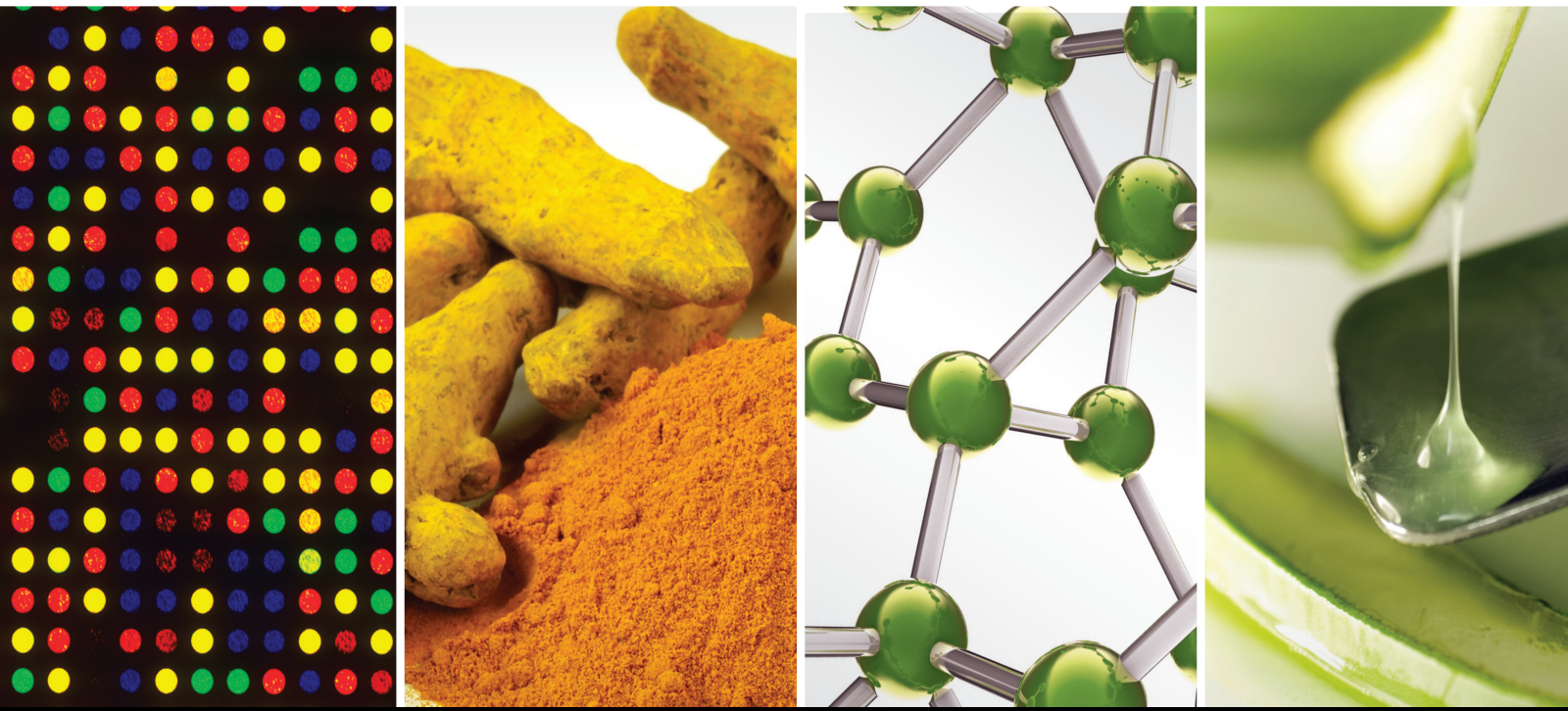
Evidence-Based Complementary and Alternative Medicine

Complementary and Alternative Therapies in Cosmetics and Dermatology

Lead Guest Editor: Ângelo Luís
Guest Editors: Ana Paula Duarte, Eugenia Gallardo, and Michał Tomczyk



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Review Article

The Role of Herbal Medicine in the Treatment of Acne Vulgaris: A Systematic Review of Clinical Trials

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Over the past few decades, interest in medicinal plants and phytochemicals for the treatment of skin disorders, including acne vulgaris, has progressively increased. Acne vulgaris is a chronic inflammatory disease of the pilosebaceous unit, which mainly occurs in adolescents and young adults. The treatment focuses on the four main factors involved in its pathogenesis: increased sebum production, hyperkeratinization, overgrowth of *Cutibacterium acnes*, and inflammation. The treatment includes topical retinoids, benzoyl peroxide, antibiotics, and oral isotretinoin. In this regard, the use of herbal medicine as a complementary and alternative medicine is a promising strategy. The main objective of this study was to systematically evaluate the efficacy and safety of medicinal plants and phytochemicals in the treatment of acne vulgaris. Three scientific databases (PubMed, Web of Science, and Scopus) were searched from inception to January 2021. Clinical trials comparing herbal therapies with placebo or other medicines for the treatment of acne vulgaris were included and analyzed. Outcome measures of interest comprised acne lesions (inflammatory and noninflammatory), sebum production, acne severity, and quality of life. The risk of bias in the included randomized controlled trials (RCTs) was assessed using the Cochrane risk-of-bias tool. A total of 34 clinical trials involving 1753 participants met the inclusion criteria for this systematic review. Most trials showed that herbal medicine significantly reduces inflammatory and noninflammatory acne lesions and has a relevant effect on acne severity. Some medicinal plants revealed equal or higher efficacy to standard treatments. No significant difference between groups in sebum production and quality of life was observed and no severe adverse events were reported. This systematic review provides evidence that medicinal plants and phytochemicals are promising treatments for mild to moderate acne vulgaris. However, more quality of evidence and standardized methodologies are needed to support their effectiveness and safety claims.

1. Introduction

Acne vulgaris, one of the most common dermatological conditions, is a chronic inflammatory disease of the pilosebaceous unit, affecting more than 85% of adolescents and young adults, particularly males [1–3]. Although uncommon in adulthood, recent epidemiological data point to an increasing prevalence, around 40%, predominantly in females [3–6]. The main clinical manifestations of acne are noninflammatory and inflammatory lesions, which occur primarily on the face, neck, trunk, and back [7]. Acne is generally a mild and self-limiting condition, but in its most severe form it can result in scarring and hyperpigmentation of the skin. Sequelae have a strong impact on the quality of life of individuals and are often associated with the development of psychiatric disorders [8–11].

The pathogenesis of acne is a multifactorial process that involves four main pathophysiological factors: hyperplasia and hyperproduction of sebaceous; hyperkeratinization of the sebaceous ducts; bacterial colonization and proliferation, mainly by *Cutibacterium acnes*; and inflammatory response [12, 13].

The hormonal changes typical of puberty, particularly, the increase in androgen levels, are considered the main

triggers of the pathology [14, 15]. In the sebaceous glands, the type I 5 α-reductase enzyme reduces androgens to dihydrotestosterone, a more potent androgen, which stimulates lipogenesis and the proliferation and differentiation of sebocytes [12, 13, 16]. With increased sebum production, linoleic acid levels decrease [17], being the deficit of this compound in sebum responsible for the penetration of free fatty acids, synthesized from triglycerides, in the follicular barrier. In the follicle, fatty acids induce the production of several cytokines, such as interleukins IL-8 and IL-1α, involved in inflammation and keratinocyte proliferation [12, 16, 17]. In parallel, androgens promote the abnormal multiplication and differentiation of intrafollicular keratinocytes, which results in hyperkeratinization of the sebaceous duct [18, 19].

The gradual concentration of sebum and cells within the sebaceous duct leads to the development of the microcomedone, the microscopic precursor of all acne lesions, which transitions into a clinically visible lesion, i.e., an open or closed comedone. Subsequently, colonization of the follicle by *C. acnes* and the release of inflammatory mediators in the surrounding dermis encourage progression to an inflammatory lesion (papule, pustule, nodule, or cyst) [13, 19].

C. acnes is a Gram-positive anaerobic commensal bacterium that, through several mechanisms, stimulates the inflammatory and immune responses [13, 20]. The virulence factors secreted by this bacterium include lipases, responsible for the hydrolysis of triglycerides present in sebum; proteases and hyaluronidases, which damage the dermal and epidermal extracellular matrix; and porphyrins, molecules capable of generating reactive oxygen species and stimulating the production of IL-8 and prostaglandin PGE2 by keratinocytes [16, 21–23]. Additionally, *C. acnes* interacts with markers of the innate immune system, particularly with Toll-like receptors expressed by monocytes and keratinocytes that, once activated, secrete proinflammatory cytokines that recruit neutrophils to the pilosebaceous unit [20, 22–24]. Some recent studies have shown that *C. acnes* may reside in the pilosebaceous follicle in macrocolonies or biofilms and that these are directly related to the bacteria's resistance to antibiotics [23, 25].

According to the European guidelines, the treatment of acne vulgaris is based on the type and severity of acne, considering the patient's comorbidities and preferences [26, 27]. For mild to moderate comedogenic acne, the administration of topical agents is recommended, particularly retinoids, benzoyl peroxide, and azelaic acid [26]. Topical monotherapy treatment is usually sufficient to control the symptoms of mild acne [28]. For mild to moderate papulopustular acne, the administration of fixed combinations of benzoyl peroxide with adapalene or benzoyl peroxide with clindamycin is strongly recommended. In more severe cases, topical retinoids, namely, adapalene, can be associated with systemic antibiotics [26]. For severe papulopustular acne or moderate to severe nodular acne, treatment with oral isotretinoin monotherapy is recommended. In women, the administration of antiandrogenic hormonal therapy associated with systemic antibiotics and/or topical treatments other than antibiotics can also be considered [26].

Topical treatment includes retinoids (adapalene, tretinoin, and isotretinoin), benzoyl peroxide, azelaic acid, and antibiotics (erythromycin and clindamycin) [26]. Retinoids suppress comedogenesis, reduce sebum production, and normalize epithelial desquamation, in addition to having anti-inflammatory activity [27, 29]. Benzoyl peroxide has antibacterial and anti-inflammatory activities and exhibits mild comedolytic activity. Similarly, azelaic acid has antimicrobial, anti-inflammatory, and comedolytic properties and does not give rise to bacterial resistance [28]. Topical antibiotics have antibacterial and anti-inflammatory action, but they are not recommended in monotherapy, due to the potential development of bacterial resistance, and should be combined with benzoyl peroxide [26, 28].

Systemic treatment includes oral antibiotics, oral isotretinoin, and hormone therapy. The most used oral antibiotics are tetracyclines (doxycycline, minocycline, and lymecycline) and macrolides (erythromycin, clindamycin, and azithromycin) [27, 29]. Isotretinoin is the only drug that acts on the four pathological factors of acne, making it the most effective treatment available. It is usually reserved for cases of severe acne; however, it can be used for cases of moderate acne that do not respond to conventional therapy [29]. Finally, hormonal therapy is recommended in women with persistent inflammatory acne that is refractory to conventional treatment, with severe seborrhea, and with late-onset acne [29]. Hormonal agents include androgen receptor inhibitors (cyproterone acetate and spironolactone) and inhibitors of androgen production by the ovaries (oral contraceptives) and adrenal glands (glucocorticoids) [6, 29].

Although several therapeutic options are available for the treatment of acne, potential adverse effects, inadequate response to therapy, and the high costs associated with some treatments encourage an increased demand for alternative and complementary therapies, particularly of natural origin [30, 31]. For example, isotretinoin and its commercially available brands, although effective in the treatment of acne, can cause developmental abnormalities in the fetus (teratogenic effects) and therefore should not be used during pregnancy due to the risk of birth defects. The range and severity of associated abnormalities vary [30, 31]. Over the last few decades, there has been a growing interest in the use of medicinal plants as an alternative or adjuvant therapy in the treatment of acne vulgaris. This interest resulted from the need to minimize the increase in bacterial resistance to existing antimicrobials, eliminate or attenuate the potential adverse effects of conventional therapies, encourage adherence to therapy, and address inadequate responses to treatment [31].

Several studies have recently emerged on the use of medicinal plants and phytochemicals in the treatment of acne vulgaris, which motivated this systematic review of clinical trials. Thus, this study focused on reviewing the available studies on herbal medicine with a potential antiacne effect.

2. Methods

2.1. Search Strategy and Inclusion and Exclusion Criteria. Three electronic databases (PubMed, Web of Science, and Scopus) were searched from inception to January 2021. The

PubMed search strategy served as a reference for the development of the search strategies for the other databases. The search terms used included the MeSH term "acne vulgaris" combined with the MeSH terms "phytotherapy," "plants, medicinal," "plant extracts," and "herbal medicine" using boolean operator tools (Table 1). Studies were included if they were clinical trials evaluating the effectiveness of herbal therapies. The selected studies comprised one or more of the following outcome measures: number of acne lesions (inflammatory and noninflammatory), sebum production, acne severity, and quality of life. Two filters were used that limited the search to articles written in English and that involved humans. All studies in which the participants used oral, cutaneous, or mechanical therapies (extrinsic to the study) for the treatment of acne vulgaris during the study were excluded; studies whose therapeutic composition was not described or did not contain herbal or phytochemical products, studies where the participants had other pathologies or dermatological conditions that could interfere with the treatment or with the evaluation of the results, and studies carried out in animals were also excluded.

2.2. Study Selection. Following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) recommendations [32–34], two reviewers independently screened all titles and abstracts based on the defined inclusion criteria. Subsequently, the full text of each potentially eligible article was obtained and screened to support its inclusion in this systematic review. Any disagreement about study eligibility was solved through discussion.

2.3. Data Extraction and Synthesis. According to the PRISMA methodology [32–34], two authors independently reviewed and extracted the data using a prespecified protocol. In cases of discordance, a third reviewer was consulted to analyze discrepancies in data extraction. The data extracted from each study were synthesized and included the identification of the authors, publication year, study design and duration, study population (number of participants and classification of acne), details of the intervention (herbal medicine, pharmaceutical form(s), dose/frequency, and route(s) of administration), controls, outcome measures, and adverse effects.

2.4. Assessment of Risk of Bias. Two independent reviewers assessed the risk of bias of the included randomized controlled trials (RCTs) using the "Cochrane Guide for Review Authors on Assessing Study Quality" which is based on the "Cochrane Collaboration tool for assessing the risk of bias" [35]. The studies were classified as "low risk," "unclear risk," or "high risk" of bias regarding the following criteria: random sequence generation, allocation concealment, blinding (participants and personnel), blinding (outcome assessment), incomplete outcome data, selective reporting, and other sources of bias [36]. The results of the risk of bias assessment were presented in a risk of bias summary (review author's judgments about each risk of bias item for each included study), which were sketched using Review Manager 5.3 (Version 5.3.5).

Table 1: Search string used for this systematic review.

PubMed
(1) Plant (1082807)
(2) Plant extract (188557)
(3) Tea (30664)
(4) Herbal products (7154)
(5) Natural products (637692)
(6) 1 OR 2 OR 3 OR 4 OR 5 (1547065)
(7) Phytotherapy (39254)
(8) Treatment (10562707)
(9) Remedy (10139)
(10) Natural therapy (155217)
(11) Herbal medicine (41337)
(12) 7 OR 8 OR 9 OR 10 OR 11 (10578745)
(13) Acne vulgaris (12303)
(14) Propionibacterium acnes (4976)
(15) 13 OR 14 (16271)
(16) 6 AND 12 AND 15 (732)
(17) 16 AND Humans AND English (362)
Web of Science
(1) Plant (4958255)
(2) Plant extract (463391)
(3) Tea (125509)
(4) Herbal products (43344)
(5) Natural products (350473)
(6) 1 OR 2 OR 3 OR 4 OR 5 (5283779)
(7) Phytotherapy (39811)
(8) Treatment (9089471)
(9) Remedy (64916)
(10) Natural therapy (206773)
(11) Herbal medicine (69546)
(12) 7 OR 8 OR 9 OR 10 OR 11 (9313410)
(13) Acne vulgaris (17168)
(14) *Propionibacterium acnes* (8498)
(15) 13 OR 14 (23700)
(16) 6 AND 12 AND 15 (569)
(17) 16 AND English (547)
Scopus
(1) Plant (2340371)
(2) Plant extract (271770)
(3) Tea (64489)
(4) Herbal products (16588)
(5) Natural products (191436)
(6) 1 OR 2 OR 3 OR 4 OR 5 (2547351)
(7) Phytotherapy (40878)
(8) Treatment (7537055)
(9) Remedy (60029)
(10) Natural therapy (73955)
(11) Herbal medicine (53492)
(12) 7 OR 8 OR 9 OR 10 OR 11 (7665338)
(13) Acne vulgaris (14914)
(14) *Propionibacterium acnes* (8158)
(15) 13 OR 14 (21527)
(16) 6 AND 12 AND 15 (362)
(17) 16 AND English (338)

3. Results

3.1. Included Studies. The searches in the three databases were carried out until January 2021, with a total of 1247

records having been identified. After removing 331 duplicates, 916 records were analyzed by reading the titles and abstracts, of which 46 were selected for full reading of the text, based on the inclusion and exclusion criteria. Of the 46 studies, 9 were not included in this systematic review as it was not possible to access their full texts. Other 3 studies were also excluded due to their characteristics incompatible with the defined inclusion criteria. In total, 34 studies were included in this systematic review (Figure 1).

3.2. Characteristics of the Studies. The characteristics of the 34 studies included in this systematic review are summarized in Table 2. Through the selection process, 34 studies were obtained, of which 25 were RCTs and 9 were non-RCTs, in which 3 were controlled and 6 were noncontrolled trials. Regarding the controlled trials, 16 compared the intervention with placebo, 6 with another approved therapy for the treatment of acne vulgaris, one with another herbal therapy, and 5 used more than one control. The duration of the studies ranged from a minimum period of 21 days to a maximum of 6 months. The studies involved a total of 1753 participants.

In 24 studies, the degree of acne severity was used as an inclusion criterion. The participants' acne was classified according to the degree of severity as follows: mild, mild to moderate, mild to severe, moderate, moderate to severe, and severe. The classification systems used were quite different between studies; however, the lesion count was the most applied classification method.

Regarding the intervention, of the 34 studies, 22 investigated a single herbal medicine, 9 tested different combinations of herbal medicines, and 3 evaluated the potential of phytochemicals in the treatment of acne vulgaris. Concerning the administration routes, the cutaneous one was the most used, followed by the oral route, and by the association of the cutaneous with the oral routes. The included studies presented several outcomes, which were used in this systematic review, namely, the number of skin lesions, the time needed to reduce 50% of the number of injuries, the area occupied by the lesions, the production of sebum, the severity of the acne, the production of porphyrins, the global clinical assessment, the evaluation by the participants, and the quality of life of the participants. Finally, 26 out of 34 studies reported the occurrence or absence of adverse effects during the study.

3.3. Risk of Publication Bias. The results found in the assessment of the risk of publication bias in the 25 included RCTs are summarized in Figure 2.

In general, the included RCTs satisfied all the domains of bias defined by the Cochrane collaboration tool. Concerning the selection, performance, and detection bias, related to the allocation concealment, blinding of participants and personnel, and blinding of outcome assessment, respectively, there were several studies classified as "unclear risk," since there were doubts regarding the allocation of participants as well as about the blinding process (single or double). In addition, other sources of bias were found, which can skew the obtained results. It is important to note, however, that the assessment of the risk of publication bias is a subjective task, even when employing the Cochrane tool, because it is based on the personal judgments of the review authors.

3.4. Results of the Included Trials

3.4.1. Inflammatory Lesions. The number of inflammatory lesions decreased relative to baseline in the intervention groups in all studies that included this outcome. However, only in 14 studies, the change was considered statistically significant. Regarding the controlled trials that comprised this outcome, 16 of the 19 studies reported that the herbal intervention was substantially more effective in reducing the number of inflammatory lesions than the respective controls. When compared to placebo, herbal products (*L. digitata*, *C. sinensis*, *B. vulgaris*, *A. vera*, *G. mangostana*, and epigallocatechin-3-gallate) significantly reduced the number of lesions. Similar results were observed in the study by Kwon et al., with the administration of *Lactobacillus*-fermented *C. obtusa* [45]. Two other studies had better results in the intervention group than in the control one, but the changes induced by the herbal medicines, *C. sinensis* and *C. mukul*, were not statistically different from those caused by placebo and tetracycline, respectively.

In the studies by Enshaieh et al., Sharquie et al., and Mazzarello et al., the inflammatory lesions, papules, and pustules were counted individually [39]. The first two studies reported considerable differences between the intervention group and the control group in the reduction of the two types of injuries [39, 55]. In the study by Mazzarello et al., although the herbal combination under study significantly reduced the number of papules and pustules in the participants, when compared to erythromycin, the difference between the two groups only reached statistical significance in reducing the number of papules [64].

In contrast to the above results, in 3 studies, the herbal intervention was less effective in reducing the number of inflammatory lesions than the control [37, 46, 63]. In the study by Lee et al., the difference between the results achieved by the group that administered the formulation containing *Rosa* extract and the results obtained in the group that applied adapalene was not statistically significant, although the reduction in the number of lesions was higher in the control group [46]. In the studies by Bassett et al. and Lubtikulthum et al., benzoyl peroxide, administered as a control in both studies, was superior to tea tree oil and the herbal combination in reducing the number of inflammatory lesions [37, 63]. However, only the first study reported that the difference between the two groups was statistically significant [37].

3.4.2. Noninflammatory Lesions. The number of noninflammatory lesions was reduced from baseline in the intervention groups in all studies that used it as an outcome. Of these studies, only 12 reported that the reduction was statistically significant. Seventeen controlled trials considered this outcome, of which 13 achieved greater reductions in the

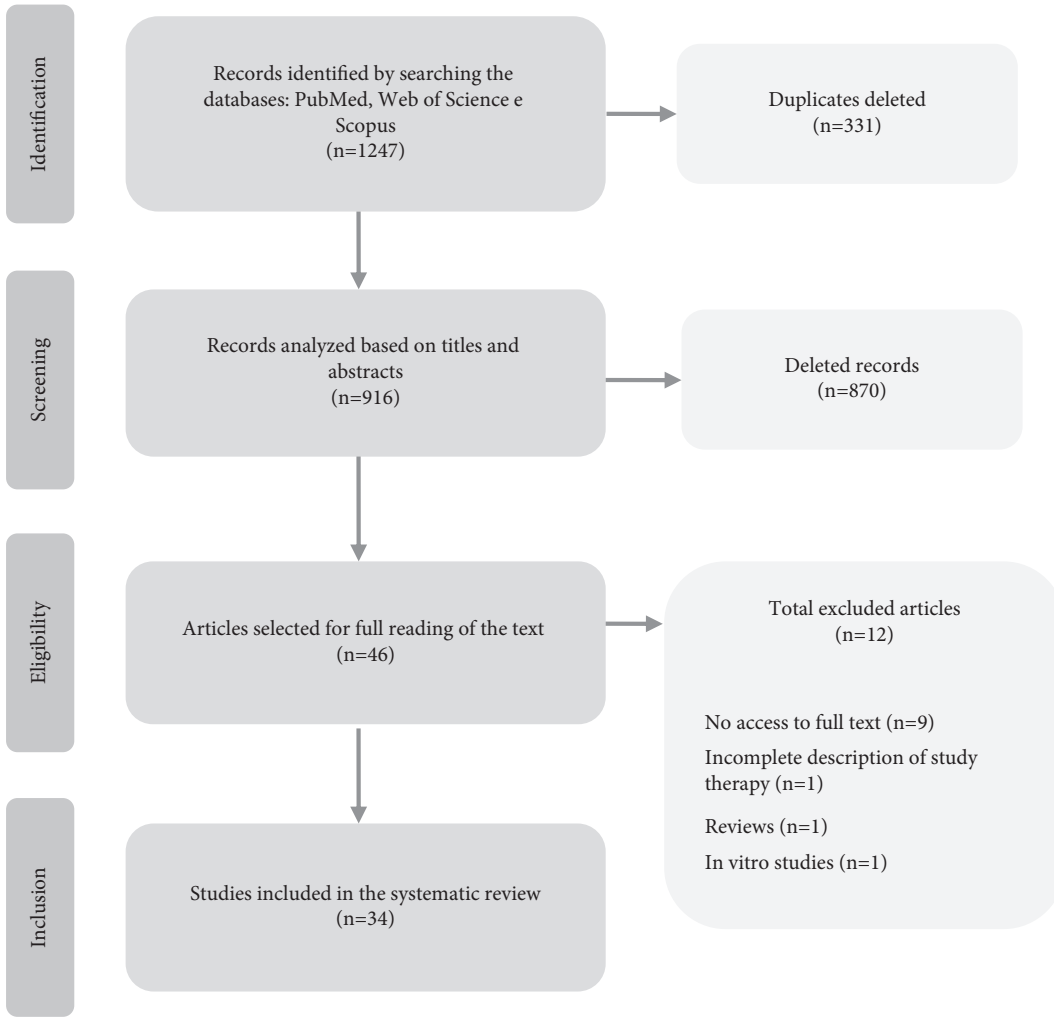

Figure 1: Flow diagram of the database search, trial selection, and articles included in this systematic review.

number of noninflammatory lesions in the intervention group than in the control. The changes induced by the herbal medicines *L. digitata*, tea tree oil, *B. vulgaris*, *A. vera*, and epigallocatechin-3-gallate were statistically significant when compared to placebo. Similar results were observed with the administration of *Lactobacillus*-fermented *C. obtusa* when compared to tea tree oil [45]. In the studies by Forest and Rafikhah and Kim et al., the difference between the two groups did not reach statistical significance, although the reduction in the number of lesions was higher in the intervention group [40, 61]. It should be noted that in the study by Mazzarello et al., the herbal combination under study provided a greater reduction in the number of noninflammatory lesions than placebo, being this reduction lower when compared to erythromycin [64].

In 4 studies, the reduction observed in the control group was higher than the reduction achieved in the intervention group. In these studies, tea tree oil, herbal combination, formulations containing *Rosa* extract, and *C. sinensis* were compared with benzoyl peroxide, adapalene, and placebo, respectively. However, in 2 of these studies, the difference between the two groups did not reach statistical significance [46, 47].

3.4.3. Total Number of Lesions. The total number of lesions, resulting from the sum of the number of inflammatory lesions with the number of noninflammatory lesions, was reduced relative to the beginning of the study in the intervention groups and in all studies that included it as an outcome. Still, only 7 studies mentioned that the change was statistically significant. In 5 of the 9 controlled trials that integrated this result, the herbal medicines tea tree oil, *C. sinensis*, *B. vulgaris*, and *A. vera* significantly reduced the total number of lesions when compared to placebo. Similar results have been reported with the administration of the propolis-tea tree oil-*A. vera* formulation compared to erythromycin [64]. In the study by Sutono, although the reduction in the total number of lesions was higher in the group that administered *G. mangostana*, the difference between the reduction achieved in this group and the reduction achieved in the group that administered placebo was not statistically significant [57].

Diverging from other results, in the studies by Lee et al., Lu and Hsu, and Lubtikulthum et al., the herbal medicines *Rosa*, *C. sinensis*, and the herbal combination were less effective than adapalene, placebo, and benzoyl peroxide, respectively, in reducing the total number of lesions

Table 2: Main characteristics of the included studies in this systematic review.

Author, year	Study design, duration	Participants		Intervention	Control	Outcomes	Adverse effects
		N (intervention group/control group)	Acne classification (severity degree; classification system)	Herbal medicine; pharmaceutical form(s); dose/frequency; route(s) of administration			
Plant extracts							
Bassett et al., 1990 [37]	RCT, 3 months	61/63	Mild to moderate; leeds system	Tea tree oil 5%; gel; cutaneous	Benzoyl peroxide 5%	Number of inflammatory and noninflammatory lesions	Intervention group: 44% of the participants reported dryness, itching, burning, and redness of the skin. Control group: 79% of the participants reported the same adverse effects.
Capitanio et al., 2012 [38]	RCT, 8 weeks	30/30	Mild; leeds system	A complex of zinc and an oligosaccharide derived from the seaweed *Laminaria digitata*; cream; twice a day; cutaneous	Placebo	Number of inflammatory and noninflammatory lesions; sebum production	Absence of irritation and skin peeling.
Enshaieh et al., 2007 [39]	RCT, 45 days	30/30	Mild to moderate; injury count	Tea tree oil 5%; gel; twice a day; cutaneous	Placebo	Number of total lesions; number of inflammatory and noninflammatory lesions; acne severity (ASI)	Intervention group: itching ($N = 3$); burning ($N = 1$); desquamation ($N = 1$). Control group: itching ($N = 2$); burning ($N = 2$).
Forest and Rafikhah, 2014 [40]	RCT, 30 days	18/16	Mild to moderate; leeds system	*Camellia sinensis* (aqueous extract of green tea); capsule; 500 mg/3 times per day; oral	Placebo	Number of total lesions; number of inflammatory and noninflammatory lesions	Without adverse effects
Fouladi, 2012 [41]	RCT, 4 weeks	25/25	Moderate to severe; injury count	*Berberis vulgaris* (aqueous extract of dried fruit); capsule; 200 mg/3 times per day; oral	Placebo	Number of total lesions; number of inflammatory and noninflammatory lesions; acne severity (Michaelson's acne severity score)	Without adverse effects
Hajheydari et al., 2014 [42]	RCT, 8 weeks	30/30	Mild to moderate; GAGS	*Aloe vera* topical gel combined with tretinoin cream 0.025%; gel; twice a day; cutaneous	Placebo + tretinoin	Number of total lesions; number of inflammatory and noninflammatory lesions; acne severity (ASI)	The intervention group reported fewer adverse effects than the control group

Table 2: Continued.

