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Review article

Mechanisms of single herbs and herbal pairs in the treatment of mammary gland hyperplasia: An integrated review☆

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ABSTRACT

Background: The pathogenesis of hyperplasia of mammary glands (HMG) is a complex process, involving multiple links and systems within the body. Current clinical research indicates that traditional Chinese medicine (TCM) demonstrates a significant therapeutic effect in treating HMG. Single herbs or herbal pairs (two herbs) are the basic units of preventing and treating HMG. It is of great significance to explore the mechanism of single herbs or herbal pairs in treating HMG for clarifying the mechanism of preventing HMG with TCM.

Purpose: This study aimed to review the literature, summarize the known mechanisms of single herbs and herbal pair therapy for treating hyperplasia of mammary glands (HMG), and elucidate the relevant substances involved within and outside the body during these treatments.

Study design: In this study, the action mechanism of single herbs or herbal pairs in treating HMG was selected as the research object. English articles were mainly selected and Chinese articles were supplemented. We conducted a literature search in PubMed, CNKI, WanFang Database, etc , including full-text studies published between January 1992 and December 31, 2022. The primary literature was carefully screened, and the mechanism of action was explored by logical analysis.

Methods: We conducted a literature review focusing on basic studies that explored the mechanisms underlying the effects of herbal treatments for mammary gland hyperplasia. The literature search was performed in PubMed, CNKI, and WanFang Database, covering full-text articles published from January 1992 to 31 December 2022, using various keywords (e.g., hyperplasia of mammary glands, single herb, herbal pair, effect, mechanism, inclusion criteria). Exclusion criteria were also set. We employed methods such as literature measurement, literature research, and content analysis to logically analyze, induce, and deduce the findings of the collected literature.

Results: This review reveals that several distinct mechanisms contribute to the beneficial effects of single herbs or herbal pairs on the recovery of mammary gland hyperplasia. Regarding hormone levels, Chinese herbs can decrease hormones such as Estradiol(E2) and Prolactin(PRL), increase Progesterone(P) levels, balance the E2/P ratio, reduce the expression of sex hormone receptors, and lessen the self-sensitivity of breast tissue under the influence of E2. Histologically, Chinese herbs can inhibit breast neovascularization and alleviate blood viscosity. At the cellular level, Chinese herbs can modulate the expression of apoptosis genes and proteins, decrease cell proliferation activity, and ultimately inhibit or even reverse breast hyperplasia. From a

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pharmacological perspective, Chinese herbs exhibit analgesic, anti-inflammatory, antioxidant, and immune-regulating properties.
Conclusion: The evidence in this review demonstrates the effectiveness of single herbs or herbal pairs in preventing and treating mammary gland hyperplasia, with precise underlying mechanisms.

Abbreviations

androgen receptors AR
bone morphogenetic protein BMP
epidermal growth factor receptor EGFR
estrogen receptors ER
estrogen receptor 1 ESR1
estrogen receptor α ERα
Follicle stimulating hormone FSH
glucocorticoids GR
G protein-coupled receptor GPER
Human breast cancer cells MCF7
hyperplasia of mammary glands HMG
interleukin-6 IL-6
Monoamine oxidase MAO
Malondialdehyde MDA
mannose 6-phosphate/insulin-like growth factor II M6P/IGF-II
nitric oxide NO
phosphatidylinositol 3-kinase PI3K
Protein Kinase B AKT
protein tyrosine kinase family dimer ErbB
prolactin receptor PRLR
Progesterone receptor PR
prostaglandin E2 PGE2
traditional Chinese medicine TCM
tumor necrosis factor-α TNF-α
Tumor Necrosis Factor TNF
Vascular endothelial growth factor A VEGFA
basic fibroblast growth factor bFGF
cyclooxygenase2 COX2
estradiol E2
estrogen receptors ESR
estrogen receptor 2 ESR2
estrogen receptor β ERβ
Ginsenoside Rd GSRD
glutathione peroxidase GSH-px
growth factor receptor 2 HER2
5-hydroxytryptamine 5-HT
interleukin-1β IL-1β
insulin growth factor receptor IGFR
inducible nitric oxide synthase iNOS
mitogen-activated protein kinase MAPK
nuclear transcription factor-κB NF-κB
phosphatidylinositol 3,4,5-trisphosphate PIP3
phosphatase and tensin homolog PTEN
Protein-protein interaction PPI
Prolactin PRL
Progesterone P
progesterone receptors PGR
superoxide dismutase SOD
transforming growth factor-β1 TGF-β1
transcriptional regulation pathway of AP-2 TFAP2
Vascular endothelial growth factor VEGF

1. Introduction

The pathogenesis of hyperplasia of mammary glands (HMG) is a complex process, involving multiple links and systems within the body. Current clinical research indicates that traditional Chinese medicine (TCM) demonstrates a significant therapeutic effect in treating HMG, offering advantages such as improved patient compliance and fewer adverse reactions.

This study aims to conduct a comprehensive literature review, summarizing the known mechanisms underlying the treatment of HMG using single herbs or herbal pairs (two herbs) and detailing the substances related to these mechanisms both within and outside the body. The evidence in this review reveals that various distinct mechanisms contribute to the beneficial effects of single herbs or herbal pairs on HMG rehabilitation.

2. Materials and methods

Our literature review was designed to encompass basic studies addressing the mechanisms underpinning the therapeutic effects of herbs on mammary gland hyperplasia. The primary researches on HMG treatment with herbs were integrated, and the discussion on single herbs and herbal pairs was conducted. We conducted a literature search in PubMed, CNKI, and the WanFang Database, including full-text studies published between January 1992 and December 31, 2022. The following keywords were utilized.

- Hyperplasia of mammary glands
- Single herb
- Herbal pair
- Effect
- Mechanism

2.1. Inclusion criteria included

- Specific mechanisms describing the effects of single herbs or herbal pairs
- Hyperplasia of mammary glands type
- Exclusive use of single herbs or herbal pairs for treating mammary glands hyperplasia
- Related studies cited in the included articles

2.2. The exclusion criteria are as follows :

- Literature review
- In combination with other herbs
- In combination with other drugs such as chemical or biological drugs
- In combination with other treatments

3. Results

Using these parameters, we identified 226 published articles. Of these, only 122 met the inclusion criteria and were reviewed. The references of these 122 articles led to an additional 88 articles that also fulfilled our inclusion criteria. Consequently, a total of 210 articles were included in the final review.

4. Discussion

4.1. Single herb used alone exerted specific effect

4.1.1. Ginseng can improve the body immunity by nourishing vitality

Ginseng (*Panax ginseng* C. A. Meyer) is derived from the root of the perennial herb *Panax ginseng*, a member of the *Araliaceae* family and *Panax* genus. It typically thrives in deciduous broad-leaved forests or mixed coniferous broad-leaved forests at elevations of several hundred meters above sea level. Exhibiting a mild nature with a sweet and slightly bitter taste, *Ginseng* is associated with the lung and spleen meridians. Renowned as the "king of tonic medicines," ginseng's fleshy root is a potent tonic herb known for its capacity to enhance vitality, improve blood circulation, fortify the spleen and lungs, promote fluid secretion, stabilize the mind, and boost intellect. Clinically, ginseng is used to regulate blood pressure, restore heart function, and prevent and treat neurasthenia.

Ginsenoside Rd (GSRD) is the primary active component of ginseng responsible for preventing and treating mammary gland hyperplasia. As the main active monomer isolated from ginseng, GSRD has been shown to significantly alleviate pathological hyperplasia of the mammary gland and the associated pain. Its pharmacological activities include modulating immunity, enhancing microcirculation, relieving pain, and exerting anti-inflammatory effects. The pharmacological mechanism underlying its treatment of mammary gland hyperplasia primarily involves bolstering in vivo antioxidant capacity, regulating the expression of Bcl-2 and p53 proteins, and

inducing apoptosis of hyperplastic mammary gland cells [1–6]. (Fig. 2).

4.1.1.1. Treating HMG by enhancing antioxidant capacity and preventing inflammation. GSRD addresses mammary gland hyperplasia by augmenting in vivo antioxidant capacity. The hyperplasia of breast tissue is accompanied by a rise in oxygen free radical generation. An excess of oxygen free radicals triggers lipid peroxidation of unsaturated fatty acids on the biofilm, leading to increased tissue peroxide levels, a subsequent release of inflammatory cytokines, and damage to the cell membrane structure, thereby exacerbating tissue damage. Malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-px) are crucial indicators of in vivo antioxidant capacity. In mammary gland hyperplasia model rats, serum MDA levels were significantly elevated, while SOD content and GSH-px activity were markedly reduced. GSRD has been found to increase serum SOD levels, enhance GSH-px activity, and decrease MDA content. Consequently, GSRD can significantly improve pathological mammary gland hyperplasia and alleviate associated pain symptoms. Relevant experiments have also confirmed that GSRD can markedly inhibit mice's writhing response induced by glacial acetic acid and the foot-licking response elicited by formalin, demonstrating a significant inhibitory effect on inflammatory pain [7–10].

