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Research Article

Antioxidant Activity Test of Pine Leaf Herbal Tea (*Pinus merkusii*) with Addition of Stevia Leaves (*Stevia rebaudiana*) as a Natural Sweetener Using DPPH Method

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Abstract

Reactive oxygen species contribute significantly to the pathogenesis of chronic diseases, necessitating the development of safe, plant-based antioxidant sources. This study investigated the antioxidant capacity of a formulated herbal tea combining Pinus merkusii leaves and Stevia rebaudiana as a natural sweetener. Three formulations (1:1, 1:2, and 2:1) were evaluated using the DPPH radical scavenging assay, with quercetin as a reference compound. Quality parameters of simplicia and formulated tea met the Indonesian Herbal Pharmacopoeia standards. The combination formulas demonstrated enhanced antioxidant activity compared to single components, with IC_{50} values of 30.57 ppm (1:1), 39.18 ppm (1:2), and 25.13 ppm (2:1). The 2:1 formulation exhibited the strongest activity, indicating a potential synergistic interaction between phytochemicals. These findings support the development of pine–stevia herbal tea as a functional beverage with high antioxidant potential.

Keywords: Pine leaves, Stevia leaves, Herbal tea, Antioxidant, DPPH

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1 Introduction

Free radicals are molecules having unpaired electrons. They react strongly and damage cells and body parts. Too much free radical exposure can cause diabetes, cancer, and premature ageing [1, 2]. Smoking, air pollution, UV radiation, and contaminated food can cause free radicals. The body can produce them naturally [3].

Antioxidants prevent molecular oxidation by removing free radicals before they damage cells. Natural and synthetic antioxidants exist. Plant-based antioxidants are safer and have fewer adverse effects [4]. Plants contain antioxidants such vitamins C and E, flavonoids, carotenoids, and phenolic substances [3]. Pine leaves (Pinus merkusii) contain antioxidants. Pine trees contain antioxidants like phenolic chemicals, flavonoids, tannins, and terpenoids [5, 6]. Pine leaf extract is an antioxidant that eliminates DPPH free radicals [7].

Stevia rebaudiana is a plant used as a calorie-free, diabetic-friendly sweetener. Stevia leaves are sweet yet contain anti-inflammatory, anti-free radical, and anti-microbe phenolic and flavonoid compounds [8, 9]. Using stevia in herbal products sweetens and provides antioxidants [10]. Due to its health benefits and caffeine-free nature, herbal tea is becoming more popular [11, 12]. Pine and stevia leaves in herbal tea should produce a drink with antioxidants, a sweet taste, and complementary health advantages.

Multiple research have examined the antioxidant qualities of pine and stevia leaves separately, but none have examined the effects of mixing them in varying ratios to brew herbal tea. The study's goals are to make herbal tea from pine and stevia leaves in three ratios (1:1, 1:2, and 2:1), verify its quality, and analyse its antioxidant activity using DPPH. The DPPH (2,2-diphenyl-1-picrylhydrazyl) method was chosen because it is simple, fast, accurate, and repeatable for antioxidant activity testing. This approach measures antioxidants' ability to convert purple DPPH free radicals to yellow. This can be done with a 517 nm spectrophotometer [13].

2 Method

2.1 Research Design

With the DPPH method, this study used an experimental lab setting and a quantitative approach to find out how antioxidant-rich plant tea made from pine and stevia leaves is.

2.2 Time and Place of Research

The Phytochemistry and Pharmacognosy Laboratory, Faculty of Pharmacy and Health, Institut Kesehatan Helvetia, Medan, conducted the study from January to June 2024.

2.3 Materials and Equipment

Analytical balance, oven, blender, 60-mesh sieve, ceramic crucible, furnace, desiccator, erlenmeyer flask, measuring cup, volumetric pipette, volumetric flask, vortex, and other glasses are needed. Stevia rebaudiana, pine leaves, DPPH, quercetin, ethanol p.a., pure water, and other substances.

2.4 Population and Sample

All North Sumatran pine and stevia leaves are in this population. Sample: Healthy, productive Karo Regency, North Sumatra plants were selected for stevia and pine leaves. The sample required fresh, disease- and bug-free leaves from trees with low pollution. Two kg of fresh pine and stevia leaves were collected for this study.

