Spinach (*Amaranthus sp.*) and Pineapple (*Ananas comosus*) Probiotic Drink (PROSPLE) with Antioxidant Property: A Potential Antidepressant

William Ben Gunawan[1*], Alexander Ivan Gunawan[2]

[1] Department of Nutrition Science, Faculty of Medicine, Diponegoro University
Jl. Prof. Sudarto No. 13, Tembalang, Semarang, Jawa Tengah, Indonesia
[*]**Correspondence** : Email: wbwilliambenwb@gmail.com, Phone +628557636711
[2] Department of Psychology, Faculty of Social and Political Sciences, Brawijaya University
Jl. Veteran, Ketawanggede, Lowokwaru, Malang, Jawa Timur, Indonesia

ABSTRACT

As many as 39% of global suicides were found in Southeast Asia, with depression being the most common psychiatric disorder in suicide offenders. Depression clinically can be caused by free radicals that disrupt the balance of the monoaminergic and glutamatergic systems. Recent studies have suggested that spinach and pineapple are beneficial for mental health (antidepressants) due to their antioxidant content, active amino acids (L-tryptophan), and epicatechin. Probiotics, also known as psychobiotics, are microorganisms that can improve the balance of gut microbiota when consumed adequately and have the potential to be antidepressants. This study processed spinach and pineapple into probiotic drinks (PROSPLE), then determined the best formula based on antioxidant activity and vitamin C content. All ingredients were mixed using a blender and then inoculated with Lactobacillus paracasei 5% w/v for 14 days under anaerobic conditions. The content of vitamin C was determined using the iodometric titration method, while antioxidant activity was expressed as a percentage of inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Statistical analysis consists of a normality test (Shapiro-Wilk) and a comparison test (Analysis of Variance). S3 was the best formula with a vitamin C content of 127.40 ± 0.28 mg/100 g and antioxidant activity of 37.24 ± 0.19% which was significantly different from the other two formulas ($p<0.05$). The content of vitamin C may regulate the monoaminergic and glutamatergic systems as well as intracellular acidification and produce antidepressant and neuroprotective effects. Antioxidants can attenuate inflammation and prevent nerve cell apoptosis. The potential of PROSPLE antidepressants may also be influenced by the content of serotonin and probiotics that modulate the gut-brain axis mechanism. PROSPLE contains vitamin C and has antioxidant activity so it can be developed as a probiotic drink with antidepressant potential.

Keywords: *Antidepressant, Antioxidant, Spinach (Amaranthus sp.), Pineapple (Ananas comosus), Probiotic*

INTRODUCTION

As many as 39% of global suicides occur in Southeast Asia, with depression being the most common psychiatric disorder in suicide offenders (1). The incidence of depression is known to be associated with oxidative stress levels. Excess free radicals trigger oxidative stress which can cause inflammation of nerve cells, mitochondrial dysfunction, and macromolecular damage, leading to nerve cell dysfunction and the development of depressive conditions (2). Studies show that consuming vegetables and fruits high in fiber and micronutrients (3) and probiotics (4) affects mental state and depression.

Probiotics are known as living microorganisms that can provide health benefits by balancing the gut microbiota when consumed sufficiently. Probiotics are categorized as functional foods that contain immune boosters, bioactive compounds, vitamins, and minerals that help fight viral infections (5). Probiotics have many roles in fighting infections, controlling the gut microbiota, mucosal immune response, and immune enhancers (6). Probiotics are also known as psychobiotics (7) which can act as an antidepressant through gut-brain axis mediation that regulates immune, humoral,

neural, and metabolic pathways (4,8). Indonesia has a variety of vegetable and fruit commodities that are probiotics, especially pineapple and spinach.

