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Incorporation of gambir catechin crude extract in robusta instant coffee made from different coffee processing methods

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ABSTRACT

This research aimed to enhance the antioxidant properties of instant gambir coffee by adding gambir catechin crude extract during coffee processing using natural anaerobic, full wash, and honey methods. The experiment used a completely randomized non-factorial design (RALNF), with each treatment replicated five times. The treatments consisted of nine formulations (F), namely F1= natural anaerobic 87.5% (w/w): gambir catechin crude 5% (w/w), F2 = natural anaerobic 82.5% (w/w): gambir catechin crude 10% (w/w), F3 = natural anaerobic 77.5% (w/w), gambir catechin crude 15% (w/w), F4 = honey 87.5% (w/w): gambir catechin crude 5% (w/w), F5 = honey 82.5% (w/w): gambir catechin crude 10% (w/w); F6 = honey 77.5% (w/w): gambir catechin crude 15% (w/w) F7 = full wash 87.5% (w/w): gambir catechin crude 5% (w/w); F8 = honey 82.5% (w/w): gambir catechin crude 10% (w/w), and F9 = honey 77.5% (w/w): gambir catechin crude 15% (w/w). The results showed that the formulation treatments significantly affected the instant gambir coffee's water content, solubility percentage, acidity level (pH), total phenols, and IC50. The characteristics of the resulting instant gambir coffee included water content, solubility percentage, pH, total phenols, and IC50 with values of 6.56-7.02%, 94.35-96.55%, 5.25-5.75, 24.91-40.35 mgGAE/g, and 31.46-121.75 mg/mL, respectively.

Keywords: gambir, honey, full wash, coffee, natural anaerobic

INTRODUCTION

Coffee has become immensely popular worldwide, including in Indonesia, leading to a rapid growth of coffee shops and the increasing circulation of instant coffee packets. Generally, coffee served in cafes or marketed in packet form prioritizes flavor, with only a small proportion offering antioxidant properties, which naturally compounds such as chlorogenic acid. It is coffee's main bioactive compound with antioxidant properties **[1]**. Robusta coffee contains higher chlorogenic acid and caffeine levels than arabica coffee **[2]**. There is a decrease in chlorogenic acid content by 37-59% due to the high-temperature roasting process **[3]**.

The addition of gambir catechin crude to enhance the antioxidant properties of coffee was affected by caffeine levels and coffee acidity **[4]**. Adding gambir extract to the naturally processed robusta coffee powder resulted in a 45% increase in antioxidant properties **[5]**. Caffeine is associated with catechin compounds, affecting the free catechin in coffee. Therefore, the higher the caffeine content, the more catechins are bound, and the fewer free catechins in the coffee. Previous investigations showed that the amount of free catechin in a food system determines its antioxidant properties, indicating the more free catechins, the higher the antioxidant properties, and vice versa. In addition to caffeine levels, coffee pH affects antioxidant properties because catechin compounds are stable under acidic conditions. This indicated that adding crude catechin in coffee with a high pH level will produce more stable catechin compounds and enhance the coffee's antioxidant properties.

Generally, there are three commonly used coffee processing methods, natural anaerobic, full wash, and honey, which significantly affect caffeine content and pH. The full wash and honey methods are known as wet processing,

while natural anaerobic is a dry process. Natural anaerobic involves fermentation without going through the pulping process. The pulp's juice is used by acid bacteria during fermentation, resulting in a more acidic coffee with a sharper aroma (high caffeine). Coffee processed with the full wash method contains low caffeine content with a high pH level, while the honey method contains low caffeine and pH levels. Incorporating gambir catechin crude extract into instant robusta coffee processed with the natural anaerobic, full wash, and honey methods can produce gambir instant coffee with good physical, chemical, and antioxidant properties.

Scientific hypothesis

Incorporating gambir catechin crude extract into instant robusta coffee processed with the natural anaerobic, full wash, and honey methods can produce gambir instant coffee to increase the functional properties, especially its antioxidant activity.