Author, year	Study design, duration	Participants		Intervention	Control	Outcomes	Adverse effects
		N (intervention group/control group)	Acne classification (severity degree; classification system)	Herbal medicine; pharmaceutical form(s); dose/frequency; route(s) of administration			
Hou et al., 2018 [43]	Uncontrolled trial, 4 weeks	20	Mild to moderate; NR	*Panax ginseng* (hydrophobic fraction in red ginseng ethanol extract); cream; 2 twice a day; cutaneous	—	Number of inflammatory and noninflammatory lesions; sebum production	NR
Khan and Akhtar, 2014 [44]	RCT, 12 weeks	(Female 1) 25/25 (Female 2) 25/25	Moderate; leeds system	(F1) *Hippophae rhamnoides*; (F2) *Cassia fistula*; emulsion; 500 mg twice a day; cutaneous; each powdered plant was extracted with 70% methanol solution	Placebo	Sebum production; global clinical evaluation	NR
Kwon et al., 2014 [45]	RCT, 8 weeks	34/34	Mild to moderate; modified leeds system	*Chamaecyparis obtusa* fermented by *Lactobacillus*; cream; twice a day; cutaneous	Tea tree oil	Number of inflammatory and noninflammatory lesions; sebum production; acne severity (modified leeds system)	Intervention group: mild erythema ($N = 2$); skin dryness ($N = 2$). Control group: slight skin dryness ($N = 4$); moderate erythema and desquamation ($N = 6$).
Lee et al., 2011 [46]	RCT, 12 weeks	50/47	Mild to moderate; KAGS	*Rosa* combined with hexamidine disethionate 0.05% and retinol 0.03%; once a day; cutaneous	Adapalene 0.1%	Number of total lesions; number of inflammatory and noninflammatory lesions; acne severity (KAGS); global clinical evaluation; participants evaluation (TR)	The intervention group reported fewer adverse effects than the control group. However, by the end of the study, the difference between the two groups became negligible.
Lu and Hsu, 2016 [47]	RCT, 4 weeks	40/40	Moderate to severe; IGA	*Camellia sinensis* (decaffeinated green tea extract); capsule; 500 mg/3 times per day; oral	Placebo	Number of total lesions; number of inflammatory and noninflammatory lesions; life quality (CADI)	Intervention group: constipation ($N = 1$); abdominal discomfort ($N = 2$). Control group: polydipsia ($n = 1$); insomnia ($N = 1$).

Table 2: Continued.

Author, year	Study design, duration	Participants		Intervention	Control	Outcomes	Adverse effects
		N (intervention group/control group)	Acne classification (severity degree; classification system)	Herbal medicine; pharmaceutical form(s); dose/frequency; route(s) of administration			
Lueangarun et al., 2019 [48]	RCT, 12 weeks	28/28	Moderate to severe; GAGS	*Garcinia mangostana* (topical mangosteen extract in nanoparticle loaded gel, containing α-mangostin); gel; twice a day; cutaneous	Clindamycin 1%	Number of inflammatory and noninflammatory lesions; porphyrins production; clinical global evaluation; participants evaluation (TS)	Similar adverse effects in both groups. After 4-weeks of treatment, no participant had adverse effects on both sides of the face.
Malhi et al., 2017 [49]	Uncontrolled trial, 12 weeks	18	Moderate to severe; injury count and IGA	Tea tree oil; gel; twice a day; cutaneous	—	Number of total lesions; acne severity; participants evaluation (TR)	Well tolerated treatment. Moderate desquamation ($N = 2$); moderate skin dryness ($N = 1$).
Miglani and Manchanda, 2014 [50]	Uncontrolled trial, 6 months	34	NR; GAGS	*Arctium lappa*; 4 pills/4 times per day for 7 days followed by 7 days of placebo; oral	—	Number of total lesions; number of inflammatory and noninflammatory lesions; acne severity (GAGS); life quality (Acne-QoL)	NR
Orafidiya et al., 2002 [51]	RCT, 4 weeks	112/ (1) 7 (2) 7	NR; injury count	*Ocimum gratissimum* essential oil; 0.25 cm^3/twice a day; cutaneous	(1) Benzoyl peroxide 10% (2) Placebo	Time necessary to reduce 50% of the total number of lesions (days)	Adverse effects are minimal and tolerable
Pan-In et al., 2015 [52]	RCT, 4 weeks	10/10	NR; injury count	*Garcinia mangostana* (cellulose-based nanoparticles as nano-reservoir and α-mangostin, an active component isolated from the edible *Garcinia mangostana* fruit); gel; twice a day; cutaneous	Placebo	Number of inflammatory lesions; acne severity (ASI)	NR
Pécastaings et al., 2018 [53]	Controlled trial, 56 days	60	Mild to moderate; GEA	*Myrtus communis* leaf extract; cream; twice a day; cutaneous	Healthy volunteers, free of facial or dorsal acne and of any facial dermatosis	Acne severity; porphyrins production	Without adverse effects

Table 2: Continued.

Author, year	Study design, duration	Participants		Intervention	Control	Outcomes	Adverse effects
		N (intervention group/control group)	Acne classification (severity degree; classification system)	Herbal medicine; pharmaceutical form(s); dose/frequency; route(s) of administration			
Shafiq et al., 2014 [54]	RCT 45-days	25/25	NR; injury count	*Casuarina equisetifolia* bark extract 5% with 90% methanol; cream; twice a day; cutaneous	Benzoyl peroxide	Acne severity (Cook's Acne Grading Scale); global clinical evaluation	Intervention group: without adverse effects. Control group: 17% of participants reported skin irritation and redness.
Sharquie et al., 2006 [55]	RCT, 2 months	30/30	Mild to moderate; injury count	*Camellia sinensis*; lotion; twice a day; cutaneous	Placebo	Number of inflammatory lesions; participants evaluation (TS)	Without adverse effects
da Silva et al., 2012 [56]	Controlled clinical trials, 21 days	10/10	Mild; NR	*Copaifera langsdorffii* essential oil; gel; twice a day; cutaneous	Placebo	Area occupied by the inflammatory lesions (mm^2)	Without adverse effects
Sutono, 2013 [57]	RCT, 3 weeks	45/41	Mild to moderate; Lehman criteria	*Garcinia mangostana* (extract of mangosteen rind); capsule; 400 mg/3 times per day; oral	Placebo	Number of total lesions; number of inflammatory and noninflammatory lesions	Without adverse effects
Thappa and Dogra, 1994 [58]	RCT, 3 months	10/10	Severe (nodulocystic); injury count	*Commiphora mukul* (gugulipid, equivalent to 25 mg guggulsterone); 1 pill twice a day; oral	Tetracycline oral (500 mg)	Number of inflammatory and noninflammatory lesions	Without adverse effects
Combinations of plant extracts							
Beltrami et al., 2001 [59]	Controlled clinical trials, 90 days	15/15	Mild to severe; NR	*Krameria trianda*, *Serenoa repens*, and *Centella asiatica*; cutaneous	Topical treatment + placebo (oral)	Sebum production	Intervention group: burning (resolved with continued treatment).
Lone et al., 2012 [60]	Uncontrolled trial, 45 days	25	NR; Cook's system of acne grading	Unani formulation: Irsa (*Iris florentina*), barghe neem (*Azadirachta indica* leaves), poste saras (*Acacia speciosa* bark), ghungchi safaid (*Abrus precatorious*), and *Namake Sambhar* (Lake salt) 50 grams each; 6 to 10 g/once a day; cutaneous	—	Acne severity (Cook's Acne Grading Scale)	Without adverse effects

Table 2: Continued.

Author, year	Study design, duration	Participants		Intervention	Control	Outcomes	Adverse effects
		N (intervention group/control group)	Acne classification (severity degree; classification system)	Herbal medicine; pharmaceutical form(s); dose/frequency; route(s) of administration			
Kim et al., 2019 [61]	RCT, 8 weeks	28/28	NR; injury count	Cheongsangbangpoong-tang formulation: *Schizonepeta tenuifolia* (0.5 g), *Coptis japonica* makino (0.5 g), *Mentha arvensis* var. *iperascens* (0.5 g), *Ponciri Fructus Immaturus* (0.5 g), *Glycyrrhiza uralensis* FISCH (0.5), *Gardenia augusta* (1.0 g), *Cnidium officinale* (1.0 g), *Scutellaria baicalensis* (1.0 g), *Forsythia koreana* (1.0 g), *Angelica dahurica* (1.0 g), *Platycodon grandiflorum* (1.0 g), *Ledebouriella seseloide*s (1.0 g), corn starch (1.2 g), lactose hydrate (2.3 g); granulated; 5 g/3 times per day; oral	Placebo	Number of inflammatory and noninflammatory lesions acne severity (KAGS; IGA)	Intervention group: Digestion discomfort ($n = 3$). There were no serious adverse effects.
Lalla et al., 2001 [62]	RCT, 4 weeks	(G1) 23 (G2) 23 (G3) 5 (G4) 2	Mild to severe; Leeds system	Ayurvedic formulation (soft extracts of *Aloe barbadensis* Miller, *Azardirachta indica* Juss, *Curcuma longa* Linn, *Hemidesmus indicus* Linn, *Terminalia chebula* Retzr, *Terminalia arjuna* Rob, and *Withania somnifera* Linn (one part of the extract approximately representing four parts of dried/fresh plant material); 2 pills/twice a day + topical preparation (gel (G1) or cream (G2))/twice a day oral and cutaneous	(G3) Placebo (topical preparation) (G4) placebo (oral and topical preparation)	Participants evaluation (TR)	Mild itching ($N = 2$); increased gastric motility ($N = 2$). Reported adverse effects decreased with continued treatment.

Table 2: Continued.

Author, year	Study design, duration	Participants		Intervention	Control	Outcomes	Adverse effects
		N (intervention group/control group)	Acne classification (severity degree; classification system)	Herbal medicine; pharmaceutical form(s); dose/frequency; route(s) of administration			
Lubtikulthum et al., 2019 [63]	RCT, 12 weeks	39/38	Mild to moderate; modified leeds system	*Allium cepa*, *Lavandula*, *Garcinia mangostana*, *Aloe vera*, *Morus papyrifera*, and *Melaleuca alternifolia*; gel; 1 g/twice a day; cutaneous	Benzoyl peroxide 2.5%	Number of total lesions; number of inflammatory and noninflammatory lesions; life quality (DLQI); porphyrins production; participants evaluation (TS)	Most common adverse effect: Skin irritation. The intervention group reported fewer adverse effects (skin desquamation and erythema) than the control group.
Mazzarello et al., 2018 [64]	RCT, 30 days	(PTA) 20 (1) 20 (2) 20	Mild to moderate; injury count	Propolis 20%, tea tree oil 3%, and *Aloe vera* 10%; cream; twice a day; cutaneous	(1) Erythromycin 3% (2) Placebo	Number of total lesions; number of inflammatory and noninflammatory lesions; acne severity (ASI); sebum production	NR
Orafidiya et al., 2004 [65]	RCT, 4 weeks	48/ (1) 12 (2) 12 (3) 12	NR; injury count	*Ocimum gratissimum* essential oil 2%, and *Aloe vera* (25%, 50%, and 100%); lotion; 0.25 cm^3/twice a day; cutaneous	(1) Placebo (2) Negative control (*A. vera*) (3) Positive control (clindamycin)	Time necessary to reduce 50% the number of inflammatory lesions (days)	Intervention group: mild and tolerable adverse effects—96% of participants reported feeling a slight burning sensation on the skin.
Paranjpe and Kulkarni, 1995 [66]	RCT, 6 weeks	67/15	Moderate; injury count	4 ayurvedic formulations; pills; 500 mg/3 times per day; oral	Placebo	Number of inflammatory and noninflammatory lesions; participants evaluation (TR)	NR
Parveen et al., 2009 [67]	RCT, 2 months	20/10	NR; IGA	Unani formulation; cream; twice a day; cutaneous	Placebo	Acne severity (IGA)	NR
Phytochemicals							
Fabbrocini et al., 2011 [68]	Controlled clinical trials, 60 days	20/20	NR; GAGS	Resveratrol (0.01%, w/v); gel; once a day; cutaneous	Placebo	Acne severity (GAGS)	Without adverse effects
Jung et al., 2012 [69]	Uncontrolled trial, 8 weeks	30	Mild to moderate; injury count (inflammatory and noninflammatory lesions)	Polyphenon-60: catechin from green tea and is the representative green tea extract compound (20 mg/mL); lotion; twice a day; cutaneous	—	Number of inflammatory and noninflammatory lesions	NR

Table 2: Continued.

Author, year	Study design, duration	Participants		Intervention	Control	Outcomes	Adverse effects
		N (intervention group/control group)	Acne classification (severity degree; classification system)	Herbal medicine; pharmaceutical form(s); dose/frequency; route(s) of administration			
Yoon et al., 2013 [70]	RCT, 8 weeks	(Epigallocatechin-3-gallate 1%) 17/17 (Epigallocatechin-3-gallate 5%) 18/18	NR; modified Leeds system	Epigallocatechin-3-gallate 1% and 5%; solution; twice a day; cutaneous	Placebo	Number of inflammatory and noninflammatory lesions; acne severity (modified leeds system)	Intervention group (5%): erythema and skin irritation ($N = 4$). Intervention group (1%): without adverse effects.

ASI: Acne Severity Index; CADI: Cardiff Acne Disability Index; DLQI: Dermatology Life Quality Index; GAGS: Global Acne Grading System; GEA: Global Acne Severity Scale; IGA: Investigator's Global Assessment; KAGS: Korean Acne Grading System; NR: not eported; TR: treatment response; TS: treatment satisfaction.

[46, 47, 63]. However, the first two studies reported that the difference between the two groups was not statistically significant [46, 47].

3.4.4. Time Needed to Reduce 50% the Number of Lesions. The time needed to reduce 50% the number of lesions was used as an outcome in 2 studies, which included 210 participants [51, 65]. In the first study, several formulations were administered with increasing concentrations of the *O. gratissimum* essential oil (0.5%, 1%, 2%, and 5%) dispersed in different bases (polysorbate 80, cetomacrogol, petrolatum, and alcohol) [51]. In addition to being compared to each other, the different preparations were compared with benzoyl peroxide and with placebo. It was found that the reduction in the number of pustules was faster in preparations with high concentrations (2% and 5%) of *Ocimum* oil and with bases containing cetomacrogol or alcohol in their composition. These preparations were statistically more effective than benzoyl peroxide and placebo in reducing the number of pustules ($p < 0.05$) [51].

The second study evaluated the effect of *A. vera* on the activity of the *O. gratissimum* essential oil [65]. In the preparation, *Ocimum* oil was dispersed in increasing concentrations (0%, 25%, 50%, and 100%) of *A. vera*, which were later compared with placebo, with negative control (*A. vera* gel), and with positive control (clindamycin). The results achieved with the administration of the preparations with lower concentrations (0% and 25%) of *A. vera* were similar to the results presented by the group that administered clindamycin, whereas the preparations with higher concentrations (50% and 100%) of *A. vera* gave significantly better results than the positive control ($p < 0.05$) [65]. The number of inflammatory lesions decreased by 50% or more in all participants who administered *Ocimum* oil within a period of 2 to 5 days. The group that administered the negative control (*A. vera* gel) did not show a significant reduction in inflammatory lesions when compared to the groups that administered the herbal preparations and the group that applied the placebo did not achieve a 50% reduction in the number of lesions [65].

3.4.5. Occupied Area by the Lesions. Only one study, which involved 20 participants, evaluated the effect of an herbal formulation on the area occupied by the inflammatory lesions [56]. The results revealed that in the areas where the essential oil of *C. langsdorffii* was administered, there was a significant decrease ($p < 0.01$) in the extension affected by the lesions. On the other hand, in the areas where the placebo was applied, an increase in the surface occupied by the lesions was verified in several participants [56].

3.4.6. Sebum Production. In total, 6 studies, involving 254 participants, investigated the action of herbal medicine in the production of sebum. In all studies, the amount of cutaneous sebum, determined using a Sebumeter®, was reduced compared to the start of the study. However, only 2 studies reported that the reduction was statistically significant ($p < 0.05$) [43, 59].

Of the 5 controlled trials that integrate this outcome, 2 achieved a statistically significant difference between the intervention and the control groups ($p < 0.05$). In these

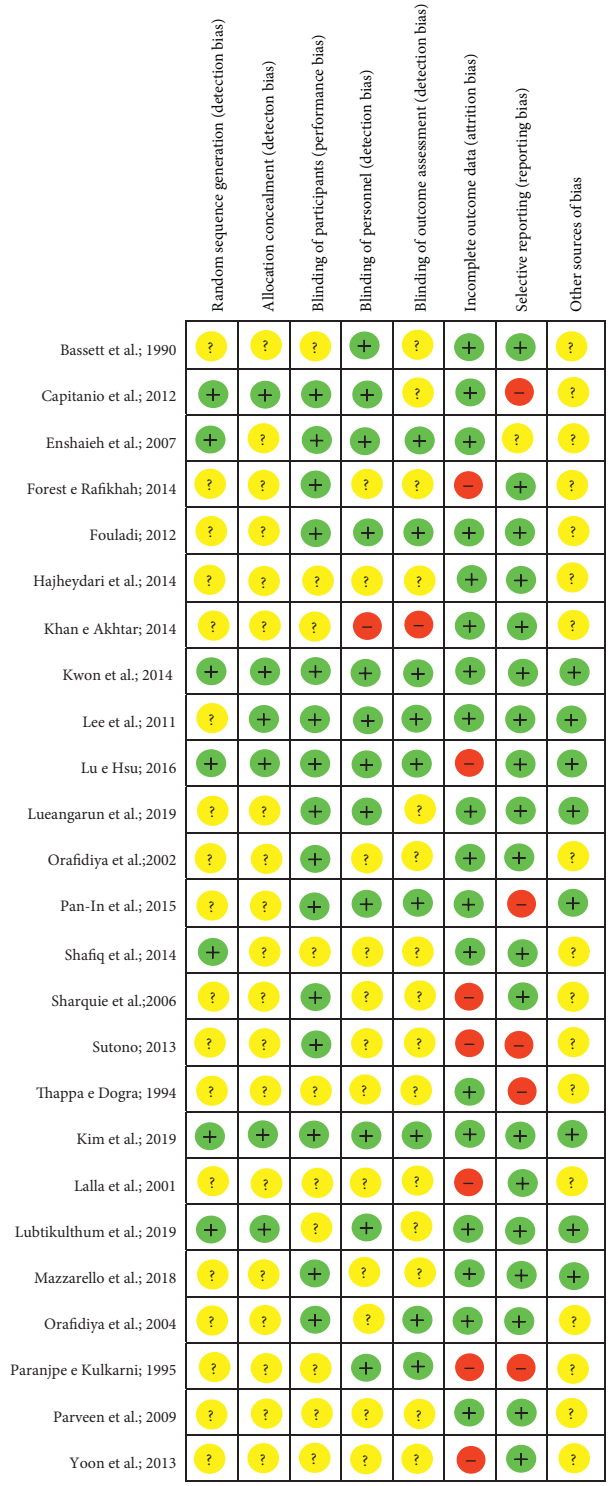

FIGURE 2: Results of risk of bias assessment regarding the methodological quality of included studies—the risk of bias summary. Review the author's judgments about each risk of bias item for each included study.

studies, the herbal medicines *H. rhamnoides*, *C. fistula*, and *Lactobacillus*-fermented *C. obtusa* were more effective in reducing sebum production than placebo and tea tree oil, respectively [44, 45]. In the remaining studies, the difference between the intervention group and the control group was not considered significant, as the decrease in sebum production was similar in both groups.

3.4.7. Acne Severity. In order to assess the effectiveness of herbal medicine in the treatment of acne vulgaris, 16 studies, which included 699 participants, used the alteration of the degree of acne severity. The studies that integrated this outcome used several classification systems, based on clinical examinations and photography.

In all studies, the degree of acne severity was reduced in the intervention group, relatively to the beginning of the study. However, only 11 studies considered the change to be statistically significant. In total, 12 controlled studies included this outcome, of which 7 reported that the herbal medicines tea tree oil, *B. vulgaris*, *A. vera*, *G. mangostana*, and Unani formulation, and the phytochemicals resveratrol and epigallocatechin-3-gallate were significantly more effective than placebo in reducing in the degree of severity of acne ($p = 0.000$; $p < 0.001$; $p = 0.001$; $p = 0.042$; $p < 0.0001$; $p < 0.001$; $p < 0.05$, respectively) [39, 41, 42, 52, 67, 68, 70].

In the study by Kwon et al., participants in the group that administered the formulation containing *Lactobacillus*-fermented *C. obtusa* considerably reduced the degree of acne severity when compared to those who administered tea tree oil ($p < 0.05$) [45]. In the study by Mazzarello et al., the group that administered the propolis-tea tree oil-*A. vera* formulation achieved a greater reduction in severity than the groups that administered placebo and erythromycin [64]. The difference between the results achieved in the intervention group and in the group that administered erythromycin was statistically significant ($p = 0.0368$) [64]. In the study by Kim et al., the *Cheongsangbangpoong-tang* formulation promoted a reduction in the severity of acne, but the results were not statistically different from those of the group that administered the placebo [61]. Similarly, in the study by Shafiq et al., the results achieved by the group that administered the herbal medicine *C. equisetifolia* were not significantly different from the results presented by the group that applied benzoyl peroxide [54].

On the other hand, in the study by Lee et al., the reduction in acne severity of the participants who administered the formulation containing the *Rosa* extract was minor than the reduction achieved with the administration of adapalene; however, the difference between the two groups was not considerable ($p = 0.641$) [46].

3.4.8. Porphyrin Production. The concentration of porphyrins, which indirectly reveals the amount of *C. acnes* in the skin, was used by 3 studies as an outcome, including 165 participants. Different quantification methods were employed in the various studies, namely examination using Wood's lamp, image analysis based on UV photography, and the VISIA® analysis system.

All herbal medicines, *G. mangostana*, *M. communis*, and the combination of *A. cepa*, *Lavandula*, *G. mangostana*, *A. vera*, *M. papyrifera*, and *M. alternifolia* significantly reduced ($p < 0.001$; $p < 0.0001$; $p = 0.003$, respectively) the concentration of porphyrins, in relation to the beginning of the study, thus demonstrating their antibacterial properties [48, 53, 63]. Additionally, the efficacy of *G. mangostana* and the herbal combination was compared with that of clindamycin and benzoyl peroxide, respectively. In both studies, the difference between the changes observed in the intervention group and in the control group was not statistically significant ($p = 0.649$ and $p = 0.425$) [48, 63].

3.4.9. Global Clinical Evaluation. Six studies, involving 360 participants, described the overall response to treatment as an outcome. The response to treatment was assessed by specialists who were guided by scales defined by each of the studies.

In the study by Khan and Akhtar, the response to treatment with the herbal medicines, *H. rhamnoides* and *C. fistula,* was classified as "excellent," "good," or "undefined," relative to the beginning of the study. At the end of the study, of the 31 participants with Grade I (mild) acne, 9 had an "excellent" response, and 17 had a "good" response to treatment. As for the 19 participants with Grade II (moderate) acne, 4 responded "excellent" to the treatment, and 13 responded "good" [44]. Similarly, in the study by Shafiq et al., the response to treatment with *C. equisetifolia* was also categorized [54]. The study results demonstrated that the number of participants who achieved a response rated "excellent" or "good" was higher in the intervention group than in the benzoyl peroxide group [54]. Additionally, in the study by Lalla et al., the response to treatment was rated from "excellent" to "poor". Several conclusions were drawn from the results of this study: (1) the two groups of participants who administered the ayurvedic formulation, orally and dermally, had a higher number of excellent responses to treatment than the group of participants who administered the ayurvedic formulation orally only; (2) of the two groups that administered the ayurvedic formulation orally and topically, the group that administered the cream formulation had a higher number of excellent responses than the group that administered the gel formulation (57.89% vs 31.58%); (3) the control group that simultaneously administered placebo preparations orally and topically did not obtain any response [62]. In the study by Paranjpe and Kulkarni, only one of the Ayurvedic formulations, called Sunder Vati, gave rise to significant changes in relation to the beginning of the study. Approximately two-thirds of participants who administered this formulation exhibited a "good" to "excellent" clinical response at the end of the study [66].

According to the study by Lee et al., the formulation containing *Rosa* extract provided a considerable improvement in acne in 84% of participants, compared to the beginning of the study. However, the results did not differ significantly from the group that administered adapalene ($p = 0.303$), which generated a significant response in 97% of

participants [46]. Finally, in the study by Lueangarun et al., *G. mangostana* promoted the regression of acne more markedly than clindamycin. The difference between the two groups was statistically significant ($p = 0.004$) [48].

3.4.10. Participants' Evaluation. In total, 5 studies, which included 280 participants, used the opinion of individuals as a method of evaluating the effectiveness of treatment. Thus, in the studies by Lee et al. and Malhi et al., the participants evaluated the evolution of acne during treatment [46, 49]. In the first study, 77% of participants treated with a formulation containing *Rosa* extract said that their acne significantly improved compared to the baseline, but the results were not statistically different from those reported by participants who administered adapalene ($p = 0.314$) [46]. In the second study, at the end of each week of tea tree oil treatment, participants looked at whether the severity of acne had changed from the previous week. The most frequent answers were that the acne was the same (46%) or slightly better (43%) [49].

The remaining studies assessed participants' satisfaction with the treatment. In the study by Lueangarun et al. (2019), the participants showed high satisfaction ($p < 0.001$) with the administration of the formulation containing *G. mangostana*, as well as with the administration of the clindamycin gel, with no statistically significant difference being reached between the two treatments ($p = 0.714$) [48]. Regarding the study by Sharquie et al., the participants who administered the herbal medicine *C. sinensis* revealed levels of satisfaction higher than those who used placebo [55]. Finally, in the study by Lubtikulthum et al., the satisfaction with the treatment efficacy was similar in both groups ($p = 0.391$); however, the participants expressed greater satisfaction with the administration of the herbal combination than with the administration of benzoyl peroxide, which resulted in a difference statistically significant ($p = 0.011$) [63].

3.4.11. Participants' Quality of Life. Three studies, with a total of 191 participants, evaluated the impact of herbal treatment on the participants' quality of life. In the study by Lu and Hsu, the quality of life of the participants, determined using the Cardiff Acne Disability Index (CADI) questionnaire, did not vary significantly in relation to the beginning of the study ($p = 0.28$). Furthermore, the difference between the results obtained with the herbal medicine *C. sinensis* and with the placebo did not reach statistical significance ($p = 0.83$) [47].

In the remaining studies, the herbal *A. lappa* and the combination of extracts from *A. cepa*, *Lavandula*, *G. mangostana, A. vera*, *M. papyrifera*, and *M. alternifolia* promoted a significant improvement ($p < 0.001$) in the quality of life of the participants, regarding the beginning of the study, according to the questionnaires used [50, 63]. The results obtained with the administration of the herbal combination were also compared with those of benzoyl peroxide, but there were no statistically significant differences between the two groups ($p = 0.344$) [63].

4. Discussion

This systematic review included 34 studies with a total of 1753 participants, which evaluated the efficacy of herbal medicine in the treatment of acne vulgaris. The evidence presented by the studies suggests that herbal and phytochemical formulations can be effective in the treatment of acne vulgaris, as demonstrated by the reduction in the number of lesions, the production of sebum, the severity of the pathology, and the production of porphyrins, as well as for the improvement of the participants' quality of life, observed in the intervention group in several studies. In most controlled trials, the intervention group achieved results equal to or better than the control group, with some studies showing that the difference between groups was statistically significant.

The different therapeutic strategies employed showed the versatility with which herbal products can be introduced in the daily treatment of acne vulgaris. Monotherapy was the most used strategy, followed by the association of herbal medicine with standard acne treatments. This last strategy, called adjuvant therapy, proved to be promising as it allowed to reduce the initial dose of certain drugs and, therefore, the adverse effects associated with their administration. Additionally, several studies have reported synergistic therapeutic effects when different herbal medicines were combined.

Considering the results of the studies and the quality of the evidence presented, the botanical species *Melaleuca alternifolia*, *Camellia sinensis*, *Berberis vulgaris*, and *Chamaecyparis obtusa* fermented by *Lactobacillus*, *Garcinia mangostana*, and *Aloe vera*, were the most employed in the included clinical trials.

Concerning some adverse effects that are reported in clinical trials included in this systematic review, the overall results of the studies employing tea tree oil revealed that it is as effective as benzoyl peroxide in reducing inflammatory lesions, but benzoyl peroxide has a faster onset of action [37, 39, 49]. Still, subjects who administered tea tree oil experienced fewer adverse effects (dryness, itching, burning, and flushing) than those who administered benzoyl peroxide [37]. Following these results, tea tree oil presents itself as an alternative therapy to conventional treatments of mild to moderate acne vulgaris, acting simultaneously as an antibacterial and anti-inflammatory. Given its broad-spectrum antibacterial activity, tea tree oil may be a viable option in the treatment of therapy-resistant acne. The minimal adverse effects associated with its administration and the absence of teratogenicity encourage its use in the treatment of acne vulgaris [39].

Tea tree oil is an essential oil extracted from the plant native to Australia, *Melaleuca alternifolia* [71]. Considered as a medicinal essential oil, it has been used for several decades in the treatment of skin disorders [49, 72]. Consisting of more than 100 components, tea tree oil has terpinen-4-ol as its major compound, which corresponds to at least 35% of the oil [71, 73]. Terpinen-4-ol has strong antimicrobial and anti-inflammatory activity and properties that support the use of tea tree oil in the treatment of acne vulgaris [71, 74, 75]. The antimicrobial mechanism of action

of this oil involves structural and functional changes in the bacterial membrane [75]. Several studies investigated the antimicrobial activity of the essential oil on *C. acnes*, having reported that the minimum inhibitory concentration (MIC) of the oil for the bacterium is between 0.3 and 0.6% and the minimum bactericidal concentration (MBC) is between 0.25 and 0.5% [45,76–78]. The second property of tea tree oil that contributes to its therapeutic efficacy is its anti-inflammatory activity. *In vitro*, the main constituent of the oil reduced the production of inflammatory mediators, such as TNF-α, IL-1β, IL-8, IL-10, and prostaglandin (PG) E2 [79]. Additionally, the water-soluble fractions of tea tree oil, terpinene-4-ol, and α-terpineol suppressed monocyte superoxide production [80].