4.1.1.2. Treating HMG by hormonal regulation and hemorheological improvement. Mammary gland hyperplasia in model rats is characterized by increased whole blood viscosity, plasma viscosity, hematocrit, and fibrinogen content. GSRD administration significantly reduces these indicators, suggesting that it improves hemorheology in model rats, thereby exerting an anti-mammary gland hyperplasia effect. Estrogen is known to promote lipid peroxidation, leading to increased MDA levels and decreased SOD and GSH-px levels. GSRD effectively reduces estrogen levels in the blood of rats with mammary gland hyperplasia and enhances its in vivo antioxidant effect by regulating estrogen [1,11].

4.1.1.3. Treating HMG through regulation of protein expression. The initiation and progression of mammary gland hyperplasia result from increased proliferation and reduced apoptosis of mammary epithelial cells. Bcl-2 family proteins and p53 protein play critical roles in the apoptotic signal transduction process. Bcl-2 protein, the encoded product of the Bcl-2 proto-oncogene, is an anti-apoptotic gene that extends cell survival, inhibits apoptosis, and localizes in the mitochondria, endoplasmic reticulum, and continuous perinuclear membrane. Bcl-2 protein is expressed in mammary duct epithelial cells and participates in the pathological process of mammary gland hyperplasia. Overexpression of Bcl-2 in hyperplastic mammary glands of rats correlates with the degree of hyperplasia [12–19].

The p53 gene, a tumor suppressor gene located on chromosome 17, encodes a 53-kDa nuclear protein. Wild-type p53 inhibits cell proliferation, whereas mutant p53 promotes cell proliferation and contributes to tumorigenesis. Research indicates that mutant p53 protein expression is elevated in mammary gland hyperplasia, while wild-type p53 gene expression is deficient and mutated in breast ductal hyperplasia tissues [12–17].

In model group rats, Bcl-2 protein expression in mammary epithelial cells is increased, while wild-type p53 protein expression is reduced. GSRD induces apoptosis by decreasing Bcl-2 protein expression and increasing p53 protein expression, thereby ameliorating the hyperplastic state of breast tissue and achieving a therapeutic effect [18,19].

4.1.2. Prunella vulgaris can remove breast lumps by detoxifying, clearing heat, eliminating phlegm stagnation

Prunella vulgaris, a *Lamiaceae* plant, is characterized by its dried fruit ear, pungent and bitter taste, and cold nature. It selectively affects the liver and gallbladder meridians and possesses therapeutic properties such as clearing away heat, purging fire, improving eyesight, dispersing stagnation, and reducing swelling [20]. The principal constituents of *Prunella vulgaris* include *ursolic acid, luteolin, methyl rosmarinate, β-amyrinol, urticine, scopolamine, dihydrochelerythrine, isoorientin, Prunellaside A, Prunellaside B,* etc. (Fig. 3)

4.1.2.1. Treating mammary gland hyperplasia via anti-inflammatory and antibacterial mechanisms. Prunella Vulgaris is predominantly employed in the treatment of mammary gland hyperplasia, leveraging its anti-inflammatory and antibacterial properties. The primary component responsible for this therapeutic effect is hyperin, which significantly inhibits the phosphorylation of NF-κB p65 and P38 mitogen-activated protein kinase (MAPK), subsequently downregulating TNF-α (Tumor Necrosis Factor), interleukin-1β (IL-1β), interleukin-6 (IL-6), and mRNA levels of iNOS to achieve anti-inflammatory effects [21].

Various solvent extracts from *Prunella vulgaris* fruit, including n-hexane, petroleum ether, butanol, chloroform, and water, have been shown to inhibit endotoxin-stimulated production of nitric oxide (NO) and prostaglandin E2 (PGE2), as well as gene expression related to inducible nitric oxide synthase (iNOS), cyclooxygenase2 (COX2), and tumor necrosis factor-α (TNF-α). These findings demonstrate the plant's potent anti-inflammatory activity, with the n-hexane extract displaying the most pronounced effect. The observed effects are associated with the inhibition of nuclear transcription factor-κB (NF-κB) activation and the nuclear translocation of NF-κB's p50 and p65 subunits [22].

Prunella vulgaris exerts its anti-inflammatory effects primarily by inhibiting the expression and deposition of inflammatory factors through the following mechanisms: (1) suppressing cyclooxygenase-2 expression, which in turn impacts arachidonic acid metabolism and reduces prostaglandin E2 production; (2) downregulating TNF-α levels and inhibiting IkappaB activation to suppress the nuclear factor-κb pathway; (3) inhibiting the phosphorylation of p38 MAPK, which further impedes the activation of transcription factor NF-κB as well as the nuclear translocation of p50 and p65 subunits, thereby reducing the synthesis and release of downstream inflammatory mediators and regulating oxidative stress; and (4) exerting an anti-inflammatory effect by inhibiting *Staphylococcus aureus* [23].

The antibacterial mechanisms of *Prunella vulgaris* primarily involve: (1) affecting bacterial cell wall permeability and inhibiting bacterial growth; (2) damaging cell membrane permeability, leading to the leakage of proteins and carbohydrates from the cytoplasm and hindering bacterial metabolic growth; and (3) interfering with DNA replication by inhibiting the activity of DNA polymerase [24].

4.1.2.2. Treating mammary gland hyperplasia through hormone regulation. Imbalances in estrogen and progesterone secretion are the primary causes of breast hyperplasia (Fig. 1). The proliferative effect of estrogen relies on the ratio of estrogen receptors (ER). Estradiol, the biologically active component of estrogen, binds to the ligand-binding domain of estrogen receptor α (ERα) in breast tissue cells. This binding activates downstream pathways and induces transcription [25].

Progesterone exhibits bidirectional effects. It can both synergize with estrogen to activate the bone morphogenetic protein (BMP) signaling pathway, promoting an increase in breast ducts and branches, and inhibit mammary gland tissue proliferation by reducing ER concentration, enabling hyperplastic breast tissue to undergo reversion [26,27].

Another ER, estrogen receptor β (ERβ), is present in breast tissue. It binds to estrin, exerting inhibitory effects. The ratio of ERα and ERβ in breast tissue determines the proliferative effect of estrogen.

Prunella vulgaris intervention significantly reduced serum estradiol content, while increasing progesterone content and 5-hydroxytryptamine (5-HT) levels. *Prunella vulgaris* may inhibit mammary gland tissue proliferation or induce reversion in proliferative mammary gland tissue by modulating estrogen and progesterone levels, influencing the effect of these hormones on ERα and ERβ, or regulating blood lipid levels, thereby contributing to the treatment of breast hyperplasia [28].

Furthermore, patients with mammary gland hyperplasia may experience neuro-endocrine-immune imbalances. *Prunella vulgaris* can regulate immune function and alleviate immune disorders, thereby enabling accurate identification and removal of diseased cells and enhancing the breast tissue's ability for timely and efficient recovery [29].

4.1.2.3. Treating mammary gland hyperplasia by regulation of signaling pathways and induction of apoptosis. The relationship between the primary active components, their targets, and related biological signaling pathways of *Prunella vulgaris* in relation to mammary gland hyperplasia was investigated using network pharmacology. Enrichment analysis of key targets revealed the critical signaling pathways involved in the inhibition of mammary gland hyperplasia by *Prunella vulgaris*. These include the breast cancer pathway, endocrine resistance pathway, transcriptional regulation pathway of AP-2 (TFAP2) in the transcription factor family, epithelial cell proliferation pathway, AKT signaling pathway activated by PIP3, and estradiol stress pathway. *Prunella vulgaris* exerts its therapeutic effects by acting on these targets and influencing the associated signaling pathways.

Ursolic acid, a component of *Prunella vulgaris*, can bind to Akt1/VEGFA, inhibiting mammary gland hyperplasia via the estrogen or ErbB (protein tyrosine kinase family dimer) pathway, while its β-sitosterol binds to EGFR/MYC to inhibit mammary gland hyperplasia through the estrogen or ErbB pathway. Prunella vulgaris extract inhibits breast cell proliferation and induces apoptosis by upregulating miR-195 and inhibiting the VEGF/PI3K/Akt signaling pathway [30–33].

Prunella vulgaris contains several anti-proliferative active ingredients, such as *ursolic acid, luteolin,* and *methyl rosmarinate,* which exert their effects through various synergistic mechanisms, such as promoting pro-apoptotic processes or inhibiting the activity of *cysteine-containing aspartate proteolytic enzymes-3 (caspase-3)* and *caspase-9* to regulate cell division [34–38].

