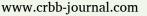


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# Comparative study of ultrasonic and maceration extraction in enhancing antioxidant and SPF properties of green coffee beans serum

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# ABSTRACT

This study investigates the efficiency of maceration and ultrasonic extraction methods for obtaining antioxidants from Arabica, Robusta, and Liberica green coffee beans. Ultrasonic extraction demonstrated higher yields and enhanced antioxidant activity, with Liberica exhibiting the most potent radical scavenging potential (lowest IC<sub>50</sub> values), followed by Robusta and Arabica in 45.3706 ppm, 46.6647 ppm, 49.4257 ppm. Formulated serums derived from these extracts were evaluated for compliance with SNI 16-4399-1996 standards. Both methods produced serums with acceptable texture, homogeneity, pH levels, and active ingredient retention. However, ultrasonic-derived serums displayed superior microbial safety profiles, with significantly lower total plate counts. Viscosity analysis revealed higher values for maceration-derived serums, while Sun Protection Factor (SPF) evaluation indicated that serum of Liberica extract provided the highest UV protection. These findings emphasize the potential of ultrasonic extraction and Liberica green coffee beans in developing high-value cosmetic and pharmaceutical products, paving the way for further research into optimized extraction techniques and broader applications.

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# 1. Introduction

Anti-aging serums are a rapidly evolving field in medicine and cosmetics, focusing on preventing aging and addressing age-related issues (Chaudhari and Patil, 2024). Aging is characterized by a decline in organ function, including the skin, through intrinsic (chronological) processes, often accelerated by extrinsic factors such as UV radiation, pollutants, and smoking (Preston and Biddell, 2021). These factors trigger oxidative stress via reactive oxygen species (ROS), accelerating skin aging. Antioxidants are widely used in anti-aging cosmetics to counteract ROS effects (Pourzand et al., 2022).

Natural antioxidants have gained significant attention for their biological potential in preventing oxidative stress-related diseases (Cardoso and Fazio, 2020). Plant-based sources, such as spices, fruits, and vegetables, are rich in antioxidant compounds, including phenols and vitamin C (Chibuye et al., 2024; Lourenço et al., 2019). Among these, green coffee beans are notable for their high levels of chlorogenic acids (CGA), which exhibit strong antioxidant activity (Leta et al., 2021).

Indonesia is one of the top coffee producers globally, contributing approximately 7% of the world's coffee production, with an annual export volume exceeding 600,000 tons (BPS, 2024). Coffee (*Coffea* sp.) is a vital agricultural commodity, providing significant economic and social benefits to the country. The primary species cultivated in Indonesia are Arabica (*Coffea arabica*),

Robusta (*Coffea canephora*), and Liberica (*Coffea liberica*) (Lim et al., 2019). Studies have shown that green coffee beans possess significant antioxidant properties, with light roasting of Arabica beans yielding the highest activity (Jelena and Yustiantara, 2022). These findings suggest the potential of green coffee beans as valuable sources of antioxidants to combat oxidative stress (Leta et al., 2021). The utilization of local industries in the development of coffee beans-based serum provides a great opportunity to increase economic value as well as environmental sustainability. Through research and development of innovative products, both from coffee beans and waste, local industries can contribute to public health and environmental preservation (Arya et al., 2022).

Traditional extraction techniques used to isolate antioxidants from plant materials include maceration, infusion, digestion, decoction, and soxhlet extraction (Rasul, 2018). These methods rely on solvent interactions to extract bioactive compounds but are often limited by efficiency and environmental considerations. Recent advancements have introduced eco-friendly extraction techniques, such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and enzymeassisted extraction (EAE), which enhance yield and process efficiency (Achmad et al., 2022). These methods, particularly UAE, have shown promise in isolating antioxidant components from green coffee beans efficiently (Bravo et al., 2013; Gligor et al., 2023). While numerous studies have examined extraction methods for antioxidants, there is a paucity of research investigating their subsequent usage in cosmetic formulations, especially in anti-aging serums. The extraction method determines both the yield and the composition and efficacy of the produced bioactive chemicals. The efficacy of these substances is generally assessed via antioxidant activity assays, such as the DPPH assay, to ascertain their capacity to neutralize reactive oxygen species (ROS). Moreover, their integration into serum compositions must provide stability and bioavailability while maintaining the intended protective benefits against oxidative stress and UV radiation. An integrated approach that connects the extraction process, measurement of antioxidant activity, and formulation quality is crucial for the successful development of functional cosmetic products.

