# Efficacy and Toxicity of Nanoencapsulation of *Peronema canescens* Extract in Reducing ARDS Inflammation *In-vivo*

Tonny Cortis Maigoda[1*], Judiono Judiono[2], Felix Zulhendri[3,4], Meriwati Meriwati[1], Rustam Aji[1]

## Abstract

Background: Acute respiratory distress syndrome (ARDS) is a severe condition frequently observed in intensive care units, particularly exacerbated during the COVID-19 pandemic. Excessive inflammation, often seen in ARDS, poses significant treatment challenges. Peronema canescens, known for its anti-inflammatory and immunomodulatory properties, offers potential therapeutic benefits. This study investigates the impact of nanoencapsulation of P. canescens leaf extract on inflammation in ARDS. Methods: Male Wistar rats were used to model ARDS through intratracheal administration of lipopolysaccharide (LPS). The experimental groups included normal controls, negative controls (LPS only), Imboost, Vitamin C, P. canescens extract, and three doses of nanoencapsulated P. canescens extract (nPC). Clinical observations, histopathological analyses, and serum TNF-α levels were assessed over a 14-day period. Nanoencapsulation involved the use of pectin, chitosan, and Na-tripolyphosphate, and the encapsulated products were characterized for particle size and encapsulation efficiency. Results: The nanoencapsulation efficiency of P. canescens extract was 86.16%, with an average particle size of 496.3 nm. Clinical observations indicated reduced activity and kyphosis in negative control and nPC groups. Macroscopic and histopathological analyses showed significant inflammation and lung damage in these groups compared to normal, Imboost, Vitamin C, and P. canescens extract groups. Serum TNF-α levels were significantly lower in the P. canescens extract group compared to the negative control on day 14, but no significant difference was observed between the nPC and negative control groups. Conclusion: While P. canescens extract demonstrated efficacy in reducing inflammation and improving lung health in ARDS rats, the nanoencapsulated form did not enhance therapeutic outcomes. The potential reasons include poor bioactive compound release and possible adverse interactions with encapsulation materials. Further research is needed to explore the kinetics of bioactive compound release and the potential toxicity of nanoencapsulation materials.

**Keywords:** Acute Respiratory Distress Syndrome (ARDS), Peronema canescens, Nanoencapsulation, Anti-inflammatory, Bioactive compounds

***Significance*** | This study demonstrated nanoencapsulation for effective delivery of Peronema canescens extract to reduce ARDS-related inflammation offers potential advancements in respiratory disease management.

*Correspondence. Tonny Cortis Maigoda, Nutrition Department, Poltekkes Kemenkes Bengkulu.
E-mail: tony@poltekkesbengkulu.ac.id, meriwati.mahyudin@poltekkesbengkulu.ac.id

Editor Md Shamsuddin Sultan Khan, And accepted by the Editorial Board Jun 23, 2024 (received for review Apr 16, 2024)

## Introduction

Respiratory tract inflammation is a hallmark symptom of acute respiratory distress syndrome (ARDS), a condition that affected approximately 10% of patients in intensive care units (ICUs) during the global COVID-19 pandemic (Fiala et al., 2020). While

**Author Affiliation.**
[1] Nutrition Department, Poltekkes Kemenkes Bengkulu, Indonesia.
[2] The Health Polytechnic of Bandung, Ministry of Health Republic of Indonesia, Indonesia.
[3] Department of Pharmacology and Clinical Pharmacy, Universitas Padjadjaran, Sumedang, West Java, Indonesia.
[4] Kebun Efi, Kabanjahe, Sumatera Utara, Indonesia, Indonesia.
[5] Department of Nursing, Poltekkes Kemenkes Bengkulu, Indonesia.

**Please cite this article.**
Tonny Cortis Maigoda, Judiono Judiono et al. (2024). Efficacy and Toxicity of Nanoencapsulation of Peronema canescens Extract in Reducing ARDS Inflammation in-vivo, Journal of Angiotherapy, 8(6), 1-10, 9753

inflammation is a natural response to infections, in cases like SARS-CoV-2, avian influenza, and other acute infectious diseases, an excessive inflammatory response can be detrimental and sometimes fatal. The treatment and management of ARDS present significant challenges, particularly during the COVID-19 pandemic and similar infectious outbreaks. In this context, Peronema canescens leaves, known for their flavonoid, saponin, and polyphenol content, have shown promise due to their anti-inflammatory and immunomodulatory properties (Latief et al., 2021; Maigoda et al., 2022; Oktiansyah et al., 2023). Peronema canescens, commonly known as Sangkareho or Sandpaper Tree or Daun Sungkai, is a notable plant species indigenous to Southeast Asia, particularly in Indonesia. Historically, it has been an integral part of functional food and/or traditional medicine systems in this region, utilized for its health promoting and therapeutic properties. The extract of Peronema canescens, derived from its leaves and bark, has recently gained attention for its potential health benefits. The traditional use of Peronema canescens spans several centuries, with local communities employing it for immune-booster and inflammation (Latief et al., 2021; Maigoda et al., 2022; Oktiansyah et al., 2023; Putranto, 2014).

The bioactive compounds present in Peronema canescens, such as phenolics, flavonoids, tannins, and other phytochemicals, are believed to contribute to its medicinal properties (Maigoda et al., 2022). These compounds are known for their antioxidant and anti-inflammatory activities, which are crucial in the prevention and management of various chronic diseases. Recent studies have focused on the biochemical properties of these compounds in relation to human health. For instance, the antioxidant properties of the flavonoids found in Peronema canescens are linked to their ability to scavenge free radicals, thereby mitigating oxidative stress, a key factor in the pathogenesis of chronic conditions like cardiovascular diseases and cancer (Elfita et al., 2022). Furthermore, the anti-inflammatory properties of Peronema canescens have been suggested to be beneficial as functional food in managing inflammatory disorders (Maigoda et al., 2022).