Prunella vulgaris extract can reduce the expression of estrogen-responsive genes in MCF-7 (Human breast cancer cells) cells at the mRNA level and decrease the expression of ERα protein, thereby inducing apoptosis in MCF-7 cells and potentially treating mammary gland hyperplasia [39].

4.1.3. Lychee nucleus can remove stasis, promote Qi circulation, relieve pain

Lychee nucleus, derived from the dried mature seed of the *Litchi chinensis* Sonn. plant, is a warm and sweet, slightly bitter substance that belongs to the *Sapindaceae* family. Primarily cultivated in Fujian, Guangdong, and Guangxi, lychee seed has been shown to have selective effects on liver and kidney meridians. It is known to promote *Qi* movement, dispel evil stagnation, alleviate cold, and relieve pain. The primary chemical constituents of lychee seed include phlorizin, naringin, rutin, naringenin-7-*O*-rutinoside, proanthocyanidin A2, *p*-hydroxybenzoic acid, protocatechuic aldehyde, *p*-hydroxybenzaldehyde, and protocatechuic acid [40]. (Fig. 5).

4.1.3.1. Treating mammary gland hyperplasia by regulating hormone. Mammary gland hyperplasia is a condition that has been directly linked to endocrine disorders. Abnormal estrogen secretion can lead to prolonged stimulation of breast tissue, resulting in hyperproliferation when progesterone is unable to provide proper protection. Estrogen, a steroid hormone (primarily estradiol), is prevalent in human target tissues and is responsible for various regulatory functions. Estrogen exerts its effects on the body by binding to the estrogen receptor (ER). Progesterone receptor (PR) is induced and synthesized by estradiol (E2) and functions as an ER, increasing its sensitivity due to E2 stimulation. Elevated ER and PR levels enhance mammary epithelial cell sensitivity to estrogen, which can trigger mammary gland hyperplasia. When the ER signaling pathway becomes dysregulated, intracellular protein function may be altered, leading to an imbalance in gene expression and resulting in mammary gland cell hyperproliferation or apoptosis arrest [41,42].

Lychee nucleus saponin has been demonstrated to significantly improve nipple hyperplasia and the pathological morphology of mammary gland hyperplasia in a rat model. It reduces abnormally elevated estradiol levels in the serum of affected rats, increases progesterone content, and downregulates ER and PR expression in hyperplastic mammary gland tissue by modulating estrogen levels. Consequently, the sensitivity of breast tissue to estrogen is diminished, and the biological effect of estrogen on target cells is weakened [43,44].

4.1.3.2. Treating mammary gland hyperplasia by inhibiting the formation of new blood vessels. The hyperplasia of breast tissue necessitates continuous neovascularization to supply adequate blood flow. Effective treatment can be achieved by inhibiting the formation of new blood vessels. Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are specific factors that contribute to the neovascularization process [44]. Lychee seed has been found to inhibit neovascularization in breast tissue and prevent breast hyperplasia by reducing the expression of VEGF [45].

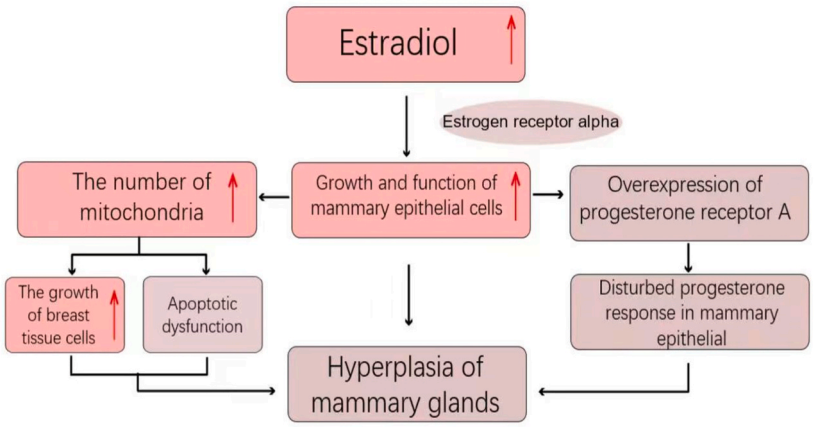
4.1.4. Centella asiatica can clear away heat, eliminate dampness, reduce lumps, remove toxic and pathogenic factors

Centella asiatica, a dried whole grass from the *Umbelliferae* plant family, possesses a cylindrical root with a light yellow or grayish-yellow surface. Characterized by its bitter and pungent taste, as well as its cold nature, this plant selectively affects the liver, spleen, and kidney meridians. *Centella asiatica* exhibits various therapeutic effects, such as clearing away heat, eliminating dampness, reducing lumps, and removing toxic and pathogenic factors. It is primarily found in regions such as Jiangsu, Anhui, Zhejiang, and Jiangxi.

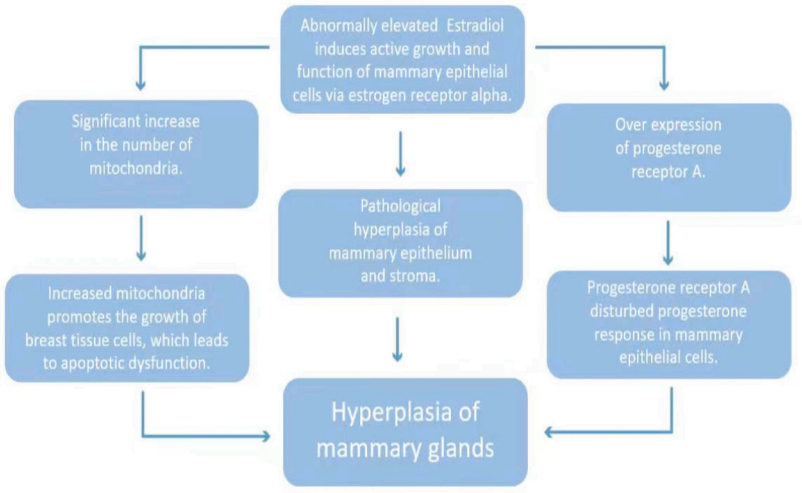
The chemical composition of *Centella asiatica* encompasses numerous compounds, including *α-aromatic resin alcohol type triterpene acids* and *saponins, caryophyllene, farnesol, 3-eicosyne, longifolene, α-Lycaene, β-Caryophyllene, chlorogenic acid, vanillic acid, succinic acid* , etc. Among these constituents, *glycosides* are the active ingredient responsible for treating breast hyperplasia [46]. (Fig. 4).

4.1.5. Treating mammary gland hyperplasia by promoting cell apoptosis

Centella asiatica total glycosides' therapeutic mechanism for breast hyperplasia involves direct action on mammary duct epithelial cells and mammary gland lobe cells, thus hindering mammary gland cell hyperplasia or causing atrophic hyperplastic breast tissue. Additionally, *Centella asiatica glycosides* inhibit breast cell proliferation by reducing serum estrogen levels, thereby decreasing estrogen-induced stimulation of breast tissue [47,48].



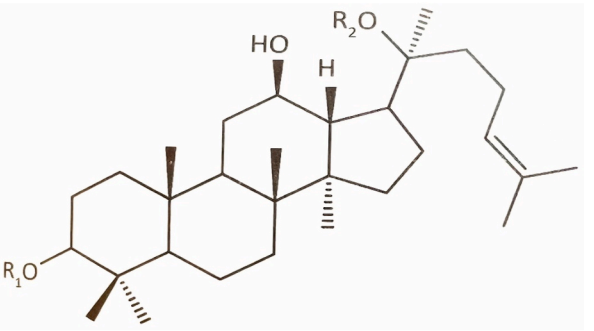
Effects of hormone on mammary gland hyperplasia (Estradiol)



Effects of hormone on mammary gland hyperplasia (Progesterone)

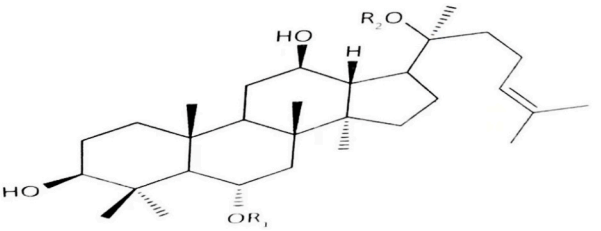
Fig. 1. Pathogenesis of mammary gland hyperplasia.