In product development, delineating the active components and validating their efficacy is a crucial preliminary phase to guarantee that the extracted antioxidants maintain their potency in the final formulation. The Quality by Design (QbD) methodology emphasizes the necessity of formulating products that preserve the stability and effectiveness of active components, guaranteeing that the end serum adheres to established quality criteria. Nonetheless, current research seldom offers a thorough assessment of the impact of various extraction techniques on the performance and regulatory adherence of the finished product (Aru et al., 2024).

This study seeks to assess the efficacy of maceration and ultrasonic extraction techniques in extracting antioxidants from Arabica, Robusta, and Liberica green coffee beans. The study will develop an antioxidant serum utilizing these extracts and evaluate its microbiological safety, antioxidant efficacy, sun protection factor (SPF), and adherence to SNI requirements. This research aims to improve the formulation of high-value cosmetic and pharmaceutical goods by combining modern extraction techniques with stringent quality evaluation, utilizing green coffee bean extract as a natural antioxidant source. A comprehensive assessment of extraction and formulation parameters guarantees that the final serum adheres to industry standards and offers good defense against oxidative damage and UV exposure.

# 2. Materials and methods

### 2.1. Materials

# 2.1.1. Material collection and simplisia preparation

This investigation utilized Arabica green coffee bean, Robusta green coffee beans, Liberica green coffee beans. For Arabica and Robusta green coffee beans are taken from farmers in Temanggung Regency, Central Java, and Liberica green coffee beans are taken from Arjuno Mountain, East Java. The beans were dried, cleaned, and pulverized to prepare the simplisia.

# 2.1.2. Chemical reagents

The reagents used in this study include 96% Ethanol, Propylene Glycol, Refined Glycerine, Carbomer 940, D-Glucitol, Dimethyloldimethyl (DMDM) Hydantoin, Hyaluronic Acid, Niacinamide, L-Ascorbic Acid (LAA), Rose Hydrosol, Distilled Water. All chemicals were of analytical grade from Luwei Pharmaceutical Group Co., Ltd, China.

# 2.2. Methods

#### 2.2.1. Extraction process

Two extraction methods were employed: maceration and ultrasonic extraction.

#### 2.2.1.1. Maceration extraction

In sample preparation each coffee bean variety was weighed to 100 grams and ground into smaller fragments. Then the ground coffee beans were macerated in 96% ethanol at a 1:6 ratio (w/v)

for 10 days with occasional stirring to ensure adequate solvent interaction. The mixture was filtered to separate the liquid extract, which was then concentrated using a rotary evaporator at a temperature not exceeding  $60^{\circ}$ C. The extraction yield was calculated using the formula:

%Yield = 
$$\frac{\text{weight of viscous extract (g)}}{\text{weight of simplisia (g)}} \times 100\%$$

#### 2.2.1.2. Ultrasonic extraction

Similar to maceration, the coffee beans were ground and weighed to 100 grams. The ground beans were mixed with 96% ethanol (1:6 ratio, w/v). The mixture was sonicated using an ultrasonic probe device FS-300N at a frequency of 20 kHz, amplitude of 100%, and temperature of  $50^{\circ}$ C for 10 minutes. The extract was filtered and concentrated using the same rotary evaporation process as in maceration. The yield was calculated using the same formula.

#### 2.2.2. Serum formulation

Anti-aging serums were prepared using the following formulation (in percentage by weight): distilled water (65,61%), propylene glycol (10%), refined glycerin (12%), D-glucitol (1,19%), DMDM hydantoin (0,5%), carbomer 940 (0,1%), rose hydrosol (0,15%), LAA (3%), niacinamide (3%), hyaluronic acid (0,4%), and coffee beans extract (3%). Then, all ingredients were mixed thoroughly until homogeneous. The serum was transferred into dark, airtight containers and followed to settle for 1-3 hours to eliminate foaming. The pH of the serum was adjusted and verified. The process was repeated for extracts derived from maceration and ultrasonic methods for Arabica, Robusta, and Liberica coffee beans. All chemicals were of analytical grade from Luwei Pharmaceutical Group Co., Ltd, China.

