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# Analysis of Amino Acids, Protein Profile and phyto chemical of Leaves, Green Coffee, Black Coffee, Coffee Fruit Skins Of Robusta Coffee and Its Potential For Health

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***Abstract***
*Amino acid content, protein profile and phytochemical analysis of leaves, green coffee, black coffee and fruit peels are important because they are beneficial for health. The research aimed at analyzing the amino acids and phytochemicals of leaves, green coffee, black coffee and rinds of Robusta coffee fruit originating from Jember Regency, East Java Province, Indonesia. This study used Robusta coffee from Jember district, Indonesia, which was extracted by maceration of 96% ethanol, then the ultrasonication method was carried out. Amino acid analysis was carried out by High Performance Liquid Chromatography (HPLC). Protein profiling by SDS Page electrophoresis. Phytochemical analysis using Thin-layer Chromatography (TLC) method. The result is that coffee leaves contain 4 types of essential amino acids (methionine, leucine-isoleucine, phenylalanine, valine) and 4 non-essential amino acids namely arginine, glutamic acid (not present in leaves and fruit skin), tyrosine, proline). More varied protein profile with some thicker bands (Molecular weight around 10-17, around 25. Green coffee has thin band around molecular weight 25, above 42. Fruit skin has thick band around molecular weight 25 and above 42. All contain polyphenols, flavonoids, triterpenoids/sapogenin steroids, free terpenoids/steroids, alkaloids, anthraquinones. Quantitatively polyphenols are the highest in fruit skin, followed by black coffee, green coffee and leaves. Polyphenols here include flavonoids, caffeine, chlorogenic acid, diterpenes and trigonelline The highest flavonoids are green coffee, followed by leaves, black coffee and fruit skin Conclusion, leaves, green coffee, black coffee and Robusta coffee fruit skin originating from Jember Regency, East Java Province, Indonesia contain protein, amino acids and bioactive components that can be utilized for health.*

***Key words****: Coffee; proteins; amino acid; phytochemicals; health*

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## I. Introduction

Indonesia is the largest coffee producing country in the 4th place after Brazil, Columbia and Vietnam. Meanwhile, Jember district is in second place in East Java with a total production of 11,863 tons. One type of coffee cultivated in Jember is Robusta coffee which is known to have many benefits for the body (Dewanti et al., 2019; Riastuti, 2021). In East Java there are six of the largest coffee-producing regencies/cities, namely Malang, Banyuwangi, Jember, Lumajang, Pasuruan and Bondoswoso (BPS East Java, 2011). On the other hand, a large number of coffee skins are a by-product of smallholder plantations that have not been explored optimally, so that this potential natural resource only becomes accumulated waste (Wardhana et al., 2019).

Coffee chemical compounds such as flavonoids, xanthine, antioxidants, alkaloids can function as anti-inflammatory and antibacterial. Phenol in coffee can induce platelet aggregation (Coralie et al., 2006; Natella et al., 2008). The protein content of Robusta coffee beans is 11% -13% or 10-13% (Health Secret Editor, 2012; Mulato and Edy (2015). The contents in the skin of the robusta coffee bean such as caffeine, phenol, chlorogenic acid and trigonelline compounds can act as antimicrobials. (Ullah et al., 2015; Dewanti et al., 2019; Dewanti et al., 2022). In addition, it is important to know the amino acid content in the coffee plant. It is said that all parts of the coffee bean contain protein in large enough quantities. For example, coffee grounds contain high protein (13.5–19.5 g/100 g). Coffee waste skin contains 6.67% crude protein, with 18.28% crude fiber, 1.0% fat, 0.21%

calcium, and phosphorus 0.03%.Availabilities of this amount of material in areas in Indonesia, and has not been utilized properly (Londra, 2007).

