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INVITED REVIEW

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The potential of pharmacological activities of the multi-compound treatment for GERD: literature review and a network pharmacology-based analysis

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Abstract

The prevalence of gastroesophageal reflux disease (GERD) is rapidly increasing due to the adoption of a Westernized lifestyle; at the same time, safe and efficient treatment is required due to the side effects and refractoriness of proton pump inhibitors (PPIs). The frequently used multi-compound treatment for GERD in the current traditional Korean medicine (TKM) clinical field comprises *Crassostrea gigas* Thunberg shell (CGTS), Bambusae Caulis in Taeniam (BCT), Ponciri Fructus Immaturus (PFI), *Scutellaria baicalensis* Georgi (SBG), medicated leaven (ML) and Glycyrrhizae Radix et Rhizoma (GRR). The current review was based on "Kun-Shin-Choa-Sa" theory and network analysis was conducted to explore the potential pharmacological activities, including efficacy and mechanisms of action of multi-compound treatment against GERD. Hypergeometric test results showed that the targets of multi-compound treatment are significantly associated with GERD gene sets, consistent with the literature review findings. In particular, the enrichment analysis indicated that the SBG targets are related to the IL-17 signaling pathway, bile secretion, small-cell lung cancer, and non-small cell lung cancer, corroborating the literature review, particularly concerning anti-inflammatory effect. In the literature review, CGTS and BCT, classified as "Kun," play a role in anti-acid, anti-inflammatory, and anti-oxidative effects. The complementary "Shin" herbs, PFI and SBG, showed functions related to improving the prolonged gastric emptying rate, peristalsis, and a gastric cytoprotective effect. With the role of "Choa," ML was suggested to inhibit *H. pylori* growth and diminish gastric acid secretion, consistent with the gastric acid secretion pathway in the enrichment analysis. However, the enrichment analysis did not show any significantly related pathways for CGTS and PFI, which may reflect the lack of information in the KEGG database in terms of the link between GERD, its mechanisms, and the abundance of minerals in CGTS. Despite the pharmacological potential of multi-compound treatment, this study should be corroborated by well-designed future experimental studies.

Keywords: GERD, Multi-compound treatment for GERD, Network pharmacological analysis, "Kun-Shin-Choa-Sa" theory

Introduction

The prevalence of gastroesophageal reflux disease (GERD) in the Western countries is relatively high, ranging from 10 to 20% [1]. The 2008 GERD prevalence in South Korea was 3.5–8.5%, which is lower than that in the West [2–4]. However, the recent prevalence in South Korea has been increasing due to the westernization of

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dietary lifestyle, an increase in the obese population, and rapid growth in the elderly population [5]. To ease recurrent severe symptoms that lower patient quality of life, safe and effective GERD therapy is required. Ranitidine and proton pump inhibitors (PPIs) are medications that decrease stomach acid production and are commonly used to treat peptic ulcer disease and GERD. Recently, the United States Food and Drug Administration announced the decision to withdraw all prescription and over-the-counter (OTC) ranitidine drugs from the market. Although the prescription of PPI drugs is increasing rapidly due to the "carcinogenic" concerns surrounding ranitidine, experts are warning against the long-term use of PPI drugs, which may cause more severe side effects than ranitidine [6]. Therefore, there is an increasing interest in complementary and alternative medicine (CAM) with synergistic effects and fewer side effects.

In Korea, traditional Korean medicine (TKM) practitioners can legally allowed to prescribe herbal medicines according to the pattern differentiation of traditional East Asian medicine (TEAM) for patients by combining herbs that have been used for a long time. TKM practitioners diagnose and prescribe based on the unique disease classification system of *Donguibogam* published by Dr. Heo Jun in the Joseon Dynasty (400 years ago) based on the "Kun-Shin-Choa-Sa" theory [7]. Due to their uniqueness and considerable efficacy, the treatments are still widely adopted in the current TKM clinical field. Some of the treatments may not be effective for modern patients due to lifestyle changes including diet and environment. Some established TKM practitioners have their own confidential prescriptions formulated by empirically supported treatment knowledge. In the present study, a frequently used multi-component prescription (P05), for the curative therapy of GERD in the clinical field is reviewed from a pharmacological activity and perspective. Additionally, P05 was analyzed in silico via network analysis to predict and explore the specific targets and pathways for each ingredient as a background for future assessments of P05 efficacy in in vitro, in vivo, and clinical trials.

Study design and methods

A literature review and network pharmacological analysis were conducted to elucidate the potential pharmacological activities of a multi-compound herbal mixture in GERD treatment.

Search strategy for review

A search was performed for English and Korean literature in online databases, including PubMed, Google Scholar, and ScienceDirect. Several keywords were used, including "GERD," "GERD treatment," "CAM treatment," "*Crassostrea gigas* Thunberg shell," "*Crassostrea gigas* Thunberg shell GERD [or gastrointestinal (GI) diseases]," "Bambusae Caulis in Taeniam," "Bambusae Caulis in Taeniam GERD [or gastrointestinal (GI) diseases]," "Ponciri Fructus Immaturus," " Ponciri Fructus Immaturus GERD [or gastrointestinal (GI) diseases]," "*Scutellaria baicalensis* Georgi," "*Scutellaria baicalensis* Georgi GERD [or gastrointestinal (GI) diseases]," "Medicated leaven," "Medicated leaven GERD [or gastrointestinal (GI) diseases]," "Glycyrrhizae Radix et Rhizoma," "Glycyrrhizae Radix et Rhizoma GERD [or gastrointestinal (GI) diseases]."

Network pharmacological analysis

Network pharmacological analysis was performed by predicting the targets of the ingredients in P05 and constructing a herb-compound-target network. The information on herb compounds was obtained from the TCM-MESH database (http://mesh.tcm.microbioinformatics.org). "Bambusae Caulis in Taeniam" and "Medicated leaven" were not included in the TCM-MESH database; therefore, we obtained their compound information from previous studies that were based on high-performance liquid chromatography[8, 9]. To filter out compounds that hardly play a drug role in oral administration, we employed the quantitative estimate of drug-likeness (QED) method, which measures drug-likeness based on molecular descriptors [10]. QED outperforms other drug-like classifiers, such as the Rule of 5, Ghose filter, and Veber's rule. The QED ranges from 0 to 1, and the closer the QED of a compound is to 1, the more drug-like it is. We set the QED cut-off value for the compounds at 0.35, as it is the overall average of QED for FDI-approved oral drugs. The potential targets of the compounds were obtained from STITCH (http://stitch.embl.de/) based on the combined scores of interactions between compounds and targets. We set the threshold of the combined score at 0.7, which is regarded as the criterion for filtering prediction results with high confidence. An herb-compound-target network was then constructed by linking herbs to their compounds, and compounds to their predicted targets using in-house Python (version 3.8) code and visualized using Cytoscape (version 3.8.2). Gene set enrichment analysis (GSEA) based on the Kyoto Encyclopedia of Genes and Genomes (KEGG, https://www.genome.jp/kegg/), DisGeNET (https://www.disgenet.org/search), and Human Phenotype Ontology (HPO, https://hpo.jax.org/) databases was performed to identify the relationship between herbal targets and diseases, and potential pathways associated with GERD using hypergeometric tests and Enrichr [11–14]. We calculated adjusted p-values, and combined scores using the logarithm of the multiplication of the p-values and z-scores

(note that the combined score differs from the combined score in STITCH).