	R1	R2
20(S)-protopanaxadiol	H	H
Ginsenosides Ra_1	Glc(2→1)Glc	Glc(6→1)Ara(p)(4→1)Xyl
Ginsenosides Ra_2	Glc(2→1)Glc	Glc(6→1)Ara(f)(2→1)xyl
Ginsenosides Rb_1	Glc(2→1)Glc	Glc(6→1)Glc
Ginsenosides Rb_2	Glc(2→1)Glc	Glc(6→1)Ara(p)
Ginsenosides Rc	Glc(2→1)Glc	Glc(6→1)Ara(f)
Ginsenosides Rd	Glc(2→1)Glc	Glc
Ginsenosides Rg_3	Glc(2→1)Glc	H
Ginsenosides Rh_2	Glc	H



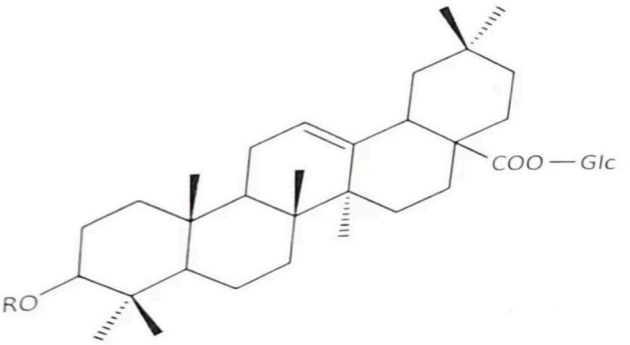
Molecular structure expression of *Panaxadiol-type ginsenosides*

	R1	R2
20(s)-protopanaxatriol	H	H
Ginsenosides Re	Glc(2→1)Rha	Glc
Ginsenosides Rf	Glc(2→1)Glc	H
Ginsenosides Rg_1	Glc	Glc
Ginsenosides Rg_2	Glc(2→1)Rha	H



Molecular structure expression of *Panax triol ginsenosides*

Ginsenosides Ro R= GlcA(2→1)Glc



Molecular structure expression of *Oleanolic acid type ginsenoside*

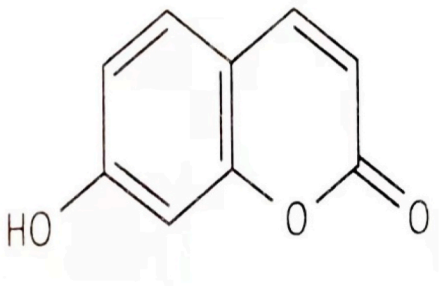
Fig. 2. Molecular structure expression of the main component of '*Ginseng* (*Ginsenosides*).

4.1.6. Enhancing autophagy through modulation of signal transduction

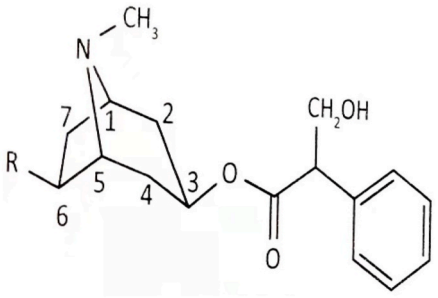
The *glycosides* enhance autophagy through the modulation of Notch1/Hes1 signaling. As a self-phagocytosis mechanism, autophagy is crucial for maintaining intracellular environmental stability by degrading misfolded proteins and damaged organelles. It also sustains energy balance and cell survival in response to nutrient and energy stress [49–52].

4.1.6.1. Treating mammary gland hyperplasia by antioxidant and anti-inflammatory. *Centella asiatica* glycoside (ASI) exhibits potent antioxidant and anti-inflammatory effects, offering diverse protective benefits. ASI can inhibit endothelial proliferation, dilate blood vessels, and ameliorate ischemia/reperfusion injury [53].

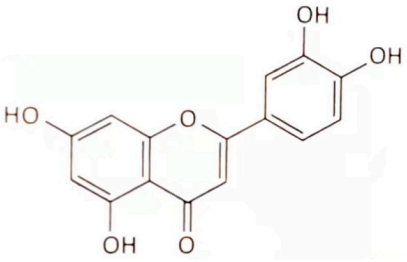
4.1.6.2. Treating mammary gland hyperplasia by regulating cellular proliferation and restoration. Notch1/Hes1 plays a pivotal role in cellular proliferation and repair, serving as a regulatory mechanism for autophagy in mammary gland hyperplasia-induced injury. Autophagy-related protein expression decreases in the HMG group, while Notch1 and Hes1 expression significantly increases. ASI effectively enhances autophagy and downregulates the expression of Notch1 and Hes1. In the HMG model, ASI inhibits the Notch1/Hes1 signal, augments autophagy, and consequently improves DCM-induced damage [49].



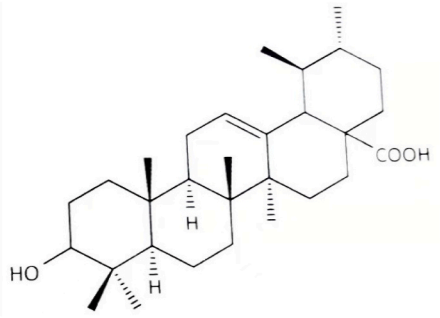
Molecular structure expression of *Umbelliferone*



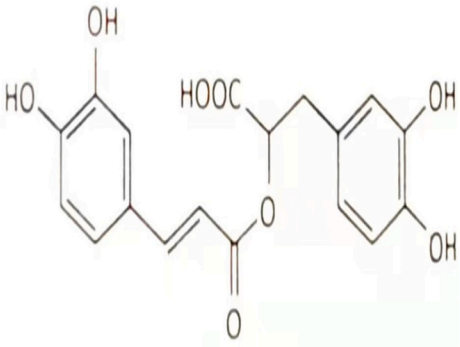
R=H Hyoscyamine（atropine）
R=OH Anisodamine



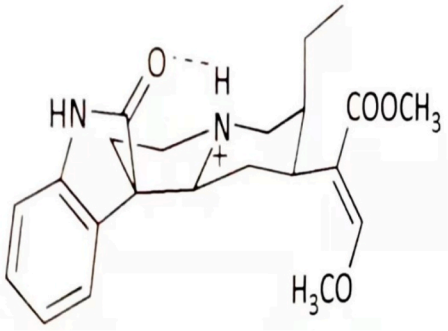
Molecular structure expression of *Luteolin*



Molecular structure expression of *Ursolicacid*



Molecular structure expression of *Rosmarinic acid*



Molecular structure expression of *Isorhynchophylline*

Fig. 3. Molecular expression of six effective components of *Prunella vulgaris* in treating mammary hyperplasia.

4.1.7. Stag horn can warm kidney-yang, fortify muscles and bones, reduce ecchymosis and tumefaction

Stag horns are the ossified horns of the *Cervidae* family (*Cervus elaphus Linnaeus or Cervus nippon Temminck*), primarily found in Xinjiang, Qinghai, and the three northeastern provinces of China. Characterized by a salty taste and warm nature, *Stag horns* selectively influence kidney and liver meridians. The key effects of *Stag horns* include warming kidney-yang, fortifying muscles and bones, and reducing ecchymosis and tumefaction. They are commonly used to treat kidney-yang deficiency, impotence, spermatorrhea, cold pain in the waist, and pain resulting from bruising and hematoma. The primary chemical constituents of *Stag horns* are *amino acids, such as serine, alanine, leucine, L-hydroxyproline, glycine, L-proline, aspartic acid, glutamic acid, histidine,* and *arginine* [54]. (Fig. 6 Fig. 7).

4.1.7.1. Treating mammary gland hyperplasia by participating in dopamine metabolism. *Stag horns* contains numerous monoamine oxidase inhibitors, such as *hypoxanthine, phosphatidylethanolamine,* and *p-hydroxybenzaldehyde. Monoamine oxidase* (MAO) is a crucial enzyme involved in dopamine metabolism, which is a key component in the antler's anti-mammary hyperplasia effects. Antlers inhibit prolactin in the blood by promoting dopamine synthesis, reducing dopamine breakdown, and maintaining elevated dopamine levels in the brain [54].

4.1.7.2. Treating mammary gland hyperplasia by modulating sex hormone receptor expression. *Stag horns* exhibit anti-mammary gland hyperplasia effects by modulating sex hormone receptor expression. They significantly reduce the protein expression of ER and PR in rat breast hyperplasia tissue, as well as the positive expression rate of ER and PR [12]. Not only do deer antlers counteract breast hyperplasia induced by external hormones, they also reduce the levels of E2, P, and PRL in the body [55].

Stag horns have a substantial regulatory effect on the imbalance of serum reproductive hormone levels in rats induced by estradiol benzoate and progesterone. They reduce estrogen levels, such as PRL, FSH (Follicle stimulating hormone), and E2, while increasing luteinizing hormone and progesterone in rats with breast hyperplasia. Stag horns significantly reduce nipple swelling in rats with breast hyperplasia, decrease the number of lobes and acinar vesicles in the mammary gland, inhibit acinar vesicle secretion, improve ductal epithelial hyperplasia, and alleviate breast hyperplasia symptoms.

The anterior pituitary gland exhibits high 2-hydroxylase activity, which can convert estrogen into catechol estrogen (2-hydroxyestrogen). Catechol estrogen competes with brain dopamine (DA) for DA receptors, interrupting the inhibitory effect of DA on prolactin (PRL) secretion. Additionally, catechol estrogen can inhibit tyrosine hydroxylase activity, reduce DA synthesis, and increase PRL secretion. This mechanism underlies the breast hyperplasia model induced by hexestrol, leading to elevated blood PRL content.

