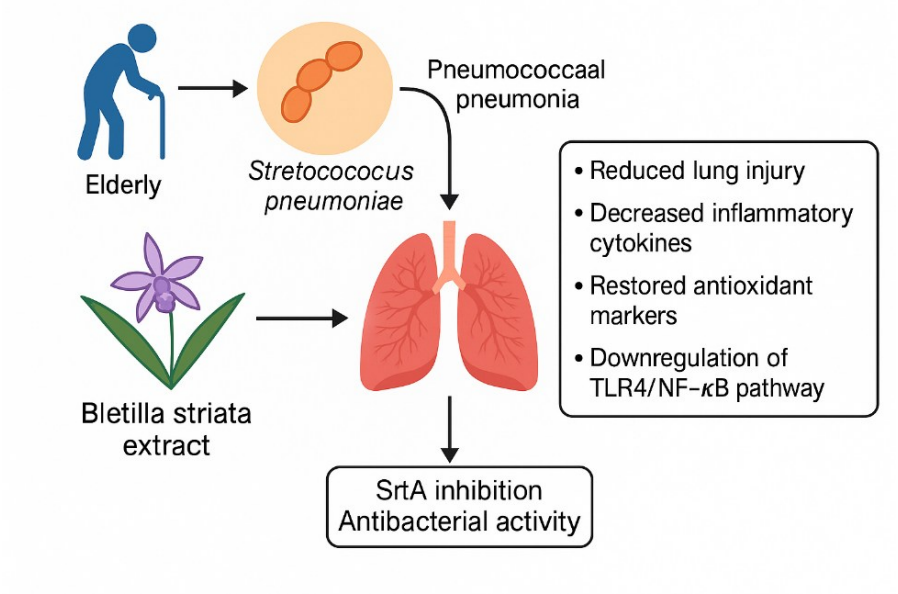
Bletilla striata Confers Antibacterial Activity and Pulmonary Protection in Senile Pneumonia through Sortase A Inhibition

Zhaojuan Ma [a,#], Qinyan An,[b,#] Yuhong Qin,[c] Haipeng Mu,[d] and Canye Zhong [c,*]
[#] *Contributed equally to the work*

* *Corresponding author: zhongcanye529@outlook.com*

DOI: 10.15376/biores.21.2.3002-3020

GRAPHICAL ABSTRACT



Bletilla striata Confers Antibacterial Activity and Pulmonary Protection in Senile Pneumonia through Sortase A Inhibition

Zhaojuan Ma [a,#] Qinyan An,[b,#] Yuhong Qin,[c] Haipeng Mu,[d] and Canye Zhong [c,*]

Pneumonia caused by *Streptococcus pneumoniae* remains a serious threat to the elderly due to weakened immunity and limited treatment efficacy. This study investigated the antibacterial activity and therapeutic potential of *Bletilla striata* ethanolic extract (BSE) in a rat model of pneumococcal pneumonia. BSE demonstrated selective antibacterial activity against *S. pneumoniae* (MIC: 1.25 mg/mL) and significantly inhibited Sortase A (SrtA), a key bacterial virulence factor. *In vivo*, BSE improved body weight, reduced lung wet/dry ratios, and lowered serum ALP and LDH levels. It also diminished inflammatory cell infiltration in bronchoalveolar lavage fluid and decreased TNF-α, IL-1β, and IL-6 levels. Antioxidant markers SOD and GSH increased, while MDA levels declined. Histological analysis showed preserved lung architecture with reduced edema and cellular infiltration. Mechanistically, BSE suppressed the TLR4/NF-κB pathway. These findings indicate that BSE can offer a dual benefit—direct antimicrobial action and modulation of inflammation and oxidative stress—highlighting its potential as a natural therapeutic for elderly patients with pneumonia.

DOI: 10.15376/biores.21.2.3002-3020

Keywords: Streptococcus pneumoniae; Antibacterial; Sortase A; BALF; Oxidative stress; TLR4; NF-κB

Contact information: a: Department of Pulmonary and Critical Care Medicine, The Second Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou Zhejiang, 310005, China; b: Department of Respiratory, Sijing Hospital of Songjiang District, Shanghai, 201601, China; c: Department of Geriatrics, Chongqing University Fuling Hospital, Chongqing, 408000, China; d: Department of Critical Care Medicine, Wuhai People's Hospital, Wuhai Inner Mongolia Autonomous Region, 016000, China;
*# Contributed equally to the work; *Corresponding author: zhongcanye529@outlook.com*

INTRODUCTION

Streptococcus pneumoniae remains the leading bacterial cause of community-acquired pneumonia worldwide, responsible for up to 60% of adult cases and half of pneumonia deaths in those over 65 (Luna *et al.* 2018; Shoji *et al.* 2018; Yesilkaya *et al.* 2022). In elderly patients, age-related immune decline, comorbidities, and impaired mucociliary clearance heighten susceptibility and worsen outcomes (Henig and Kaye 2017). The bacterium's capsule, pneumolysin, and adhesins facilitate lung invasion, inflammation, and tissue damage (Loughran *et al.* 2019). Globally, pneumococcal pneumonia affects an estimated 14 million people and causes 1.6 million deaths annually, with incidence exceeding 200 cases per 10,000 elderly in long-term care (Deb *et al.* 2022). Typical symptoms—fever, productive cough, chest pain, dyspnea—often present atypically in the senile, manifesting as confusion or functional decline and delaying diagnosis. Standard treatment with β-lactams plus macrolides or fluoroquinolones is

undermined by rising resistance—macrolide resistance now exceeds 30% in many regions—and by drug-related toxicity and interactions in polymedicated elders (Brooks and Mias 2018; Domínguez-Alegría *et al.* 2018). Although conjugate vaccines reduce disease, their efficacy is lower in the elderly and serotype replacement remains problematic (Musher *et al.* 2022). These challenges underscore the urgent need for novel therapies—targeting pneumococcal virulence, quorum sensing, or host immune enhancement—to overcome resistance, minimize adverse effects, and improve outcomes in senile pneumonia.

Medicinal plant extracts contain bioactive phytochemicals—phenolics, flavonoids, terpenoids—that exert antibacterial, anti-inflammatory and immunomodulatory effects against pneumonia pathogens, potentially overcoming rising antibiotic resistance and reducing drug toxicity (Dhama *et al.* 2014; Gupta and Birdi 2017; Keita *et al.* 2022). For example, *Lawsonia inermis* (henna) ethyl-acetate extract shows potent activity against S. pneumoniae (MIC 0.16 mg/mL) by disrupting cell walls, inhibiting biofilm formation and down-regulating autolysin (lytA) and peptidase (peZA/T) genes (Tafroji *et al.* 2022). *Hibiscus sabdariffa* extract inhibits pneumococcal growth (MIC 1.25 mg/mL) *via* membrane permeabilization and reactive oxygen species–mediated damage. Such multifaceted mechanisms—and documented synergy with antibiotics—underscore the promise of plant-derived agents as adjuncts or leads for new anti-pneumonia drugs.

Bletilla striata (Thunb.) Reichb.f., a terrestrial, rhizomatous geophyte of the Orchidaceae family, is native to China through central and southern Japan and extends into Korea, Taiwan, Vietnam, Thailand, and Myanmar, where it grows on grassy slopes, riverbanks, roadsides and in temperate woodlands (Xu *et al.* 2019). In traditional Chinese medicine, *Bletilla striata* harbors a broad spectrum of bioactive phytochemicals, including polyphenols, stilbenoids, phenanthrenes, bibenzyls, glycosides, quinones, triterpenoids, sterols, and polysaccharides. In a previous study, a comprehensive UPLC-Q-TOF analysis of 95% ethanolic tuber (ETB) and fibrous-root extracts (EFB) of *Bletilla striata* revealed 39 distinct compounds, including 24 stilbenoids (such as bibenzyl and phenanthrene derivatives), 6 glycosides, 4 phenolic acids, 3 quinones, 1 steroid, and 1 other compound (Luo *et al.* 2022). Notable phenolic acids identified include p-hydroxybenzoic acid and 3-hydroxycinnamic acid, with gastrodin glycoside observed primarily in fibrous-root fractions (ETB, 2.17×10^6; EFB, 1.43×10^6 units of relative peak area) (Luo *et al.* 2022). The *Bletilla striata* further showed stilbenoid-rich profile, including bibenzyls such as bletistrin and shancigusin C, has been correlated with antimicrobial activity, exhibiting MIC values as low as 2 to 26 μg/mL against *Staphylococcus aureus* strains (Jiang *et al.* 2020). In the reported GC–MS study of *Bletilla striata*, volatile and semi-volatile components were identified using thermal gradient separation (40 → 280 °C) and electron-impact ionization with acquisition in the 50 to 550 m/z range. (Han *et al.* 2023).