In addition, nanoencapsulation has emerged as a pivotal strategy for delivering bioactive compounds. This technique involves enclosing these compounds within nanoscale carriers, typically ranging from 10 to 1000 nanometers (Ezhilarasi et al., 2013; Pateiro et al., 2021; Rezaei et al., 2019). The primary objective of nanoencapsulation is to protect sensitive bioactive ingredients from environmental degradation and to control their release at the target site. This is particularly crucial in pharmaceuticals, food technology, and cosmetics, where maintaining the stability, solubility, and bioavailability of active ingredients is essential. Nanoencapsulation not only ensures a controlled and sustained release of bioactives but can potentially enhance the absorption and efficacy of these compounds (Ezhilarasi et al., 2013; Pateiro et al., 2021; Rezaei et al., 2019).

This study focuses on evaluating the impact of nanoencapsulation of Peronema canescens (known as Sungkai/Jati Sabrang/Ki Sabrang in Indonesia) leaf extract on the inflammation, particularly in the context of acute respiratory distress syndrome (ARDS). The research involved inducing ARDS in male Wistar strain rats using lipopolysaccharide (LPS) compounds (De Souza Xavier Costa et al., 2017; Håkansson et al., 2012). Key aspects examined in this study include clinical (preclinical) parameters, levels of proinflammatory biomarkers like TNF-α (Tumor Necrosis Factor-alpha), and histological analyses to assess lung repair and recovery following LPS induction (Faffe et al., 2000; Massey et al., 2015).

## 2. **Materials and methods**

### *2.1 Moisture content measurement.*

Moisture content testing of PC samples was conducted using the thermogravimetric method in an oven. Approximately 2 g of the sample in a crucible was oven-dried for 2 hours at 105°C, then placed in a desiccator to stabilize for 30 minutes and weighed. The sample in the crucible was oven-dried again for 1 hour, then returned to the desiccator to stabilize for another 30 minutes and weighed. This procedure was repeated until a constant weight was achieved, after which data processing was conducted to determine the moisture content percentage. Peronema canescens ethanolic extract was prepared in accordance to (Maigoda et al., 2022).

### 2.2 *Nano-encapsulation of Peronema canescens Extract*

#### *2.2.1 Preparation of Nanoencapsulation of PC extract*

The coatings used for encapsulation included 0.08% (w/v) pectin, 0.04% (w/v) chitosan, and 0.01% (w/v) Na-tripolyphosphate (NaTPP). The 0.08% pectin was prepared by dissolving pectin in hot purified water. The 0.04 % (w/v) chitosan was prepared by dissolving chitosan in 0.2% (v/v) acetic acid (pH 4.7), stirred using a magnetic stirrer for six hours with heat, and then filtered through a 0.45 µm filter paper to ensure the absence of insoluble aggregates. The 0.01% (w/v) NaTPP was prepared by dissolving NaTPP in warm purified water, stirred until homogeneous, and filtered using a 0.45 µm filter paper. All three coatings were sonicated for further homogenization.

In the encapsulation stage, the PC leaf extract was suspended in the pectin, chitosan, and NaTPP coatings using the complex coacervation method. The amount of PC leaf extract to be encapsulated was determined based on the dosage calculation of 3.94 mg/200 g body weight of rats. A total of 106.38 mg of PC leaf extract was added to the chitosan solution, stirred with a magnetic stirrer until homogeneous, then the TPP solution was added and stirred again. The resulting mixture was then combined with the pectin solution and stirred again until homogeneous using a

magnetic stirrer. The composition of the coating used was 2:20:5 (0.08% (w/v) pectin, 0.04% (w/v) chitosan, 0.01% (w/v) NaTPP).

***2.2.2 Characterization of Encapsulated Products***

***2.2.2.1 Particle Size Analysis***

The encapsulated product was analyzed for average particle size using a particle size analyzer (PSA) Horiba SZ-100 (Horiba Ltd, Japan) to confirm the formation of nano-sized particles. The dynamic light scattering type PSA used is capable of analyzing particle sizes up to < 0.5 μm. The principle of this device is the measurement of Brownian motion or random movement of particles in suspension, allowing for the determination of particle size in a sample.

***2.2.2.2 Encapsulation efficiency analysis***

The encapsulated product was tested for encapsulation efficiency to determine the amount of extract successfully encapsulated. The product suspension was centrifuged for 75 minutes at 4500 rpm. The obtained supernatant's absorbance was measured with a UV-Vis spectrophotometer at the 233 nm wavelength. The data obtained were then analyzed to determine the encapsulation efficiency percentage (%EE). The EE% was calculated by subtracting the initial amount of PC extract with the remaining PC extract after encapsulation, divided by the initial amount of PC extract, multiplied by 100%.

***2.3 In vivo experiments***

The study utilized male Wistar rats, eight weeks old and weighing around 200 grams, sourced from the iRATco Veterinary Laboratory Services' animal breeding facility in Dramaga Bogor. The rats were housed in individual cages, each measuring 625.5 $cm^2$ in area and 18.7 cm in height. Groups of five rats were placed in each cage. These cages were maintained in an environment controlled by an air handling unit, ensuring an air flow of 60 Air Changes per Hour (ACH). Temperature and humidity were regulated using an air conditioner, set at 22±3°C, with humidity levels maintained between 55-68%. The cages were lined with clean, dry, pest-free husk, sterilized using ultraviolet radiation. Artificial lighting was provided, mimicking a natural cycle with 12 hours of light and 12 hours of darkness (Poulos and Borlongan, 2000; Soares-Cunha et al., 2018). During the 7-day acclimatization period, the rats were fed standard laboratory rodent feed containing 18% (w/w) crude protein and 5.7% (w/w) fat. Clean drinking water was available ad libitum (Purkon et al., 2021; Sinam et al., 2016). In cases of death during the experiment, necropsies were performed to ascertain the causes. Organ samples were preserved in 10% neutral buffered formalin (NBF) for subsequent histopathological examination using the paraffin embedding technique (Seger et al., 2018).

