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Prebiotic Effect of Cogon Grass Root Extract on Stimulating Lactic Acid Bacteria Growth in Mice Intestine

Efek Prebiotik Ekstrak Akar Alang-alang dalam Menstimulasi Pertumbuhan Bakteri Asam Laktat pada Usus Halus Mencit

Gita W Pradini^{1^}, Nisa Fauziah^{1^}, Shinta Asarina², Neni Anggraeni³, Rini Widyastuti⁴, Mas Rizky AA Syamsunarno^{1*}

¹Department of Biomedical Science, Faculty of Medicine, Universitas Padjadjaran
Jl. Raya Jatinangor-Sumedang Km 21 Jatinangor, Sumedang

²Microbiology Laboratory, Faculty of Medicine, Universitas Padjadjaran
Jl. Eijkman No. 38 Bandung

³Doctoral program in medical science, graduate school of comprehensive human science, University of Tsukuba

1 Chome-1-1 Tennodai, Tsukuba, Ibaraki 305-8577, Jepang

⁴Department of Veterinary Product, Faculty of Veterinary, Universitas Padjadjaran
Jl. Raya Jatinangor-Sumedang Km 21 Jatinangor, Sumedang

[^]These authors have contributed equally to this work.

*Corresponding author

Email: rizky@unpad.ac.id

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Abstract

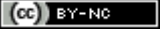
Lactic acid bacteria (LAB) are known for their numerous beneficial effects for health and their colonization within intestines is affected by prebiotic consumption. Cogon grass root is an herbal medicine which contain substantial quantities of polyphenols. Recently, many studies suggested polyphenols as a novel group of prebiotics. Therefore, this study aimed to explore prebiotic effect of cogon grass root extract (CGRE) on stimulating growth of LAB in mice intestine. Mice ($n = 7/\text{group}$) were divided into three groups; group A (control), group B and C received CGRE orally with daily dosage 90 and 115mg/kg BW, respectively. After 14 days of treatment, all mice were sacrificed. LAB was isolated and counted from ileum. Additional biochemical tests were performed to confirm the bacteria as LAB. Further identification of representatives LAB was performed with API-CHL-50 System. We found increased growth and diversity of LAB in the treatment group with optimum growth stimulation at CGRE dosage 90mg/KgBW daily (group B). Further identification showed *Lactococcus lactis* was the most frequent LAB found in all groups, with additional *Lactobacillus paracasei* and *Lactobacillus curvatus* were found in the treatment group. These findings support additional benefit of CGRE as prebiotics by stimulating LAB growth and diversity in mice intestine.

Keywords: cogon grass root; lactic acid bacteria; polyphenols; prebiotic

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Abstrak

Bakteri asam laktat (BAL) diketahui memiliki banyak manfaat untuk kesehatan dan kolonisasinya di usus halus dipengaruhi oleh konsumsi prebiotik. Akar alang-alang adalah salah satu obat herbal yang memiliki polifenol dengan jumlah yang bermakna. Banyak studi terkini yang merekomendasikan polifenol sebagai kelompok baru prebiotik. Penelitian ini bertujuan untuk mengetahui efek prebiotik dari akar alang-alang dalam menstimulasi pertumbuhan BAL dalam usus halus mencit. Mencit (n=7/kelompok) dibagi menjadi tiga kelompok: kelompok A (kontrol), kelompok B dan kelompok C (uji) yang masing-masing mendapatkan ekstrak akar alang-alang secara oral dengan dosis 90 dan 115 mg/KgBB setiap harinya. Setelah perlakuan selama 14 hari, semua mencit dikorbankan. BAL diisolasi dan dihitung koloninya dari ileum. Uji biokimiawi dilakukan untuk mengkonfirmasi bakteri yang diisolasi sebagai BAL. Identifikasi lebih lanjut dengan menggunakan API-CHL-50 *system* dilakukan untuk beberapa isolat yang representatif. Hasil penelitian menunjukkan peningkatan jumlah koloni dan diversitas BAL pada kelompok uji dengan dosis optimal 90mg/KgBB per hari (kelompok B). Identifikasi spesies BAL menunjukkan *Lactococcus lactis* merupakan BAL yang tersering ditemukan di seluruh kelompok. *Lactobacillus paracasei* dan *Lactobacillus curvatus* ditemukan hanya pada kelompok uji. Penelitian ini menunjukkan manfaat lain dari ekstrak alang-alang sebagai prebiotik dengan menstimulasi pertumbuhan dan diversitas BAL dalam usus halus mencit.