In addition to low-molecular-weight phenolics, *B. striata* contains high-molecular-weight polysaccharides (BSP) – primarily glucomannans – that have significant biomedical value. BSPs are typically extracted using aqueous or diluted ethanol precipitation, then fractionated and characterized by molecular weight, monosaccharide composition, and structure–activity relationships. They exhibit antioxidant (DPPH, ABTS, FRAP), anti-inflammatory, and hemostatic activity *via* modulation of NOX4/ROS signaling and regulation of inflammatory cytokines such as TNF-α and IL-1β (Gou *et al.* 2022; Jiang *et al.* 2023).

Polysaccharide-lacking fractions, especially chloroform or ethyl acetate subfractions, are enriched in phenanthrenes and bibenzyls with potent antimicrobial and

anti-inflammatory effects. For instance, spirostane-type steroidal saponins isolated from ethanol extracts modulate inflammatory mediators in RAW264.7 macrophages, downregulating IL-6, TNF-α, and IL-1β production at concentrations as low as 2.5 μg/mL (Wang and Meng 2015).

Taken together, BSE represents a multi-compound bioresource with synergistic potential:

- Polyphenols (stilbenoids, phenanthrenes, phenolic acids) contribute antioxidant, antimicrobial, and anti-inflammatory activities.
- Polysaccharides (glucomannans) exert immunomodulatory, antioxidant, and tissue-repairing effects.
- Steroidal saponins and quinones further enhance anti-inflammatory activity.

Pharmacologically, the dried tubers of Bletillae Rhizoma have long been used in traditional Chinese, Korean, and Japanese medicine for hemostasis, wound healing and treatment of respiratory ailments—including silicosis, tuberculosis and hemorrhagic pneumonia—reflecting potent anti-inflammatory and mucosa-protective effects in lung tissue (Xu *et al.* 2019; Devkota *et al.* 2022). Phytochemical investigations have identified over 150 compounds—polysaccharides, bibenzyls, phenanthrenes, glucosides, and phenolic acids—that underlie antioxidant, anti-cancer, antiviral, antibacterial and immunomodulatory activities; notably, *Bletilla striata* polysaccharides (BSP) attenuate acute respiratory distress by inhibiting neutrophil extracellular trap formation and reducing lung inflammation in ARDS models (Wu *et al.* 2024). Antibacterial studies demonstrate that *B. striata* extracts disrupt Gram-positive bacterial cell-wall and membrane integrity, down-regulate autolysin and peptidase genes, and inhibit biofilm formation in pathogens such as *Staphylococcus aureus* and *Cutibacterium acnes* (Li *et al.* 2014; Jiang *et al.* 2020). Given that *Streptococcus pneumoniae* is a leading Gram-positive cause of pneumonia—especially in the elderly—and faces rising antibiotic resistance, the convergence of *B. striata*'s lung-targeted anti-inflammatory properties and its membrane-disrupting antibacterial mechanisms provides strong rationale for exploring its extract as novel anti-pneumococcal agents.

.

EXPERIMENTAL

Preparation of Extract

The plant was collected from the gardens of the Wuhai People's Hospital and identified by the botanist of the Institute. The sample voucher (No. WPH/2024/C43-02) was deposited in the herbarium of the Institute for future reference. Whole *Bletilla striata* plants were shade-dried until a constant weight was achieved, then milled to a coarse powder. Fifty grams of this powdered material were subjected to extraction with 95% ethanol under reflux at 78 °C for 60 min to ensure exhaustive recovery of both polar and moderately non-polar constituents. The resulting mixture was vacuum-filtered to remove plant debris, and the filtrate was concentrated under reduced pressure in a rotary evaporator at 40 °C to remove residual ethanol. The dark-brown residue was dried to a constant weight, yielding 6.25 g of crude ethanolic extract—equivalent to a 12.5% w/w extraction yield.

Antibacterial Activity of *Bletilla striata* Ethanolic Extract (BSE)

The broth dilution technique recommended by NCCLS was used to test the antibacterial activity *via* determining the minimum inhibitory concentration (MIC) against

gram positive and gram negative ATCC microorganisms. Two-gram positive bacterial strains (*S. aureus* and *S. pneumoniae*, ATCC55115 and ATCC53819) and two-gram negative strains (*E. coli* and *P. aeruginosa*, ATCC56521, and ATCC57853, respectively) were employed as per the previously reported procedure (Singh *et al.* 2011).

Sortase A Inhibitory Activity

The inhibitory effect of the extracts on Sortase A (SrtA) enzymatic activity was assessed by monitoring the fluorescence generated from the cleavage of a fluorogenic peptide substrate labeled with 5-FAM and a QXL® quencher. The assay was performed using the SensoLyte® 520 Sortase A Activity Assay Kit (AnaSpec, San Jose, CA, USA), according to the manufacturer's instructions. Upon substrate cleavage by active SrtA, the quencher is separated from the fluorophore, resulting in an increase in fluorescence intensity, which was measured using a microplate reader. A reduction in fluorescence in the presence of the BSE indicated their potential to inhibit SrtA activity.

Animals

SPF-grade male Sprague Dawley (SD) rats weighing 180 to 220 g were selected and obtained from the institutional animal house. They were kept at 24 ± 2 °C, with 12 h alternate light/dark cycle, with relative humidity of 55%. The rats had free access to food and water. The animal study was approved by the Ethics Committee of the Chongqing University Fuling Hospital, China and was conducted in accordance with the institutional guidelines and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Induction of *S. Pneumonia* Infection in Mice

After one week of acclimation, fifty SD rats were randomized into five groups (n=10 each): control, model, and BSE treatment at 50, 100, or 200 mg/kg b.w. The model and BSE groups received an intratracheal inoculation of 0.1 mL *Streptococcus pneumoniae* suspension (1×10^8 CFU/mL) once daily for three days as per the earlier reported procedure (Han *et al.* 2021). The BSE groups also received their respective doses orally in using saline as vehicle, while the model and control groups received saline. The BSE was administered using a 16-18 gauge feeding tube about 2 to 3 inches (~5 to ~8 cm) in length (Turner *et al.* 2011). Twenty-four h after the final treatment, anesthesia was induced with 10% chloral hydrate (3 mL/kg, i.p.), and 5 mL of blood was drawn from the abdominal aorta into anticoagulant tubes. Serum was separated by centrifugation (3,000 rpm, 10 min) and stored at –20 °C. The right lung was ligated, and the left lung was lavaged twice *via* the trachea with 1.5 mL saline. BALF was collected, centrifuged (3,000 rpm, 10 min), and the supernatant stored at –20 °C. The right lung's upper and middle lobes were frozen at –80 °C, and the lower and posterior lobes were fixed in 10% formalin for further analyses. For biochemical and histological assessments, measurements were conducted on 3 randomly selected animals per group from the total of 10 Sprague-Dawley rats. The selection was performed randomly prior to data collection to avoid bias. This sample size was determined based on preliminary experiments demonstrating that 3 replicates per group provided consistent and representative results with acceptable variance for ANOVA-based statistical analysis, while adhering to the 3Rs principle (Replacement, Reduction, and Refinement) to minimize invasive sampling. The remaining animals were reserved for other planned endpoints and to account for potential experimental loss.

Bronchoalveolar Lavage Fluid (BALF) Preparation

All the rats were anesthetized by pentobarbital sodium (i.p., 50 mg/kg). Subsequently, BALF samples were collected and centrifuged and the resulting supernatants were used to measure the numbers of neutrophils, eosinophils, lymphocytes, monocytes, and total cells.

Measurements of Body Weight and Lung Wet/Dry Ratio

Following BALF collection, rats were euthanized and their body weights recorded. Fresh lung tissues were excised, and wet weights measured immediately. Samples were then dried at 60 °C for 72 h to obtain dry weights. The lung wet-to-dry weight ratio was calculated to assess tissue edema.

Analysis for Serum Biochemistry

Blood samples were collected from the retro-orbital plexus under light anesthesia and allowed to clot at room temperature. The samples were then centrifuged to separate the serum. Serum levels of alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were quantified using specific commercial assay kits, following the manufacturers' protocols. Absorbance was measured using a UV-visible spectrophotometer, and enzyme activities were calculated accordingly to assess tissue damage and systemic toxicity.

Pro-inflammatory Cytokines

For the purpose of determining the serum levels of TNF-α, IL-1B, and IL-6, the ELISA kits were utilized in accordance with the protocol provided by the manufacturer (Beyotime Institute of Biotechnology, Nanjing, China).

Oxidative Stress Factors

For the purpose of determining the levels of MDA, SOD, and GSH activities, commercial kits were utilized in accordance with the protocol that was provided by the manufacturers.

