

PHYTOCHEMICAL AND ORGANOLEPTIC CHARACTERISTIC OF *Sterculia quadrifida* R.Br. TREE BARK HERBAL TEA

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PHYTOCHEMICAL AND ORGANOLEPTIC CHARACTERISTICS OF FALOAK (*Sterculia quadrifida* R.Br.) TREE BARK HERBAL TEA. *Sterculia quadrifida* R.Br. Bark decoction is used to treat hepatitis and consumed as a tonic by Timorese in the East Nusa Tenggara Province, Indonesia. Raw herbal materials are susceptible to fungi contamination, have limitations in transportation, and have low economic value. Processing the bark into powder packed in tea bags is expected to overcome these obstacles. Stevia, ginger, and mint leaves are herbs often added to herbal drink formulas. This study aimed to evaluate the antioxidant capacity, total flavonoid content (TFC), total phenol content (TPC), physicochemical properties, and sensory properties of six formulas, namely: (F1) pure *S. quadrifida* bark; (F2) bark of *S. quadrifida* and leaves of stevia; (F3) stem bark of *S. quadrifida* and ginger; (F4) bark of *S. quadrifida*, stevia, and ginger; (F5) bark of *S. quadrifida* and mint leaves; (F6) bark of *S. quadrifida*, stevia, and mint. Total phenolic content was measured using the Folin-Ciocalteu method, and total flavonoids were determined using the AICI₃ method. Antioxidant activity was measured using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method. The TPC of *S. quadrifida* tea ranged from 14.09±0.7 to 68.20±0.95% Gallic Acid Equivalent (GAE), and the TFC ranged from 0.03±0.005 to 0.09±0.004% QE. The Ascorbic acid Equivalent Antioxidant Capacity (AEAC) of F1, F2, F5, and F6 were insignificantly different. The pure *S. quadrifida* bark formula (F1) contained the highest levels of antioxidants (1,044.3±28.48 ppm Ascorbic Acid Equivalent (AAE)). Formulas with the addition of stevia, mint, and ginger showed weaker antioxidant activity than pure *S. quadrifida*. The formula of pure *S. quadrifida* bark (F1) and *S. quadrifida* with the addition of stevia (F2) have the potential to be developed as commercial herbal teas.

Keywords: Antioxidant, faloak, herbal tea, hepatitis, peanut tree, phenolic

KARAKTERISTIK FITOKIMIA DAN ORGANOLEPTIK TEH HERBAL KULIT BATANG POHON FALOAK (*Sterculia quadrifida* R.Br.). Rebusan kulit batang *Sterculia quadrifida* R.Br. digunakan untuk mengobati hepatitis dan dikonsumsi sebagai tonik oleh orang Timor di Provinsi Nusa Tenggara Timur, Indonesia. Bahan herbal mentah rentan terhadap kontaminasi jamur, akan menghadapi kesulitan dalam pengiriman, dan memiliki nilai ekonomi yang rendah. Pengolahan kulit batang menjadi bentuk serbuk yang dikemas dalam kantong teh celup diharapkan dapat mengatasi kendala-kendala tersebut. Daun stevia, jabe, dan mint adalah beberapa tumbuhan yang sering digunakan dalam formula minuman herbal. Penelitian ini bertujuan untuk mengevaluasi kapasitas antioksidan, kandungan flavonoid total, kandungan fenol, sifat fisikokimia, dan sensori enam formula yaitu: (F1) kulit batang *S. quadrifida* murni; (F2) kulit batang *S. quadrifida* dan daun stevia; (F3) kulit batang *S. quadrifida* dan jabe; (F4) kulit batang *S. quadrifida*, stevia dan jabe; (F5) kulit batang *S. quadrifida* dan daun mint; (F6) kulit batang *S. quadrifida*, stevia dan mint. Kandungan total fenolik diukur dengan menggunakan metode Folin-Ciocalteu dan Total flavonoid ditentukan dengan menggunakan metode AICI₃. Aktivitas antioksidan diukur menggunakan metode DPPH. Kandungan fenol total teh *S. quadrifida* berkisar antara 14.09±0.7 hingga 68.20±0.95% EAG, dan kandungan flavonoid berkisar antara 0.03±0.005 hingga 0.09±0.004% EK. Kapasitas antioksidan tertinggi adalah 1,044,33±28.48 ppm EAA pada F1, dan terendah adalah

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801.81±9.46 ppm EAA pada F2 yang memiliki nilai sensori tertinggi. Formula dengan tambahan stevia, mint dan jabe memiliki aktivitas antioksidan yang lebih rendah dibandingkan dengan formula *S. quadrifida* murni. Formula kulit batang *S. quadrifida* murni (F1) dan formula *S. quadrifida* dengan tambahan stevia (F2) memiliki potensi untuk dikembangkan menjadi teh herbal komersial.