Pineapple (*Ananas comosus*) is a tropical fruit rich in vitamins and minerals (9) and various bioactive compounds (10) which is known to have high antioxidant activity and can boost immunity (11). Pineapple can affect the functioning of the nervous system, lower cholesterol, and has antiatherogenic and anti-inflammatory properties (9). Spinach (*Amaranthus sp.*) is known for its health benefits, such as having antiviral, antidepressant (12), anticancer, antiobesity, hypoglycemia (13), and hepatoprotective properties (14).

Based on the various health benefits of pineapple and spinach, these two ingredients can be incorporated into a functional drink with high antioxidant activity and have the potential to be antidepressants. Many prior studies found that utilizing plant-based ingredients as a functional drink showed good antioxidant and health-related benefits (15,16). This study aimed to process pineapple and spinach as fermented "Probiotic Spinach and Pineapple Drink" (PROSPLE). In particular, this study was conducted to find out the best formulations of PROSPLE that have the highest antioxidant activity and vitamin C content, which indirectly describes the potential for better antidepressants.

MATERIAL AND METHODS

The research included the scope of food and nutrition product development, functional food, fermentation, and local food, as well as health-related benefits such as antioxidants, vitamin C, and antidepressants. The study is a quantitative experimental study. The formulation, incubation, and testing of *in vitro* parameters related to products were carried out at the UPT Terpadu Laboratory, Diponegoro University, Semarang.

Formulation of PROSPLE

The PROSPLE formulation is based on a comparison of spinach, pineapple, and water concentration per overall volume used (Table 1). All ingredients were mixed according to the formulation and then homogenized using a blender for 15 minutes. The results in the form of PROSPLE drinks were then placed on food-grade glass bottles. Subsequently, all samples were inoculated with *Lactobacillus paracasei* 5% w/v for 14 days under anaerobic conditions by covering them using gauze and rubber.

Table 1. Composition of PROSPLE Formula

	Spinach	Pineapple	Water
S1	1	1	1
S2	2	1	1
S3	3	1	1

Antioxidant Activity Assay

The antioxidant activity was measured based on the 1,1-diphenyl2-picrylhydrazyl (DPPH) inhibition method of KK kombucha (17). For the stock solution, 24 mg of DPPH were dissolved in 100 mL of methanol. Filtration of DPPH stock solution with methanol produced a suitable combination with an absorbance of approximately 0.973 at 517 nm. Next, 3 mL of DPPH working solutions were mixed with 100 µL of KK kombucha in a test tube. The tubes were then placed in a dark room for 30 minutes. Finally, the absorbance was measured at 517 nm.

Determination of Vitamin C Content

The vitamin C content of KK kombucha was determined according to Manasa et al., 2021 (18). A conical flask was filled with 10 mL of KK kombucha

and 40 mL of 0.1 M $CuSO_4$ solution. The reaction mixture was thoroughly mixed and kept at room temperature for 30 minutes. After half an hour, 20 mL of 2 N sulphuric acid, 20 mL of 10% potassium iodide, and 10 mL of distilled water were added to the reaction mixture. Using a starch indicator, the released iodine was titrated against a 0.5 M sodium thiosulphate solution.

Data Management and Statistical Analysis

The normality of the data was carried out with the Shapiro-Wilk test since the number of samples is categorized as small (<50). The analysis of variance (ANOVA) was used to assess the differences in each parameter between all samples. Further analysis was tested using the Tukey-Kramer test. GraphPad Prism 9.4.1 was used to perform statistical analysis and visualization.

RESULT

Table 2. Quantification of Antioxidant and Vitamin C Activity of PROSPLE

Formula	Antioxidant Activity (%)	Vitamin C (mg/100 g)
S1	30,04 ± 0,58	110,3 ± 0,33
S2	33,20 ± 0,44	111,3 ± 0,36
S3	37,24 ± 0,19	127,4 ± 0,28
Mean±SD	33,49 ± 3,15	116,3 ± 8,32

Table 2 showed the value of antioxidant activity and vitamin C levels of PROSPLE which ranges from 30–37% and 110–127 mg/100 g. The highest antioxidant activity and vitamin C levels were obtained in the S3 formula with antioxidant activity of 37.24 ± 0.19% and vitamin C levels of 127.4 ± 0.28 mg/100 g. Figure 1 depicted that the S3 formulation has the best antioxidant activity and vitamin C content; which is significantly meaningful ($p<0.05$). It also revealed that there were differences in antioxidant activity and vitamin C content caused by the addition of the composition of spinach and pineapple used.

