AFSSAAE

Advances in Food Science, Sustainable Agriculture and Agroindustrial Engineering 2023 | Special Issue | 152-158
ISSN 2622-5921

ORIGINAL RESEARCH Open Access

Anti-inflammatory activity of hydrolysed glucomannan from porang (*Amorphophallus muelleri* Blume) through inhibition response of nitric oxide production in lipopolysaccharide (LPS)-activated RAW 264.7 macrophage cells

Mochamad Affandi, Zamnia Wahyuli, Sudarminto Setyo Yuwono, Simon Bambang Widjanarko*)

Department of Food Science and Biotechnology, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia.

KEYWORDS

Amorphophallus
Anti-inflammatory
Hydrolysed glucomannan
Nitric oxide
Porang

ABSTRACT

Few studies have been conducted on porang (*Amorphophallus muelleri* Blume) glucomannan (PGM) as anti-inflammatory drugs. The study aimed to evaluate the relationship between the use of sulfuric acid concentration in the hydrolysis process and the anti-inflammatory effects of PGM hydrolysates (PGMH) using the nitric oxide (NO) inhibition approach. PGMH is prepared by hydrolysing PGM with sulfuric acid (0.25N, 0.5N, and 1.0N concentration). This experimental study analysed the production of nitric oxide (NO) formation related to inflammation. Lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells were treated with PGM and PGMH (62.5, 125, 250, and 500 micrograms per millilitres). The cytotropic substance was measured by using the Griess reaction assay. The analysis showed that the PGM and PGMH possessed more potent NO inhibitory activity than the positive control. PGMH 1.0N treatment had the highest inhibitory potential of NO production with an IC_{50} value of 353.1 micrograms per milliliters. Increasing the concentration of a PGMH 1.0N was inversely proportional to the decrease in NO production. PGMH 1.0N 500 micrograms per milliliter treatment significantly suppressed the production of NO. PGM and PGMH as alternative therapies stimulate the immune system in vitro significantly. The current study might be used as a preliminary guide for choosing the best concentrations of PGMH and sulfuric acid in future studies that aim to reduce inflammation and modulate the immune system.

Introduction

Porang (*Amorphophallus muelleri* Blume) is a species of the family Araceae. This species is a local Indonesian plant that originally grew wild in the forest. This plant has great potential to be developed as a raw material for the food industry. Porang plant has great potential to be used as a source of glucomannan (GM) in Indonesia. This is because the GM content in porang tubers is relatively higher than other similar plants. Commercial production of GM is generally extracted from dried tubers (Faridah and Widjanarko, 2013).

The chemical structure of GM consists of main chains and branched chains of polysaccharide polymer sequences. The main chains of GM consists of the polysaccharide sequence M-M-G describing the β-1,4 bond among D-glucose (G) and D-mannose (M). Whereas the branched chains consists of the sequence Ac-M-G describing the β-1,6-glucosyl bond symbolizing an acetyl group bound randomly to the C-6 site of the D-mannose (M) unit. A short side chain at the C-3 site of the mannose and the presence of a random acetyl group at the C-6 site of the sugar unit are also possibilities (Behera and Ray, 2016).

Several previous studies reported that Konjac glucomannan (KGM) flour has functional properties as an immunomodulator in the development of the immune system in the digestive organs, urinary tract and respiratory system. Another study on KGM flour with a size of 75-100 µm showed functional properties as an anti-inflammatory in male rats (Onishi et al., 2007).

*Corresponding author
E-mail address: simonbw@ub.ac.id

GM has several functional properties, but in general the formulation of food products using GM still needs to be developed. Therefore, it is possible that oligosaccharides from GM have similar bioactive properties which could potentially be used more widely. Oligosaccharides from GM can be obtained by hydrolyzing the polysaccharide form using physical, chemical methods or by enzymatic treatment (Li et al., 2021).

Previous research on the production of nanocrystal form of konjac glucomannan hydrolyzate (KGMH) using sulfuric acid method and neutralization with distilled water reported that the particle shape of KGMH was more crystalline than the amorphous form of natural KGM (Huang et al., 2010).