#### 2.2.3. Serum testing based on SNI 16-4399-1996

The serum formulations were evaluated based on the SNI 16-4399-1996 standards to ensure their quality and safety. The tests included organoleptic analysis to assess texture, color, and odor, as well as homogeneity checks performed under light to confirm consistency. pH values were measured to ensure they fell within the acceptable range of 4,0 to 6,0, while viscosity was determined using a viscometer. The specific gravity of each serum was calculated using a pycnometer. Microbial contamination tests were conducted, including total plate count (ALT) and checks for the absence of pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*. These microbiological tests adhered to MA PPOM 61/MIK/06 standards, confirming the safety and stability of the formulations.

# 2.2.4. DPPH (2,2-diphenyl-1-1-picrylhydrazyl) analysis

For the antioxidant activity (DPPH 2,2-diphenyl-1-1picrylhydrazyl) (Himedia), a 0,15 mM DPPH solution was prepared in ethanol. A stock solution of the extract was prepared and diluted to varying concentrations. The samples were mixed with the DPPH solution and incubated for 30 minutes in the dark. Absorbance was measured at 512 nm using a spectrophotometer UV probe-1800 (Shimadzu, Japan). The percentage of Radical Scavenging Activity (RSA%) was calculated using the formula:

$$RSA\% = \frac{A_{blank} - A_{sample}}{A_{blank}} \ x \ 100\%$$

where,

A<sub>blank</sub>: absorbance of the control sample (96% ethanol pro analysis + DPPH in 96% ethanol).

A<sub>sample</sub>: absorbance of sample (experimental sample in 96% ethanol + DPPH in 96% ethanol).

The correlation between antioxidant activity and percentage inhibition is examined by linear regression to ascertain the  $IC_{50}$ value. A reduced  $IC_{50}$  value signifies enhanced antioxidant activity. The  $IC_{50}$  is determined using a linear regression equation, with sample concentration shown on the x-axis and percentage inhibition on the y-axis:

$$y = ax + b$$
  

$$50 = ax + b$$
  

$$(x)IC_{50} = \frac{50-a}{b}$$

where,

y: dependent variable (percentage inhibition in the context of DPPH assay)

a: slope of the line

b: constant (intercept or point of intersection on the axis)

*x*: independent variable (extract concentration)

#### 2.2.5. Sun protection factor SPF analysis

SPF values of serums were determined using Mansur's method. Samples were diluted in analytical grade ethanol, and absorbance was measured at wavelengths 290-320 nm in 5 nm increments using a UV-Vis spectrophotometer. SPF was calculated using the formula:

SPF = CF 
$$x \sum_{\lambda=290}^{320} EE x I(\lambda) x Abs(\lambda)$$

where,

CF: correction factor (10),EE: erythemal effect,I: UV intensity, andAbs: absorbance of the sample.The SPF analysis was performed in triplicate for accuracy.

 Table 1. Constant value EE x I

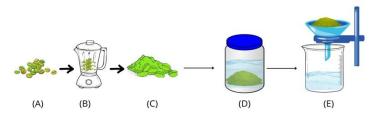
Wavelength (nm)	EE x I
290	0.015
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.018
320	0.9163

#### 3. Results and discussion

# 3.1. Outcome of extraction

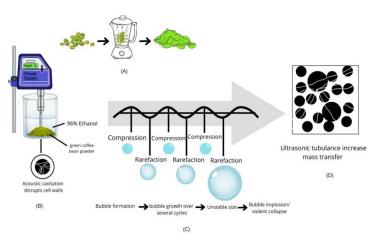
The schematic extraction procedure for coffee beans maceration commences with coarse grinding utilizing a blender to diminish the size of the coffee beans particles. This reduction enhances the surface area, promoting the release of active chemicals during extraction. The ground material is subsequently placed in an extraction vessel and immersed in 96% ethanol solvent. Periodic stirring is conducted during the soaking procedure. Stirring is essential for optimizing extraction by facilitating interaction between the solvent and coffee particles, promoting uniform solvent dispersion, and reducing sedimentation of the material at the container's base. This enhanced agitation facilitates effective mass transfer, allowing bioactive chemicals, including antioxidants, to dissolve more readily and swiftly in the solvent in Fig. 1.

