



Efficacy of *Stevia rebaudiana* extracts for promoting the growth of probiotic microorganisms



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Abstract

Stevia rebaudiana, or stevia, is a plant with many applications in the food industry. Because its phytochemical compounds that do not provide energy and are sweeter than glucose. Additionally, stevia has been found to promote the growth of beneficial bacteria in the intestines. This research extracted stevia with two types of solvents including sterile distilled water and 70% ethanol and sonication in 60°C for 60 min. The highest yield was stevia aqueous extract of 35.10%. Moreover, the total sugar was determined using the phenol sulfuric method, it was found that stevia ethanol extract had the highest sugar content equal to 3.412 mg/g extract. Thus, stevia ethanol extract and stevioside compound were used in the study for promoting the growth of probiotic microorganisms such as *Lactobacillus casei*, *L. plantarum* and *Streptomyces viridobrunneus*. The sub-MBC concentrations of stevia ethanol extract and stevioside were selected from the lowest concentration of MIC and MBC for testing. The stimulation of the growth of probiotic bacteria by stevia ethanol extract and stevioside was counted the bacteria at 0, 24, and 48 hrs by dilution drop plate technique. It was observed that after 24 hrs, *L. casei* treated with stevia extract at a concentration of 0.49 mg/mL, and *L. plantarum* treated with stevioside at a concentration of 0.25 mg/mL, exhibited significantly higher bacterial growth compared to the control ($p = 0.004$). Moreover, *L. casei* treatment with stevioside at 0.49 mg/mL was demonstrated significantly promoting the growth of bacteria compared to the control after 48 hrs ($p = 0.003$). However, neither stevia extract nor the stevioside compound stimulated the growth of *S. viridobrunneus*. Therefore, stevia extract and stevioside compound exhibited a notable ability to promote the growth of *L. casei* and *L. plantarum*.

Introduction

Stevia is one of the ingredients that many food entrepreneurs use because stevia has a sweetener that is many times more effective than glucose. Stevioside is a substance that has a carbon composition of up to 38 atoms. Given that stevia extract contains both stevioside and other important compounds, its potential as a nutrient to promote probiotic microorganisms is of interest. In this research, the researchers selected probiotics, namely *L. casei*, *L. plantarum* and *S. viridobrunneus* to test with the stevia extract. The objective was to determine whether the extract could stimulate the growth of these probiotics, thereby demonstrating its potential as a prebiotic for gut health.

Objectives

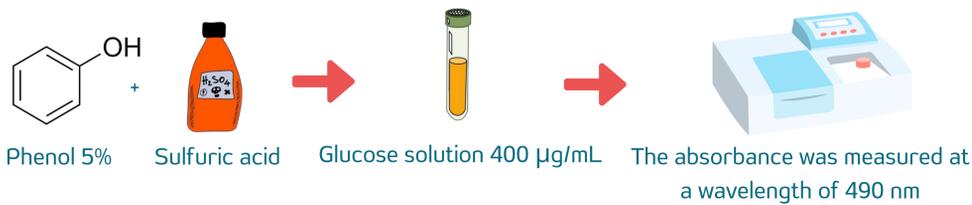
1. To determine sugar content in stevia extract
2. To study the efficacy of stevia extract for promoting the growth of probiotics

Methodology

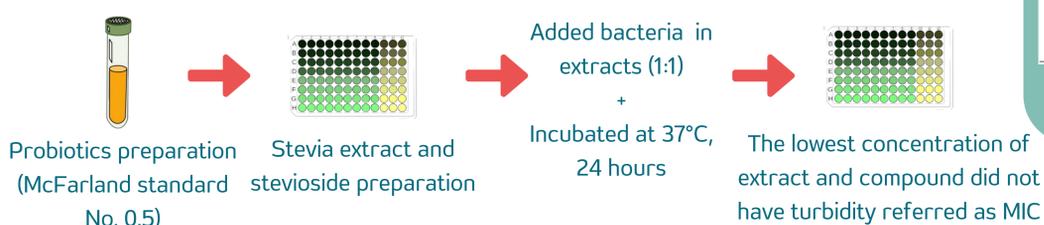
Stevia extraction



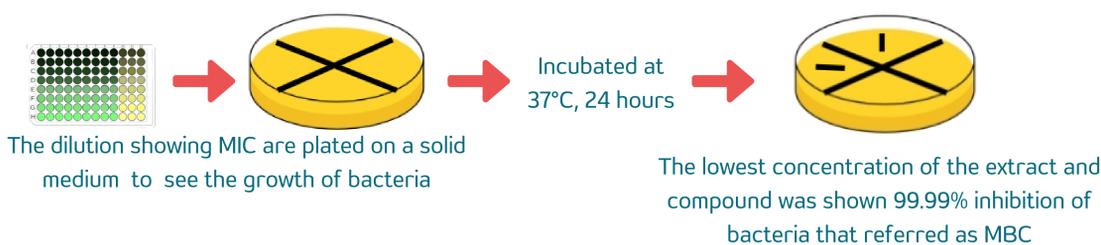
Total sugars determination by phenol sulfuric method