Histopathology Analysis

Tissues from the lower and posterior lobes of the right lung were fixed in 10% formalin, dehydrated through a graded alcohol series in an automated processor, cleared with xylene, and embedded in paraffin. Sections (4 μm) were cut, stained with hematoxylin for 5 min, counterstained with eosin, and evaluated histopathologically. The scores of histological changes in the lungs of each animal were evaluated on the average score of the given four criteria, including thickening of alveolar walls and epithelium, the numbers of infiltration cell, increase in peribronchial, and perivascular cuff area. The histological changes were scored 0 to 5.

Western Blot Analysis

The concentration of the protein was determined using a Bio-Rad Protein Assay kit (Bio-Rad Laboratories). The protein was then denatured in a sample buffer that contained 5x SDS. Molecular weight-based separation using 8 to 15% SDS-PAGE and PVDF was performed on the samples. The membranes were blocked after being rotated and left at room temperature for an h with 5% skim milk in TBST. Afterwards, the membranes were incubated at 4 degrees Celsius overnight with primary antibodies that had been diluted in a blocking buffer. The immunoreactive bands were detected using the enhanced

chemiluminescence method after three more washes (Srivastava *et al.* 2017).

Statistical Analysis

Data are expressed as means ± SEM and evaluated using one-way ANCFA. Data considered significant at a p-value < 0.05

RESULTS AND DISCUSSION

Antibacterial Activity of BSE

Determining the antibacterial activity of a novel agent against *Streptococcus pneumoniae* is particularly important in the context of senile pneumonia, as elderly patients often suffer from diminished immune defenses, comorbidities, and altered pharmacokinetics that render standard therapies less effective or more toxic (Stogova 2023). In this study, the BSE extract exhibited potent, species-selective activity against *S. pneumoniae* with a minimum inhibitory concentration (MIC) of 1.25 mg/mL. This narrow, high-affinity targeting suggests that the molecule possibly interacts with unique features of the pneumococcal cell envelope—perhaps the teichoic acid–anchored choline binding proteins or the autolysin machinery—minimizing off-target effects on commensal flora (Deb *et al.* 2022). By contrast, only moderate activity was observed against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (MICs > 5 mg/mL), indicating a reduced likelihood of disrupting gut and skin microbiota or selecting for multi-drug-resistant opportunists.

Table 1. Minimum Inhibitory Concentration of BSE against Various Bacterial Organisms

Particulars	Concentration (mg/mL)	Bacterial Strains			
BSE		*S. aureus*	*E. coli*	*P. aeruginosa*	*S. pneumoniae*
	20	-	-	-	-
	10	-	-	-	-
	5	+	+	+	-
	2.5	+	+	+	-
	1.25	+	+	+	-
	0.625	+	+	+	+
Cefixime	20	-	-	-	-
	10	-	-	-	-
	5	-	-	-	-
	2.5	-	-	-	-
	1.25	-	-	-	-
	0.625	+	+	+	+

Where, *(+)* presence of growth, *(−)* absence of growth

The antibacterial activity of cefixime, used as a standard reference drug, was also evaluated for comparison. Results indicated that BSE exhibited antibacterial potency comparable to cefixime against *Streptococcus pneumoniae*. However, against the other tested bacterial strains, the activity of BSE was lower than that of the standard drug. Clinically, a *pneumococcus*-focused agent with this profile could improve outcomes in elderly patients by rapidly clearing the primary pathogen while preserving beneficial microbial communities that support immune homeostasis. Furthermore, its modest activity

against Gram-negative and coagulase-positive staphylococci may be advantageous in polymicrobial settings, offering a degree of backup coverage without driving broad-spectrum resistance. Collectively, these findings underscore both the therapeutic promise of this agent for targeted intervention in senile pneumonia and its potential role as a precision adjunct to conventional, broader-spectrum antibiotics.

Sortase A Inhibitory Activity of BSE

Sortase A (SrtA) is a membrane-anchored transpeptidase used by Gram-positive bacteria to covalently attach surface proteins—particularly microbial surface components recognizing adhesive matrix molecules (MSCRAMMs)—to the cell wall. In the context of pneumonia, *S. pneumoniae* initiates the acute inflammatory cascade, secondary colonization by Gram-positive pathogens (*e.g.*, *Staphylococcus aureus*) is a frequent and dangerous complication (Paterson and Mitchell 2006; Gu *et al.* 2023). By anchoring adhesins that mediate epithelial attachment, biofilm formation, and immune evasion, SrtA both facilitates bacterial persistence in the injured lung and exacerbates the host inflammatory response. Consequently, SrtA has emerged as an attractive anti-virulence target: inhibition of SrtA cripples bacterial adhesion without exerting bactericidal pressure, reducing the risk of resistance development and dampening downstream inflammatory amplification.

Various plant-derived compounds have emerged as effective Sortase A (SrtA) inhibitors at pharmacologically relevant concentrations. The isoquinoline alkaloid berberine chloride, isolated from *Coptis chinensis*, inhibits *Staphylococcus aureus* SrtA (Kim *et al.* 2004). Among flavonoids, eriodictyol—derived from *Eriodictyon californicum*—acts as a reversible SrtA inhibitor, effectively blocking substrate cleavage and downstream adhesion processes (Wang *et al.* 2021). By targeting the enzyme's active or allosteric sites, these phytochemicals impede the anchoring of virulence factors without exerting bactericidal pressure, offering promising anti-virulence strategies that may circumvent conventional resistance mechanisms.

This study evaluated the ability of BSE to inhibit purified SrtA *in vitro*, measuring percent inhibition (I%) at seven logarithmically spaced concentrations (Log [C], μg/mL) after both 30- and 60-min exposures at a fixed extract dose of 50 μg/mL. As shown in Fig. 1, BSE produced a steep, concentration-dependent blockade of SrtA activity: at the lowest tested concentration (Log [C] = 0.3, ≈2 μg/mL), SrtA activity was inhibited by 55 ± 5% at 30 min and 60 ± 4% at 60 min. Increasing Log [C] to 0.6 and 0.9 yielded 30-min I% of 68 ± 4% and 80 ± 3% (60-min: 75 ± 3% and 88 ± 2%), respectively. At Log [C] = 1.2, inhibition reached 90 ± 2% at 30 min and 93 ± 1.5% at 60 min; near-complete suppression (>95%) was observed at Log [C] ≥ 1.5, with BSE achieving 98 to 100% I% at both time points for Log [C] of 1.8 and 2.1. These data demonstrate that BSE contains potent SrtA inhibitors whose effect is both concentration- and time-dependent, with maximal activity reached by 60 min even at moderate extract levels.

Mechanistically, the rapid onset and high maximal inhibition suggest that BSE's active components may bind directly to the SrtA active site or to an allosteric pocket critical for enzyme conformational dynamics. By effectively neutralizing SrtA, BSE is expected to impair Gram-positive bacterial adhesion and colonization in vivo, thereby attenuating the vicious cycle of secondary infection and inflammation in primed lungs. Together with its anti-inflammatory and antioxidant properties, SrtA inhibition positions BSE as a multifaceted therapeutic candidate for preventing and treating complex pneumonia.

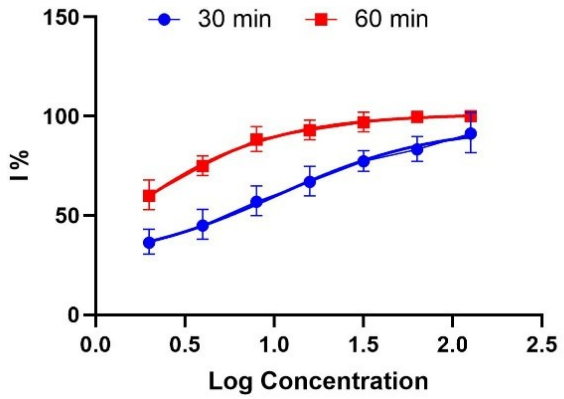


Fig. 1. Sortase A inhibitory activity of BSE extract at different time interval

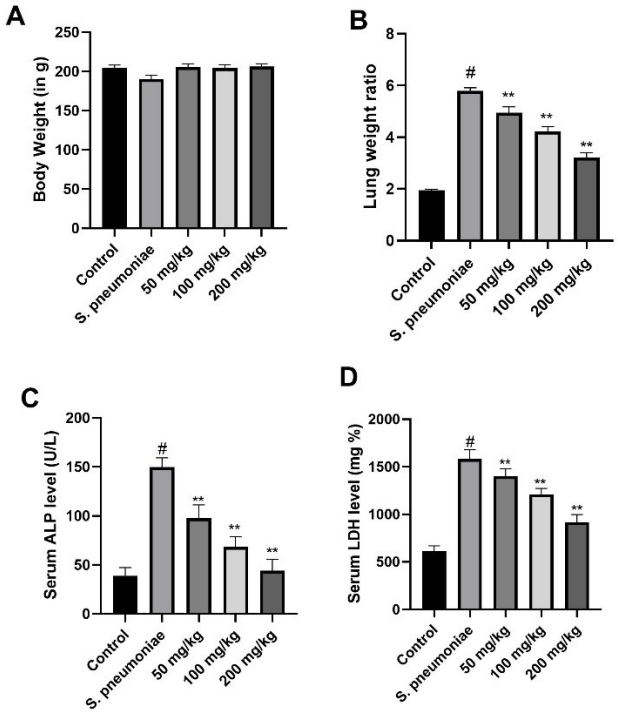


Fig. 2. Effect of BSE extract on the a) body weight, b) lung weight ratio, c) serum ALP, and d) serum LDH level. Results represent means ± SEM of three independent experiments. #p < 0.001 *vs* the control group, **p < 0.05 *vs S. pneumoniae* group.