Camellia sinensis is a plant native to Southeast Asia, from which the second most consumed beverage in the world, tea, is produced [81, 82]. From its leaves, four varieties of tea are derived, white tea, green tea, oolong tea, and black tea, whose composition varies according to the fermentation and drying processes to which the leaves are subjected [83]. White tea and green tea are not fermented, differing in the maturity of the leaf used in their production, oolong tea is partially fermented, and black tea is fully fermented [83, 84]. The fermentation process generates conformational changes in the bioactive components of tea, which results in changes in its biological properties [83, 85, 86]. Green tea is made from fresh leaves of *C. sinensis* processed to prevent oxidation of its polyphenolic compounds [87, 88]. Catechins are the main polyphenols present in green tea, representing about 30% to 42% of the water-soluble solids of this tea [89]. Its content is influenced by several factors, such as geographic location, growing conditions, and the degree of fermentation [83]. The four main catechins present in tea are epigallocatechin-gallate, epicatechin-gallate, epigallocatechin, and epicatechin [90]. Epigallocatechin-gallate is the most abundant catechin in green tea, accounting for about 59% of the total catechins, and the most important from a pharmacological point of view [91–93]. Numerous pharmacological properties have been attributed to green tea, highlighting the antioxidant, anti-inflammatory, antimicrobial, and anticancer properties [91, 94]. The antioxidant activity of green tea, mediated by catechins, occurs through the induction of antioxidant enzymes, the scavenging of free radicals, and the inhibition of lipid peroxidation [86]. This property is considered the most important of this class of polyphenols since its anti-inflammatory action derives from its action as an antioxidant [95–97]. On the other hand, its antimicrobial activity results from alterations in the bacterial membrane and from the inhibition of fatty acid synthesis and the enzymatic activity of bacteria [91, 98]. In addition to these activities, recent studies suggest that green tea reduces sebum production by inhibiting the 5α-reductase enzyme [92, 99]. Given these properties, green tea acts directly on three of the four pathological mechanisms involved in the pathogenesis of acne vulgaris. From the 3 studies included in this review that investigated the efficacy of green tea in the treatment of acne vulgaris, it is possible to conclude the following: green tea considerably reduces inflammatory lesions, but does not exert significant effects on noninflammatory lesions; green tea is more effective in treating mild to moderate acne than moderate to severe acne; oral administration of green tea is as efficient as cutaneous administration; few adverse effects are associated with the administration of green tea. Following this evidence, it is possible to state that green tea could be an alternative to conventional treatments for mild to moderate acne vulgaris.

Berberis vulgaris is a plant of the *Berberidaceae* family widely found in Europe, Asia, and America [100]. The reddish fruit of this plant is commonly included in gastronomic dishes, while the roots, stems, and bark are used in traditional medicine [101]. The medicinal properties of *B. vulgaris* are mostly attributed to berberine, an isoquinoline alkaloid that belongs to the structural class of protoberberines [102]. Berberine exhibits multiple pharmacological properties, including anti-inflammatory, antioxidant, antibacterial, antifungal, and anxiolytic properties [103]. Additionally, a study has shown that berberine considerably suppresses lipogenesis in the sebaceous glands [104]. The potential beneficial effects of *B. vulgaris* motivated the investigation of its therapeutic efficacy in the treatment of acne vulgaris. The effects of aqueous extract of the *B. vulgaris* fruit on adolescents with moderate to severe acne vulgaris were evaluated. After 4 weeks of treatment, the number of inflammatory and noninflammatory lesions, as well as acne severity were significantly reduced, with no adverse effects or associated complications. The evidence suggests that the success of the treatment resulted from the anti-inflammatory action, exerted mainly by the alkaloid fraction of *B. vulgaris*, from the antioxidant action, through the elimination of free radicals and the inhibition of lipid peroxidation, and from the anxiolytic action, since acne exacerbations are often related to bouts of anxiety and stress [105].

Chamaecyparis obtusa is a species of cypress native to Asia, which has been widely used as a cosmetic, perfume, and disinfectant [45, 106]. The essential oil extracted from its leaves contains numerous terpenes, molecules characterized by their antioxidant and anti-inflammatory properties, and specific compounds, such as β-tuiaplicin, which confer antimicrobial activity [45, 93, 106–113]. Recently, a study revealed that fermentation of *C. obtusa* by *Lactobacillus* substantially increases its antimicrobial activity, particularly against *C. acnes*, because of the increased content of dihydroxybenzoic acid, taxifolin, and quercetin [45]. Given the promising properties of this plant, Kwon et al. investigated the effect of *Lactobacillus*-fermented *Chamaecyparis obtusa* in the treatment of mild to moderate acne vulgaris and subsequently compared its efficacy with that of tea tree oil [45]. This study stands out for being the first clinical trial, to date, to compare the efficacy and safety of two herbal medicines in the treatment of acne vulgaris. The results of this study showed that the two herbal medicines were effective in reducing the number of inflammatory and noninflammatory lesions; however, *Lactobacillus*-fermented *Chamaecyparis obtusa* was significantly superior to tea tree oil. After one week of treatment with *Lactobacillus*-fermented *Chamaecyparis obtusa*, the number of inflammatory

lesions in the participants decreased considerably, indicating that *Lactobacillus*-fermented *Chamaecyparis obtusa* has a therapeutic efficacy comparable to that of topical retinoids and antibiotics, with the advantage of not having adverse effects. In contrast, tea tree oil only achieved significant reductions after four weeks of administration. Similarly, the reduction in the number of noninflammatory lesions was faster and more pronounced on the side of the face where *Lactobacillus*-fermented *Chamaecyparis obtusa* was applied. Finally, the authors elucidated the mechanism of action underlying the observed clinical results. Among the various molecules studied, the accelerated decrease in the expression of the NF-kB protein, in the area where *Lactobacillus*-fermented *Chamaecyparis obtusa* was administered, justified the stronger and faster anti-inflammatory effect of *Lactobacillus*-fermented *Chamaecyparis obtusa* compared to tea tree oil. Furthermore, sebo-suppression resulted from the reduction of the SREBP-1 protein, one of the main regulators of lipid synthesis in the sebaceous glands [45].

Mangosteen, the fruit of the *Garcinia mangostana* tree, is known as the "queen of fruits" in Southeast Asia for its distinctive flavor and numerous health benefits [114–116]. Its bark, used for centuries in the treatment of different pathologies, is currently marketed as a food supplement all over the world [115, 117, 118]. The main phytochemicals present in *G. mangostana* are xanthones, a class of secondary metabolites with biological antioxidant, anti-inflammatory, neuroprotective, antimicrobial, and antifungal effects [114, 116]. The most abundant xanthones found in this species are α-mangostine and γ-mangostine [119]. *In vitro* studies have shown that *G. mangostana*, in particular, α-mangostine, exerts strong antimicrobial activity against *C. acnes* and *Staphylococcus epidermidis*, bacteria involved in the pathogenesis of acne [120–122]. This activity, associated with its anti-inflammatory action, motivated the development of clinical studies that determined the anti-acne activity of *G. mangostana in vivo*. The studies included in this review that investigated the potential of *G. mangostana* in the treatment of acne vulgaris achieved promising results. In various studies, the number of inflammatory and noninflammatory lesions, the severity of acne, and the number of porphins were drastically reduced, with few associated adverse effects. The antimicrobial, anti-inflammatory, and antioxidant properties of *G. mangostana*, reported by in vitro studies, support the results obtained by clinical trials. Scientific studies have shown that α-mangostine, the main xanthone present in the bark of *G. mangostana*, has potent antimicrobial activity against *C. acnes*, as evidenced by a MIC of 0.039 mg/mL [121, 123]. Furthermore, α-mangostine exhibits anti-inflammatory activity, through the reduction of TNF-α and PGE2, and antioxidant activity, which results from the inhibition of reactive oxygen species [116, 124]. Taken together, these properties validate the use of *G. mangostana* as an alternative therapy in the treatment of acne vulgaris.

Aloe vera, the most popular species belonging to the genus *Aloe*, is one of the most used herbal medicines worldwide for its immeasurable health benefits [125–127]. Native to the Arabian Peninsula, *A. vera* is a xerophytic plant characterized by its long green leaves, with thorny margins, filled with a mucilaginous pulp (*A. vera* gel) rich in water and bioactive components that concentrate numerous properties [125, 128]. More than 75 different components were identified in the *A. vera* gel, including polysaccharides, anthraquinones, flavonoids, terpenes, saponins, amino acids, minerals, and vitamins [129–131]. Anthraquinones are the most important secondary metabolites present in *A. vera* gel, being responsible for the astringent, antibacterial, anti-inflammatory, antioxidant, and healing properties attributed to *A. vera* [126]. These properties, which are crucial in the treatment of skin conditions, have stimulated the investigation of the antiacne activity of *A. vera* gel *in vivo*. The *A. vera* gel minimized the adverse effects associated with the administration of tretinoin, an effect attributed to its anti-inflammatory and soothing properties. Additionally, the results of the clinical trials revealed that epigallocatechin-3-gallate is effective in reducing inflammatory and noninflammatory lesions, with few adverse effects. Taken together, the evidence from the studies suggests that epigallocatechin-3-gallate may represent a new therapeutic opportunity in the treatment of acne vulgaris.

The present systematic review has some limitations. Of the included articles, only RCTs were evaluated for the risk of bias, so the evidence from the remaining studies may be subject of high risk. Another limitation is related to the multiple acne classification and outcome assessment systems used by various studies. The absence of standardized and validated systems compromised the comparison of results between studies. Furthermore, some trials were performed for the same herbal medicine. Moreover, since the composition of the extracts studied in the clinical trials and included in the present meta-analysis is often unreported in the original paper, the obtained results may be not reproducible. Additionally, most studies investigated the effect of herbal medicines on individuals with mild to moderate acne, which made it impossible to generalize the results. Finally, since the formulation of the pharmaceutical dosage form and its physicochemical characteristics play a very important role in the efficacy of any dosage form, which is even more obvious when using medicinal plants which are usually prepared from different sources, it would be of major importance that before any clinical trial, the information regarding the suitability of the pharmaceutical dosage form from the physicochemical point of view including the extraction methods and standardization of the active raw materials was obtained. Otherwise, the results of clinical trials will be very different and unreliable due to the different quality of the applied dosage forms even produced from the same herb.

5. Conclusions

The evidence presented by the studies described suggests that herbal and phytochemical formulations can be effective in the treatment of acne vulgaris, as demonstrated by the reduction in the number of lesions, sebum production, the severity of the pathology, and the production of porphyrins, and by the improvement in the quality of life of the

participants, observed in the intervention group in several studies. In most of the controlled trials, the intervention group achieved results equal to or greater than the control group, with some studies showing that the difference between groups was statistically significant.

The different therapeutic strategies used showed the versatility with which herbal products can be introduced in the daily treatment of acne vulgaris. Monotherapy was the most used strategy, followed by the association of herbal medicines with standard acne treatments. This last strategy, known as adjuvant therapy, proved to be promising, as it allowed to reduce the initial dose of certain drugs and, therefore, the adverse effects associated with their administration. Additionally, several studies have reported synergistic therapeutic effects when different herbal medicines are combined.

Data Availability

The data presented in this study are available upon request from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Prediction of the Mechanism of Shaoyao Gancao Decoction in the Treatment of Alopecia Areata by Network Pharmacology and Its Preliminary Verification Study

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Objective. To explore the mechanism of Shaoyao Gancao decoction (SGD) in treatment of alopecia areata (AA) by network pharmacology and animal experiments. *Methods* Based on the traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP), the components and targets of SGD were determined. Then, the related targets of AA were retrieved from DrugBank, GeneCards, OMIM, and DisGeNET databases. The intersection of drug targets and disease targets was determined, and the key targets of the protein-protein interaction network were obtained with the String database. Gene Ontology (GO) biological process enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of potential key targets were carried out using the DAVID database using AutoDock for molecular docking verification. Finally, the key pathway was validated by animal experiments. *Results*. A total of 102 active components, 212 predicted targets, and 812 AA disease-related targets were obtained. Topological analysis yielded 45 key targets of SGD in the treatment of AA, including IL-6, PTGS2, TNF, VEGFA, CCL2, IL-1B, CXCL8, CASP3, MPO, and IL-10. There were 324 GO entries obtained through GO biological process enrichment analysis, and 20 pathways were obtained through KEGG pathway enrichment analysis, involving the PI3K-Akt signaling pathway, osteoclast differentiation, and Jak-STAT signaling pathway. The molecular docking results showed effective ingredients (quercetin, kaempferol, and 7-methoxy-2-methyl isoflavone) have good docking results with targets (IL-6, PTGS2, and TNF). The results of animal experiments showed that SGD can effectively upregulate the expression of PI3K and AKT proteins. *Conclusion*. This is the first in-depth study on the mechanism of SGD's treatment effect in AA using network pharmacology, and preliminary animal experiments verified that it is closely related to the PI3K/AKT signaling pathway. This finding may provide a new basis for SGD's clinical application in AA.

1. Introduction

Alopecia areata (AA) is a T-cell-mediated nonscarring alopecia characterized by an autoimmune reaction in the hair follicle. AA is associated with various factors such as genetics, neurological and psychiatric conditions, oxidative stress, and viral infections. Studies have shown that the lifetime prevalence of AA is about 2% globally and 0.27% in China [1]. AA is a disfiguring skin disease that often has a negative impact on patients' psychological health and quality of life. In terms of treatment, both glucocorticoids and immunosuppression are recommended by *The Chinese Guidelines for the Treatment of Alopecia Areata (2019)* [2], but all have the limitation of offering mainly symptom relief and not eradicating the disease; these treatments are often accompanied by various adverse effects and relapse after discontinuation. Therefore, it is crucial to find new, more durable, and effective treatments for AA.

Shaoyao Gancao decoction (SGD) is derived from Zhang Zhongjing's *Treatise on Febrile and Miscellaneous Diseases*. The whole formula consists of two herbs: Shaoyao and Gancao. Shaoyao is sour in taste and cold in property, with

the effect of nourishing blood and astringing *yin*, softening the liver and relieving pain; Gancao is sweet in taste and warm in property, strengthening the spleen and benefiting *qi* and relieving pain. The combination of the two herbs nourishes the yin with sour and sweet and harmonizes the liver and spleen as well as relieves pain. Modern pharmacological studies [3] have shown that SGD has analgesic and anti-inflammatory effects, which are commonly used clinically for the treatment of related painful and inflammatory diseases. A clinical study [4] has confirmed the safe and effective treatment of AA in adults and children with total glucosides of paeony capsules containing Shaoyao components. In addition, our group's previous study has shown that Shaoyao Gancao granule (a traditional Chinese medicine prescription made from Shaoyao and Gancao) is safe and effective in the clinical treatment of severe AA and can regulate the expression of Th1/Th2, Th17/Treg cells, and their related factors, but the specific mechanism of action is unclear [5].

Network pharmacology is an emerging systems biology research method that combines modern medicine and bioinformatics. This method uses biological databases to construct and demonstrate the relationships among diseases, targets, and drugs, thus systematically identifying the possible mechanisms of action of drugs [6]. The characteristics of this approach are consistent with the multicomponent, multitarget, and multipathway action characteristics of traditional Chinese medicine (TCM).

In this study, we investigated active components of SGD through network pharmacology, constructed a multilevel bioinformatics network of SGD for AA treatment, and successfully predicted the potential key targets and pathways of SGD for the treatment of AA, which strongly reflected the multicomponent, multitarget, and multipathway action characteristics of SGD. At the same time, molecular docking and animal experiments were used to validate the pharmacological mechanism, providing a theoretical basis for further research.

2. Materials and Methods

2.1. Preparation of SGD. The SGD granules formula consisted of 45 g of Shaoyao and 15 g of Gancao, prepared by the Pharmacy Department of the China-Japan Friendship Hospital, 10 g per bag (containing 30 g of raw medicine), in which the content of paeoniflorin was not less than 900 mg and that of glycyrrhetinic acid was not less than 75 mg.

2.2. Animals and Experimental Groups. We obtained 30 female C57BL/6J mice (22 ± 3 g), 6~8 weeks old, from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China, license no. SCXK, 2016-0006). All the animal experiments strictly followed the relevant standards and requirements of the animal experimental platform of the Institute of Clinical Medicine, China-Japan Friendship Hospital.

The 30 mice were randomly and equally divided into three groups: the control group, the model group, and the SGD group. The latter two groups were treated with topical imiquimod cream combined with 21-day chronic unpredictable mild stress to establish a mouse model of AA, after which the SGD group was given SGD (9.76 g/kg) by gavage for 21 consecutive days.

2.3. Reagents. Reagents included imiquimod cream (Sichuan Mingxin Pharmaceutical Company), phosphoinositide 3-kinase (PI3K) P85 alpha monoclonal antibody (60225-1-lg, Proteintech), AKT (60203-2-lg, Proteintech), goat-anti-mouse IgG-HRP (115-035-003, Jackson ImmunoResearch), and RIPA-containing lysis buffer (#9806, Cell Signaling Technology).

2.4. Instruments. We used a cryogenic frozen centrifuge (ThermoFisher, ID: Fresco17), Bio-Rad Mini PROTEAN Tetra, Bio-Rad Mini Trans-Blot, Bio-Rad PowerPac Basic.

2.5. Network Pharmacology-Based Mechanism for Predicting Effect of SGD on AA

2.5.1. Screening of Therapeutic Targets of Active Components Related to SGD. The chemical components of Shaoyao and Gancao were researched separately in the traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP; https://lsp.nwu.edu.cn/tcmsp.php). The screening criteria for the main chemical components were set as oral bioavailability (OB) $\geq 30\%$ and drug-like index (DL) ≥ 0.18. The Universal Protein Database (UniProt, https://www.uniprot.org/) was then used to standardize the target information.

2.5.2. Screening of Alopecia Areata Gene Targets. The keywords "alopecia areata" were used to screen for AA-related gene targets in the DrugBank database (https://www.drugbank.ca/), GeneCards database (https://www.genecards.org, Version 4.11.0), Online Mendelian Inheritance in Man (OMIM) (https://omim.org/), and DisGeNET database (https://www.disgenet.org/). The disease targets obtained from these databases were pooled and duplicate targets were removed to obtain AA disease-related targets.

2.5.3. Construction and Analysis of SGD Herbs-Compounds-Targets (H-C-T) Network. The potential target genes of SGD in the treatment of AA were the intersecting parts of the target genes corresponding to the action of active components of SGD and the target genes related to AA. The overlapping partial genes were visualized by Venn diagrams (https://bioinfogp.cnb.csic.es/tools/venny/). Cytoscape 3.8.0 software was then used to construct the network of interactions between overlapping genes, bioactive compounds, and herbs, called the H-C-T network.

2.5.4. Construction of Core Targets' Protein-Protein Interaction Network. The potential targets of SGD for AA treatment were imported into the STRING 11.0 database

(https://string-db.org/), setting the species genus as "*Homo sapiens*," the minimum interaction score as "medium confidence (0.400)," and hiding the free sites. The protein-protein interaction (PPI) network of SGD for AA was obtained using Cytoscape 3.8.0 software for network topology analysis.

2.5.5. Enrichment Analysis of Core Targets. Gene Ontology (GO) biological process enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed on the core targets for the action of SGD using the David database (https://david.ncifcrf.gov/).

2.5.6. Molecular Docking. We screened the highest five-node degree active components in the H-C-T network of SGD and the highest five-node degree core proteins in the PPI network. Then, the potential interaction of ligands in the complex molecular network was studied by molecular docking simulation. The 3D structure Protein Data Bank (PDB) format files of the target proteins were retrieved and downloaded from the database (https://www.rcsb.org/). Subsequently, AutoDock Vina was used to verify molecular docking between core components and core targets. The molecular docking results were optimized and mapped with the help of PyMOL software.

2.6. Validation of the Pharmacological Effect of SGD on AA by Western Blot Analysis. Mouse skin tissues were homogenized in RIPA-containing lysis buffer and centrifuged, and protein concentrations were determined. Total proteins were separated by electrophoresis on an 8% separation gel and electrotransferred to a 0.2 mm pore size NC membrane. After being incubated with 5% nonfat dry milk in Tris-buffered saline for 2 h at room temperature, the membranes were incubated with antibodies at 4°C overnight. Primary antibodies against AKT (60203-2-lg) and PI3K (60225-1-lg) were purchased from Proteintech (Wuhan, China). After washing three times with Tris-buffered saline with Tween 20 (TBST), the membranes were incubated with goat-anti-mouse secondary antibody (1 : 10,000), washed as previously described, and then detected by western blotting. The grayscale intensity of the protein bands was analyzed and quantified using Image Lab.

2.7. Statistical Analysis. SPSS 21.0 was used for the analysis. Normally distributed data were expressed as mean ± standard deviation, nonnormal distributions were expressed as median and quartiles, and count data were expressed as percentages. When comparing the sample means of two groups, the independent samples t-test was used for normally distributed data, the rank sum (Kruskal–Wallis H) test was used for nonnormally distributed data, and the chi-square test was used for count data. Differences were considered statistically significant when $P < 0.05$.

3. Results

3.1. Screening Results of Chemical Components of SGD. A total of 105 active ingredients were screened by searching the TCMSP database, including 13 Shaoyao roots and 92 Gancao roots, among which three compounds (MOL000211, MOL000359, and MOL000422) were repeated. After deduplication, 102 active ingredients were obtained. The results are shown in Table 1.

3.2. Construction of the SGD H-C-T Network. Among the abovementioned 102 related active compounds of SGD, nine compounds (MOL001910, MOL001921, MOL001925, MOL001928, MOL001930, MOL004860, MOL004905, MOL004917, and MOL005013) failed to match relevant targets. The remaining 93 components were matched to 1447 human gene targets in the TCMSP database, and 212 components were finally obtained after deduplication. By importing active compounds and corresponding targets into Cytoscape 3.8.0 software, the network of interactions between herbs, compounds, and target genes was constructed, with 307 nodes and 1543 edges, as shown in Figure 1.

3.3. AA Disease Target Genes. The keywords "alopecia areata" were searched in the DrugBank, GeneCards, OMIM, and DisGeNET databases. After deduplication, 812 AA disease target genes were obtained. The resulting Venn diagram of the intersection of SGD main active compounds' target genes and AA target genes is shown in Figure 2.

3.4. Construction and Analysis of the PPI Network. The PPI network (Figure 3) was constructed by importing 46 intersecting target genes into the STRING database, with 46 nodes and 465 edges (one target was not connected to other proteins, so it was not shown in PPI). The details of the 45 potential targets are listed in Table 2. The top 10 genes with degree values were IL-6, PTGS2, TNF, VEGFA, CCL2, IL-1B, CXCL8, CASP3, MPO, and IL-10. The abovementioned target genes are closely linked to other target genes and may play an important role in the treatment of AA.

3.5. GO and KEGG Enrichment Analysis Results. Through GO biological process enrichment analysis, 45 core targets yielded a total of 324 GO entries using David, including 270 biological process (BP) entries, 35 molecular function (MF) entries, and 19 cell composition (CC) entries, as shown in Figure 4.

Through the KEGG pathway enrichment analysis, 45 potential targets were mapped to a total of 60 signaling pathways using David, 20 of which were identified as $P < 0.05$, as shown in Figure 5.

3.6. Molecular Docking Results. Quercetin, kaempferol, 7-methoxy-2-methyl isoflavone, beta-sitosterol, and formononetin were molecularly docked with IL-6, PTGS2, TNF, VEGFA, and CCL2. The lower the binding energy, the

Table 1: Basic information on Shaoyao Gancao decoction (SGD) components.

Mol. ID	Compound ingredient	OB	DL	Herb
MOL001910	11-Alpha,12-alpha-epoxy-3-beta-23-dihydroxy-30-norolean-20-en-28,12-beta-olide	64.77	0.38	Shaoyao
MOL001918	Paeoniflorgenone	87.59	0.37	Shaoyao
MOL001919	(3S,5R,8R,9R,10S,14S)-3,17-dihydroxy-4,4,8,10,14-pentamethyl-2,3,5,6,7,9-hexahydro-1H-cyclopenta[a]phenanthrene-15,16-dione	43.56	0.53	Shaoyao
MOL001921	Lactiflorin	49.12	0.8	Shaoyao
MOL001924	Paeoniflorin	53.87	0.79	Shaoyao
MOL001925	Paeoniflorin_qt	68.18	0.4	Shaoyao
MOL001928	Albiflorin_qt	66.64	0.33	Shaoyao
MOL001930	Benzoyl paeoniflorin	31.27	0.75	Shaoyao
MOL000211	Mairin	55.38	0.78	Shaoyao,Gancao
MOL000358	Beta-sitosterol	36.91	0.75	Shaoyao
MOL000359	Sitosterol	36.91	0.75	Shaoyao,Gancao
MOL000422	Kaempferol	41.88	0.24	Shaoyao,Gancao
MOL000492	(+)-Catechin	54.83	0.24	Shaoyao
MOL001484	Inermine	75.18	0.54	Gancao
MOL001792	DFV	32.76	0.18	Gancao
MOL002311	Glycyrol	90.78	0.67	Gancao
MOL000239	Jaranol	50.83	0.29	Gancao
MOL002565	Medicarpin	49.22	0.34	Gancao
MOL000354	Isorhamnetin	49.6	0.31	Gancao
MOL003656	Lupiwighteone	51.64	0.37	Gancao
MOL003896	7-Methoxy-2-methyl isoflavone	42.56	0.2	Gancao
MOL000392	Formononetin	69.67	0.21	Gancao
MOL000417	Calycosin	47.75	0.24	Gancao
MOL004328	Naringenin	59.29	0.21	Gancao
MOL004805	(2S)-2-[4-Hydroxy-3-(3-methylbut-2-enyl)phenyl]-8,8-dimethyl-2,3-dihydropyrano[2,3-f]chromen-4-one	31.79	0.72	Gancao
MOL004806	Euchrenone	30.29	0.57	Gancao
MOL004808	Glyasperin B	65.22	0.44	Gancao
MOL004810	Glyasperin F	75.84	0.54	Gancao
MOL004811	Glyasperin C	45.56	0.4	Gancao
MOL004814	Isotrifoliol	31.94	0.42	Gancao
MOL004815	(E)-1-(2,4-Dihydroxyphenyl)-3-(2,2-dimethylchromen-6-yl)prop-2-en-1-one	39.62	0.35	Gancao
MOL004820	Kanzonols W	50.48	0.52	Gancao
MOL004824	(2S)-6-(2,4-Dihydroxyphenyl)-2-(2-hydroxypropan-2-yl)-4-methoxy-2,3-dihydrofuro[3,2-g]chromen-7-one	60.25	0.63	Gancao
MOL004827	Semilicoisoflavone B	48.78	0.55	Gancao
MOL004828	Glepidotin A	44.72	0.35	Gancao
MOL004829	Glepidotin B	64.46	0.34	Gancao
MOL004833	Phaseolinisoflavan	32.01	0.45	Gancao
MOL004835	Glypallichalcone	61.6	0.19	Gancao
MOL004838	8-(6-Hydroxy-2-benzofuranyl)-2,2-dimethyl-5-chromenol	58.44	0.38	Gancao
MOL004841	Licochalcone B	76.76	0.19	Gancao
MOL004848	Licochalcone G	49.25	0.32	Gancao
MOL004849	3-(2,4-Dihydroxyphenyl)-8-(1,1-dimethylprop-2-enyl)-7-hydroxy-5-methoxy-coumarin	59.62	0.43	Gancao
MOL004855	Licoricone	63.58	0.47	Gancao
MOL004856	Gancaonin A	51.08	0.4	Gancao
MOL004857	Gancaonin B	48.79	0.45	Gancao
MOL004860	Licorice glycoside E	32.89	0.27	Gancao
MOL004863	3-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-8-(3-methylbut-2-enyl)chromone	66.37	0.41	Gancao
MOL004864	5,7-Dihydroxy-3-(4-methoxyphenyl)-8-(3-methylbut-2-enyl)chromone	30.49	0.41	Gancao
MOL004866	2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-6-(3-methylbut-2-enyl)chromone	44.15	0.41	Gancao
MOL004879	Glycyrin	52.61	0.47	Gancao
MOL004882	Licocoumarone	33.21	0.36	Gancao
MOL004883	Licoisoflavone	41.61	0.42	Gancao
MOL004884	Licoisoflavone B	38.93	0.55	Gancao
MOL004885	Licoisoflavanone	52.47	0.54	Gancao
MOL004891	Shinpterocarpin	80.3	0.73	Gancao
MOL004898	(E)-3-[3,4-Dihydroxy-5-(3-methylbut-2-enyl)phenyl]-1-(2,4-dihydroxyphenyl) prop-2-en-1-one	46.27	0.31	Gancao
MOL004903	Liquiritin	65.69	0.74	Gancao

Table 1: Continued.

Mol. ID	Compound ingredient	OB	DL	Herb
MOL004904	Licopyranocoumarin	80.36	0.65	Gancao
MOL004905	3,22-Dihydroxy-11-oxo-delta(12)-oleanene-27-alpha-methoxycarbonyl-29-oic acid	34.32	0.55	Gancao
MOL004907	Glyzaglabrin	61.07	0.35	Gancao
MOL004908	Glabridin	53.25	0.47	Gancao
MOL004910	Glabranin	52.9	0.31	Gancao
MOL004911	Glabrene	46.27	0.44	Gancao
MOL004912	Glabrone	52.51	0.5	Gancao
MOL004913	1,3-Dihydroxy-9-methoxy-6-benzofurano[3,2-c]chromenone	48.14	0.43	Gancao
MOL004914	1,3-Dihydroxy-8,9-dimethoxy-6-benzofurano[3,2-c]chromenone	62.9	0.53	Gancao
MOL004915	Eurycarpin A	43.28	0.37	Gancao
MOL004917	Glycyroside	37.25	0.79	Gancao
MOL004924	(-)-Medicocarpin	40.99	0.95	Gancao
MOL004935	Sigmoidin-B	34.88	0.41	Gancao
MOL004941	(2R)-7-Hydroxy-2-(4-hydroxyphenyl) chroman-4-one	71.12	0.18	Gancao
MOL004945	(2S)-7-Hydroxy-2-(4-hydroxyphenyl)-8-(3-methylbut-2-enyl)chroman-4-one	36.57	0.32	Gancao
MOL004948	Isoglycyrol	44.7	0.84	Gancao
MOL004949	Isolicoflavonol	45.17	0.42	Gancao
MOL004957	HMO	38.37	0.21	Gancao
MOL004959	1-Methoxyphaseollidin	69.98	0.64	Gancao
MOL004961	Quercetin der.	46.45	0.33	Gancao
MOL004966	3′-Hydroxy-4′-O-methylglabridin	43.71	0.57	Gancao
MOL000497	Licochalcone a	40.79	0.29	Gancao
MOL004974	3′-Methoxyglabridin	46.16	0.57	Gancao
MOL004978	2-[(3R)-8,8-Dimethyl-3,4-dihydro-2H-pyrano[6,5-f]chromen-3-yl]-5-methoxyphenol	36.21	0.52	Gancao
MOL004980	Inflacoumarin A	39.71	0.33	Gancao
MOL004985	Icos-5-enoic acid	30.7	0.2	Gancao
MOL004988	Kanzonol F	32.47	0.89	Gancao
MOL004989	6-Prenylated eriodictyol	39.22	0.41	Gancao
MOL004990	7,2′,4′-Trihydroxy-5-methoxy-3-arylcoumarin	83.71	0.27	Gancao
MOL004991	7-Acetoxy-2-methylisoflavone	38.92	0.26	Gancao
MOL004993	8-Prenylated eriodictyol	53.79	0.4	Gancao
MOL004996	Gadelaidic acid	30.7	0.2	Gancao
MOL000500	Vestitol	74.66	0.21	Gancao
MOL005000	Gancaonin G	60.44	0.39	Gancao
MOL005001	Gancaonin H	50.1	0.78	Gancao
MOL005003	Licoagrocarpin	58.81	0.58	Gancao
MOL005007	Glyasperins M	72.67	0.59	Gancao
MOL005008	Glycyrrhiza flavonol A	41.28	0.6	Gancao
MOL005012	Licoagroisoflavone	57.28	0.49	Gancao
MOL005013	18α-Hydroxyglycyrrhetic acid	41.16	0.71	Gancao
MOL005016	Odoratin	49.95	0.3	Gancao
MOL005017	Phaseol	78.77	0.58	Gancao
MOL005018	Xambioona	54.85	0.87	Gancao
MOL005020	Dehydroglyasperins C	53.82	0.37	Gancao
MOL000098	Quercetin	46.43	0.28	Gancao

better the docking results. The results showed that the binding energy of each component to the target protein was less than −5 kcal/mol, among which quercetin had the best combination with PTGS2 (−9.2 kcal/mol), as shown in Figure 6. The molecular docking simulation diagram is shown in Figure 7.