MATERIAL AND METHODOLOGY

Samples

Instant coffee with a percentage of the processing method according to the treatment incorporated with gambir catechin extract.

Chemicals

The materials used consist of distilled water, tannic acid, 96% ethanol, 2,2-diphenyl-1-picrylhydrazil (DPPH), folin-ciocalteu, methanol, and Na_2CO_3 obtained from the Laboratory of Chemical Agricultural Products, Faculty of Agriculture, Sriwijaya University, Indonesia.

Biological Material

Gambier powder from Babat Toman Village, Musi Banyuasin Regency, South Sumatra, Indonesia. Robusta coffee powder from JagadRaye Coffee, a micro and small enterprise in Pagar Alam, South Sumatra, Indonesia.

Pasak bumi powder from the Laboratory of Chemical Agricultural Products, Faculty of Agriculture, Sriwijaya University, Indonesia.

Instruments

The tools used include an autoclave, blender (Philips, Holland), hot plate, incubator (Memmert, Germany), filter paper, laminar airflow (LAF), brand analytical balance (Kenko, Japan), drying oven (Memmert, Germany), pH meter (Eutech, Malaysia), micropipette (Dragon Lab, China), rotary vacuum evaporator, 80 mesh filter, spectrophotometer (A and E Lab, USA), and vortex (Digisystem, Taiwan).

Laboratory Methods

The parameters evaluated include water content **[6]**: measurement of water content using the gravimetric method. Soluble speed **[6]**: Dissolve 100 g of instant coffee in 200 mL of water. Then, the time instant coffee dissolves in water is calculated as the speed at which it dissolves in water using a stopwatch. Acidity Degree (pH) **[7]**: Total phenol **[8]**: Determination of total phenol content was carried out using a spectrophotometric method using Folin Ciocalteu reagent. Antioxidant activity **[9]**: Antioxidant testing using the DPPH method (2,2 diphenyl-1- picrylhydrazyl) was used.

Description of the Experiment

Sample preparation:

1. Preparation of gambir catechin crude extract. The dried gambir product was ground using a blender and sieved with a 60-mesh sieve. Subsequently, 100 g of gambir powder was macerated with ethanol solvent (1:3) for 24 hours. The crude extract of gambir catechin was filtered using filter paper and evaporated with a rotary evaporator at 60 °C until the ethanol evaporated (no ethanol aroma present). The crude extract of gambir catechin was dried using an oven dryer at 85 °C for ±15 hours. The dried sample was ground using a blender and sieved with a 60-mesh sieve, making it ready for application.
2. Preparation of instant robusta coffee powder Robusta coffee powder (natural, honey, full wash) was added to 100 °C water for 2 minutes with a coffee powder-to-water ratio of 1:4 and allowed to rest for 10 minutes. The coffee suspension was filtered using a filter cloth to obtain a filtrate. Maltodextrin (20% w/w) and Tween 80 (0.3% v/v) were added to the coffee filtrate and stirred using a mixer for 10 minutes at high speed to form foam. The foam was poured and levelled in an aluminium tray lined with polypropylene plastic with a thickness of 1 cm, dried using a food dehydrator at 60 °C for 8 hours, ground using a blender, and sieved with a 60-mesh sieve to obtain an instant coffee powder.
3. Preparation of instant coffee incorporated with gambir catechin crude extract. The ingredients used were prepared: instant coffee, crude extract of gambir catechin, and instant Javanese ginseng. According to the treatment, instant coffee with a percentage of the processing method was added with a crude extract of gambir catechin. Subsequently, instant Javanese ginseng at 7% (w/w) was added to each treatment combination. The

instant coffee incorporated with gambir catechin crude extract, weighing 30 g, was packed in an aluminium foil package and prepared for analysis.

Number of samples analyzed: We analyzed 9 samples.

Number of repeated analyses: All measurements of instrument readings were performed five times.

