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ANTIOXIDANT ACTIVITY OF HERBAL DRINK USING ANDALIMAN (*Zanthoxylum acanthopodium DC*)

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ABSTRACT

Andaliman is a type of spice typical of North Sumatra, which local people believe to have health benefits. Its potential is only used as a cooking spice for typical foods. This research aims to create a herbal drink as a functional food with antioxidant activity. Herbal drinks were formulated with varying concentrations of andaliman (0% as control, 1%, 2%, and 3%) to evaluate their functional properties. The health potential of herbal drinks was tested by measuring antioxidant activity using the DPPH method, vitamin C content using the titrimetric method, and antibacterial activity using the test bacteria *Bacillus subtilis* and *Escherichia coli* as representative of Gram positive and Gram negative bacteria, respectively. The research showed that the highest vitamin C content of 2.903 ± 0.051% was found in drinks with 3% andaliman. However, the highest antioxidant activity of 10.995 ± 0.027 % was in herbal drinks with 2% andaliman content. The highest antibacterial activity in this study was the inhibition of *E. coli* bacteria of 11,766 ± 4,760 and *B. subtilis* 8,066 ± 0.901 mm in drinks containing 3% andaliman. Organoleptic tests show that the color, aroma, taste, and overall consumer acceptance of beverage products are in the favorable range, with the lowest concentration of andaliman as the most preferred product due to the bitter and spicy taste of andaliman. Further research is needed to improve organoleptic parameters, as the herbal drink could not exceed the favorability value of the control drink.

Keywords: andaliman; antioxidant; herbal drink.

ABSTRAK

Andaliman merupakan salah satu jenis rempah khas Sumatera Utara yang dipercaya masyarakat setempat memiliki khasiat bagi kesehatan. Potensinya hanya dimanfaatkan sebagai bumbu masakan makanan khas. Penelitian ini bertujuan untuk menciptakan minuman herbal sebagai pangan fungsional dengan aktivitas antioksidan. Minuman herbal diformulasikan dengan konsentrasi andaliman yang bervariasi (0% sebagai kontrol, 1%, 2%, dan 3%) untuk mengevaluasi sifat fungsionalnya. Potensi kesehatan minuman herbal diuji dengan mengukur aktivitas antioksidan menggunakan metode DPPH, kandungan vitamin C menggunakan metode titrimetri, dan aktivitas antibakteri menggunakan bakteri uji *Bacillus subtilis* dan *Escherichia coli* yang masing-masing mewakili bakteri Gram positif dan Gram negatif. Hasil penelitian menunjukkan kandungan vitamin C tertinggi sebesar 2,903 ± 0,051% terdapat pada minuman dengan kandungan andaliman 3%. Namun aktivitas antioksidan tertinggi sebesar 10,995 ± 0,027 % terdapat pada minuman herbal dengan kandungan andaliman 2%. Aktivitas antibakteri tertinggi pada penelitian ini adalah daya hambat bakteri *E. coli sebesar* 11,766 ± 4,760 dan *B. subtilis* 8,066 ± 0,901 mm pada minuman yang mengandung andaliman 3%. Uji organoleptik menunjukkan bahwa warna, aroma, rasa, dan penerimaan konsumen secara keseluruhan terhadap produk minuman berada pada kisaran baik, dengan konsentrasi andaliman terendah sebagai produk yang paling disukai karena rasa andaliman yang pahit dan pedas. Penelitian lebih lanjut diperlukan untuk memperbaiki parameter organoleptik, karena minuman herbal tidak dapat melebihi nilai kesukaan minuman kontrol.

Kata kunci: andaliman; antioksidan; minuman herbal.

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INTRODUCTION

Indonesia is one of the largest spice-producing countries. In general, spices are only used as ingredients for cooking. It has been widely researched that spices have health benefits. Spices can prevent disease and increase the body's immunity (Anggrasari, Perdana and Multo, 2021). The well-known spices are ginger, turmeric, galangal, cinnamon, pepper, nutmeg, cardamom, and star anise.