BSE Protects Lung Tissues against Pneumonia

Body weight loss, pulmonary edema, and elevated bronchoalveolar lavage fluid (BALF) markers such as ALP and LDH are well-established indicators of systemic inflammation, increased vascular permeability, and epithelial–endothelial injury in models of induced pneumonia (Davidson *et al.* 2020). As shown in Fig. 2 a-e, intratracheal *S. pneumoniae* caused a significant reduction in body weight relative to controls, an increase in lung wet/dry ratio, in BALF ALP activity (149 ± 15 U/L vs. 38± 8 U/L), and a 2.6-fold elevation in LDH activity (1583 ± 35 U/L vs. 613 ± 20 U/L), reflecting both systemic catabolism and acute lung injury. Pretreatment with BSE mitigated these changes in a dose-dependent manner: at 50 mg/kg, a significant improvement in weight loss was observed,

and lung edema was reduced (0.85-fold *vs S. pneumoniae*), while ALP and LDH fell to 97 ± 8 U/L and 1399 ± 42 U/L, respectively. Administration of 100 mg/kg BSE further attenuated weight loss, edema and enzyme release of ALP and LDH, and the highest dose (200 mg/kg) nearly normalized all parameters. These findings demonstrate that BSE preserves body mass by counteracting systemic inflammation, strengthens the alveolar–capillary barrier to limit edema, and protects pulmonary cells from *S. pneumoniae* induced injury, highlighting its therapeutic potential in acute inflammatory lung disorders.

BSE Reduces BALF Cell Counts in Pneumonia Rat

Bronchoalveolar lavage fluid (BALF) cellularity is a sensitive, informative readout of lung inflammation in pneumonia, reflecting the recruitment and activation of multiple leukocyte populations at the site of injury (Davidson *et al.* 2020; Ahmadinejad *et al.* 2022). Upon endotoxin exposure, neutrophils rapidly extravasate into the alveolar space, where they release proteolytic enzymes and reactive oxygen species that compromise the integrity of the alveolar–capillary barrier, perpetuating fluid leak and tissue damage. Eosinophils—traditionally linked to allergic inflammation—also accumulate, releasing cytotoxic granule proteins (*e.g.*, major basic protein and eosinophil cationic protein) and lipid mediators such as leukotrienes that exacerbate bronchoconstriction and airway hyperresponsiveness (Fulkerson and Rothenberg 2013). In parallel, lymphocytes migrate into the lung and orchestrate antigen-specific immune responses: $CD4^+$ T cells secrete cytokines such as IFN-γ and IL-17 to shape macrophage function and neutrophil survival, while $CD8^+$ T cells can directly lyse infected or damaged epithelial cells (Ahmadinejad *et al.* 2022). Monocytes entering the alveoli differentiate into macrophages, which perform a dual role—engulfing cellular debris and pathogens, yet also secreting pro-inflammatory cytokines (TNF-α, IL-1β) that sustain the inflammatory milieu if unchecked (Cheng *et al.* 2020; Sánchez-Tarjuelo *et al.* 2020). The total BALF cell count provides an integrated, quantitative measure of pulmonary leukocyte burden and thus serves as a powerful index for evaluating both disease severity and the efficacy of anti-inflammatory therapies such as BSE.

In this study, intratracheal *S. pneumoniae* administration provoked a dramatic influx of inflammatory cells into the alveolar space, with neutrophils rising roughly three-fold over sham-treated controls, eosinophils, and monocytes by approximately four-fold, lymphocytes by nearly two-fold, and total cellularity by over two-and-a-half-fold (all $p < 0.001$ versus control). Pretreatment with BSE attenuated this leukocyte recruitment in a clear dose-dependent fashion. At 50 mg/kg, BSE reduced neutrophil counts by ~30%, eosinophils by ~16%, lymphocytes by ~15%, monocytes by ~28%, and total cells by ~18% (all $p < 0.05$ versus *S. pneumoniae* alone). The 100 mg/kg dose produced even greater inhibition, whereas, at the highest dose (200 mg/kg), BSE nearly normalized cellularity: neutrophils fell ~59%, eosinophils ~53%, lymphocytes ~41%, monocytes ~49%, and total cell counts ~53% compared to the *S. pneumoniae* group, restoring BALF leukocyte profiles close to baseline.

These results demonstrated that BSE powerfully inhibited the recruitment of both innate and adaptive immune cells into the lung in response to endotoxin, likely by suppressing chemokine production and vascular adhesion molecule expression. The pronounced dose-dependency underscores BSE's potential as a modulatory therapy for acute inflammatory lung injury, warranting further mechanistic studies on its effects on chemotactic signaling pathways.

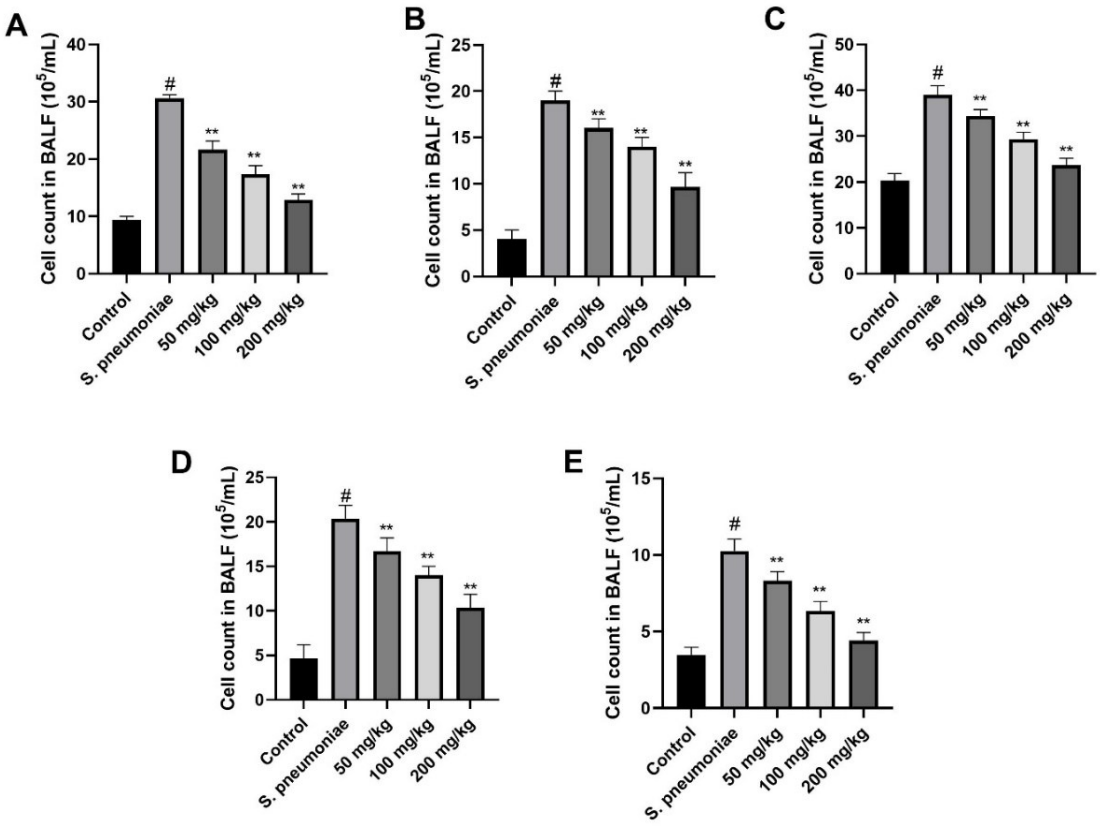


Fig. 3. Effect of BSE extract on a) neutrophils, b) eosinophils, c) lymphocytes, d) monocytes, and e) the number of total cells in BALF. Results represent means ± SEM of three independent experiments. $^{\#}p < 0.001$ *vs* the control group, $^{**}p < 0.05$ *vs S. pneumoniae* group.