The acute respiratory distress syndrome (ARDS) model, representing Covid-19, was established by intratracheally administering 5 mg/kg body weight (BW) of lipopolysaccharide (LPS; Escherichia coli 0111: B4, L 2630 from Sigma-Aldrich) to animals experienced in veterinary procedures. This in-vivo preclinical testing model was based on the methodology described by Lee et al. (2019) [18]. The following protocol was applied: (a). Rats were anesthetized using 90 mg/kg BW of ketamine and 10 mg/kg BW of xylazine, followed by disinfection of the underside skin of the neck with 70% (v/v) alcohol. (b) A small incision was made in the skin to expose the trachea. (c) LPS was diluted in 0.2 mL saline to a concentration of 5 mg/kg BW, was slowly injected into the trachea.

The experimental protocol was carried according to Lee et al. (Lee et al., 2019). The administered dose of lipopolysaccharide (LPS), used to induce inflammation in the respiratory system, was 5 mg/kg body weight. Imboost®, an immunomodulatory medication manufactured by PT. Soho Global Health (Indonesia), comprises 250 mg of Echinacea purpurea extract and 10 mg of zinc picolinate per tablet. Additionally, leaves of Peronema canescens were sourced from the Bengkulu Province on Sumatra Island, Indonesia. Thirty Wistar strain rats, acquired from the iRATco Veterinary Laboratory in Bogor, were utilized as test animals. These rats were randomly assigned into six groups, following a Randomized Clinical Trial (RCT) experimental design. There were five rats in each group. The specifics of the group divisions were as follows: (a) untreated/ normal rats were given 2 mL physiological NaCl, negative control rats: LPS 5 mg/kg bw and 2 mL physiological NaCl, Imboost rats: 5 mg/kg bw LPS and 78.4 mg/kg bw of Imboost, Vitamin C rats: 5mg/kg BW LPS and 78.4 mg/kg Vitamin C, PC extract rats: LPS 5mg/kg BW and PC extract 78.4 mg/kg BW, nano-encapsulated PC (1) (nPC (1)): LPS 5mg/kg BW and PC nano-emulsion 39.2 mg/kg BW, nano-encapsulated PC (2) (nPC (2)): LPS 5mg/kg BW and PC nano-emulsion 78.4 mg/kg BW, nano-encapsulated PC (3) (nPC (3)): LPS 5 mg/kg BW and PC nano-emulsion 156.8 mg/kg BW.

***2.4 Clinical observations, macroscopic and histopathological analyses***

The rats were observed for 14 days. Daily clinical assessments were conducted before and after ARDS induction to evaluate the test material's effectiveness in mitigating ARDS severity. Assessments included monitoring behavior, motor activity, respiratory rate, and signs of pneumonia, such as nasal discharge. At day 14, the animals were terminated for clinical observations, macroscopic and histopathological analyses. In addition, the weight of the animals was measured at day 7 and 14.

Moreover, at day 14, the measurement of the percentage of relative weight of the lungs relative to the body weight was carried out. We also measured the edema index by weighing the portion of lungs that exhibited edema and divided by the total weight of the lungs, multiplied by 100%. In addition, lung capacity was measured by scoring the observation of 20 fields of microscopic view, divided by the total area of the lungs and multiplied by 100%. The area of the healthy portion of the lungs was also calculated by observing the

non-edema portion divided by the total area of the lungs multiplied by 100%. All color-related analyses were processed and calculated using ImageJ software.

***2.5 Serum TNF-α analysis***

Blood samples were collected on day 7 and day 14 for serum TNF-α analysis. Blood was drawn from the retroorbital plexus into an EDTA tube for hematological tests and a tube without anticoagulant for serum collection. Serum was separated by centrifuging at 2500 rpm for 10 minutes. TNF-α cytokine levels were measured using ELISA as per the kit's instructions (Laksmitawati et al., 2017). Histopathological analysis was also carried out. Lung tissues were extracted on the fourteenth day, the final day of treatment. Observations for lung damage, including hemorrhage, inflammation, and fibrosis, were made based on the ARDS model (Koksel et al., 2004).

***2.6 Statistical Analysis***

The version 3.6.1 of R software was used for the statistical processing. One-way ANOVA and Tukey post-hoc test were used to analyze the data, where $p<0.05$ was considered significant.

## 3. Results

***3.1 Characterization of leaf extract and the nano-encapsulated extract***

The moisture content of the leaf extract 18.85%. In addition, the encapsulation efficiency, particle size diameter, and PI values are 86.16%, 496.3 nm, and 0.49, respectively (Table 1).

***3.2 Clinical examination of ARDS rats***

Table 2 shows the clinical analysis of the treated rats. Upon analyzing the clinical and locomotor activity abnormalities, it was observed that both the test groups and the normal groups exhibited typical behavior. However, in the negative control group (100%) and the nPC groups (60%), the test animals appeared noticeably less active. Additionally, these animals displayed a curvature in their spine, resulting in an abnormally rounded or bent upper back, a condition known as kyphosis. This suggests that the test animals in the negative control group and nPC groups likely experienced symptoms akin to pain, following the induction with lipopolysaccharides (LPS). There is no difference in terms of body weight in all rats (Figure 1).

***3.3 Macroscopic examination and histopathological analysis of the lungs***

Figure 2 illustrates the macroscopic observations of the lungs of the tested animals. The untreated controls, Imboost, vitamin C groups exhibit normal and healthy lungs. The PC-treated lungs appear to show relatively small areas of edema and bleeding. However, the negative controls, nPCs- treated lungs exhibit abnormal appearance such as distortion, erythema, atrophy, and bleeding. Figure 3 illustrates the histopathologic analysis. The infiltration of inflammatory cells, congestion, and pneumonia along with desquamation in the bronchial epithelium are apparent in the negative controls and nPC extract-treated rats. Conversely, the untreated controls, Imboost-treated, vitamin-C treated, and PC-treated lungs appear to be in healthier conditions. In addition, microscopic histopathological analysis shows similar characteristics. The untreated controls, Imboost-treated, vitamin C-treated, and PC-treated lungs show significantly less inflammatory cell infiltrates compared to the negative controls and nPCs-treated rats (Figure 3).

There is no significant difference in terms of the relative weight of the lungs to the body weight (Figure 4), edema index (Figure 5), lung capacity (Figure 6), and areas of healthy lungs (Figure 7) in untreated controls, Imboost, vitamin C, and PC-treated animals. However, there is a significant difference in terms of all parameters in the negative controls and nPC extract-treated rats. The negative controls and nPC extract-treated rats exhibit poorer results in terms of those parameters measured.