GERD and its pathophysiology

GERD is a complex clinical condition whose definition was standardized in 2006 with the Montreal Consensus. The relationship between symptoms and stomach content reflux is complex due to the difficulty distinguishing between GERD and functional esophageal disorders, which requires comprehensive classification [15].

The primary symptoms of GERD contributing to esophageal mucosal injury are heartburn, acid regurgitation, dysphagia, and globus [16, 17]. GERD is accompanied by a wide range of complications such as erosive esophagitis, esophageal stricture, Barrett's esophagus, and esophageal adenocarcinoma [18]. Understanding the pathophysiology of GERD plays a significant role in exploring future treatment targets, since proton pump inhibitor resistance is a common problem [19]. Multiple factors are involved in the pathogenesis of GERD, including transient lower esophageal sphincter relaxation and other lower esophageal sphincter (LES) pressure abnormalities. Furthermore, hiatal hernia, injured esophageal clearance, prolonged gastric emptying, and impaired mucosal defensive factors are responsible for the pathophysiology of GERD. Hiatal hernia promotes LES dysfunction, and injured esophageal clearance contributes to delayed acid exposure of the mucosa. Prolonged gastric emptying leads to gastric distension, and consequently, postprandial GERD [20]. In addition to the factors mentioned above, increased distensibility of the esophagogastric junction, obesity, and acid pocket, layers of highly acidic gastric secretion, can be risk factors for GERD. Multiple mechanisms have an impact on the perception of GERD symptoms. With regard to the involvement of *Helicobacter pylori* infection, the relationship is still subject to debate. Meanwhile, it has been reported that *H. pylori* eradication in GI acid production changes is not an important concomitant factor for GERD, according to the Maastricht V/Florence Consensus report [6].

Typically, when food is swallowed, the LES allows food to flow into the stomach and subsequently, tightens again to maintain the stomach content in the stomach via the esophagogastric junction (EGJ) [21]. The EGJ is composed of the smooth muscle of the LES surrounded by oblique gastric fibers and the crural diaphragm acting as an anti-reflux barrier [22]. When LES pressure is lower than the intragastric pressure, reflux occurs. Specifically, the frequency of transient lower esophageal sphincter relaxation (TLESR) is augmented when the intragastric pressure increases.

Treatment for GERD and its shortcomings

Patients with GERD need appropriate medical care to alleviate the recurrent and refractory symptoms that severely deteriorate their quality of life and burden them with medical expenses. Proton pump inhibitors (PPIs) effectively suppress gastric acid secretion, manage the esophageal mucosa, alleviate GERD symptoms, and prevent complications among the available GERD medications [23]. PPI medications function by irreversibly hindering an H $_{þ}$/K $_{þ}$ adenosine triphosphatase in the stomach's parietal cells, which inhibits hydrogen (protons) from being pumped into the gastric lumen to produce hydrochloric acid. Commonly available PPIs include omeprazole (Prilosec), esomeprazole (Nexium), lansoprazole (Prevacid), dexlansoprazole (Dexilant), pantoprazole (Protonix), and rabeprazole (Aciphex), with omeprazole being the most prescribed among those [21].

Although PPIs have become a major therapy for GERD, approximately 20–40% of GERD patients do not respond to PPIs [23, 24]. Refractory GERD is a widespread clinical condition that presents a lack or absence of response after 4–8 weeks of twice-daily PPI treatment [25]. In gastroenterology, refractory GERD is a frequent cause for medical care. Several GERD patients continue to have clinical manifestations despite PPI use.

Furthermore, PPIs have side effects, such as headache, diarrhea, nausea, and vomiting. According to a previous report, PPIs are associated with chronic kidney disease, cardiovascular disease, and gastric, esophageal, and liver cancers. One Washington University-based researcher warned that long-term PPI use over several months or years is unsafe [21]. Nutrient deficiencies as PPI side effects have also been reported, especially of micronutrients, including magnesium, vitamin B_{12}, calcium, iron, and vitamin C, whose absorption is negatively affected [26].

Taken together, dietary and lifestyle changes as a behavioral treatment are recommended for GERD patients because these have no side effects, and obesity is a significant risk factor for GERD. In fact, previous research has indicated that a reduced body mass index can lead to a reduction in GERD symptom frequency. In general, losing weight, elevating the head of the bed, avoiding sleeping on the right side of the body, avoiding eating at least three hours before bedtime, avoiding fatty and spicy meals, avoiding excessive eating, etc., are recommended as dietary and lifestyle interventions [21]. However, this is not a fundamental solution for GERD; thus, effective and safe therapy for GERD is still necessary.

Multi-compound treatment and the Kun-Shin-Choa-Sa theory

It has been reported that the refractoriness and several side effects of long-term PPI use, regarded as the primary treatment, have invariably increased needs for CAM-based natural drugs. Therefore, in clinical practice, several GERD patients turn to TEAM as they experience recurrent GERD symptoms despite PPI treatment [23]. Unlike conventional medications, herbal mixtures in TEAM are based on a multi-component and multi-target approach derived from a holistic philosophy [27]. To deal with complex diseases, multi-component and multi-target approaches are potentially meaningful despite the challenges in elucidating the complex mechanisms involved. Among TEAM, TKM practitioners diagnose according to pattern identification by observing the symptoms and signs exhibited by patients based on *Donguibogam* written by Dr. Heo Jun in the Joseon Dynasty (400 years ago) [7]. In TEAM, GERD is classified into three different syndromes (patterns), including "Disharmony of Liver and Stomach," referring to the failure of smooth flow of liver qi and stomach qi descending; "Deficiency-Cold of Spleen and Stomach, a part of the digestive system and sources of qi and blood in TEAM," meaning the deficiency of qi and blood and dysfunction of spleen-stomach [28, 29].

After diagnosis by defining a phenotype that can guide treatment selection as a unique diagnostic system, TKM practitioners subsequently prescribe using the "Kun-Shin-Choa-Sa" theory translated as "king-minister-assistant-ambassador" for its synergistic effects. In the past, ancient Koreans thought that taking care of the human body is philosophically similar to running a nation. The "Kun" refers to a major medicine possessing the main drug efficacy, which is supported by three different types of medicines: the "Shin" (minister), which boosts and complements the Kun's efficacy; "Choa" (assistant), which reduces the side-effects caused by the "Kun", the "Sa" (ambassador), which facilitates delivery of the "Kun" [30].

A previous study indicated that despite the intrinsic complexity of herbal mixtures, medicinal plants have the advantage of synergistic interactions among their multiple-components and poly-pharmacological effects [27]. Since TKM considers the human body as one, which is a complex interacting system, with each cell, tissue, and organ connected to each other, therapeutic drugs to restore energy and overall body balance are also prescribed by TKM practitioners according to the "Kun-Shin-Choa-Sa" theory. Consequently, it is believed that a single active ingredient or component made for a single target, such as PPIs, can cause severe side effects, as it arbitrarily controls one organically connected part. Here, a multi-component herbal mixture prescribed by TKM practitioners based on the "Kun-Shin-Choa-Sa" principle using medicinal plants that have been used for a long time is presented. The frequently used multi-compound herbal medicine, P05, has been modified for modern GERD patients based on 30 years of knowledge. P05 comprises a basic composition for GERD therapy and other specific herbs. Six P05 ingredients can be added according to specific symptoms and signs since TKM is an individualized treatment for different patients with the same disease [29, 31].

