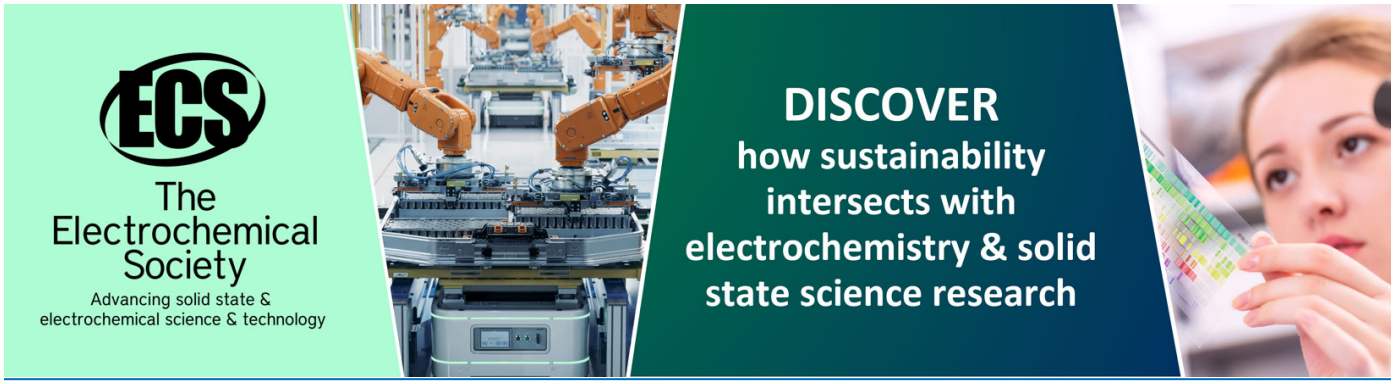
Promoting sustainability of herbs and spices from Simalungun based on its bioactive compound in traditional food *tinuktuk*



Promoting sustainability of herbs and spices from Simalungun based on its bioactive compound in traditional food *tinuktuk*

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Abstract. Bioactive compounds are associate with oxidative stress resistance and inflammation. Tinuktuk is a traditional Simalungun food made from herbs and spices. By knowing the bioactive compounds of tinuktuk traditional food, local heritage will not be lost and the sustainability of herbs and spices can maintain species loss. The purpose of this study was to promote the sustainability of herbs and spices from Simalungun based on bioactive compounds in tinuktuk. The ingredients that used in three types of tinuktuk formulations consisted of various herbs and spices. In addition, various chemicals according to the procedure for determining flavonoid, phenol, alkaloid and saponin levels. The results concluded that tinuktuk of formulation B has the highest levels of flavonoids which was 5.137 ± 0.236 Mg QE/g extract, meaning that in every gram of tinuktuk extract, there was flavonoid equivalent to 5.137 mg of quercetin. Alkaloids content which was 34.085 ± 0.665%, saponins content which was 1.989 ± 0.139%, and the highest phenolic content test result was formulation B, which was 22.913 ± 0.474 Mg GAE / g extract, meaning that in every gram of tinuktuk extract, there were phenolics equivalent to 22.913 mg of gallic acid.

1. Introduction

A special feature of plants is their ability to synthesize bioactive compounds which are important tools to avoid and defend against nuisance organisms such as insects, herbivores, microbes (bacteria, fungi), and viruses [1]. Bioactive compounds are associated with the combating against oxidative stress and inflammation. Oxidative damage and inflammatory processes at the cellular and subcellular levels are important in cardiovascular disease processes, inflammation, carcinogenesis, and aging, intake of dietary antioxidants can prevent these diseases while also increasing immunity [2].

Spices have been used in medicine for a long time, such as garlic and shallot contributing to the Egyptian Pyramids, ginger in Chinese medicine, pepper in Ayurvedic medicine. The father of medicine, Hippocrates was familiar with cinnamon, coriander, mint, saffron, and thyme. In those days spices were as important as medicine, embalming, preserving food, and masking bad odors. Herbs and spices have been used for thousands of years for flavor and aroma as well as food coloring [3].

Bioactive compounds in herbs and spices are a natural source of antioxidants. The antioxidant and anti-inflammatory properties of herbs and spices can serve to improve the overall health system [4]

Bioactive compounds that reported such as polyphenols, quinine, organosulfur compounds, flavonoids, alkaloids, polypeptides, and the other. and these compounds have diverse pharmacological activities [5] Phenolic compounds are the main antioxidants found in spices and there is a linear relation between total phenolic content and antioxidant properties of spices [2].