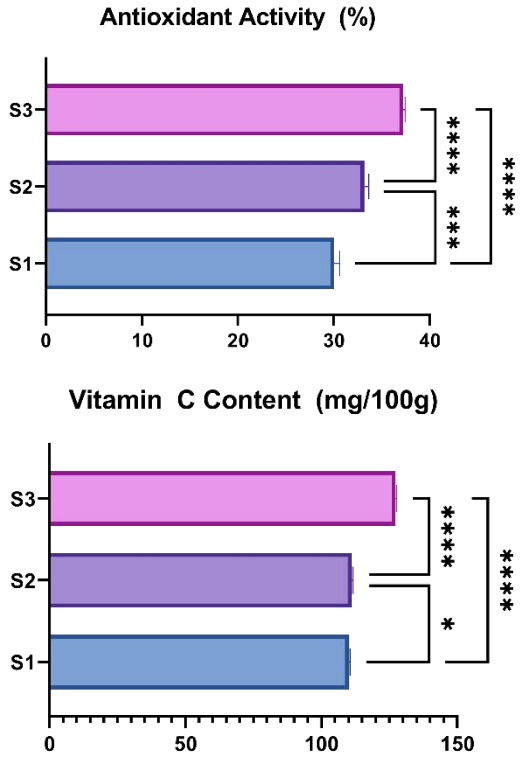

Figure 1. Antioxidant Activity and Vitamin C Content of PROSPLE
(****p=<0.0001; ***p=0.0003; *p=0.0198)

DISCUSSION

It is known that the fermentation process will increase antioxidant activity, vitamin content, and bioactive compounds contained in a plant-based food product (19). The antioxidant activities of PROSPLE are better than the results found by Rompies et al., 2021 (15.55–22.10%) but lower than the results obtained by Kepel et al., 2021 (30.60–44.95%) (20,21). Differences were also found in the parameters of PROSPLE vitamin C levels against other studies (20,21). Several factors can affect the health benefit parameters of a fermented food product. Different ingredients will produce different antioxidant potentials (22); likewise with

the use of different strains of probiotics (23).