Design of the experiment: The samples This research used a wholly randomized non-factorial design (CRND) with coffee processing methods and gambir catechin crude extract treatments, which were repeated five times. The treatments consisted of nine formulations (F), namely F1 = natural anaerobic 87.5% (w/w): gambir catechin crude extract 5% (w/w), F2 = natural anaerobic 82.5% (w/w): gambir catechin crude extract 10% (w/w), F3 = natural anaerobic 77.5% (w/w): gambir catechin crude extract 15% (w/w), F4 = honey 87.5% (w/w): gambir catechin crude extract 5% (w/w), F5 = honey 82.5% (w/w): gambir catechin crude extract 10% (w/w), F6 = honey 77.5% (w/w): gambir catechin crude extract 15% (w/w), F7 = full wash 87.5% (w/w): gambir catechin crude extract 5% (w/w), F8 = honey 82.5% (w/w): gambir catechin crude extract 10% (w/w), and F9 = honey 77.5% (w/w): gambir catechin crude extract 15% (w/w).

Statistical Analysis

This study used a factorial, completely randomized design. The treatment with a significant effect was further tested using the honest real difference test (HSD) at = 5%. The data were analysed using the SAS software version of Windows 9 to analyse of variance.

RESULTS AND DISCUSSION

The water content of instant gambir coffee ranged from 6.56-7.02%. Among all treatments, natural anaerobic 77.5% (w/w): gambir catechin crude extract 15% (w/w) (F3) resulted in the highest water content, while honey 87.5% (w/w): gambir catechin crude extract 5% (w/w) (F4) had the lowest were presented in Figure 1.

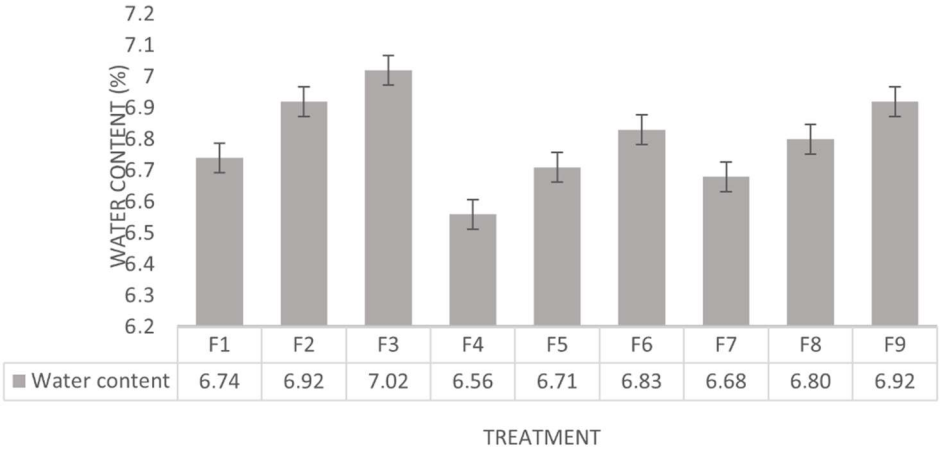

Figure 1 Water content of instant gambir coffee.

Analysis of variance showed that the coffee formulation treatment significantly influenced the water content. The results of the posthoc test BNJ 5% on water content, solubility percentage, and pH level of instant gambir coffee were presented in Table 1. Table 1 showed that treatment F3 had the highest water content compared to others and was not significantly different from treatments F2, F6, and F7. Theoretically, this occurred because naturally anaerobic-processed coffee contains more glucose, as pulping is not performed during the processing. The presence of glucose affected the amount of bound water in coffee. The gambir catechin crude extract also influenced the water content due to the several hydroxyl (OH) groups in catechin compounds. This indicated that the higher the gambir catechin crude extract content, the more OH groups that can bind water, thereby affecting the increase in water content. Haile and Kang **[10]** explained that coffee beans processed using dry processing methods such as natural anaerobic and wine have a heavier, finer, sweeter, and more complex character. The gambir catechin crude extract contained acidic catechin compounds despite having many OH groups **[11]**.

Table 1 The results of the test BNJ 5% on the water content, solubility percentage, and pH of instant gambir coffee produced.