Indonesia is rich in natural resources that have bioactive ingredients, which have great opportunities to be developed. but many of it still have not been scientifically revealed [6]. Various medicinal plants to cure diseases are found in ethnic Simalungun, but have not been passed on to the younger generation. Traditional foods that have beneficial effects, innovations, and healthy holistic processing and consumption methods must be documented [7].

In Simalungun community, it is known and commonly consumed *tinuktuk*, which is usually given to women who have just given birth to increasing fitness [8]. The process of making it by pounding it on a wooden or stone mortar, does not use water. *Tinuktuk* is usually consumed by mixing in soup, mixing in hot drinks such as sweet tea or eaten with rice and other side dishes.

The ingredients for making *tinuktuk* are various herbs and spices. Based on research [9] the ingredients used to make *tinuktuk* are as follows: yellow bitter ginger, red ginger, candlenut, sand ginger, shallot, java turmeric, sichuan pepper, roasted rice, black pepper, garlic, turmeric, galangal, tamarind four kinds and salt. While the results of the study stated as many as 15 types of mandatory herbs and spices. The results of research obtained 6 formulations of *tinuktuk* commonly processed and traded by Simalungun community. The types of ingredients and composition of ingredients differ from one formulation to another. Based on antioxidant analysis, the three best formulations are formulation A, B, and C.

Various studies on *tinuktuk's* ingredients prove that these ingredients have their respective functions, namely antioxidant, antibacterial, anti-fungal, antichamir, antiseptic, anticancer, anti-inflammatory, and antibiotic. However, the younger generation is less familiar with *tinuktuk*, they did not want to process and consume it. The use of herbs and spices has begun to be abandoned, and local culture is replaced by modernization [8]. In addition, information about *tinuktuk* is still very limited, and claims that *tinuktuk* is beneficial to health have not been scientifically proven. So, it is necessary to look for the advantages of *tinuktuk* in terms of health, namely its bioactive compounds to promote the sustainability of the herbs and spices that make *tinuktuk*. The utilization of herbs and spices derived from plants can maintain species lost. The purpose of this research was to promote the sustainability of herbs and spices from Simalungun based on the bioactive compounds in *tinuktuk.*

2. Materials and Method

2.1 Place and time of research

The research was carried out at the Food Technology Laboratory, Department of Nutrition, Poltekkes Medan and the Biology Laboratory, Faculty of Pharmacy, University of North Sumatra (Medan). This research was conducted in July-August 2022.

2.2 Tools

The equipment used in this study were knives, cutting boards, basins, pans, spatulas, winnows, sieves, blenders, spoons, stone mortar, a wooden pestle, digital scales. test tube, spatula, test tube rack, glass beaker, dropper, Erlenmeyer, porcelain cup, Erlenmeyer with lid, stand-up cooler, vacuum evaporator, separatory funnel, water bath, parchment paper, aluminum foil, stirrer, refrigerator (Toshiba), cupboard dryer, micropipette (Eppendorf), microscope (Olympus), analytical balance (Metler AE 200), oven (Fischer scientific), water bath, paper cup, tweezers, dropper, tube rack, rotary evaporator (Haake D), spatula and test tube (Pyrex), furnace (Naberthem), and UV-vis spectrophotometry.

2.3 Ingredient

The ingredients of *Tinuktuk* were red ginger, white ginger, shallot, garlic, sand ginger, candlenut, black pepper, java turmeric, sichuan pepper, lime, salt, lemongrass, galangal, torch ginger fruit, pumpkin

seeds, cloves, rice, cucumber seeds, and chives. Chemical analysis ingredients were distilled water, 10% glacial acetic acid, ammonia, quercetin, 10% $AlCl_3$, 10% sodium acetate, petroleum ether, ethyl acetate, butanol, methanol, diethyl ether, sodium acetate, sodium carbonate, hexahydrate hydrochloric acid, gallic acid, and folin 10%.

2.4 Tinuktuk making

The A formulation contained 15 ingredients: red ginger, sand ginger, shallot, garlic, black pepper, candlenut, salt, ginger, sichuan pepper, torch ginger fruit, and dried pumpkin seeds, cloves, lemongrass, rice, and galangal. The following was the processing method: salt, pepper, dried pumpkin seeds, and roasted cloves, ground and sifted into flour (mixture 1). Milled roasted candlenuts, and rice (mixture 2). Shallots, garlic, red ginger, sand ginger, galangal, lemongrass, sichuan pepper were cleaned, pounded one at a time, and then the mixture was gradually added. Add mixture 2 and mash it until it was distributed evenly. The torch ginger fruit was washed, pounded, squeezed out the water, filtered, and added to the *tinuktuk* mixture. Stirring was done until it was thoroughly combined.