***3.4 TNF-α analysis***

Figure 8 shows the expression of TNF-α. On day 7, there is no difference in the serum TNF- α in all groups. However, there is a significant difference between the negative control and the untreated controls, Imboost-treated, vitamin-C treated, and PE extract-treated lungs ($p<0.05$) at day 14. There is no difference between the negative control and the nPC group, again demonstrating the inefficacy of nPC in reducing inflammation.

## 4. Discussion

P.canescens, also known in Indonesia as Sungkai or Jatibarang, is a member of the Verbenaceae family. This plant species is commonly found in Bengkulu Province and several other regions in Indonesia, often used as an ornamental plant around homes. The Dayak tribe in East Kalimantan, one of the indigenous tribes of Indonesia, traditionally uses the leaves of P. canescens as a functional food and herbal remedy (Jamu) to boost immune functions, treat fever, influenza virus infections, stomachaches, and for antibacterial purposes on the skin and around the mouth (Putranto, 2014; Ramadenti et al., 2017). In South Sumatra and Lampung Province, the young leaves of P. canescens are utilized for their anti-plasmodial, anti-pyretic (anti-fever), and anti-inflammatory properties. The leaves of P. canescens contain a variety of secondary metabolites, including phenolic compounds, flavonoids, and terpenoids, which offer numerous health benefits (Putranto, 2014; Ramadenti et al., 2017).

The total polyphenols and flavonoid of PC extract used in this study were reported in our previous study (Maigoda et al., 2022). Our previous study also demonstrated that PC extract was effective in reducing inflammation associated with ARDS in animal models (Maigoda et al., 2023). The ethanol extract derived from P. canescens leaves has been shown to be effective in treating