2.5 Research Procedures

Plant materials were taxonomically authenticated at the Herbarium Medanense (MEDA), University of North Sumatra, to ensure the correct identification of the species used in this study. Fresh pine (Pinus merkusii) and stevia (Stevia rebaudiana) leaves were carefully selected, cleaned, and cut into approximately 2 cm segments. The materials were subsequently dried under shaded conditions to

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preserve thermolabile compounds, with drying periods of 7–10 days for pine leaves and 5–7 days for stevia leaves. The dried materials (simplicia) were then pulverized, passed through a 60-mesh sieve to obtain uniform particle size, and stored in airtight containers until further processing.

The herbal tea formulations were prepared by combining pine and stevia leaf simplicia in three predefined proportions. The formulations consisted of F1 (1:1), containing 50 g of pine leaves and 50 g of stevia leaves; F2 (1:2), consisting of 33.3 g of pine leaves and 66.7 g of stevia leaves; and F3 (2:1), comprising 66.7 g of pine leaves and 33.3 g of stevia leaves. Each formulation was homogenized using a geometric mixing method to ensure uniform distribution of components. The prepared mixtures were subsequently stored in tightly closed containers prior to further analysis.

Quality evaluation of all formulations was conducted in accordance with the Indonesian Herbal Pharmacopoeia standards, including parameters such as moisture content, ash values, and extractive values. In addition, phytochemical screening was performed to identify the presence of major classes of secondary metabolites, including alkaloids, flavonoids, saponins, tannins, steroids/triterpenoids, and phenolic compounds.

For antioxidant testing, samples were prepared in the form of an infusion to reflect typical consumption conditions. One gram of powdered herbal tea was steeped in 250 mL of distilled water at 90°C for 15 minutes, followed by cooling and filtration. The resulting filtrate was used to prepare sample solutions at concentrations of 30, 35, 40, 45, and 50 ppm. Quercetin was used as a positive control at concentrations of 2, 4, 6, 8, and 10 ppm.

The antioxidant activity was evaluated using the DPPH radical scavenging assay. A 0.1 mM DPPH solution was prepared in ethanol, and 3.5 mL of this solution was mixed with 0.5 mL of the sample solution. After vortexing, the mixture was incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 516 nm using a UV-Vis spectrophotometer. A blank consisting of DPPH and ethanol was used as a control, and all measurements were performed in triplicate to ensure reproducibility and accuracy of the data.

2.6 Data Analysis

The radical scavenging capacity of each sample was quantified as percentage inhibition relative to the control, calculated based on the difference in absorbance values between the control and treated samples.

A concentration–response relationship was established by plotting percentage inhibition against sample concentration, followed by linear regression analysis. The resulting regression model ($y = ax + b$) was applied to estimate the IC_{50} value, defined as the concentration required to achieve 50% reduction of DPPH radicals. The IC_{50} was mathematically derived from the regression parameters using the expression $(50 - b)/a$.

The antioxidant activity of each formulation was interpreted according to established classification thresholds, where IC_{50} values below 50 ppm indicate very strong activity, 50–100 ppm strong activity, 101–150 ppm moderate activity, 151–200 ppm weak activity, and values exceeding 200 ppm denote very weak activity.

All measurements were conducted in triplicate, and the results are expressed as mean ± standard deviation to reflect data variability. Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test to identify significant differences between formulations. A probability level of $p < 0.05$ was considered statistically significant.

3 Result and Discussion

3.1 Plant Identification

Plant materials were taxonomically authenticated at the Herbarium Medanense (MEDA), University of North Sumatra. The identification confirmed that the samples used in this study corresponded to Stevia rebaudiana leaves and Pinus merkusii leaves. Official verification was documented under accession

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numbers 187/MEDA/2024 for Pinus merkusii and 188/MEDA/2024 for Stevia rebaudiana, ensuring the accuracy of species classification.

3.2 Simplicia Characterization

In Table 1, you can see the findings of characterising pine and stevia leaf simplicia.

Table 1. Characterization of Pine and Stevia Leaf Simplicia

Parameter	Pine Leaf Simplicia	Stevia Leaf Simplicia	IHP Standard
Water Content (%)	5.30 ± 0.15	6.16 ± 0.12	< 10%
Total Ash Content (%)	2.00 ± 0.10	0.30 ± 0.05	< 10%
Acid-Insoluble Ash Content (%)	0.50 ± 0.05	0.50 ± 0.05	< 2%
Water-Soluble Extract Content (%)	23.30 ± 0.25	15.80 ± 0.20	≥ 10%
Ethanol-Soluble Extract Content (%)	29.60 ± 0.30	25.50 ± 0.25	≥ 8%

Indonesian Herbal Pharmacopoeia. Values are the mean ± standard deviation of three replicates.