The results of the hydrolysis of KGM with sulfuric acid (sulphated KGM) and neutralization with $NaHCO_3$ showed its function as an anti-HIV compound (HIV virus) and as an anti-coagulant compound (Bo et al., 2013). Porang glucomannan hydrolysate (PGMH) with HCl and neutralization with NaOH positively inhibited the interaction of ACE2 (Covid-19 virus receptor) with spike protein (S1) from the outer layer of the Covid-19 virus membrane (Ulayya et al., 2022).

Glucomannan hydrolyzate (GMH) has a smaller molecular size. The study of hydrolysis of GM from konjac, indicating that the molecular size of GMH is effective for suppressing Immunoglobulin E (IgE) production both in vivo and in vitro (Suzuki et al., 2010).

Polysaccharides of plant origin are ideal candidates for immunomodulatory therapeutic agents due to their relatively low toxicity (Yin et al., 2019). They can increase the viability of macrophage cells. Polysaccharides with a high proportion of galactose residues in their structure significantly promoted the proliferation of RAW264.7 cells (Ma et al., 2019).

The results of the KGM depolymerization test (DKGM) produced short fatty acid chains (RALP) which would lower the pH of the stomach. RALP is a substrate for gastric microbes and shows positive effects for the treatment of inflammation and tumor formation in the stomach or other organs (Tester et al., 2012). Nitric oxide (NO) gas was used as a parameter to measure the anti-inflammatory effectiveness of oxidized KGM (U-OKGM) by ultrasonic method on RAW264.7 macrophage cells. Where the production of iNOS was significantly inhibited by the administration of U-OKGM (Zheng et al., 2019).

Nitric oxide (NO) plays an important role as a signaling molecule in many parts of the organism as well as a cytotoxic effector molecule of nonspecific immune responses (Kröncke et al., 1997). Measurement of NO as a inflammation marker is also performed to detect organ dysfunction (Galhardo et al., 2020). This compound is synthesized by many cell types involved in immunity and inflammation. NO is important as a toxic defense molecule against infectious organisms, and regulates the functional activity, growth and death of various types of immune and inflammatory cells including macrophages cells (Coleman, 2001). Anti-inflammatory refers to the properties of a substance that can reduce inflammation or swelling. Inflammation is a biological response to noxious stimuli such as pathogens that cause tissue and cell damage (De Cássia da Silveira e Sá et al., 2014). Anti-inflammatory activities could be evaluated via inhibition against NO production (Geng et al., 2014; Chien et al., 2016; Chien, et al., 2016).

Currently, research on the effect of acids other than HCl and neutralizing bases other than NaOH in the manufacture of PGMH on anti-inflammatory and immunomodulatory properties is very limited. According to the literature review mentioned above, it is viable to produce PGMH using sulfuric acid approach in an effort to develop food products that can be used in the human immune system.

Research Methods

Time and Location

The research was conducted in several laboratories from October 2021 to February 2022: Pilot Plant Laboratory of the Faculty of Agricultural Technology, Central Laboratory of Life Sciences (LSIH), and Biomolecular Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Brawijaya.

Materials

The material used in the production of PGMH is porang glucomannan (PGM) flour from local varieties provided by Pilot Plant Laboratory of the Faculty of Agricultural Technology Universitas Brawijaya. Chemicals used in the production of

PGMH is include H_2SO_4 (PA), $Ca(OH)_2$ (PA), distilled, aquabides (double distilled water), ethanol 99% (PA). The medium used for cell culture was Dulbecco's Modified Eagle Medium (DMEM) High-Glucose. Fetal Bovine Serum (FBS) was used as a nutrient to support cell life, and Penicillin - Streptomycin (Penstrep) was used as an antibiotic.

Preparation of Glucomannan Hydrolysate

Preparation of GMH was conducted based on acid method (Suzuki et al., 2010). Various concentrations of strong acid (0.25N, 0.5N, and 1.0 N) was used to hydrolyze PGM. The modification was carried out by using sulfuric acid as a strong acid and calcium hydroxide as a neutralizer. About 2% of PGM flour dissolved in distilled water and put in shaker water-bath for 20 minutes at 75^{o}C, then hydrolyzed with H_2SO_4 (0.25N, 0.5N, and 1.0 N). Hydrolysis process conducted on a magnetic stirrer for 60 minutes at 75^{o}C. The PGMH solution then neutralized using 4N $Ca(OH)_2$ suspension at room temperature followed by addition of 1x volume double distilled water. The solution then filtered in order to collect the supernatant. The supernatant was added with twice of the volume ethanol 99% until the PGMH precipitated. The PGMH was collected and dried using an oven dryer at 50^{o}C.