Several publications indicate that the amino acid composition of proteins in coffee beans has unusual potential. Branched chain amino acids are widely used as supplements and for faster post-workout recovery. However, the evidence for its benefits is still inconclusive. Coffee protein is also reported to have a high Fischer ratio (branched-chain amino acids/aromatic amino acids). Such proteins are reported to help sufferers of malnutrition associated with cancer, burns, trauma, and liver failure and may help treat children with chronic or acute diarrhea or milk protein allergies (Bhattarai et al., 2022). Previous research stated that in general the concentration of amino acids in Robusta coffee beans is higher than Arabica coffee (Murkovic and Karin, 2006). The main amino acids in coffee are asparagine, alanine, glutamic acid, lysine and aspartic acid. Coffee is also rich in caffeine, trigonellin, theophylline and theobromine. Processing of coffee beans impacts their active compounds and pharmacological activity (Pratiwi, 2021). Previously, we used the skin of the fruit and Robusta coffee beans to make toothpaste. The concentration of our research is utilizing fruit peels and coffee beans. In this research we are for health, especially toothpaste. However, to obtain a quality product, we want to know the amino acid components and bioactive components (flavonoids, polyphenols, steroid triterpenoids/sapogenins, free terpenoids/steroids, alkaloids, anthraquinones) found in Robusta coffee plants (leaves, green coffee, black coffee)., seed coat) in Jember district. We do amino acid analysis, because amino acids play a major role in the health sector. Meanwhile, the qualitative analysis is very essential to identify the phytochemical constituents present in medicinal plants. Previously we also carried out a phytochemical analysis of fruit peels and coffee beans (qualitatively), quantitatively with the results of the levels of polyphenols (393.0166 ± 85.5224 mg GAE/g and flavonoids (0.788592 ± 0.114787 mb QE/g) using 425 nm wavelength spectrophotometry with comparison of Quercetin (flavonoid) and gallic acid (polyphenol) (Dewanti et al., 2022).

## II. Materials and Methods

### 2.1. Manufacture of plant extracts (leaves, seeds, skin) of Robusta coffee

Coffee bean extract is made by aerating the coffee bean husks to dry (900 grams), pulverizing them using a blender and then filtering them using a sieve to obtain the powder. 200 gr of coffee bean skin powder was weighed, then put into a 1000 ml Erlenmeyer. Maceration was carried out using 96% ethanol 700 ml, stirred for 2-3 minutes and closed. Ultrasonication was carried out for 1 hour and filtered with filter paper. Dregs/residue added 96% ethanol 350 ml. Do the stirring 2-3 minutes and closed. Ultrasonic 1 hour and filtered. Dregs/residue added 96% ethanol 350 ml. Do the stirring 2-3 minutes and closed. Ultrasonic 1 hour and filtered. Dregs/residue added 96% ethanol 350 ml. Do the stirring 2-3 minutes and closed. Filter until the residue/dregs are dry. Concentration of the filtrate using a rotary evaporator at 50oC 120 rpm until there is no ethanol dripping in the condensation. Extract concentration using an oven (water bath) at 50oC to constant weight. After that, the extract was weighed.

### 2. 2. Amino acid analysis

Amino acid analysis was carried out using High Performance Liquid Chromatography (HPLC) which utilizes a pre-column reaction of primary amino groups in alkaline conditions containing mercaptoethanol to form fluorescent compounds that can be detected with a fluorescence detector. Potassium borate buffer solution pH 10.4 was added to the sample (1:1 ratio) to obtain a sample solution ready for analysis. The 10 µl sample solution is mixed with 25 µl of ortophthaaldehyde (OPA) reagent, as well as the standard amino acid solution. The mixed solution (both sample and standard) is allowed to stand for 1 minute so that the derivatiation can take place perfectly. Next, 5 µl of the standard solution was injected into the HPLC column, waiting until the separation of all amino acids was complete (Sari et al., 2017).

### 2.2. Protein Profile Analysis

Determination of protein content was carried out using the Bradford method. Then extraction was carried out by: 0.25gram sample was crushed and 0.75 ml of extraction buffer was added containing 50mm MOPS-NaOH (Ph7.5), 1mm EDTA, 10mm MgCl and 1mm Phenyl Methyl Sulfonyl Fluoride (PMSF). The scale samples after being crushed were treated with a sonicator for 15 minutes. The homogenate was centrifuged (12,000 rpm 10 minutes 0C). The supernatant was transferred to a microtube and stored at -80Oc. SDS-PAGE is done using the method according to Laemmli. The solution resulting from the heatstability test was added with buffer loading (Tris-Cl0, 5M Ph 6.8; SDS 10%; glycerol 10%; bromophenol blue) and denatured by heating at 1000C for 3 minutes. Protein samples were put into the gel wells and then separated using (SDS-PAGE) 15% acrylamide concentration. Separation of proteins with SDS-PAGE was carried out with an electric current of 50-95V for 5 hours in an electrode buffer (glycine 192mm, Trisbase 25mm, SDS0.1%). Separated proteins were stained with Coomassie Brilliant Blue (CBB). The protein marker used the Blue Prestained Protein Standard brand Nacalai 02525 in the amount of 3 µl (Garcia and Baez, 2018).