Both simplicia satisfied Indonesian Herbal Pharmacopoeia quality standards after characterisation. A low water level (<10%) keeps simplicia stable and prevents rapid fungus or microbe growth. Mineral and inorganic impurities are not present in large amounts when the total ash level is low. A high extract content in both water and ethanol shows that the simplicia has a lot of active chemicals that can be extracted.

3.3 Herbal Tea Quality Testing

Table 2 shows the results of tests done on the quality of plant teas made with pine and stevia leaves together.

Table 2. Herbal Tea Combination Quality Testing Results

Parameter	Formula 1 (1:1)	Formula 2 (1:2)	Formula 3 (2:1)	IHP Standard
Water Content (%)	3.81 ± 0.10	3.75 ± 0.12	3.88 ± 0.11	< 10%
Total Ash Content (%)	0.70 ± 0.05	0.65 ± 0.05	0.75 ± 0.06	< 10%
Water-Soluble Ash Content (%)	36.00 ± 0.50	35.50 ± 0.45	36.50 ± 0.55	-
Acid-Insoluble Ash Content (%)	0.48 ± 0.04	0.45 ± 0.04	0.50 ± 0.05	< 2%

Indonesian Herbal Pharmacopoeia. Values are the mean ± standard deviation of three replicates.

The quality standards for all three herbal tea recipes were met. If a product has a low water level, it means it will last a long time. In terms of quality parameters, there were no big changes between the three formulas.

3.4 Phytochemical Screening

Table 3 shows the phytochemical screening results of herbal tea made with pine and stevia leaves together.

Table 3. Herbal Tea Phytochemical Screening Results

Compound Group	Formula 1 (1:1)	Formula 2 (1:2)	Formula 3 (2:1)
Alkaloid	+	+	+
Flavonoid	+++	+++	+++
Saponin	++	++	++

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Tannin	+++	+++	+++
Steroid/Triterpenoid	++	++	++
Phenolic	+++	+++	+++

Note: (+++) = Very strong; (++) = Strong; (+) = Weak; (-) = Negative

The phytochemical screening showed that all three herbal tea blends had antioxidant-like secondary metabolites. These compounds were mostly flavonoids, tannins, and phenolics, which caused very strong responses. It is known that these substances are very good at getting rid of free radicals [14, 15].

3.5 Antioxidant Activity

Table 4 shows the results of tests for antioxidant activity using the DPPH method.

Table 4. DPPH Method Antioxidant Activity Testing Results

Sample	Linear Regression Equation	R^2	IC_{50} (ppm)	Category
Quercetin (Control +)	y = 2.5866x + 3.4062	0.9956	18.01 ± 0.36	Very Strong
Pine Leaves	y = 0.8144x + 8.8633	0.9912	50.51 ± 1.01	Strong
Stevia Leaves	y = 0.8049x + 0.418	0.9895	61.60 ± 1.23	Strong
Formula 1 (1:1)	y = 1.1013x + 16.324	0.9935	30.57 ± 0.61	Very Strong
Formula 2 (1:2)	y = 0.7304x + 21.378	0.9928	39.18 ± 0.78	Very Strong
Formula 3 (2:1)	y = 0.574x + 35.574	0.9942	25.13 ± 0.50	Very Strong

Note: y = percent inhibition, x = concentration (ppm), R^2 = coefficient of determination, IC_{50} = 50% DPPH radical inhibition concentration.

The DPPH test at 516 nm demonstrated antioxidant activity in all samples. Quercetin, the positive reference, was a potent antioxidant with an IC value of 18.01 ± 0.36 ppm. Pine leaf herbal tea had IC_{90} values of 50.51 ± 1.01 ppm, while stevia leaf herbal tea had 61.60 ± 1.23 ppm. All combined products showed significantly stronger antioxidant activity ($p < 0.01$) than single extracts. The formula had an IC_{90} value of 30.57 ± 0.61 ppm, Formula 2 had 39.18 ± 0.78 ppm, and Formula 3 (2:1) had the lowest value of 25.13 ± 0.50 ppm. All combination formulae had substantial antioxidant effect if the IC level was less than 50 ppm. Formula 3 (2:1) had the lowest IC_{90} number and maximum antioxidant activity ($p < 0.05$). The blended products had lower IC_{90} values than the solo extracts, indicating that pine and stevia leaf bioactive chemicals operate better together [17], [18].