Upon completion of the soaking and stirring phase, the mixture is subjected to filtration to segregate the particles from the extract solution. The filtration process guarantees that the filtrate is gathered in a sterile container for other procedures, such as evaporation. The evaporation procedure eliminates the solvent, resulting in a concentrated extract abundant in bioactive chemicals. Regular agitation throughout the maceration process is essential for optimizing the extraction efficiency of critical chemicals from coffee beans, hence improving the overall efficacy of the process (Myo and Khat-udomkiri 2022).



**Fig. 1.** Protocol for maceration extraction procedure. (a) green coffee beans, (b) green coffee beans fining using a blender, (c) green coffee beans powder, (d) maceration of green coffee beans with 96% ethanol, and (e) perform filtration to separate the result with marc.

Depicts a flowchart of the ultrasonic extraction technique for coffee beans in Fig. 2. The procedure commences with the coarse grinding of coffee beans utilizing a blender, which diminishes particle size and enhances surface area to promote effective extraction. The resultant ground material is subsequently combined with 96% ethanol solvent within the extraction vessel. High-frequency ultrasonic waves are then delivered into the mixture via an ultrasonic instrument. These waves cause acoustic cavitation, a phenomenon in which cycles of compression and rarefaction occur in the liquid due to the sound waves, hence improving the extraction process.



**Fig. 2.** Protocol for ultrasonic extraction procedure. (a) prepared the green coffee beans for ultrasound extraction, (b) process the ultrasound, (c) illustration of how ultrasonic devices work, and (d) ultrasonic device use effect results.

It is a process defined by the formation of small cavitation bubbles that expand and collapse in exceedingly brief intervals. This abrupt collapse generates high pressure and liquid microjets that impact material particles, resulting in cell wall rupture or disintegration (Sieber et al., 2022). This mechanism facilitates the fast release and dissolution of bioactive chemicals into the solvent. Ultrasonication is a proficient technology for improving mass transfer, reducing extraction duration, and producing superior yields in comparison to traditional processes such as maceration (He et al., 2021; Sasidharan et al., 2011). Following sonication, the mixture is subjected to filtration to isolate the coffee grounds from the extract solution. The filtrate can undergo additional processing using methods like evaporation to concentrate the extract. This technology, owing to ultrasonic cavitation effects, demonstrated superior efficiency in extracting bioactive components from coffee beans, yielding outstanding results in a notably little timeframe.

Fig. 3 presents a comparative analysis of the yields obtained from two extraction methods: Maceration Extraction and Ultrasonic Extraction, applied to three coffee varieties: Arabica, Robusta, and Liberica. The yield is presented at two distinct phases: initial extraction yield and final yield post-evaporation. Fig. 3 displays the results of maceration extraction and ultrasonic extraction of Arabica, Robusta, and Liberica green coffee beans

This research indicates that Ultrasonic Extraction yields superior results compared to Maceration Extraction across all coffee varieties. The yields from ultrasonic extraction are marginally superior to those from maceration in all three coffee varieties tested: Arabica, Robusta, and Liberica in Fig. 3. Following the evaporation process in both maceration and ultrasonic procedures, the volume of the extraction yield has significantly diminished, with the red and purple bars markedly less than the initial extraction yield. This indicates that the majority of the extracted liquid has evaporated, resulting in a reduced quantity of concentrate.

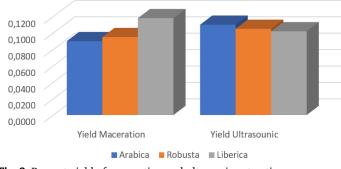


Fig. 3. Percent yield of maceration and ultrasonic extraction

This pattern exhibits uniformity across the three coffee kinds. The extraction yield analysis for maceration and ultrasonic methods demonstrates distinct differences across Arabica, Robusta, and Liberica green coffee beans. Ultrasonic extraction consistently yields higher volumes compared to maceration due to the efficiency of cavitation-induced cell wall disruption, enhancing the release of bioactive compounds. The results align with prior findings that highlight the superior efficiency of ultrasonic techniques in botanical extractions.



**Fig. 4.** (a) Outcomes of maceration extraction for robusta, liberica, and arabica coffee beans; (b) outcomes of ultrasonic extraction for arabica, liberica, and robusta coffee beans.