Treatment	Water Content (%)	Solubility (%)	pH
F1 = 87.5% *natural*: Gambir 5%	6.74 ±0.02abc	95.85 ±0.22ab	5.75 ±0.02f
F2 = 82.5% *natural*: Gambir 10%	6.92 ±0.02cd	95.13 ±0.42ab	5.63 ±0.03e
F3 = 77.5% *natural*: Gambir 15%	7.02 ±0.08d	94.35 ±0.88a	5.56 ±0.02d
F4 = 87.5% *honey* : Gambir 5%	6.56 ±0.12a	97.55 ±0.59c	5.64 ±0.02e
F5 = 82.5% *honey*: Gambir 10%	6.71 ±0.11ab	95.68 ±0.62ab	5.53 ±0.01d
F6 = 77.5% *honey*: Gambir 15%	6.83 ±0.06bcd	94.95 ±0.08ab	5.45 ±0.02e
F7 = 87.5% *fullwash*: Gambir 5%	6.68 ±0.04ab	96.28 ±0.51bc	5.47 ±0.02e
F8 = 82.5% *fullwash*: Gambir 10%	6.80 ±0.07bc	95.47 ±0.44ab	5.33 ±0.03b
F9 = 77.5% *fullwash*: Gambir 15%	6.92 ±0.07cd	94.57 ±0.57a	5.25 ±0.02a

Note: Numbers followed by the same letter in the same column are not significantly different.

The water content of instant gambir coffee produced in this research was higher than the Indonesian National Standard (SNI) No 2983 of 2014, set at 5%. The value was also greater than **[4]** at 3.84-4.81%, instant coffee from Tungkal Jambi, which has a content of 1.57-1.61% **[12]**, and cold brewed instant coffee at 2.43% **[13]**.

The solubility percentage of instant gambir coffee produced ranged from 94.35-97.55%, where the lowest value was obtained in Treatment F3 (natural anaerobic 77.5%: gambir catechin crude extract 15%). In comparison, F4 (honey 87.5%: gambir catechin crude extract 5%) had the highest, as presented in Figure 2.

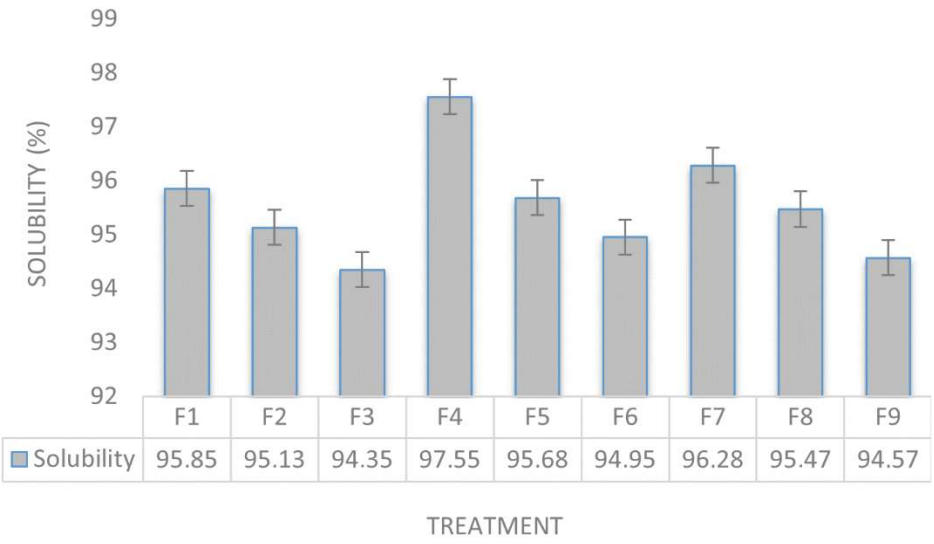

Figure 2 Solubility percentage of instant gambir coffee.