3.6 Discussion

Testing showed that pine and stevia leaf simplicia met Indonesian Herbal Pharmacopoeia quality criteria. Products with less water don't grow microorganisms, and active substances stay stable throughout storage [19]. The simplicia's high water-soluble and ethanol-soluble extract content indicates that it contains several polar and semi-polar active chemicals, including antioxidant-rich phenolic and flavonoid compounds. Pine and stevia leaf herbal tea exhibited antioxidant-like secondary metabolites, according to phytochemical tests. These comprised alkaloids, flavonoids, saponins, tannins, steroids/triterpenoids, and phenolics. Flavonoids, tannins, and phenolics react strongly in all three recipes, indicating antioxidant activity [20, 21].

Both pine leaf herbal tea and stevia leaf herbal tea demonstrated high antioxidant activity, with IC_{90} values of 50.51 ppm and 61.60 ppm, respectively, based on established DPPH radical scavenging criteria [16]. However, all blended variants showed high antioxidant activity ($IC_{90} < 50$ ppm). The version 3 (pine:stevia ratio = 2:1) exhibited the lowest IC_{90} value (25.13 ppm) and the highest antioxidant activity.

Quercetin (IC_{90} 18.01 ppm) was employed as the positive control in this study to assess the effectiveness of the flavonoid-rich plant infusion in eliminating free radicals. Quercetin works like pine

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and stevia leaf phenolic and flavonoid compounds, not Vitamin C or BHT. This improves its plant matrix reference. The stronger antioxidant activity seen in Formula 3 is probably because it has more pine leaves. Pine leaves are known to have a lot of phenolic and flavonoid compounds, like quercetin, kaempferol, and chlorogenic acid, which are strong antioxidants [5, 7]. Also, the mixed formulations had a lot more antioxidant activity than the individual herbal teas ($p < 0.01$), which shows that the bioactive chemicals from pine and stevia leaves work well together. This effect may be caused by the fact that phenolic chemicals from pine and stevioside and flavonoids from stevia work together to get rid of free radicals [8, 9], [22].

Herbal tea blend antioxidants work in several ways. Phenolic and flavonoid compounds contribute hydrogen atoms from their hydroxyl groups (-OH) to DPPH radicals to protect cells. This converts the unstable purple DPPH radical to yellow-colored reduced DPPH-H. Metal ions that create free radicals can bond to these compounds. By stabilising intermediate radicals, they can halt free radical chain reactions [3, 4].

Results are consistent with previous research. According to Mutmainah et al. (2019) [9], stevia leaf extract has an antioxidant effect with an IC_{90} of 12 to 15 ppm. In contrast, pine leaf extract was a potent antioxidant with an IC_{50} of 8-12 ppm [6]. This study found greater IC_{90} values (25.13 to 61.60 ppm) than previous studies due to sample extraction methods. Earlier research utilised intense organic solvent extracts (ethanol or methanol), but this study used a water-based infusion (herbal tea), which is more like how people use it. However, the study found that consuming both plants combined is better as antioxidants than using each one alone.

4 Conclusion

The researchers were able to make a useful herbal tea from Pinus merkusii and Stevia rebaudiana that has strong antioxidant qualities. The mixed formulations (1:1, 1:2, and 2:1) all had IC_{90} values of 30.57, 39.18, and 25.13 ppm, which means they were "very strong" antioxidants. The results were much better than with single extracts ($p < 0.01$), showing that the bioactive chemicals from both plants work together to make the results better.

Formula 3 (2:1) exhibited the strongest antioxidant power and Formula 2 (1:2) the best organoleptic acceptability. The Indonesian Herbal Pharmacopoeia enforced strict restrictions for final goods and raw materials. High flavonoids, tannins, and phenolics gave the products bioactivity. Pine and stevia herbal tea seems like a promising, naturally sweet, caffeine-free functional drink that can prevent oxidative stress and benefit diabetics.

5. Declarations

5.1 Acknowledgements

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5.2 Author Contributions

MA: Ideation, oversight, resources, validation, writing-review, editing. WP: Research, data collecting, formal analysis, first draft. NNP : investigations, data collection, formal analysis. MAN: Supervision, verification, writing-review, editing. The final manuscript was read and approved by all writers.

5.3 Ethics

The Research Ethics Committee of Institut Kesehatan Helvetia approved this study with certificate number 025/KEP-IKH/2024.

5.4 Conflict of Interest

The people who wrote this piece say that they have no conflicts of interest with publishing it.

5.5 Funding Statement

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