3.7. Validation of Potential Therapeutic Mechanism of SGD by Western Blot. The AKT protein level of mice in the model group was 0.99 ± 0.22, and the result was lower compared with that of the control group of 1.89 ± 0.39 ($P = 0.021$). The AKT protein level in mice in the SGD group was 1.92 ± 0.45, and the result was higher compared with the model group ($P = 0.021$). The AKT protein levels of mice in the control and SGD groups were similar, with no significant statistical difference ($P = 0.773$).

The PI3K protein level of mice in the model group was 1.24 ± 0.11, which was lower than that of mice in the control group, 1.90 ± 0.38 ($P = 0.029$). The PI3K protein level of mice in the SGD group was 1.98 ± 0.57, which was higher than that of the model group ($P = 0.021$). The PI3K protein

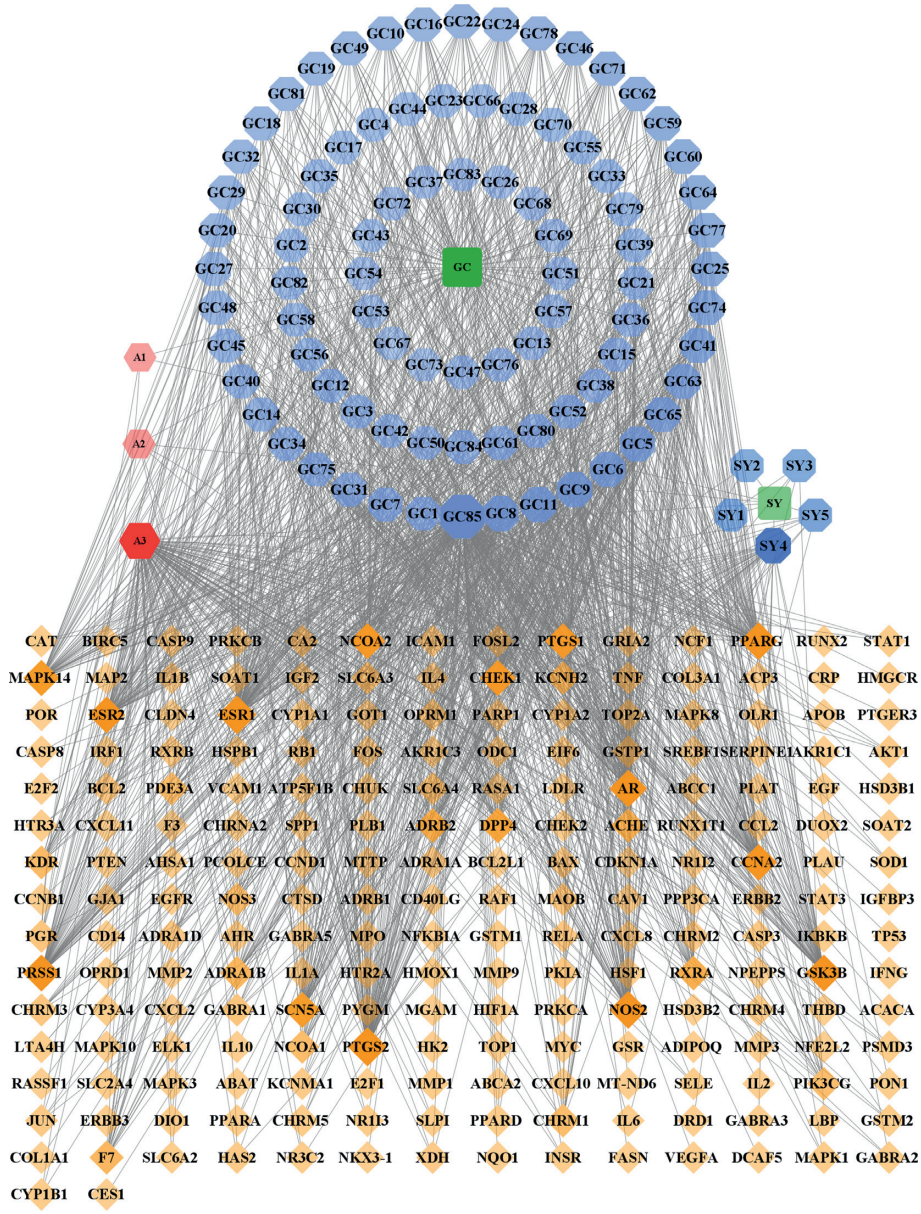

Figure 1: Shaoyao Gancao decoction (SGD) herbs, compounds, targets (H-C-T) network. Blue hexagons at the top represent active components of Gancao; blue hexagons on the upper right represent active components of Shaoyao; red hexagons represent active components shared by both Shaoyao and Gancao; orange squares represent target genes; and green squares represent drugs.

levels of mice in the control and SGD groups were similar, and no clear statistical difference was observed ($P = 0.773$). The results are shown in Figure 8.

4. Discussion

Chinese medicine holds that the main causes of AA are liver and kidney deficiency, along with a deficiency of "essence" and blood. In addition, AA is also associated with endogenous wind produced by heated blood, liver stagnation, blood stasis, splenic deficiency, and blood weakness. Modern medical research has found that psychosomatic factors play an important role in the pathogenesis of AA [7], which coincides with the theory of liver stagnation and spleen deficiency in TCM. "Hair is the remainder of blood," and blood is made of the essence of water and food, according to TCM. Although the spleen is the source of *qi* and blood, the liver is the master of draining and regulating *qi*, and the function of blood in the human body depends on the smooth regulation of *qi*. If one is worried, irritated, or overworked, the liver will become depressed and the spleen will be unable to transport and transform. Therefore, there is not enough *qi* and blood so that they cannot moisten the hair root and the hair falls off in pieces. SGD is often used clinically in the treatment of inflammatory and painful diseases. Relevant clinical studies [8] have shown that total glucosides of paeony capsules (TGPC) and compound glycyrrhizin tablets (CGT) containing Shaoyao or Gancao components are safe and effective in the treatment of AA alone or in combination. Paeoniflorin and glycyrrhetinic acid are the main active

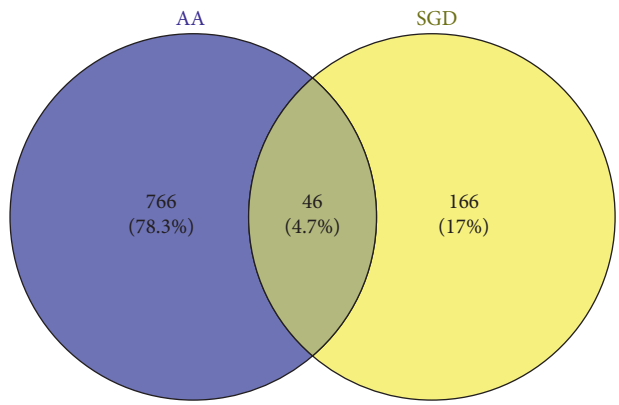

Figure 2: Venn diagram of Shaoyao Dancao decoction (SGD) and alopecia areata (AA) common targets.

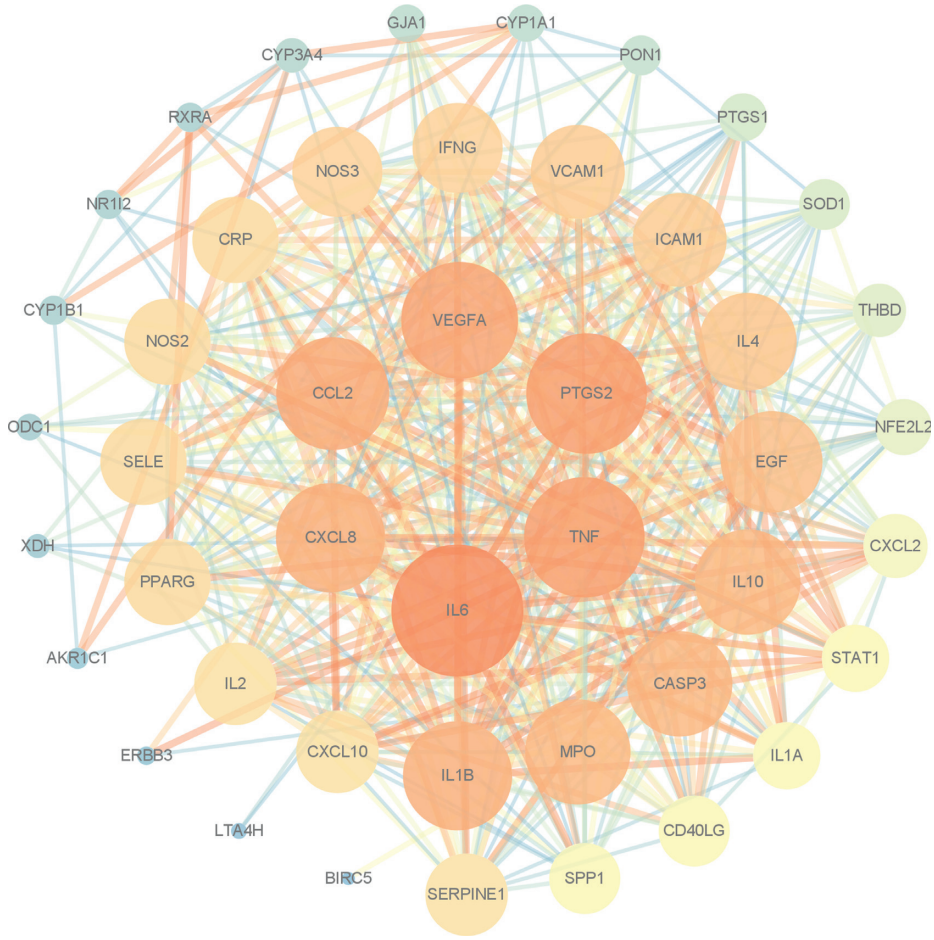

Figure 3: Protein-protein interaction (PPI) network diagram of key targets. Node size is positively correlated with degree, and node color changing from blue to orange corresponds to an increasing degree; the thickness of the edge is positively correlated with the binding fraction between proteins.

components in these two Chinese patent medicines, which have anti-inflammatory, immunomodulatory, and anti-anxiety effects. However, most of the relevant studies have focused on the active ingredients of single herbs, and there is a lack of network pharmacological studies on SGD.

According to the H-C-T network, the active components of SGD with high values include quercetin, kaempferol, 7-methoxy-2-methyl isoflavone, beta-sitosterol, and formononetin. Quercetin has anti-inflammatory, antiviral, antitumor, hypoglycemic, and immunomodulatory effects [9]. It was found that quercetin significantly inhibited the production of IL-6, MCP-1, IP-10, RANTES, GM-CSF, G-CSF, TNF-α, LIF, LIX, and VEGF [10], reduced proinflammatory cytokines (IL-1β and IL-6), and increased anti-inflammatory cytokines (IL-4, IL-10, and transforming growth factor-β 1) [11], thus exerting anti-inflammatory

Table 2: Details of the 45 potential core targets.

Number	Target	Degree
1	IL-6	38
2	TNF	35
3	PTGS2	35
4	VEGFA	34
5	CCL2	33
6	CXCL8	32
7	IL-1B	32
8	IL-10	31
9	MPO	31
10	CASP3	31
11	EGF	30
12	IL-4	29
13	VCAM1	28
14	ICAM1	28
15	NOS3	27
16	IFNG	27
17	NOS2	26
18	SELE	26
19	PPARG	26
20	CRP	26
21	CXCL10	25
22	IL-2	25
23	SERPINE1	25
24	SPP1	22
25	CD40LG	22
26	IL-1A	21
27	STAT1	21
28	CXCL2	20
29	NFE2L2	17
30	THBD	16
31	SOD1	15
32	PTGS1	14
33	PON1	11
34	GJA1	10
35	CYP1A1	10
36	CYP3A4	9
37	RXRA	7
38	NR1I2	7
39	CYP1B1	7
40	ODC1	6
41	XDH	5
42	AKR1C1	4
43	ERBB3	3
44	LTA4H	2
45	BIRC5	1

effects. Quercetin can exert immunomodulatory effects by inhibiting lymphocyte activation and proliferation [8]. Kaempferol has potent anti-inflammatory properties and has been found to inhibit inflammation-associated signaling pathways and suppress the release of inflammation-related factors, such as mitogen-activated protein kinases (MAPK), protein kinase C (PKC), phosphoinositide 3 kinase C (PKC), phosphoinositide 3-kinases (PI3K), and Janus kinase (JAK)/signal transducer and activator of transcription (STAT), thus exerting an anti-inflammatory effect [12]. In addition, kaempferol significantly inhibits the early activation of T lymphocytes and suppresses the proliferation of ConA-stimulated T cells, which have immunomodulatory effects [13]. β-sitosterol has biological activities such as anti-inflammatory, antioxidant, antitumor, antibacterial, antidepressant, and antihair loss [14]. Liao et al. [15] found that β-sitosterol can inhibit the activation of NLRP3, the production of CAS1, and the activation of the MAPK signaling pathway, leading to a significant decrease in cellular TNF-α, IL-1β, IL-6, and IL-8. In terms of immunomodulation, Alappatl and Valerio [16] showed that the combination of β-sitosterol and vitamin D3 can enhance the immune effects of macrophages. As an antidepressant, β-sitosterol can reduce the symptoms of depression by increasing norepinephrine, 5-serotonin, and its metabolite 5-hydroxyindole acetic acid in the brains of mice [17]. Formononetin can improve intrinsic immune function, inhibit apoptosis, and increase the conversion rate of splenocytes in mice [18]. For paeoniflorin, the main active ingredient of Shaoyao, experiments in various animal models have found that it may improve depression-like behavior and cellular damage caused by neurotoxicity associated with depression through mechanisms such as increasing monoamine neurotransmitters, improving hypothalamic-pituitary-adrenal axis activation, and attenuating neuroinflammatory factor damage and antiapoptotic cell death [19]. All the studies mentioned previously suggest that the main active components of SGD have anti-inflammatory, immunomodulatory, and anti-anxiety-depression effects, and all of these play an important role in the treatment of AA, reflecting its multicomponent synergistic effects.

Based on the results of the PPI network diagram analysis, it is evident that targets such as IL-6, PTGS2, TNF, VEGFA, CCL2, IL-1B, CXCL8, CASP3, MPO, and IL-10 are potential key targets of SGD in the treatment of AA. Proinflammatory factors such as IL-6 and IL-1B are essential for the differentiation of Th1 and Th17, which in turn affect the development of AA by influencing Th1 and Th17 cells involved in the development of AA [20]. Prostaglandin endoperoxide synthase 2 (PTGS2), also known as cyclooxygenase-2, is a proinflammatory response-inducing enzyme that plays an important role in pain and inflammatory mechanisms. TNF-α is a potent inhibitor of proliferation. TNF-α, IL-1α, and IL-1β were found to inhibit the growth of cultured hair follicles in vitro, leading to the pathogenesis of AA through activation of T cells [21]. Hair follicle immune privilege (HF IP) is an important link in the pathogenesis of AA, and related triggers can cause the release of IFN-γ and TNF-α, leading abnormal expression of MHC class I molecules in hair follicles during the anagen phase and disrupting HF IP, thus leading to the pathogenesis of AA. IL-10 is a Th2 cytokine that inhibits the synthesis of cytokines such as IFN-γ, IL-2, and TNF-α [22]. It was found that increased secretion of IL-10 could inhibit the expression of MHC-I and MHC-II, the action of IFN-γ, and promote the re-establishment of HF IP, which facilitates hair regrowth [23]. CCL2 is a member of the CC subfamily of chemokines, which recruits inflammatory cells to the site of the lesion and induces the synthesis of cytokines such as IL-2 and IL-6, thus exerting inflammatory suppressive and immunomodulatory effects. Elevated levels of Th1-related markers such as CCL2 have been found in patients with long-term AA [24], so it may be involved in the pathogenesis of AA by participating in the imbalance of Th1/Th2 cell subsets.

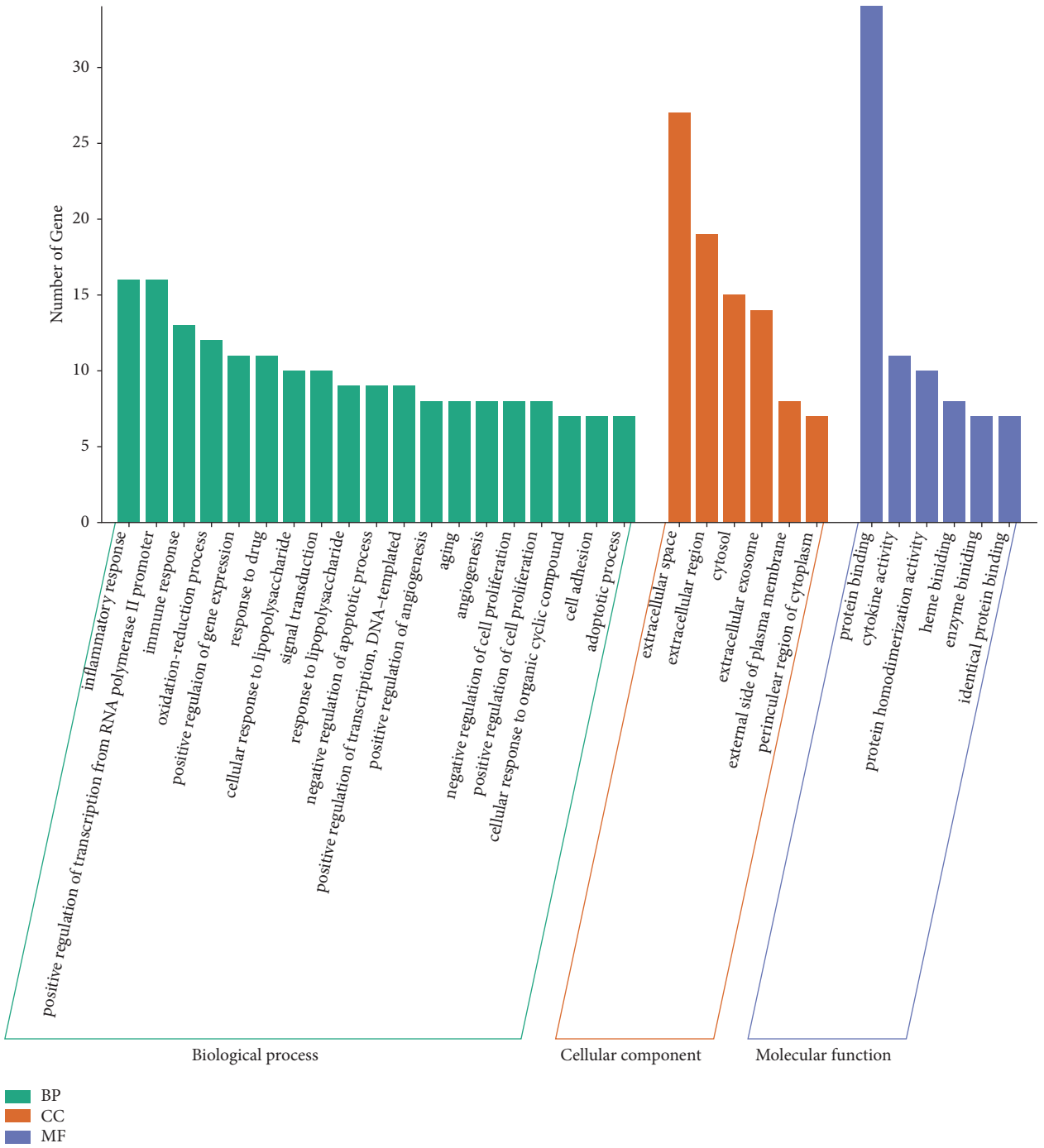

Figure 4: Gene ontology (GO) enrichment biological process map.

The results of the GO analysis showed that the biological processes involved in AA treatment with SGD were mainly inflammatory response, immune response, oxidation-reduction process, etc. Previous studies showed that SGD had anti-inflammatory and immunomodulatory effects, inhibiting the production of prostaglandin E2 (PGE2), nitric oxide (NO), and interleukin-6 (IL-6) [25], as well as regulating the ratio of CD4+CD25+Foxp3+ regulatory T cells [26]. Meanwhile, IL-6 was shown to be involved in AA pathogenesis as a proinflammatory factor; PGE2 inhibited macrophage function, reduced Thl cell proliferation and IL-1 and TNF-α synthesis, increased IL-4 production, and downregulated the effects of type I cytokines in treating AA by modulating immunity and anti-inflammation [27].

In the KEGG enrichment analysis of target genes, the PI3K-AKT signaling pathway, osteoclast differentiation, hepatitis B, and Jak-STAT signaling pathways were enriched to a higher degree and were closely related to AA. It was found that many cytokines involved in the pathogenesis of autoimmune and inflammatory diseases transmit intracellular signals through the JAK/STAT signaling pathway; for example, IL-6 can bind to JAK1 to exert biological effects [28].

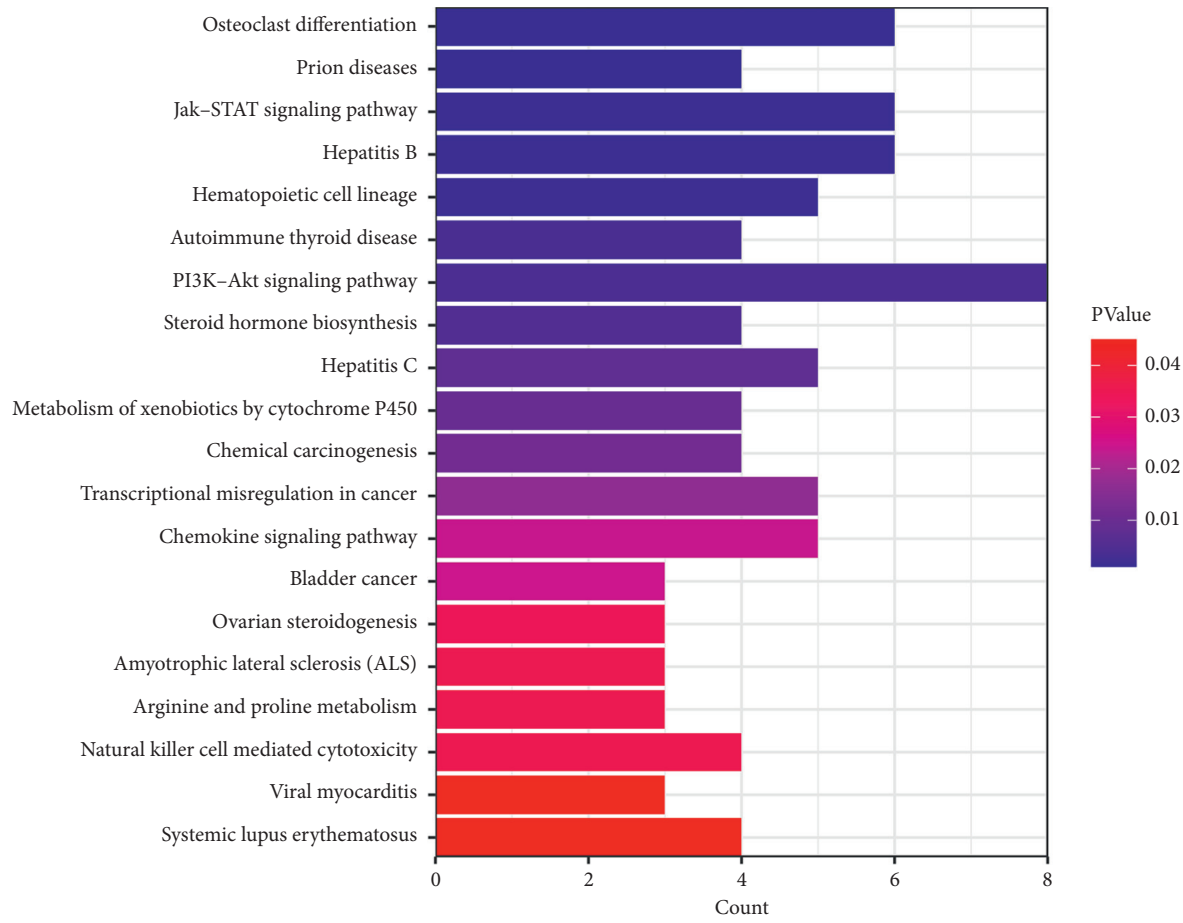

Figure 5: Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment map.

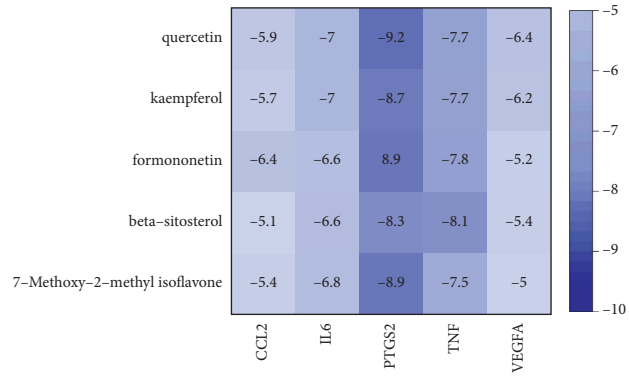

Figure 6: Heat map of core active components and core targets' binding energy.

Meanwhile, biopsies of AA skin lesions showed JAK3 overexpression and mildly elevated JAK1 and JAK2 expression, suggesting an important role for the JAK/STAT pathway in the pathogenesis of AA [29]. The emergence of JAK inhibitors in recent years has been a breakthrough in the treatment of AA. Although JAK inhibitors have not yet been approved by the U.S. Food and Drug Administration for the treatment of AA, several clinical studies have shown that JAK inhibitors such as tofacitinib can effectively treat AA [30–32]. The PI3K-AKT signaling pathway is widely present in cells and is involved in the regulation of cell growth, proliferation, and differentiation [33]. Current studies have focused on its relationship with the development, progression, treatment, and regression of various types of malignancies. Wachstein et al. [34] found that specific inhibitors of PI3K/AKT reduced the immunosuppressive function of HSP-70-treated T-regs cells by increasing the secretion of IFN-γ, TNF-α, IL-10, and TGF-β. Studies of AA patients [35] suggest that Th17/Treg cell imbalance and related cytokines are involved in the development of AA. This leads to the speculation that the PI3K-AKT signaling pathway may play a role in AA pathogenesis through immunomodulatory, anti-inflammatory, and

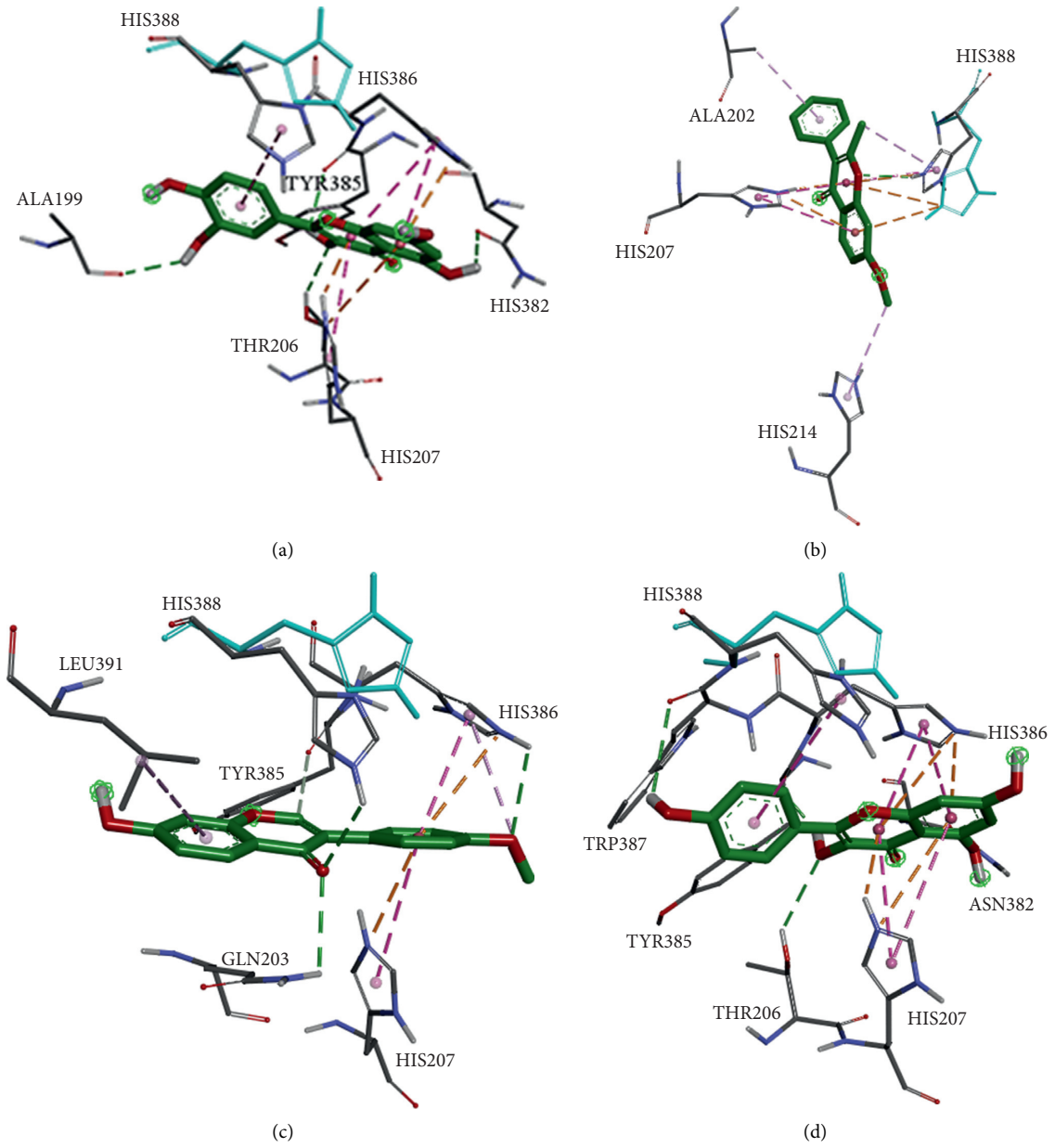

Figure 7: Molecular docking pattern of core active components and core targets. The four docking pattern diagrams representing higher binding energy are (a) PTGS2-quercetin, (b) PTGS2-7-methoxy-2-methyl isoflavone, (c) PTGS2- formononetin, and (d) PTGS2-kaempferol.

antiapoptotic effects. However, the pathways of osteoclast differentiation and hepatitis B, which are at the top of the enrichment index, have not been reported in the literature to be associated with AA pathogenesis.