The herbal mixture (P05) consists of *Crassostrea gigas* Thunberg shell (CGTS), Bambusae Caulis in Taeniam (BCT), Ponciri Fructus Immaturus (PFI), *Scutellaria baicalensis* Georgi (SBG), medicated leaven (ML) and Glycyrrhizae Radix et Rhizoma (GRR). Among the complex P05 components, CGTS and BCT play the role of "Kun", while the roles of "Shin", "Choa", and "Sa" are played by PFI and SBG, ML, and GRR, (Fig. 1).

The bioactive components of P05 for GERD treatment are presented in Table 1. Previous reports have shown that P05 can improve GERD symptoms through anti-inflammation, apoptosis modulation, hormone levels regulation, antioxidant activity, acid suppression, pepsin secretion reduction, and mucosal protection.

Crassostrea gigas Thunberg shell as one of "Kun-Kings"

Oysters shell, Ostreae Concha/*Crassostrea gigas* Thunberg shell (CGTS), has been commonly used with other herbs in TEAM prescriptions to alleviate diverse symptoms, including abdominal mass, tinnitus, insomnia, dizziness, palpitations, scrofula, and subcutaneous nodules. It has been reported that immune system reinforcement, anti-acid, anti-gastric ulcer, sedation, anti-tumor, and anti-virus properties are among the pharmacological effects of oyster shell [32]. Calcium carbonate (more than 95% content) and calcium phosphate have been confirmed to be representative components of oyster shell. Oyster shell has been widely used as a primary ingredient in calcium supplements, pharmaceuticals, and animal feed, owing to its calcium carbonate content. Taurine and betaine have been reported as components of oyster muscle blocks in oyster shell. Various biological functions of taurine, such as bile acid conjugation, antioxidation, membrane stabilization, and modulation of calcium signaling, have been reported. Taurine and betaine are known to improve diabetes and have lipid-lowering effects [33]. Additionally, calcium sulfate and proteins such as conchiolin are CGTS components, and the anti-inflammatory effect of CGTS extract has been reported [34, 35].

Bambusae Caulis in Taeniam as one of "Kun-Kings"

Bamboo shavings, Zhu Ru/Bambusae Caulis in Taeniam (BCT), medicinal herb in TEAM, is derived from the

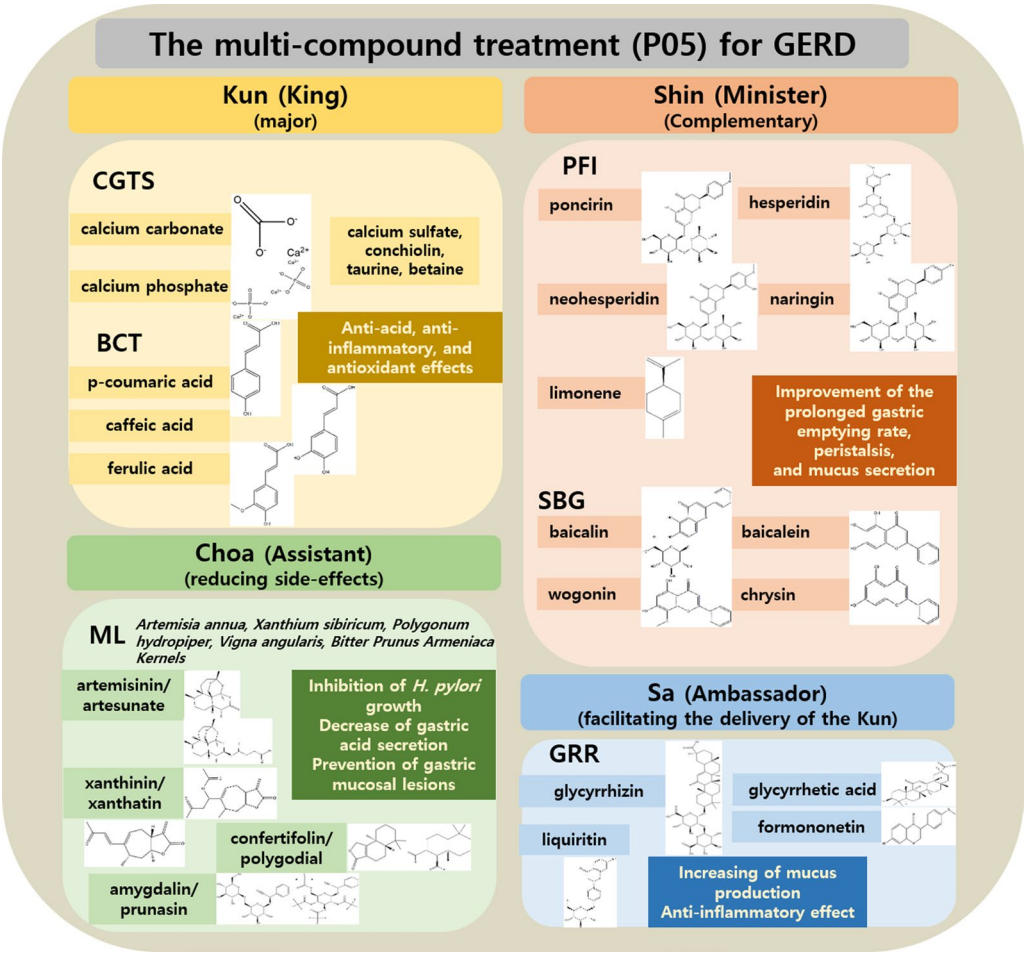


Fig. 1 Components and major roles of multi-compound treatment based on the "Kun-Shin-Choa-Sa" theory. CGTS and BCT correspond to "Kun", PFI and SBG correspond to "Shin", ML to "Choa", and GRR to "Sa". *CGTS Crassostrea gigas* Thunberg shell, *BCT* Bambusae Caulis in Taeniam, *PFI* Ponciri Fructus Immaturus, *SBG Scutellaria baicalensis* Georgi, *ML* medicated leaven, *GRR* Glycyrrhizae Radix et Rhizoma

inner bark of *Phyllostachys nigra* var. *henosis,* and has been reported to have various pharmacological activities. It has been widely used to treat diarrhea, fever, and chest inflammation. A recent study indicated a beneficial effect on cigarette smoke-induced pulmonary and intestinal inflammation, asthma, and neurodegenerative diseases [36, 37]. The major constituents of BCT are *p*-coumaric acid, which prevents oxidative gastric damage; caffeic acid, which has an anti-inflammatory effect on gastric mucosal damage via the nitric oxide (NO) pathway; ferulic acid, which decreases the total and free acidity of gastric contents [38–40]. A previous study pointed out that phenolic acids such as *p*-coumaric, caffeic, and ferulic acids play a significant role by displaying anti-secretory and effects, anti-histaminic effects, down-regulating parietal cell H þ/K þ adenosine triphosphatase, enhancing mucosal defensive factors, and cytoprotective effect by increasing the prostaglandin content and mucus formation in the gastric mucosa [38].