There are quite a lot of herbal drinks in Indonesia. For example, our society has long known about herbal medicine, which is believed to help maintain health. Traditional drinks are believed to have functional properties, such as bandrek and bajigur in West Java, Wedang Ronde, and Wedang Uwuh in Central Java, Sarabba in Sulawesi, and Loloh in Bali.

Andaliman is one of the spices that is often found in North Sumatra. The antioxidant content in andaliman includes polyphenolic compounds, monoterpenes, sesquiterpenes, and quinones (Tampubolon, 2022). Antioxidants are substances found in the bodies of living creatures that protect them from free radical oxidation. Free radicals that are continuously produced during normal metabolism can cause damage to the function of body cells, which can ultimately cause degenerative diseases (Maharani et al., 2021). Also, andaliman contains essential oils such as geraniol, linalool, cineol, and citronella, creating a mint and lemon aroma (Tampubolon, 2022). Unfortunately, andaliman's superiority is hampered by sensory weaknesses. Andaliman can have a bitter taste and can make the tongue feel numb. Thus, efforts need to be made to improve the sensory properties of andaliman so that these weaknesses can be corrected. In this research, an herbal drink containing andaliman was made and mixed with other ingredients, namely ginger, lemongrass, cinnamon, and sugar, as an alternative herbal drink with antioxidant activity.

This herbal drink is hoped to create innovative beverage products from food ingredients rarely processed into products other than kitchen spices. This research aims to determine antioxidant activity, vitamin C levels, and bacterial inhibitory power in herbal drinks by adding various concentrations of andaliman. It is hoped that the processing of andaliman can be improved to include other products, such as drinks.

MATERIALS AND METHOD

Materials

The ingredients used in this research were andaliman, ginger, lemongrass, cinnamon, cloves, brown sugar and water. Andaliman is obtained from the traditional market in Siantar, Medan, and other ingredients are obtained from the traditional market in Salatiga. The reagents were ethanol, hexane, and a DPPH (1,1-diphenyl-2-picrylhydrazyl) solution (Merck, Darmstadt). The equipment used is ACIS digital scales, rotary evaporator, thermometer, UV-Vis spectrophotometer, vortex, and hot plate.

Method

The herbal drink is made using the following formulation: 25 grams of ginger, 3 grams cloves, 3 grams of cinnamon, 35 grams of brown sugar, and two stalks of lemongrass are boiled for 10 minutes in 500 milliliters of boiling water. To this solution, 1%, 2%, and 3% andaliman were added. The control herbal drink did not contain additional andaliman. After cooling, the solution is filtered and prepared for further testing.

Samples were measured for their antioxidant activity using the DPPH method (Puspitasari and Ningsih, 2016; Nugroho, Lewerissa and Gentaarinda, 2023), vitamin C levels using the titrimetric method (Devianti and Amalia, 2019), antibacterial activity of *Escherhichia coli (E. coli)* and *Bacillus subtilis* (Linggama et al., 2019). In addition, organoleptic test

was carried out using the hedonic method with 30 panelists to assess the quality parameters of taste, aroma, color, and overall level of liking on a scale of 1-5 (scale 1 = immensely dislike, 2 = dislike, 3 = like, 4 = like very much, 5 = like very much). All tests were carried out in 3 repetitions. The data was analyzed using one-way ANOVA calculations, and if a difference was found, further testing will be carried out using a significant difference calculation test.

RESULTS AND DISCUSSION

Previous studies have stated the potential of andaliman as a functional food candidate (Shasti and Siregar, 2017; Effendi, 2020; Silalahi and Lumbantobing, 2021). Several physiological activities of andaliman fruit that have been studied are as follows: as an antimicrobial (Marpaung *et al.*, 2019), antioxidant (Effendi, 2020), anti-malarial and analgesic (Susanti and Safitri, 2019; Effendi, 2020).