Kata kunci: Antioksidan, faloak, teh herbal, hepatitis, peanut tree, fenolik

I. INTRODUCTION

Sterculia quadrifida R.Br., or "Peanut tree," is a medicinal plant of the Malvaceae family. This plant can be found in Indonesia, Australia, Pakistan, Timor Leste, and India (Cowie, 2006; Karthikeyan, Shanthi, Saravanan, & Saranya, 2014; Akter, 2016; Manirujjaman, 2019; Siswadi et al., 2021). The plant is known as "Faloak" in East Nusa Tenggara province, Indonesia. *S. quadrifida* bark decoction is a traditional remedy for hepatitis, lumbago, ulcers, and malaria and is consumed as a tonic (Siswadi, Raharjo, Pujiono, Rianawati, & Saragih, 2016). To treat health problems, the bark is boiled, and the decoction is consumed several times daily.

Sterculia quadrifida bark is harvested from trees that naturally grow along roadsides, yards, gardens, green spaces, and forests. The high number of debarked *S. quadrifida* trees in the region indicated the high demand for this herbal remedy (Siswadi et al., 2021). *Sterculia quadrifida* bark extract exhibits high antioxidant capacity (Lulan, Fatmawati, Santoso, & Ersam, 2018; Saragih & Siswadi, 2019) and has the potential to be used as a mixture of breast cancer drugs

(Rollando & Prilianti, 2017) and diabetes mellitus treatment (Lalong, Zubaidah, & Martati, 2022). It is also immunomodulatory (Hertiani et al., 2019; Winanta, Hertiani, Purwantiningsih, & Siswadi, 2019) and has the potential to inhibit HVC (Dean, Handajani, & Khotib, 2019; Sola, 2019), and is hepatoprotective (Darojati, 2022; Renggani, 2022). The bark extract also has low toxicity since the LD₅₀ was higher than 2,000 mg/kg BW (Siswadi & Saragih, 2018; Noviyannah, Hertiani, Murwanti, Siswadi, & Setyowati, 2021)).

Sterculia quadrifida bark is sold in traditional markets in Kupang without post-harvesting treatment (Figure 1). Raw herbal materials risk fungi contamination, have limitations in transportation and have low economic value (Li et al., 2015; Setiarso, Rusijono & Kusumawati, 2018; Singh, Tiwari, Maurya, Kumar, & Dubey, 2022). Moreover, the decoction has a woody aroma and chelate taste. Some ingredients are commonly added to herbal drinks, including stevia leaves (*Stevia rebaudiana* B.), ginger (*Zingiber officinale* Rosc.), and mint (*Mentha piperita* L.) This study processed the bark into tea bag packaging



Figure 1. *Sterculia quadrifida* bark is sold in traditional markets in Kupang city.

and combined it with dried stevia leaves (*Stevia rebaudiana* B.), ginger (*Zingiber officinale* Rosc.), and mint (*Mentha piperita* L.). It was aimed to provide functional *S. quadrifida* bark herbal preparation and improve the taste and hygiene since herbal mixtures can improve the taste of herbal preparation (Guimarães, Barros, Carvalho, & Ferreira, 2011).

An analysis of water content, microbial and heavy metals contamination was conducted to determine the quality of herbal tea formulas. Moreover, the formulas' total phenol, total flavonoid content, and antioxidant capacity were also analyzed. This study was carried out to determine the phytochemical, antioxidant activity, and sensory properties of *S. quadrifida* herbal tea and the blending of *S. quadrifida* with stevia, ginger, and mint.

II. MATERIAL AND METHOD

A. Study Site

Sterculia quadrifida bark was harvested in Kupang city, the capital of Nusa Tenggara Timur province, Indonesia. This region has a semi-arid climate with low rainfall and long dry season.

B. Methods

1. Preparation of *S. quadrifida* bark tea

S. quadrifida bark was obtained from trees far from residential areas and roadside in Kupang City. The bark was cleaned, then chopped into small pieces ($\pm 0.5 - 2$ cm), and oven-dried at 55°C for 48 hours. Dry bark was powdered using a grinder. The powder was re-dried for 8 hours using the same temperature to ensure that the powder's moisture content was less than 10%. *S. quadrifida* powder was then sieved

to obtain a powder with a particle size between 0.42 – 1.41 mm.