The formulation treatment significantly influences the solubility percentage of instant gambir coffee produced. The results of the BNJ 5% test are shown in Table 1. Table 1 shows that formulation F4 had the highest solubility percentage, 97.55%, but not significantly different from F7. This can be explained based on the principle of like dissolved likes, where non-polar compounds dissolve in non-polar solvents, and conversely, polar compounds are soluble in polar solvents. Previous investigations showed that the catechin crude extract of gambir contains semi-polar catechin compounds. Therefore, the solubility level decreases as the concentration of catechin crude extract of gambir increases and vice versa. Coffee processing methods also affected the solubility level of coffee because, according to Herawati **[14]**, honey coffee has the highest level of a polar compound **[15]**, namely chlorogenic acid, compared to other processing. The solubility percentage of instant gambir coffee produced was higher than the solubility level of 93.79% for instant coffee made from robusta beans processed using a vacuum dryer, as reported by Matanari **[16]**. However, the value was lower than Tungkal Jambi Liberika instant coffee, which had a content of 97.95-98.20% **[12]**.

The pH level of instant gambir coffee produced ranged from 5.25-5.75, with the lowest and highest levels found in formulations F9 (honey 77.5% (w/w): gambir catechin crude extract 15%) and F1 (natural anaerobic 87.5% (w/w): gambir catechin crude extract 5% (w/w), respectively were presented in Figure 3 .

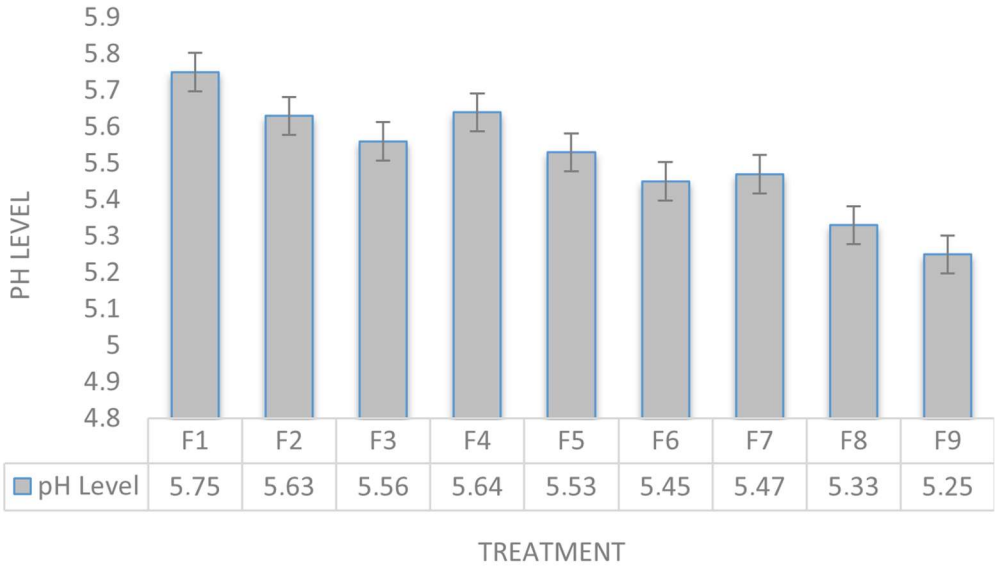

Figure 3 pH level of instant gambir coffee.

The analysis of variance showed that the formulation treatment significantly influenced the pH level of instant gambir coffee produced. The results of the LSD test at a 5% significance level for the pH level of instant gambir coffee are shown in Table 1. Formulation F9 produced the lowest pH level and significantly differed from other formulations as presented in Table 1 by the LSD test at a 5% significance level. This occurred because the full-wash coffee involved fermentation, during which aliphatic acids such as citric, malic, and quinic acids are formed **[17]**, thereby affecting the pH level of the coffee produced. The pH level of this instant coffee was also influenced by the crude extract content of gambir catechin, as catechin compounds are acidic, easily oxidized at neutral pH, and stable at low pH **[11]**. Therefore, the higher the concentration of crude extract of gambir catechin, the greater the pH level or the lower the pH value. The pH level of instant gambir coffee produced in this research is similar to robusta coffee at 5.47 **[18]**, instant mangosteen peel coffee at 5.26-5.63 **[19]**, brewed robusta coffee at 5.16-5.69 **[20]**, and fermented robusta coffee, which ranged from 5.25-5.37 **[21]**.