Kata kunci: akar alang-alang; bakteri asam laktat; polifenol; prebiotik

Introduction

Gastrointestinal microbiota is known to have a significant contribution to both health and disease in humans. The balance of gastrointestinal microbiota diversity is very important for intestinal homeostasis, and it has an impact on human health and longevity.¹⁻³ Microbiota of the gastrointestinal tract are diverse, it include the beneficial lactic acid bacteria (LAB) which contribute significantly to maintain intestinal homeostasis.⁴ LAB is known to have beneficial effect for improving gastrointestinal integrity, prevent gastrointestinal infection, relieving constipation, reduce inflammatory conditions, and promote general wellness.⁵⁻⁶ LAB mostly inhabit jejunum and ileum, with *Lactobacilli* and *Streptococci* as dominant genera.^{7,8} The LAB is colonized and attached to the mucus layer of the intestines and acts as an immunomodulator.⁷ The colonization and abundance of these bacteria are affected by dietary patterns, particularly by prebiotic consumption.^{8,9} During a prebiotic deficient diet, it was revealed that the condition lead to development of hypertensinogenic gut microbiota, thus increased risk for hypertension and its complications in the future.¹⁰

Recently, evidence suggested polyphenols can act as prebiotics.¹¹⁻¹³ Without being absorbed in the small intestines, their presence in the lumen of the gastrointestinal tract can modulate gastrointestinal microbiota.^{12,14,15} Interestingly, dietary polyphenols can act differently

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on LAB and pathogenic bacteria, it enhances LAB adhesion on intestinal epithelial cells but reduces the adhesion of pathogenic bacteria.^{12,13,16} The main sources of polyphenols in humans are derived from plants such as fruit, vegetables, herbs, cocoa and beverages (e.g. tea, coffee, and wine).^{9,13,17} Other sources for polyphenols are traditional herbal medicines.^{12,18-20} The root of Cogon grass (*Imperata cylindrica* (L.)), is a common traditional medicine in Indonesia,²¹ has been proven to have antioxidant and anti-inflammatory properties due to its polyphenols contents.²²⁻²⁵ Previous study found pretreatment of mice with oral cogon grass root extract (CGRE) for two weeks, significantly reduced serum cholesterol levels after acute olive oil loading.²⁵ There is evidence polysaccharide from root of *I. cylindrica* can reduce cholesterol level,²⁶ and we want to know if it also has impact on LAB. Therefore, the objective of this study is to explore whether the CGRE has prebiotic effects to stimulate LAB growth in mice intestine.

Methods

Plant Material and Extract

CGRE preparation was conducted as previously described.²⁶ Briefly, authenticated cogon grass roots (*Imperata cylindrica* (L.) Beauv.) from local region of Java was dried and powdered. The grass root powder was macerated by 96% ethanol for 72 hours to extract its active compounds. The macerated pulp then filtered and evaporated to remove ethanol excess. The extract then diluted with 0.5% carboxyl methyl cellulose (CMC).

Animal Study

The study was approved by the Ethics Review Committee Faculty of Medicine Universitas Padjadjaran Number 1263/UN6.C10/PN/2017. Adult male DDY-mice with aged 8-12 weeks were obtained from PT. Bio Farma (Bandung, Indonesia). Mice were housed in a room with 12/12 h light and dark cycle with good air circulation. Food and drinking water were provided with no restriction.

