Effect of boiling temperature and time on total flavonoids, total phenols, and radical scavenging activity of decoction water fresh gotu kola (Centella asiatica (L.) Urb.)

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***Abstract*-. Gotu kola (Centella asiatica (L.) Urb.) possesses significant pharmacological effects as a versatile herb, particularly in wound healing, neuroprotection, and cardiovascular health. Some people derive the benefits of medicinal plants by boiling and consuming them. The boiling process significantly impacts the biochemical composition, including total phenols, total flavonoids, and antioxidant activity. This study aims to investigate the impact of temperature and time on total flavonoids, total phenols, and radical scavenging activity during the boiling process of fresh C. asiatica. Three different temperature treatments (75°C, 85°C, 95°C) and four different boiling time treatments (5, 10, 15, and 20 minutes) were used to assess the total flavonoid content, total phenolics, and radical scavenging activity using the DPPH method. Quantitative data analysis employed DMRT and PCA techniques to ascertain the impact and correlation among factors. The results showed that temperature and boiling time significantly influenced total flavonoids, total phenols, and radical scavenging activity. Maximum levels of total flavonoids, total phenols, and radical scavenging activity were achieved in C. asiatica boiling water after boiling at 95 °C for 20 minutes. Temperature, total flavonoids, and total phenols have a strong correlation, where total flavonoids and total phenols increase as the boiling temperature increases.**

Keywords; Boiling Process, Centella asiatica (l.) Urb., Total Flavonoid Content, Total Phenol

I. INTRODUCTION

Centella asiatica (L.) Urb., also known as Gotu Kola and Pegagan in Indonesia, is a perennial of the Apiaceae family with a remarkable therapeutic application as an herb [1]. The activity of this agent can be explained in a way that the bioactive constituents contribute substantially to the therapeutic effects, including triterpenes like asiaticoside, madecassoside, and their acid forms, which possess antioxidant, anti-inflammatory, and neuroprotective properties [2–4]. Significantly, these compounds significantly augment the plant's capacity to scavenge free radicals, leading to reduced oxidative damage and raised levels of cellular protective mechanisms [5–7].

The use of medicinal has been orthodox for ages in most of the societies of the earth's surface, for instance, in Wawo District Bima Regency, where it has been used together with the land boiled plant, which is then taken orally by the people [8,9]. Furthermore, the Dayak Kanayatn people residing in Tonang Village, Sengah Temila District, Landak Regency, West Kalimantan, have relied on traditional medicine, primarily mostly by ingesting manufactured herbal mixtures[10]. These long-standing customs have been transmitted over generations, offering crucial insight into how the local population has been harnessing the medicinal resources of nature to fulfil their healthcare requirements [11,12].

Optimization of total phenols, total flavonoids, and radical scavenging activity based on boiling temperature and time has been the subject of numerous investigations leading to complicated conclusions considering numerous factors, such as the type of plant material and specific boiling parameters [13,14]. Apart from this, the boiling general effects on total phenolics and more so on flavonoid contents in different plant extracts, who reported that after boiling bamboo shoots for thirty minutes, the total phenolics and flavonoids concentrations decreased together with the radical scavenging activity, thereby suggesting that extreme temperature for a long duration can cause breakdown of these bioactive substances [15]. In about the same "state," boiling celery roots resulted in a significant reduction in total phenolic content and

antioxidant activity, which is related to the loss of water-soluble phenolics in boiling water [16]. This phenomenon is explained that oxygen may still reach the reactant even during heat treatment by heat-induced boiling and leaching and, therefore, phenolic acids and flavonoids may be reduced [17].

Some studies indicate that boiling can also enhance the extraction of certain phenolic compounds, where a 3% rise in the overall phenolic acid content was observed in white beetroot after boiling for 60 minutes, suggesting that the boiling process can sometimes facilitate the release of phytochemicals from plant matrices [18]. Previous studies have demonstrated that longer boiling times typically result in a decline in antioxidant capacity and reported a significant reduction in antioxidant activity of sweet potato leaves after boiling, with a decrease of 63.82% observed [19]. Similarly, a 50-60% reduction in quercetin levels in onions after 60 minutes of boiling is documented, which directly correlates with diminished antioxidant activity [20]. Alternatively, several studies propose specific cooking methods, leading new antioxidant compounds, where boiling chestnuts resulted in a total phenolic content comparable to that of baked chestnuts, suggesting the possibility of improved antioxidant properties under certain circumstances [21].