Depicts the variations in extraction yields for Arabica, Robusta, and Liberica green coffee beans utilizing maceration and ultrasonic extraction techniques in Fig. 4a illustrates the results of the maceration process, whereas Fig. 4b depicts the outcomes of ultrasonic extraction. The analysis demonstrates that ultrasonic extraction consistently attains superior extraction yields for all coffee varieties. This benefit is ascribed to the cavitation effects produced by ultrasonic waves, which efficiently rupture cell walls and promote the release of bioactive substances into the solvent. Maceration, by contrast, depends on passive diffusion, which naturally constrains the efficiency and velocity of the process. These findings indicate that ultrasonic extraction not only expedites the extraction process but also markedly improves the recovery of valuable bioactive components, establishing it as a superior approach for extracting antioxidants from green coffee beans.

The extraction yield analysis for maceration and ultrasonic methods demonstrates distinct differences across Arabica, Robusta, and Liberica green coffee beans. Ultrasonic extraction consistently yields higher volumes compared to maceration due to the efficiency of cavitation-induced cell wall disruption, enhancing the release of bioactive compounds. This aligns with prior findings highlighting the superior efficiency of ultrasonic techniques in botanical extractions.

Maceration offers simplicity in operation, making it suitable for small-scale extractions. Additionally, it relies less on sophisticated equipment, thereby reducing initial costs. However, this method has significant drawbacks, including prolonged extraction times that limit its scalability for industrial applications. The higher solvent consumption not only increases production costs but also has a negative environmental impact. Furthermore, the higher viscosity in the resulting extract poses challenges for cosmetic formulations that require lighter textures.

In contrast, ultrasonic extraction significantly reduces extraction time through cavitation, leading to enhanced efficiency. This method also produces lower-viscosity extracts, making it advantageous for serum formulations. Moreover, it requires less solvent, which contributes to eco-friendliness and cost efficiency. Despite these advantages, ultrasonic extraction involves a high initial investment in equipment and carries the risk of bioactive compound degradation if ultrasonication settings are not properly controlled.

# 3.2. Antioxidant activity of green coffee beans by maceration and ultrasonic extraction

The DPPH test of green coffee beans extract seeks to assess its antioxidant potential. The measurement of Arabica, Robusta, Lierica green coffee beans extracted from DPPH test findings was conducted using a spectrophotometer, yielding the % inhibition value for each concentration (IC<sub>50</sub>). The % inhibition values are presented in Table 2.

Fig. 5 and Table 2 provide comprehensive insights into the relationship between the concentration of green coffee beans extracts and the percentage of inhibition (% inhibition), as well as the effectiveness of the extraction methods, namely maceration and ultrasonic extraction.

In Fig. 5a, the linear regression demonstrates a strong relationship between concentration (ppm) and % inhibition, with  $R^2$  values exceeding 0.99 for all varieties (Arabica, Robusta, and Liberica). The linear equation for Arabica (y= 0.5865x + 8.4494) reveals a positive intercept, indicating the presence of initial inhibitory activity even at lower concentrations. Robusta (y = 0.6189x + 10.671) shows a steeper slope, suggesting a higher increase in inhibition per unit concentration compared to Arabica. Liberica (y= 0.6597x + 3.842) exhibits the highest slope, highlighting its superior inhibitory effectiveness in the maceration method.

In contrast, the Fig. 5b shows significant improvements in inhibitory activity with the ultrasonic method. Arabica's linear equation (y=0.9631x + 5.0587,  $R^2=0.9982$ ) indicates a much steeper slope compared to the maceration method, reflecting greater extraction efficiency of bioactive compounds. Robusta (y=1.0288x - 0.8485) demonstrates an even higher slope, emphasizing the method's effectiveness for this variety. Liberica maintains its superior performance with the equation y=0.9884x + 5.1534 ( $R^2 = 0.9993$ ), consistently showing the highest inhibition potential across both methods.