The number animal tested for each group was determined using formula of minimum and maximum sample size for group comparison one-way ANOVA. The formula consist of minimum number of animals per group: $n = (10/k) + 1$ and maximum number of animals per group: $n = (20/k) + 1$. The "k" represents number of treatment group.²⁷

Based on the formula, we include seven mice for each group. Mice were divided into three groups, which are: group A as control, group B and group C as treatment group which received CGRE orally with daily dosage 90 and 115mg/kg BW in 200 μ l of 0.5 % CMC, respectively. CGRE was given once a day orally for 14 days. At the end of the experiment, mice

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were euthanized, and ileum was aseptically isolated (± 3.5 cm) from each mouse and placed into a sterile tube containing 2 ml of sterile NaCl 0.85%. Samples were immediately stored in the -40°C freezer until it is ready to perform LAB isolation and counting.²⁸

Isolation of Lactic Acid Bacteria (LAB)

MRS (de Man, Ragosa, and Sharpe) agar plates with the addition of CaCO_3 1% (MRS+ CaCO_3 1%) were used for LAB isolation. CaCO_3 1% was added to distinguish acid-producing bacteria, which was identified by the presence of a clear zone surrounding the suspected colony. Stored ileum samples were thawed and aseptically cut and minced using sterile scissors and homogenized using a vortex. Homogenized samples were then serially diluted from 10^{-1} to 10^{-4} fold in sterilized water. One hundred microliters from each serial dilution were spread onto the surface of MRS+ CaCO_3 1% agar plates and incubated at 37°C under microaerophilic conditions (5% CO_2) for 48 hours. Colonies of acid-producing bacteria were identified by the presence of a clear zone around each colony. The number of LAB colonies from each sample was calculated by using the total plate count method. Five colonies from each intestine sample were selected randomly from plates containing between 30-300 colonies, each colony was Gram stained to ensure the purity of the Gram-positive colony. Each colony was sub-cultured and incubated at 37°C for 48 hours under microaerophilic conditions. Purified LAB colonies were stored at -80°C in MRS broth with 10% glycerol (v/v) until it is ready for further identification of LAB characteristics.

Morphological, Biochemical and Physiological Test of Probiotics LAB

The purified LAB colony was grown on MRS+ CaCO_3 1% agar for 48 hours at 37°C . Morphology, gram staining response, biochemical and physiological tests were observed afterward. Catalase activity was confirmed by placing a loopful of the LAB colony on the glass slide and mixing with a drop of 3% H_2O_2 . LAB was identified for its negative catalase activity. Gas production from glucose fermentation was observed by incubating a loopful of LAB colony into 5 ml phenol red glucose broth (glucose 1%, peptone 1%, NaCl 0.5%, and phenol red 0.4%) in Durham tube test for 48 hours at 37°C . Gas production indicated for heterofermentative LAB, whereas homofermentative LAB did not produce gas.²⁹ Physiological characterization of probiotics LAB (acid and salt resistance) was observed with growth at pH 3.0, 3.5, 4.0, 4.5 and 7.0 in MRS broth after incubation at 37°C for 2 and 4 days. Salt resistance of probiotics LAB was tested in MRS broth containing 3.0 and 6.5% NaCl at 37°C for 2 and 4 days.³⁰ All incubation for LAB growth was performed under microaerophilic conditions.

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Lactic Acid Production

The purified colony of LAB was grown in MRS broth for 24 hours at 37°C under microaerophilic condition. Approximately 10% v/v of each purified colony was inoculated into 40 ml glucose broth 37°C. After 48 hours of fermentation, lactic acid production was quantified with acid-base titration method. Two replicates were made for each treatment.

Confirmation of Lactic Acid Bacteria Species

Carbohydrates fermentation of twenty LAB isolates were determined using API 50 CHL in conjunction with API 50 CHL medium (bioMérieux, France) for identification of LAB with *L. plantarum* as control. The APILAB PLUS database identification software was used to interpret the result.

Statistical Analysis.

Statistical analysis using ANOVA was performed to find significant differences in LAB count between control, 90 mg dose and 115 mg dose of CGRE treatment. A significance level of $p < 0.05$ was used.

Results

Lactic Acid Bacteria (LAB) Growth

Based on LAB growth on MRS+CaCO₃ 1% agar, sample group A (control) had mean colony counting of $210(\pm 247) \times 10^3$ cfu/ml, group B (dosage 90 mg/KgBW) had mean colony counting of $5,811(\pm 5595) \times 10^3$ cfu/ml and group C (dose 115 mg/KgBW) had mean colony counting $1,871 (\pm 2038) \times 10^3$ cfu/ml (Figure 1). From this result, the optimum growth stimulation for LAB in mice intestines is after supplementation with CGRE with daily dosage 90mg/KgBW for 14 days.