Preparation of RAW 264.7 Cell Culture

Stock RAW 264.7 cells stored at -80ºC were thawed at room temperature in Laminar Air Flow (LAF). The DMEM medium was then mixed with FBS and Penstrep in a ratio of 10% and 1% of the total DMEM used. DMEM which has been added with FBS and Penstrep is then homogenized. Furthermore, 5 mL of complete media was given into a sterile 60 mm culture dish. The thawed RAW 264.7 cells were then transferred to a culture dish containing media. The plates containing cells were then incubated in an incubator at 37ºC, 95% RH, and 5% CO_2 content for 2 days. If the cells were not confluent or full of the 60 mm ± 80% culture plate when observed under the microscope, then only the media was replaced. If the cells are confluent, they can be harvested according to the needs of treatment or testing.

Harvesting and Counting of RAW 264.7 Cell

RAW 264.7 cells are cells that grow attached to a culture dish. Cells were harvested by removing the media first, then adding 1 mL of culture media. The RAW 264 cells were then scraped using a sterile scraper slowly until the white part attached to the bottom of the cup was not visible. RAW 264.7 cells that had been scraped were then counted in the hemocytometer. The cell suspension was taken as much as 5 µL of cell suspension and then added with 95 µL of methylene blue and then homogenized with a pipette. Live cell counts were carried out using a counting chamber in a hemocytometer. Dead cells will be blue while living cells are colorless or clear. The number of living cells is counted in 5 medium-sized squares, then the cells are counted using the equation:

Cell Count = Cell count x 5 x dilution factor x 10^4 cells/ml ..... (1)

If the number of cells is sufficient for treatment, RAW 264.7 cells are ready to be used in the treatment and assay of anti-inflammatory activity.

Nitric Oxide (NO) Analysis

RAW 264.7 cells that had met the required number were implanted into a 24-well microplate with a density of $1x10^5$ per well. The cells were then incubated in a CO_2 incubator for 1x24 hours. After 1x24 hours, the culture medium was replaced with a treatment medium (except for the control group) as much as 1 mL of PGMH (62.5 ppm, 125 ppm, 250 ppm and 500 ppm). Each well except the negative control was added 1-2 g/mL lipopolysaccharide (LPS) to stimulate RAW 264.7 cells. Microplates were then incubated for 1x 24 hours. Measurement of NO formation was carried out using a spectrophotometer after being reacted with Griess method (Möller et al., 2019; Kang, 2018; Liao et al., 2014; Bryan and Grisham, 2007). The NO level test was carried out by taking 75 µL of media in a microplate, then transferred to a new 96-well microplate. Griess reagent was prepared by making stock solution A consisting of 1% sulfanilamide in 5% phosphoric acid and solution B consisting of 0.1% N-1-napthylethylenediamine dihydrochloride in H_2O. Solutions A and B were then mixed with a ratio of 1:1 (v/v) and homogenized with a vortex. The NO level test was then carried out by giving the prepared Griess Reagent into the microplate with a ratio of 1:1 (v/v). The standard solution to make a standard nitrite curve is to make the serial concentrations of $NaNO_2$ in the media 25, 50, 75, and 100 µM respectively. The culture media used were considered as blank. The microplate was then read using a microplate reader at a wavelength of 571 nm.

Half-Maximal Inhibitory Concentration (IC_{50}) Analysis

IC_{50} value is calculated through the regression equation y = ax + b between the log sample concentration used and the percentage of Nitric oxide (NO) obtained (Martinez-Morales et al., 2020) (Scherer and Godoy, 2009) (Science Gateway, 2022).

Statistical Analysis

Statistical analysis was carried out using one way ANOVA (Analysis of Variance) with a confidence interval of 95% for any significant difference.

Results and Discussion

Effect of Acid Concentration on IC_{50} Value of NO

The IC_{50} is the concentration of compound required for 50% inhibition. IC_{50} is an operational term dependent on the assay conditions (Swinney, 2011). The IC_{50} value was calculated using measurements of NO compound formation inhibition. The addition of LPS to macrophage cells caused an inflammatory process that resulted in the formation of NO compounds. Effect of H_2SO_4 concentrations in PGM hydrolysis shown as a significant factor for inhibition activity of NO production (Figure 1).