The molecular docking results showed that the binding energy of the main active components of SGD to the core protein was less than −5 kcal/mol, among which quercetin had the best binding property to PTGS2 (−9.2 kcal/mol), thus verifying the effect of SGD for the treatment of AA at the molecular level.

Through animal experiments, we initially verified the possible mechanism of SGD in the treatment of AA. First, unlike previous studies that reported upregulation of anti-apoptotic protein p-Akt expression in lymph node cells of AA mice [36], this study found that PI3K and AKT protein levels were significantly lower in the AA model group compared with the control group of mice, suggesting a significant correlation between the onset of AA and the PI3K/AKT pathway. In addition, by comparing the PI3K and AKT protein levels between the AA model group and the SGD group, we found that SGD could effectively upregulate the expression of these two proteins; thus it was hypothesized that SGD could affect the secretion of related factors and improve cellular immune function through the PI3K/AKT pathway.

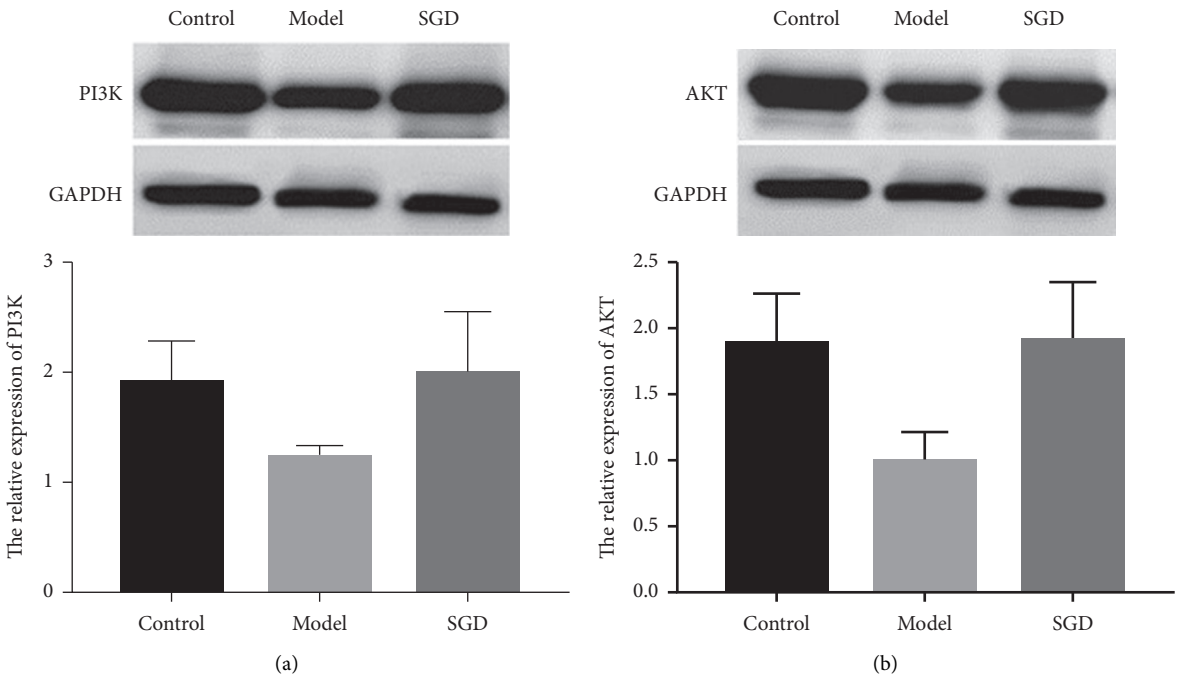

Figure 8: Effects of Shaoyao Gancao decoction (SGD) on PI3K (a) and AKT (b) protein expression.

5. Conclusions

Utilizing network pharmacology's multilevel (drug-component-target-pathway) characteristics, this study determined the features of SGD responsible for drug synergy and the holistic concept of TCM and successfully predicted possible pharmacological mechanisms of SGD for the treatment of AA. Furthermore, the potential key pathway, the PI3K-AKT signaling pathway, was verified by animal experiments, providing a basis for exploring mechanisms of SGD in AA treatment.

In this study, network pharmacology findings suggested that the possible mechanism of SGD in AA treatment is through acting on IL-6, PTGS2, TNF, CCL2, IL-1B, IL-10, and other targets, mainly regulating the PI3K-AKT signaling pathway and Jak-STAT signaling pathway, thus exerting anti-inflammatory, immunomodulatory, and antidepressant functions. However, network pharmacology cannot predict the specific regulation of drugs on the targets, and false-positives may occur, so further experiments are needed to verify the results. Our subsequent animal experimental results showed that SGD could effectively upregulate PI3K and AKT protein expression in AA mice, which strongly supports the prediction of network pharmacology.

However, network pharmacology is based on predicting the main active components of traditional Chinese medicine, as a result of which we cannot analyze products of traditional Chinese medicine after absorption in the body. In addition, the experiment in this study was validated at the protein level, and subsequent further studies can investigate the changes in factors related to the PI3K/AKT pathway affected by SGD at the molecular level to explore the possible mechanism in greater depth.

Data Availability

The data are available from the Universal Protein Database (UniProt, https://www.uniprot.org/), the Drugbank database (https://www.drugbank.ca/), the GeneCards database (https://www.genecards.org; version 4.11.0), the Online Mendelian Inheritance in Man (OMIM) (https://omim.org/), and the Disgenet database (https://www.disgenet.org/).

Conflicts of Interest

The authors report no conflicts of interest.

Authors' Contributions

Shuying Lv proposed the idea, designed this study, carried out the animal experiments, and wrote the manuscript. Lei Wang and Yuhang Duan participated in data analysis. Dan Huang carried out the literature survey. Dingquan Yang improved the manuscript. All authors read and approved the final manuscript.

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Research Article

A Novel Effective Formulation of Bioactive Compounds for Wound Healing: Preparation, *In Vivo* Characterization, and Comparison of Various Postbiotics Cold Creams in a Rat Model

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The wound is a break in the integrity of the skin produced by injury, illness, or operation. Wound healing is an essential dynamic biological/physiological process that occurs in response to tissue damage. The huge health, economic, and social effects of wounds on patients and societies necessitate the research to find novel potential therapeutic agents in order to promote wound healing. Postbiotics, the newest member of the biotics family, are valuable functional bioactive substances produced by probiotics through their metabolic activity, which have several beneficial properties, including immunomodulatory, anti-inflammatory, antimicrobial, and angiogenesis characteristics, resulting in acceleration of wound healing. In the current study, three topical cold cream formulations containing postbiotics obtained from *Lactobacillus fermentum*, *Lactobacillus reuteri,* or *Bacillus subtilis* sp. *natto* probiotic strains were prepared. The effectiveness and wound healing activity of the developed postbiotics cold cream formulations were investigated compared to cold cream without postbiotics and no treatment via wound closure investigation, hydroxyproline content assay, and histological assessment in 25 Sprague Dawley rats divided into five groups. Interestingly, analysis of the results revealed that all three formulations containing postbiotics significantly accelerated the wound healing process. However, in general, the *Bacillus subtilis natto* cold cream manifested a better wound healing property. The pleasing wound healing characteristics of the topical postbiotics cold creams through the *in vivo* experiment suggest that formulations containing postbiotics can be considered as a promising nominee for wound healing approaches.

1. Introduction

A term wound is defined as a cut or break in the integrity of the skin produced by injury, illness, or operation [1–3]. Wounds can happen due to a disease or as a consequence of an accidental or intentional reason [4, 5]. The principal function expressed for the skin is to provide a protective barrier for the body against the surrounding environment [6]. Loss of skin unity provides an appropriate context for various microorganisms to contaminate the wound surface [2, 7]. As intact skin is vital to protect the body against the environment, regenerative mechanisms (healing) need to be initiated and progressed to resolve the existing defect [1, 8].

Cutaneous wound healing is a dynamic complex biological phenomenon that commences following tissue injury. The critical goal of wound healing is inhibition against

infections, restoring skin tissue function and strength [1, 4, 9]. A wound as a tissue injury stimulates a regulated and coordinated response, and the wound healing process is attained through 4 principal precise physiological phases of homeostasis, inflammation, proliferation, and remodeling [1, 2, 4, 10]. In the first phase of the healing cascade that is hemostasis, platelets are activated, and growth factors and cytokines along with other substances are secreted, which in turn stimulates the mechanisms of tissue repairing resulting in inflammation, proliferation, angiogenesis, deposition of extracellular matrix (ECM), and finally tissue remodeling [2, 9, 11, 12].

Many items can interfere with the wound healing process leading to delayed or impaired wound healing, which represents a significant cause of patient morbidity, mortality, and poor cosmetic consequence [2, 4, 13, 14]. Furthermore, wounds' health, economic burden, and social effects are among other substantial problems requiring special consideration [1, 2, 15, 16]. Wounds represent a major worldwide challenge for patients and their families, health institutions, and caregivers [17, 18]. Regarding the economic aspect, the annual cost of wound-related complications in the United States alone is more than 1 billion dollars [4, 13]. Consequently, higher levels of attention and research are needed to investigate the novel potential therapeutic agents that can fulfill one of the primary goals of wound treatments, speeding the process of wound healing [1, 2, 4, 6].

Probiotics are live microorganisms used in the appropriate amounts, which positively impact host health [19–23]. The advantages of probiotic bacteria for wound healing have been proposed extensively via induction of the immune system, decrease of inflammation, angiogenesis, and antimicrobial properties [2, 3, 20, 24–29]. Recently, there has been an increasing interest in the newest member of the biotics family, postbiotics [19, 30]. Postbiotics are functional bioactive substances produced through the metabolic activity of the probiotics during fermentation, which directly and/or indirectly exert beneficial effects on the host cells. Postbiotics can include many components such as metabolites, cell fractions, cell lysates, short-chain fatty acids (SCFAs), extracellular polysaccharides (EPS), teichoic acid, proteins, and peptidoglycan-derived muropeptides as well as pili type structures [19, 23, 31]. Although postbiotics do not have live microorganisms, they show a beneficial impact on host health by similar mechanisms that are features of probiotics, diminishing the possible risks accompanying their intake. Therefore, postbiotics seem to be safe due to the lack of any possible side effects that may be introduced for live microorganisms while preserving similar effectiveness like probiotics [30, 31].

Topical drug delivery is applying a formulation to the skin tissue to treat a cutaneous disorder or improvement of the cutaneous appearance of a disease. Creams, topical semisolid preparations, are among the widely used therapeutic or cosmetic preparations in many skin conditions. They can be utilized to any part of the body and by all age groups easily and efficiently [32].

Postbiotics' topical application can be considered as a novel therapeutic approach in wound research to accelerate the healing process. The present study aimed to examine the impact of postbiotics formulations (prepared in the form of cream) on the wound healing process. Accordingly, three novel formulations of cold cream containing postbiotics were developed to enhance the wound healing process possibly. The efficacy of the prepared postbiotics creams was investigated through *in vivo* assessments, including wound sizes, wound healing percentages, hydroxyproline content assay, and histopathological evaluation in a rat model.

2. Materials and Methods

2.1. Materials. Tryptic soy broth (TSB), De Man, Rogosa & Sharpe (MRS) broth, and yeast extract were purchased from Himedia (India). Soy-peptone was from Quelab (Canada), and magnesium sulfate, potassium hydrogen phosphate, maltose, and glucose were obtained from Merck (Germany). N-chloro tosylamide (chloramine-T), p-dimethyl amino benzaldehyde, pure L-hydroxyproline, perchloric acid, and n-propanol were also purchased from Merck (Germany). All the other reagents, solvents, and salts used for buffer solution preparations were of analytical grade and acquired from Merck (Germany).

2.2. Preparation of Postbiotics. The three utilized probiotic bacteria in the present study were *Bacillus subtilis* sp. *natto* (*B.S.* natto, ATCC 15245), *Lactobacillus reuteri* (*L. reuteri*, ATCC 23272), and *Lactobacillus fermentum* (*L. fermentum*, ATCC 9338) strains.

The *L. reuteri* and *L. fermentum* bacteria were first cultured consuming MRS broth medium at 37°C for 48 h under microaerophilic conditions until the achievement of the stationary phase. *B.S. natto* strain was cultured utilizing soy-peptone (10 g), magnesium sulfate (1 g), potassium hydrogen phosphate (2 g), maltose (20 g), glucose (2 g), and yeast extract (10 g) and incubated at 37°C for 48 h. The pH was adjusted to 7.2. The inoculum of each strain was prepared at an approximate density of 1×10^8 to 1×10^9 CFU/mL. The number of viable bacteria was measured via plate counts utilizing MRS agar, and the bacteria were then harvested using Eppendorf 5810R centrifuge (Germany) at 4000 rpm for 20 min at 4°C. Filtration of the supernatants was performed using a membrane filter of 0.2 μm to omit the remaining bacteria and other remains. The filtered supernatants were lyophilized using an Alpha 1-2LD Plus lyophilizer (Martin Christ, Germany) and stored at −20°C. There was not any evidence of lactobacilli growth in bacterial counting of MRS agar plates. The existence of lipopolysaccharide in L.S. had been investigated via a diagnostic kit from Cambrex Corporation (East Rutherford, NJ).

2.3. Preparation of Cold Cream. The cold cream is based on water in oil (w/o) emulsion. Beeswax (15% w/w) and liquid paraffin (45% w/w) were taken in a beaker and heated up to 70°C using a water bath to prepare the oily phase. As an aqueous phase, borax (1% w/w) was dissolved in water (qs to 100) in another beaker and heated to 75°C. The aqueous phase was slowly added to the oily phase with continuous

stirring at −4°C until a cream consistency was obtained (20 g cream). The prepared cream was packed in a suitable container and stored in a cool and dry place for further use.

2.4. Preparation of Postbiotics Cold Creams. Each lyophilized postbiotic at the amount of one milligram was added to 10 grams of the prepared cold cream and mixed for 5 min at room temperature to develop three different postbiotics cold cream formulations as follows:

Formulation 1: *Lactobacillus fermentum* postbiotic cold cream; Formulation 2: *Lactobacillus reuteri* postbiotic cold cream; and Formulation 3: *Bacillus subtilis* sp. *natto* postbiotic cold cream.

2.5. Animal Study

2.5.1. Study Design. Twenty-five mature Sprague Dawley rats were purchased from the Center of Comparative and Experimental Medicine, Shiraz University of Medical Science, possessing a bodyweight in the range of 200 to 300 g. The rats were housed in a standard cage wherein an ordinary standard rodent's pellet chow diet (RoyanFeed®, Isfahan, Iran) and tap water were available. Before initiation of the experiment, the animals were maintained in the new provided situation at the temperature of 25°C ± 1°C and 12 h/12 h light/dark photo schedule along with relative humidity of 40% ± 10% for 15 days to remove any impacts of stress on them. During the acclimatization period, the rats were examined for their health by a veterinarian. After this period, the rats were equally allotted into five groups (Table 1) containing five rats in each.

2.5.2. Wound Creation. Each rat was then anesthetized using an anesthesia mixture including ketamine and xylazine at the amount of 80 mg/kg and 10 mg/kg, respectively, followed by shaving the needed area to remove hair, cutting a skin layer, and creating an excision wound of 226 mm^2 square with 2 mm depth.

2.5.3. Wound Healing Activity Measurement. Topical postbiotics formulations, prepared at a concentration of 1% W/W postbiotic in cold cream, were administered to the created wounds once a day repeated for two weeks (14 days). The rats receiving nothing and the rats receiving the formulation without any postbiotics were considered as control groups. The diameters of the excised wounds in each rat group were recorded on days of 1 to 14. The following equation calculated wound healing percentage:

$$\text{wound Healing\%} = \left(1 - \frac{\text{WSt}}{\text{WS0}}\right) \times 100, \quad (1)$$

where WS_t and WS_0 are the wound size on a specific day and day 0, respectively.

2.5.4. Hydroxyproline Assay. Hydroxyproline (HP) content was measured as an index of collagenesis according to the method first described by Woessner [33] with further modifications [34]. Briefly, at the end of Day 14, a piece of skin tissue from the healed wound zone was collected and analyzed for the hydroxyproline content. Each skin sample was dried at 60°C to obtain and record a constant dry weight. Skin tissue homogenate at a concentration of 20% w/v was prepared in phosphate-buffered saline (PBS, pH = 7.4), and 500 μl of the mixture was hydrolyzed in 1 ml of 6 N HCl (hydrochloric acid). Following incubation in a sealed tube for 8 h at 120°C, the hydrolysate (25 μl) was mixed with 25 μl of citrate-acetate buffer (pH = 6) to be neutralized and was subjected to 500 μl of chloramines-t-solution (56 Mm). The resulting mixture was allowed to remain at room temperature for 20 min. Afterward, 500 μl of Ehrlich's reagent (15 g of p-dimethyl amino benzaldehyde in 2 : 1 v/v n-propanol/perchloric acid) was added, followed by incubation for 15 min at 65°C. Perchloric acid and Ehrlich's reagent were used as reaction terminators and color developers, respectively. After cooling, the absorbance values of the developed pink color were measured at the wavelength of 550 nm using a spectrophotometer (Ultrospec 2000®UV, Pharmacia Biotech, Sweden). The experiment was performed similarly for all the rat groups receiving different formulations.

2.5.5. Wound Histopathological Evaluation and Scoring. The rats were first anesthetized and then sacrificed by spinal cord injury. Full-thickness wound skin tissues (each wound at dimensions of 3.5 cm × 1.2 cm) were detached. After preparation of the paraffin-embedded sections, they (each with 2 mm thickness) were cut vertically to the width of the skin surface followed by staining with a combination of two histological stains called hematoxylin-eosin [35–38]. The hematoxylin precisely stains cell nuclei, while eosin stains the extracellular matrix and cytoplasmic components. Then, the histological alterations of skin tissue through investigation of different phases of epithelialization, fibrosis, inflammation, and granulation were evaluated for all the samples. To quantify the wound healing process, degrees of epithelialization, fibrosis, inflammation, and granulation were blindly scored by a professional pathologist, as presented in Table 2.

2.6. Statistical Analysis. Data analysis was performed using GraphPad software version 8 (v8.4.0, GraphPad Software Inc., San Diego, CA). Quantitative variables were expressed as mean ± standard deviation (SD). The comparisons were carried out by analysis of variance (ANOVA) with Tukey's comparison post hoc test. Statistical significance was defined as P values less than 0.05 ($P < 0.05$). Scores of histopathological skin changes are presented as median and quartiles, and the Kruskal-Wallis, followed by the Mann–Whitney U test, was employed to analyze the skin tissue histopathological changes.

3. Results

3.1. Wound Healing Activity Measurement. Various rat groups were topically treated with three different postbiotics cold creams (G3, G4, and G5), cold cream without postbiotic

Table 1: Different rat groups treated with various topical postbiotics formulations.

Rat groups	The topical formulation administered to the rat group
Group 1	No treatment (control)
Group 2	Cold cream without postbiotics
Group 3	Formulation 1 (*Lactobacillus fermentum* postbiotic cold cream)
Group 4	Formulation 2 (*Lactobacillus reuteri* postbiotic cold cream)
Group 5	Formulation 3 (*Bacillus subtilis* sp. *natto* postbiotic cold cream)

Table 2: Scores of the changes in histopathological features of the skin.

Score	Changes in histopathological features of skin tissue
–	There is no apparent change
++	Mild changes
++	Moderate changes
+++	Severe changes

(G2), and no treatment (G1) to evaluate their wound healing process as well as compare their abilities to improve the rate of wound healing through measurement of wound sizes and wound healing percentages in different days of treatment. The qualitative trend of wound healing in treated rat groups was demonstrated in Figure 1. As it is clear, the process of wound healing was typically initiated and progressed in all groups. However, the rate of wound healing is more in all three groups treated with postbiotics cold cream formulations (G3, G4, and G5) than in the group with no treatment (G1) and the group treated with cold cream alone (G2). The quantitative wound healing trends of all rat groups obtained from wound size against days of treatment and wound healing percentage against days of treatment were illustrated in Figures 2 and 3, respectively. Moreover, the healing percentages were summarized in Table 3.

The wound size was 22.5 ± 1.00 mm on the first day (Day 0) for each rat in all the groups, equivalent to 0% wound healing percentage. The trend of wound sizes (Figure 2) and wound healing percentages (Figure 3) decreased and increased, respectively, in all rat groups. Wound sizes decreased significantly (Figure 2, $P < 0.0001$), and wound healing percentages increased significantly (Figure 3, $P < 0.0001$) from Day 2 to Day 14 in all five rat groups of G1 to G5. There was not any significant difference ($P > 0.05$) between the group receiving no treatment (G1) and the group receiving cold cream without postbiotics (G2) during almost all days of the treatment period (from Day 0 to Day 14) regarding wound size (Figure 2) and wound healing percentage (Figure 3). From Day 4 to the end of the treatment (Day 14), all the three postbiotics cold creams (G3, G4, and G5) showed smaller wound sizes (Figure 2) and higher wound healing percentages (Figure 3) significantly in comparison with the untreated group (G1) and the group treated with cold cream without postbiotics (G2) ($P < 0.0001$). The administration of cold cream alone did not significantly enhance the wound healing process compared to the treatment with postbiotics cold creams ($P > 0.0001$). The wound sizes were the smallest (Figure 2, $P < 0.001$), and the wound healing percentages were the highest (Figure 3, $P < 0.001$) significantly from Day 4 to Day 14 of treatment in the groups receiving the *L. reuteri* cold cream (G4) and *B.S.* sp. *natto* cold cream (G5) followed by *L. fermentum* cold cream (G3). The wound healing process in two groups receiving *L. reuteri* cold cream (G4) and *B.S.* sp. *natto* cold cream (G5) was completed by Day 14 in which the wound sizes obtained were 0 (Figure 2), and the healing percentages reached 100% (Figure 3). The wound size and wound healing percentage in the group receiving *L. fermentum* cold cream (G3) were measured 2.000 ± 0.100 (Figure 2) and 91.150 ± 1.000 (Figure 3 and Table 3) by Day 14. The healing process in the group receiving no treatment (G1) and the group receiving cold cream without postbiotics (G2) was not completed at the end of the experiment (Day 14) (Figures 2 and 3 and Table 3) with the respective wound sizes of 5.800 ± 0.250 and 5.600 ± 0.210 and the respective wound healing percentages of 74.336 ± 4.000 and 75.221 ± 3.030.

3.2. Hydroxyproline Assay. Hydroxyproline is a basic component of collagen, and its measurement can be used as a biomarker for collagenesis in skin tissue [39]. The calculated hydroxyproline content related to various rat groups receiving various postbiotic cold creams, cold cream without postbiotic, and no treatment is shown in Figure 4. The hydroxyproline content in wound tissue of the groups treated with each of three postbiotics cold creams (G3 to G5) significantly increased ($P < 0.0001$) in comparison with the wound tissues of the groups receiving no treatment (G1) or cold cream without postbiotic (G2) (Figure 4). Interestingly, all three postbiotics cold creams showed a significantly higher amount of hydroxyproline than the untreated and the cold cream alone groups ($P < 0.0001$). Among three postbiotics cold creams, the produced hydroxyproline was the highest for the group treated with *B.S. natto* cold cream (G5; 296 ± 4, $P < 0.0001$). Moreover, the result of comparing the other two postbiotics formulations demonstrated that the hydroxyproline content of the group treated with *L. fermentum* cold cream (G3) was significantly higher (252 ± 4, $P < 0.05$) than the group treated with *L. reuteri* cold cream (G4) (236 ± 3). The hydroxyproline content in the untreated group (G1) and the group treated with cold cream alone (G2) was the least of all ($P < 0.0001$) without any significant difference between them ($P > 0.05$).

3.3. Wound Histopathological Evaluation. The histological assessment at the end of the treatment (Day 14) with various postbiotics cold creams, cold cream without postbiotic, and no treatment was performed to determine the histological characterizations of wound healing. Skin tissue histopathological changes are demonstrated in Figure 5, and the

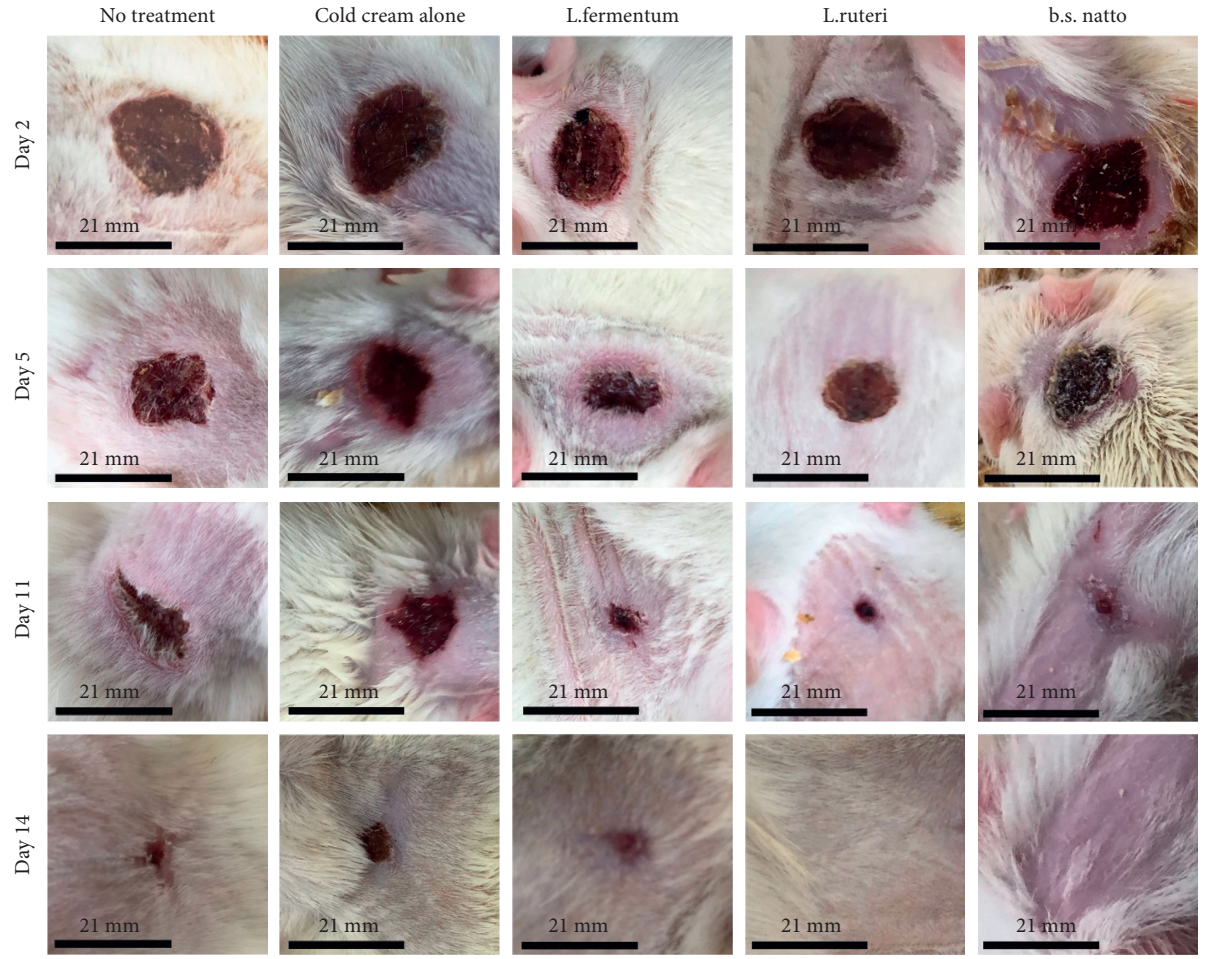

Figure 1: Qualitative trend of wound healing process of different rat groups receiving no treatment (control), cold cream alone, *Lactobacillus fermentum* postbiotic cold cream, *Lactobacillus Reuteri* postbiotic cold cream, or *Bacillus Subtilis* sp. *natto* postbiotic cold cream. The photos were taken at Day 2, Day 5, Day 11, and Day 14 of treatment.

scores by an expert given to different processes of epithelization, inflammation, granulation, and fibrosis are presented in Table 4.

As illustrated in Figure 5 and expressed in Table 4, the epithelialization process was complete in the groups of rats receiving *L. reuteri* cold cream (G4) and *B.S. natto* cold cream (G5), while the epithelization in groups receiving *L. fermentum* cold cream (G3), cold cream without postbiotics (G2), and no treatment (G1) was incomplete (super), incomplete (deep), and incomplete, respectively.

According to the inflammation process (Figure 5 and Table 4), the groups treated with cold cream without postbiotics (G2) and the group receiving no treatment (G1) as well as *L. fermentum* cold cream (G3) possessed the highest degree of inflammation (defined as moderate inflammation) in comparison to the other groups. The skin inflammation differed among the groups treated with various postbiotics cold creams (G3, G4, and G5). The group treated with *B.S. natto* cold cream (G5) showed a mild degree of inflammation. However, treatment of the rats with *L. reuteri* cold cream (G4) resulted in no inflammation.

The degree of granulation (Figure 5 and Table 4) was moderate for the groups treated with cold cream without postbiotics (G2) and the group receiving no treatment (G1), which was the highest among the studied groups, followed by the group receiving *L. fermentum* cold cream (G3) which was defined as mild granulation. The groups were treated with *L. reuteri* cold cream (G4), and *B.S. natto* cold cream (G5) did not demonstrate any histological alterations regarding granulation.