Stag horns extract (*Stag horns polypeptide*), can significantly reduce PRL in female mouse plasma and pituitary cell culture solution in both in vitro and in vivo experiments. This extract exhibits a notable inhibitory effect on breast hyperplasia in female mice. The mechanism through which this occurs involves increasing the DA content in the brain and decreasing the PRL content.

4.2. Herbal pairs combine two herbs to exert strong effects

Herbal pair is made according to the principle of enhancing efficacy, under the guidance of TCM theory. Herbal pair embodies the core of prescription compatibility law, is the simplest prescription expression, and is also the foundation of prescription composition.

Network pharmacology serves as an optimal tool for investigating the prevention and treatment of breast hyperplasia using traditional Chinese medicine. This study elucidates the molecular mechanisms of various herbal pairs in addressing breast hyperplasia through network pharmacological approaches [56–59].

4.2.1. Herbal pair (bupleurum + angelica) can sooth liver, eliminate depression, tonify blood, promote blood circulation, relieve pain

4.2.1.1. Treating mammary gland hyperplasia by modulating oxidative stress response, inflammatory response, anti-angiogenesis, cell proliferation, and apoptosis. The molecular functions of the *Bupleurum* and *Angelica* herbal pair in treating HMG involve cytokine receptor binding and transcription cofactor binding. Key biological processes include reactive oxygen species metabolism and oxidative stress response. The primary cellular components encompass the cell membrane region and cytoplasmic membrane raft. The herbal pair affects signaling pathways, including the IL-17, TNF, and MAPK pathways. By constructing an "active ingredient-key target-key pathway" network, breast hyperplasia-related compounds, such as *quercetin, kaempferol, petunidin*, and *3,3″,4″,5,5″,6,7-*

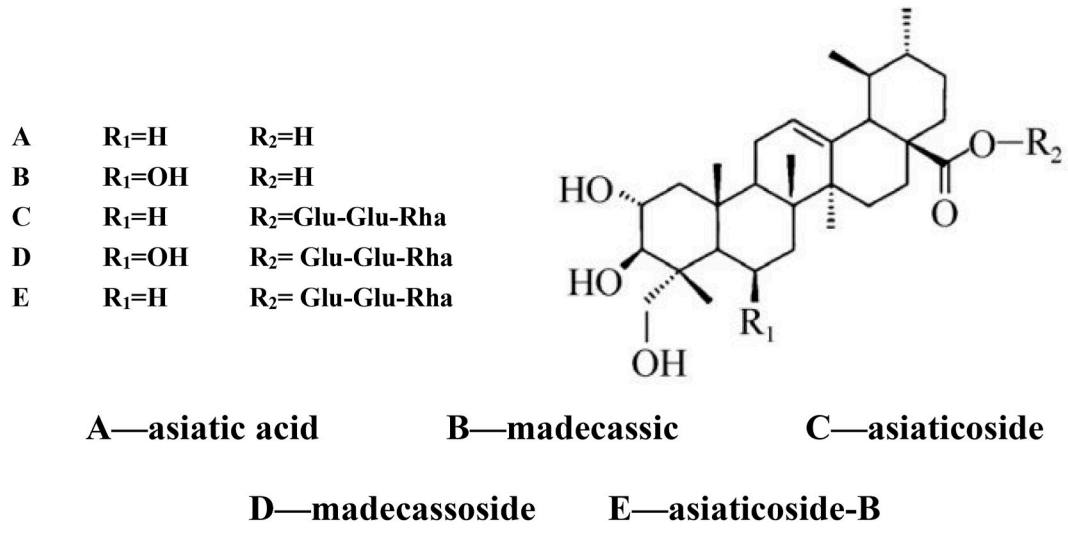


Fig. 4. Molecular expression of five effective components of *Centella* in treating mammary hyperplasia.

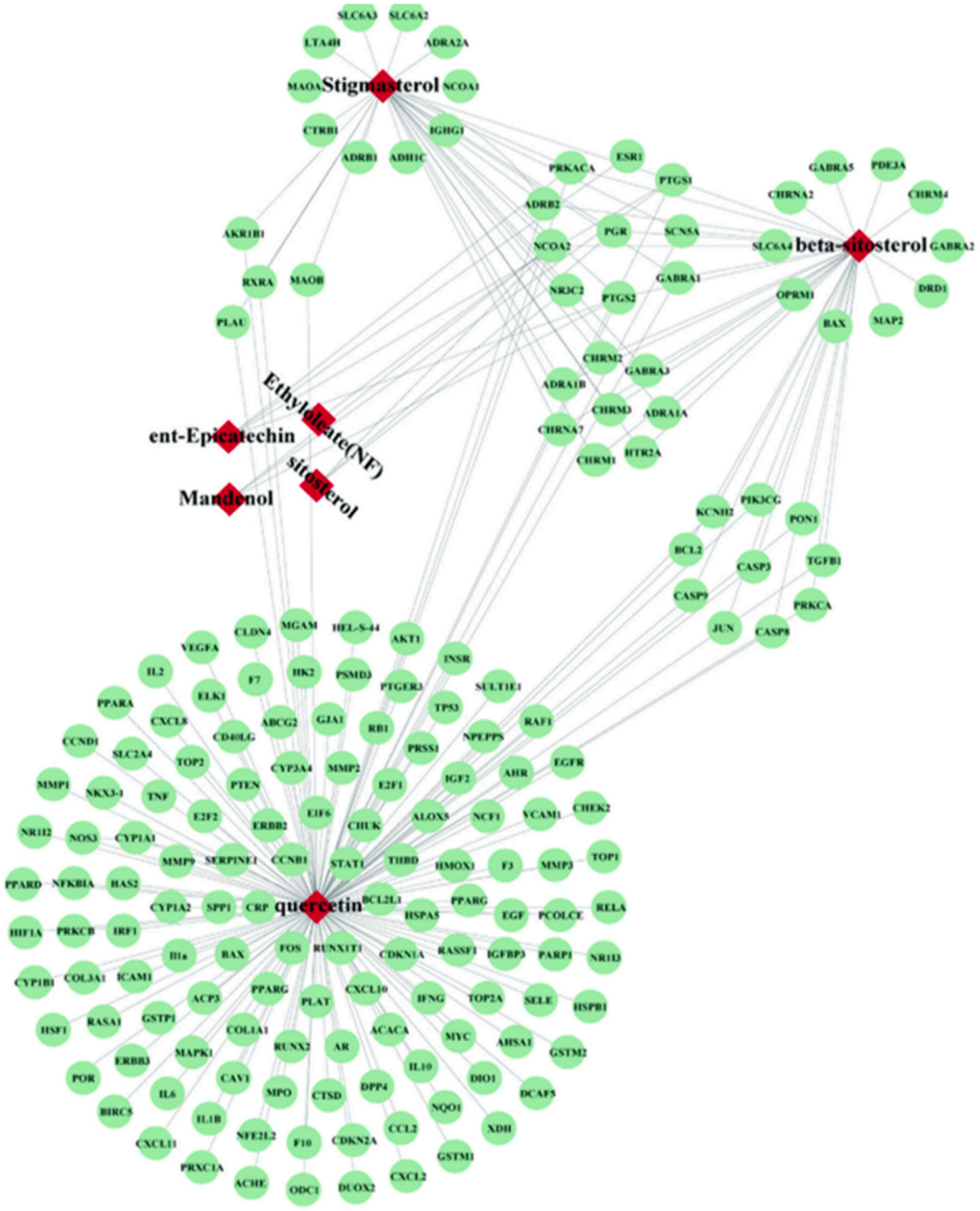


Fig. 5. The network of active components-potential targets of *Lychee nucleus*.

hexamethoxyflavonoids, are identified. flavonoids like *quercetin, kaempferol*, and *3,3″,4″,5,5″,6,7-hexamethoxyflavonoids* exhibit estrogen-like or anti-estrogen effects [60]. (Fig. 8).

PPI topology analysis indicates that AKT1, IL-6, TP53, VEGFA, and TNF have higher degree values, suggesting they are potential core targets of the Bupleurum and Angelica herbal pair for breast hyperplasia treatment. The herbal pair addresses breast hyperplasia by regulating these core targets and participating in IL-7, TNF, MAPK, and HIF-1 signaling pathways, thus modulating oxidative stress response, inflammatory response, anti-angiogenesis, cell proliferation, and apoptosis. AKT1 is involved in cell survival and cell cycle regulation, with delayed breast hyperplasia degeneration associated with increased phosphorylation at the Thr308 site of AKT1 [61].