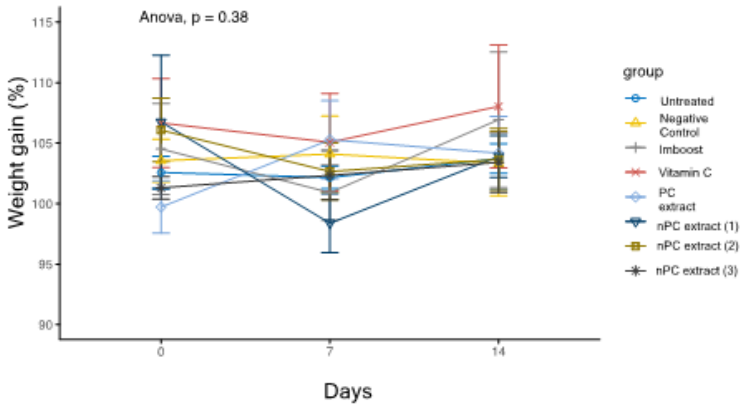


**Figure 1.** The weight of the rats on day 7 and day 14.

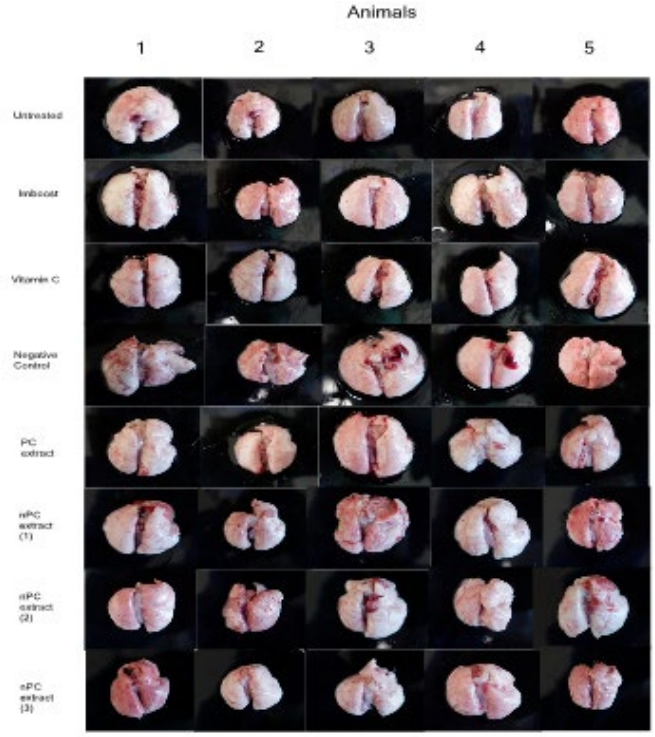


**Figure 2.** The macroscopic photos of the lungs of the rats.

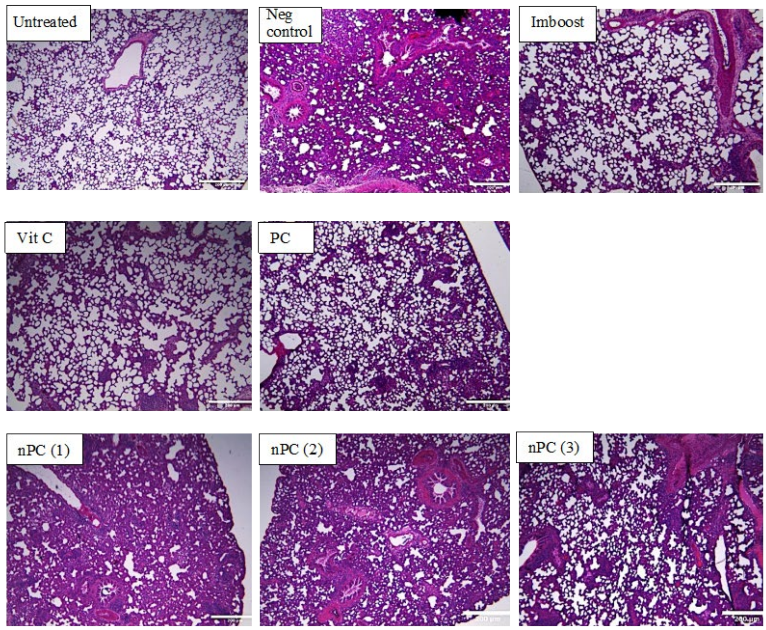


**Figure 3**. Histopathology analysis of the lungs of the rats

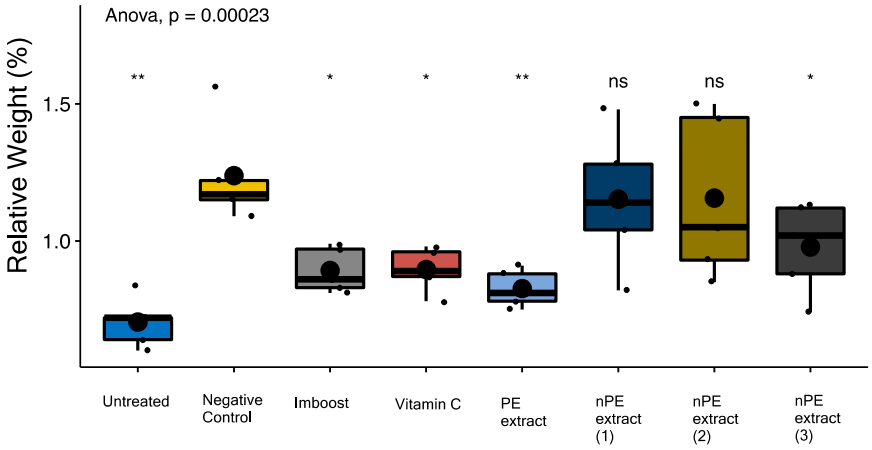


**Figure 4.** The relative weight of the lungs compared to the body weight.

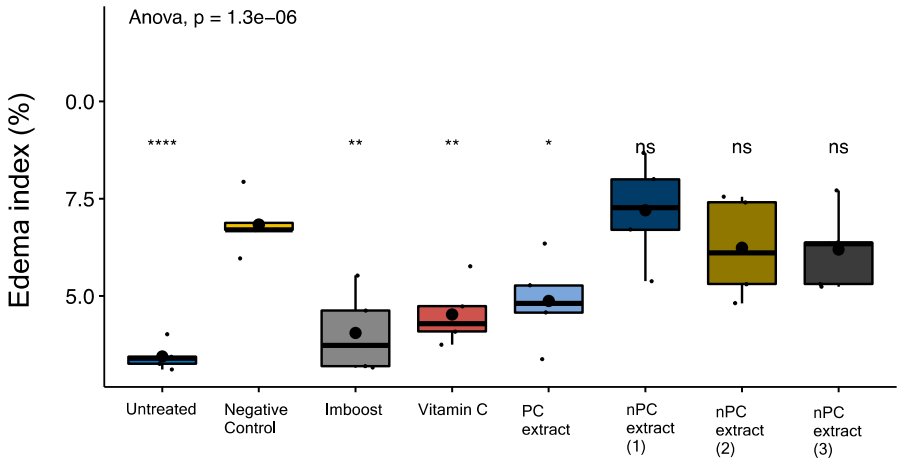


**Figure 5.** The edema index of the treated animals.

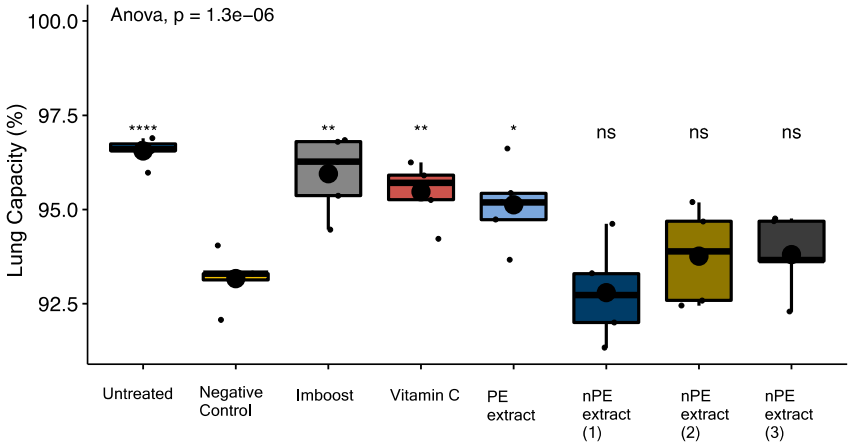


**Figure 6**. The lung capacity of the treated animals

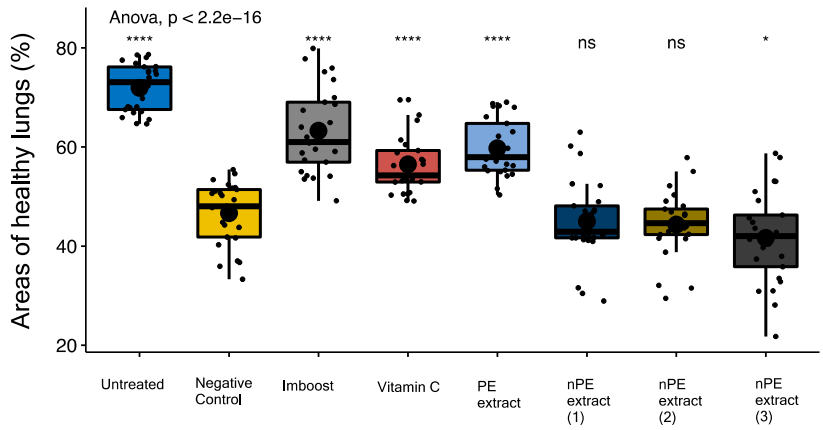


Figure 7. Areas of the healthy lungs.