Poncirus Fructus Immaturus as one of "Shin-Ministers"

Poncirus immature fruit, Ponciri Fructus Immaturus (PFI), is the dried immature fruit of the trifoliate orange (*Poncius trifoliate*). It has been traditionally applied to treat diverse diseases, including ulcers, dyspepsia, constipation, gastritis, and several inflammation-associated ailments in TEAM [32]. A recent study also pointed out a beneficial effect against hyperlipidemia [33]. A growing body of evidence suggests the anti-inflammatory and gastroprotective effects of PFI [41]. The representative constituents of PFI are poncirin, which attenuates HCI-induced gastric lesions; hesperidin, which improves the delayed gastric emptying rate; neohesperidin, which stimulates mucus secretion; naringin, which prevents

Table 1 Bioactive components in an herbal mixture (P05) for GERD

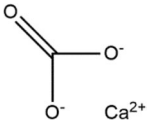
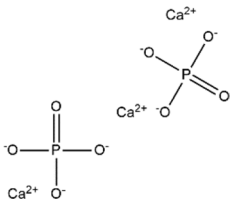
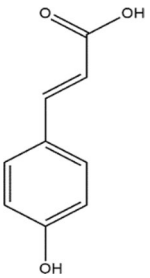
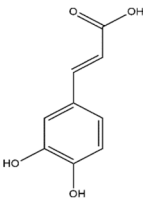
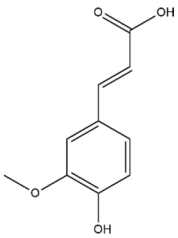
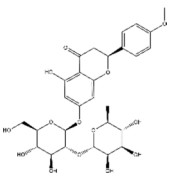
English name	Latin names	Main components	Chemical structure	Primary mechanism of action	References
Oyster shell	*Crassostrea gigas* Thunberg shell	Calcium carbonate		Anti-acid • Neutralization of esophageal pH by increasing plasma gastrin levels • Inhibition of pepsin and bile acid	[73, 75, 76]
		Calcium phosphate		Anti-acid • The phosphate ions likely react with hydrochloric acid to neutralize the pH	[77, 78]
Bamboo shavings	Bambusae Caulis in Taeniam	*p*-Coumaric acid (p-CA)		Prevents oxidative gastric damage by: • Attenuating the ulcer-elevated levels of malondialdehyde • Restoring the ulcer-depleted levels of reduced glutathione and the antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase	[38, 79]
		Caffeic acid		• Attenuation of gastric mucosal damage via the NO pathway	[39, 79]
		Ferulic acid		• Increase in gastric pH • Decrease in total acidity, free acidity, and lipid peroxidation • Inhibition of gastric acid secretion by diminishing H^+-K^+ATPase enzyme	[40, 79]
Poncirus immature fruit	Ponciri Fructus Immaturus	Poncirin		• Attenuation of HCl/ethanol-induced gastric lesions • Inhibition of iNOS, COX-2, TNF-α, and IL-6 expression via the down-regulation of NF-κB binding activity	[52, 53]

Table 1 (continued)

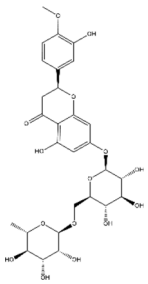
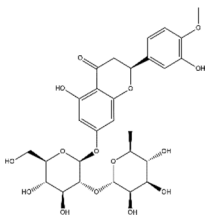
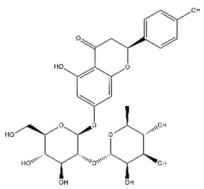
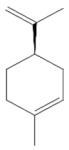
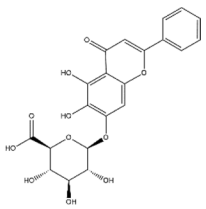
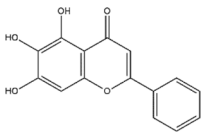
English name	Latin names	Main components	Chemical structure	Primary mechanism of action	References
		Hesperidin		• Improvement of the delayed gastric emptying rate • Inhibition of *H. pylori* growth	[53, 55]
		Neohesperidin		• Prevention of HCl/ethanol-induced gastric lesions • Stimulation of mucus secretion • Increase in gastric pH by decreasing in gastric secretion and gastric acid output	[53]
		Naringin		• Prevention of gastric ulcer via inhibiting inflammation markers, including TNF-α, IL-6, CRP, iNOS, and caspase-3 levels	[80]
		Limonene		Neutralization of the gastric acid effect by: • Coating the stomach wall • Protecting the mucosal lining from gastric acid exposure • Improvement of healthy peristalsis	[74]
Scutellariae root	*Scutellaria baicalensis* Georgi	Baicalin		• Down-regulation of TRPV1 in DRG neurons	[42, 45]
		Baicalein		• Decrease of acute ulcers via α2-adrenoreceptors, SH compounds, NO, PG, and KATP channels • Increases in gastric mucus secretion • Inhibition of gastric acid secretion by suppressing the histaminergic pathway and H^+, K^+-ATPase activity	[43]

Table 1 (continued)

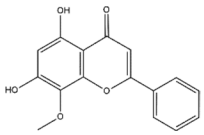
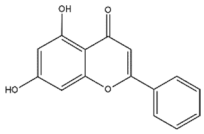
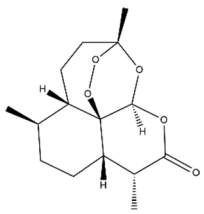
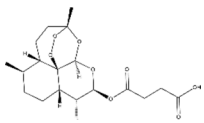
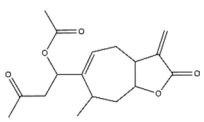
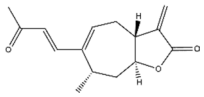
English name	Latin names	Main components	Chemical structure	Primary mechanism of action	References
		Wogonin		• Anti-gastric inflammatory agent via arachidonic acid metabolism such as induction of prostaglandin D2 and suppression of 5S-hydroxyeicosatetraenoic acid (5S-HETE) • Preventive induction of profuse apoptosis in the stomach	[44]
		Chrysin		Anti-inflammation • Increase of cell proliferation effect via EGF • Reduction of cellular apoptosis by modulating caspase-3 • Reduced TNF-α, NF-κB p65 unit, interleukin-1β (IL-1β), IL-17A, interferon-gamma (IFN-γ), IL-12 and IL-6 • Inhibitory agent against PPAR-γ • Down-regulates pro-inflammatory enzymes, such as COX-2, myeloperoxidase (MPO), iNOS, prostanoids, and phospholipase A2 • Blocks activation of NF-κB and degradation of IL-1β-stimulated IκB-α	[49, 81]
Medicated leaven	*Artemisia annua*	Artemisinin		• Inhibition of *H. pylori* growth • Suppression of *H. pylori*-induced NF-κB activation	[54]
		Artesunate		• Decrease in gastric acid secretion • Inhibition of oxidative stress markers such as malondialdehyde, glutathione, and superoxide dismutase activity • Reversing expression of pro-inflammation markers, including TNF-α, IL-1β, IL-6, NF-κB, myeloperoxidase, and COX-2	[58, 59]
	Xanthium sibiricum	Xanthinin		No previous research	
		Xanthatin		• Expressing anti-inflammation effect via suppression of NO production, PGE2 synthesis, and 5-lipoxygenase activity	[60, 61]