AKT1 is involved in cell survival and cell cycle regulation. Breast hyperplasia's delayed degeneration is linked to increased phosphorylation at AKT1's Thr308 site. AKT1 plays a role in regulating breast cell growth. KEGG analysis reveals that the PI3K-AKT signaling pathway is associated with endocrine drug resistance. By inhibiting PI3K or AKT activity, it can enhance ER expression,

influence endocrine drug resistance, and treat breast hyperplasia [62].

The MAPK signaling pathway is implicated in endocrine regulation, inflammatory response, and oxidative stress response to address breast hyperplasia. This pathway regulates estrogen and progesterone receptors ER and PR, affecting breast cell proliferation. IL-1β, IL-6, IFN-γ, and TNF-α synergistically contribute to the pathogenesis of autoimmune diseases through the MAPK signaling pathway, enhancing immunity and alleviating breast hyperplasia.

TNF, an inflammatory factor, can mitigate breast hyperplasia inflammation and oxidative stress by reducing the expression of oxidative stress-related proteins 8-OHdG and NT. By regulating TNF expression, the *Bupleurum* and *Angelica* herbal pair can enhance immune function and alleviate breast hyperplasia. The TNF signaling pathway affects VEGF expression through TNF-α secreted by macrophages, influencing new blood vessel formation in breast tissue [63].

The HIF-1 signaling pathway is a hypoxia adaptive pathway involved in regulating cell apoptosis, cell cycle, immune response, new blood vessel generation, and tissue hypoxia tolerance. HIF-1α is the main target whose stability increases in hypoxic conditions. The cross-regulation of the HIF-1 pathway and other signaling pathways plays a crucial role in breast hyperplasia treatment. Under hypoxia, ERK phosphorylation in cells promotes HIF-1 expression. The MAPK signaling pathway can regulate HIF-1 expression to treat breast hyperplasia. Furthermore, the HIF-1 signaling pathway is related to the NF-κB-mediated inflammatory signaling pathway. Inflammatory stimuli can increase HIF-1 gene and protein expression, regulate inflammatory response by modulating the NF-κB-mediated inflammatory signaling pathway, and thereby exert anti-breast hyperplasia effects [64].

4.2.1.2. Treating mammary gland hyperplasia by reducing vascular endothelial growth factor A. Breast tissue hyperplasia demands new angiogenesis for nutrient delivery. Vascular endothelial growth factor A (VEGFA) and its receptors cooperatively regulate endothelial cell differentiation and angiogenesis through paracrine pathways, stimulating angiogenesis and promoting cell growth. As hyperplasia aggravates, VEGFA expression increases. The Bupleurum and Angelica herbal pair reduce VEGFA expression, inhibiting neovascularization in breast hyperplasia tissue and treating breast hyperplasia [65].

4.2.1.3. Treating mammary gland hyperplasia by estrogen-like or anti-estrogenic effects. The herbal pair of *Bupleurum* and *Angelica* contains flavonoids such as *quercetin, kaempferol,* and *3,3″,4″,5,5″,6,7-hexamethoxyflavonoids,* which can exhibit estrogen-like or anti-estrogenic effects [60]. Estrogen plays a critical role in the development of breast hyperplasia by promoting breast cell proliferation through the synthesis of growth factors. Flavonoids can reduce the production of estrogen metabolites by modulating the expression of estrogen metabolism genes. Quercetin can exert anti-estrogenic effects and inhibit breast epithelial cell survival at high concentrations, thereby contributing to breast hyperplasia treatment [66].

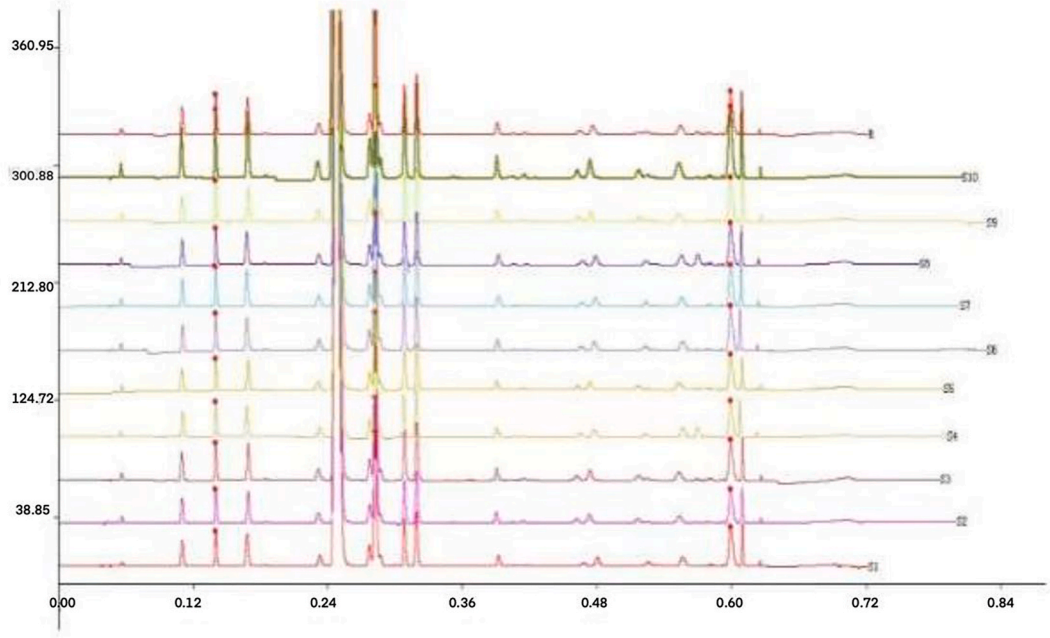


Fig. 6. Fingerprint of *hydrolyzed amino acids* from *Stag horn*.

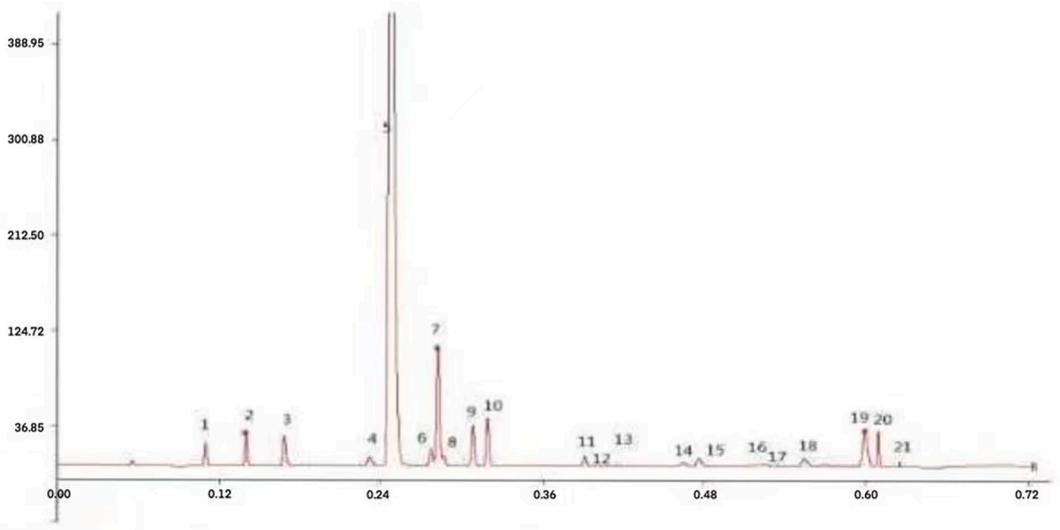


Fig. 7. Common pattern comparison map of *amino acid* fingerprints of *Stag horn*.

4.2.2. Herbal pair (frankincense + myrrh) can reduce swelling, relieve pain, promote the circulation of blood and Qi

4.2.2.1. Herbal pair (frankincense + myrrh) contains many active ingredients for inhibiting breast hyperplasia, its intervention for breast hyperplasia through a variety of mechanisms. The herbal pair of *Frankincense* and *Myrrh* contains 51 main active ingredients known for inhibiting breast hyperplasia, including *Gansyl, taxene, boswellic acid, incenol, α-boswellic acid, acetyl-α-boswellic acid, β eleminolic acid,* among others. In the context of breast hyperplasia intervention, the herbal pair targets 271 action sites. The top 10 targets include androgen receptor, cytochrome P450 19A1, microtubule-associated tau, estrogen receptor α, estrogen receptor β, sodium-dependent norepinephrine transporter, steroid 17α-hydroxylase/17,20 lyase, glucocorticoid receptor, mineralocorticoid receptor.

The intervention mechanism of the *Frankincense* and *Myrrh* herbal pair on mammary gland hyperplasia primarily involves inhibiting hyperplasia of mammary cells and promoting apoptosis and autophagy. Additionally, the mechanism includes suppressing inflammatory reactions and angiogenesis [67].