Dried ginger, mint, and stevia were purchased from the local market. All ingredients were dried in the oven at 55°C for 12 hours. Afterward, the ingredients were weighed, mixed with *S. quadrifida* bark powder, and packed in tea bags. Formula F1, F2, F3, F4, F5, and F6 were herbal tea formulas made from the main ingredient of *S. quadrifida* bark with ginger, stevia, and mint (Table 1). The tea bags were steeped in hot water ($\pm 90^\circ$ C) for 5 minutes before being served.

2. Physicochemical and Microbiological properties of *S. quadrifida* herbal tea ingredients

According to the Regulation of the Indonesian National Agency of Drug and Food Control (BPOM) Number 32 of 2019 concerning Traditional Medicine Safety and Quality Requirements, several parameters must be analyzed to determine the quality of raw materials in herbal tea production.

The moisture contents were determined according to the methods described in the Indonesian National Standard (abbreviated SNI) 01-2891-1992 Food and Beverages Test Methods (National Standardization Agency of Indonesia, 1992). The contents of lead (Pb), Cadmium (Cd), Mercury (Hg), and Arsenic (As) were determined according to the method described by AOAC 999.11 Official Method 999.11 (2001); SNI 01-2896-1998 Test Method for Arsenic Pollutant in Food (1998); SNI 01-4866-1998 Test Method for Metal Pollutant in Food (1998); Test Method for Arsenic Pollutant in Food (1998); SNI 01-2896-1998 Test Method for Metal Pollutant in Food (1998).

Table 1. The formula of herbal teas tested

Ingredients	Formula					
	F1	F2	F3	F4	F5	F6
<i>S. quadrifida</i> bark	100%	90%	90%	80%	70%	60%
Stevia		10%		10%		10%
Ginger			10%	10%		
Mint					30%	30%

Microbiological analysis was carried out using the following methods: total plate count at 30°C for 72 hours using the ISO 4833:2003 and ISO 7218:2012; method from Bacteriological Analytical Manual (BAM) 2002 Chapter 4 for the detection of *Escherichia coli*; *Pseudomonas aeruginosa* pour plate method with *Pseudomonas* agar base; ISO 6579: 2002 for the detection of *Salmonella* in food samples; method from BAM 2001 chapter 12 for the detection of *Staphylococcus aureus* (Sandra, Bennett, & Lancette, 2019; Tournas, Stack, Mislivec, Koch, & Bandler, 2001).

3. Extraction

Aqueous extraction was conducted using a modification of the method described by (Kim, Goodner, Park, Choi, & Talcott, 2011). A tea bag containing 4 grams of each formula was steeped in 200 mL hot water at a temperature of $\pm 90^\circ\text{C}$ for 5 minutes. Brewed teas were filtered using filter paper, and then 5-10 mL was taken for the total phenolic content, flavonoid content, and antioxidant activity analysis.

4. Total Flavonoid Content (TFC)

The calibration curve was prepared by using quercetin. Quercetin (Sigma Aldrich) standard solution was prepared by weighing 10 mg quercetin diluted with 96% ethanol to a volume of 10 mL. Stock solution pipetted of 1 mL added by 10 mL ethanol 96%. The concentration of the resulting solution was made above concentrations of 3.75, 5, 6.25, 7.5, 8.75, and 10 ppm; then added with 3 mL of 96% ethanol, added 0.2 mL of 10% AlCl_3 (Merck) solution, added 0, 2 mL of 1 M acetic acid (Merck EMSURE®), added up to 10 mL sterile distilled water allowed for 30 minutes at room temperature. The measured wavelength of maximum absorbance at 415 nm, against sterile distilled water as the blank and 10% AlCl_3 created a calibration curve with linearity of ($r = 0.99$).

Total flavonoids were determined using the AlCl_3 method (Ahmad, Wisdawati, & Asrifa, 2014). One mL of 2% AlCl_3 (Merck) and 8 mL

of 5% acetic acid (Merck EMSURE®) were added to 1 mL of 10,000 ppm sample solution, which was then allowed to stand for 30 minutes at room temperature. The absorbance of the sample solution was read at a wavelength of 415 nm using a Thermo Scientific™ GENESYS 10S UV-Vis spectrophotometer. TFC was determined from extrapolation of calibration curve, which was made by preparing quercetin solution as described earlier. The total flavonoids were expressed as Quercetin Equivalents (QE).