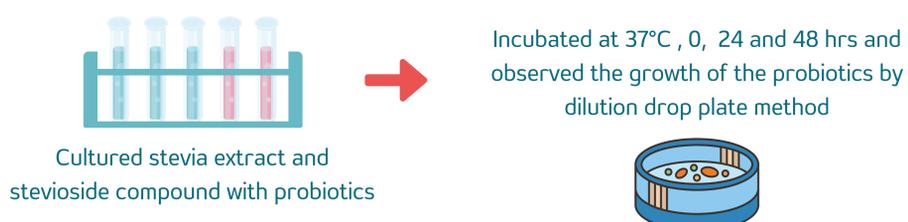
Minimum inhibitory concentration (MIC)



Minimum bactericidal concentration (MBC)



Effect of stevia extract on the growth of probiotics



Results

Stevia extracts

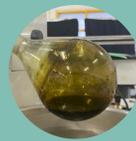


Fig 1. Stevia extracted by 70% ethanol



Fig 2. Stevia extracted by water
Yield of extract: 19.71% Yield of extract: 35.10%

Total sugar content

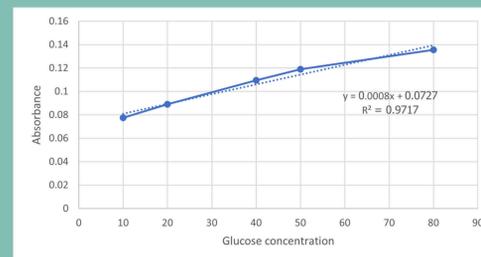


Fig 3. Standard curve of glucose

Table 1. Total sugar content of stevia extracts

Extracts	Total sugar (mg/g extract)
Extracts from Ethanol	3.412 ± 0.804
Extracts from DW water	1.888 ± 0.120

MIC/MBC

Table 2. MIC and MBC of extracts and solution against tested bacteria

Extracts/stevioside	Concentrate (mg/mL)					
	<i>Lactobacillus casei</i>		<i>Lactobacillus plantarum</i>		<i>Streptomyces viridobrunneus</i>	
	MIC	MBC	MIC	MBC	MIC	MBC
Extracts from Ethanol	31.25	62.5	62.5	125	0.98	0.98
Extracts from DW water	31.25	125	62.5	125	0.98	0.98
Stevioside solution	125	>125	>125	125	125	125

Promoting the growth of probiotics

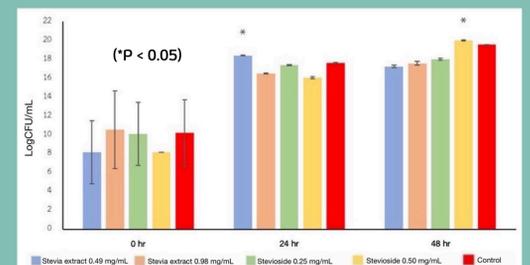


Fig 4. Effect of stevia extract and stevioside on the growth of *L. casei*

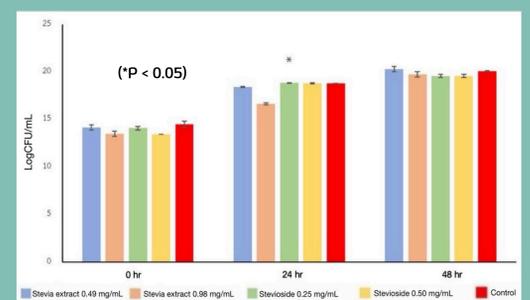


Fig 5. Effect of stevia extract and stevioside on the growth of *L. plantarum*

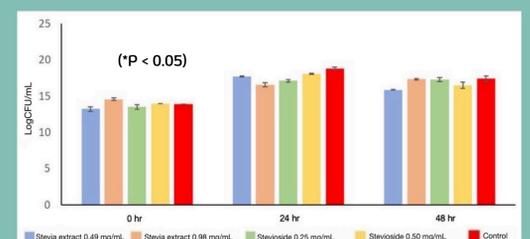


Fig 6. Effect of stevia extract and stevioside on the growth of *S. viridobrunneus*

Conclusion

- Stevia ethanol extract showed the highest total sugar content.
- Stevia extract and stevioside compound exhibited a notable ability to promote the growth of *L. casei* and *L. plantarum*.

References

- McDermott A. 2020. Everything You Need to Know About Stevia: <https://www.healthline.com/health/food-nutrition/stevia-side-effects>
- Medeot, D. B., Nilson, A., Miazzo, R. D., Grosso, V., Ferrari, W., Jofré, E., & Peralta, M. F. 2023. Stevia as a natural additive on gut health and cecal microbiota in broilers. *Veterinary and Animal Science*, 22, 100322.

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