Although the fibrosis process was detected in all the studied groups, it is significantly higher in the group receiving no treatment (defined as moderate fibrosis). Mild fibrosis was observed in the other four groups (G2, G3, G4, and G5) (Figure 5 and Table 4).

4. Discussion

The wound is a break or cut in the skin, and wound healing is a dynamic, complex physiological reaction that initiates following skin injuries. The enormous health, social, and economic challenges associated with wounds lead to finding novel therapeutic agents that can enhance the wound healing process. Postbiotics, functional bioactive substances produced by probiotics, have recently attracted a great deal of interest due to many beneficial characteristics. In the present

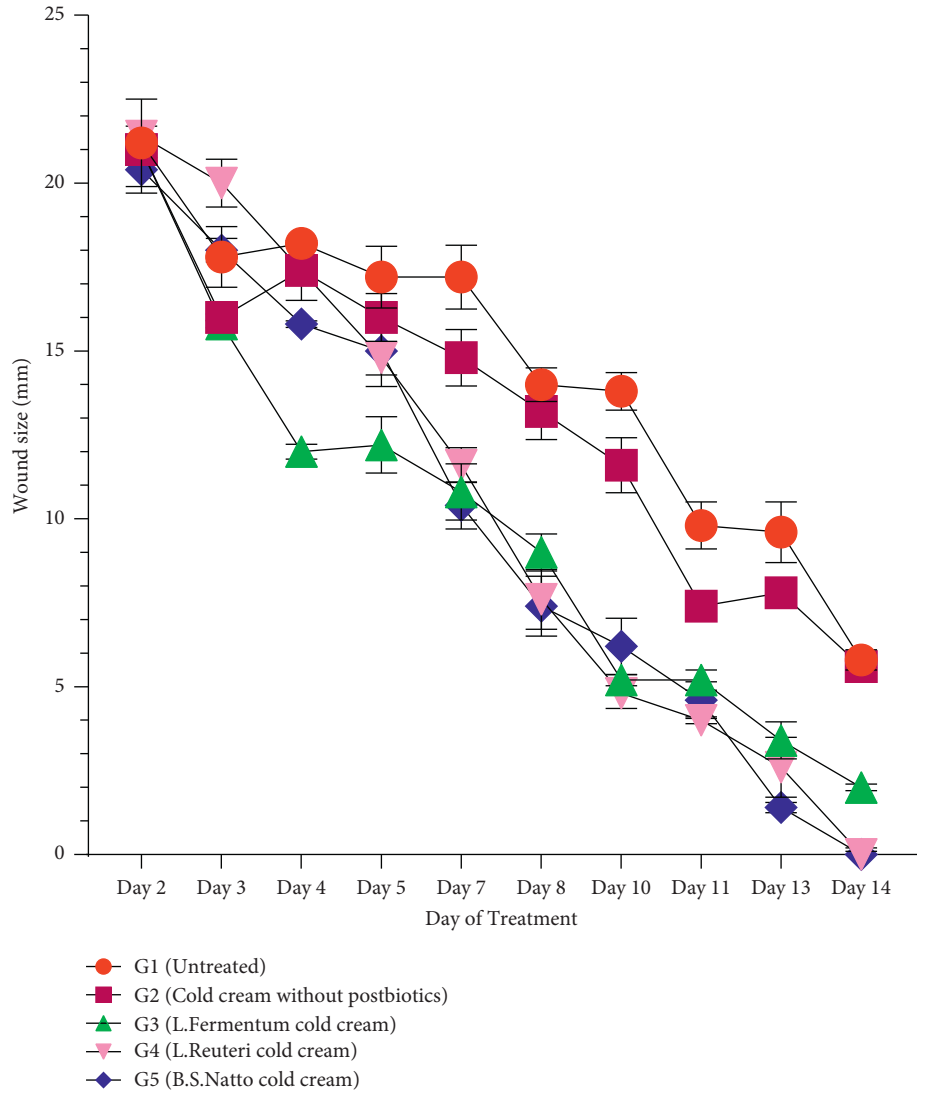

Figure 2: Wound sizes (mm) following treatment of different rat groups (G1 to G5) with various formulations. Data are expressed as mean ± SD (standard deviation).

study, three new formulations (postbiotics cold creams) were developed to study their efficacy in the wound healing process via *in vivo* experiment in a rat model.

According to the wound sizes (Figure 2) and wound healing percentages (Figure 3 and Table 3), at the end of a 14-day treatment, wound sizes decreased (Figure 2), and wound healing % increased significantly (Figure 3 and Table 3) by increasing day of treatment in all five groups (G1 to G5) but in different rates which demonstrates that the process of wound healing was initiated and progressed regardless of the healing rate. However, wound sizes were smaller and wound healing percentages were higher from Day 4 to Day 14 in all three groups which received postbiotics cold creams (G3, G4, and G5) than the group with no treatment (G1) and the group treated with cold cream (G2), which indicates the higher rates of wound healing in all the three postbiotics groups than the controls. Administration of cold cream without postbiotics (G2) did not increase the wound healing process significantly (which was the same as no treatment group) (Figures 2 and 3 and Table 3) in comparison to the treatment with postbiotics cold creams (G3 to G5), which confirms that the higher activity of wound healing factors was associated with the postbiotics substances in these formulations. The smallest wound sizes (Figure 2) and the highest wound healing percentages (Figure 3) in Day 4 to Day 14 of treatment were allocated to the groups receiving the *L. reuteri* cold cream (G4) and *B.S.* sp. *natto* cold cream (G5), followed by *L. fermentum* cold cream (G3). Moreover, the wound healing process in groups receiving *L. reuteri* cold cream (G4) and *B.S.* sp. *natto* cold cream (G5) was completed by Day 14 (Figures 2 and 3 and Table 3). The wound healing process in the group receiving *L. fermentum* cold cream (G3) reached near completion (90%) by Day 14 (Figures 2 and 3 and Table 3), while the healing process in the group receiving no treatment (G1) and the group receiving cold cream without postbiotics (G2) was not completed even at the end of the treatment (Day 14) (Figures 2 and 3 and Table 3). It reveals that the *L. reuteri* and *B.S.* sp. *natto* postbiotics could accelerate the healing

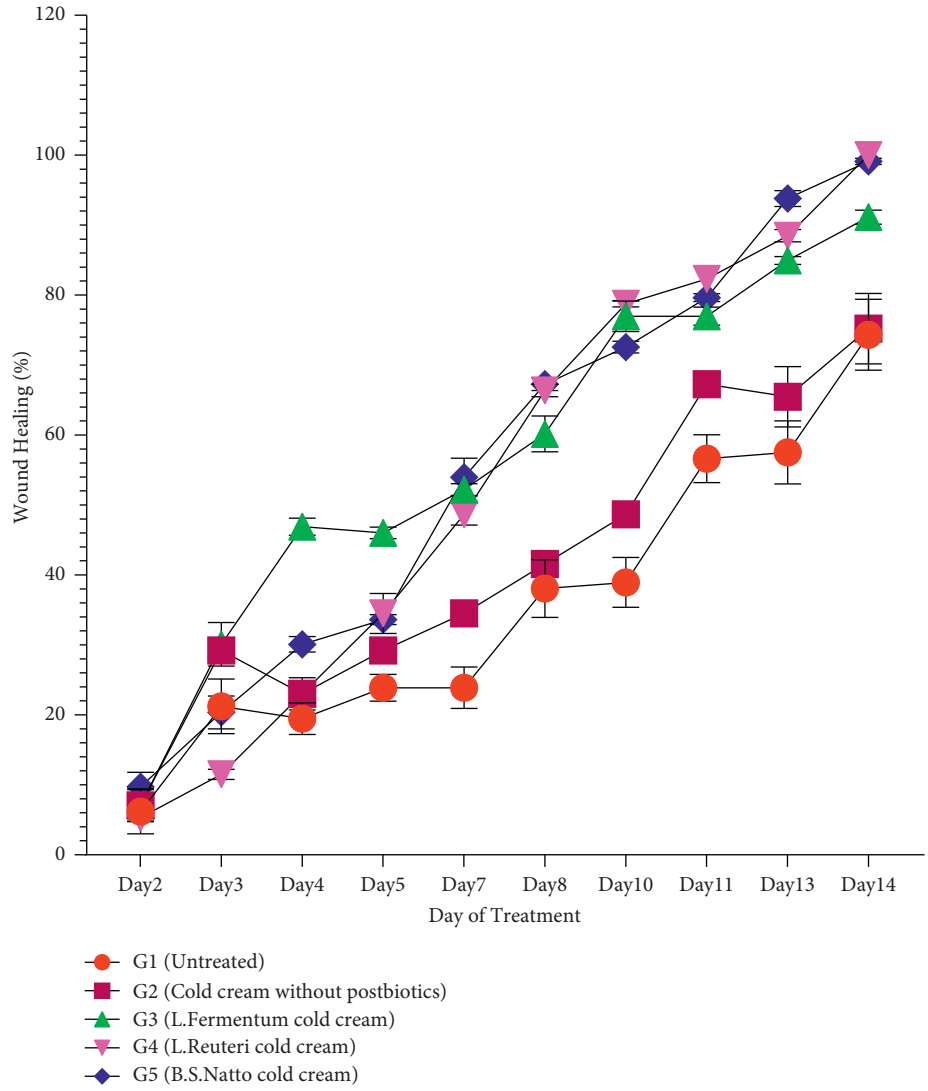

FIGURE 3: Wound healing percentages following treatment of different rat groups (G1 to G5) with various formulations. Data are expressed as mean ± SD (standard deviation).

process more than the others regarding wound sizes and wound healing percentages, followed by *L. fermentum*. Previous studies demonstrated that the administration of *L. reuteri* and could significantly enhance the wound healing process through exerting various properties such as anti-inflammatory and antipathogenic ones [40–42]. In consistent with the findings of this study, some studies showed the postbiotics as probiotic metabolites can effectively boost the wound healing process. However, the amount of their efficacy may depend on bacterial strain, amount of administration, and other factors.

Collagen is the major structural component of granulation tissue, strengthening the extracellular matrix. It was demonstrated that a collagen sponge improves the formation of the connective tissue and increases the vascularization related to the repaired tissue. As a result, collagen is effectively able to increase the healing process [43, 44]. The hydroxyproline amount of wound tissues is assessed to estimate the amount of produced collagen in the wound healing process. Because the amino acid proline is a crucial component of the collagen fiber, hydroxyproline can be considered an index of collagenesis. Accordingly, the higher amount of hydroxyproline positively indicates the higher progression of wound healing [17, 39, 45]. Therefore, the higher amount of hydroxyproline in postbiotics formulations (Figure 4) suggests that all the 3 postbiotics cold creams, regardless of their bacterial source, can enhance the wound healing process. Among three postbiotics cold creams, the produced hydroxyproline was the highest for the group treated with *B.S. natto* cold cream (G5) (Figure 4), suggesting its highest wound healing as a consequence of the highest collagenesis. Previous studies also have demonstrated that their studied formulations showed higher collagen amount and therefore could promote the wound healing process [46–49].

The histological evaluation can reveal important facts about the process of wound healing. Wound healing consists of several organized mechanisms and is affected by different

Table 3: Wound healing percentages in various days of a 14-day treatment of five rat groups with three postbiotic cold cream formulations, cold cream formulation alone, and no treatment (control).

Rat groups Day	No treatment (control)	Cold cream alone	L. *fermentum* cold cream	L. *reuteri* cold cream	*B. S. natto* cold cream
2	6.195 ± 0.330	7.800 ± 0.350	7.080 ± 0.600	5.310 ± 0.300	9.734 ± 0.400
3	21.239 ± 1.000	29.203 ± 1.000	30.088 ± 0.900	11.504 ± 0.710	20.354 ± 0.350
4	19.469 ± 0.890	23.009 ± 0.890	46.902 ± 1.210	23.009 ± 1.300	30.088 ± 1.100
5	23.894 ± 0.710	29.203 ± 0.710	46.017 ± 0.840	34.513 ± 2.000	33.628 ± 0.710
7	24.1 ± 0.840	34.513 ± 0.840	52.212 ± 0.840	48.672 ± 1.520	53.982 ± 2.700
8	38.053 ± 0.800	41.593 ± 0.800	60.177 ± 2.550	66.372 ± 0.890	67.256 ± 0.890
10	38.938 ± 1.500	48.672 ± 1.820	76.991 ± 2.170	78.761 ± 0.450	72.566 ± 0.840
11	56.637 ± 1.340	67.257 ± 1.340	76.995 ± 1.300	82.301 ± 0.100	79.646 ± 0.550
13	57.522 ± 3.000	65.487 ± 3.320	84.956 ± 0.550	88.495 ± 0.890	93.805 ± 1.150
14	74.336 ± 4.000	75.221 ± 3.030	91.150 ± 1.000	100.00 ± 0.010	100.00 ± 0.850

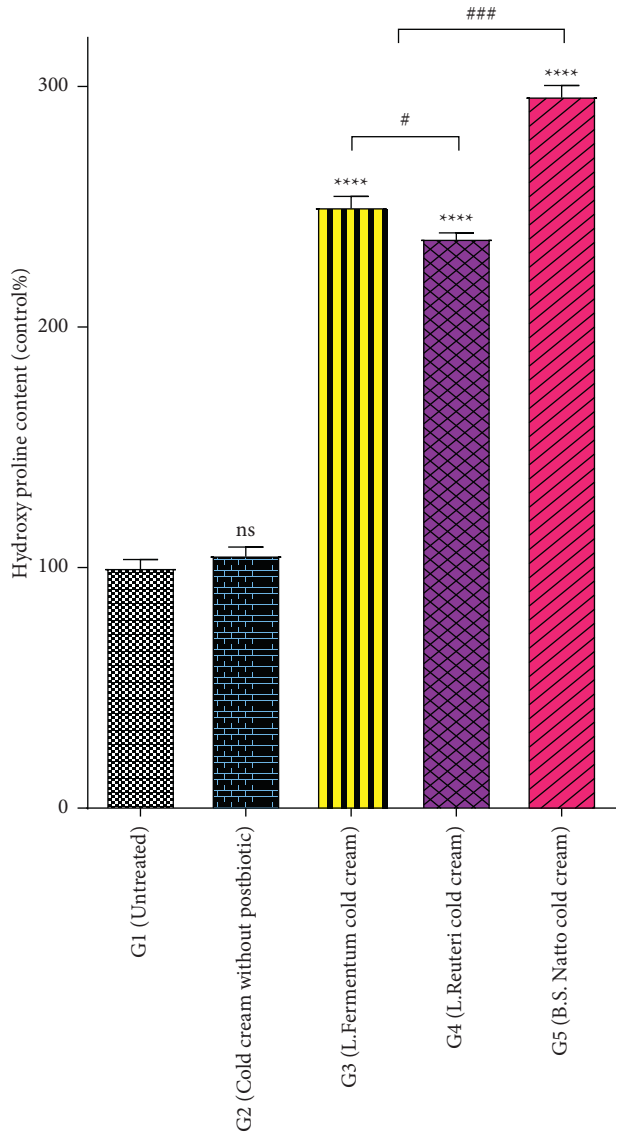

Figure 4: Determination of wound hydroxyproline production as an indicator of collagen levels measured at the end of Day 14 following administration of three different postbiotics cold creams (*Lactobacillus fermentum* postbiotic cold cream, *Lactobacillus Reuteri* postbiotic cold cream, or *Bacillus subtilis* sp. *natto* postbiotic cold cream), cold cream without postbiotics, and no treatment (control) on the excised wounds in rat model. Result values are expressed as means ± standard deviation. $^{****}P$ value < 0.0001, $^{***}P$ value < 0.001, and $^{*}P$ value < 0.05; ns denotes not significant compared to untreated wound tissue. $^{\#\#\#}P$ value < 0.001 and $^{\#}P$ value < 0.05.

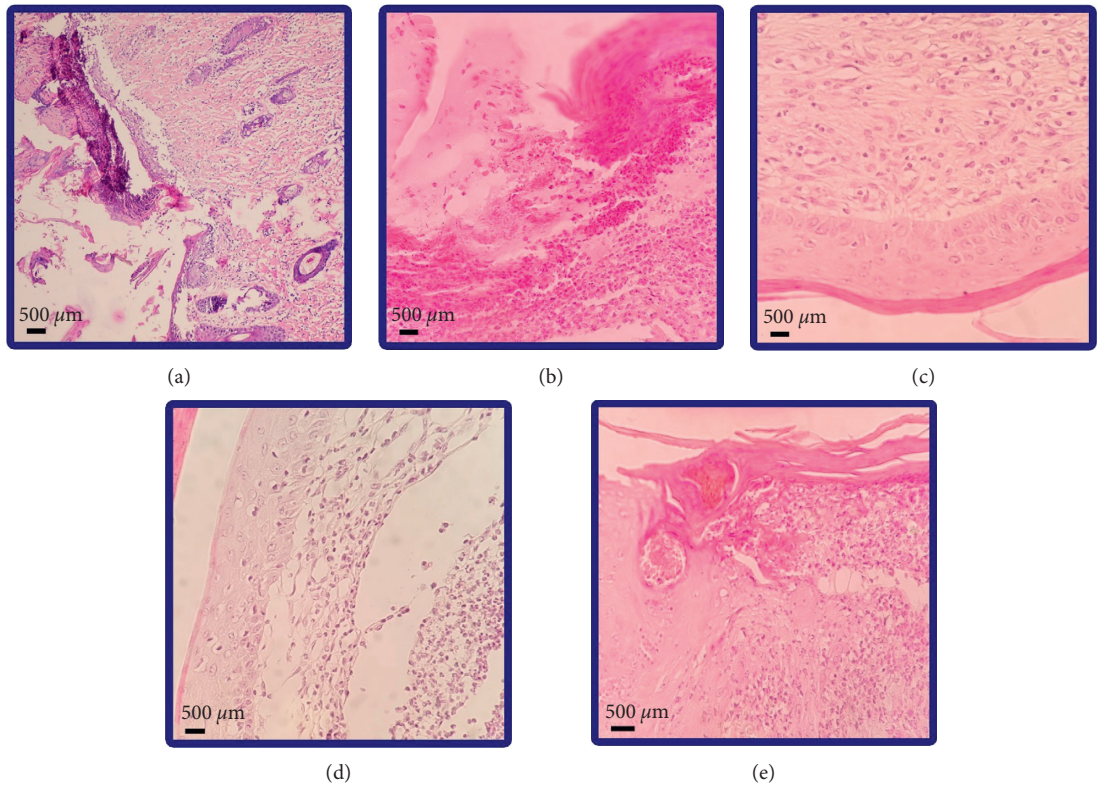

Figure 5: Skin tissue histopathological assessment of different rat groups at the end of Day 14 through hematoxylin-eosin staining. (a) Rat group receiving no treatment (control). (b) Rat group receiving cold cream alone. (c) Rat group receiving *Lactobacillus fermentum* postbiotic cold cream. (d) Rat group receiving *Lactobacillus Reuteri* postbiotic cold cream. (e) Rat group receiving *Bacillus subtilis* sp. *natto* postbiotic cold cream.

Table 4: Skin histopathological changes at the end of Day 14 in different rat groups following 14-day administration of various formulations on wound tissues.

	No treatment (control)	Cold cream	*L. fermentum* cold cream	*L. reuteri* cold cream	*B.S. natto* cold cream
Epithelization	Incomplete	Incomplete (deep)	Incomplete (super)	Complete	Complete
Inflammation	++	++	++	−*	+*
Granulation	++	++	+*	−*	−*
Fibrosis	++	+*	+*	+*	+*

– means no significant change ($P > 0.05$); + and ++ mean mild ($P < 0.05$) and moderate ($P < 0.001$) histopathological significant changes, respectively. *Significant histopathological changes in comparison to the no-treatment group (control) ($P < 0.05$).

factors. Epithelialization, a major component of wound healing, happens in the proliferative phase and is used as a defining parameter for healing success. In the absence of reepithelialization, a wound is not able to be healed [50]. Epithelialization is a process in which epithelial cells migrate upwards and renovate the wounded zone. Skin stem cells located in the epidermis contribute to the reepithelialization when the skin is injured. The epithelialization process is activated by the inflammatory signal. After that, the keratinocyte migrates and differentiates to close the skin defect [51]. Epithelialization was complete in the rat groups receiving *L. reuteri* cold cream (G4) and *B.S. natto* cold cream (G5), while the epithelization observed in other groups (G1, G2, and G3) was incomplete, demonstrating that *L. reuteri* and *B.S. natto* resulted in probable higher wound healing as the accelerated epithelization means promoted wound healing. Previous studies have also shown that accelerated epithelization led to boosting the wound healing process [25, 42, 47, 48]. At the beginning of injury, the inflammation is activated to stimulate the wound healing process. In the inflammation phase, the mediators contribute to the infiltration of immune cells into the inflammation site. However, the apoptosis of the immune cells and clearance of the apoptotic cells by macrophages results in the end of inflammation, and by a decrease of the inflammation, the wound healing process initiates and progresses [52]. According to the inflammation process, the groups treated with cold cream without postbiotics (G2) and the groups receiving no treatment (G1) and *L. fermentum* cold cream (G3) had the highest degree of inflammation among all five groups (Figure 5 and Table 3) which propose that these three groups possessed lower wound healing than *L. reuteri* (G4)

and *B.S. natto* (G5). Besides, the inflammation was the weakest for *L. reuteri* (G4) and *B.S. natto* (G5) postbiotics (Figure 5 and Table 3) with the observation of no inflammation and mild inflammation, respectively, demonstrating better wound healing in these two postbiotics groups. Previous studies have shown similar results regarding epithelization in the wound healing process [25, 42, 46, 47, 53]. Regarding granulation, groups treated with *L. reuteri* cold cream (G4) and *B.S. natto* cold cream (G5) did not show any histological changes (Figure 5 and Table 3), and the group receiving *L. fermentum* cold cream (G3) showed mild granulation (Figure 5 and Table 4), while observed granulation for the groups treated with cold cream without postbiotics (G2) and the group receiving no treatment (G1) was moderate. The results suggest that the healing process is better in *L. reuteri* (G4) and *B.S. natto* postbiotics followed by *L. fermentum*. The fibrosis process obtained was mild in all groups (G2 to G5) except for the no-treatment group (G1), which resulted in moderate fibrosis (Figure 5 and Table 4).

In the present study, the conventional cream, cold cream, was used, which itself did not show any efficacy towards the promotion of wound healing. Consequently, the positive effect of postbiotics cold creams on wound healing was related to the nature of postbiotics. The efficacy of postbiotics in wound healing may be due to the metabolites [30]. Postbiotics include many substances such as cell fractions, cell lysates, short-chain fatty acids (SCFAs), extracellular polysaccharides (EPS), teichoic acid, and proteins [19]. These secreted metabolites can stimulate proteoglycans deposition, angiogenesis, reduction of inflammation through reduction of the expression of proinflammatory cytokines, and secretion of growth factors like EGF [25, 26, 40, 54–59]. All these effects can be responsible for the good efficacy of postbiotics in the improvement of wound healing. In the past, researchers thought that probiotics were only advantageous in gastrointestinal diseases [60]. However, extensive research leads to understanding their importance in daily life and many disorders [24, 27, 61]. There are some studies relating to the effects of probiotics on wound healing [40, 42, 62, 63], but there are few studies evaluating postbiotics, particularly for wound healing because its recognition does not go far in the past, and it is regarded as a new member of biotics family. Various novel formulations such as hydrogels [46, 47, 53, 64], chitosan nanogels [65], microspheres [64, 66], nanoparticles [64, 67–69], liposomes [64], asymmetric membranes [70], and a lot more have been studied in wound healing. Interestingly, it might be possible to use these novel formulations in combination with postbiotics biocompounds in order to enjoy the probable synergic efficacy in the enhancement of wound healing. Moreover, pH of the wounds is acidic. pH of the postbiotics is also acidic, which can be compatible with wound situations. Therefore, this matter can open new frontiers for developing smart or targeted formulations of postbiotics such as pH-sensitive ones in wound healing. Accordingly, this novel postbiotics formulation may open a new horizon for the treatment of wound healing in the future.

5. Conclusion

The results revealed that wound treatment with formulations of postbiotics cold creams in a rat model accelerated a wound healing rate in comparison to no-treatment and cold cream without postbiotics-treated rat groups. According to the wound sizes and wound healing percentages, *B.S. natto* and *L. reuteri* were the best. Regarding hydroxyproline content, *B.S. natto* produced the highest amount of hydroxyproline, and histological characterization manifested the best wound healing for *L. reuteri* and *B.S. natto*. Generally, the results propose that the prepared novel postbiotics formulation can be considered a supporting wound healing therapy.

Data Availability

All the data generated or analyzed during this study are included in this article.

Ethical Approval

The animal management and welfare accepted guidelines provided by the Helsinki University (Finland) were applied to conduct the study and treat the rats in the experiment. All the experimental protocols and procedures were accomplished according to the regulations of the Animal Ethics Committee approved by Shiraz University of Medical Sciences under code number I.R.SUMS.REC.1399.758.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Ahmad Gholami, Yousef Ashoori, and Seyedeh Narjes Abootalebi designed the study. Data acquisition was performed by Yousef Ashoori, Reza Heidari, Navid Omidifar, and Milad Mohkam. Data analysis was performed by Nasim Golkar, Reza Heidari, and Navid Omidifar. The manuscript was written by Nasim Golkar and edited by Ahmad Gholami. Graphical abstract was drawn by Nasim Golkar. All the authors approved the final manuscript.

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Review Article

Efficacy and Safety of Oral Herbal Drugs Used as Adjunctive Therapy for Melasma: A Systematic Review and Meta-Analysis of Randomised Controlled Trials

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Background. Melasma is an acquired disorder of facial pigmentation. Its etiology is multifactorial; thus, the management is usually challenging. As a complementary therapy, herbal drugs are often used in the management of melasma. This work was aimed to investigate the efficacy and safety of herbal drugs on melasma in female patients. *Methods*. This study followed Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A comprehensive search was conducted, and all randomised controlled trials (RCTs) on the use of oral herbal drugs as complementary therapy for melasma in female patients were included. A meta-analysis was conducted according to the guidelines of the Cochrane Collaboration using Review Manager 5.4. *Results*. Ten eligible trials, with 1015 female melasma patients, were included. All of the included RCTs had some concerns for risk of bias for different reasons, especially for that most of included trials were unblinded. Pooled data suggested phytotherapy plus routine therapy had significantly better efficacy on melasma than routine therapy, in terms of response rate (OR: 4.49, 95% CI: 3.25 to 6.20, $p < 0.00001$), reduction of skin lesion score (SMD: −0.56, 95% CI: −0.79 to −0.33, $p < 0.00001$), and improvement of serum E2 levels (SMD: −1.58, 95% CI: −2.62 to −0.55, p 0.003). In addition, there was no significant difference in the incidence of AEs between phytotherapy plus routine therapy and routine therapy (OR: 0.92, 95% CI: 0.53 to 1.58; p 0.76). Overall, herbal drugs used as an adjunct to routine therapy significantly enhanced the efficacy for the treatment of melasma but with a comparable safety profile. *Conclusion*. These findings have implications for recommending herbal drugs as a viable complementary treatment option for melasma.

1. Introduction

Melasma is an acquired disorder of facial pigmentation characterized by irregular tan or brown macules on the forehead, cheeks, and upper lip. Epidemiologic studies have estimated the prevalence of melasma in different populations, and it varies according to skin types, ethnic composition, and levels of UV exposure [1–4]. It is estimated that the prevalence of melasma in the general population is 1%, while in the high-risk population it is 9–50% [1–4]. The melasma-prone population includes East Asians (Chinese, Korean, and Japanese), Indians, Pakistanis, and Middle Easterners [5]. Although the exact pathogenesis of melasma has not yet been elucidated, hormone secretion, genetic factors, and chronic ultraviolet (UV) exposure are reported to play important roles in its occurrence [6–8]. Other studies also suggest that skin inflammation is involved in the pathogenesis of melasma [5, 9]. Melanin synthesis is a tyrosinase-dependent process consisting of tyrosine hydrolysis to L-DOPA, DOPA oxidation to quinine, and quinine oxidation to melanin [6–8]. Tyrosinase-related proteins (TRP) include tyrosinase, TRP-1 and TRP-2. Microphthalmia-associated transcription factor (MITF) plays a fundamental role in the transcriptional regulation of these genes. A

thorough understanding of the pathogenesis of melasma is crucial to the appropriate management of melasma.

Since the etiology of melasma is multifactorial, the management of this condition is usually challenging. Currently, the gold standard treatment for melasma is topical hydroquinone cream and broad-spectrum sunscreens. Hydroquinone is a tyrosinase inhibitor for blocking the conversion of DOPA to melanin. However, hydroquinone has been associated with the development of exogenous ochronosis and mutagenicity [10, 11]. Off-label tranexamic acid has emerged as a potential treatment for melasma since it was suggested to inhibit melanin synthesis by blocking the interaction between melanocytes and keratinocytes [12, 13]. Other commonly used agents include vitamin C and glutathione. Although various treatment modalities have been used, with inconsistent results, the efficacy is often insufficient.

Herbal medicines have been used empirically in topical therapy since ancient times. Both patients and physicians are increasingly welcoming the use of herbal medicines as an adjunct to routine therapy in view of their presumed high tolerance and efficacy. The herbal prescription for oral administration has been reported to function to improve endocrine secretion, tone the kidneys, relieve the depressed liver, and regulate the circulation of blood, which has been increasingly used as an adjunctive treatment of various skin conditions [14, 15]. Recently, randomised controlled trials (RCTs) have been conducted on the effects of using herbal drugs as an adjunctive therapy on the improvement of melasma. However, there is a lack of sufficiently pooled evidence on their efficacy and safety. In this study, we conducted a systematic review and meta-analysis of RCTs to investigate the efficacy and safety of applying herbal drugs as adjunctive therapy in patients with melasma.

2. Materials and Methods

This study was conducted and reported according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [16]. The study protocol was prospectively registered on PROSPERO (CRD42021 283700).

2.1. Search Strategy. Seven databases (PubMed, Embase, the Cochrane Library, Web of Science, Scopus, CNKI, and WANFANG data) were systematically searched from the inception of the databases until October 2021. This search was conducted by two independent reviewers (X. R. Liu and Y. Zou). Studies on melasma were identified with the terms *melanosis* (as a medical subject heading (MeSH) and a free text term), and *melasma, chloasma, melanose$, melanism*, or *freckle* (as free text terms). These were combined using the set operator and with studies identified with the terms: *herbal medicine, medicinal plants, herbal drugs, plant extracts, or phytotherapy* (as MeSH terms and free text terms), and the following terms: *herb$, plant*, botanical, natural product, weed*, algae, fungi, or fungus* (as free text terms). A manual citation check of included articles was also performed to find any additional studies.