Principal Component Analysis (PCA) is a robust multivariate statistical method that clarifies the correlations among total phenols, total flavonoids, and radical scavenging capacity in various plant extracts [22]. Research highlights the utility of PCA in differentiating phenolic compounds and antioxidant activity across various tea types, demonstrating that PCA can effectively capture the variance in phenolic content and its relationship with antioxidant capacity [23]. Similarly, there is a strong correlation between total phenolic and flavonoid contents and radical scavenging activities, indicating that these compounds significantly contribute to the antioxidant potential of plant extracts [24]. This relationship is reinforced by research indicating that more significant amounts of phenolic compounds are linked to enhanced antioxidant activity, as demonstrated by the positive regression coefficients documented in several analyses [25,26].

An analysis of intricate datasets related to medicinal herbs and spices supports the idea that Principal Component Analysis (PCA) may accurately summarize the connections between several variables, including total phenols, flavonoids, and antioxidant activity [27]. There was a high linear correlation between anthocyanins and antioxidant capacity, suggesting that phenolic compounds are crucial in determining antioxidant activity [28]. This comprehensive understanding can guide future research and applications in natural antioxidants to determine the effect and relationship between total flavonoids, total phenols, and radical scavenging activity parameters in studying the effect of temperature and boiling time of fresh *C. asiatica*.

II. MATERIAL AND METHODS

Sample praparation

The materials used were *C. asiatica* planted in the Medicinal Plant Garden of UPF Dr Sardjito in Karangpandan District, Karanganyar Regency, Central Java Province. The tools used were analytical scales (Adam PWZ 14), a Sonicator (Elma S40H), and a Spectrophotometer (Thermo Scientific Multiskansky).

Heat 1000 ml of water on a hot plate. Add 100 grams of fresh *C. asiatica* sample to the pot until it can be consumed while stirring. Turn off the hot plate. The sample is then filtered, and 25 mL of water is taken. The experimental design used was completely randomized, with three levels of temperature treatments, namely T1 (75 °C), T2 (85 °C), and T3 (95 °C), and four levels of boiling time treatment (D1 (5 minutes), D2 (10 minutes), D3 (15 minutes), and D4 (20 minutes). The samples were then tested for total flavonoids, total phenols, and radical scavenging activity using a spectrophotometer.

Total flavonoid content (TFC)

Analysis of total flavonoid content was conducted using the Widodo technique. In methanol, the extract was dissolved at a concentration of 1,250 µg/mL. The experimental mixture comprised 500 µL of the sample, 150 µL of a 0.1 M AlCl3 solution (in a control group without AlCl3 and substituted with 150 µL of methanol), 350 µL methanol, 250 µL of acetate buffer (pH 3.8), and methanol to a total volume of 1250 µL. A 30-minute incubation at 37 °C was conducted on the reaction mixture. A 150 microlitre test solution was placed into the microplate and the absorbance was quantified using a spectrophotometer at a wavelength of 398 nanometres. Quercetin is employed as a standard by constructing a standard curve. The compound Quercetin was dissolved in a constant volume of methanol at concentrations of 0, 50, 100, 300, 400, and 500 µg/mL. The quantification of flavonoids is expressed in µg of Quercetin Equivalents (RE) per gramme of extract [29].

Total phenol content (TPC)

Quantification of total phenol content was conducted using the Widodo method with certain adjustments [30]. In its final state, the reaction mixture comprises 40 mg of extract, 4% methanol, 10% FCR, and 5% NaCO3. A 40 µL sample of extract (1 mg/mL: 1 mg dissolved in 1 mL methanol using a sonicator for 15 minutes) was combined with 360 µL of deionised water and 100 mL of fresh distilled water. After homogenising the solution, it was let to stand for 2 minutes. Neutralisation of the reaction was achieved by adding 500 mL of sodium carbonate (10% w/v)

thoroughly mixed and then incubated at 37 °C for 35 minutes. The test solution, measuring 150 µL, was prepared and placed onto the microplate. The absorbance of gallic acid was measured using a spectrophotometer at a wavelength of 732 nm, which corresponds to the highest achievable absorbance. Gallic acid was employed as a standard with concentration series ranging from 0 to 32 µg/mL. Expression of total phenol content is in µg Gallic Acid Equivalent (GAE) per gramme of extract.