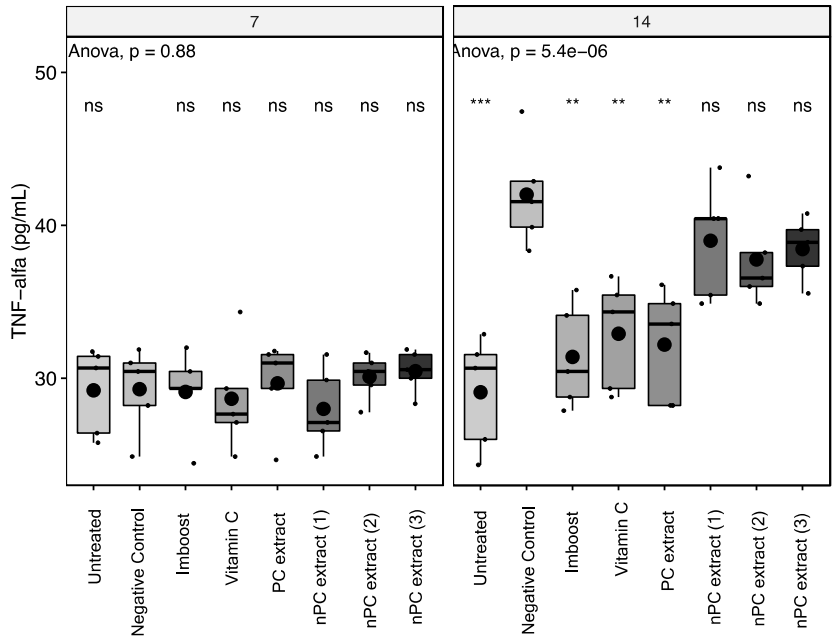


**Figure 8.** The serum concentration of TNF-α on days 7 and 14.

**Table 1.** Characteristics of *Peronema canescens* extract and its nano-encapsulated products

| **Parameters** | **Measurements** |
|---|---|
| Moisture content | 18.85 ± 0.12 % |
| **Nanoencapsulation** | |
| Encapsulation efficiency | 86.16 ± 0.43 % |
| Z-average/ particle size diameter | ~496.3 nm (range 130 nm to 510 nm) |
| PI | ~0.49 |

**Table 2.** Clinical analysis of the test animals.

| **Treatment groups** | **Clinical symptoms** | | | | | |
|---|---|---|---|---|---|---|
| | **Normal** | **Kyphosis and inactivity** | **Pain** | **Bulging eyes (exophthalmos)** | **Spasm** | **Death** |
| Untreated | 5/5 | - | - | - | - | - |
| Negative control | - | 5/5 | - | - | - | - |
| Imboost | 5/5 | - | - | - | - | - |
| Vitamin C | 5/5 | - | - | - | - | - |
| PE extract | 5/5 | - | - | - | - | - |
| nPE extract (1) | 2/5 | 3/5 | - | - | - | - |
| nPE extract (2) | 2/5 | 3/5 | - | - | - | - |
| nPE extract (3) | 2/5 | 3/5 | - | - | - | - |

pneumonia and in reducing lung damage caused by ARDS. This efficacy is evident from macroscopic observations, hematological and biochemical analyses, and histological examinations conducted on rats. The effect was repeated and confirmed in the present study. Figures 2-8 illustrate the efficacy of PE extract in reducing inflammation based on various macroscopic, histopathological analyses, and TNF-α serum, compared to vitamin C and Imboost. PC extract is shown to reduce infiltration of inflammatory cells, congestion, and pneumonia. In terms of clinical symptoms, none of the PE extracted-treated rats exhibited kyphosis, exophthalmos, pain, and/or spasm (Table 2).

This study aims to investigate the potential use of nano-encapsulated extract of PC in reducing inflammation in animal models of ARDS. Chitosan-TPP mixture was chosen as the primary encapsulation materials. The combination of chitosan with TPP often results in the formation of biocompatible, cross-linked structures based on chitosan. These structures are effective for the transportation of bioactive compounds such as phytochemicals including polyphenols (Di Santo et al., 2021). However, it appears that all three concentrations of nano-encapsulated PE did not result in any significant improvement in efficacy. In fact, the nano-encapsulated PC performed significantly less in terms of reducing the clinical symptoms and/or inflammation in ARDS animal models to the level where there was no significant difference compared to the negative controls (Figures 2-8).

We postulated that the bioactive compounds in the nano-encapsulated forms were not delivered or released during the experiments. Furthermore, the ingredients used in the nano-encapsulation solution could potentially interact or diminish the efficacy of PC extract. Moreover, the adverse effects of the nano-encapsulation materials might play a role in dampening the therapeutic effect of PC extract. Chitosan has been shown to induce lung inflammation (Huang et al., 2005).

Nanoencapsulation of bioactive compounds is a rapidly evolving area in the field of nanotechnology, particularly in pharmaceuticals, food science, and biotechnology. This technique involves encapsulating active ingredients in nanoscale carriers to enhance their delivery and effectiveness. Despite its numerous advantages, such as improved solubility, stability, controlled release, and targeted delivery, nanoencapsulation can sometimes result in diminished pharmacological activity compared to non-encapsulated bioactive compounds (Bazana et al., 2019).

However, there are several reasons that nano-encapsulation might reduce the efficacy of the bioactive compounds. One of the primary reasons for reduced efficacy post nanoencapsulation is the potential alteration in the chemical structure of the bioactive compound. During the encapsulation process, factors such as heat, pressure, and shear forces can cause degradation of sensitive compounds (Pisoschi et al., 2018). Although one of the objectives of nanoencapsulation is to enhance bioavailability, in some cases, the opposite effect can be observed. The encapsulating material might not degrade properly and therefore the release of the bioactive compounds does not commence (Bazana et al., 2019; Pisoschi et al., 2018).

The interaction between the bioactive compound and the encapsulating material can also lead to reduced activity. In some cases, the encapsulating material might bind too strongly with the compound, preventing it from being released in an active form (Domingues et al., 2022; Taouzinet et al., 2023). Additionally, the encapsulating material might interact with the compound in a way that inhibits its biological activity. This interaction can be particularly problematic when the encapsulating material is not biocompatible or when it interferes with the compound's controlled release, a key advantage of nanoencapsulation, can sometimes result in limited bioavailability of the bioactive compound. If the release is too slow or incomplete, it can lead to sub-therapeutic levels at the target site. This can be particularly challenging in treating acute conditions where rapid onset of action is required (Domingues et al., 2022; Taouzinet et al., 2023). In addition, nanocarriers can sometimes elicit an immune response or exhibit toxicity, which can overshadow the beneficial effects of the bioactive compound. The size, shape, surface chemistry, and composition of the nanoparticles can influence their interaction with biological systems (Huang et al., 2017).