2.2. Study Selection. Inclusion criteria were as follows: RCTs describing the efficacy and safety of oral herbal medicines used as an adjunct in the treatment of melasma in females. Participants included in trials should be healthy adults with melasma diagnosed by dermatological examination. We excluded studies that provided no gender information of participants and studies that included participants with pregnancy or breastfeeding. According to the defined criteria, 2 authors (Q. T. Tang and H. J. Yang) independently selected records based on the title and abstract and then performed an eligibility assessment based on the full text. Any discrepancies between investigators were resolved by discussion to reach consensus.

2.3. Study Outcomes. The primary outcome was physician-assessed improvement in melasma reported as a response rate. The secondary outcomes included melasma improvement evaluated through the changes of Melasma Area and Severity Index (MASI) score, skin lesion score, and serum levels of estradiol (E2), as well as AEs occurring as a result of therapy.

2.4. Data Extraction. For each included trial, 2 authors (Q. T. Tang and H. J. Yang) independently extracted information on the first author, publication year, country, study design, characteristics of patients, description of intervention, outcomes, and duration of follow-up. These data were extracted using a standard data extraction form, with any disagreement resolved by discussion to reach a consensus. For missing data, the first author of the report was contacted when possible.

2.5. Risk of Bias Assessment. The risk of bias and methodological quality of included RCTs were assessed using the Cochrane Collaboration's "risk of bias" tool as outlined in the Cochrane Handbook for Systematic Reviews of Interventions [17]. Two investigators (Q. T. Tang and H. J. Yang) independently performed the assessment of each study, with any disagreement resolved by discussion to reach a consensus.

2.6. Data Synthesis and Analysis. Data collected from trials were preprocessed within Microsoft Excel. Meta-analyses were conducted using Review Manager (RevMan) Version 5.4 (Cochrane Collaboration). The outcomes including the response rate and AEs were dichotomous data while the outcomes including the changes of MASI score, skin lesion score, and E2 levels were continuous data. Pooled dichotomous data were expressed as odds ratio (OR) with 95% confidence interval (CI). Pooled continuous data were expressed as standardized mean difference (SMD) with 95% CI. Heterogeneity was assessed by virtually examining the forest plot to detect nonoverlapping CIs using the χ^2 test of heterogeneity (with $p < 0.1$ indicating statistical significance) and the I^2 statistic of inconsistency (with <30%, 30%–60%, and >60%, respectively, representing low, moderate, or high heterogeneity). A p value of <0.05 was considered significant

for the test of overall effect. Funnel plots were used to assess potential small study effects when at least 10 trials were available within a comparison. Meanwhile, publication bias was also assessed by the Egger test, with $p < 0.05$ indicating statistical significance.

3. Results

3.1. Literature Search. The literature search yielded 3528 records in which 2083 records were retained after removing duplicates. After the first screening based on title and abstract, 2065 records were excluded. The full text of 18 studies was reviewed for inclusion. After excluding studies that did not meet inclusion criteria, 10 studies were deemed appropriate and were included for meta-analysis [18–27]. The process for study selection is shown in Figure 1.

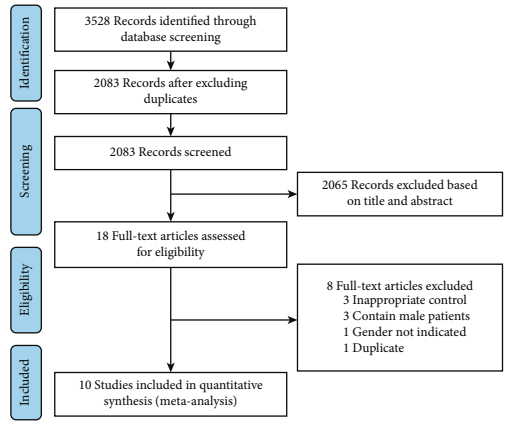

Figure 1: Study flow diagram.

3.2. Study Characteristics. The characteristics of the included studies are summarized in Table 1. Ten trials enrolled 1015 female participants. All the study participants were adults with ages ranging from 19 to 55 years. All trials were completed in China during 2013–2019. Of 10 trials, 9 trials reported the duration of melasma in patients [18, 19, 21–27], while 1 trial did not [20]. Across the included trials, the routine regimens for melasma treatment varied. Topical therapies alone were applied in 2 trials [18, 20], oral administration alone was used in 4 trials [21, 23, 27], and topical therapies combined oral administration were applied in 4 trials [19, 24–26]. Among routine regimens, the predominant topical drug was hydroquinone cream, and the predominant oral drug was glutathione tablet. As an adjunct to routine therapy, the regimens of oral herbal medicines differed among these studies. Of 10 trials, 3 trials used Danggui Shaoyao preparation as an adjuvant [18, 22, 27], 3 trials used Honghua Xiaoyao tablet [19, 21, 26], 1 trial used Bazhen capsule [20], 1 trial used Jingtian Quban capsule [23], 1 trial used Tiaogan Jianpi Quban powder [24], and 1 trial used Tiaochong Xiaoban decoction [25]. Among these drugs, Honghua Xiaoyao tablet was administered three times daily, while other drugs were administered twice-daily. The duration of intervention was 12 weeks for all trials. A total of 5 trials reported the incidence of AEs [19, 21, 26, 27]. Across studies, the outcome measures included physician assessment, melasma area and color score, serum levels of sex hormones and biochemical indexes, MASI score, and dermatology life quality index (DLQI). A descriptive summary of study outcomes and efficacy is shown in Table 2.

3.3. Risk of Bias Assessment. Figure 2 shows the detailed assessment of the risk of bias. All of the included RCTs had some concerns for risk of bias for different reasons. All studies claimed to be randomised and presented similar baseline characteristics between groups, but failed to provide adequate information on allocation concealment. Of the 10 RCTs included, only 1 study claimed to be single-blind [24] while the others were unblinded. Furthermore, all studies did not provide adequate information to determine whether the outcome assessors were blinded or not. One study provided incomplete outcome data due to the absence of serum biochemical data at baseline [23]. Of 10 RCTs included, 5 studies used herbal drugs produced from commercial industries, but did not provide adequate information on funding [19, 21, 23, 26]. In summary, the quality of the included studies varied but was generally poor.

3.4. Meta-Analysis of Efficacy Outcome. A total of 10 studies reported the response rates were included in the meta-analysis for the primary outcome. In total, there were 1004 patients, 504 of whom received routine therapy with phytotherapy as an adjunct, and another 500 received routine therapy alone. As shown in Figure 3, pooling analysis suggested a significant difference in response rates between phytotherapy plus routine therapy and routine therapy, showing a pooled OR of 4.49 (95% CI 3.25 to 6.20, $p < 0.00001$). Subgroup analysis was also conducted according to the types of adjunct drugs. The effect size of Honghua Xiaoyao (OR: 2.89, 95% CI: 1.61 to 5.21, and p 0.004) was smaller than overall effect size, while the effect size of Danggui Shaoyao (OR 8.10, 95% CI 4.58 to 14.33, $p < 0.00001$) was larger than overall effect size. The heterogeneity across studies was found to be low ($\chi^2 = 10.20$, df = 9, $p = 0.33$, $I^2 = 12\%$). The tolerable symmetry of funnel plot (Supplementary Figure S1) and the result of Egger test ($p = 0.003$) suggested low risk of publication bias or other small study effects.

Three studies containing 158 participants in the phytotherapy plus routine therapy group and 155 in the routine therapy group, reported the efficacy through the changes of skin lesion score [25–27]. Pooled analysis suggested that combination therapy had a greater effect on the reduction of skin lesion score than routine therapy (SMD −0.56, 95% CI −0.79 to −0.33, $p < 0.00001$; Figure 4). Likewise, the meta-analysis from 2 studies [22, 24] showed that MASI reduction was greater in the phytotherapy plus routine therapy group compared with the routine therapy group (SMD: −2.20, 95% CI: −3.92 to −0.48, and p 0.01; Supplementary Figure S2). In 3 studies [18, 22, 25], the changes of serum E2 levels were reported between baseline and end-point. Phytotherapy plus routine therapy caused a greater reduction of E2 levels versus routine therapy alone (SMD: −1.58, 95% CI: −2.62 to −0.55, and p 0.003; Figure 5).

Table 1: Characteristics of included studies.

Study	Study design	Sample size	Age (years)	Duration (years)	Interventions		Adverse events	Dropout (reason)
					Experiment group (E)	Control group (C)		
Chen et al. 2016	Randomized, controlled trial	96 (48E, 48C)	19–43 E: 32.6 ± 6.4; C: 31.8 ± 6.9	E: 2.6 ± 1.4; C: 2.8 ± 1.5	Danggui Shaoyao powder, twice-daily + ultrasonic therapy with L-vitamin C, once-weekly for 12 weeks	Ultrasonic therapy with L-vitamin C, once-weekly for 12 weeks	NR	0
Ji et al. 2013	Randomized, controlled trial	100 (50E, 50C)	28–50 40.33 ± 6.35	2–22 13.2 ± 2.1	Honghua Xiaoyao tablet, three times daily + vitamin C, three times daily, and hydroquinone cream, twice-daily for 12 weeks	Vitamin C, three times daily, and hydroquinone cream, twice-daily for 12 weeks	E: 9 × gastrointestinal reaction, 6 × burning and erythema; C: 9 × burning and erythema	0
Jiang et al. 2019	Randomized, controlled trial	90 (45E, 45C)	29–51 E: 45.52 ± 4.39; C: 45.63 ± 4.42	NR	Ba Zhen capsule, twice-daily + hydroquinone cream, twice-daily for 12 weeks	Topical hydroquinone cream, twice-daily for 12 weeks	E: 1 × gastrointestinal reaction; C: 1 × pruritus and 2 × erythema	0
Lu et al. 2017	Randomized, controlled trial	82 (41E, 41C)	29–55 E: 42.4 ± 6.5; C: 41.1 ± 7.7	E: 8.9 ± 2.8; C: 10.0 ± 2.3	Honghua Xiaoyao granule, three times daily + tranexamic acid tablet, twice-daily for 12 weeks	Tranexamic acid tablet, twice-daily for 12 weeks	E: 7 × gastrointestinal reaction and 3 × menoxenia; C: 8 × gastrointestinal reaction and 7 × menoxenia	0
Luo et al. 2019	Randomized, controlled trial	156 (78E, 78C)	21–45 E: 34.6 ± 2.5; C: 33.7 ± 2.4	E: 4.2 ± 1.9; C: 5.3 ± 1.7	Danggui Shaoyao powder, twice-daily + glutathione, three times daily, and vitamin C, three times daily, and vitamin E, once-daily for 12 weeks	Glutathione tablets three times daily, and vitamin C tablets, three times daily, and vitamin E capsules, once-daily for 12 weeks	0	0
Wang et al. 2018	Randomized evaluator-blinded clinical trial	94 (47E, 47C)	24–49 E: 36.36 ± 7.43; C: 35.61 ± 6.69	E: 3.46 ± 2.62; C: 3.52 ± 2.25	Tiaogan Jianpi Quban powder, twice-daily + vitamin C tablet, three times daily, and vitamin E cream, once-daily for 12 weeks	Vitamin C tablets, three times daily and vitamin E cream, once-daily for 12 weeks	0	E: 5 (unknown reasons); C: 6 (unknown reasons)
Xie et al. 2016	Randomized, controlled trial	126 (63E, 63C)	30–48 E: 37.3 ± 9.1; C: 38.1 ± 8.6	E: 1.6 ± 0.9; C: 1.7 ± 1.0	Tiaochong Xiaoban decoction, twice-daily + glutathione, and vitamin C and vitamin E tablets, three times daily, and hydroquinone cream, twice-daily for 12 weeks	Glutathione, vitamin C, and vitamin E tablets, three times daily, and hydroquinone cream, twice-daily for 12 weeks	NR	0

Table 1: Continued.

Study	Study design	Sample size	Age (years)	Duration (years)	Interventions			Dropout (reason)
					Experiment group (E)	Control group (C)	Adverse events	
Xu et al. 2019	Randomized, controlled trial	102 (52E, 50C)	29–53	E: 1–20; C: 1–19	Honghua Xiaoyao tablets, three times daily + tranexamic acid, twice-daily, and hydroquinone cream, twice-daily 12 weeks	Tranexamic acid, twice-daily, and hydroquinone cream, twice-daily 12 weeks	E: 2 × gastrointestinal reaction and 1 × erythema; C: 1 × gastrointestinal reaction, 2 × menoxenia, and 1 × facial tingling	0
Zhu et al. 2016	Randomized, controlled trial	85 (43E, 42C)	20–50 E: 34.67 ± 4.28; C: 34.81 ± 1.15	E: 4.2 ± 1.9; C: 5.3 ± 1.8	Danggui Shaoyao decoction, twice-daily + glutathione tablet, once-daily for 12 weeks	Glutathione tablet, once-daily for 12 weeks	E: 2 × gastrointestinal reaction and 1 × pricking; C: 2 × pricking and 1 × peeling	0
Wang et al. 2019	Randomized, controlled trial	84 (42E, 42C)	23–47 E: 31.54 ± 3.11; C: 30.86 ± 3.25	E: 4.61 ± 1.19; C: 4.58 ± 1.23	Jingtian Quban capsule, twice-daily + glutathione tablet, three times daily for 12 weeks	Glutathione tablet, three times daily for 12 weeks	NR	0

NR: not reported.

Table 2: Outcome measures and description of efficacy across studies.

Study	Outcome measures (measurement points)	Efficacy (PT + RT vs RT)
Chen et al. 2016	Melasma area and color score, physician assessment, and serum levels of α-MSH and E2 (12th week)	% of patients with "cure" or "improvement": clinical response: 85.4% vs. 39.6% ($p < 0.05$); % improvement in melasma area score: 71.7% vs. 33.6% ($p < 0.05$); % improvement in melasma color score: 81.9% vs. 36.2% ($p < 0.05$); % improvement in serum levels of α-MSH: 38.2% vs. 2.4% ($p < 0.05$) and E2: 31.6% vs. 19.4% ($p < 0.05$)
Ji et al. 2013	Physician assessment (12th week)	% of patients with "cure" or "improvement": clinical response: 84% vs. 58% ($p < 0.05$)
Jiang et al. 2019	Physician assessment, recurrence rate (8th and 12th weeks, 3-month follow-up)	At 8th week, % of patients with "cure" or "improvement": clinical response: 42.2% vs. 37.8% ($p > 0.05$). At 12th week, % of patients with "cure" or "improvement": clinical response: 66.7% vs. 55.6% ($p < 0.05$), recurrence rate: 8.9% vs. 20% ($p = 0.025$)
Lu et al. 2017	Physician assessment (12th week)	% of patients with "cure" or "improvement": clinical response: 85.4% vs. 61.0% ($p < 0.05$)
Luo et al. 2019	Physician assessment, MASI score, and serum levels of sex hormones (12th week)	% of patients with "cure" or "improvement": clinical response: 60.0% vs. 26.9% ($p < 0.05$); % improvement in MASI score: 77.1% vs. 41.2% ($p < 0.05$); % improvement in serum levels of E2: 71.6% vs. 48.4% ($p < 0.05$), testosterone: 133.8% vs. 52.3% ($p < 0.05$), progesterone: 1.4% vs. 1.4% ($p > 0.05$), FSH: 62.0% vs. 36.7% ($p < 0.05$), and LH: 53.0% vs. 30.8% ($p < 0.05$)
Wang et al. 2018	Physician assessment, MASI score, DLQI, serum levels of SOD, and MDA (12th week and 3-month follow-up)	At 12th week % of patients with "cure" or "improvement": clinical response: 50.0% vs. 30.0% ($p < 0.05$); % improvement in MASI score: 76.3% vs. 48.5% ($p < 0.05$); % improvement in DLQI: 67.6% vs. 51.4% ($p < 0.05$); % improvement in serum levels of SOD: 86.3% vs. 48.8% ($p < 0.05$) and MDA: 55.2% vs. 36.5% ($p < 0.05$); 3-month follow-up % improvement in MASI score: 76.1% vs. 42.1% ($p < 0.05$); % improvement in DLQI: 66.1% vs. 24.0% ($p < 0.05$)
Wang et al. 2019	Physician assessment and serum levels of E2, LH, and FSH (12th week)	% of patients with "cure" or "improvement": clinical response: 81.0% vs. 52.4% ($p < 0.05$); improvement in serum levels of E2, LH and FSH ($p < 0.05$)

Table 2: Continued.

Study	Outcome measures (measurement points)	Efficacy (PT + RT vs RT)
Xie et al. 2016	Physician assessment, skin lesion score, DLQI, and serum levels of E2, progestogen, SOD, MDA, and LPO (12th week)	% of patients with "cure" or "improvement": clinical response: 68.3% vs. 52.4% ($p < 0.05$); % improvement in skin lesion score: 75.5% vs. 64.3% ($p < 0.01$); % improvement in DLQI: 79.5% vs. 64.4% ($p < 0.01$); % improvement in serum levels of E2: 19.1% vs. 8.0% ($p < 0.01$), progestogen: 27.9% vs. 13.0% ($p < 0.01$), SOD: 36.7% vs. 16.1% ($p < 0.01$), MDA: 26.3% vs. 21.5% ($p < 0.01$), and LPO: 41.9% vs. 25.9% ($p < 0.01$)
Xu et al. 2019	Physician assessment and skin lesion score (12th week)	% of patients with "cure" or "improvement": clinical response: 80.8% vs. 62.0% ($p < 0.05$); % improvement in skin lesion score: 55.1% vs. 46.4% ($p < 0.05$)
Zhu et al. 2016	Physician assessment, skin lesion score, and serum levels of SOD and MDA (12th week)	% of patients with "cure" or "improvement": clinical response: 67.4% vs. 50.0% ($p < 0.05$); % improvement in skin lesion score: 45.4% vs. 31.9% ($p = 0.045$); % improvement in serum levels of SOD: 34.0% vs. 7.0% ($p < 0.001$) and MDA: 41.1% vs. 27.5% ($p = 0.001$)

PT + RT: phytotherapy plus routine therapy, RT: routine therapy, E2: estradiol, MSH: melanocyte-stimulating hormone, FSH: follicle-stimulating hormone, LH: luteinizing hormone, DLQI: dermatology life quality index, and LPO: lipid hydroperoxide.

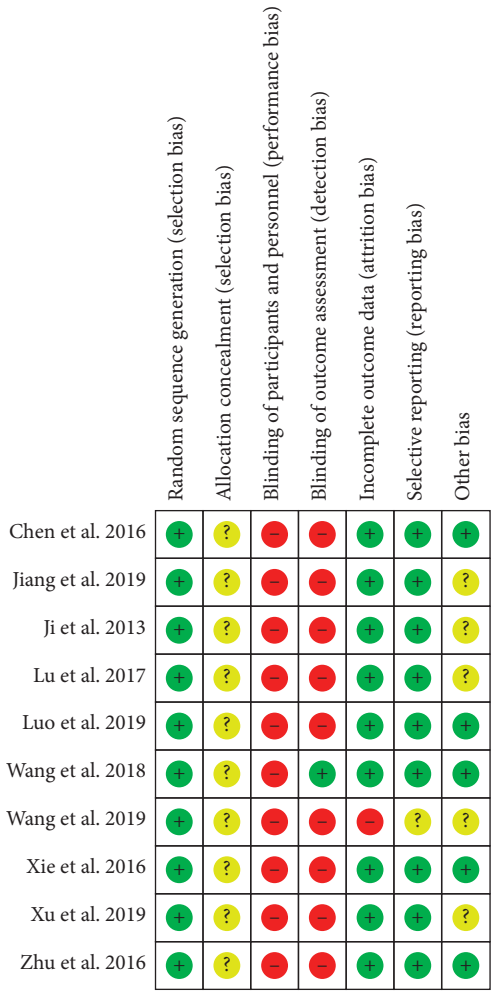

Figure 2: Risk of bias assessment.

3.5. Meta-Analysis of Safety Outcome. In 10 trials, AEs were identified in 5 studies (Table 1), but no serious AEs were reported. The majority of AEs were gastrointestinal reactions which were reported in 5 studies [19, 21, 26, 27], and others were pruritus [20], erythema [19, 20], pricking [27], peeling [27], menoxenia [21, 26] and burning [19]. There were 32

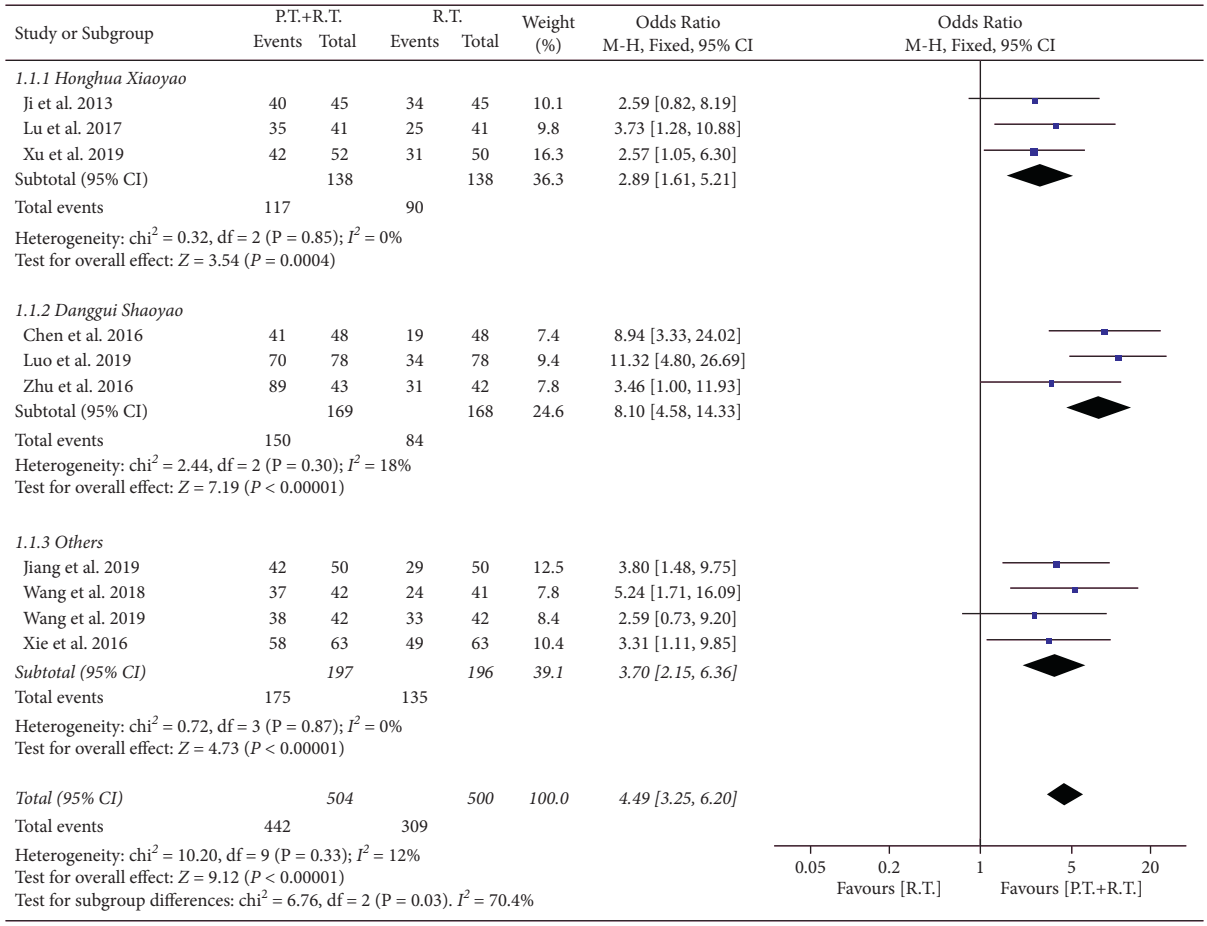

Figure 3: Forest plot of response rate in the overall analysis. Subgroup analysis was stratified according to the types of adjunct drugs. PT + RT = phytotherapy plus routine therapy; RT = routine therapy.

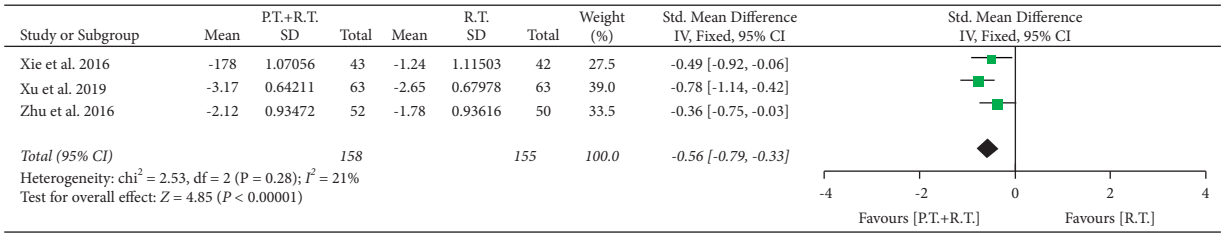

Figure 4: Forest plot of change in skin lesion score in the overall analysis. PT + RT = phytotherapy plus routine therapy; RT = routine therapy.

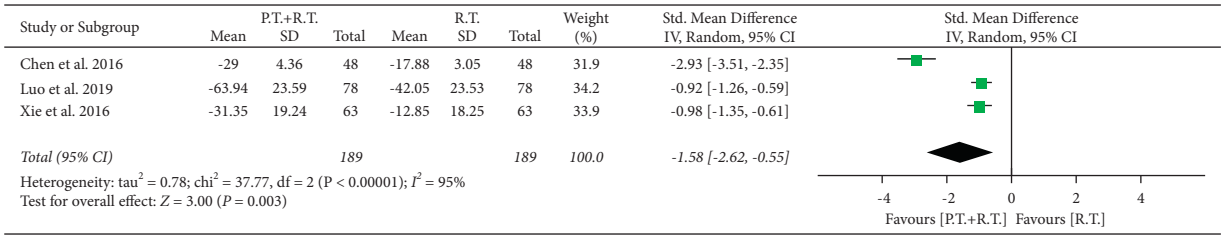

Figure 5: Forest plot of change in serum estradiol (E2) levels in overall analysis. PT + RT = phytotherapy plus routine therapy; RT = routine therapy.

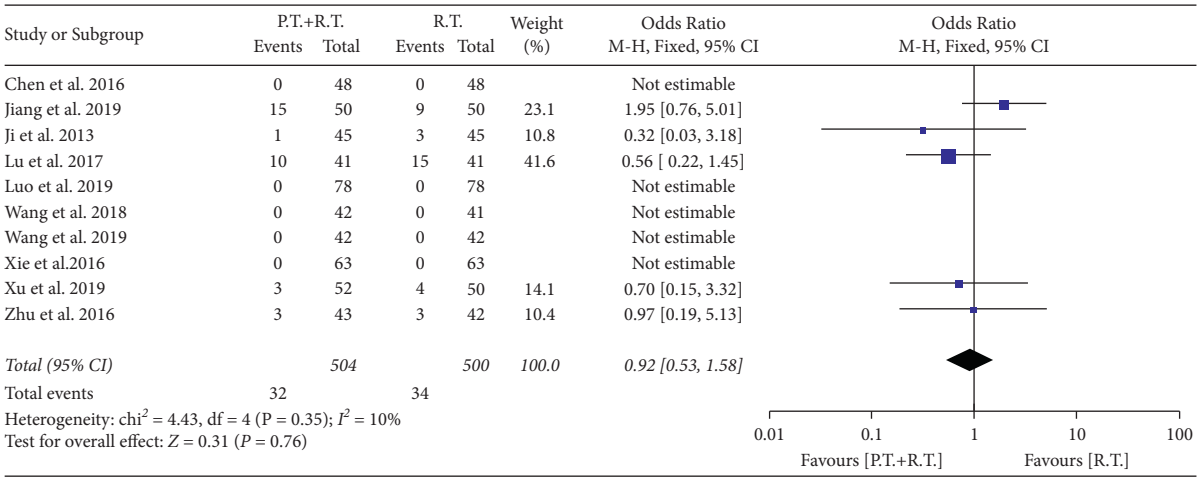

Figure 6: Forest plot of adverse events (AEs) in the overall analysis. PT + RT = phytotherapy plus routine therapy; RT = routine therapy.

(6.3%) of 504 patients receiving combination therapy who experienced AEs, compared with 34 (6.8%) of 500 patients receiving routine therapy. Pooled data showed that there was no significant difference in the incidence of AEs between patients receiving phytotherapy plus routine therapy and routine therapy (OR: 0.92, 95% CI: 0.53 to 1.58; p 0.76; Figure 6). Overall, phytotherapy plus routine therapy was well tolerated across studies. The symmetry of the funnel plot (Supplementary Figure S3) and the Egger test (p 0.7432) suggested the small potentiality of publication bias.

4. Discussion

Although various therapy options have been offered, no regimen guarantees satisfactory results. Currently, management of melasma remains challenging, and the exploitation of safe and effective therapies is imperative. Herbal medicines have been used empirically as therapeutic agents since ancient times. Treating aesthetically displeasing skin disorders using herbal drugs as an adjunct is gaining interest due to their high tolerance and efficacy as well as their perception of safety [28–30]. Only recently, though, the clinical efficacy of some of these herbal drugs has been substantiated through clinical studies. In this regard, our aim was to examine the efficacy and safety of various herbal drugs used as adjuncts to routine agents in the treatment of melasma. We found that combination therapy consisting of phytotherapy and routine therapy significantly increased the response rate when compared with routine therapy alone. In addition, the meta-analysis from 3 studies [25–27] suggested that the effect sizes of combination therapy for skin lesion scores were larger than routine therapy alone. Besides those subjective outcome measures, objective outcome measures, such as serum levels of sex hormones, were also used in several studies [18, 19, 22, 24, 25, 27]. From the pooled results of 3 studies [18, 22, 25], we found that there was a significant benefit associated with the use of herbal drugs as an adjunct to routine therapy in serum E2 levels when compared with routine therapy alone. These results were consistent with the findings from pooled subjective outcomes. Regarding the safety of herbal drugs, our systematic review demonstrated that herbal drugs were well tolerated, with only a small proportion of patients receiving herbal drugs experiencing AEs. In addition, our meta-analysis also suggested that rates of AEs for routine therapy with herbal drugs as an adjunct were comparable to those for routine therapy alone.