Radical scavenging activity (RSA)

The assay employs the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method with several modifications [29]. The DPPH were prepared in methanol p.a. at 350 µM, respectively. Samples were prepared in a 1500 µL tube by combining 40 µL of the sample, 760 µL of methanol, and 200 µL of DPPH. The control comprised 40 µL of the sample and 960 µL of methanol. The tube underwent vortexing for 10 seconds and was subsequently incubated at 37 °C for 35 minutes in a dark incubator, with inversion occurring every 10 minutes. Absorbance was measured using a spectrophotometer at a wavelength of 515 nm. The DPPH radical scavenging assay was conducted on three separate occasions.

The calculation of radical inhibitory activity was performed using the following formula (1):

$$\% \text{ Inhibition} = \frac{[(\text{Abs control} - \text{Abs blank}) - (\text{Abs sampel} - \text{Abs control sample})]}{(\text{Abs control} - \text{Abs blank})} \times 100\% \quad (1)$$

Data analyst

The data obtained in the previous stage were analyzed statistically with DMRT at the 5% level to determine the effect of treatment on parameters. The correlation between parameters was tested using the Principal Component Analysis (PCA) method. Data processing in this study used the help of Past4.13.exe software.

III. RESULT AND DISCUSSION

Total flavonoid content at the level of temperature and time in fresh *C. asiatica* boiled water (Figure 1) shows that the higher the temperature and the longer the boiling time, the higher the total flavonoid content. Boiling at 75 °C for 5 minutes has the lowest flavonoid content compared to other treatments which is 0.17 ± 0.05 µg QEq/mL. At 95 °C boiling for 20 minutes, the highest flavonoid content was obtained at 1.00 ± 0.09 µg QEq/mL. Total flavonoid content increased by over 40% at 95 °C, boiling for 5 to 20 minutes. Similar research on *Muntingia calabura* leaves where total flavonoid levels increased up to a boiling time of 20 minutes but decreased at boiling for 30 minutes. Because of density, temperature can impact a compound's solubility. This is most likely because a prolonged boiling time damages the flavonoid components in the heat-sensitive water extract of *M. calabura* leaves, resulting in a drop in overall flavonoid concentration [31].

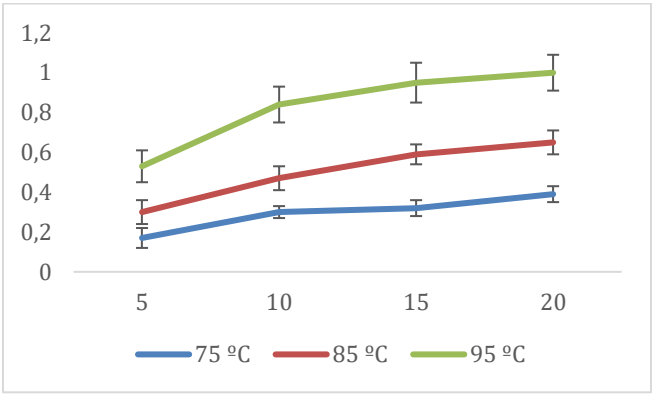

Figure 1. Total flavonoid content of *Centella asiatica (L.) Urb.* decoction water.

The highest total phenol content in *C. asiatica* leaf cooking water was obtained at 95 °C. Figure 2 shows that the higher the boiling temperature, the higher the total phenol content. The total phenol content also increases with the longer the boiling time. Figure 2 illustrates that the total phenol content at a boiling temperature of 75 °C did not increase significantly. Boiling *C. asiatica* for 15 minutes to 20 minutes, there is no significant difference. There was an increase in total phenol content in the boiled water of *C. asiatica* leaves, reaching three times the boiling water with a temperature of 95 °C. Extending the heating time of black tea has yielded similar results in terms of an increase in total phenolic content. This is due to the possibility that the boiling procedure destroyed the effects of the cell wall structure on the solvent's ability to extract more phenolic chemicals [14,32].