5. Total Phenolic Content (TPC)

Total phenolic content was measured using the Folin-Ciocalteu's method with gallic acid as standard (Singleton, Orthofer, & Lamuela-Raventós, 1999). The analysis began with dissolving 1 mL of the standard solution or sample in 5 mL of distilled water and 0.5 mL of reagent solution Folin-Ciocalteu's (Sigma-Aldrich). The solution was then incubated for 5 minutes in a dark room, then 1 mL of Na_2CO_3 solution was added, and it was incubated again in the darkroom for 1 hour. After incubation, the solution was vortexed (Thermo Scientific™ 88882012 Vortex Mixer), and a GENESYS 10S UV-Vis spectrophotometer (Thermo Scientific™) measured its absorbance at a wavelength of 725 nm. The spectrophotometer blank solution is distilled water. The total phenolic content was expressed as Gallic Acid Equivalents (GAE).

6. Ascorbic Acid Equivalent Antioxidant Capacity (AEAC)

The extract's antioxidant activity was measured using the DPPH (1,1-diphenyl-2-picrylhydrazyl) (Sigma-Aldrich) free radical (Molyneux, 2004). Ascorbic acid was used as the standard for comparing the antioxidant activity of the samples. The herbal tea formula antioxidant activity was calculated based on its equivalence with ascorbic acid's (AAE) antioxidant activity expressed in ppm AEAC.

The analysis was started by mixing 2 ml of buffered acetate solution (pH 5.5), 3.75 mL of

methanol with 200 μ L of DPPH 3 mM solution in methanol, then vortexed (Thermo Scientific™ Vortex Mixer). After that, 50 μ l of sample solution or standard solution of antioxidants was added to the mixed solution. The solution was then incubated at 37°C using a water bath for 30 minutes. The absorbance of the solution was then measured with a Thermo Scientific™ Genesys 10S UV-Vis spectrophotometer at $\lambda=517$ nm. The spectrophotometer blank solution was distilled water.

7. Sensory Analysis

The sensory evaluation was approved by the Ministry of Environment and Forestry of the Republic of Indonesia and conducted in the Faculty of Public Health, Nusa Cendana University, The State Agricultural Polytechnic of Kupang, and Kupang Education and Training Agency of Environment and Forestry. There were 40 untrained panellists aged 20 to 56 years that participated voluntarily. In each questionnaire, the panellists were asked to rate using 7-point hedonic scales (1= 'Strongly dislike'; 2 = 'Moderately dislike'; 3 = 'Slightly dislike'; 4 = 'neither like nor dislike'; 5 = 'Slightly like'; 6 = 'Moderately like', and 7 = 'Strongly like') about their satisfaction degree on sensory attributes (colour, taste, and aroma) of the teas (Granato, De Castro, Ellendersen, & Masson, 2010). Each tea bag was filled with 4 g of herbal tea formula and brewed with 200 mL of hot water at a temperature of 90°C. Then 40 mL of each herbal tea formula was poured into a coded plastic cup (Figure 2).

C. Statistical Analysis

The antioxidant, TFC, and TPC measurements were performed in triplicate. The data were tabulated and presented as mean \pm SD (standard deviation). All data were checked for normality and homogeneity by the Kolmogorov–Smirnov and Levene's test. One-way ANOVA and Tukey Honestly Significant Difference (HSD) test were used to test differences among the formula's antioxidant, TPC, and TFC. The non-parametric Independent-Samples Kruskal-Willis test was used to test differences among the formula's organoleptic scores, and then further analyzed by a pairwise comparison test to see the significance between the formulas. The Pearson correlation coefficient was used to examine the correlation between total phenolic and flavonoid content and antioxidant activity. Significance was set at a 95% confidence level ($p < 0.05$). Statistical data were analyzed using SPSS IBM 25.

III. RESULT AND DISCUSSION

A. Result

1. Physicochemical and Microbiological Properties

In general, all ingredients of *S. quadrifida* herbal tea formulas met the requirements of the Indonesian National Agency for Drug and Food Control, WHO, and European standards (WHO, 2007; THIE, 2018; BPOM, 2019) (Table 2). The moisture content requirement for brewed herbal is less than 10%. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* sp. were absent in all herbal materials.