Table 2. Table of percent inhibition of extract coffee beans utilizing the maceration and ultrasonic extraction method

Type of green coffee	Maceration process				Ultrasonic process			
	Concentration (ppm)	Average of percent inhibition	Standard deviation	IC <sub>50</sub> (ppm)	Concentration (ppm)	Average of percent inhibition	Standard deviation	IC <sub>50</sub> (ppm)
	20	20.644	0.174		20	24.583	0.174	
	40	31.364	0.114		40	44.167	0.237	46.6647
Arabica	50	37.652	0.174	70.4417	50	52.841	0.114	
Arabica	60	44.484	0.174	/0.441/	60	61.439	0.174	
	80	53.750	0.114		80	83.030	0.174	
	100	68.068	0.114					
	20	22.273	0.227	63.5459	20	19.356	0.174	49.4257
	40	34.280	0.237		40	39.773	0.114	
Dalareta	50	42.917	0.174		50	51.364	0.114	
Robusta	60	49.318	0.114		60	61.894	0.174	
	80	60.455	0.114		80	80.568	0.227	
	100	71.402	0.286					
	20	16.023	0.114		20	24.886	0.301	
Liberica	40	29.811	0.237		40	44.091	0.114	45.3706
	50	37.803	0.174	69.6462	50	54.886	0.114	
	60	44.848	0.174		60	65.303	0.174	
	80	56.629	0.174		80	83.712	0.174	
	100	68.826	0.174					

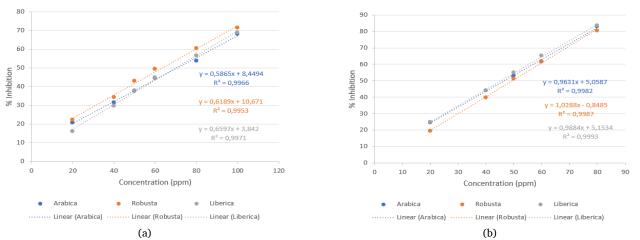
Based on the IC<sub>50</sub> values presented in the Table 2, the ultrasonic extraction method significantly reduces the IC50 values compared to maceration, indicating more effective inhibitory activity. For Arabica, the IC<sub>50</sub> decreases from 70.4417 ppm with maceration to 46.6647 ppm with ultrasonic extraction. Robusta also demonstrates a notable reduction from 63.5459 ppm to 49.4257 ppm, while Liberica achieves the lowest IC<sub>50</sub> among all varieties, dropping from 69.6462 ppm with maceration to 45.3706 ppm with ultrasonic extraction. These reductions confirm the superiority of the ultrasonic method in extracting active compounds responsible for inhibition. The antioxidant potential of green coffee beans extracts was evaluated using the DPPH assay. The IC<sub>50</sub> values for ultrasonic extraction were significantly lower than those for maceration across all coffee beans varieties, indicating enhanced radical scavenging activity. Liberica exhibited the highest antioxidant activity, followed by Robusta and Arabica. This trend underscores the potential of Liberica as a potent source of antioxidants, particularly when extracted using ultrasonic methods. The reduced IC50 values reflect the ability of ultrasonic techniques to preserve and concentrate active compounds.

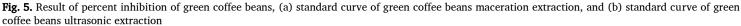
# 3.3. Antioxidant activity serum of green coffee beans by maceration and ultrasonic extraction

The DPPH test of serum green coffee beans seeks to assess its antioxidant potential. The serum of Arabica, Robusta, Liberica green coffee beans from DPPH test findings was conducted using a spectrophotometer, yielding the % inhibition value for each concentration (IC<sub>50</sub>). The % inhibition values are presented in Table 3.

The data presented in Table 3 and the associated graphs illustrate the inhibitory potential of green coffee beans (Arabica, Robusta, and Liberica) under two extraction methods: maceration and ultrasonic processes. The inhibition values, expressed as  $IC_{50}$  (the concentration required to inhibit 50% of the activity), provide crucial insights into the efficacy of each extraction method. For the maceration process, Arabica beans demonstrated an  $IC_{50}$  value of 2212.42 ppm, indicating moderate inhibition activity compared to Robusta (2163.56 ppm) and Liberica (2487.63 ppm). This trend signifies that Robusta exhibits the most potent inhibitory effect among the three, as indicated by its lowest  $IC_{50}$ . Similarly, for the ultrasonic extraction process, Robusta retained its superior activity with an  $IC_{50}$  of 2266.80 ppm).

The graphical representation (Fig. 6a and 6b) further substantiates these findings. The linear regression equations derived from the standard inhibition curves (y = mx + c) provide a visualization of the relationship between concentration and percent inhibition. Notably, the slope of the regression lines for Robusta consistently exhibits a steeper gradient, affirming its enhanced inhibition efficiency compared to Arabica and Liberica across both extraction methods.