Across included studies, there was no sufficient data for assessing the efficacy of phytotherapy plus routine therapy at different end-points. However, in one trial [20], we noted an increase in response rate at the 12th week when compared with the 8th week, suggesting the duration of intervention is important for combination therapy to achieve better therapeutic effect. As previously reported, melasma is easy to recrudesce making efficacy maintenance challenging [31]. One of the included trials reported the recurrence rate at 3 months' follow-up, and the recurrence rate was significantly lower in the combination therapy group than in the routine therapy group [20]. Moreover, another trial demonstrated that phytotherapy plus routine therapy caused a significant decrease in MASI and a significant increase in DLQI at the 12th week, with no rebound within 3 months of combination therapy cessation [24]. By contrast, within 3 months of routine therapy cessation, the reduction of MASI and increase of DLQI were receded [24]. These results suggested that phytotherapy plus routine therapy could have an advantage over routine therapy in efficacy maintenance.

Of the included trials, the agents used in routine therapy mainly included hydroquinone, tranexamic acid, glutathione, and vitamin C. The action mechanisms underlying the efficacy of routine therapy vary with the contained agents. Hydroquinone can bind to the active site of tyrosinase and inhibit the conversion of 3,4-dihydroxyphenylalanine (DOPA) to melanin [31]. Tranexamic acid decreases the generation of arachidonic acid, which leads to a reduction in melanocyte-stimulating hormone and a decrease in pigmentary production [32]. Vitamin C possesses antioxidant activity to scavenge free radicals, while glutathione is thought to act as an antioxidant that decreases inflammation [33]. Differ to the abovementioned agents, the efficacy of herbal drugs were suggested to be derived

from the synergetic and holistic functions of active ingredients rather than the effect on a single target. The herbal drugs were reported to improve the functions of the liver and spleen, dredge the channels and collaterals, and promote blood circulation to remove blood stasis so as to extinct the facial pigmented spots [18, 19, 21, 22, 26]. In addition, herbal drugs could improve internal secretion to regulate the levels of progesterone, E2, and testosterone [18, 22, 25]. Some studies suggested that active ingredients from herbal drugs exerted antioxidant and anti-inflammatory effects which could contribute to the inhibition of melanocyte proliferation [18, 19, 21, 22]. In summary, the function and underlying mechanism of herbal drugs were believed to be complementary to routine therapy, thus resulting in a synergistic effect on the improvement of melasma.

There were some limitations deserving for discussion in this work. First, the quality of included trials was generally poor. Secondly, the included trials and enrolled participants for each meta-analysis were relatively small. Thirdly, the duration of intervention was short, within 12 weeks, there was a lack of data on long-term efficacy. Despite the discussed limitations, this work provides a snapshot of the best level of evidence currently available on the use of oral herbal drugs as an adjunct in the treatment of melasma.

In conclusion, our systematic review and meta-analysis investigated the efficacy and safety of oral herbal drugs used as adjunctive therapy for melasma. Herbal drugs are increasingly popular supplements to standard therapy agents. Evidence-based knowledge of the efficacy and safety will be helpful for dermatologists who may prescribe these herbal drugs. Our results demonstrated that herbal drugs used as an adjunct to routine therapy significantly enhanced the response rate in the treatment of melasma (OR: 4.49, 95% CI: 3.25 to 6.20, $p < 0.00001$). In addition, such combination therapy was also well tolerated with a comparable safety profile to routine therapy alone (OR: 0.92, 95% CI: 0.53 to 1.58; p 0.76). These findings might have implications for recommending herbal drugs as a viable complementary treatment option for melasma. However, the poor design of these included studies has hampered the reliability of these findings. Moreover, none of the included studies provided long-term effects of the interventions and incorporated participant assessment data. Therefore, more rigorous clinical studies with longer follow-up durations need to be conducted to strengthen the reliability of these findings.

Data Availability

The data are available on request to the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Qingti Tang and Hongjie Yang contributed equally to this work.

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Supplementary Materials

Figure S1: funnel plot of trials reporting response rate outcome. Figure S2: forest plot of Melasma Area and Severity Index (MASI). Figure S3: funnel plot of trials reporting adverse events (AEs). (*Supplementary Materials*)

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Review Article

Pharmacological Effects of *Centella asiatica* on Skin Diseases: Evidence and Possible Mechanisms

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The medicinal herb *Centella asiatica* (L.) Urban known as gotu kola has been reported to exhibit a wide range of pharmacological activities. In particular, a significant body of scientific research exists on the therapeutic properties of preparations of *C. asiatica* or its triterpenes in the treatment of skin diseases. The present study is aimed to provide a comprehensive overview of the beneficial effects of *C. asiatica* on skin diseases. Peer-reviewed articles on the potent dermatological effects of *C. asiatica* were acquired from PubMed, Web of Science, Scopus, ScienceDirect, and SciFinder. This review provides an understanding of pharmacological studies which confirm the potent dermatological effects and underlying molecular mechanisms of *C. asiatica*. This medicinal plant and its triterpenes include asiaticoside, madecassoside, and their aglycones, asiatic acid and madecassic acid. These compounds exert therapeutic effects on dermatological diseases such as acne, burns, atopic dermatitis, and wounds via NF-κB, TGF-β/Smad, MAPK, Wnt/β-catenin, and STAT signaling in *in vitro* and *in vivo* studies. However, additional rigorously controlled long-term clinical trials will be necessary to confirm the full potential of *C. asiatica* as a therapeutic agent.

1. Introduction

Centella asiatica (L.) Urban is a perennial plant that grows in swampy areas of tropical and subtropical regions of India, Southeast Asia, and Malaysia, as well as some temperate regions of China, Korea, Japan, and Taiwan [1]. The herb, also known as gotu kola or Indian pennywort, is a valued medicinal plant widely used in the Orient to treat infectious skin diseases and accelerate the healing of skin ulcers and wounds. In addition, internal preparations have been applied to treat dysentery, gastric ulcers, and syphilitic lesions [2].

Secondary metabolites found in the aerial parts of *C. asiatica* are classified into pentacyclic triterpenoids, sesquiterpenes, plant sterols, and saponins [3]. The herb is rich in pentacyclic triterpenoids (C_{30}), of which the most abundant bioactive substances include asiaticoside, madecassoside, and their aglycones, asiatic acid and madecassic acid [1]. Essential oils from plants contain high levels of sesquiterpenes (C_{15}) and monoterpenes (C_{10}), including α-humulene, β-caryophyllene, myrcene, bicyclogermacrene, and germacrene-D [4]. Other constituents found in the aerial part of *C. asiatica* have been characterized as chlorogenic acids, isomeric dicaffeolyl esters, and flavonoids such as catechin, epicatechin, kaempferol, and quercetin [5].

A growing body of research suggests therapeutic potentials of *C. asiatica* in treatment of neurological, endocrine, cardiovascular, digestive, respiratory, and dermatological diseases [6]. Investigations have reported that *C. asiatica* enhances the functions of the nervous system; *C. asiatica* and its triterpenes have a positive effect in relieving symptoms of Parkinson's disease [7] and Alzheimer's disease [8]. In addition, *C. asiatica* extracts have been reported to be effective in the treatment of endocrine diseases such as type 2 diabetes [9–11] and obesity [12]. Asiaticoside and asiatic acid, the active compounds of *C. asiatica,* have positive effects on cardiovascular diseases including hypertension [13, 14] and atherosclerosis [15, 16]. *C. asiatica* extract and its triterpenes exert protective effects against liver injury [17–20] and gastrointestinal tract damage [21, 22]. Asiaticoside and asiatic acid also have therapeutic effects on respiratory disorders such as pulmonary fibrosis

[23], chronic obstructive pulmonary disease [24], and acute lung injury [25]. In particular, several *in vitro* and *in vivo* studies suggest that *C. asiatica* extracts and their triterpenes hold great promise as natural remedies that mitigate acne, burns, atopic dermatitis, and wounds [6, 26].

The aim of the current study was not only to review the pharmacological effects of *C. asiatica* on skin diseases but also to elucidate the possible underlying mechanisms of dermatological activity. Although a review paper had discussed the benefits of *C. asiatica* in dermatology a decade ago [26], it is necessary to include reports published after that time. Database searches using PubMed, Web of Science, Scopus, ScienceDirect, and SciFinder were performed until August 2021 to include up-to-date documented information in the present review. For data mining, the following descriptors were applied in the databases mentioned above: *C. asiatica*, triterpene, asiaticoside, madecassoside, asiatic acid, madecassic acid, therapeutic effect, treatment, prevention, skin disease, acne, burns, atopic dermatitis, and wounds. In almost all cases, original articles were obtained and the relevant data were extracted.

2. Pharmacological Effects of *Centella asiatica* on Skin Diseases

The therapeutic effect of *C. asiatica* on skin diseases has been reported to treat or relieve acne (Table 1), burns (Table 2), atopic dermatitis (Table 3), and wounds (Table 4) [6, 26].

2.1. Acne. Acne is a common chronic inflammatory skin disease that affects the pilosebaceous units of the skin [27]. The four main pathological factors involved in the development of acne include increased sebum production, irregular follicular desquamation, *Propionibacterium acnes* proliferation, and inflammation [28]. In particular, *P. acnes* proliferation may trigger the release of chemostatic factors such as neutrophils, which may cause follicular damage and rupture and leakage of bacteria, fatty acids, and lipids into the surrounding dermis. This process results in the formation of inflammatory lesions [29].

The methanol extract of *C. asiatica* was assayed for antibacterial activity using a disk diffusion assay [30]. The inhibition zones for the disks with 60 μL of 15 mg/mL *C. asiatica* extract were <15 mm against *P. acnes*, indicating that the *C. asiatica* extract showed little antibacterial activity against *P. acnes*. Although the herbal mixture containing the *C. asiatica* extract exhibited high antimicrobial activity against *P. acnes* with 31.25 μg/mL of minimum inhibitory concentration (MIC), it did not support the antibacterial activity against *P. acnes* [31]. Purified madecassoside is a major pentacyclic triterpene saponin from *C. asiatica*. In contrast to the low antimicrobial activity of *C. asiatica* extract against *P. acnes*, the purified madecassoside significantly inhibited the production of proinflammatory cytokine IL-1β, TLR2 expression, and nuclear translocation of NF-κB in *P. acnes*-stimulated THP-1 human monocytic cells [32].

2.2. Burns. Burns result in the development of a dysregulated inflammatory and stress host response characterized by elevated levels of cytokines, chemokines, and acute phase proteins [33]. Following the inflammatory response, activation of keratinocytes and fibroblasts via various cytokines and growth factors helps restore vascular perfusion and further promotes wound healing. The next phase of healing involves wound remodeling, in which collagen and elastin are deposited and continuously transform fibroblasts into myofibroblasts. Over time, a delicate balance between contraction of myofibroblasts and reepithelialization determines the quality and pliability of the repaired wounds and determines the extent of scar formation, which is characterized by fibrous malposition of collagen fibers [34].

One study confirmed the burn wound-healing properties of asiaticoside and madecassoside [35]. Topical treatment with asiaticoside and madecassoside not only induced collagen synthesis, proliferation, and cell growth but also stimulated burn wound healing in male ICR mice. Cytol Centella® is a commercial cream formulated with a titrated extract of *C. asiatica*. Topical application of Cytol Centella® significantly stimulated burn wound contraction by facilitating collagen synthesis in male Wistar rats [36].

Topical application with Centiderm ointment made from *C. asiatica* ethanol extract significantly improved the objective (pliability, vascularity, pigmentation, height, and visual acuity scores) and subjective (dryness, itching, and irritation) signs in patients with second-degree burn wounds on their limbs [37]. In addition, the means of reepithelialization and complete healing were significantly better in the Centiderm group than in the control group. Polyester coated with herbal extract (5% *C. asiatica* extract and 2.5% *Aloe vera* extract) dressings on the burn wound area facilitated burn wound healing, reduced the sizes of burn wounds with higher % epithelialization, and decreased pain scores in another clinical trial [38].

2.3. Atopic Dermatitis. Atopic dermatitis (AD) is the most common inflammatory skin disorder that induces intense itching, edema, erythema, thickening, severe pruritus, and eczematous skin lesions. The pathogenesis of AD is multifactorial, involving immunologic processes including type 1 IgE dysfunction, defects in cell-mediated immune responses, and changes related to barrier dysfunction [39].

The therapeutic effect of a test material on AD is usually evaluated using phthalic anhydride (PA)-induced AD animal models. Titrated extract of *C. asiatica* (TECA) treatment attenuated the development of PA-induced AD by inhibiting the expression of iNOS and COX-2, NF-κB activity, and the release of TNF-α, IL-1β, IL-6, and IgE [40]. Another similar report supports the therapeutic effect of *C. asiatica* on AD in PA-induced AD animal models. Topical treatment with *C. asiatica* phytosome inhibited the expression of iNOS and COX-2, the activity of NF-κB, and the release of TNF-α, IL-1β, and IgE, leading to the suppression of inflammatory cell infiltration [41]. Furthermore, TECA mixed with astaxanthin augmented the inhibitory effect of TECA on PA-induced morphological changes in skin and ear thickness [42]. Interestingly, both topical and oral administration of *C. asiatica* ethanol extract decreased mast cell infiltration in the ear tissue and reduced the expression of various

Table 1: Pharmaceutical effect of *C. asiatica* on acne.

In vitro				
Material tested	Cell line/assay system	Maximum concentration	Effect	Reference
C. asiatica methanol extract	Disk diffusion assay	15 mg/ml	Low antibacterial activity against *P. acnes*	[30]
Herbal mixture containing *C. asiatica* extract	Disk diffusion assay	ND	MIC for *P. acnes* = 31.25 μg/ml	[31]
Purified madecassoside	*P. acnes*-stimulated THP-1 human monocytic cell line	500 μM	TLR2 expression and nuclear translocation of NF-κB $\downarrow$	[32]

ND: not determined; MIC: minimum inhibitory concentration.

Table 2: Pharmaceutical effect of *C. asiatica* on burns.

In vivo				
Material tested	Animal model	Dose, duration	Effect	Reference
Each of asiaticoside and madecassoside	Male SD rats	0.5 μl on the area of burning wounds, 14 d	Collagen synthesis and cell proliferation $\uparrow$; burn wounds $\downarrow$	[35]
Cytol Centella® (titrated extract of *C. asiatica*)	Male Wistar rats	0.13 mg/mm^2 on the area of burning wounds, 33 d	Burn wound contraction $\uparrow$; collagen synthesis $\uparrow$	[36]
Clinical trial				
Material tested	Study design/volunteer (n)	Dose, duration	Effect	Reference
Centiderm ointment containing *C. asiatica* ethanol extract	RCT, DB/patients with second-degree burn wounds on their limbs ($n = 60$)	Appropriate amounts on the area of burning wounds, 25 d	Objective and subjective signs $\uparrow$; mean of reepithelialization and healing completion $\uparrow$	[37]
Polyester coated with herbal extracts (5% *C. asiatica* extract and 2.5% *Aloe vera* extract)	RCT, DB/patients with second-degree burn wounds ($n = 35$)	Covering the area of burning wounds with the dressings with change every 3 days, 21 d	Burn wound healing $\uparrow$; sizes of burn wounds with higher % epithelialization $\downarrow$; pain scores $\downarrow$	[38]

RCT: randomized controlled trial; DB: double blind; objective: pliability, vascularity, pigmentation, height, and visual acuity score; subjective: dryness, itching, and irritation.

Table 3: Pharmaceutical effect of *C. asiatica* on atopic dermatitis.

In vivo				
Material tested	Animal model	Dose, duration	Effect	Reference
Titrated extract of *C. asiatica* (TECA)	Phthalic anhydride-induced AD model	40 or 80 μg/cm^2, 3 times a week for 4 wk	Development of AD $\downarrow$; hyperkeratosis and inflammatory cell infiltration $\downarrow$	[40]
C. asiatica phytosome	Phthalic anhydride-induced AD model	20 μl/cm^2, 3 times a week for 4 wk	Inflammatory cell infiltration $\downarrow$; expression of iNOS and COX-2 $\downarrow$; activity of NF-κB and release of TNF-α, IL-1β, and IgE $\downarrow$	[41]
TECA and astaxanthin combination ointment	Phthalic anhydride-induced AD model	20 μg/cm^2, 3 times a week for 4 wk	Phthalic anhydride-induced skin morphological changes and ear thickness $\downarrow$	[42]
C. asiatica ethanol extract	2,4-Dinitrochlorobenzene-induced AD model	80 μg/cm^2 (topical) or 200 mg/kg/d (oral), 14 d	Mast cell infiltration $\downarrow$; expression of various cytokines $\downarrow$	[43]

proinflammatory cytokines such as TNF-α, IL-4, IL-5, IL-6, IL-10, and IL-17 [43].

2.4. Wounds. Skin wounds are characterized by injury to the skin due to trauma, tears, cuts, or contusions. Wound healing is a physiological process that restores skin integrity and repairs the damaged tissues. Skin wound healing proceeds in four phases: hemostasis, inflammation, proliferation, and remodeling [44].

Asiaticoside was found to promote normal human skin cell migration, attachment, and growth in an *in vitro* wound healing model. ECa 233, a standardized extract of *C. asiatica*, induced keratinocyte migration and promoted wound healing through the activation of FAK, Akt, and MAPK signaling pathways [45].

Table 4: Pharmaceutical effect of *C. asiatica* on skin wounds.

In vitro				
Material tested	Cell line/assay system	Maximum concentration	Effect	Reference
Standardized extract of *C. asiatica* (ECa 233)	Human keratinocyte cell line (HaCaT)	100 μg/ml	Cell migration ↑ wound healing activity ↑	[45]
In vivo				
Material tested	Animal model	Dose, duration	Effect	Reference
C. asiatica hydrogel	New Zealand white albino rabbits for an incision model	Appropriate amounts on the area of incisional wounds, 12 d	Wound healing ↑; formation of a thick epithelial layer, keratin, granulation tissues, fibroblasts, and collagen ↑	[46]
Gelatin membranes containing *C. asiatica* methanol extract	Male SD rats for an incision model	Covering the wound surfaces, 14 d	Wound healing ↑; collagen deposition and angiogenesis ↑	[47]
Topical spray containing *C. asiatica* methanol extract	Male Wistar rats for excision wound model	2.5 ml, once daily for 14 d	Wound healing ↑	[48]
Asiaticoside nitric oxide gel	Male SD rats for an incision model	0.2 ml, twice daily for 14 d	Healing rate of diabetic cutaneous ulcer wounds ↑; growth of bacteria in the wound surface ↓	[49]
Clinical trial				
Material tested	Study design/volunteer (n)	Dose, duration	Effect	Reference
Standardized extract of *C. asiatica* (ECa 233 gel)	RCT, DB/patients with bilateral atrophic facial acne scars ($n = 30$)	Appropriate amount on half-side of the face, twice daily for 3 mo	Post-laser-resurfacing wound healing ↑	[50]

RCT: randomized controlled trial; DB: double blind.

Asiaticoside-rich hydrogel made from aerial parts of *C. asiatica* facilitated skin wound healing faster than the commercial cream and the untreated wounds in rabbits in an incision model [46]. The accelerated wound healing with *C. asiatica* hydrogel treatment may be owing to the formation of a thick epithelial layer, keratin, granulation tissues, fibroblasts, and collagen. The wound areas of rat skin treated with gelatin membranes containing *C. asiatica* methanol extract exhibited collagen deposition and a high number of capillaries, leading to enhanced skin wound healing [47].

In an *in vivo* excision wound model, there was significant wound healing in the animal group treated with topical spray containing *C. asiatica* methanol extract than in the untreated group [48]. Interestingly, asiaticoside nitric oxide gel promoted the healing rate of diabetic cutaneous ulcer wounds by regulating the Wnt/β-catenin signaling pathway and inhibiting the growth of bacteria on the wound surface in a rat wound model with diabetic cutaneous ulcers [49].

In contrast to the abundant *in vivo* evidence of the wound-healing effect of *C. asiatica*, clinical studies are sparse. Yet, one clinical trial showed a significant improvement in post-laser-resurfacing wound healing in patients with bilateral atrophic facial acne scars treated with ECa 233 gel for three months compared to the patients in the control group [50].

2.5. Other Skin Diseases. Vitiligo is a depigmenting skin disorder characterized by the selective loss of melanocytes which leads to pigment dilution in affected areas of the skin. It is the result of genetics and environmental causes in addition to metabolic stress, oxidative stress, and cell detachment abnormalities [51].

Madecassoside has attenuated mitochondrial damage caused by H_2O_2-induced oxidative stress in human epidermal melanocytes, suggesting that it could be a promising treatment for vitiligo mainly caused by oxidative stress (Table 5) [52].

Additionally, TECA promoted three-dimensional dermal papilla sphere formation by inhibiting the STAT activation in human dermal papilla cells, indicating TECA treatment may provide a useful strategy for promoting hair growth [53].

3. Underlying Mechanisms for the Pharmacological Effects of *Centella asiatica* on Skin Diseases

It has been reported that the feasible molecular mechanisms involved in the pharmacological effects of *C. asiatica* on skin diseases are as follows: NF-κB, TGF-β/Smad, MAPK, Wnt/β-catenin, and STAT signaling.

3.1. NF-κB Signaling. Nuclear factor-kappa B (NF-κB) is a family of dimeric transcription factors that coordinate inflammatory responses, innate and adaptive immunity, and cellular differentiation, proliferation, and survival in almost all multicellular organisms [54]. The NF-κB system is tightly regulated, and misregulation of NF-κB has been implicated in a wide range of diseases ranging from cancer to inflammatory and immune disorders. As a result, the NF-κB regulatory network and its dynamics offer a multitude of promising therapeutic targets that remain to be fully explored and translated into clinical use [55].

Table 5: Pharmaceutical effects of *C. asiatica* on other skin diseases.

In vitro				
Material tested	Cell line/assay system	Maximum concentration	Effect	Reference
Madecassoside	Human epidermal melanocytes	100 μg/ml	Damage of mitochondria ↓; oxidative stress ↓	[52]
TECA	Human dermal papilla cells	25 μg/ml	Potential of hair inductive capacity ↑	[53]

Titrated extract of *C. asiatica* (TECA) treatment suppressed NF-κB activity and subsequently inhibited the expression of TNF-α, IL-1β, and IL-6 in a phthalic anhydride (PA)-induced atopic dermatitis (AD) animal model [40]. Furthermore, the addition of astaxanthin to TECA augmented the NF-κB-mediated anti-inflammatory activity of TECA [42]. Another similar report supports the therapeutic effect of *C. asiatica* on AD via NF-κB signaling. Topical treatment with *C. asiatica* phytosome inhibited the translocation of NF-κB into the nucleus and the release of TNF-α, IL-1β, and IgE, leading to the suppression of inflammatory cell infiltration [41]. In an *in vitro* assay, purified madecassoside, a major pentacyclic triterpene from *C. asiatica*, significantly inhibited the nuclear translocation of NF-κB and IL-1β production as well as TLR2 expression in *Propionibacterium acnes*-stimulated THP-1 human monocytic cells [32].

3.2. TGF-β/Smad Signaling. Transforming growth factor-β (TGF-β) is considered a crucial mediator in tissue fibrosis and causes tissue scarring largely by activating its downstream small mothers against decapentaplegic (Smad) signaling [56]. TGF-β plays a pivotal role in producing the myofibroblast phenotype, which is responsible for massive collagen deposition and contraction of wounds [57]. Since the TGF-β-induced Smad-dependent pathway is critical for the pathogenesis of all fibrotic diseases, various therapeutic strategies have been investigated to target the signaling pathway to attenuate aberrant skin scar formation [58].

Among the triterpenoid compounds of *C. asiatica*, the glycosides (asiaticoside and madecassoside) themselves, rather than their corresponding metabolites, asiatic acid and madecassic acid, are recognized as the main active constituents of *C. asiatica* herbs responsible for burn wound healing. Oral administration of both asiaticoside and madecassoside enhanced collagen type III synthesis by activating skin fibroblasts via the TGF-β/Smad pathway and facilitated burn wound healing in male ICR mice [59].

3.3. MAPK Signaling. Mitogen-activated protein kinase (MAPK) is an important signaling pathway in living beings in response to extracellular stimuli. There are five main subgroups manipulated by a set of sequential actions: ERK (ERK1/ERK2), c-Jun N (JNK/SAPK), *p*38 MAPK (*p*38α, *p*38β, *p*38γ, and *p*38δ), and ERK3/ERK4/ERK5 [60]. When stimulated, these groups have long been linked to multiple biological processes such as cell proliferation, differentiation, death, migration, invasion, and inflammation [61]. ECa 233, a standardized extract of *C. asiatica*, induced keratinocyte migration and subsequently promoted wound healing through the activation of FAK, Akt, and MAPK signaling pathways [45].

3.4. Wnt/β-Catenin Signaling. The Wnt/β-catenin pathway is one of the main processes in the regulation of a variety of biological processes including cell proliferation, apoptosis, and differentiation. It is also considered an important pathway in the healing of skin wounds. [62]. Asiaticoside nitric oxide gel promoted the healing rate of diabetic cutaneous ulcer wounds by regulating the Wnt/β-catenin signaling pathway and inhibiting the growth of bacteria on the wound surface in a rat model with diabetic cutaneous ulcers [49].

3.5. STAT Signaling. The signal transducer and activator of transcription (STAT) signaling pathway is a universally expressed intracellular signal transduction pathway and is involved in many crucial biological processes, including cell proliferation, differentiation, apoptosis, and immune regulation [63]. Hair growth can be induced from resting mouse hair follicles by topical application of Janus kinase (JAK) inhibitors, suggesting that JAK/STAT signaling is required for maintaining hair follicle stem cells in a quiescent state [64]. The titrated extract of *C. asiatica* (TECA) promoted three-dimensional dermal papilla sphere formation by inhibiting STAT activation in human dermal papilla cells, indicating that TECA treatment may provide a useful strategy for promoting hair growth [53].

4. Pharmacokinetic Properties of Active Compounds of *Centella asiatica*

The complex composition of *C. asiatica* renders pharmacokinetic studies of its multiple active compounds in humans or animal models particularly challenging. To date, pharmacokinetic studies on the active compounds of *C. asiatica* have primarily focused on *in vivo* absorption, distribution, metabolism, and elimination (ADME), as well as the bioavailability of *C. asiatica*-specific triterpenoids asiaticoside, madecassoside, and their aglycones asiatic acid and madecassic acid.

One pharmacokinetic study on asiatic acid was conducted after oral administration of an encapsulated water-soluble extract obtained from the aerial parts of *C. asiatica* in beagle dogs [65]. The main pharmacokinetic parameters of asiatic acid obtained from beagle dog plasma were $T_{1/2}$, 4.29 h; T_{max}, 2.70 h; C_{max}, 0.74 μg/ml; AUC_{0-t} and $AUC_{0-\infty}$, 3.74 and 3.82 μg h/ml. Another pharmacokinetic study suggested that the absolute oral bioavailability of asiatic acid in rats is very low (16.25%), which may result from poor

solubility and rapid metabolism [66]. Asiaticoside is converted *in vivo* to asiatic acid by hydrolytic cleavage of the sugar moiety, leading to little asiaticoside within the plasma after oral administration because of a proposed complete biotransformation into asiatic acid and its glucuronide and sulphate conjugates in a human study [67]. When pure madecassoside was administered orally as a single compound in rats, the pharmacokinetic parameters of madecassoside were T_{max} and $T_{1/2}$ of 0.90 ± 0.14 h and 3.47 ± 0.68 h, respectively [68]. After a single oral dosing in rats, madecassoside was widely distributed in the heart, liver, spleen, lungs, and kidneys of rats and the levels of madecassoside in the liver and kidney were relatively higher than in other organs with primary elimination via the feces [69]. Notably, Anukunwithaya et al. conducted a disposition kinetic study on ECa 233, a standardized extract of *C. asiatica*, containing madecassoside (53.1%) and asiaticoside (32.3%) in rats [70]. Madecassoside and asiaticoside were rapidly absorbed, reaching maximum levels within 5–15 min after oral administration in rats, whereas madecassic and asiatic acids were found in negligible amounts. Both triterpenoid glycosides were extensively distributed in the brain, stomach, and skin within 1 h and remained there for at least 4 h after dosing. Interestingly, madecassoside and asiaticoside in ECa 233, administered orally at a dose of 100 mg/kg, were rapidly distributed to the skin with an $AUC_{(0-4)}$ of 667.22 ± 121.06 and 114.50 ± 12.07 ng × h/g of skin tissue, respectively.

Few studies have examined the tissue distribution of triterpenes following oral administration or topical application, particularly the skin levels of these compounds. Although one study mentioned above [70] reported the dermal distribution of madecassoside and asiaticoside in a standardized extract of *C. asiatica* after oral dosing in rats, the cutaneous absorption rate of the active principles of topical application of *C. asiatica* has yet to be to be measured. Thus, further investigation is needed to understand the dermal distribution of these compounds and their metabolites.

5. Conclusions and Perspectives

Several *in vitro* and *in vivo* studies have demonstrated the therapeutic potential of *Centella asiatica* in the treatment of acne, burns, atopic dermatitis, and wounds. It has been suggested that the feasible molecular mechanisms involved in the pharmacological effects of *C. asiatica* on skin diseases include NF-κB, TGF-β/Smad, MAPK, Wnt/β-catenin, and STAT signaling. However, further intensive clinical trials are required to confirm its efficacy as a therapeutic agent for treating skin diseases.

Medicinal products containing *C. asiatica* preparations are authorized and marketed in some of the European countries including Belgium, France, Greece, Italy, Portugal, and Spain. For external use, cutaneous cream 1% and powder 2% are recommended to support the local treatment of moderate or benign problems in wound formation and to aid in the local treatment of cutaneous ulcerations. In addition, oral tablets containing the titrated extract of *C. asiatica* (TECA) are authorized as potent wound-healing agents [71]. Since various formulations of *C. asiatica* have already been authorized and marketed as wound-healing therapeutics such as Madecassol®, Centellase®, and Blastoestimulina®, it could be considered that there is little need for clinical trials to confirm its effect on wound healing. However, extensive clinical studies are necessary to verify the healing effect of *C. asiatica* in subjects with various types and severities of wounds as well as the effect on skin diseases other than wounds, such as acne, burns, and atopic dermatitis.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The author declares that there are no conflicts of interest regarding the publication of this paper.

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