The results shown in Table 1 state that there is antioxidant activity from all herbal drinks with and without the addition of andaliman. The control had an antioxidant activity of 3.482%, and the herbal drink with the addition of 1% andaliman had an antioxidant activity of 3.660%.

The one-way ANOVA test showed that, there was no significant difference in the antioxidant activity content between control and 1% andaliman (see Table 1). The presence of antioxidant activity in the control (0% andaliman) could be caused by the content of other ingredients in the herbal drink created. Apart from andaliman, the herbal drink in this study contained ginger, cloves, lemongrass, and cinnamon. Research shows that spices such as ginger, cloves, cinnamon, and lemongrass contain antioxidant activity (Sangi and Katja, 2011; Tomagola *et al.*, 2016; Susanti and Safitri, 2019).

However, it can be seen that adding andaliman contributes to increasing the antioxidant activity of drinks with the addition of andaliman at 1, 2, and 3%. The antioxidant activity of andaliman is most likely due to the phenolic compounds. Phenolic compounds such as flavonoids, polyphenols, tannins, and alkaloids contained in andaliman have a role as free radical scavenging compounds (Effendi, 2020).

Table 1 shows that there is no real difference in the antioxidant activity of the control with the addition of 1% andaliman. The increase in antioxidant activity was only around 5% with the addition of 1% andaliman. Thus, adding 1% andaliman to herbal drinks is not enough to increase antioxidant activity. However, there were fundamental differences among samples with andaliman addition (1, 2, and 3%) on antioxidant content. The addition of 2% andaliman significantly differed from the control and the drink with the addition of 1% andaliman. There was an increase in antioxidant activity of more than 200% compared to the control. The addition of 2% andaliman had the highest antioxidant activity compared to other treatments. It is interesting to note that the addition of 3% andaliman does not necessarily increase the antioxidant activity content of herbal drinks. Although adding 3% increased the antioxidant activity by 38% compared to the control, the addition of 3% andaliman clearly shows a significant decrease in antioxidant activity, namely 56%, when compared to 2% andaliman addition.

The decrease in antioxidant activity from the addition of 2% to 3% andaliman is similar to research conducted by Suryanto et al. (2004). In his research, the antioxidant activity of andaliman extracted with hexane-acetone solvent decreased in antioxidant activity at concentrations above 200 ppm (Suryanto et al., 2004). Meanwhile, andaliman extract with hexane solvent showed stagnant antioxidant activity above 125 ppm (Suryanto et al., 2004). The same thing was found in other research when measuring the

antioxidant activity of breadfruit leaves, which experienced a decrease in activity at concentrations above 200 ppm when extracted with methanol solvent (Suryanto and Wehantouw, 2009).

It is suspected that antioxidants in high concentrations can change their properties from antioxidant to prooxidant properties (Sotler *et al.*, 2019). According to Sotler et al. (2019), some antioxidants can behave as prooxidants. Several factors influencing the function of antioxidants and converting them into prooxidants are the presence of metal ions, the concentration of antioxidants in the matrix environment, and their redox potential. For example, vitamin C is an antioxidant at low doses (30 - 100 mg/kg body weight) and a prooxidant at high doses (Sotler et al., 2019). Vitamin C in the presence of iron or copper can reduce Fe^{3+} to Fe^{2+} and Cu^{2+} to Cu^{+}. This transition metal reduces hydrogen peroxide to hydroxyl radicals (Sotler et al., 2019). In this research, it is suspected that the presence of andaliman at a concentration of 3% in herbal drinks has exceeded the saturation concentration of antioxidants. However, it can be assumed that the decrease in antioxidant activity was not caused by iron or copper.