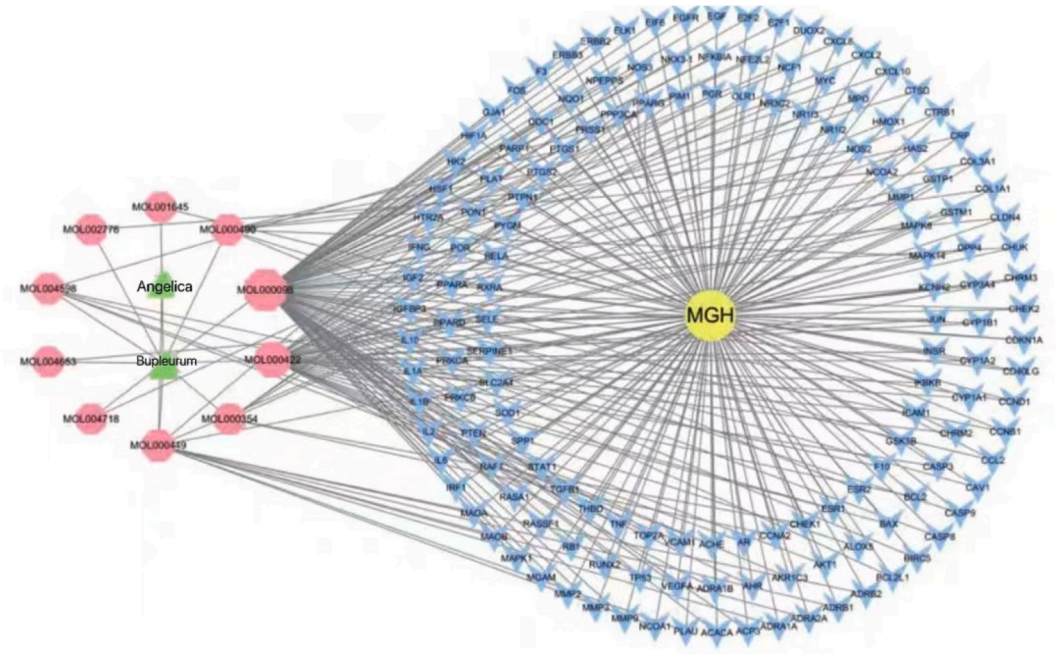


Fig. 8. Active ingredient-function target-disease network diagram (*Bupleurum* + *Angelica*).

4.2.2.2. Treating mammary gland hyperplasia by regulating signal pathways. The *Frankincense* and *Myrrh* herbal pair regulates mammary gland hyperplasia through six signaling pathways: VEGF signaling pathway, ErbB signaling pathway, mTOR signaling pathway, steroid hormone biosynthesis, androgen and estrogen metabolism, and steroid biosynthesis. Target signaling pathway enrichment analysis indicates that the herbal pair inhibits breast hyperplasia by modulating signaling pathways involved in breast cell proliferation, inflammation, angiogenesis, apoptosis, and autophagy.

4.2.2.3. Treating mammary gland hyperplasia by regulating hormone and receptor expression. This herbal pair can also regulate hormone and receptor expression levels, enhance antioxidant capacity and immune function, and improve proliferative tissues. Active ingredients such as guggulsterone and naringenin regulate steroid-related signaling pathways and other biological processes, including the mTOR signaling pathway, steroid biosynthesis, response to estrogen stimulation, estrogen metabolism, sterol biosynthesis, and response to corticosteroid stimulation. Through these mechanisms, this herbal pair effectively inhibits breast hyperplasia [68,69].

4.2.2.4. Treating mammary gland hyperplasia by hormonal regulation. The biologically-driven process of breast hyperplasia regulation involving the herbal pair *Frankincense* and *Myrrh* is characterized by responses to hormone and corticosteroid stimulation, regulation of hormone levels and metabolism processes, and modulation of steroid hormone receptor signaling pathways. This includes involvement in estrogen biosynthesis, steroid and sterol biosynthesis, and participation in steroid and sterol metabolism processes.

The mechanism of action for the *Frankincense* and *Myrrh* herbal pair in intervening the onset and progression of mammary gland hyperplasia is demonstrated through the regulation of targets such as androgen receptors (AR), estrogen receptor 1 (ESR1), and estrogen receptor 2 (ESR2), which can be modulated by at least 15 active ingredients in the herbal pair [67]. Frankincense and myrrh can target estrogen receptor α (ERα), promoting the degradation of ERα and blocking its transport to the nucleus by encouraging dissociation from HSP90, thereby exerting an estrogen-blocking effect.

4.2.2.5. Treating mammary gland hyperplasia by affecting protein synthesis and decomposition. Frankincense and *Myrrh* can induce dissociation of ERα from other proteins by binding to HSP90. Guggulsterone exhibits a high affinity for steroid hormone receptors and can interact with them to produce corresponding effects on *androgens (AR), glucocorticoids (GR), progesterone receptors (PGR),* and *estrogen receptors (ESR)*. Naringenin can downregulate the expression of ERα66 and GPR30 while upregulating the expression of ERβ and ERα36 [69,70]. This herbal pair can attenuate the overexpression of cyclooxygenase-2 and inducible nitric oxide synthase, inhibit the formation of 8-hydroxydeoxyguanosine and nitrotyrosine, and play an anti-hyperplasia role by regulating the expression of protein kinase B and stress-activated protein kinase in breast tissue, ultimately reducing oxidative stress and inflammation.

4.2.3. Herbal pair (bupleurum + atractylodes) can invigorate spleen, eliminate dampness, enhance Qi and blood function

4.2.3.1. Treating mammary gland hyperplasia by controlling and regulating estrogen. Estrogen receptor signaling plays a crucial role in regulating various physiological processes such as breast growth, development, and apoptosis. Imbalances in the estrogen receptor signaling pathway can lead to excessive proliferation or inhibited apoptosis of breast cells, resulting in breast hyperplasia [71].

The PI3K/AKT signaling pathway is involved in promoting breast cell proliferation, inhibiting apoptosis, and stimulating angiogenesis. Its function is achieved by influencing a range of downstream effector molecules [72]. The PI3K/AKT/mTOR pathway is one of many downstream channels of the PI3K/AKT pathway. When activated, AKT directly phosphorylates mTOR, exerting anti-apoptotic effects and promoting breast cell proliferation.

Upregulation of BMP2 and BMP4 expression encourages the proliferation and differentiation of breast stem cells, leading to an increase in breast ducts and branches, ultimately causing breast epithelial hyperplasia. The MAPK signaling pathway, which includes ERK1/2, JNK, and p38 MAPK pathways, serves to transmit extracellular signals to cells. This pathway plays a crucial regulatory role in breast cell proliferation, differentiation, apoptosis, and metastasis, impacting the development of breast hyperplasia and other breast diseases.

The herbal pair comprising *Bupleurum* and *Atractylodes macrocephala* works to maintain the stability of estrogen receptors through the estrogen pathway, allowing estrogen to perform normal physiological functions and maintain regular growth, development, and apoptosis of the mammary gland. Simultaneously, this herbal pair blocks the PI3K/AKT/mTOR pathway, inhibiting the transmission of anti-apoptotic signals, promoting mammary glandular cell apoptosis, and suppressing mammary gland hyperplasia. In terms of the therapeutic effect on mammary gland hyperplasia through the RAS signaling pathway, the herbal pair primarily reduces malignant transformation and hyperplasia of breast cells, effectively preventing the progression of mammary gland hyperplasia and its deterioration.

4.2.3.2. Treating mammary gland hyperplasia by regulating signal paths. The *isorhamnetin* in the herbal pair *Bupleurum* and *Atractylodes* exhibits inhibitory effects on breast cell proliferation by suppressing the PI3K/AKT pathway and the membrane receptor tyrosine protein kinase signaling pathway. Concurrently, it mitigates excessive mammary gland hyperplasia by downregulating the expression of the anti-apoptotic factor Bcl-2 and upregulating the pro-apoptotic factor Bax. As a plant hormone, high concentrations of quercetin can induce exogenous apoptosis by promoting the cleavage of polyribose polymerase, which upregulates the levels of caspase-8 and caspase-3 proteins, thus inhibiting the onset and progression of breast hyperplasia [73].

The *Bupleurum* and *Atractylodes macrocephala* herbal pair can inhibit the expression of BMP-related proteins by blocking the BMP signaling pathway, subsequently reducing breast stem cell proliferation and differentiation. This herbal combination also prevents

extracellular proliferation signals from being transmitted to mammary cells by modulating the MAPK pathway, inhibiting mammary glandular cell proliferation. Additionally, the herbal pair promotes the transmission of apoptosis signals, increasing breast cell apoptosis and effectively treating mammary gland hyperplasia [74,75].