Effect of BSE on Pro-Inflammatory Cytokines

S. pneumoniae triggers a robust inflammatory cascade in the lung by activating Toll-like receptor 4 (TLR4), which leads to downstream NF-κB signaling and rapid production of key pro-inflammatory cytokines—namely, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6 (Duan *et al.* 2020; Guo *et al.* 2024). TNF-α acts as a master regulator in the innate response, promoting endothelial activation, neutrophil recruitment *via* upregulation of adhesion molecules and chemokines, and induction of other cytokines and acute-phase proteins. IL-1β operates in concert with TNF-α to potentiate NF-κB activation and amplify chemokine expression such as KC and MIP-2, critically supporting neutrophil influx. IL-6, produced predominantly by activated macrophages and epithelial cells, orchestrates acute-phase responses, stimulates hepatic synthesis of CRP, and sustains neutrophil production, while also exerting context-dependent regulatory control on inflammation (Leemans *et al.* 2002).

In the *S. pneumoniae* -induced pneumonia model, BALF concentrations of TNF-α, IL-1β, and IL-6 were all markedly elevated compared to controls (all $p < 0.001$) in both BALF and serum, signifying a pronounced innate immune activation. Pretreatment with BSE led to a dose-dependent suppression of these cytokines in both BALF and serum compared with *S. pneumoniae* alone ($p < 0.001$), while the highest reduction was achieved in the case of 200 mg/kg treated group (Fig. 4).

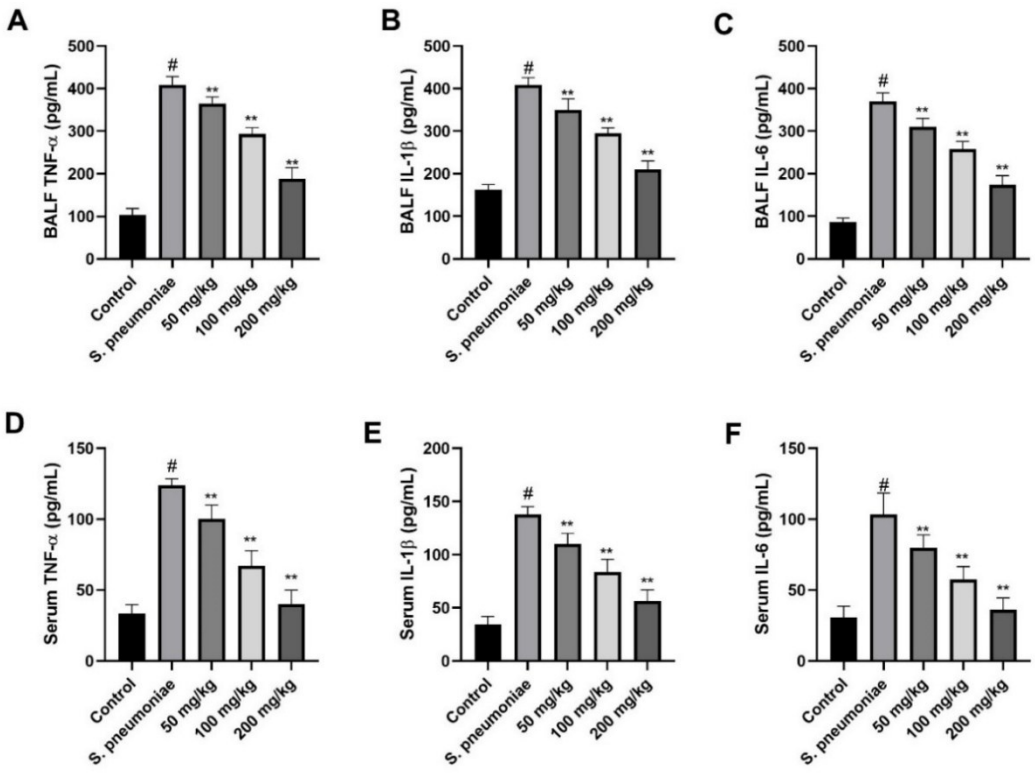


Fig. 4. Effect of BSE on the level of various pro-inflammatory cytokines in BALF and serum. Results represent means ± SEM of three independent experiments. #p < 0.001 *vs* the control group, **p < 0.001 *vs. S. pneumoniae* group

These results indicate that BSE effectively interrupts the TLR4–NF-κB–cytokine axis, curbing both initiation (TNF-α/IL-1β) and amplification (IL-6) phases of inflammation. By simultaneously targeting multiple pro-inflammatory nodes, BSE demonstrates a comprehensive immunomodulatory capacity with therapeutic potential against acute inflammatory lung injury.

Effect of BSE on Oxidative Stress

S. pneumoniae exposure in the lung generates a burst of reactive oxygen species (ROS) *via* activation of NADPH oxidase and mitochondrial dysfunction, overwhelming endogenous antioxidant defences and leading to peroxidation of membrane lipids—measured as elevated malondialdehyde (MDA) levels—and depletion of key antioxidants such as superoxide dismutase (SOD) and reduced glutathione (GSH) (Blokhina *et al.* 2003; Hernandez-Morfa *et al.* 2023). MDA serves as a reliable marker of lipid peroxidation and cellular oxidative injury, correlating with alveolar–capillary barrier disruption and edema formation in acute lung injury (Janero 1990; Zhang *et al.* 2021; Wu *et al.* 2022). SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide and oxygen, acting as the first enzymatic line of defence against ROS; its activity is markedly suppressed in *S. pneumoniae* -induced pneumonia, exacerbating oxidative damage (Hsieh *et al.* 2006; Zahlten *et al.* 2015). GSH, a tripeptide thiol, is the principal non-enzymatic antioxidant that directly scavenges free radicals and serves as a cofactor for glutathione peroxidase; depletion of GSH under endotoxin challenge impairs ROS detoxification and promotes inflammatory signaling (Sekhar *et al.* 2011; Kwon *et al.* 2019).

As shown in Fig. 5, *S. pneumoniae* markedly increased lung tissue MDA by approximately 2.5-fold over the control (from 2.06 ± 0.5 to 5.33 ± 0.7 nmol/mg protein; $p < 0.001$), while SOD activity fell by approximately 72% (from 11 ± 3 to 3 ± 1 U/mg protein; $p < 0.001$) and GSH content dropped by 58% (from 0.31 ± 0.4 to 0.13 ± 0.2 μmol/g tissue; $p < 0.001$).

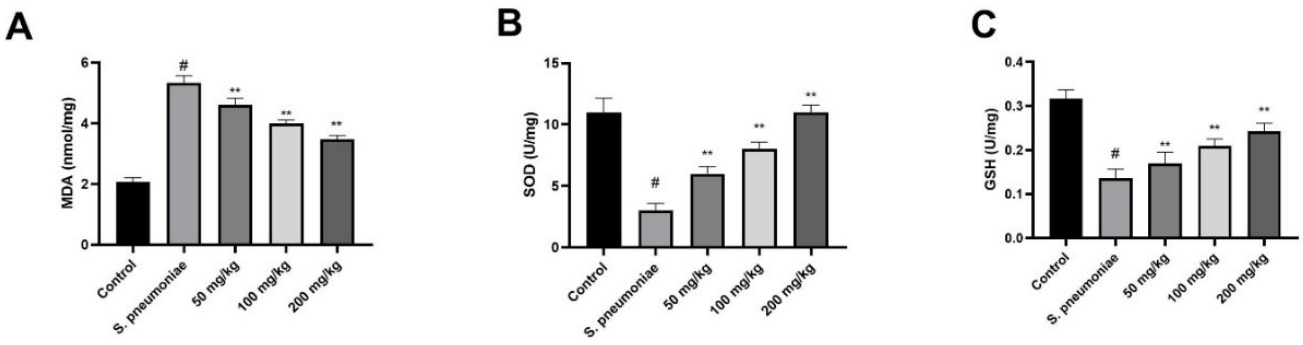


Fig. 5. Effect of BSE on the level of various oxidative stress biomarkers (a) MDA, (b) SOD, and (c) GSH. Results represent means ± SEM of three independent experiments. #p < 0.001 *vs.* the control group,**p < 0.001 *vs. S. pneumoniae* group.