Natural PGM is shown on the bar graph with zero normality sulfuric acid concentration or without acid addition. While the other bar graphs are PGMH samples resulting from acid hydrolysis with increasing concentrations of H_2SO_4 sequentially (0.25N, 0.5N, and 1.0 N). Sulfuric acid compounds are expected to break down natural PGM polymer chains into shorter PGMH chains through chemical reactions. Beside from chemical processes, research on GM derivative products with shorter chains or oligosaccharides can be obtained by enzymatic processes (Li et al., 2021).

In general, PGMH samples inhibited the formation of NO compounds better than natural PGM. This is possible because PGMH product compounds with shorter chains can play a larger role in the mechanism of inhibition of NO production than natural PGM. Polysaccharides have numerous biological functions in the human body, including antioxidants, immunomodulators, and antitumor activity. However, these properties are highly correlated with the structure of the polysaccharide polymer, namely molecular weight, monosaccharide composition, type of glycosidic bond, and degree of chain branching (Drira et al., 2021). Polysaccharides with a higher molecular weight will stimulate NO production more effectively (Nie et al., 2018).

The histogram shows that increasing the concentration of H_2SO_4 produces PGMH with greater activity in suppressing NO production. Higher acid concentration is possible to produce PGMH with lower molecular weight. PGMH with 1N H_2SO_4 treatment had the highest inhibitory potential of NO production with an IC_{50} value of 353.1 micrograms per milliliters. Previous studies using konjac flour showed that the lower molecular size of GM hydrolysate products has a significant role in immune system function and anti-inflammatory capabilities (Suzuki et al., 2010; Zheng et al., 2019).

Effect of Hydrolyzed Glucomannan Concentration on Anti-Inflammatory Activity by Inhibition of NO Production

Because of the best IC_{50} value of NO carried out by the highest acid concentration (1N H_2SO_4), the analysis was continued to determine the effect of PGMH (1N H_2SO_4) concentration on the anti-inflammatory activity by inhibition measurement of NO production. The treatment with the addition of sulfuric acid in PGM hydrolysis is expected to form sulfated polysaccharide complexes that have bioactive activity. Increasing the concentration of PGMH (1N H_2SO_4) was inversely proportional to the decrease in NO production (Figure 2). This is possible because the higher the concentration of PGMH flour used in the treatment, the more possibility of sulfated polysaccharide products that play a role in suppressing the inflammatory process.

Positive control with LPS treatment is shown on the bar graph with zero micrograms per milliliters PGMH concentration or without PGMH addition. While the other bar graphs are PGMH (1N H_2SO_4) samples with increasing concentrations sequentially (62.5, 125, 250, and 500 micrograms per milliliters). Effect of PGMH (1N H_2SO_4) concentrations in NO production shown as a significant factor for NO inhibition (Table 1). Based on the values in Table 1, it shows the significance level of the different treatments in the different columns of each PGMH (1N H_2SO_4) concentration. Different letter nominations show significantly differences treatment. PGMH (125, 250, and 500 micrograms per milliliters) treatment significantly suppressed the production of NO compared to the positive control. PGMH 500 micrograms per milliliters treatment had the highest inhibitory potential of NO production.