**2.3. Phytochemical Analysis**

Steroid/triterpenoid sapogenins: 50.5 g of extract plus 5 ml of 2N HCl, boiled and covered with a funnel containing wet cotton for 2 hours to hydrolyze saponins. After cooling, neutralized with ammonia, extracted with 3 mL of n-hexane. Evaporated until only 0.5 ml remained. Smeared on the TLC plate (Silica gel 60 F254). Evaluated with the mobile phase n-hexane-ethyl acetate (4:1). Sprayed with anisaldehyde sulfate reagent. Heated at 115oC for 5-10 minutes. The presence of sapogenins or triterpenoids, tripenoids or free steroids is indicated by the formation of purplish-red stains.

Anthraquinone group: extract added with ethanol, stirred until smooth, smeared on a TLC plate, eluted in toluene-ethyl acetate-acetic acid as mobile phase (75:24:1). Sprayed with 10% KOH reagent stain remover in methanol. The appearance of yellow, brown yellow, purple red or green purple stains indicates the presence of anthraquinone compounds.

Polyphenolic compounds: 0.1 g of extract plus 3 ml of hot water, stirred and allowed to come to room temperature. Added 2 drops of 10% NaCl, stirred and filtered. Smeared on a TLC plate, delusional on toluene-acetone-formic acid (6:6:1) mobile phase. Sprayed with stain remover, it will be black.

Flavonoids: 0.1 g of extract, shaken with 1 ml of n-hexane many times until the extract is colorless. The residue was dissolved in a few drops of ethanol, smeared on the TLC plate, eluted with butanol-glacial acetic acid-water as the mobile phase (4:1:5). Sprayed with a stain remover on the citroborak reagent. Flavonoids are marked in yellow.

Alkaloids: 0.1 g of extract plus 2 ml of 2N HCl, heated over a water bath for 2-3 minutes while stirring. After chilling, 0.1 g of NaCl was added, stirred, filtered. The filtrate was added 2 mL of 2N HCl, added with 28% NH4OH until the solution became alkaline. Set aside for at least 15 minutes, extractedwith 5 ml of water-free chloroform. The filtrate was evaporated to dryness, dissolved in a few drops of methanol, smeared on the TLC plate, eluted with acyl acetate-methanol-water as the mobile phase (9:2:2). Sprayed with Dragendorf reagent stain spotter. Alkaloids are marked orange.

## III. Results

**3.1. Amino Acid Analysis**

**Table 1**. Amino acid content of black coffee, fruit skin, green coffee, leaves of Robusta coffee

| Sample | Sample Weight (mg) | Extract volume (ml) | Leucine-Isoleucine (mM) | Leucine-Isoleucine (ppm) | Leucine-Isoleucine (%) |
|---|---|---|---|---|---|
| Leaves | 200 | 25 | 0,2890 | 37,908 | 0,474 |
| Green coffee | 200 | 25 | 0,2900 | 38,039 | 0,475 |
| Black coffee | 200 | 25 | 0,2230 | 29,251 | 0,366 |
| Fruit skin | 200 | 25 | 0,2360 | 30,956 | 0,387 |

| Sample | Sample Weight (mg) | Extract volume (ml) | Valine (mM) | Valine (ppm) | Valine (%) |
|---|---|---|---|---|---|
| Leaves | 200 | 25 | 0,0273 | 3,198 | 0,040 |
| Green coffee | 200 | 25 | 0,0579 | 6,783 | 0,085 |
| Black coffee | 200 | 25 | 0,0432 | 5,061 | 0,063 |
| Fruit skin | 200 | 25 | 0,0305 | 3,573 | 0,045 |