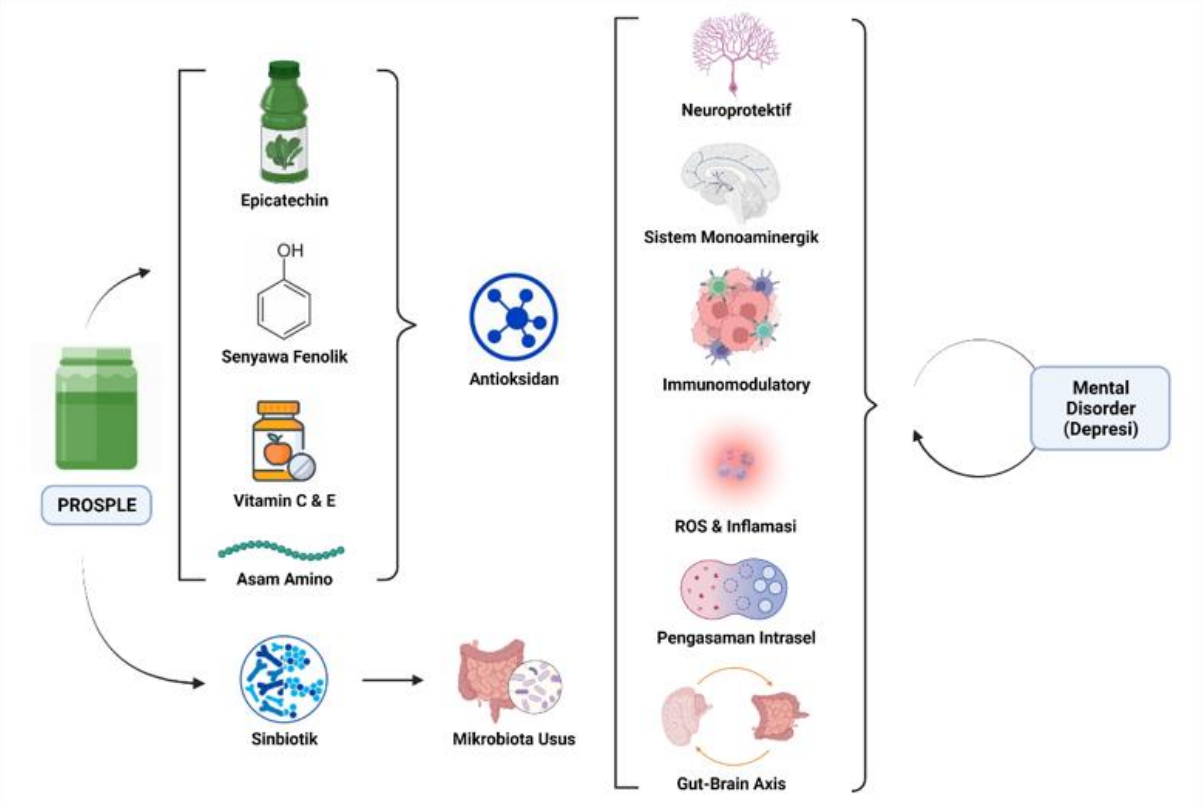

Figure 2. Summarized Antidepressant Potential Mechanisms of PROSPLE

Antidepressant effects can be caused by antioxidant activity (24). Excess free radicals (peroxides) can cause damage to macro-cellular molecules such as fatty acids, proteins, DNA, RNA, and mitochondria; which can contribute to neuron apoptosis and depressive events (25). Excess pro-inflammatory cytokines and chronic oxidative stress can lower serotonin levels (7) as well as lowering neurogenesis in the brain, thus contributing to the decline in nervous system function and the incidence of depression (26,27). Clinical research shows that administering vitamin C in patients with major depressive disorder decreases symptoms related to depression; both in adult patients (28) as well as in children (29). This is also in line with proof that the consumption of foods that are high in antioxidants and vitamin C is also associated with a better mood status (30). Another possible mechanism is that vitamin C affects depressive symptoms through increased acidity in cells that activate the N-methyl D-aspartate (NMDA) receptor (31).

Various bioactive compounds, bioactive peptides, amino acids, minerals, and the modulation ability of the gut microbiota of probiotics, spinach, and pineapple also contribute to PROSPLE's potential as an antidepressant agent. Probiotics can affect nervous system function (synthesis of serotonin, neuropeptides, and neurotransmitters; regulation of systemic inflammation and immunity) through the mediation of the gut-brain axis (7,27). Along with antioxidant activity, it is estimated that various mechanisms synergistically support the potential of PROSPLE antidepressants. The potential

antidepressant mechanisms of PROSPLE are summarized in Figure 2.

This study is a preliminary study with an *in vitro* approach. Therefore, there are limitations to the parameters tested, namely antioxidant activity and vitamin C content which reflects the initial picture of the antidepressant potential of PROSPLE. In-depth testing of several *in vitro* parameters such as polyphenol and tryptophan quantification in PROSPLE can be added to strengthen the evidence. The next exploration that can be done on the potential of PROSPLE antidepressants is through *in silico*, *in vivo*, and clinical trials since the health benefits of a new food product can be claimed if they have a significant effect in the clinical testing phase.