Table 1 (continued)

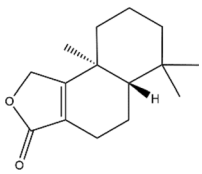
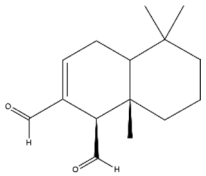
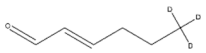
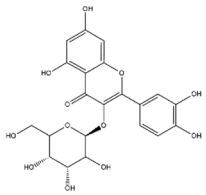
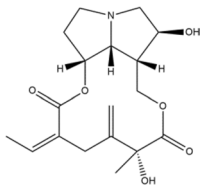
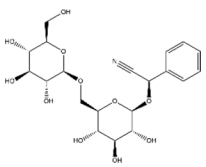
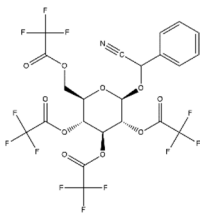
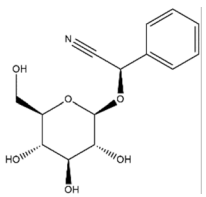
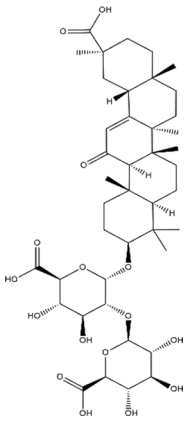
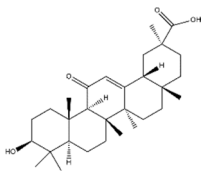
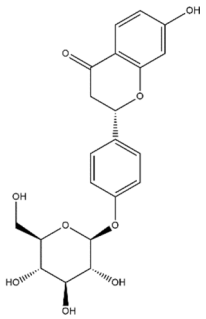
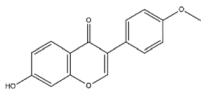
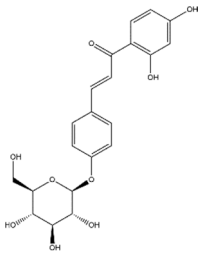
English name	Latin names	Main components	Chemical structure	Primary mechanism of action	References
	Polygonum hydropiper	Confertifolin		No previous research	
		Polygodial		• Prevention of gastric mucosal lesions by regulating endogenous prostaglandins, NO, sulfhydryl compounds, and vanilloid receptor	[82, 83]
		(2E)-hexenal		• Inhibition of *H. pylori* growth	[84]
		Isoquercetrin		• Prevention of eosinophilic esophagitis through a decrease in eosinophil, neutrophil, and IL-5 levels	[85]
	Vigna angularis	Angularin		• Anti-inflammatory effects through mitigation of NO production	[54]
	Bitter *Prunus armeniaca* Kernel	Amygdalin		• Inhibition of gastric ulcer via diminishing NO production and TNF-α expression	[86]
		Prunasin		• Anti-inflammatory activity via attenuation of IL-6, IL-8, IL-23, HSP70 and ICAM-1 expression	[57]
					

Table 1 (continued)

English name	Latin names	Main components	Chemical structure	Primary mechanism of action	References
Licorice root	Glycyrrhizae Radix et Rhizoma	Glycyrrhizin		• Expressing anti-inflammatory effect through inhibiting the generation of ROS by neutrophils	[57, 58]
		Glycyrrhetic acid		• Inhibition of *H. pylori* growth • Inhibition of inflammatory effect via attenuating pro-inflammatory markers such as iNOS, COX-2, TNF-α, IL-1β, and IL-6	[59, 87]
		Liquiritin		• Anti-inflammatory effects via attenuating pro-inflammatory markers such as iNOS, COX-2, TNF-α, IL-1β, and IL-6	[87]
		Formononetin		• Decrease in gastric secretion • Increase in mucus production	[60]
		Isoliquiritin		Antioxidant: • Activation of Nrf2 pathway Anti-inflammation: • Suppression of NF-κB pathway	[61]

gastric ulcers, and limonene, which enhances healthy peristalsis.

Scutellaria baicalensis Georgi as one of "Shin-Ministers"

In TEAM, the root of Scutellariae, *Scutellaria baicalensis* Georgi (SBG), plays a significant role in clearing dampness and heat, resolving phlegm, purging fire, and detoxification.

SBG has been used for treating allergic and inflammatory diseases, and a variety of beneficial effects against cancer, psychiatric disorders, infectious and, circulatory diseases, among others, have been reported. In particular, concerning gastrointestinal (GI) diseases, it has been reported that baicalin has a treatment effect on *H. pylori* infection. At the same time, baicalein increases gastric mucus secretion, and wogonin acts as an anti-gastric inflammatory agent—these three constitute the major bioactive ingredients of SBG [42–45]. SBG has also been reported to be beneficial for improving liver fibrosis [46]. The representative constituents of SBG include baicalin, with an anti-inflammatory activity which occurs by communication with baicalein and oroxylin A via intestinal microflora through inhibiting NF-κB activation [47]; baicalein with antioxidant, anti-inflammatory, and anti-allergic effects and enhances intestinal barrier function [48]; wogonin, which has a gastric cytoprotective effect by enhancing PGD_2 biosynthesis accompanied by decreased 5-HETE biosynthesis in alcohol-induced gastric mucosa injury and also has a strong anti-inflammatory effect [44]; chrysin, which has anti-inflammatory and antitoxic functions by inhibiting AGS human gastric cancer cell line growth [49, 50].

Medicated leaven as a "Sa-Ambassador"

Medicated leaven (ML) is a fermented mixture of wheat flour, *Artemisia annua*, *Xanthium sibiricum*, *Polygonum hydropiper*, *Vigna angularis*, and bitter *Prunus armeniaca* Kernel. ML, Shen qu, has been used to treat gastrointestinal (GI) diseases for thousands of years, such as reducing food stagnation, strengthening the stomach (stomach cold with food stagnation or accumulation, with epigastric and abdominal fullness or distention), lack of appetite, borborygmus, and diarrhea [51]. The primary components and related specific bioactive constituents of ML are described in the following sections.

Artemisia annua-Artemisinin, Artesunate

Artemisia annua inhibits *H. pylori* growth and decreases gastric acid secretion.

Xanthium sibiricum-Xanthinin, xanthatin

Xanthium sibiricum has been used to treat inflammation, pain, and infection, and traditionally known to dispel wind and dampness, detoxify, alleviate rheumatic pain [52].

Polygonum hydropiper-confertifolin, polygodial, (2E)-hexenal, isoquercitrin

Polygonum hydropiper is widely used as a traditional remedy for gastroenteritis and dysentery to eliminate phlegm, dampness, and inflammatory activity [53].

Vigna angularis (adzuki bean)

A previous study highlighted that angularin, the main component of *Vigna angularis*, has an anti-inflammatory effect by mitigating NO production [54].