Pretreatment with BSE significantly attenuated oxidative injury in a dose-dependent manner: at 50 mg/kg, MDA was reduced by ~13%, SOD increased by ~95%, and GSH by ~31% *versus S. pneumoniae* alone ($p < 0.001$). Moreover, on further increasing the concentration to 100 mg/kg and 200 mg/kg, the MDA levels approached baseline, while SOD and GSH were restored to near-control levels with ($p < 0.001$). These findings demonstrate that BSE robustly counteracts *S. pneumoniae* -induced oxidative stress by both suppressing lipid peroxidation and bolstering enzymatic and non-enzymatic antioxidant systems, thereby preserving redox homeostasis and limiting downstream inflammatory injury.

Effect of BSE on the Histopathology of Lung Tissues

Histopathological examination of lung sections provides a direct visual and quantitative measure of alveolar–capillary barrier integrity, inflammatory cell infiltration, and edema in *S. pneumoniae* -induced pneumonia (Aeffner *et al.* 2015; Long *et al.* 2022). In histopathological analysis, the control animals, alveolar septa were thin and patent, with minimal perivascular or interstitial inflammatory cells and intact alveolar spaces with the injury score of 0.31 ± 0.028. Conversely, *S. pneumoniae* challenge produced pronounced architectural disruption: extensive neutrophilic infiltration in alveolar lumina and interstitium, thickening of alveolar walls, focal hemorrhage, and proteinaceous edema filling airspaces, accompanied by occasional hyaline membrane formation, which was further confirmed by injury score of 4.63 ± 0.14. Pretreatment with BSE markedly ameliorated these histopathological changes in a dose-dependent fashion, and it reduced the lung injury score near to normal. These findings indicate that BSE protects lung parenchyma from *S. pneumoniae* -induced structural damage—likely through its combined anti-inflammatory, antioxidant, and barrier-stabilizing effects—and underscore its therapeutic potential for mitigating acute lung injury at the tissue level.

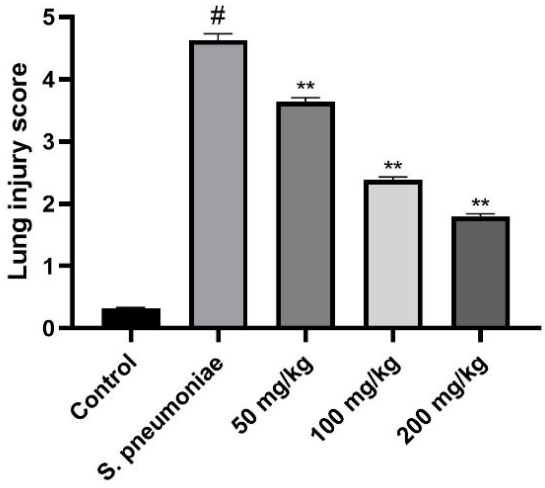


Fig. 6. Effect of BSE on the lung injury score of rats. Results represent means ± SEM of three independent experiments. #p < 0.001 *vs.* the control group,**p < 0.001 *vs. S. pneumoniae* group.

Western Blot Analysis

Toll-like receptor 4 (TLR-4) is the principal pattern-recognition receptor for *S. pneumoniae* on alveolar macrophages and airway epithelial cells (Bäckhed and Hornef 2003). Upon *S. pneumoniae* binding, TLR-4 dimerizes and recruits the adaptor proteins MyD88 and TRIF, triggering downstream activation of the IκB kinase (IKK) complex. IKK phosphorylates IκBα, earmarking it for proteasomal degradation and liberating NF-κB (p65/p50) to translocate into the nucleus.

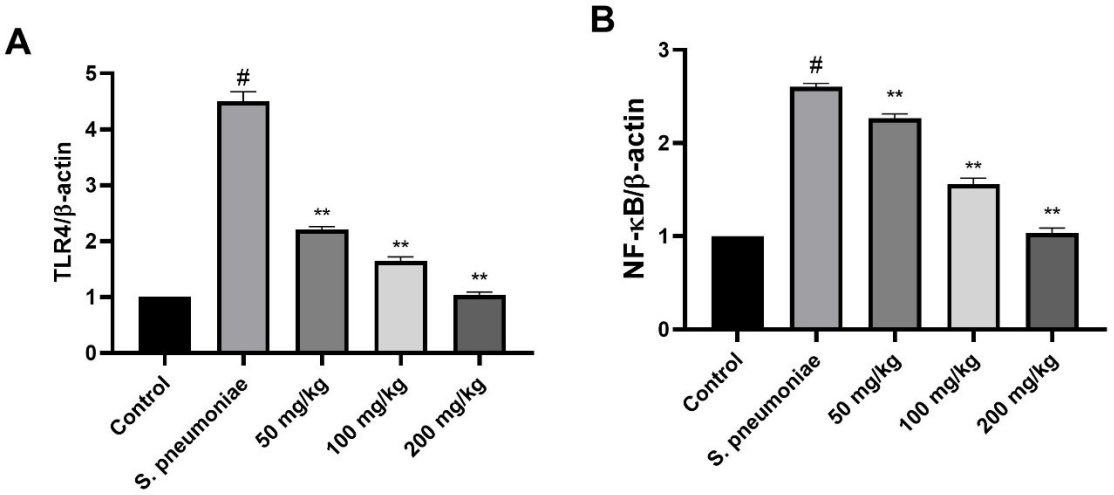


Fig. 7. Western blot analysis of BSE on TLR4 and NF-κB. Results represent means ± SEM of three independent experiments. #p < 0.001 *vs.* the control group,**p < 0.001 *vs. S. pneumoniae* group.

Nuclear NF-κB then drives transcription of dozens of pro-inflammatory mediators—including TNF-α, IL-1β, IL-6, and key chemokines—that orchestrate neutrophil and monocyte recruitment, increase vascular permeability, and amplify oxidative injury in the lung parenchyma (Bäckhed and Hornef 2003; Ding *et al.* 2005; Li and Wu 2021). In *S. pneumoniae* -induced pneumonia, excessive or sustained

TLR-4/NF-κB signaling underlies the cytokine storm and tissue damage characteristic of acute lung injury and acute respiratory distress syndrome.

As shown in Fig. 7, the western blot analysis of lung homogenates confirmed that intratracheal *S. pneumoniae* markedly upregulated both TLR-4 expression and NF-κB p65 phosphorylation compared to controls. Pretreatment with BSE attenuated this signaling cascade in a clear dose-dependent manner, with highest activity achieved in the case of the 200 mg/kg group. These findings indicate that BSE disrupts the *S. pneumoniae* –TLR-4–NF-κB axis at multiple nodal points, thereby curbing the upstream trigger of cytokine overproduction and mitigating downstream inflammatory injury in the lung.

CONCLUSIONS

1. **Targeted Anti-Pneumococcal Action:** *Bletilla striata* extract (BSE) exhibits selective antibacterial activity against *S. pneumoniae*, effectively inhibiting Sortase A—disrupting bacterial adhesion and colonization without broad-spectrum disruption of the microbiota.
2. **Multifaceted Lung Protection:** BSE significantly reduces pulmonary inflammation, oxidative stress, and tissue injury, while preserving alveolar architecture—highlighting its dual role in both antimicrobial defense and tissue repair.
3. **Molecular Pathway Modulation:** The extract suppresses the TLR4/NF-κB signaling axis, thereby halting the cytokine cascade that drives acute lung injury—demonstrating its role as a natural immunomodulator.
4. **A Promising Therapeutic Alternative:** With its anti-virulence strategy, antioxidant effects, and safety in vivo, BSE emerges as a compelling phytotherapeutic candidate for senile pneumonia, especially in an era of rising antibiotic resistance.

REFERENCES CITED

Aeffner, F., Bolon, B., and Davis, I. C. (2015). "Mouse models of acute respiratory distress syndrome," *Toxicologic Pathology* 43(8). https://doi.org/10.1177/0192623315598399

Ahmadinejad, M., Mohammadzadeh, S., Pak, H., Hashemiyazdi, S., Soltanian, A., Rahimi, M., and Ahmadinejad, I. (2022). "Bronchoalveolar lavage of ventilator-associated pneumonia patients for antibiotic resistance and susceptibility test," *Health Science Reports* 5(1), article e472. https://doi.org/10.1002/hsr2.472

Bäckhed, F., and Hornef, M. (2003). "Toll-like receptor 4-mediated signaling by epithelial surfaces: Necessity or threat?," *Microbes and Infection* 5(11), 951-959. https://doi.org/10.1016/S1286-4579(03)00189-8

Blokhina, O., Virolainen, E., and Fagerstedt, K. V. (2003). "Antioxidants, oxidative damage and oxygen deprivation stress: A review," *Annals of Botany* 91, 179-194. https://doi.org/10.1093/aob/mcf118

Brooks, L. R. K., and Mias, G. I. (2018). "*Streptococcus pneumoniae*'s virulence and host immunity: Aging, diagnostics, and prevention," *Frontiers in Immunology* 9. https://doi.org/10.3389/fimmu.2018.01366