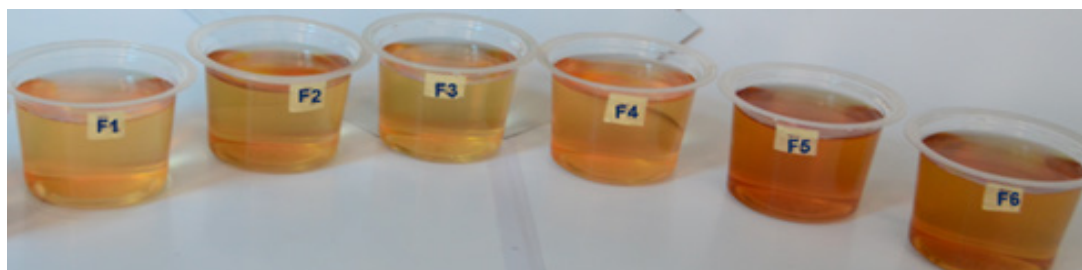


Figure 2. Six *S. quadrifida* formulas for the organoleptic test

This study showed that lead, cadmium, mercury, and arsenic in examined herbal materials were within permissible limits. The total plate count of stevia, ginger, and mint exceeded the limit the National Agency of Drug and Food Control required but was lower than the permissible limit of WHO and Tea & Herbal Infusions Europe association (THIE).

2. Phytochemical and Antioxidant Properties

The highest TPC was measured in the *S. quadrifida* bark and mint formula F5 (68.20±0.95% GAE) but the formula also has the lowest TFC (0.03±0.005% EQ) and is significantly different from other formulas. Meanwhile, formula F6 has the highest TFC. The phenolic content of all formulas was significantly different. In two formulas with the addition of stevia, the antioxidant decreased from 851.95±19.21 in F5 and to 801.81±9.46 ppm AAE in F6. The addition of stevia in the F5 formula reduced TPC but increased TFC. Likewise, the TPC of the F3 formula that contains *S. quadrifida* and ginger is 17.89±1.47% GAE, but then with the addition of stevia in F4, the TPC decreased to 14.09±0.72% GAE.

The antioxidants, TFC, and TPC, of the six herbal tea formulas are presented in Table 3. The TFC, TPC, and antioxidants in the six tea formulas ranged from 0.03±0.005 to 0.09±0.004% QE, 14.09±0.7 to 68.20±0.95% GAE, and 801.81±9.46 to 1,044.33±28.48 ppm AAE, respectively. The antioxidant capacity of the F1 formula was significantly different from those of the F2, F3, F4, F5, and F6 formulas. Formula F5 and F6, with the addition of mint leaves, had the lowest AEAC capacity (801.81±9.46 and 851.95±9.46 ppm AAE). The AEAC of F1, F2, F5 and F6 were insignificantly different. The pure *S. quadrifida* bark formula (F1) contained the highest levels of antioxidants (1,044.3±28.48 ppm AAE). The addition of stevia in the F2 formula also decreased the AEAC activity from 1,044.33±28.48 in F1 to 889.76±10.66 ppm AAE in F2.

TFC levels correlated negatively with TPC, showing very low correlation coefficient ($r = -0.22$). Meanwhile, very low correlation was also shown by TFC with AEAC ($r = 0.05$) and TPC with AEAC ($r = 0.167$) (Table 4).

Table 2. Heavy metal content and total microbial plate count for the raw material for the herbal tea formulation

Parameter	<i>S. quadrifida</i>	Stevia	Ginger	Mint	Indonesian FDA Standard B POM (2019)	WHO and European standards (THIE, 2018); WHO, 2007)
Moisture content (%)	6.71	5.39	7.94	15.2	< 10	<12
Total aflatoxins (µg/kg)	< 9.94	< 9.94	< 9.94	< 9.94	≤ 20	≤ 20
Heavy metals contamination						
- Lead (Pb) (mg/kg)	< 0.031	< 0.031	< 0.040	0.21	≤ 10	10
- Cadmium (Cd) (mg/kg)	0.03	< 0.004	< 0.005	< 0.007	≤ 0.3	0.3
- Mercury (Hg) (mg/kg)	< 0.005	< 0.005	< 0.005	< 0.005	≤ 0.5	0.2
- Arsen (As) (mg/kg)	< 0.013	< 0.013	< 0.003	< 0.013	≤ 5	5
Microbial contamination						
- Total Plate Count (CFU/g)	6.3 x 10 ²	1.0 x 10 ⁵	5.2 x 10 ⁵	4.8 x 10 ⁴	3 x 10 ³	≤ 10 ⁷
- <i>E. coli</i> (APM/g)	< 3	< 3	< 3	< 3	Negative	≤ 10
- <i>Salmonella</i> sp.	Negative	Negative	Negative	Negative	Negative	Negative
- <i>S. aureus</i> (CFU/g)	0	0	0	0	Negative	≤ 10 ⁵
- <i>Pseudomonas aeruginosa</i>	0	0	0	0	Negative	≤ 10 ⁷