We postulate that there are three events that potentially occurred in our study. Firstly, the potential toxicity of the encapsulation materials such as chitosan. Secondly, the encapsulating materials did not degrade as intended when delivered into the rats and therefore, the bioactive compounds of PC were not released. Thirdly, there was an unintended interaction and/or formation of complexes between the PC bioactive compounds and encapsulating material and therefore preventing the release of PC bioactive compounds. We observe that the nPC-treated animals demonstrate slight improvement in terms of lung capacity and TNF-α serum level compared to negative controls. However, they do not reach any statistical significance. In addition, there are improvements in terms of the numbers of rats showing clinical deficiencies, i.e. kyphosis in nPC-treated rats and the negative control groups. We suggest future studies investigating the potential toxicity and the kinetics of the release of the bioactive compounds of PC are carried out.

## 5. **Conclusions**

The extract from PC showed promising results in treating ARDS. This efficacy was confirmed through various analyses, including macroscopic and microscopic examinations, clinical symptoms, and serum TNF-α. The nano-encapsulation of PC did not improve the therapeutic effect of PC due to several unforeseen factors such

as the failure of the bioactive compound delivery and/or possibly toxicity. Further research investigating the toxicity of the nanoencapsulation materials and/or the kinetics of the PC bioactive compound release is warranted.

## Author contributions

T.C.M. conceptualized and drafted the manuscript. J.J., F.Z., M.M., and R.A. contributed to data analysis, provided critical revisions, and participated in the review and editing process. All authors reviewed and approved the final version of the manuscript.

## Acknowledgment

Author was grateful to their department.

## Competing financial interests

The authors have no conflict of interest.

## References

Bazana, M.T., Codevilla, C.F., de Menezes, C.R., 2019. Nanoencapsulation of bioactive compounds: challenges and perspectives. Curr. Opin. Food Sci. 26, 47–56. https://doi.org/10.1016/j.cofs.2019.03.005

De Souza Xavier Costa, N., Ribeiro, G., Dos Santos Alemany, A.A., Belotti, L., Zati, D.H., Frota Cavalcante, M., Veras, M.M., Ribeiro, S., Kallás, E.G., Saldiva, P.H.N., Dolhnikoff, M., Da Silva, L.F.F., 2017. Early and late pulmonary effects of nebulized LPS in mice: An acute lung injury model. PLoS One 12. https://doi.org/10.1371/journal.pone.0185474

Di Santo, M.C., D' Antoni, C.L., Domínguez Rubio, A.P., Alaimo, A., Pérez, O.E., 2021. Chitosan-tripolyphosphate nanoparticles designed to encapsulate polyphenolic compounds for biomedical and pharmaceutical applications – A review. Biomed. Pharmacother. 142. https://doi.org/10.1016/j.biopha.2021.111970

Domingues, C., Santos, A., Alvarez-Lorenzo, C., Concheiro, A., Jarak, I., Veiga, F., Barbosa, I., Dourado, M., Figueiras, A., 2022. Where Is Nano Today and Where Is It Headed? A Review of Nanomedicine and the Dilemma of Nanotoxicology. ACS Nano 16, 9994–10041. https://doi.org/10.1021/acsnano.2c00128

Elfita, Oktiansyah, R., Mardiyanto, Widjajanti, H., Setiawan, A., 2022. Antibacterial and antioxidant activity of endophytic fungi isolated from Peronema canescens leaves. Biodiversitas 23, 4783–4792. https://doi.org/10.13057/biodiv/d230946

Ezhilarasi, P.N., Karthik, P., Chhanwal, N., Anandharamakrishnan, C., 2013. Nanoencapsulation Techniques for Food Bioactive Components: A Review. Food Bioprocess Technol. 6, 628–647. https://doi.org/10.1007/s11947-012-0944-0

Faffe, D.S., Seidl, V.R., Chagas, P.S.C., Gonçalves de Moraes, V.L., Capelozzi, V.L., Rocco, P.R.M., Zin, W.A., 2000. Respiratory effects of lipopolysaccharide-induced inflammatory lung injury in mice. Eur. Respir. J. 15, 85–91. https://doi.org/10.1183/09031936.00.15108500

Fiala, A., Slagle, C., Legband, N., Aghabaglou, F., Buesing, K., Borden, M., Harris, S., Terry, B., 2020. Treatment of a Rat Model of LPS-Induced ARDS via Peritoneal Perfusion of Oxygen Microbubbles. J. Surg. Res. 246, 450–456. https://doi.org/10.1016/j.jss.2019.09.017

Håkansson, H.F., Smailagic, A., Brunmark, C., Miller-Larsson, A., Lal, H., 2012. Altered lung function relates to inflammation in an acute LPS mouse model. Pulm. Pharmacol. Ther. 25, 399–406. https://doi.org/10.1016/j.pupt.2012.08.001

Huang, Y.C., Vieira, A., Huang, K.L., Yeh, M.K., Chiang, C.H., 2005. Pulmonary inflammation caused by chitosan microparticles. J. Biomed. Mater. Res. - Part A 75, 283–287. https://doi.org/10.1002/jbm.a.30421

Huang, Y.W., Cambre, M., Lee, H.J., 2017. The Toxicity of Nanoparticles Depends on Multiple Molecular and Physicochemical Mechanisms. Int. J. Mol. Sci. 18. https://doi.org/10.3390/ijms18122702

Koksel, O., Cinel, I., Tamer, L., Cinel, L., Ozdulger, A., Kanik, A., Ercan, B., Oral, U., 2004. N-acetylcysteine inhibits peroxynitrite-mediated damage in oleic acid-induced lung injury. Pulm. Pharmacol. Ther. 17, 263–270. https://doi.org/10.1016/j.pupt.2004.05.002