There were 11 ingredients for the B formulation, namely: red ginger, sand ginger, shallot, garlic, black pepper, candlenut, salt, lime, torch ginger fruit, dried pumpkin seeds, dried cucumber seeds. The following was the processing method: add salt after the shallot and garlic have been cleaned, cut, roasted separately, and pounded (mixture 1). Then sliced, roasted, and ground Candlenuts (mixture 2). Roasted, crushed, and sieved pumpkin seeds and cucumber seeds (mixture 3). Ginger and sand ginger should be cleaned, not roasted, and ground with salt added afterward (mixture 4). black pepper was pulverized then sifted it into flour (mixture 5). put the entire mixture in the mortar and pound until well combined. Mix thoroughly after adding lime juice and torch ginger fruit.

There were 11 ingredients for the C formulation, namely: white ginger, sand ginger, shallot, village garlic, black pepper, candlenut, salt, lime, dried pumpkin seeds, chives, and rice. The following was the processing method: washed, sliced, then crushed, shallots, village garlic, chives, sand ginger, white ginger (mixture 1). Rice was roasted with salt, then pepper was added and simmered (until it sounds done), then pounded (mixture 2). roasted, crushed, and sifted pumpkin seeds (mixture 3), roasted and mashed candlenuts (mixture 4). Mixtures 1, 2, 3, and 4 were put in a mortar and pounded again until well combined. After the ingredients are thoroughly combined, add the lime juice and whisk until evenly mixed.

2.5 Extract manufacture

Blended *tinuktuk* and heated water to 50°C. 50 grams of *tinuktuk* were mixed with 50 cc of warm water, left to stand for 6 hours, and then stirred for 5 minutes. Use Whatman Paper No. 1 for drying after 6 hours. The residual dregs are combined two more times with warm water.

2.6 Compound setting

2.6.1 Determination of alkaloid levels. 2.5 g of a sample were weighed in an Erlenmeyer. Put 50 ml of a 10% acetic acid solution in (acetic acid in ethanol). After 4 hours of stirring with a stirrer, the solution was filtered. When the filtrate has evaporated, ammonium hydroxide is drip-treated until an alkaloid precipitate forms. The precipitate was filtered and then washed with a solution of 1% ammonium hydroxide after the empty filter paper had been weighed. The precipitate-containing filter paper was dried in an oven for 30 minutes at 60°C. The filter paper was weighed to a constant weight after cooling.

$$\%\text{Alkaloid} = \frac{(\text{weight of residue} + \text{weight of filter paper}) - \text{weight of filter paper}}{\text{weight of sampel}} \; x \; 100\% \qquad (1)$$

2.6.2 Determination of saponin levels. In a closed Erlenmeyer, a sample weighing 1.25 grams was measured. added 50 ml of Petroleum ether, refluxed for 30 minutes at 60–800C. Separate the petroleum ether solution from the residue once it has cooled. 50 cc of ethyl acetate was added to the remaining

residue before it was moved to a separating funnel. Distinguish the solution from the residue. The remaining substance was dissolved using 50 ml each of 1 butanol 3 times. A rotary evaporator is used to mixed and evaporate the entire butanol solution. 10 milliliters of methanol were added to the residual evaporation. Then, while using a stirrer, this solution was dripped into 50 cc of diethyl ether. Filter paper with a known weight was used to remove the precipitate that had formed. The precipitate on filter paper was then weighed repeatedly until the weight remained constant.

$$\%\text{Saponin} = \frac{(\text{weight of residue} + \text{weight of filter paper}) - \text{weight of filter paper}}{\text{weight of sampel}} x\ 100\% \quad (2)$$

2.6.3 Determination of flavonoid levels. Gallic acid standard master solution preparation. A concentration of 500 ppm was achieved by dissolving a total of 25 mg of gallic acid in 1 mL of methanol, followed by dilution with distilled water to a volume of 50 mL.

Determination of Gallic Acid's Maximum Wavelength. A concentration of 20 ppm was achieved by pipetting 0.4 mL into a 10 mL volumetric flask filled with a standard solution of 500 ppm gallic acid. After adding 0.5 mL and 0.5 mL Folin-Ciocalteau and vortexing for about a minute, 1 mL of Na_2CO_3 20% was added. The mixture was then incubated for 35 minutes. Using a UV-Vis spectrophotometer, the maximum wavelength was determined to be between 400 and 800 nm.

Determining operating time 500 ppm Gallic acid standard solution. Pipetted 0.5 mL added, followed by 0.5 mL Folin-Ciocalteau, vortexed for approximately one minute, and added 1 mL Na_2CO_3 20%, Using a UV-Vis spectrophotometer, the maximum wavelength of 742 nm was determined.