Table 3: The Total Flavonoid Content (TFC), Total Phenolic Content (TPC), and AEAC activity of six *S. quadrifida* herbal tea formulas

Formula	TFC (% QE)	TPC (% GAE)	AEAC ppm AAE)
F1	0.07±0.020a	47.66±0.82a	1,044.33±28.48a
F2	0.06±0.012a	21.89±1.57b	889.76±10.66b
F3	0.04±0.008a	17.89±1.47c	870.71±15.52bc
F4	0.05±0.005a	14.09±0.72d	893.57±4.58b
F5	0.03±0.005b	68.20±0.95e	851.95±19.21c
F6	0.09±0.004a	29.24±0.89f	801.81±9.46d

Remarks:

*F1 = *S. quadrifida* bark powder 100%; F2 = 90% *S. quadrifida* bark and 10% stevia leaf; F3 = *S. quadrifida* bark and ginger; F4 = *S. quadrifida* bark, ginger, and stevia; F5 = *S. quadrifida* bark and mint; F6 = *S. quadrifida* bark, mint, and stevia. The different letter indicates significant differences at $p < 0.05$

Table 4. Pearson's correlation coefficients between TFC, TPC, and AEAC of *S. quadrifida* herbal tea ($p > 0.05$)

Variable	TFC	TPC	AEAC
TFC	1	0.226	0.059
TPC	0.226	1	0.167
AEAC	0.059	0.167	1

3. Sensory characteristics

In each parameter, different formulas obtained the highest score. Formula F2 obtained the highest score for taste (5.74 ± 1.30) and color (5.98 ± 0.65). The F3 formula is the most preferred for aroma (5.63 ± 1.02). The aroma score is divided into two groups: slightly like and neutral. The slightly liked formulas were F2 (5.28 ± 1.30), F3 (5.63 ± 1.02), and F4 (5.47 ± 1.00), and the nor like nor dislike formulas were F1 (4.88 ± 1.19), F5 (4.00 ± 1.60), and F6 (4.67 ± 1.75). The formula with the highest color scores was F2 (5.98 ± 0.65). While, the F6 formula combination of stevia and mint (F6) showed the lowest color score (4.62 ± 1.52) (Table 5).

S. quadrifida bark formula with stevia leaf (F2) had the highest overall score. The score indicates that the combination of *S. quadrifida* bark and stevia is preferred. Meanwhile, formulas with the addition of mint leaf had the lowest score. *S. quadrifida* and ginger formula,

and *S. quadrifida*, ginger, and stevia formula have the same overall score (Figure 3).

B. Discussion

1. Physicochemical and microbiological properties

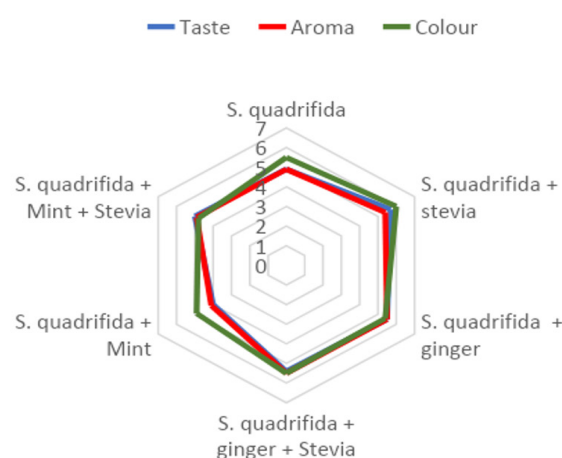
The moisture content of raw materials can affect physicochemical properties, antioxidant, and sensory test results (Asikin et al., 2014). Mint is the only herbal material with a water content higher than 10%. Mint leaves naturally have high water content, reducing moisture content and bacteria total plate count (Haile, Admassu, & Fisseha, 2015). Heat treatment of 70°C for at least 2 min is sufficient to kill bacteria (James et al., 2021). Heavy metal contamination is often found in herbal medicines (Meseret, Ketema, & Kassahun, 2020; Luo et al., 2021). Cadmium, mercury, lead, and arsenic is concerned because of their potential toxicity even at low concentrations. Accumulating heavy metals can lead to human metabolism dysfunction (Fu & Xi, 2020). This study was in accordance with