| Sample | Sample Weight (mg) | Extract volume (ml) | Tyrosine (mM) | Tyrosine (ppm) | Tyrosine (%) |
|---|---|---|---|---|---|
| Leaves | 200 | 25 | 0,1770 | 32,069 | 0,401 |
| Green coffee | 200 | 25 | 0,1300 | 23,553 | 0,294 |
| Black coffee | 200 | 25 | 0,0740 | 13,407 | 0,168 |
| Fruit skin | 200 | 25 | 0,0910 | 16,487 | 0,206 |

| Sample | Sample Weight (mg) | Extract volume (ml) | Arginine (mM) | Arginine (ppm) | Arginine (%) |
|---|---|---|---|---|---|
| Leaves | 200 | 25 | 0,0190 | 3,310 | 0,041 |

| | | | | | |
|---|---|---|---|---|---|
| Green coffee | 200 | 25 | 0,0270 | 4,703 | 0,059 |
| Black coffee | 200 | 25 | 0,0120 | 2,090 | 0,026 |
| Fruit skin | 200 | 25 | 0,0280 | 4,878 | 0,061 |

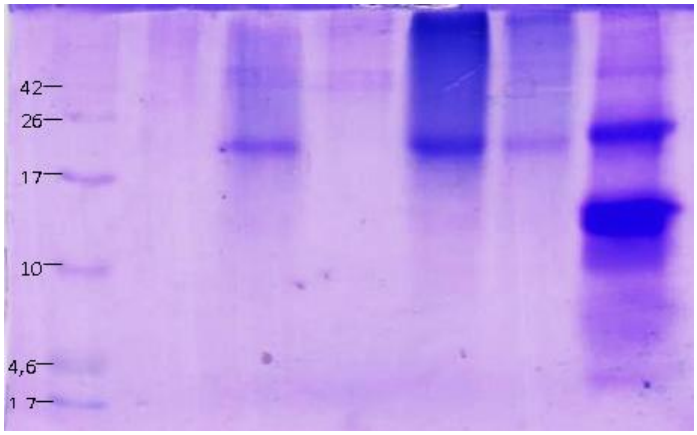
| Sample | Sample Weight (mg) | Extract volume (ml) | Methionine (mM) | Methionine (ppm) | Methionine (%) |
|---|---|---|---|---|---|
| Leaves | 200 | 25 | 0,0335 | 4,999 | 0,062 |
| Green coffee | 200 | 25 | 0,0628 | 9,370 | 0,117 |
| Black coffee | 200 | 25 | 0,0492 | 7,341 | 0,092 |
| Fruit skin | 200 | 25 | 0,0392 | 5,849 | 0,073 |

| Sample | Sample Weight (mg) | Extract volume (ml) | Proline (mM) | Proline (ppm) | Proline (%) |
|---|---|---|---|---|---|
| Leaves | 200 | 25 | 0,0265 | 3,051 | 0,038 |
| Green coffee | 200 | 25 | 0,0407 | 4,686 | 0,059 |
| Black coffee | 200 | 25 | 0,0302 | 3,477 | 0,043 |
| Fruit skin | 200 | 25 | 0,0259 | 2,982 | 0,037 |

| Sample | Sample Weight (mg) | Extract volume (ml) | Glutamic Acid (mM) | Glutamic Acid (ppm) | Glutamic Acid (%) |
|---|---|---|---|---|---|
| Leaves | 200 | 25 | 0,0000 | 0,000 | 0,000 |
| Green coffee | 200 | 25 | 0,0390 | 5,738 | 0,072 |
| Black coffee | 200 | 25 | 0,0280 | 4,120 | 0,051 |
| Fruit skin | 200 | 25 | 0,0000 | 0,000 | 0,000 |

| Sample | Sample Weight (mg) | Extract volume (ml) | Phenylalanine (mM) | Phenylalanine (ppm) | Phenylalanine (%) |
|---|---|---|---|---|---|
| Leaves | 200 | 25 | 0,2510 | 41,463 | 0,518 |
| Green coffee | 200 | 25 | 0,2200 | 36,342 | 0,454 |
| Black coffee | 200 | 25 | 0,2280 | 37,663 | 0,471 |
| Fruit skin | 200 | 25 | 0,1480 | 24,448 | 0,306 |