Table 2 The results of the 5% BNJ follow-up test on total phenol and IC_{50} of the resulting instant coffee.

Treatment	Total Phenol (mgGAE/g)	IC_{50}(ppm)	Clear zona (mm)
F1 = 87.5% *natural*: Gambir 5%	25.75 ±0.30b	86.02 ±0.17d	0.71 ±0.02a
F2 = 82.5% *natural*: Gambir 10%	30.53 ±0.73abc	44.54 ±0.19b	0.77 ±0.01a
F3 = 77.5% *natural*: Gambir 15%	34.38 ±0.38cd	42.23 ±0.23b	0.81 ±0.02b
F4 = 87.5% *honey*: Gambir 5%	24.91 ±4.30a	121.75 ±7.26e	0.71 ±0.01bc
F5 = 82.5% *honey*: Gambir 10%	27.01 ±3.80ab	65.10 ±0.13c	0.76 ±0.03bc
F6 = 77.5% *honey*: Gambir 15%	40.35 ±1.39d	31.46 ±0.39a	0.83 ±0.01cd
F7 = 87.5% *fullwash*: Gambir 5%	27.16 ±0.77ab	88.25 ±0.12d	0.77 ±0.02d
F8 = 82.5% *fullwash*: Gambir 10%	31.56 ±1.46bc	64.72 ±2.95c	0.83 ±0.02d
F9 = 77.5% *fullwash*: Gambir 15%	40.18 ±1.38d	49.89 ±3.78b	0.93 ±0.02e

Note: Numbers followed by the same letter in the same column are not significantly different.

The total phenol content of this instant gambir coffee ranged from 24.91-40.35 mgGAE/g, with the lowest and highest levels found in formulations F4 (honey 87.5% (w/w): gambir catechin crude extract 5% (w/w) and F6 (honey 77.5% (w/w): gambir catechin crude extract 15% (w/w), respectively were presented in Figure 4.

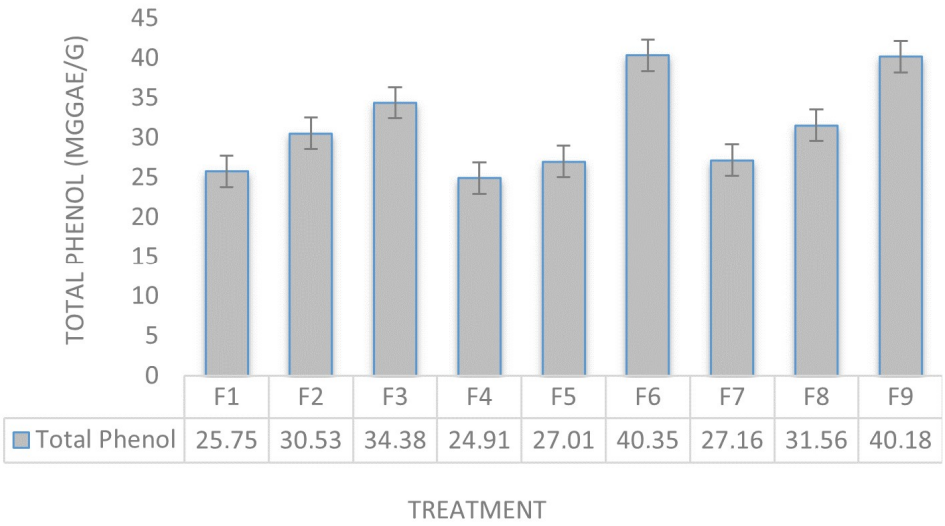

Figure 4 Total phenol of instant gambir coffee.