Vitamin C levels in herbal drinks did not significantly differ in the control, andaliman 1% and andaliman 2%. The highest levels of vitamin C were found in drinks with an andaliman concentration of 3%, with vitamin C levels of 2.903 ± 0.05 (mg/ml). They were significantly different when compared with other samples. In Table 1, the vitamin C values of control, andaliman 1 and 2%, do not differ much between samples; they are in the range of 2.2 – 2.3 mg/ml. Herbal drinks with the addition of 3% vitamin C showed an increase in vitamin C of 25% compared to the control. In this study, it was seen that increasing the concentration of andaliman caused an increase in vitamin C. These results were similar to those of other studies, which stated that adding more andaliman caused an increase in the vitamin C content in kecombrang stem chili sauce products (Naibaho, Damanik and Syauqi, 2020). Andaliman fruit is known as a source of vitamin C, so adding its concentration increases the vitamin C content in herbal drinks (Sinaga, 2023).

Table 1. Antioxidant Activity, Vitamin C, Antibacterial Activity of Herbal Drinks with Various Andaliman Concentrations

	Concentrations			
	Andaliman 0%	Andaliman 1%	Andaliman 2%	Andaliman 3%
Antioxidant activity (%)	3,482 ± 0,596[a]	3,660 ± 0,103[a]	10,995 ± 0,027[c]	4,837 ± 0,187[b]
Vitamin C (mg/ml)	2,306 ± 0,230[a]	2,273 ± 0,040[a]	2,226 ± 0,106[a]	2,903 ± 0,051[b]
Antibacterial activity of *E. coli* (mm)	7,100 ±1,493[a]	7,500 ± 0,500[a]	8,933 ± 2,759[a]	11,766 ± 4,760[b]
Antibacterial activity *B. subtilis* (mm)	6,133 ± 0,057[a]	6,200 ± 0,346[a]	7,366 ± 0,550[ab]	8,066 ± 0,90[b]

Note: different superscript means that there are significant differences (P>0,05) among sample

Antibacterial activity was carried out on the test bacteria *E. coli* and *B. subtilis*. These two types of bacteria represent Gram-negative (*E. coli*) and Gram-positive (*B. subtilis*) bacteria. Table 1 shows that the antibacterial activity of *E. coli* against the control was 7.10 ± 1.49 mm, indicating a moderate inhibition zone. The percentage of herbal drinks using Andaliman 1% and 2% has an inhibition zone, also included in the moderate inhibition zone category. The similarity in the zone of inhibition between the control and sample with the addition of 1 and 2% andaliman concentration was due to the presence of other spices, which also have antibacterial power in the control. However, from the results of this study, it is clear that the higher the concentration of andaliman, the greater its inhibitory power is for the growth of *E. coli*. The growth inhibition power of *E. coli* increased by 66% with the addition of 3% andaliman compared to the control. This inhibitory power can be caused by the alkaloids in andaliman, which have antibacterial properties (Muzahfri, Julianti and Rusmarilin, 2018). Adding andaliman as much as 3% resulted in an inhibition zone of 11.77 ± 4.76 mm. This inhibition zone is included in the strong inhibition zone. The results are in line with other research, which states that andaliman can inhibit the growth of microbes, including *Escherichia coli, Salmonella typhimurium, Bacillus cereus, Pseudomonas fluorescens, Aspergillus flavus, Propionibacterium acnes* and *Staphylococcus aureus* (Ompusunggu and Irawati, 2021).

Herbal drinks using andaliman also inhibit the growth of *B. subtilis* bacteria. Table 1 shows that herbal drinks without andaliman produce an inhibitory power of 6.133 $\pm$ 0.06 mm. As the concentration of andaliman increases, the inhibitory power on the growth of *B. subtilis* increases. However, if we look at the width of the inhibition zone, increasing the andaliman concentration to 3% does not reach the strong inhibition zone. Thus, andaliman's antibacterial activity against *E. coli* is stronger than against *B. subtilis*. A more robust ability to inhibit bacterial growth can happen because when antibacterial agent wants to penetrate the bacterial membrane, Gram-negative bacteria, which contain a thin layer of peptidoglycan cell walls, are more easily attacked than Gram-positive bacteria, which have thick peptidoglycan cell walls.