### 3.2. Protein profile of leaves, green coffee bean, black coffee bean, fruit skin of Robusta coffee




Marker Black Coffee Fruit skin GreenLeaves coffee

**Figure 1.**SDS-PAGE of black coffee, fruit skin, green coffee, leaves of Robusta coffee

### 3.3. Phytochemical analysis of of leaves, green coffee bean, black coffee bean, fruit skin of Robusta coffee

**Table 2.** Phytochemical analysis of Robusta coffee fruit skin

| No | system used | Result | description |
|---|---|---|---|
| 1 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>Butanol mobile phase: acetic acid : water (4 : 1 : 5)<br>Detection: cytoboric reagent | There are intense yellow stains | Flavonoids (++) |
| 2 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>mobile phase: methanol : water (6 : 4)<br>Detection: reactor FeCl3 | There are black stains | Polyphenols (+) |
| 3 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>mobile phase: n-hexane : ethyl acetate (4 : 1)<br>Detection: reactor anisaldehyde-sulfate | There are purple stains | Triterpenoid/sapogenin steroids (+) |
| 4 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>mobile phase: n-hexane : ethyl acetate (4 : 1)<br>Detection: reactor anisaldehyde-sulfate | There are puple stains | free terpenoids/steroids (+) |
| 5 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>mobile phase: ethyl acetate : methanol : water (9 : 2 : 2)<br>Detection: reactor Dragoendorf | There are orange stains | Alkaloids (+) |
| 6 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>mobile phase: hexane : ethyl acetate (3 : 7)<br>Detection: reactor ethanolic KOH | There are brown yellow stains | Anthraquinone (+) |

the levels of polyphenols (393.0166 ± 85.5224 mg GAE/g and flavonoids (0.788592 ± 0.114787 mb QE/g) using 425 nm wavelength spectrophotometry with comparison of Quercetin (flavonoid) and gallic acid (polyphenol) (Dewanti et al., 2022).

**Table 3.** Green coffee phytochemical analysis

| No | system used | Result | description |
|---|---|---|---|
| 1 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>Butanol mobile phase: acetic acid : water (4 : 1 : 5)<br>Detection: cytoboric reagent | There are intense yellow stains | Flavonoids (++) |
| 2 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>mobile phase: methanol : water (6 : 4)<br>Detection: reactor FeCl3 | There are black stains | Polyphenols (+) |
| 3 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>mobile phase: n-hexane : ethyl acetate (4 : 1)<br>Detection: reactor anisaldehyde-sulfate | There are purple stains | Triterpenoid/sapogenin steroids (+) |
| 4 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>mobile phase: n-hexane : ethyl acetate (4 : 1)<br>Detection: reactor anisaldehyde-sulfate | There are puple stains | free terpenoids/steroids (+) |
| 5 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>mobile phase: ethyl acetate : methanol : water (9 : 2 : 2)<br>Detection: reactor Dragoendorf | There are orange stains | Alkaloids (-) |
| 6 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>mobile phase: hexane : ethyl acetate (3 : 7)<br>Detection: reactor ethanolic KOH | There are brown yellow stains | Anthraquinone (+) |

The total level of green coffee bean flavonoids was 7.285479 ± 1.454318 mg QE/g extract. Total polyphenol content 68.17539 ± 4.901761 mg GAE/g extract.

**Table 4**. Black coffee beans phytochemical analysis

| No | system used | Result | description |
|---|---|---|---|
| 1 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>Butanol mobile phase: acetic acid : water (4 : 1 : 5)<br>Detection: cytoboric reagent | There are intense yellow stains | Flavonoids (+) |
| 2 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>mobile phase: methanol : water (6 : 4)<br>Detection: reactor FeCl3 | There are black stains | Polyphenols (+) |
| 3 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>mobile phase: n-hexane : ethyl acetate (4 : 1)<br>Detection: reactor anisaldehyde-sulfate | There are purple stains | Triterpenoid/sapogenin steroids (+) |
| 4 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>mobile phase: n-hexane : ethyl acetate (4 : 1)<br>Detection: reactor anisaldehyde-sulfate | There are puple stains | free terpenoids/steroids (+) |
| 5 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>mobile phase: ethyl acetate : methanol : water (9 : 2 : 2)<br>Detection: reactor Dragoendorf | There are orange stains | Alkaloids (-) |
| 6 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>mobile phase: hexane : ethyl acetate (3 : 7)<br>Detection: reactor ethanolic KOH | There are brown yellow stains | Anthraquinone (+) |

Total flavonoid content of black coffee bean extract: 4,643327 ± 0,400277 mg QE/g extract. Total content of black coffee bean extract: 311,8249 ± 18,53496 mg GAE/g extract.