Morphological, biochemical and physiological properties of LAB isolated from mice intestines

A total of 25, 27 and 33 LAB isolates (total n=85) were collected from mice groups A, B, and C respectively. Further LAB characterization was done as in table 1. All presumptive LAB isolates were Gram-positive and catalase-negative. To confirm the isolates as a lactic acid producer, lactic acid production was measured resulting in mean lactic acid concentration from all group ranged between 9.4 mM–10.4 mM. Salt and acid tolerability were measured to

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characterized functional properties of isolated bacteria, the result showed most isolates were acid tolerable at pH 4.0 and salt tolerable for up to 4 days. Sugar fermentation types from all isolates were homofermentative (no gas produced from glucose fermentation).²⁹

Confirmation of LAB Species

To confirm the species of LAB isolated from mice intestines, 16 representative samples from the control group (n=9) and treatment group (group B, n=7) were randomly selected to further identified with API CHL 50 system (table 2).

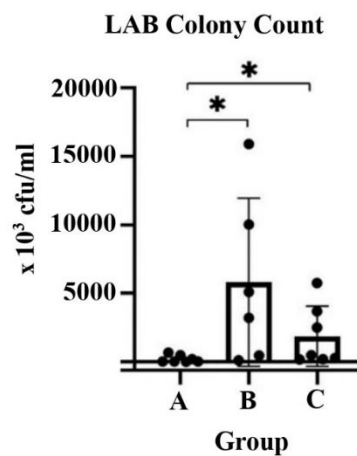


Figure 1 The LAB Colony Counting

Legend: A (control), B (CGRE dosage 90 mg/KgBW), C (CGRE dosage 100 mg/KgBW). Asterix (*) indicate p<0.05 (statistically significant different between 2 groups: A vs B and A vs C)

Table 1 Characterization of Isolated LAB

Characteristics	Group A		Group B		Group C	
Number of Isolates	27		25		33	
Morphology	100% Cocci		12% Cocobacilli 8% Bacilli 80% Cocci		94% Cocci 6% Bacilli	
Gram stain	All positive		All positive		All positive	
Catalase activity	All negative		All negative		All negative	
Fermentation type	Homofermentative		Homofermentative		Homofermentative	
Gas production	All negative		All negative		All negative	
Lactic acid concentration (mean±SD)	10.4 ± 1.1 mM		10.1 ± 2.7 mM		9.4 ± 1.1 mM	
Growth in NaCl	Day 2	Day 4	Day 2	Day 4	Day 2	Day 4
3%	100%	100%	96%	96%	100%	100%
6.5%	100%	100%	96%	96%	100%	100%
Growth at pH	Day 2	Day 4	Day 2	Day 4	Day 2	Day 4
3.0	40.7%	0	28%	0	9%	0
3.5	88.8%	25.9%	60%	0	75.6%	6%
4.0	100%	100%	92%	88%	97%	94%

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4.5	100%	100%	96%	88%	100%	97%
7.0	100%	100%	100%	100%	100%	100%

Discussion

Marked changes on gastrointestinal microbiota diversity or dysbiosis have linked to major human diseases, including autoimmune and inflammatory diseases (obesity, hypertension, cardiovascular disease, and type 2 diabetes mellitus).^{2-3,31} Modulation of gastrointestinal microbiota to a healthy state could ease many health problems, and it can be achieved by consumption of prebiotics.³² Herbal medicine could exert its benefit through its polyphenols compound by modulating gastrointestinal microbiota. Its polyphenols not only support the growth of beneficial bacteria, but also suppress pathogenic bacterial growth within intestine.^{15,33} Our study found administration of herbal medicine CGRE, with daily dosage of 90mg/KgBW, intraorally for 14 days significantly increase the growth of LAB in mice intestine (figure 1), suggesting its effect as prebiotics. Therefore, supporting application of CGRE as an alternative for dietary source of prebiotics and might be beneficial to alleviate gastrointestinal problems caused by prebiotics deficiency.