Gallic Acid Calibration Curve Preparation To create solutions with concentrations of 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm, a standard solution of 500 ppm gallic acid was pipetted into each 10 mL volumetric flask in increments of 0.4 mL, 0.6 mL, 0.8 mL, 1 mL, and 1.25 mL. Pipetting up to 0.5 mL from each concentration, adding 1 mL of distilled water, 0.5 mL of Folin-Ciocalteau, vortexing for about a minute, and then adding 1 mL of 20% Na_2CO_3, followed by 35 minutes of incubation. The 742 nm maximum wavelength was measured. The calibration curve for gallic acid and the linear regression equation y = ax + b was obtained.

Total content determination Extract of phenol. *Tinuktuk* thick extract weighing a total of 10 mg was dissolved in 1 mL of methanol and then enough distilled water was added to reach a concentration of 1000 ppm. Following a 0.5 mL pipette of the 1000 ppm concentration solution, 1 mL of distilled water, 0.5 mL of Folin-Ciocalteau, and a vortex for about a minute, 1 mL of 20% Na_2CO_3 was added, and the mixture was incubated for 35 minutes. By using UV-Vis spectrophotometry at a maximum wavelength of 742 nm, the absorbance of each solution concentration was evaluated in comparison to the reagent used (blank).

2.6.4. Determination of phenol levels. Gallic Acid Standard Motherboard Solution Preparation. 25 mg of gallic acid were weighed, 1 methanol was used to dissolve it, and 50 mL of distilled water was added to make the solution, resulting in a 500 ppm concentration. Calculation of Gallic Acid's Maximum Wavelength. A pipette of 0.4 mL from a standard solution of 500 ppm gallic acid was used to transfer the 20 ppm concentration into a 10 mL flask. Add 0.5 mL pipette, 0.5 mL Folin-Ciocalteau, vortex for about a minute, then add 1 mL of 20% Na_2CO_3 and incubate for 35 minutes. Using a UV-Vis spectrophotometer, the maximum wavelength was determined to be between 400 and 800 nm. determining operating time Gallic acid standard solution 500 ppm. Add 0.5 mL pipette, 0.5 mL Folin-Ciocalteau, vortex for about a minute, and then add 1 mL of 20% Na_2CO_3. With a UV-Vis spectrophotometer, the maximum wavelength is 742 nm.

Developing calibration curves for gallic acid. Pipette amounts of 0.4 mL, 0.6 mL, 0.8 mL, 1 mL, and 1.25 mL from a standard solution of 500 ppm gallic acid were added to each 10 mL volumetric flask to create solutions with concentrations of 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm. Pipetting was used to combine 0.5 mL of each concentration with 1 mL of distilled water, 0.5 mL of Folin-Ciocalteau, and

then to vortex the mixture for about a minute before adding 1 mL of 20% Na_2CO_3 and incubating it for 35 minutes. The maximum wavelength detected is 742 nm. The linear regression line equation y = ax + b and the gallic acid calibration curve were obtained.

Determining the content of the whole phenol extract. To achieve a concentration of 1000 ppm, 10 mg of a thick sungkai leaf extract were weighed, diluted in 1 mL of methanol, and then mixed with 10 mL of distilled water. A total of 0.5 mL of a solution with a concentration of 1000 ppm was pipetted, 1 mL of distilled water, 0.5 mL of Folin-Ciocalteau, and vortexed for about a minute before adding 1 mL of 20% Na_2CO_3 and 35 minutes of incubation. UV-Vis spectrophotometry was used to determine the absorbance of each concentration of the solution to the reagent employed (blank) at a maximum wavelength of 742 nm.

2.7. Data analysis

Based on the mean and standard deviation, the results of the research into the concentrations of flavonoids, phenolics, alkaloids, and saponins were presented and descriptively analyzed.

3. Results and Discussion

Sample extraction was carried out using warm water, with the aim of attracting the bioactive contained in *tinuktuk.* The choice of solvent using water because *tinuktuk* is food that will be consumed directly.

3.1. Determination of flavonoid level

The determination of flavonoid levels is based on the calculation of the regression equation from the quercetin absorption curve. The regression equation is Y = 0.01129X + 0.0334 and the coefficient value is 0.9941 which means that 99.41% absorption is affected by concentration

In Table.1, it can be seen the results of the flavonoid level test in *tinuktuk.* The highest flavonoid content was the B formulation, which was 5.137±0.236 Mg QE/g extract, meaning that in every gram of *tinuktuk* extract there was a flavonoid equivalent to 5.137 mg of quercetin. One of the ingredients in *tinuktuk* is black pepper and black pepper is rich in flavonoids [10].