Bitter Prunus armeniaca Kernel (bitter apricot kernels)-Amygdalin, prunasin

According to literature, the kernel of *P. armeniaca* L. has reported remarkable pharmacological effects such as strong antioxidant, antimicrobial, anti-inflammatory, anticancer, hepatoprotective, and cardioprotective activities [55].

Glycyrrhizae Radix et Rhizoma as a "Choa-Assistant"

Glycyrrhizae Radix et Rhizoma (GRR), licorice root, has diverse pharmacological activities, including antiviral, anti-inflammatory, anti-tumor, antimicrobial, anti-oxidative, anti-allergic, and hepatoprotective activities [56]. The primary components of GRR are glycyrrhizin, which has an anti-inflammatory effect by inhibiting the generation of ROS; glycyrrhetic acid, which inhibit *H. pylori* growth and inflammatory activity; liquiritin, with anti-inflammatory effects; formononetin, which decreases gastric secretions and increases mucus production; isoliquiritin, with anti-inflammatory activity [42, 57–61].

The results of network pharmacological analysis for P05

We conducted a network pharmacological analysis to elucidate the system-level effects and mechanisms of P05. To investigate whether the targets of P05 are associated with GERD, hypergeometric tests were performed based on DisGeNET and HPO. Our results showed that the targets of P05 were statistically associated with GERD gene sets($p = 0.0050$), esophagitis ($p = 2.3 \times 10^{-6}$), and esophageal neoplasm($p = 7.6 \times 10^{-10}$) (Table 2). These

results indicate that the targets of P05 may be related to the pathophysiology of GERD and the GERD therapeutic effects.

Next, to explore the potential mechanisms of P05 in GERD, enrichment tests were conducted based on KEGG pathways. The pathways obtained from literature can be categorized into three groups: (1) pathways related to the digestive system: gastric acid secretion, epithelial cell signaling in *H. pylori* infection, pancreatic secretion, and bile secretion; (2) pathways related to cytokines, including TNF signaling pathway, IL-17 signaling pathway, inflammatory mediator regulation of TRP channels, NF-κB signaling pathway, and MAPK signaling pathway; and (3) pathways related to intrathoracic pressure: small-cell lung cancer, non-small cell lung cancer, tuberculosis, asthma, fatty acid degradation, and thermogenesis [62–65]. Figure 2 shows that P05 and its herbs were significantly associated with pathophysiologically important pathways. P05 is associated with all the digestive system-related pathways. Specifically, SBG and GRR are related to bile secretion and signaling in *H. pylori* infection, and ML is simultaneously related to gastric acid secretion, indicating that P05 herbs complementarily affect the digestive system to control GERD. It also shows that P05 is related to multiple cytokine pathways, suggesting that P05 can alleviate esophagitis by modulating cytokine signaling pathways and that ML is significantly related to obesity-related pathways; otherwise, SBG, GRR, and BCT are related to respiratory diseases. These results indicate that P05 herbs can also modulate other diseases that aggravate GERD in a complementary manner. CGTS and PFI were not significantly related to these pathways.

To identify therapeutic compounds in P05 focused on digestive system-related pathways more directly involved in GERD, we isolated targets associated with digestive system-related pathways and visualized the herb-compound-target network. Among the pathways, the relationship between GERD and *H. pylori* is controversial: some argue that *H. pylori* infection can cause a decrease in gastric acid secretion and erosive esophagitis [66, 67], but others insist that there is no significant relationship between presence of *H. pylori* and prevalence or the symptom profiles of GERD [68, 69]. In this study, we included the *H. pylori*-related pathway in network pharmacological analysis not to miss probable therapeutic mechanisms of P05 for GERD. As shown in Fig. 3, palmitic, hexanoic, and lauric acids, which are compounds of ML, and nobiletin, a PFI compound, are mainly associated with digestive fluid secretion, including bile, pancreatic, and gastric acid secretions. This finding corroborates the recognition of 'ML' and 'PFI' in traditional Asian medicine, as dyspepsia remedies. At the same time, several SBG and GRR compounds are related to signaling in *H. pylori* infection. Their targets, NFκB1, CASP3, and others, are associated with inflammation and the cell cycle, suggesting that SBG and GRR modulate cellular physiology in *H. pylori* infection. Of note is, baicalein, which is a well-known SBG compound, broadly affecting pathways linked to the digestive system, implying that baicalein itself modulates GERD's digestive system control. We noted that the targets of each herb are usually separated, supporting the notion that herbs in GERD can complimentarily affect digestive system-level pathology. Among these pathways, the relationship between GERD and *H. pylori* is controversial, some arguing that *H. pylori* infection can cause a decrease in gastric acid secretion and erosive esophagitis [66, 67]. In contrast, others insist that there is no significant relationship between *H. pylori* and prevalence or the symptom profiles of GERD [68, 69]. In the present study, we included the *H. pylori*-related pathway to avoid omitting potential therapeutic mechanisms. In our results, CGTS and PFI were not significantly related to these pathways.

Discussion

The current review and network analysis revealed that the multi-compound treatment, P05, might be a potential therapeutic agent for GERD. The hypergeometric tests' results showed that the targets of P05 were significantly associated with GERD gene sets. This is consistent with the results of a review based on accumulated literature demonstrating that the herbal mixture, P05, prescribed based on the "Kun-Shin-Choa-Sa" theory, has comprehensive therapeutic effects on GERD. The major medicinal plants of the "Kun-Shin-Choa-Sa" theory-based P05 are *Crassostrea gigas* Thunberg shell (CGTS), Bambusae

Table 2 Enrichment analysis based on GERD gene sets and GERD-related diseases

Diseases	The number of disease-related genes	The number of targets of P05	Overlap	Adjusted *P*-value
GERD	485	680	38	0.0050
Esophagitis	109	680	18	2.3×10^{-6}
Esophageal neoplasms	1185	680	108	7.6×10^{-10}