Cheng, H. R., Song, J. Y., Zhang, Y. N., Chen, Y. Bin, Lin, G. Q., Huang, G. Q., He, J. C., and Wang, Z. (2020). "High monocyte-to-lymphocyte ratio is associated with stroke-associated pneumonia," *Frontiers in Neurology* 11, article 575809. https://doi.org/10.3389/fneur.2020.575809

Davidson, K. R., Ha, D. M., Schwarz, M. I., and Chan, E. D. (2020). "Bronchoalveolar lavage as a diagnostic procedure: A review of known cellular and molecular findings in various lung diseases," *Journal of Thoracic Disease* 12(9), 4991-5019. https://doi.org/10.21037/jtd-20-651

Deb, A., Podmore, B., Barnett, R., Beier, D., Galetzka, W., Qizilbash, N., Haeckl, D., Mihm, S., Johnson, K. D., and Weiss, T. (2022). "Clinical and economic burden of pneumococcal disease among individuals aged 16 years and older in Germany," *Epidemiology and Infection* 150, article e204. https://doi.org/10.1017/S0950268822001182

Devkota, H. P., Logesh, R., Adhikari-Devkota, A., and Paudel, M. R. (2022). "Orchids of genus *Bletilla*: Traditional uses, phytochemistry, bioactivities, and commercial importance," in: *Orchids Phytochemistry, Biology and Horticulture. Reference Series in Phytochemistry*, J. M. Mérillon and H. Kodja (Eds), Springer, Cham, Switzerland, p. 573-590. https://doi.org/10.1007/978-3-030-38392-3_36

Dhama, K., Tiwari, R., Chakraborty, S., Saminathan, M., Kumar, A., Karthik, K., Wani, M. Y., Amarpal, Singh, S. V., and Rahal, A. (2014). "Evidence based antibacterial potentials of medicinal plants and herbs countering bacterial pathogens especially in the era of emerging drug resistance: An integrated update," *International Journal of Pharmacology* 10(1), article 43. https://doi.org/10.3923/ijp.2014.1.43

Ding, K., Shibui, A., Wang, Y., Takamoto, M., Matsuguchi, T., and Sugane, K. (2005). "Impaired recognition by Toll-like receptor 4 is responsible for exacerbated murine Pneumocystis pneumonia," *Microbes and Infection* 7(2), 195-203. https://doi.org/10.1016/j.micinf.2004.10.010

Domínguez-Alegría, A. R., Pintado, V., and Barbolla, I. (2018). "Treatment and prevention of invasive pneumococcal disease," *Revista Clinica Espanola* 218(5), 244-252. https://doi.org/10.1016/j.rce.2018.01.004

Duan, Q., Jia, Y., Qin, Y., Jin, Y., Hu, H., and Chen, J. (2020). "Narciclasine attenuates LPS-induced acute lung injury in neonatal rats through suppressing inflammation and oxidative stress," *Bioengineered* 11(1), 801-810. https://doi.org/10.1080/21655979.2020.1795424

Fulkerson, P. C., and Rothenberg, M. E. (2013). "Targeting eosinophils in allergy, inflammation and beyond," *Nature Reviews Drug Discovery* 12, 117-129. https://doi.org/10.1038/nrd3838

Gou, K., Li, Y., Qu, Y., Li, H., and Zeng, R. (2022). "Advances and prospects of *Bletilla striata* polysaccharide as promising multifunctional biomedical materials," *Materials and Design* 223, article 111198. https://doi.org/10.1016/j.matdes.2022.111198

Gu, K., Ding, L., Wang, Z., Sun, Y., Sun, X., Yang, W., Sun, H., Tian, Y., Wang, Z., and Sun, L. (2023). "Wogonin attenuates the pathogenicity of *Streptococcus pneumoniae* by double-target inhibition of Pneumolysin and Sortase A," *Journal of Cellular and Molecular Medicine* 27(4), 563-575. https://doi.org/10.1111/jcmm.17684

Guo, Q., Jin, Y., Chen, X., Ye, X., Shen, X., Lin, M., Zeng, C., Zhou, T., and Zhang, J. (2024). "NF-κB in biology and targeted therapy: New insights and translational implications," *Signal Transduction and Targeted Therapy* 9(53).

https://doi.org/10.1038/s41392-024-01757-9

Gupta, P. D., and Birdi, T. J. (2017). "Development of botanicals to combat antibiotic resistance," *Journal of Ayurveda and Integrative Medicine* 8(4), 266-275. https://doi.org/10.1016/j.jaim.2017.05.004

Han, X., Song, Z., Liu, J., Zhang, Y., Wu, M., and Liu, H. (2023). "Effects of different drying methods on the quality of *Bletilla striata* scented tea," *Molecules* 28(6), article 2438. https://doi.org/10.3390/molecules28062438

Henig, O., and Kaye, K. S. (2017). "Bacterial pneumonia in older adults," *Infectious Disease Clinics of North America* 31(4), 689-713. https://doi.org/10.1016/j.idc.2017.07.015

Hernandez-Morfa, M., Olivero, N. B., Zappia, V. E., Piñas, G. E., Reinoso-Vizcaino, N. M., Cian, M. B., Nuñez-Fernandez, M., Cortes, P. R., and Echenique, J. (2023). "The oxidative stress response of *Streptococcus pneumoniae*: Its contribution to both extracellular and intracellular survival," *Frontiers in Microbiology* 141, article 269843. https://doi.org/10.3389/fmicb.2023.1269843

Hsieh, C. L., Cheng, C. Y., Tsai, T. H., Lin, I.-h., Liu, C. H., Chiang, S. Y., Lin, J. G., Lao, C. J., and Tang, N. Y. (2006). "Paeonol reduced cerebral infarction involving the superoxide anion and microglia activation in ischemia-reperfusion injured rats," *Journal of Ethnopharmacology* 106(2), 208-215. https://doi.org/10.1016/j.jep.2005.12.027

Janero, D. R. (1990). "Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury," *Free Radical Biology and Medicine* 9(6), 515-540. https://doi.org/10.1016/0891-5849(90)90131-2

Jiang, G., Wang, B., Wang, Y., Kong, H., Wang, Y., Gao, P., Guo, M., Li, W., Zhang, J., Wang, Z., and Niu, J. (2023). "Structural characteristics of a novel *Bletilla striata* polysaccharide and its activities for the alleviation of liver fibrosis," *Carbohydrate Polymers* 313, article 120781. https://doi.org/10.1016/j.carbpol.2023.120781

Jiang, S., Wang, M. Y., Yuan, H. W., Xie, Q., Liu, Y., Li, B. S., Jian, Y. Q., Liu, C. X., Lou, H. Y., Atta-Ur-Rahman, Pan, W. D., and Wang, W. (2020). "Medicinal plant of *Bletilla striata*: A review of its chemical constituents, pharmacological activities, and quality control," *World Journal of Traditional Chinese Medicine*. 6(4), 393-407. https://doi.org/10.4103/wjtcm.wjtcm_58_20

Keita, K., Darkoh, C., and Okafor, F. (2022). "Secondary plant metabolites as potent drug candidates against antimicrobial-resistant pathogens," *SN Applied Sciences* 4(209). https://doi.org/10.1007/s42452-022-05084-y

Kim, S. H., Shin, D. S., Oh, M. N., Chung, S. C., Lee, J. S., and Oh, K. B. (2004). "Inhibition of the bacterial surface protein anchoring transpeptidase sortase by isoquinoline alkaloids," *Bioscience, Biotechnology and Biochemistry* 68(2), 421-424. https://doi.org/10.1271/bbb.68.421

Kwon, D. H., Cha, H. J., Lee, H., Hong, S. H., Park, C., Park, S. H., Kim, G. Y., Kim, S., Kim, H. S., Hwang, H. J., and Choi, Y. H. (2019). "Protective effect of glutathione against oxidative stress-induced cytotoxicity in RAW 264.7 macrophages through activating the nuclear factor erythroid 2-related factor-2/heme oxygenase-1 pathway," *Antioxidants* 8(4), article 82. https://doi.org/10.3390/antiox8040082

Leemans, J. C., Vervoordeldonk, M. J. B. M., Florquin, S., Van Kessel, K. P., and Van Der Poll, T. (2002). "Differential role of interleukin-6 in lung inflammation induced by Lipoteichoic acid and peptidoglycan from *Staphylococcus aureus*," *American Journal of Respiratory and Critical Care Medicine* 165(10), 1445-1450.

https://doi.org/10.1164/rccm.2106045

Li, D., and Wu, M. (2021). "Pattern recognition receptors in health and diseases," *Signal Transduction and Targeted Therapy* 6(291). https://doi.org/10.1038/s41392-021-00687-0