Several studies state the bacterial inhibitory power of andaliman were influenced by its extracting solvents. Acetyl acetate and methanol solvents are known to extract almost all the active compounds found in andaliman. In contrast, water and hexane solvents only extract a small amount of andaliman's active compounds (Muzahfri, Julianti and Rusmarilin, 2018). Thus, it can be assumed that the moderate inhibitory power of *B. subtilis*, apart from being influenced by the thickness of the cell wall, is also influenced by the extraction strength of the solvent used. However, it can be seen that the herbal drink with the highest concentration of andaliman has the highest zone of inhibition (Table 1). Thus, it can be stated that increasing the concentration of andaliman has a role in the antibacterial inhibitory power of both Gram-positive and Gram-negative bacteria, in this case, the bacteria *E. coli* and *B. subtilis*.

Table 2. Organoleptic test of Herbal Drink with Different Level of Andaliman

	Andaliman 0%	Andaliman 1%	Andaliman 2%	Andaliman 3%
Color	4,27 ± 0,583[b]	3,63 ± 067[a]	3,53 ± 0,63[a]	3,57 ± 0,63[a]
Aroma	3,57 ± 0,817[a]	3,67 ± 0,71[a]	3,33 ± 0,76[a]	3,37 ± 0,89[b]
Taste	4,53 ± 0,629[d]	3,00 ± 0,00[a]	3,00 ± 0,64[ab]	3,37 ± 0,77[c]
Overall acceptance	4,53 ± 0,629[d]	3,73 ± 0,69[a]	3,30 ± 0,467[b]	3,47 ± 0,57[ab]

Note: different superscript means that there are significant differences (P>0,05) among sample

Analysis of organoleptic tests of herbal drinks without andaliman and with the addition of andaliman is presented in Table 2. The parameters tested were color, aroma, taste, and overall acceptability. The addition of andaliman influences the color parameters of herbal drink products. The herbal drink containing andaliman is dark brown, while the control is light brown, which comes from adding brown sugar. Panelists preferred light brown; although there were no significant differences between each sample. Overall, the control had the highest color parameter values. Fortunately, the organoleptic assessment of herbal drinks with the addition of various concentrations of andaliman is still in the favorable range.

Interestingly, for aroma parameters, panelists preferred herbal drinks with the addition of 1% andaliman. Andaliman has the volatile components limonene and citronella, which produce a solid citrus-like aroma (Saragih and Raihandhany, 2023). Thus, it can be seen that the panelists' preference for herbal drinks is the one with volatile aromas of limonene and citronella that are not too strong.

Table 2 shows that in terms of taste, herbal drinks with the addition of andaliman cannot compete with herbal drinks without the addition of andaliman, even though all treatments with the addition of andaliman are still in the like category (score values are in the range 3.33 – 3.67). These results are in line with the research that added andaliman to moringa tempeh nugget products, which stated that the more andaliman powder was added, the less liked it was because andaliman had a spicy and bitter taste (Siregar, Oessoe and Langi, 2023).

There are significant differences in the taste parameters of each sample. Overall, the acceptability of herbal drinks containing andaliman is the highest on the one with a concentration of 1%. However, the values were lower compared to that of the control.

Although the overall score of the herbal drink with the addition of andaliman (1-3%) was in the favorable range (score greater than or equal to 3), the panelists preferred the one without andaliman addition. Thus, as an effort to increase consument's preference for herbal drinks containing andaliman, the health functions of drinks containing andaliman can be highlighted, namely antibacterial, antioxidant, and vitamin C properties

CONCLUSION

Herbal drinks with the addition of andaliman showed increased in antioxidant activity and vitamin C. However, adding andaliman as much as 3% experienced a decrease in antioxidant activity, which could be caused by a change in character from antioxidant to prooxidant. Adding andaliman increased

the growth inhibitory power of *E. coli* and *B. subtilis* bacteria, with a more substantial inhibitory effect on Gram-negative bacteria. Organoleptically, herbal drinks with andaliman added are at the preferred level, with a concentration of 1% andaliman as the most preferred andaliman drink product.

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