GERD gastroesophageal reflux disease

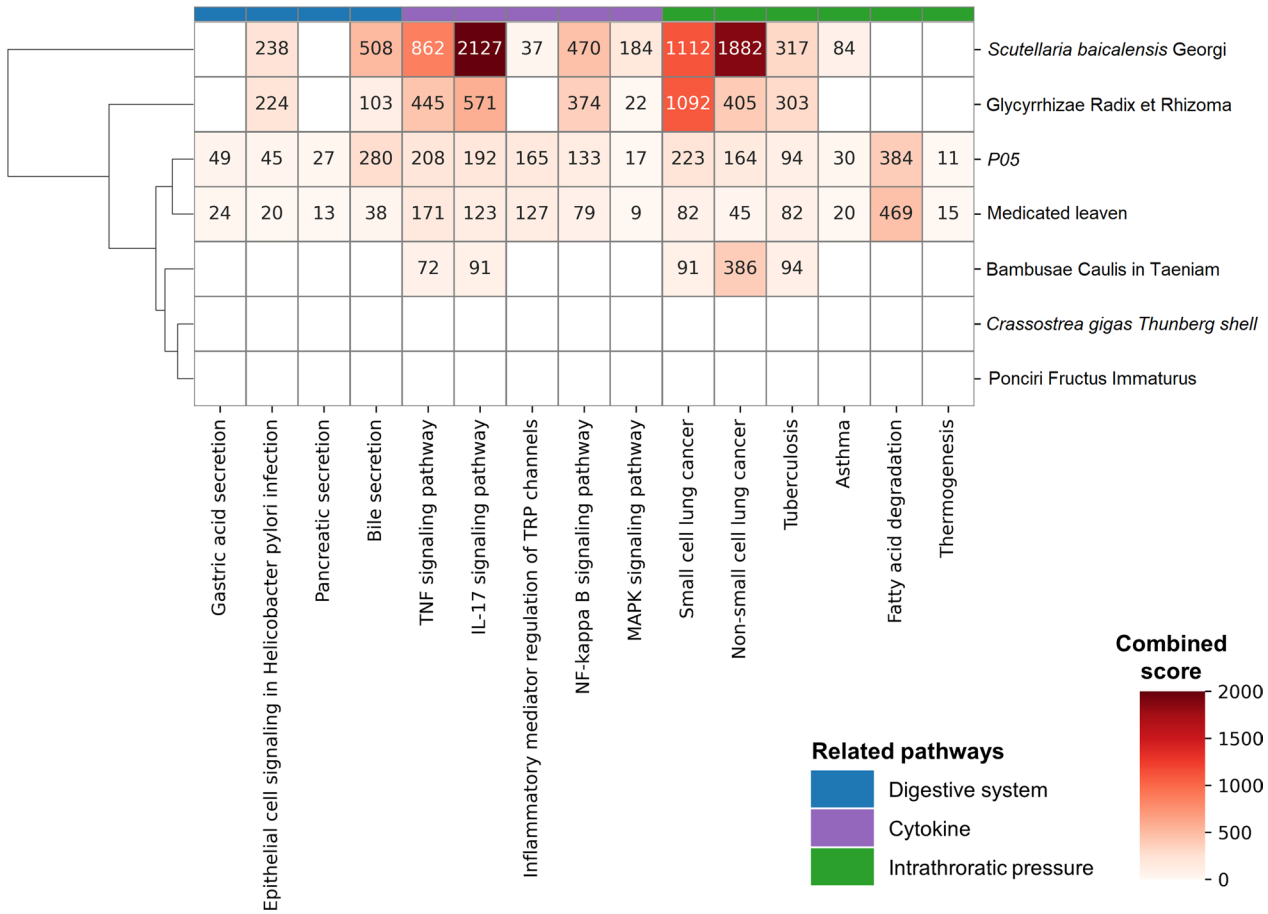


Fig. 2 Enrichment pathways associated with GERD by P05 targets and its constituent herbs. Colors of cells indicate combined scores that represent reliability of predicted associations between herbs and pathways. Colors of columns indicate categories of pathways. Combined scores with corresponding adjusted $p > 0.05$ are masked

Caulis in Taeniam (BCT), Ponciri Fructus Immaturus (PFI), *Scutellaria baicalensis* Georgi (SBG), medicated leaven (ML), and Glycyrrhizae Radix et Rhizoma (GRR). Among the complex components of P05, CGTS and BCT play "Kun" role, whiles the roles of "Shin," "Choa," and "Sa" are fulfilled by PFI and SBG, ML, and GRR, respectively. P05 prescription emanates from diagnosis based on the TKM theory that phlegm, a significant pathological agent, is the primary cause of GERD. Zhu Zhenheng, a renowned TEAM practitioner from the Yuan dynasty, stated that nine out of ten diseases are caused by phlegm [70]. In TEAM, the dysfunction of fu organs that should send food down occurs due to phlegm, which leads to GERD as one of "damjeok syndrome" (damjeok means phlegm mass) [71]. P05 was prescribed based on the pathological diagnosis of GERD modified for modern individuals from *Donguibogam.* As a matter of course, the prescription will be changed in more detail by adding more medicinal herbs according to the specific symptoms and signs of the patients.

According to literature, two medicinal herbs, CGTS and BCT, corresponding to "Kun-king," have medicinal components playing a strong role in decreasing gastric acidity and antioxidant and anti-inflammatory activities. Consistent with our findings, CGTS has been widely used as a food supplement for calcium supplementation in the body [72]. Previous studies have suggested that the calcium carbonate extracted from oyster shell transforms into calcium oxide, which can be used as an anti-acid agent [73]. Calcium carbonate is also efficiently absorbed by the intestine and helps improve bone mineral density in Japan's elderly population [74]. However, there was no significant association between CGTS and GERD-related pathways in the enrichment pathways analysis based on KEGG, which may be because CGTS mainly contains minerals such as calcium carbonate compared with other

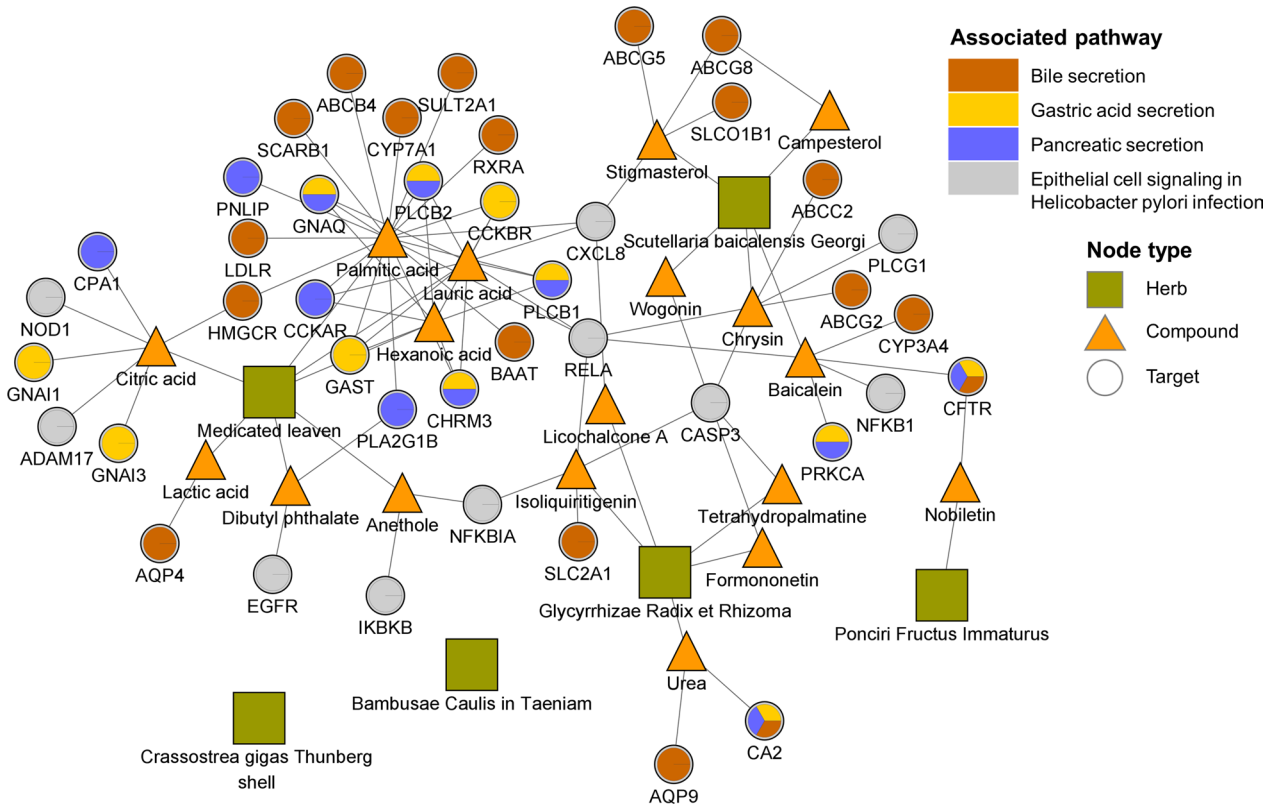


Fig. 3 Herb-compound-target network of P05 in digestive system-related pathways. Targets are color-coded by their associated pathways. Shapes of nodes (square, triangle, or circle) indicate the types of nodes (herb, compound, or target). Colors of target nodes indicate KEGG pathways that the targets were associated with. Edges between herb nodes and compound nodes indicate that the herbs contain the compounds, and edges between compound nodes and target nodes indicate that the compounds were predicted to target the genes