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Table 3. Table of percent inhibition of serum coffee beans utilizing the maceration and ultrasonic extraction method

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True of succes	Maceration Process			Ultrasound Process					
Type of green coffee	Concentration (ppm)	Average of percent Inhibition	Standard Deviation	IC <sub>50</sub> (ppm)	Concentration (ppm)	Average of percent inhibition	Standard Deviation	IC <sub>50</sub> (ppm)	
	1	29.205	0.227		1	29.053	0.237		
	1.5	36.326	0.286		1,5	33.750	0.227		
Anahiaa	2	46.894	0.237	0010 40	2	42.879	0.237		
Arabica	2,5	54.659	0.114	2212.42	2.5	50.644	0.174	2582.50	
	3	65.152	0.459		3	57.879	0.174		
	5	80.417	0.174		5	67.311	8.201		
	1	28.144	0.174		1	19.356	0.174		
	1.5	37.386	0.114	2162 56	1.5	39.773	0.114		
Dalareta	2	48.636	0.114		2	51.364	0.114	2266.80	
Robusta	2.5	57.386	0.114	2163.56	2.5	61.894	0.174		
	3	64.773	0.114		3	80.568	0.227		
	5	81.894	0.174		5	70.568	9.940		
	1	24.773	0.114		1	28.068	0.227		
Liberica	1.5	33.939	0.237	2487.63	1.5	36.705	0.227		
	2	41.742	0.174		2	45.114	0.227	2202 50	
	2.5	50.606	0.286		2.5	53.371	0.174	2302.59	
	3	59.508	0.286		3	62.955	0.114		
	5	74.129	0.237		5	76.818	0.114		

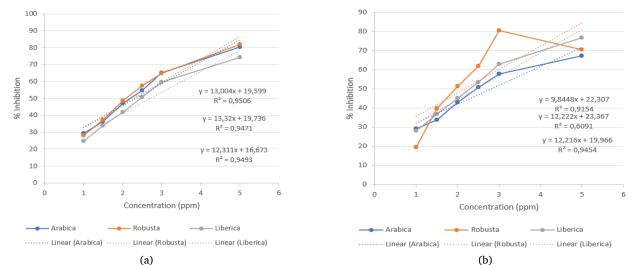


Fig. 6. Result of percent inhibition of green coffee beans, (a) standard curve of serum green coffee beans maceration extraction, and (b) standard curve of serum green coffee beans ultrasonic extraction

The comparison between maceration and ultrasonic extraction highlights the impact of methodology on bioactive compound availability. While both processes demonstrated a similar trend in  $IC_{50}$  ranking, the ultrasonic process achieved slightly higher inhibition percentages at equivalent concentrations. This suggests that ultrasonic extraction may enhance the release or activity of inhibitory compounds in green coffee beans.

The findings emphasize that both extraction methods are effective in isolating bioactive compounds from green coffee beans, with Robusta displaying the highest inhibition potential. However, the ultrasonic method showed marginally better results in terms of inhibition efficiency, likely due to its ability to enhance compound extraction. This research underscores the significance of extraction techniques and beans variety in determining inhibitory activity, providing a basis for further exploration into optimizing extraction methods for therapeutic applications.

# 3.4. Outcome serum testing based on SNI 16-4399-1996

The results of serum testing based on SNI 16-4399-1996 standards demonstrate the overall quality and compliance of formulations derived from Arabica, Robusta, and Liberica green coffee beans using maceration and ultrasonic extraction methods. All serum samples exhibited homogeneous textures, meeting the standard for product consistency. The pH values ranged from 4.0 to 5.0, aligning well with the acceptable range of 4.0-6.0, ensuring suitability for safe application on the skin. Specific gravity values, which ranged between 0.9464 and 1.0, also adhered to the specified standards, indicating consistent formulation characteristics (SNI, 1996).

In terms of viscosity, there were notable differences between the two extraction methods and coffee beans types. The maceration process generally produced serums with higher viscosities, with Liberica achieving the highest value of 4493 cP. In contrast, the ultrasonic process yielded serums with comparatively lower viscosities, with Liberica again having the highest value at 2433 cP. These differences suggest that the maceration process retains more structural integrity of bioactive compounds contributing to viscosity, whereas ultrasonic extraction may result in slightly thinner formulations.