Li, Q., Li, K., Huang, S. S., Zhang, H. L., and Diao, Y. P. (2014). "Optimization of extraction process and antibacterial activity of *Bletilla striata* polysaccharides," *Asian Journal of Chemistry* 26(12), article 16500. https://doi.org/10.14233/ajchem.2014.16500

Han, L., Kou, J., Hu, K., Wang, Y., Tang, Z., Wu, Z., and Song, X. (2021). "Protective effects of Re-yan-ning mixture on *Streptococcus pneumonia* in rats based on network pharmacology," *Pharmaceutical Biology* 59(1), 209-221. https://doi.org/10.1080/13880209.2021.1872653

Long, M. E., Mallampalli, R. K., and Horowitz, J. C. (2022). "Pathogenesis of pneumonia and acute lung injury," *Clinical Science*. DOI: 10.1042/CS20210879

Loughran, A. J., Orihuela, C. J., and Tuomanen, E. I. (2019). "*Streptococcus pneumoniae*: Invasion and inflammation," *Microbiology Spectrum* 7(2). https://doi.org/10.1128/microbiolspec.gpp3-0004-2018

Luna, C. M., Pulido, L., Niederman, M. S., Casey, A., Burgos, D., Leiva Agüero, S. D., Grosso, A., Membriani, E., Entrocassi, A. C., Rodríquez Fermepin, M., Vay, C. A., Garcia, S., and Famiglietti, A. (2018). "Decreased relative risk of pneumococcal pneumonia during the last decade, a nested case-control study," *Pneumonia* 10(9). https://doi.org/10.1186/s41479-018-0053-6

Luo, Y., Wang, J., Li, S., Wu, Y., Wang, Z., Chen, S., and Chen, H. (2022). "Discovery and identification of potential anti-melanogenic active constituents of *Bletilla striata* by zebrafish model and molecular docking," *BMC Complementary Medicine and Therapies* 22(9). https://doi.org/10.1186/s12906-021-03492-y

Musher, D. M., Anderson, R., and Feldman, C. (2022). "The remarkable history of pneumococcal vaccination: An ongoing challenge," *Pneumonia* 14(5). https://doi.org/10.1186/s41479-022-00097-y

Paterson, G. K., and Mitchell, T. J. (2006). "The role of *Streptococcus pneumoniae* sortase A in colonisation and pathogenesis," *Microbes and Infection* 8(1), 145-153. https://doi.org/10.1016/j.micinf.2005.06.009

Sánchez-Tarjuelo, R., Cortegano, I., Manosalva, J., Rodríguez, M., Ruíz, C., Alía, M., Prado, M. C., Cano, E. M., Ferrándiz, M. J., de la Campa, A. G., Gaspar, M. L., and de Andrés, B. (2020). "The TLR4-MyD88 signaling axis regulates lung monocyte differentiation pathways in response to *Streptococcus pneumoniae*," *Frontiers in Immunology* 11, article 2120. https://doi.org/10.3389/fimmu.2020.02120

Sekhar, R. V., Patel, S. G., Guthikonda, A. P., Reid, M., Balasubramanyam, A., Taffet, G. E., and Jahoor, F. (2011). "Deficient synthesis of glutathione underlies oxidative stress in aging and can be corrected by dietary cysteine and glycine supplementation," *American Journal of Clinical Nutrition* 94(3), 847-853. https://doi.org/10.3945/ajcn.110.003483

Shoji, H., Vázquez-Sánchez, D. A., Gonzalez-Diaz, A., Cubero, M., Tubau, F., Santos, S., García-Somoza, D., Liñares, J., Yuste, J., Martí, S., and Ardanuy, C. (2018). "Overview of pneumococcal serotypes and genotypes causing diseases in patients with chronic obstructive pulmonary disease in a Spanish hospital between 2013 and 2016," *Infection and Drug Resistance* 11, 1387-1400. https://doi.org/10.2147/IDR.S165093

Singh, U. P., Singh, R. K., Bhat, H. R., Subhashchandra, Y. P., Kumar, V., Kumawat, M. K., and Gahtori, P. (2011). "Synthesis and antibacterial evaluation of series of novel tri-substituted-s-triazine derivatives," *Medicinal Chemistry Research* 20(9), 1603-1610. https://doi.org/10.1007/s00044-010-9446-7

Srivastava, J. K., Pillai, G. G., Bhat, H. R., Verma, A., and Singh, U. P. (2017). "Design and discovery of novel monastrol-1,3,5-triazines as potent anti-breast cancer agent via attenuating epidermal growth factor receptor tyrosine kinase," *Scientific Reports* 7(1), article 5851. https://doi.org/10.1038/s41598-017-05934-5

Stogova, N. A. (2023). "Features of clinical and morphological manifestations of pneumonia complicated by exudative pleuritis in senile patients," *Pulmonologiya* 33(3), 350-356. https://doi.org/10.18093/0869-0189-2023-33-3-350-356

Tafroji, W., Margyaningsih, N. I., Khoeri, M. M., Paramaiswari, W. T., Winarti, Y., Salsabila, K., Putri, H. F. M., Siregar, N. C., Soebandrio, A., and Safari, D. (2022). "Antibacterial activity of medicinal plants in Indonesia on *Streptococcus pneumoniae*," *PLoS ONE* 17(9 September). https://doi.org/10.1371/journal.pone.0274174

Turner, P. V., Brabb, T., Pekow, C., and Vasbinder, M. A (2011). "Administration of substances in laboratory animals: routes of administration and factors to consider," *Journal of the American Association for Laboratory Animal Science* 50(5), 600-613.

Wang, L., Li, Q., Li, J., Jing, S., Jin, Y., Yang, L., Yu, H., Wang, D., Wang, T., and Wang, L. (2021). "Eriodictyol as a potential candidate inhibitor of sortase A protects mice from methicillin-resistant *Staphylococcus aureus*-induced pneumonia," *Frontiers in Microbiology* 12, article 635710. https://doi.org/10.3389/fmicb.2021.635710

Wang, W., and Meng, H. (2015). "Cytotoxic, anti-inflammatory and hemostatic spirostane-steroidal saponins from the ethanol extract of the roots of *Bletilla striata*," *Fitoterapia* 101, 12-18. https://doi.org/10.1016/j.fitote.2014.11.005

Wu, Q., Qu, M., Zhong, P., Zeng, Y., Wang, J., Zhang, Q., Wang, T., Liu, D., Yang, L., Zhou, J., and Wang, T. (2022). "Anti-inflammatory and anti-oxidant activity of ultra-short wave diathermy on LPS-induced rat lung injury," *Bulletin of Experimental Biology and Medicine* 172(4), 423-429. https://doi.org/10.1007/s10517-022-05407-4

Wu, Q., Zhou, M., Chen, Y., Zhu, B., Zhou, F., Ye, X., Huang, Y., and Ding, Z. (2024). "*Bletilla striata* polysaccharides protect against ARDS by modulating the NLRP3/caspase1/GSDMD and HMGB1/TLR4 signaling pathways to improve pulmonary alveolar macrophage pyroptosis," *Journal of Ethnopharmacology* 319, article 117361. https://doi.org/10.1016/j.jep.2023.117361

Xu, D., Pan, Y., and Chen, J. (2019). "Chemical constituents, pharmacologic properties, and clinical applications of *Bletilla striata*," *Frontiers in Pharmacology* 10, article 1168. https://doi.org/10.3389/fphar.2019.01168

Yesilkaya, H., Oggioni, M. R., and Andrew, P. W. (2022). "Streptococcus pneumoniae: 'captain of the men of death' and financial burden," *Microbiology (United Kingdom)* 168(12), article 1275. https://doi.org/10.1099/mic.0.001275

Zahlten, J., Kim, Y. J., Doehn, J. M., Pribyl, T., Hocke, A. C., García, P., Hammerschmidt, S., Suttorp, N., Hippenstiel, S., and Hübner, R. H. (2015). "Streptococcus pneumoniae-induced oxidative stress in lung epithelial cells depends on pneumococcal autolysis and is reversible by resveratrol," *Journal of Infectious Diseases* 211(11), 1822-1830. https://doi.org/10.1093/infdis/jiu806

Zhang, L., Ouyang, S., Zhang, H., Qiu, M., Dai, Y., Wang, S., Wang, Y., and Ou, J. (2021). "Graphene oxide induces dose-dependent lung injury in rats by regulating autophagy," *Experimental and Therapeutic Medicine* 21(5), article 462. https://doi.org/10.3892/etm.2021.9893

Article submitted: June 22, 2025; Peer review completed: July 19, 2025; Revised version received and accepted: October 2, 2025; Published: February 8, 2026.
DOI: 10.15376/biores.21.2.3002-3020