CONCLUSION

The S3 formula of PROSPLE has the highest antioxidant activity and vitamin C levels compared to S2 and S1 significantly ($p<0.05$). The antioxidant activity and vitamin C content in PROSPLE demonstrate its potential as an antidepressant probiotic drink. This research needs to be developed further to be able to support the claim that PROSPLE can become an antidepressant functional drink, both through the determination of phenol and tryptophan content, *in silico* testing, determination of sensory parameters, and observation of the effects of PROSPLE administration *in vivo*, especially with S3 formulations as a basis for preclinical and clinical trials.

REFERENCES

1. Ahmed HU, Hossain MD, Aftab A, Soron TR, Alam MT, Chowdhury MWA, et al. Suicide and depression in the World Health Organization South-East Asia Region: A systematic review. WHO South-East Asia J Public Heal. 2017;6(1):60–6.
2. Xu Y, Wang C, Klabnik JJ, O'Donnell JM. Novel therapeutic targets in depression and anxiety: antioxidants as a candidate treatment. Curr Neuropharmacol. 2014 Mar;12(2):108–19.
3. Ljungberg T, Bondza E, Lethin C. Evidence of the Importance of Dietary Habits Regarding Depressive Symptoms and Depression. Int J Environ Res Public Health. 2020 Mar;17(5).
4. Ansari F, Pourjafar H, Tabrizi A, Homayouni A. The Effects of Probiotics and Prebiotics on Mental Disorders: A Review on Depression, Anxiety, Alzheimer, and Autism Spectrum Disorders. Curr Pharm Biotechnol. 2020;21(7):555–65.
5. Alkhatib A. Antiviral functional foods and exercise lifestyle prevention of coronavirus. Nutrients. 2020;12(9):1–17.
6. Sundararaman A, Ray M, Ravindra P V., Halami PM. Role of probiotics to combat viral infections with emphasis on COVID-19. Appl Microbiol Biotechnol. 2020;104(19):8089–104.
7. Cheng LH, Liu YW, Wu CC, Wang S, Tsai YC. Psychobiotics in mental health, neurodegenerative and neurodevelopmental disorders. J Food Drug Anal. 2019;27(3):632–48.
8. Liu RT, Walsh RFL, Sheehan AE. Prebiotics and probiotics for depression and anxiety: A systematic review and meta-analysis of controlled clinical trials. Neurosci Biobehav. 2019;102:13–23.
9. Ali MM, Hashim N, Aziz SA, Lasekan O. Pineapple (Ananas comosus): A comprehensive review of nutritional values, volatile compounds, health benefits, and potential food products. Food Res Int. 2020;137(September):109675.
10. Sayago-Ayerdi S, García-Martínez DL, Ramírez-Castillo AC, Ramírez-Concepción HR, Viuda-Martos M. Tropical Fruits and Their Co-Products as Bioactive Compounds and Their Health Effects: A Review. Foods. 2021;10(8):1952.
11. Yang F, Zhang Y, Tariq A, Jiang X, Ahmed Z, Zhihao Z, et al. Food as medicine: A possible preventive measure