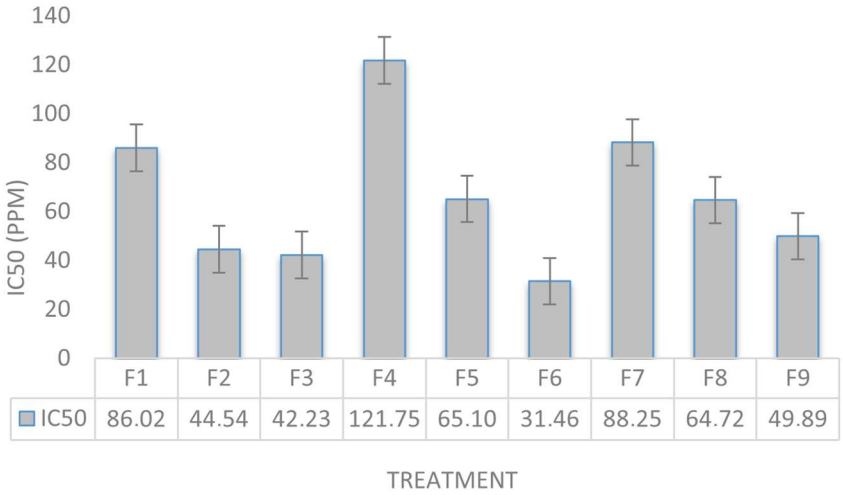

Figure 5 IC_{50} of instant gambir coffee.

The analysis of variance showed that the formulation treatment significantly affected the total phenol content of instant gambir coffee produced. The results of the LSD test at a 5% significance level for the effect of formulation treatment on total phenol content and IC_{50} of instant coffee produced were shown in Table 2. Table 2 showed that instant gambir coffee formulation F6 contained the highest total phenols and was not significantly different from formulations F3 and F9. This can be attributed to the coffee processing methods, which did not affect total phenols, but rather the concentration of coffee and gambir catechin crude extract. As the coffee concentration decreases, the caffeine content also reduces, while an increase in the gambir catechin crude extract, will lead to higher catechin compounds. Caffeine can bind to catechin compounds, however. due to the higher concentration of catechin compounds, the amount of free catechin compounds in instant gambir coffee is greater, causing an increase in total phenol values. Kamsina and Firdausni **[22]** reported that the addition of gambir catechin extract increased total phenols in wet noodles by 84%, while **[23]** added that gambir powder contains catechin compounds of 62.13%. The total phenol values of this instant gambir coffee are higher compared to baked coffee at 16.66 mg/mLGAE **[24]**, and cinnamon coffee of 34.46 mg/mLGAE **[25]**. However, the coffee produced from this research was lower compared to roasted arabica coffee, with a total phenol content of 49.90 mg/mLGAE **[26]**, raw and roasted robusta coffee with a value of 208.89 mg/mLGAE and 119.22 mg/mLGAE, respectively, as well as branded coffee in Indonesia at 46.27 mg/mLGAE **[27]**.

The antioxidant activity of the produced instant gambir coffee can be categorized as strong, as indicated by the IC_{50} value below 50 ppm, which ranged from 31.46-121.75 mg/mL. were presented in Figure 5. The analysis of variance showed that the formulation treatment significantly affected the IC_{50} value of the produced instant gambir coffee. The results of the 5% BNJ test in Table 2 showed that the honey coffee formulation with a concentration of 77.5% and 15% gambir catechin crude extract (F6) had the highest anti-oxidant activity, as indicated by the

lowest IC50 value. This formulation treatment differed significantly from others. The IC50 value was inversely proportional to the total phenol value, therefore, the lower the IC50 value, the higher the total phenol value, and vice versa. The IC_{50} value of the produced instant gambir coffee was similar to that of encapsulated green coffee extract **[28]**, cold-brewed green coffee **[13]**, and dried green coffee using a foam mat **[29]**, namely 87.65 ppm, 71.97-83.21 ppm, and 25.187 ppm, respectively. However, compared to green coffee from Ethiopia **[1]**, robusta coffee **[30]**, and non-instant gambir coffee **[4]**, at 167 ppm, 426-294 ppm, 710-2210 ppm, and 40.10-583.06 ppm, respectively, the IC_{50} value of the instant gambir coffee is lower.