4.2.3.3. Treating mammary gland hyperplasia by inhibiting cell proliferation and promoting cell apoptosis. The *Bupleurum* and *Atractylodes* herbal pair promotes breast cell apoptosis by suppressing the expression of PRKCE, PRKCA, and PIK3CA. Simultaneously, the pair enhances the cytotoxic effects of other drugs on breast cells, thereby inhibiting breast hyperplasia. Furthermore, this herbal pair decelerates breast hyperplasia progression by inhibiting the expression of AKT1, reducing estrogen receptor phosphorylation, and regulating endocrine imbalances within the body. Additionally, the pair prevents breast hyperplasia or its deterioration by decreasing the mutation rate of the HRas gene, inhibiting abnormal proliferation of mammary epithelial cells.

4.2.3.4. Treating mammary gland hyperplasia by estrogen-like effect. The *Bupleurum* and *Atractylodes* herbal pair can have an estrogen-like effect, increasing uterus quality and elevating estradiol levels. For conditions caused by excessive estrogen, such as breast hyperplasia, this herbal pair can counteract estrogen, decrease serum estradiol levels, and regulate progesterone content [76].

4.2.4. Herbal pair (Rhizoma Cyperi + Radix curcumae) can sooth liver, promote the circulation of Qi and blood, relieve pain

4.2.4.1. Treating mammary gland hyperplasia by inhibiting cell proliferation. The herbal pair *Rhizoma Cyperi* and *Radix Curcumae* contains phytosterols (*sitosterol, stigmasterol)*, which can maintain breast cells in the DNA synthesis phase of cell division, inhibit cell proliferation, and promote cell apoptosis [77,78].

The flavonoids (*isorhamnetin*) present in the *Rhizoma Cyperi* and *Radix Curcumae* herbal pair can not only promote cell apoptosis when combined with autophagy/mitotic inhibitors but also treat breast hyperplasia by inhibiting cell growth, inducing cytotoxicity, increasing oxidative stress, arresting the cell cycle, and suppressing breast cell proliferation [79].

4.2.4.2. Treating mammary gland hyperplasia by augmenting immune response. This herbal pair enhances the proliferation of peripheral blood lymphocytes and T cells, thereby augmenting the immune response and preventing the further progression of mammary gland hyperplasia from an immunosuppressive standpoint.

4.2.4.3. Treating mammary gland hyperplasia by participating in signaling pathways. The *Rhizoma Cyperi* and *Radix Curcumae* herbal pair also contains *kaempferol* (a flavonoid of the flavonol subgenus), which participates in various signaling pathways such as EGFR/PI3K/AKT and Notch1/PI3K/AKT. This compound upregulates pro-apoptotic proteins Bax and caspase-3, downregulates anti-apoptotic proteins Bcl-2, thereby blocking the cell cycle and promoting apoptosis [80]. (Fig. 9).

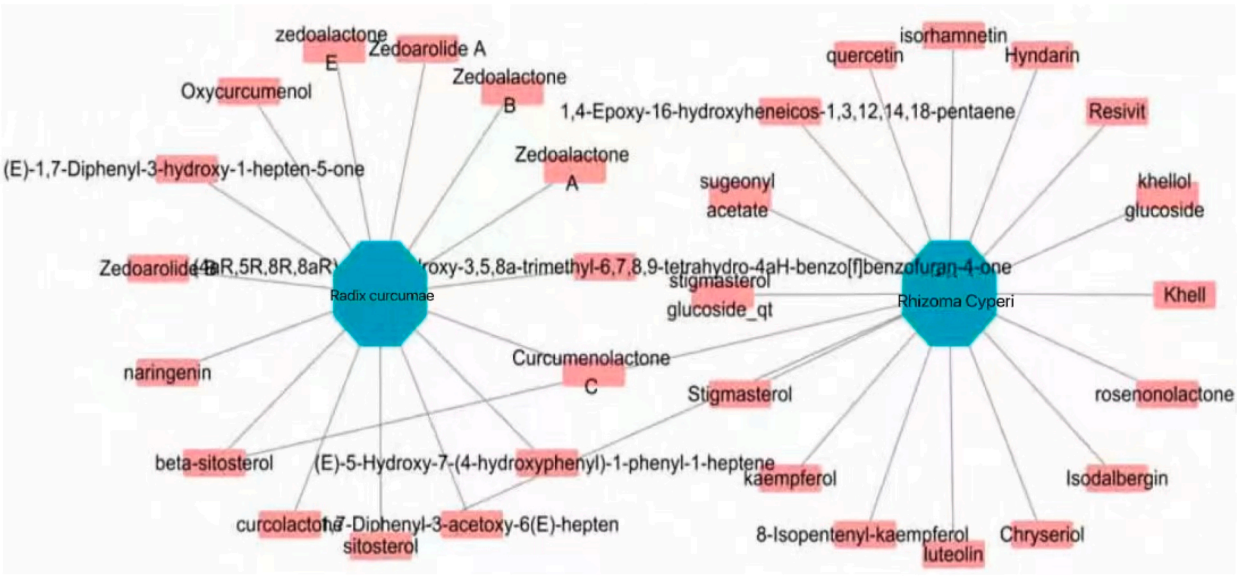


Fig. 9. Herb - Chemical compound network (*Rhizoma Cyperi + Radix curcumae*).

4.2.4.4. Treating mammary gland hyperplasia by regulating hormone levels. Herbal pair *Rhizoma Cyperi* and *Radix Curcumae* contains *quercetin*, a natural phytohormone with estrogenic activity. Its structure resembles estradiol (E2), allowing it to bind with the ER and regulate hormone levels. Quercetin modulates the mammary epithelial cell cycle and induces apoptosis by downregulating the anti-apoptotic factor Bcl-2 and upregulating pro-apoptotic factors Bax and Bak [81,82].

4.2.5. Herbal pair (Perfume lemon + gecko) can clear away stasis, dissipate sputum, eliminate heat and toxin, promote circulation of Qi and blood

4.2.5.1. Treating mammary gland hyperplasia through signal pathway regulation. The herbal pair consisting of *Perfume lemon* and *Gecko* has been found to effectively treat breast hyperplasia through its involvement in the prolactin signaling pathway, vascular endothelial growth factor signaling pathway, and estrogen signaling pathway. By binding to the prolactin receptor (PRLR), the prolactin signaling pathway activates downstream pathways such as JAK/STAT, PI3K/AKT/mTOR, and RAS/RAF/MAPK, which contribute to the regulation of various tissues. The JAK/STAT pathway, primarily activated by PRL-PRLR, influences breast epithelial cell proliferation and milk protein synthesis.

Furthermore, the RAS/RAF/MAPK pathway plays a role in prolactin-mediated breast epithelial cell proliferation. The herbal pair of *Perfume lemon* and *Gecko* can directly or indirectly inhibit prolactin expression by impacting gene expression in the PRL-PRLR downstream pathway, thus suppressing breast tissue hyperplasia [83].

Components of this herbal pair, such as *bergamot lactone, D-limonene, limonin, hesperidin,* and *amino acids,* modulate the prolactin signaling pathway, vascular endothelial growth factor signaling pathway, and estrogen signaling pathway. This modulation occurs through interactions with targets like ER, AR, CYP19A1, PTGS2, and CCND1, illustrating the multi-component, multi-target, and multi-pathway mechanism for treating breast hyperplasia.

Bergamot lactone, present in the herbal pair, can induce ER consumption in breast hyperplasia cells via SMAD4 protein-mediated ubiquitination. Additionally, *bergamot lactone* promotes the formation of autophagosomes in mammary hyperplasia cells by upregulating the expression of phosphatase and tensin homolog (PTEN) in the PI3K/AKT signaling pathway [84].

4.2.5.2. Treating mammary gland hyperplasia by growth factors' regulation. Limonin, found in the *Perfume lemon* and *Gecko* herbal pair, inhibits human mammary cell (ER+, ER-) proliferation by activating anti-caspase-7. Another component, *D-Limonene*, exhibits anti-breast hyperplasia properties by upregulating transforming growth factor-β1 (TGF-β1) and mannose 6-phosphate/insulin-like growth factor II (M6P/IGF-II) [85].

5. Conclusion

In this comprehensive study, we have gathered and systematically analyzed the research findings on the mechanisms of single herbs and herbal pairs in the prevention and treatment of breast hyperplasia over the past 20 years. Our analysis indicates that significant progress has been made in understanding the mechanisms of Chinese herbs in treating breast hyperplasia. However, the current knowledge has not yet reached a level that fully aligns with the scientific, rational, and safe principles of clinical medication.

Moving forward, it is imperative to leverage systems biology technology to further elucidate the mechanisms underlying Chinese herbal treatments for breast hyperplasia. This will provide a more robust foundation for highlighting the advantages of Chinese herbal treatments within the context of traditional Chinese medicine's holistic approach to breast hyperplasia management.

CRediT authorship contribution statement

Xujie Yang: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Writing – original draft, Writing – review & editing. **Pei Xiaohua:** Formal analysis, Methodology. **Zhang Hong:** Data curation. **Zhang Wanyue:** Software, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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