Table 1. Results of determination of *tinuktuk* flavonoid levels.

Sample	Flavonoid compounds Mg QE/g extract ± SD
Formulation A	5.014±0.272
Formulation B	5.137±0.236
Formulation C	4.593±0.064

3.2. Determination of phenolic levels

Determination of phenolic content is based on the calculation of the regression equation from the gallic acid absorption curve. The regression equation is Y = 0.01263X + 0.0406 and the coefficient value of r = 0.990 which means that 99.0% absorption is affected by concentration. This figure is close to 1 which indicates a linear calibration curve and there was a correlation between the concentration of gallic acid solution and the absorption value.

In Table.2 it can be seen the results of the *tinuktuk's* phenol level test. The highest phenolic content was the B formulation, which was 22,913±0.474 Mg GAE/g extract, meaning that in every gram of *tinuktuk* extract there was phenolic equivalent to 22,913 mg gallic acid. Phenol content is mainly influenced by processing techniques and extraction methods [11].

Table 2. Results of determination of *tinuktuk* phenolic content.

Sample	Fenolic compounds Mg GAE/g extract ± SD
Formulation A	22.863±0.314
Formulation B	22.913±0.474
Formulation C	19.013±0.068

3.3. Determination of alkaloid levels

The method used in determining the levels of alkaloids is the gravimetric method, the gravimetric method is a method that has the advantage that there is no comparison substance (standard alkaloid) and the simplest method of analysis compared to other methods, because in the gravimetric method the amount of substance is determined by direct weighing. the mass of the separated substance [12]. The results of the determination of the alkaloid content can be seen in Table.3. It turns out that the highest alkaloid content was the B formulation, which is 34.085±0.665 %.

Table 3. Results of determination of *tinuktuk* alkaloid content.

Sample	Alkaloid compounds % ± SD
Formulation A	31.475±0.327
Formulation B	34.085±0.665
Formulation C	33.721±0.282

3.4. Determination of saponin levels

Saponins are detergents where surface active properties are amphiphilic or soluble in polar and non-polar, where the large molecular weight and structure of saponins consists of steroid or triterpene aglycones called sapogenins or glycans containing one or more sugar chains [13]. The results of the determination of saponin levels can be seen in Table.4. It turns out that the highest alkaloid content was the B formulation, which is 1.989±0.139%.

Table 4. Results of determination of *tinuktuk* saponin levels.

Sample	Saponin compounds % ± SD
Formulation A	1.369±0.047
Formulation B	1.989±0.139
Formulation C	0.938±0.500

Tinuktuk is prepared from various herbs and spices, which have been studied for their bioactive compounds. Ginger, black pepper, shallots, garlic, turmeric, cloves contain flavonoids and phenolics. In addition, shallot and garlic contain alkaloids and saponins [14]. Aromatic ginger contains phenolics and flavonoids [15]. Sichuan pepper contains phenols and flavonoids [16]. alkaloids and saponins. Torch

ginger contains flavonoids, saponins and tannins. Pumpkin seeds contain phenolics, tocopherols, and phytosterols. Flavonoid and phenolic contents are lower in fresh samples than in dried samples.

The potential of phytochemicals in spices was studied and if consumed in high amounts may offer antioxidant properties and regulate key digestive enzymes that may lead to the prevention or reduction of disease progression such as cancer, diabetes and cardiovascular disease. Foods that can prevent or reduce degenerative diseases and have a positive effect on health are called functional foods. The known levels of bioactive compounds in *tinuktuk* offer opportunities and potential to be developed as functional food with product innovation. Thus, the sustainability of *tinuktuk* can be reintroduced as a functional food to modern society and the younger generation

4. Conclusion

Among the three tinuktuk's formulations, it is concluded that tinuktuk of formulation B has the highest levels of flavonoids, phenolics, alkaloids and saponins. Formulation B used 11 types of herbs and spices in its preparation, namely red ginger, aromatic ginger, shallot, garlic, black pepper, candlenut, salt, lime sour, torch ginger, dried pumpkin seeds, dried cucumber seeds. Differences in the composition of herbs and spices and how to process tinuktuk formulation turned out to provide different levels of bioactive compounds. By knowing the bioactive compounds in tinuktuk, the sustainability of Simalungun agriculture with a variety of unique herbs and spices can be maintained. The existence of species of herbs and spices can be continued to be introduced to future generations. Further research needs to be done to standardize processing techniques, make innovative product creations. In addition, it is necessary to analyze the benefits to health for the sustainability of traditional food spices and herbs, tinuktuk as functional food.

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