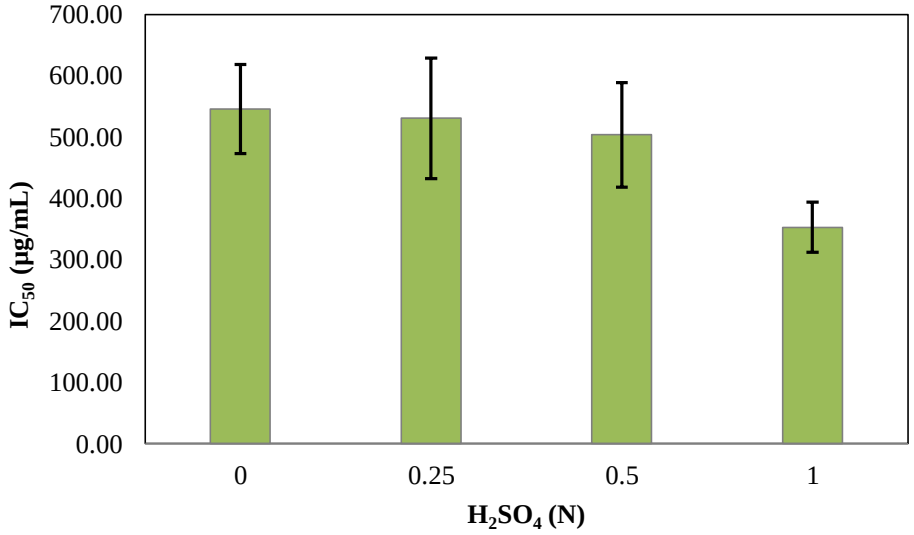

Figure 1. Effect of acid concentration on IC_{50} value of NO

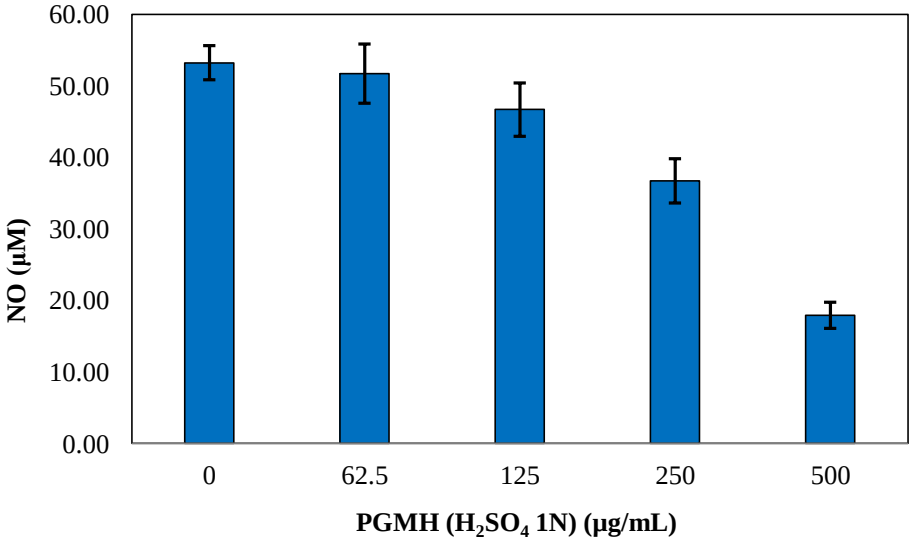

Figure 2. Effect of PGMH (1N H_2SO_4) concentration on inhibition of Nitric Oxide (NO) production

Table 1. Anti-Inflammatory Activity by Inhibition of Nitric Oxide Production

Materials		Nitric Oxide (µM)
LPS (g/mL)	PGMH (H_2SO_4 1N) (µg/mL)	
-	-	1.8±0.5[a]
1	0.0	55.0±2.4[e]
1	62.5	53.5±4.1[de]
1	125	48.5±3.7[d]
1	250	38.5±3.1[c]
1	500	19.7±1.8[b]

Remarks: [a-e] Mean values within a column followed by the differences letters are significantly different at $p < 0.05$ according to Duncan's Test.

Previous studies have shown that the higher the concentration of sulfuric acid used, the more likely it is to form more mannose residues and sulfated polysaccharides. Sulfated polysaccharides have a high potential for immunological activity (Huang et al., 2019). Numerous polysaccharides that exhibit immunomodulatory activity contain large amounts of mannose and galactose residues in their structure (Nie et al., 2018; Tan et al., 2015). NO production in macrophages increased in a concentration-dependent manner upon treatment with various concentrations of polysaccharides (Huang et al., 2016). It can be concluded that the higher concentration of mannose residues and sulfated polysaccharides used, the better effect to inhibition of NO production.

Conclusion

By inhibiting the generation of NO, natural porang glucomannan and porang glucomannan hydrolisate both had stronger anti-inflammatory effect than the positive control. Higher H_2SO_4 concentrations resulted in porang glucomannan hydrolisate that had more NO production-suppression action. NO generation decreased in inverse proportion to an increase in porang glucomannan hydrolisate (1N H_2SO_4) concentration.

Acknowledgement

Acknowledgments to the Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia for their ongoing support on this research.

Declarations

Conflict of interests The authors declare no competing interests.