**Table 5.**leaves extract of Robusta coffeephytochemical analysis

| No | system used | Result | description |
|---|---|---|---|
| 1 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>Butanol mobile phase: acetic acid : water (4 : 1 : 5)<br>Detection: cytoboric reagent | There are intense yellow stains | Flavonoids (+) |
| 2 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>mobile phase: methanol : water (6 : 4)<br>Detection: reactor FeCl3 | There are black stains | Polyphenols (+) |
| 3 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>mobile phase: n-hexane : ethyl acetate (4 : 1)<br>Detection: reactor anisaldehyde-sulfate | There are purple stains | Triterpenoid/sapogenin steroids (+) |
| 4 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>mobile phase: n-hexane : ethyl acetate (4 : 1)<br>Detection: reactor anisaldehyde-sulfate | There are puple stains | free terpenoids/steroids (+) |
| 5 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>mobile phase: ethyl acetate : methanol : water (9 : 2 : 2)<br>Detection: reactor Dragoendorf | There are orange stains | Alkaloids (-) |
| 6 | Thin-layer chromatography/TLC<br>Stationary phase: TLC silica gel 60 F254<br>mobile phase: hexane : ethyl acetate (3 : 7)<br>Detection: reactor ethanolic KOH | There are brown yellow stains | Anthraquinone (+) |

Total flavonoid content of black coffee bean extract: 6,683168 ± 0,740198 mg QE/g extract. Total content of black coffee bean extract: 185,9972 ± 33,2616 mg GAE/g extract.

## IV. Discussion

### 4.1. Amino acid

Coffee has 4 types of essential amino acids (methionine, leucine-isoleucine, phenylalanine, valine) and 4 non-essential amino acids namely arginine, glutamic acid (not present in leaves and fruit skin), tyrosine, proline). The amino acid glutamate (the largest in green coffee) is included as a non-essential which functions to help metabolize sugar and fat, transport potassium, brain energy, improve personality disorders, overcome epilepsy, ulcers, give a savory taste to food, increase salivary secretion, suppress obesity, nourish the brain. The amino acids leucine and isoleucine (the largest in green coffee), are essential amino acids for muscle recovery, proteinogenesis, forming antibodies, activating various hormones, providing energy, making ketones and glucose. Valine (the largest is green coffee), including hydrophobic aliphatic essential amino acids which function in regulating the absorption of other types of amino acids. The amino acid valine functions to regulate blood sugar, prevent muscle damage, remove excess nitrogen, cure liver and bile contents diseases, maintain body energy, maintain mental function. Arginine (the largest seed coat), is a semi-essential amino acid that functions to protect the liver, skin, joints and muscles. Arginine can strengthen the immune system, regulate hormones and blood sugar, increase male fertility. Arginine also improves blood circulation, treats impotence and heart. Methionine is a sulfur-containing essential amino acid that is not nucleophilic and will react with several electrophilic centers. Its function is to remove toxins, improve cardiovascular health, help the liver process fat, make creatine (a natural nutrient for muscles, heart and blood vessel function), formation of nails, skin and connective tissue, reduce inflammation, reduce allergies. Tyrosine (leaves) is also non-essential which plays a role in the production of thyroid hormones T3 and T4.

### 4.2. Protein Analysis

SDS_PAGE results green coffee, black coffee, fruit skin, Robusta coffee leaves contain proteins with different molecular weights. Coffee leaves have a more varied protein profile with some thicker bands (Molecular weight around 10-17, around 25. Green coffee has thin bands around molecular weight 25, over 42. Fruit skin has thick bands around molecular weight 25 and above 42. Black coffee has a rather thick band of about 25. It can be said to be a major protein fraction, in which the protein has greater thickness and color intensity than other proteins. A thick band indicates a large concentration, while a thin band indicates protein content The difference between thick and thin bands is due to differences in the number of migrated molecules,

thick bands are the fixation of several bands. Bands that have greater ionic strength will migrate farther than bands with low ionic strength. Green leaves produce a less varied profile, but has a larger molecular weight than leaves, green coffee and black coffee.