LAB has been proved to have beneficial effects on human health through metabolism regulation, infection control, and inflammation/allergy modulation.^{34,35} According to phenotypic characteristics data, we found more diverse LAB morphology in groups who received CGRE than the control group (table 1). Representative samples were chosen from treatment group (B) and control (A) to confirm the species of LAB. All representative samples which are found from the control group were identified as *Lactococcus lactis ssp lactis* 1 (n=9), while from the treatment group (B) *Lactobacillus curvatus* (n=1), *Lactocobacillus paracasei ssp. paracasei* 3 (n=4), and *Lactococcus lactis ssp lactis* 1 (n=2) were found. This finding represents criteria for defining a prebiotic, which is the ability to stimulate selectively the growth and/or activity of intestinal bacteria associated with health and wellbeing.⁸ Similar evidence was also found from a study in metabolic syndrome patients who consumed polyphenol-rich red wine and presenting a higher number of intestinal barrier protector bacteria (*Bifidobacterium* and *Lactobacillus* genera) and butyrate-producing bacteria (*Faecalibacterium prausnitzii* and *Roseburia*) while the number of *E. coli* and *E. cloacae* were decreasing.³³

Table 2 Species Identification of Representatives LAB Samples

Group	Species	Isolates (n)
Control (Group A)	<i>Lactococcus lactis ssp lactis</i> 1	9
	<i>Lactobacillus curvatus</i>	1
Treatment (Group B)	<i>Lactococcus lactis ssp lactis</i> 1	2
	<i>Lactocobacillus paracasei ssp paracasei</i> 3	4

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These findings suggest an effect of polyphenols to promote growth and diversity of beneficial bacteria, including LAB. Increased diversity of LAB within the treatment group might also be stimulated with cross-feeding phenomena, which is the utilization of prebiotics by-products (e.g. exopolysaccharides, short-chain fatty acids) produced by certain microbes as substrates for growth of other microbes within the same community.³² LAB can synthesize bioactive molecules, including polysaccharides, such as exopolysaccharides.³⁵ Biosynthesis of polysaccharides is increased with the presence of polyphenols which might provide the metabolic source for another sub-dominant beneficial LAB genera within intestines.³³ Thus, increasing the amount and diversity of the beneficial LAB. Although much evidence shows positive stimulation of polyphenols on growth and functional properties of LAB,^{16,33,36} other studies also found inhibition of polyphenols on certain LAB growth which usually depend on LAB strain, concentration and type of polyphenols given.³⁹ Our study found a higher dose of CGRE (115 mg/kg BW daily) reduced the LAB colony within the intestine. This finding confirmed polyphenol concentration-dependent on LAB growth stimulation.

This study is limited by CGRE used in this study which is crude extract and it had not been characterized for specific polyphenols content. Nevertheless, another study found twelve phenolic compounds present in the cogon-grass root that belongs to the group of flavonoids, simple phenols, phenolic acids, coumarins, and lignans.³⁸ Moreover, the impact of CGRE on LAB growth might be affected by polysaccharides that naturally present in cogon-grass roots.³⁹ Polysaccharides, particularly with prebiotic properties, can modify intestinal microbiota with growth stimulation of beneficial microbiota, including lactic acid bacteria.⁴⁰

Conclusion

This study revealed another benefit of CGRE, a traditional herbal medicine, as prebiotics. The prebiotic effects showed by CGRE stimulation on LAB growth and diversity in mice intestines with optimal daily dosage at 90 mg/kg BW for 14 days. Our findings complete the knowledge of CGRE's beneficial effect and its mechanism, particularly in modulating gut microbiota through the stimulation of LAB growth.