References

Behera, S. S., and Ray, R. C. (2016) 'Konjac glucomannan, a promising polysaccharide of Amorphophallus konjac K. Koch in health care', *International Journal of Biological Macromolecules*, 92, pp. 942–956

Bo, S., Muschin, T., Kanamoto, T., Nakashima, H., and Yoshida, T. (2013) 'Sulfation and biological activities of konjac glucomannan', *Carbohydrate Polymers*, 94(2), pp. 899–903

Bryan, N. S., and Grisham, M. B. (2007) 'Methods to detect nitric oxide and its metabolites in biological samples', *Free Radical Biology and Medicine*, 43(5), pp. 645–657

Chien, R. C., Lin, L. M., Chang, Y. H., Lin, Y. C., Wu, P. H., Asatiani, M. D., Wasser, S. G., Krakhmalnyi, M., Agbarya, A., Wasser, S. P., and Mau, J. L. (2016) 'Anti-inflammation properties of fruiting bodies and submerged cultured mycelia of culinary-medicinal higher basidiomycetes mushrooms', *International Journal of Medicinal Mushrooms*, 18(11), pp. 999–1009

Chien, R.-C., Yang, Y.-C., Lai, E. I., and Mau, J.-L. (2016) 'Anti-inflammatory effects of extracts from the medicinal mushrooms *Hypsizygus marmoreus* and *Pleurotus eryngii* (Agaricomycetes)', *International Journal of Medicinal Mushrooms*, 18(6), pp. 477-487

Coleman, J. W. (2001) 'Nitric oxide in immunity and inflammation', *International Immunopharmacology*, 1(8), pp. 1397–1406

De Cássia da Silveira e Sá, R., Andrade, L. N., Dos Reis Barreto de Oliveira, R., and De Sousa, D. P. (2014) 'A Review on anti-inflammatory activity of phenylpropanoids found in essential oils', *Molecules*, 19(2), pp. 1459-1480

Drira, M., Hentati, F., Babich, O., Sukhikh, S., Larina, V., Sharifian, S., Homai, A., Fendri, I., Lemos, M. F. L., Félix, C., Félix, R., Abdelkafi, S., and Michaud, P. (2021) 'Bioactive carbohydrate polymers—between myth and reality', *Molecules*, 26, pp. 1-36

Faridah, A., and Widjanarko, S. B. (2013) 'Optimization of multilevel ethanol leaching process of porang flour (*Amorphophallus muelleri*) using response surface methodology', *International Journal on Advanced Science Engineering Information Technology*, 3(2), pp. 74–80

Galhardo, L. F., Ruivo, G. F., de Oliveira, L. D., Parize, G., Santos, S. S. F. Dos, Pallos, D., and Leão, M. V. P. (2020) 'Inflammatory markers in saliva for diagnosis of sepsis of hospitalizes patients', *European Journal of Clinical Investigation*, 50(5), pp. e13219

Geng, Y., Zhu, S., Lu, Z., Xu, H., Shi, J. S., and Xu, Z. H. (2014) 'Anti-inflammatory activity of mycelial extracts from medicinal mushrooms',

International Journal of Medicinal Mushrooms, 16(4), pp. 319–325

Huang, J., Gao, S., and Shen, X. (2010) 'Konjac glucomannan nanocrystals prepared by acid hydrolysis', *E-Polymers*, 10(1), pp. 1-8

Huang, K., Li, Y., Tao, S., Wei, G., Huang, Y., Chen, D., and Wu, C. (2016) 'Purification, characterization and biological activity of polysaccharides from dendrobium officinale', Molecules, 21, pp. 1-17

Huang, L., Shen, M., Morris, G. A., and Xie, J. (2019) 'Sulfated polysaccharides: Immunomodulation and signaling mechanisms', *Trends in Food Science and Technology*, 92, pp. 1–11

Kang, H. (2018) 'Anti-inflammatory mechanism of Isodon japonicas (Burm) Hara on lipopolysaccharide-induced neuroinflammation', *Tropical Journal of Pharmaceutical Research*, 17(1), pp. 85–89

Kröncke, K.-D., Fehsel, K., and Kolb-Bachofen, V. (1997) 'Nitric oxide: Cytotoxicity versus cytoprotection— how, why, when, and where? *Nitric Oxide*, 1(2), pp. 107–120

Li, F., Sun, X., Yu, W., Shi, C., Zhang, X., Yu, H., and Ma, F. (2021) 'Enhanced konjac glucomannan hydrolysis by lytic polysaccharide monooxygenases and generating prebiotic oligosaccharides', *Carbohydrate Polymers*, 253, pp. 117241