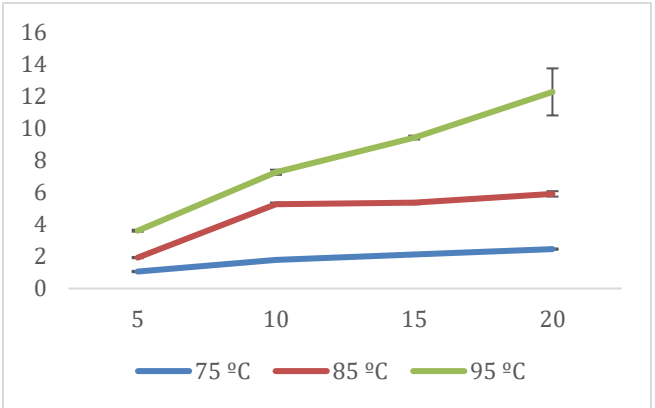

Figure 2. Total phenol content of *Centella asiatica (L.) Urb.* decoction water

Figure 3 shows the radical scavenging activity of *C. asiatica* decoction water at a concentration of 2000 µg/mL using the DPPH method. The highest activity of *C. asiatica* leaf cooking water was also obtained at a temperature of 95 °C with a duration of 20 minutes (µg/ml). Figure 3 shows that the higher the boiling temperature, the higher the radical scavenging activity. The radical scavenging activity also increases with the length of boiling time. Figure 3 illustrates that the total phenol content at boiling temperatures of 85 and 95 °C has reached more than 50% inhibition. At 85 °C boiling time of 10 to 20 minutes, there was a sharp increase of 412% from the original inhibition. The radical scavenging activity remained at 15 to 20 minutes of boiling. The results differ from research on turmeric, where the longer the boiling time, the more antioxidant activity decreases due to temperature damage [33].

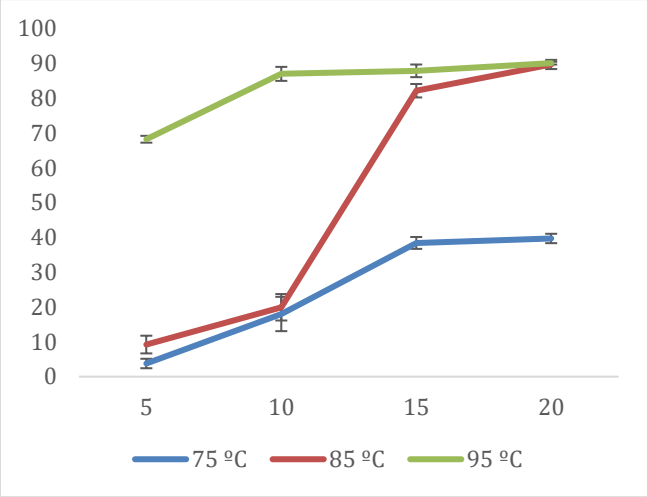

Figure 3. Radical scavenging activity of *Centella asiatica (L.) Urb.* decoction water.

Table 1 shows the results of DMRT analysis on the parameters of total flavonoid content, total phenolics and radical scavenging activity of fresh *C. asiatica* boiling process at specific temperatures and time levels. The analysis showed that the temperature and boiling time significantly influenced the total flavonoids, total phenols and radical scavenging activity of fresh *C. asiatica*. The higher the temperature and the longer the boiling time, the higher the total flavonoids, total phenols and radical scavenging activity levels. However, the levels of total phenols in boiling for 15 minutes and 20 minutes did not differ significantly. Increase in total phenol content after heating because cooking inactivates the enzyme polyphenol oxidase, inhibiting the degradation of polyphenols [34].

Table 1. Total phenol content, total flavonoids, and radical scavenging activity of *Centella asiatica (L.) Urb.* decoction water

Sample Code	TPC (mg GAE/g)		TFC (µg QEq/mL)		RSA (µg/ml)	
T1D1	1.06 ± 0.04	a	0.17 ± 0.05	a	3.78 ± 1.36	a
T1D2	1.78 ± 0.02	ab	0.3 ± 0.03	b	18.00 ± 4.93	c
T1D3	2.12 ± 0.02	b	0.32 ± 0.04	b	38.41 ± 1.71	d
T1D4	2.46 ± 0.04	b	0.39 ± 0.04	bc	39.69 ± 1.35	d
T2D1	1.93 ± 0.04	b	0.30 ± 0.06	b	9.22 ± 2.54	b
T2D2	5.27 ± 0.10	d	0.47 ± 0.06	cd	19.93 ± 3.80	c
T2D3	5.36 ± 0.10	d	0.59 ± 0.05	ef	82.13 ± 1.93	f
T2D4	5.91 ± 0.17	d	0.65 ± 0.06	f	89.67 ± 1.32	g
T3D1	3.61 ± 0.06	c	0.53 ± 0.08	de	68.19 ± 0.98	e
T3D2	7.26 ± 0.16	e	0.84 ± 0.09	g	86.97 ± 2.01	g
T3D3	9.43 ± 0.12	f	0.95 ± 0.10	gh	87.84 ± 1.82	g
T3D4	12.28 ± 1.47	g	1.00 ± 0.09	h	90.05 ± 0.40	g

Remarks : The value is average value ± deviation standar; n=3. The same abjad on the same column shows no significant difference at 5%.