**4.3. Phytochemicals**

Based on the phytochemical analysis of all parts (leaves, fruit skin, green coffee, black coffee contains polyphenols, flavonoids, triterpenoids/sapogenin steroids, free terpenoids/steroids, alkaloids, anthraquinones. For polyphenols the highest is fruit skin, followed by black coffee, green coffee and leaves Polyphenols here include flavonoids, caffeine, chlorogenic acid, diterpenes and trigonelline. The highest flavonoids are green coffee, followed by leaves, black coffee and fruit skin. This content can be affected by the type of variety, processing (Dao et al., 2022). Flavonoids are a group of compounds bioactive secondary metabolites which play a role in antioxidant activity because they are able to donate hydrogen atoms to capture free radicals.Compounds in the flavonoid class are known to have functions to protect cell structures, increase the effectiveness of vitamin C, anti-inflammation and antibiotics.Positive tests on tannins show that roasted coffee contains compounds. This compound is incorporated in k polyphenol group which is responsible for the bitter and chelating taste (Adzkiya and Agung, 2022). Sholichah et al (2019) also stated that the skins of Robusta and Arabica coffee beans were sources of polyphenols. Polyphenol compounds function as antioxidants through primary antioxidant mechanisms, namely breaking the chain of oxidation processes. The hydroxyl group (-OH) in flavonoids, especially in ring B position 2; 3; and 4 is an active group to capture free radicals. Polyphenols apart from potentially protecting against oxidative stress which prevents cell damage from occurring, can also act as an anti-inflammatory. The presence of polyphenols can prevent cardiovascular disease (lowering cholesterol), lowering blood sugar levels, inhibiting aging, preventing cancer. However, the high levels of polyphenols in coffee protein concentrate may contribute to its low protein digestibility, giving the protein concentrate a bitter or astringent taste (Bhattarai et al., 2022). Antioxidants inhibit nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in the mitochondria and reduce the production of reactive oxygen species (ROS) and affect endothelial nitric oxide (NO) production (Yamagata, 2018). The content of coffee beans such as phenolic compounds, namely flavonoids, works by damaging the bacterial cell walls.

Research by Nuhu et al (2013), Dewanti et al (2022) explained that other ingredients such as trigonelline in the skin of Robusta coffee beans have a positive correlation with the reduction of biofilm formation in Streptococcus mutans due to its bacteriostatic action. Caffeine as one of the alkaloids can inhibit the growth of bacteria. The caffeine content in Robusta coffee beans ranges from 1.6% -2.4% (Rahman, 2021). Alkaloids are a group of naturally occurring chemical compounds that mostly contain a nitrogen atom base. The term "alkaloid" is derived from the Arabic word "al-qali" which means ash containing potassium carbonate of plant material. Traditionally, alkaloids are defined as heterocyclic nitrogenous compounds that are biosynthesized from amino acids; however, many other substances that do not quite conform to this rule are classified as alkaloids. In addition to carbon, hydrogen and nitrogen, alkaloid molecules may contain sulfur and rarely chlorine, bromine or phosphorus (Geyter, 2012). The alkaloid content in coffee includes caffeine and trigonellin. Caffeine is a purine alkaloid, has antimicrobial activity, which inhibits esterase enzymes along with DNA and RNA polymerase, inhibits cellular respiration, plays a role in DNA intercalation, causes damage and lysis. Alkaloid compounds have a base group that contains nitrogen which will react with amino acid compounds that make up the bacterial cell wall and bacterial DNA, so that the cell wall layer is not formed completely and causes cell death (Dahliati, 2014; Dewanti, 2022).

Steroid saponins show structural similarities to the insect moulting hormone ecdysteroid 20E. Triterpenes are a large group of structurally and biogenetically diverse natural compounds derived from active isoprene. Triterpenes, especially pentacyclic ones, are secondary metabolites that are widely distributed in plants and are found in leaves, bark, fruits and roots. The curative potential of triterpenes is very high but is still little known. A number of in vitro and in vivo studies have revealed its properties as anti-cancer, antioxidant, anti-inflammatory, anti-atherosclerotic or antiviral (Nazaruk and M. Borzym, 2015).

## V. Conclusion

Leaves, Green Coffee, Black Coffee and Robusta Coffee Fruit Skin Derived from Jember Regency, East Java Province, Indonesia contain protein, amino acids and bioactive components that can be used for health.

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