medicinal herbs. Several pathways are directly associated with GERD, including the gastric acid secretion pathway, epithelial cell signaling in the *H. pylori* pathway, pancreatic secretion pathway, bile secretion, and inflammation-related pathways. BCT is associated with cytokine signaling pathways, such as the TNF and IL-17 signaling pathways, which is consistent with the anti-inflammatory effect of BCT described in the current review.

The medicinal herbs equivalent to the "Shin-minister" that complement the "Kun" are PFI and SBG. Accumulating evidence points to the improvement of the delayed gastric emptying rate and peristalsis, attenuation of HCI induced gastric lesions, stimulation of mucus secretion, and a gastric cytoprotective effect of each of the PFI and SBG components. From the perspective of TKM, the fu organs, including the stomach, intestine, and bladder, are responsible for send foods down by peristalsis. Thus, GERD is thought to occur when the process of sending food down is disrupted by phlegm derived from the disharmony of body fluid. It is intriguing that the pathological diagnosis of GERD as a dysfunction of the digestion process, including the delayed gastric emptying rate in TKM, is consistent with the efficacy of these medicinal herbs based on previous evidence. In contrast, PFI did not show a significant association with any pathway in the pathways enrichment analysis. This might be due to the lack of information regarding the mechanism of action of PFI and GERD-related pathways in the KEGG database, which requires further research. The enrichment analysis indicated that the targets of SBG, the other "Shin" component, are significantly associated with the IL-17 signaling pathway, bile secretion, respiratory pathways, including small-cell lung cancer and non-small cell lung cancer. The literature review indicated that SBG has anti-inflammatory and gastric cytoprotective effects. Taken together, the anti-inflammatory effect of SBG was shown in both the review findings and enrichment analysis.

Medicinal leaven (ML) consists of *Artemisia annua*, *Xanthium sibiricum, Polygonum hydropiper, Vigna angularis,* Bitter *Prunus armeniaca* kernels, which have been traditionally used for treating GI tract diseases for thousands of years. A recent study suggested that ML could be a new therapeutic agent for GI disorders due to its role

in digestive function and its secondary metabolites, powerful antioxidant and anti-inflammatory activities [51]. ML assists "Kun" as "Choa" in P05 by ameliorating the side-effects of "Kun." According to literature, the ingredients in "Choa" have anti-inflammatory effects, reduce gastric acid secretion, and prevent GI mucosal damage, although it has not been established whether "Choa" is directly involved in reducing the side effects. Based on the pathway analysis, results indicated that ML mainly acts on gastric acid secretion, which is in accordance with the review findings. However, as shown in Fig. 3, the relationship between palmitic, hexanoic, and lauric acids in ML and digestive system-related pathways was not found in the literature review. Thus, further meticulous studies should be conducted to explore the relationship, and by extension, the differences between each ML component and the combined ML mixture's efficacy and underlying mechanisms.

The "Sa" that mediates "Kun's" efficacy in P05 is GRR, the most frequently used medicinal herb in TKM. In the enrichment analysis, the results implied that GRR is significantly related to epithelial cell signaling in the *H. pylori* infection pathway, bile secretion, inflammation-related pathways such as the TNF signaling pathway, etc. This is consistent with the current review findings that the main components of GRR have an anti-inflammatory effect and increase mucus production.

Lastly, the integrated efficacy, safety, and the active mechanisms of the multi-compound herbal mixture (P05) and their roles from a "Kun-Shin-Choa-Sa" theory perspective should be investigated in the future through diverse designed studies such as in vitro, in vivo, and clinical trials.

This is the first study to review and analyze the mechanisms of action of the P05 multi-component herb mixture, *Crassostrea gigas* Thunberg shell (CGTS), Bambusae Caulis in Taeniam (BCT), Ponciri Fructus Immaturus (PFI), *Scutellaria baicalensis* Georgi (SBG), medicated leaven (ML), and Glycyrrhizae Radix et Rhizoma (GRR), for GERD treatment using network analysis. However, there are several limitations to this study. The specific extraction conditions of the herbal mixture and proportions of P05 components were not elucidated in this study because we were unable to obtain specific information on P05 from TKM practitioners. Future studies are required to explore the synergetic effects and unknown mechanisms of action of each herb in GERD treatment. In addition, the pharmacological mechanisms observed based on the network analysis should be experimentally verified via in vitro and in vivo studies. Furthermore, the P05 components we investigated should be considered in bioavailability and complex metabolic processes, as well as the pharmacological actions of metabolites. As seen in the literature review, each ingredient in P05 has not been equally studied. Some of herbal ingredients in P05 have been studied thoroughly, on the other hand, accumulated evidence for some herbal plants was scare. Therefore, there is a possibility that the findings on efficacy and relevance regarding each P05 component may be skewed according to the number of accumulated studies. This potential bias may also influence network pharmacological analysis using accumulated database based on existing research results. Lastly, even though natural products have been used since time immemorial, if any side effects of the natural herbs exist, even considerably weak side effects, they should be considered and dealt with.

Abbreviations

GERD: Gastroesophageal reflux disease; PPIs: Proton pump inhibitors; OTC: Over-the-counter; CAM: Complementary and alternative medicine; TKM: Traditional Korean medicine; TEAM: Traditional East Asian medicine; GI: Gastrointestinal; TLESR: Transient lower esophageal sphincter relaxation; LES: Lower esophageal sphincter; EGJ: Esophagogastric junction; *H. pylori*: *Helicobacter pylori*; CGTS: *Crassostrea gigas* Thunberg shell; BCT: Bambusae Caulis in Taeniam; PFI: Ponciri Fructus Immaturus; SBG: *Scutellaria baicalensis* Georgi; ML: Medicated leaven; GRR: Glycyrrhizae Radix et Rhizoma; NO pathway: Nitric oxide pathway.

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Authors' contributions

Conceptualization: S-HC and KSK; methodology, TJC, DJ and C-EK; investigation, JP and HMP; writing—original draft preparation, JP and DJ; writing—review and editing, KSK; supervision, S-HC, SL, KSK; project administration, KSK. All authors have read and agreed to the published version of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article [and its Additional files].

Declarations

Competing interests

The authors declare that they have no competing interests.

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