Table 2. Principal Component Analysis extraction result of *Centella asiatica* (L.) Urb. decoction water

PC	Eigenvalue	% variance
1	0.366335	85.818
2	0.0364748	8.5446
3	0.0234268	5.488
4	0.00060634	0.14204
5	3.11621E-05	0.0073001

Table 2 shows that the 5 variables extracted with 2 components already represent 94.3626% of the overall parameters. For example, there are 85.818% in component 1 and 8.5446% in component 2. In addition, there is a graph that explains the results of determining the number of factors, the graph can be seen in Figure 4 Scree Plot. In this study, 2 PC were taken because 2 PC had reached 90% of the overall parameters.

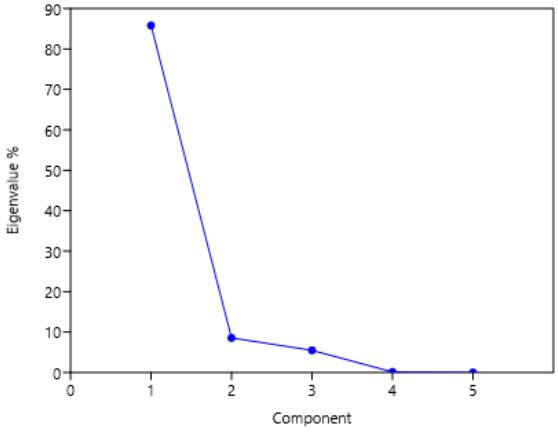

Figure 4. Scree plot Principal Component Analysis of *Centella asiatica* (L.) Urb. decoction water

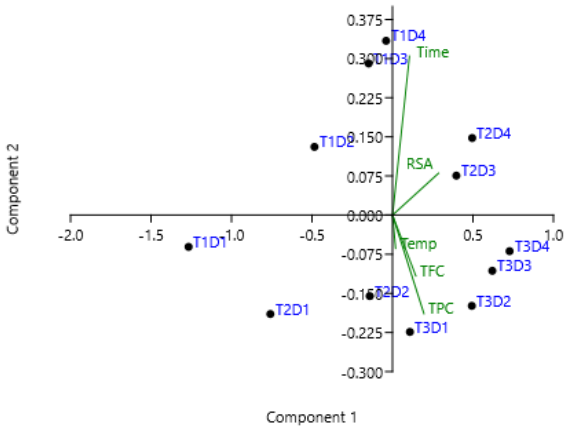

Figure 5. Biplot Principal Component Analysis of *Centella asiatica* (L.) Urb. decoction water

The PCA Biplot graph shows the relationship of the main components of all variables that can make a difference (Figure 5). Samples T1D1 and T1D2 had the most different properties from the other samples based on the variables of temperature and boiling time. TFC and TPC parameters have a positive correlation characterized by the angle formed between with the angle formed between the two parameters is very small less than 45°. TFC and TPC parameters also have a positive correlation relationship to the boiling temperature variable. Research in 2016 had similar results where TFC and TPC had a positive correlation in boiling Egyptian sweet and chilli pepper [35]. Sample T3D1 has similar properties to the TPC parameter so that it can be concluded that sample T3D1 is dominant to the TPC parameter. Meanwhile, samples T2D4 and T2D3 have a tendency with RSA parameters. Samples T1D4 and T1D3 have the same tendency with the variable boiling time.

IV. CONCLUSION

The amount of total flavonoids, total phenols, and radical scavenging activity were all highly impacted by the boiling temperature and duration. Boiling at 95°C for 20 minutes produced the maximum levels of total flavonoids, total phenols, and radical scavenging activity in *C. asiatica* heated water. There was a substantial relationship between temperature, total flavonoids, and total phenols, with total flavonoids and total phenols rising with rising boiling temperatures. The metrics of total flavonoids content and total phenols content correlated positively. The time parameter, on the other hand, correlated positively with radical scavenging activity.

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