Liao, H., Banbury, L., Liang, H., Wang, X., Lü, X., Hu, L., and Wu, J. (2014) 'Effect of honghua (*Flos Carthami*) on nitric oxide production in RAW 264.7 cells and α-glucosidase activity', *Journal of Traditional Chinese Medicine*, 34(3), pp. 362–368

Ma, L., Jiao, K., Luo, L., Xiang, J., Fan, J., Zhang, X., Yi, J., and Zhu, W. (2019) 'Characterization and macrophage immunomodulatory activity of two polysaccharides from the flowers of *Paeonia suffruticosa Andr*', *International Journal of Biological Macromolecules*, 124, pp. 955–962

Martinez-Morales, F., Alonso-Castro, A. J., Zapata-Morales, J. R., Carranza-Álvarez, C., and Aragon-Martinez, O. H. (2020) 'Use of standardized units for a correct interpretation of IC50 values obtained from the inhibition of the DPPH radical by natural antioxidants', *Chemical Papers*, 74(10), pp. 3325–3334

Möller, M. N., Rios, N., Trujillo, M., Radi, R., Denicola, A., and Alvarez, B. (2019) 'Detection and quantification of nitric oxide–derived oxidants in biological systems', *Journal of Biological Chemistry*, 294(40), pp. 14776–14802

Nie, C., Zhu, P., Ma, S., Wang, M., and Hu, Y. (2018) 'Purification, characterization and immunomodulatory activity of polysaccharides from stem lettuce', *Carbohydrate Polymers*, 188, pp. 236–242

Onishi, N., Kawamoto, S., Suzuki, H., Santo, H., Aki, T., Shigeta, S., Hashimoto, K., Hide, M., and Ono, K. (2007) 'Dietary pulverized konjac glucomannan suppresses scratching behavior and skin inflammatory immune responses in NC/Nga Mice', *International Archives of Allergy and Immunology*, 144(2), pp. 95–104

Scherer, R., and Godoy, H. T. (2009) 'Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method', *Food Chemistry*, *112*(3), pp. 654–658

Science Gateway. (2022). *How to calculate IC50* [Online]. Available at https://www.sciencegateway.org/protocols/cellbio/drug/hcic50.htm. (Accessed: 23 November 2022)

Suzuki, H., Oomizu, S., Yanase, Y., Onishi, N., Uchida, K., Mihara, S., Ono, K., Kameyoshi, Y., and Hide, M. (2010) 'Hydrolyzed konjac glucomannan suppresses ige production in mice b cells', *International Archives of Allergy and Immunology*, 152(2), pp. 122–130

Swinney, D. C. (2011) 'Chapter 18 - molecular mechanism of action (MMoA) in drug discovery', *Annual Reports in Medicinal Chemistry*, 46, pp. 301-317

Tan, L. H., Zhang, D., Yu, B., Zhao, S. P., Wang, J. W., Yao, L., and Cao, W. G. (2015) 'Antioxidant activity and optimization of extraction of polysaccharide from the roots of Dipsacus asperoides', *International Journal of Biological Macromolecules*, 81, pp. 332–339

Tester, R., Al-Ghazzewi, F., Shen, N., Chen, Z., Chen, F., Yang, J., Zhang, D., and Tang, M. (2012) 'The use of konjac glucomannan hydrolysates to recover healthy microbiota in infected vaginas treated with an antifungal agent', *Beneficial Microbes*, 3(1), pp. 61–66

Ulayya, A. H., Mubarok, A. Z., Widodo, W., Widjanarko, S. B., and Marlita, M. (2022) 'The importance of natural ace2 inhibitor: potency of porang (*Amorphophallus muelleri*) glucomannan as anti-SARS-Cov-2', *Electronic Journal of General Medicine*, 19(1), pp. 2–5

Yin, M., Zhang, Y., and Li, H. (2019) 'Advances in research on immunoregulation of macrophages by plant polysaccharides', *Frontiers in Immunology*, 10, pp. 1-9

Zheng, Q., Li, W., Liang, S., Zhang, H., Yang, H., Li, M., and Zhang, Y. (2019) 'Effects of ultrasonic treatment on the molecular weight and anti-inflammatory activity of oxidized konjac glucomannan', *CyTA - Journal of Food*, 17(1), pp. 1–10