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References

1. Olvera-Rosales LB, Cruz-Guerrero AE, Ramírez-Moreno E, Quintero-Lira A, Contreras-López E, Jaimez-Ordaz J, et al. Impact of the Gut Microbiota Balance on the Health-disease Relationship: The Importance of Consuming Probiotics and Prebiotics. *Foods*. 2021;10(6):1261.
2. Ding RX, Goh WR, Wu RN, Yue XQ, Luo X, Khine WWT, et al. Revisit Gut Microbiota and Its Impact on Human Health and Disease. *JFDA*. 2019;27(3):623-31.
3. Valdes AM, Walter J, Segal E, Spector TD. Role of the gut microbiota in nutrition and health. *BMJ*. 2018;361:36-44.
4. Lu S. The role of Lactic Acid Bacteria in Gut Microbiota and Mucosal Immune System. *South Fla*. 2021;2(3):3918-25.
5. Qin D, Ma Y, Wang Y, Hou X, Yu L. Contribution of Lactobacilli on Intestinal Mucosal Barrier, and Diseases: Perspectives and Challenges of *Lactobacillus casei*. *Life*. 2022;12(11):1910.
6. Dempsey E, Corr SC. *Lactobacillus spp.* for Gastrointestinal Health: Current and Future Perspectives. *Front Immunol*. 2022;6(13):840245.
7. Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. *Nat. Rev. Microbiol*. 2015;14(1):20–32.
8. Nishiyama K, Sugiyama M, Mukai T. Adhesion properties of lactic acid bacteria on intestinal mucin. *Microorganisms*. 2016;4(3).
9. Wang S, Xiao Y, Tian F, Zhao J, Zhang H, Zhai Q, et al. Rational use of prebiotics for gut microbiota alterations: Specific bacterial phylotypes and related mechanisms. *J. Funct. Foods*. 2020; 66:103838.
10. Kaye DM, Shihata WA, Jama HA, Tsyganov K, Ziemann M, Kiriazis H, et al. Deficiency of Prebiotic Fiber and Insufficient Signaling Through Gut Metabolite-Sensing Receptors Leads to Cardiovascular Disease. *Circulation*. 2020;141(17):1393-403.
11. Pickard JM, Zeng MY, Caruso R, Núñez G. Gut microbiota: Role in pathogen colonization, immune responses, and inflammatory disease. *Immunol. Rev*. 2017;279(1):70–89.
12. Rodríguez-Daza MC, Pulido-Mateos EC, Lupien-Meilleur J, Guyonnet D., Desjardins Y, Roy D. Polyphenol-Mediated Gut Microbiota Modulation: Toward Prebiotics and Further. *Front. Nutr*. 2021;28(8):689456.
13. Gwiazdowska D, Juś K, Jasnowska-Małecka J, Kluczyńska K. The Impact of Polyphenols on Bifidobacterium growth. *Acta Biochim Pol*. 2015;62(4):895–901
14. Plamada D, Vodnar DC. Polyphenols-gut microbiota interrelationship: A transition to a new generation of prebiotics. *Nutrients*. 2022;14(1):137.
15. Milutinović M, Dimitrijević-Branković S, Rajilić-Stojanović M. Plant Extracts Rich in Polyphenols as Potent Modulators in the Growth of Probiotic and Pathogenic Intestinal Microorganisms. *Front Nutr*. 2021;30(8):688843.
16. de Souza EL, de Albuquerque TMR, dos Santos AS, Massa NML, de Brito Alves JL. Potential interactions among phenolic compounds and probiotics for mutual boosting of their health-promoting properties and food functionalities-A review. *Crit. Rev. Food Sci Nutr*. 2019;59(10):1645–59.
17. Abbas M, Saeed F, Anjum FM, Afzaal M, Tufail T, Bashir MS, et al. Natural polyphenols: An overview. *Int. J Food Prop*. 2017;20(8):1689–99.
18. Hano C, Tungmunnithum D. Plant Polyphenols, more than Just Simple Natural Antioxidants: Oxidative Stress, Aging and Age-Related Diseases. *Medicines*. 2020;7(5):26
19. Klinder A, Shen Q, Heppel S, Lovegrove JA, Rowland I, Tuohy KM. Impact of increasing fruit and vegetables and flavonoid intake on the human gut microbiota. *Food Funct*. 2016;7(4):1788–96.
20. Singh AK, Cabral C, Kumar R, Ganguly R, Rana HK, Gupta A, et al. Beneficial effects of dietary polyphenols on gut microbiota and strategies to improve delivery efficiency. *Nutrients*. 2019;11(9):2216.
21. Susanto AH, Dwiati M. Assessment of cogon grass (*Imperata cylindrica* (L.) P. Beauv.) genetic variation in Java, Indonesia using atpB-rbcL and trnL-F intergenic spacer. *Biodiversitas*. 2022;23(5):2760-7.
22. Sytar O, Hemmerich I, Zivcak M, Rauh C, Brestic M. Comparative analysis of bioactive phenolic compounds composition from 26 medicinal plants. *Saudi J. Biol. Sci*. 2018;25(4):631–41.
23. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines*. 2018;5(3):93.
24. Hidayat S, Rachmadiyah AN. Utilization of Alang-Alang (*Imperata cylindrica* (L.) Raeusch.) as Traditional Medicine in Indonesian Archipelago. *Proceedings The 1st SATREPS Conference*. 2017; 1:82-89.
25. Indriyanti RA, Ariyanto EF, Usman HA, Effendy RR, Dhianawaty D. Quantification of total polyphenols and flavonoids, antioxidant activity, and Sinensetin and Imperatorin contents of *Imperata cylindrica* root ethanol extract. *Pharmacogn J*. 2022;14(4):327–37.
26. Anggraeni N, Syamsunarno MRAA, Mukarromah GR, Zada A, Triatin RD, Pamela Y, et al. Low Serum Cholesterol in Mice Pre-treated with *Imperata cylindrica* (L.) after Acute Olive Oil Gavage. *KnE Life Sci*. 2017;3(6):460.
27. Arifin WN, Zahiruddin WM. Sample size calculation in animal studies using resource equation approach. *Malays J Med Sci*. 2017;24(5):101–5.
28. Bayu HH, Isolation and Identification of Lactic Acid Bacteria from *Channa sp.* as Potential Probiotic. *Jurnal Pembelajaran dan Biologi Nukleus*. 2023;9(1):75-84.