Table 5: Sensory score of *S. quadrifida* herbal tea formulas

Formula	Taste	Aroma	Color
F1	4.84±1.19a	4.88±1.19b	5.44±0.86ab
F2	5.74±1.30b	5.28±1.30a	5.98±0.65a
F3	5.47±1.20b	5.63±1.02a	5.37±1.07ab
F4	5.49±1.34b	5.47±1.00a	5.51±0.95ab
F5	3.74±1.41a	4.00±1.60b	5.16±1.46b
F6	4.97±1.75b	4.67±1.75b	4.62±1.52b

Remarks:

F1 = *S. quadrifida* bark powder 100%; F2 = 90% *S. quadrifida* bark and 10% stevia leaf; F3 = *S. quadrifida* bark and ginger; F4 = *S. quadrifida* bark, ginger, and stevia; F5 = *S. quadrifida* bark and mint; F6 = *S. quadrifida* bark, mint, and stevia. Means with different letters in the same column indicate significant differences ($p < 0.05$). Data are means of triplicates ($n = 3$) \pm SD

Figure 3. The radar chart shows the overall acceptance of *S. quadrifida* herbal tea formulas

another study showing that heavy metals in the herbal materials were below the required limits (Keshvari et al., 2021). A study showed lead, mercury, and cadmium contamination in herbal medicine, but within considerably safe limits (Wang, Wang, Wang, Li, & Li, 2019). *S. quadrifida* bark in this study was obtained from locations far from residential areas and roads. Therefore, the trees were not exposed to heavy metals pollution from human activities.

2. Phytochemical and antioxidant properties

The flavonoid content in all *S. quadrifida* herbal tea formulas is lower than in black teas ranging from 2.3 to 4.3% (Bansode, 2015). Other studies showed similar total flavonoid content. The highest flavonoid content of brewed *Rosmarinus officinalis* was 0.03% (Kılıç, Can, Yılmaz, Yıldız, & Turna, 2017). Total

flavonoid content is varied, even within the same species. The flavonoid content of *S. quadrifida* bark differs between the colours of the bark, diameter classes, and the elevation of their habitat (Siswadi, Faridah, & Hertiani, 2021). The bark's colour, the harvested tree's diameter class, and its habitat's altitude determine the flavonoid content of *S. quadrifida*. In brewed herbal, the water temperature affected the flavonoid content (Kılıç, et al., 2017).

Phenolic compounds contribute to the biological activity of medicinal plants (Nguyen & Chuyen, 2020; Ambriz-Pérez, Leyva-López, Gutierrez-Grijalva, & Heredia, 2016). The TPC of the brewed combination of *S. quadrifida*, and ginger was lower ($14.09 \pm 0.72\%$) than the TPC of all the tested herbal teas in this experiment. Another study on the total phenolic content of several types of herbal tea in China also

resulted in lower TPC that ranged from 0.0032 to 0.1395% (Fu et al., 2011). The variation of TPC may be caused by the parts used (Saragih & Siswadi, 2019), tree age, harvesting season (Aregay et al., 2021), processing technique (Rekasih, Muhandri, Safithri, & Wijaya, 2021), and fermentation (Rahmi, Khairiah, Rufida, Hidayati & Rahmi, 2020).

Antioxidant activity of herbal tea is mainly determined by the plant's bioactive component and the post-harvesting processing (Piljac-Žegarac, Šamec, & Piljac, 2013). Studies have processed *S. quadrifida* bark and measured the antioxidant activity of the product. A study made syrup from *S. quadrifida* extract. The result showed that *S. quadrifida* bark syrup has weak antioxidant activity (IC₅₀ = 1,042 ppm) (Tenda, 2018). It is similar to the antioxidant activity of the instant drink of *S. quadrifida* bark mixed with ginger which has a very weak IC₅₀ value ranging from 2,044.2 - 2,077.1 ppm (Tenda, Hilaria, & Wijaya, 2019). However, fermentation of the bark powder of *S. quadrifida* for 14 days can increase its antioxidant activity by 11% (Lalong et al., 2022). The drying technique is among the post-harvest treatments that affect the antioxidant content of herbal substances. Drying was said to reduce antioxidants. TPC and AEAC in *Vitex negundo* were better than those in oven-dried leaves (Rabeta & Vithyia, 2013). The study also showed that the higher the temperature in the brewing process, the lower the antioxidant content.