against coronavirus disease (COVID-19). Phytother Res. 2020;34(12):3124–36.
12. Son H, Jung S, Shin J, Kang M, Kim H. Anti-Stress and Anti-Depressive Effects of Spinach Extracts on a Chronic Stress-Induced Depression Mouse Model through Lowering Blood Corticosterone and Increasing Brain Glutamate and Glutamine Levels. J Clin Med. 2018;7(11):406.
13. Roberts JL, Moreau R. Functional properties of spinach (Spinacia oleracea L.) phytochemicals and bioactives. Food Funct. 2016;7(8):3337–53.
14. Mokhtari E, Farhadnejad H, Salehi-Sahlabadi A, Najibi N, Azadi M, Teymoori F, et al. Spinach consumption and nonalcoholic fatty liver disease among adults: a case–control study. BMC Gastroenterol. 2021;21(1):1–9.
15. Gunawan W Ben, Basoeki LEAS. Incorporating Watermelon Rind into Jackfruit Seed Drink: A Functional Drink Rich in Antioxidant and Phenolic Compounds. EDUFORTECH. 2022;7(2):100–7.
16. Gunawan W Ben, Priambodo AS, Winarti D, Nurohma A, Wijayanti LO. Proximate and Sensory Analysis of Functional Drink from Jackfruit Seed Extract with Citrulline Fortification As A Potential Antidiabetic. J Food Heal. 2021;1(2):56–64.
17. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, et al. Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa. Molecules. 2022;27(4).
18. Manasa C, Sudhir A, Thimmappa SP, Rai KML. Iodometric System for Determining Vitamin C using Cu (II). J Appl Chem. 2021;10(3):302–7.
19. Hur SJ, Lee SY, Kim YC, Choi I, Kim GB. Effect of fermentation on the antioxidant activity in plant-based foods. Food Chem. 2014;160:346–56.
20. Rompies R, Nurkolis F, Natanael H, Manoppo JIC, Mayulu N, Kawengian S, et al. Probiotic drink from the fermentation of banana flower (Ontong) with addition of kluthuk banana leaves (Musa balbisiana). Proc Nutr Soc. 2021;80(OCE2):2021.
21. Kepel BJ, Nurkolis F, Kawengian S, Assa YA, Mayulu N, Natanael H, et al. Functional drink formulation from the fermentation of banana tree (Musa parasidica) rich in antioxidant and food fibre. Proc Nutr Soc. 2021;80(OCE2):2021.
22. Skrovankova S, Sumczynski D, Mlcek J, Jurikova T, Sochor J. Bioactive compounds and antioxidant activity in different types of berries. Int J Mol Sci. 2015;16(10):24673–706.
23. Hoffmann A, Kleniewska P, Pawliczak R. Antioxidative activity of probiotics. Arch Med Sci. 2021;17(3):792–804.
24. Chigurupati S, Shaikh SA, Mohammad JI, Selvarajan KK, Nemala AR, Khaw CH, et al. In vitro antioxidant and in vivo antidepressant activity of green synthesized azomethine derivatives of cinnamaldehyde. Indian J Pharmacol. 2017;49(3):229–35.
25. Liu T, Zhong S, Liao X, Chen J, He T, Lai S, et al. A meta-analysis of oxidative stress markers in depression. PLoS One. 2015;10(10):1–17.
26. Huang Q, Liu H, Suzuki K, Ma S, Liu C. Linking What We Eat to Our Mood: A Review of Diet, Dietary Antioxidants, and Depression. Antioxidants (Basel, Switzerland). 2019 Sep;8(9).
27. Godos J, Currenti W, Angelino D, Mena P, Castellano S, Caraci F, et al. Diet and Mental Health: Review of the Recent Updates on Molecular Mechanisms. Vol. 9, Antioxidants . 2020.
28. Sahraian A, Ghanizadeh A, Kazemeini F. Vitamin C as an adjuvant for treating major depressive disorder and suicidal behavior, a randomized placebo-controlled clinical trial. Trials. 2015;16(1):4–11.
29. Amr M, El-Mogy A, Shams T, Vieira K, Lakhan S. Efficacy of Vitamin C as an Adjunct to Fluoxetine Therapy in Pediatric Major Depressive Disorder. Clin Nutr. 2013;33–50.
30. Shafiee M, Ahmadnezhad M, Tayefi M, Arekhi S, Vatanparast H, Esmaeili H, et al. Depression and anxiety symptoms are

associated with prooxidant-antioxidant balance: A population-based study. J Affect Disord. 2018;238(April):491–8.
31. Bonnet U. The sour side of vitamin C might mediate neuroprotective, anticonvulsive and antidepressant-like effects. Med Hypotheses. 2019;131(January):109320.