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29. Mejia-Gomez CE, Balcázar N., Isolation, characterisation and continuous culture of *Lactobacillus spp.* and its potential use for lactic acid production from whey. *Food Sci. Technol.* 2020;40(4):1-8.
30. Ni K, Wang Y, Li D, Cai Y, Pang H. Characterization, identification, and application of lactic acid bacteria isolated from forage paddy rice silage. *PLoS One.* 2015;10(3):e0121967.
31. Elias-Oliveira J, Leite JA, Pereira ÍS, Guimarães JB, Manso GM da C, Silva JS, et al. NLR and Intestinal Dysbiosis-Associated Inflammatory Illness: Drivers or Dampers? *Front Immunol.* 2020;11(11):1810.
32. Holscher HD. Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut Microbes.* 2017;8(2):172–84
33. Moreno-Indias I, Sánchez-Alcoholado L, Pérez-Martínez P, Andrés-Lacueva C, Cardona F, Tinahones F, et al. Red wine polyphenols modulate fecal microbiota and reduce markers of the metabolic syndrome in obese patients. *Food Funct.* 2016;7(4):1775–87
34. Moorthy M, Sundralingam U, Palanisamy UD. Polyphenols as prebiotics in the management of high-fat diet-induced obesity: A systematic review of animal studies. *Foods.* 2021;10:299
35. Hakim BNA, Xuan NJ, Oslan SNH. A Comprehensive Review of Bioactive Compounds from Lactic Acid Bacteria: Potential Functions as Functional Food in Dietetics and the Food Industry. *Foods.* 2023;12(15):2850.
36. Chan CL, Gan RY, Shah NP, Corke H. Polyphenols from selected dietary spices and medicinal herbs differentially affect common food-borne pathogenic bacteria and lactic acid bacteria. *Food Control.* 2018;92:437–43.
37. dos Santos AS, de Albuquerque TMR, de Brito Alves JL, de Souza EL. Effects of Quercetin and Resveratrol on in vitro Properties Related to the Functionality of Potentially Probiotic *Lactobacillus* Strains. *Front. Microbiol.* 2019;10:2229.
38. Nayim P., Mbaveng AT., Sudhir K., Wamba BEN., Sanjukta M., et al. Phytochemistry and pharmacology of *Imperata cylindrica*: A comprehensive review. *Invest Med Chem Pharmacol.*2023;6(1):76
39. Rosnizar R., Muliani F., Ramli IM., Eriani K. The Immunostimulant Effects of Alang-Alang (*Imperata cylindrica*) Roots Extract on BALB/c Male Mice (*Mus musculus*). *Adv. Biol. Sci. Res.* 2022;22
40. Álvarez-Mercado AI., Plaza-Díaz J. Dietary Polysaccharides as Modulators of the Gut Microbiota Ecosystem: An Update on Their Impact on Health. *Nutrients* 2022;14(19):4116