The antibacterial test is carried out by measuring the diameter of the clear zone formed around the disc. The barrier zone formed in instant coffee ranges from 0.71 mm to 0.93 mm. The smallest resistance zone is in the F1 treatment (87.5% natural: Gambir 5%) while the largest resistance zone is in the F9 treatment (77.5% fullwash: Gambir 15%). The results of measuring the antibacterial activity value of functional instant coffee can be seen in Figure 6.

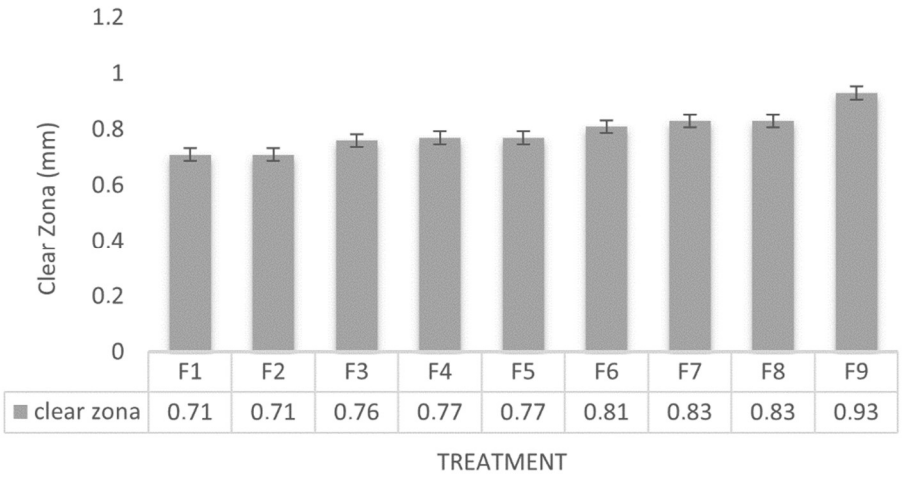

Figure 6 Clear zona of instant gambir coffee.

The results of the analysis of the diversity of antibacterial activity show that instant coffee formulation and catechin extract from gambir have a significant effect on the antibacterial activity of functional instant coffee.The results of the BNJ 5% further test showed that the F1 treatment (87.5% natural: Gambir 5%) was significantly different from the F9 treatment (77.5% fullwash: Gambir 15%). This shows that the addition of gambier catechin extract can increase the antibacterial activity of instant coffee. The flavonoid content in gambier catechin extract is able to inhibit the growth of gram-positive bacteria (*Staphylococcus aureus*). This is because the flavonoids and peptidoglycan layer in gram-positive bacteria are polar so that flavonoids will more easily penetrate the cell wall layer which can cause lysis of bacterial cells **[31]**. The more concentration of gambier catechin added, the antibacterial activity of instant coffee will increase. Robusta coffee also contains compounds that have antibacterial properties, namely caffeine. Caffeine is an alkaloid compound. Alkaloid compounds work by inhibiting cell wall synthesis which can cause lysis. Chlorogenic acid also acts as an antibacterial. The mechanism of this compound is to enter the bacterial cell nucleus and damage the cell wall structure. Apart from that, phenolic compounds in the form of flavonoids in coffee are also able to inhibit bacterial growth **[32]**.

CONCLUSION

The addition of crude gambir catechin extract to coffee processed using all the methods used can increase the antioxidant properties. Reducing the concentration of coffee in each processing method and increasing the concentration of crude gambir catechin extract had a significant effect on increasing the antioxidant properties.The formulation treatment significantly affected the water content, solubility percentage, pH, total phenol, and IC50 of the produced instant gambir coffee. The characteristics of the produced instant gambir coffee included water content, solubility percentage, pH degree, total phenol, and IC50 with values of 6.56-7.02%, 94.35-96.55%, 5.25-5.75, 24.91-40.35 mgGAE/g, and 31.46-121.75 mg/mL, respectively.

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