Adding ginger, stevia, and mint was expected to increase the antioxidant activity of brewed pure *S. quadrifida* bark. Stevia is a natural sweetener safe for consumption that contains flavonoids with antidiabetic potential (Zaidan, Zen, Amran, Shamsi, & Abd Gani, 2019). Dietary flavonoids exhibit antioxidant capacity (Khan et al., 2021). Ginger is known to have antiviral, antidiabetic, and antioxidant properties (Hussain, Zamir, Javed, Munawar, & Batool, 2020). It is also recommended as a food flavouring agent (Kiyama, 2020; Shafique, 2019). Mint contains flavonoids, antioxidants, and antibacterial compounds (Jurić et al.,

2021). Mint leaves showed antiviral, anti-inflammatory, and antioxidant activity and contained high levels of flavonoid and phenolic acid (Figuerola-Pérez, Rocha-Guzmán, Perez-Ramirez, Mercado-Silva, & Reynoso-Camacho, 2014; Li et al., 2017). However, in this study, formulas with the addition of stevia, mint, and ginger showed weaker antioxidant activity than pure *S. quadrifida*. As another study suggests, combining several herbal ingredients lowered the antioxidant activity even if each ingredient has a high antioxidant value (Guimarães et al., 2011). The effect of interaction can be the results of different doses (Sonam & Guleria, 2017; Pan et al., 2018) and chemical composition (Nedamani, Mahoonak, Ghorbani, & Kashaninejad, 2015).

TFC, TPC, and antioxidant activity of *S. quadrifida* herbal tea formulas are uncorrelated. This is similar to a previous study that found a very weak correlation between TPC, TFC, and antioxidant ethanol extract of *S. quadrifida* parts (Saragih & Siswadi, 2019). The result in this study is also in accordance with a study of TPC, TFC, and antioxidant activity of cocoa beans which did not find a correlation between TPC and antioxidant activity (Othman, Ismail, Ghani & Adenan, 2007). Another study also did not detect a significant correlation between TPC and DPPH (Othman, Mukhtar, Ismail & Chang, 2014). The antioxidant activity of *S. quadrifida* bark aqueous extract could be due to other compounds besides phenolics and flavonoids, which are not soluble in water. Another study found a moderate negative correlation between TFC and TPC and a weak positive correlation between TFC and antioxidant activity in *M. malabathricum* methanol extract (Danladi et al., 2015). It is possible that flavonoids and phenolics are not the compounds that contribute to the antioxidant activity of *S. quadrifida* herbal tea formulas. Moreover, the reagents used in the analysis may become a reason for the inaccuracy of TFC and TFC estimation (Othman et al., 2014). The Ministry of Health in Supplement II of the Indonesian Herbal Pharmacopoeia Edition I (2011) also

recommends stock solution be used as the blank solution, so the reading only resulted in the target compounds.

3. Sensory characteristics

Pure *S. quadrifida* formula (F1) has a light astringent taste like commercial black tea. Therefore, the taste of F1 is acceptable for people who used to drink black tea. The addition of stevia leaf adds a mild sweet taste. The formulas with stevia addition have a high taste score, except for the formula with mint. The addition of 30% mint results in a strong mint aroma and taste. The addition of mint leaves decreased the panellists' preference, while stevia leaves increased the panellists' preference (Testiningsih, 2015). Mint taste is identical to the taste of a sore throat lozenge, and panellists were not used to mint flavour drinks. The panellists' also liked the addition of ginger since it gave a familiar fresh taste.

S. quadrifida bark contains tannin (Dillak, Kristiani & Kasmiyati, 2019). Tannins are a phenolic compound usually identified with a bitter taste (Hariyadi, Tedja, Zubaidah, Yuwono, & Fibrianto, 2020). The tannin content can produce brownish-red colour (Widyawati, Budianta, Utomo, & Harianto, 2016). The addition of ginger results in a significant difference in the acceptance of taste but not in the aroma and colour (Wahyunus & Tamrin, 2017). The addition of too much (20%) stevia or very little (5%) does not increase the acceptance of herbal teas (Siagian, Bintoro, & Nurwantoro, 2020).

Pure *S. quadrifida* bark tea and *S. quadrifida* bark with stevia tea were patented by the Directorate General of Intellectual Property in 2021 (Siswadi & Saragih, 2021; Siswadi & Saragih, 2021a).

IV. CONCLUSION

Heavy metals and microbe contamination in some ingredients were below the permissible levels. *S. quadrifida* and mint formulas have the highest TPC and TFC but obtained the lowest organoleptic scores. The pure *S. quadrifida* bark

(F1) and the formula with stevia (F2) have the highest antioxidant activity and are more preferred. However, further research is needed to determine the shelf life of *S. quadrifida* bark herbal tea and sustainable bark harvesting of *S. quadrifida* are also needed.

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