Laksmitawati, D.R., Widyastuti, A., Karami, N., Afifah, E., Rihibiha, D.D., Nufus, H., Widowati, W., 2017. Anti-inflammatory effects of Anredera cordifolia and piper crocatum extracts on lipopolysaccharide-stimulated macrophage cell line. Bangladesh J. Pharmacol. 12, 35–40. https://doi.org/10.3329/bjp.v12i1.28714

Latief, M., Sari, P.M., Fatwa, L.T., Tarigan, I.L., Rupasinghe, H.P.V., 2021. Antidiabetic Activity of Sungkai (Peronema canescens Jack) Leaves Ethanol Extract on the Male Mice Induced Alloxan Monohydrate. Pharmacol. Clin. Pharm. Res. 6, 64. https://doi.org/10.15416/pcpr.v6i2.31666

Lee, S.A., Lee, S.H., Kim, J.Y., Lee, W.S., 2019. Effects of glycyrrhizin on lipopolysaccharide-induced acute lung injury in a mouse model. J. Thorac. Dis. 11, 1287–1302. https://doi.org/10.21037/jtd.2019.04.14

Maigoda, T., Judiono, J., Purkon, D., Haerussana, A., 2023. Peronema canescens ethanol extract attenuates inflammatory biomarkers and lung damage in ARDS rats animal models induced by LPS. J. Appl. Pharm. Sci. 13, 123–131.

Maigoda, T., Judiono, J., Purkon, D.B., Haerussana, A.N.E.M., Mulyo, G.P.E., 2022. Evaluation of Peronema canescens Leaves Extract: Fourier Transform Infrared Analysis, Total Phenolic and Flavonoid Content, Antioxidant Capacity, and Radical Scavenger Activity. Open Access Maced. J. Med. Sci. 10, 117–124. https://doi.org/10.3889/oamjms.2022.8221

Massey, V.L., Poole, L.G., Siow, D.L., Torres, E., Warner, N.L., Schmidt, R.H., Ritzenthaler, J.D., Roman, J., Arteel, G.E., 2015. Chronic Alcohol Exposure Enhances Lipopolysaccharide-Induced Lung Injury in Mice: Potential Role of Systemic Tumor Necrosis Factor-Alpha. Alcohol. Clin. Exp. Res. 39, 1978–1988. https://doi.org/10.1111/acer.12855

Oktiansyah, R., Elfita, E., Widjajanti, H., Setiawan, A., Mardiyanto, M., Nasution, S.S.A., 2023. Antioxidant and Antibacterial Activity of Endophytic Fungi Isolated from The Leaves of Sungkai (Peronema canescens). Trop. J. Nat. Prod. Res. 7, 2596–2604. https://doi.org/10.26538/tjnpr/v7i3.20

Pateiro, M., Gómez, B., Munekata, P.E.S., Barba, F.J., Putnik, P., Kovačević, D.B., Lorenzo, J.M., 2021. Nanoencapsulation of promising bioactive compounds to improve their absorption, stability, functionality and the appearance of the final food products. Molecules 26. https://doi.org/10.3390/molecules26061547

Pisoschi, A.M., Pop, A., Cimpeanu, C., Turcuş, V., Predoi, G., Iordache, F., 2018. Nanoencapsulation techniques for compounds and products with antioxidant and antimicrobial activity - A critical view. Eur. J. Med. Chem. 157, 1326–1345. https://doi.org/10.1016/j.ejmech.2018.08.076

Poulos, S.G., Borlongan, C. V., 2000. Artificial lighting conditions and melatonin alter motor performance in adult rats. Neurosci. Lett. 280, 33–36. https://doi.org/10.1016/S0304-3940(99)00997-0

Purkon, D.B., Iwo, M.I., Soemardji, A.A., Rahmawati, S.F., Fadhlillah, F.M., Nadhifah, A., 2021. Immunostimulant Activity of Marchantia paleacea Bertol. Herb Liverwort Ethanol Extract in BALB/c Mice. Indones. J. Pharm. 32, 464–473. https://doi.org/10.22146/ijp.2128

Putranto, A.M.H., 2014. EXAMINATION OF THE SUNGKAI'S YOUNG LEAF EXTRACT (Peronema canescens) AS AN ANTIPIRETIC, IMMUNITY, ANTIPLASMODIUM AND TERATOGENITY IN MICE (Mus.muculus). Int. J. Sci. Eng. 7. https://doi.org/10.12777/ijse.7.1.30-34

Ramadenti, F., Sundaryono, A., Handayani, D., 2017. Test of Ethyl Acetate Fraction of Peronema canescens Leaves against Plasmodium berghei in Mus musculus. Alotrop J. Pendidik. dan Ilmu Kim. 2, 89–92.

Rezaei, A., Fathi, M., Jafari, S.M., 2019. Nanoencapsulation of hydrophobic and low-soluble food bioactive compounds within different nanocarriers. Food Hydrocoll. 88, 146–162. https://doi.org/10.1016/j.foodhyd.2018.10.003

Seger, S., Stritt, M., Vezzali, E., Nayler, O., Hess, P., Groenen, P.M.A., Stalder, A.K., 2018. A fully automated image analysis method to quantify lung fibrosis in the bleomycininduced rat model. PLoS One 13. https://doi.org/10.1371/journal.pone.0193057

Sinam, G., Sahu, V., Pandey, N., Asthana, A.K., 2016. Effect of arsenic on biochemical and antioxidant enzymes in two species of Marchantia L. (Marchantiophyta): Role of enzymes in stress acclimatization 79–85.

Soares-Cunha, C., Coimbra, B., Borges, S., Domingues, A.V., Silva, D., Sousa, N., Rodrigues, A.J., 2018. Mild prenatal stress causes emotional and brain structural modifications in rats of both sexes. Front. Behav. Neurosci. 12. https://doi.org/10.3389/fnbeh.2018.00129

Taouzinet, L., Djaoudene, O., Fatmi, S., Bouiche, C., Amrane-Abider, M., Bougherra, H., Rezgui, F., Madani, K., 2023. Trends of Nanoencapsulation Strategy for Natural Compounds in the Food Industry. Processes 11. https://doi.org/10.3390/pr11051459