All serum formulations successfully retrieved active ingredients in accordance with Ministry of Health Regulation No.376/Menkes/Per/VIII/1990 and met preservative requirements, ensuring product stability and efficacy. However, in terms of microbial safety, slight differences were observed. While all formulations complied with microbial standards for *Pseudomonas*  *aeruginosa, Staphylococcus aureus*, and Aerobic Plate Counts (APM), the maceration-derived Liberica serum showed the presence of mold. Notably, Total Plate Count (ALT) values for the ultrasonic-extracted serums were significantly lower (<10 cfu/mL), highlighting the effectiveness of this method in minimizing microbial contamination.

Overall, the formulated serums were assessed for compliance with SNI 16-4399-1996 standards. Both extraction methods produced serums meeting texture, homogeneity, and pH

Table 4. Result serum testing based on SNI 16-4399-1996

requirements (4.0-6.0). However, microbial contamination analysis revealed minor discrepancies, with ultrasonic-derived serums showing lower total plate counts, affirming their superior safety profile.

Viscosity differences were noted between the two extraction methods, with maceration-derived serums exhibiting higher viscosities. This could be attributed to the retention of structural polysaccharides in maceration. Nonetheless, all formulations remained within acceptable viscosity ranges.

Criteria	Standard SNI	Maceration process			Ultrasound process		
Criteria	Standard Sini	Arabica	Robusta	Liberica	Arabica	Robusta	Liberica
Shifting	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH	4.0-6.0	4.5	4.5	4.5	4.5	4.5	4.5
Specific Gravity	0.95 – 1.05 g/ml	0.9648	1.0047	0.9642	0.958	0.9746	0.9832
Viscosity	2000 - 50000 Cp As per	4371	3893	4493	3595	2779	2433
Active Ingredients	Minister of Health Regulation. No.376/Menkes/P er/VIII/1990	Retrieved	Retrieved	Retrieved	Retrieved	Retrieved	Retrieved
Preservative	As per Minister of Health Regulation. No.376/Menkes/P er/VIII/1990	Retrieved	Retrieved	Retrieved	Retrieved	Retrieved	Retrieved
Microba contaminant							
mold	Negative (colonies/gr)	-	+	-	-	-	-
ALT	Maximal 1.0 x 10 <sup>2</sup> colonies/g (colonies/g = cfu/ml)	1.5 x10 <sup>1</sup>	3.6 x 10 <sup>1</sup>	7.0 x 10 <sup>1</sup>	<10 cvu/ml	2.4 x 10 <sup>1</sup>	9.0 x 101
Pseudomonas aeruginosa	Negative	-	-	-	-	-	-
Staphylococcus aureus	Negative	-	-	-	-	-	-
APM	< 3 APM/g	< 3.6	> 1100	< 3.6	< 3.6 APM/ml	< 3.6	< 3.6

#### 3.5. Sun protection factor value

The data presented in Tables 5 and 6 highlight the UV absorption properties and Sun Protection Factor (SPF) values of serums derived from Arabica, Robusta, and Liberica green coffee beans, extracted using maceration and ultrasonic methods. The UV intensity (III) values across wavelengths of 290-320 nm indicate the ability of these serums to absorb UV radiation, which is a key factor in determining their potential as sunscreen agents.

In the maceration process, Liberica exhibited the highest UV intensity values across most wavelengths, peaking at 1.086 at 320 nm. Robusta consistently followed with slightly lower values, while Arabica displayed the lowest UV absorption among the three. For the ultrasonic process, the trend shifted slightly, with Robusta showing higher UV intensity at most wavelengths, particularly at 320 nm with a value of 0.949. Liberica, although slightly reduced in UV intensity compared to its maceration counterpart, still maintained competitive absorption levels, while Arabica again exhibited the lowest UV intensity.

#### Table 5. UV intensity (i) value

Wave	Mac	Maceration Process			Ultrasound Process		
Length	Arabica	Robusta	Liberica	Arabica	Robusta	Liberica	
290	0.814	0.893	0.929	0.761	0.768	0.670	
295	0.835	0.904	0.918	0.758	0.765	0.662	
300	0.853	0.921	0.920	0.763	0.773	0.660	
305	0.859	0.937	0.915	0.764	0.780	0.651	
310	0.878	0.969	0.928	0.781	0.802	0.660	
315	0.954	1.054	0.995	0.840	0.868	0.705	
320	1.030	1.152	1.086	0.919	0.949	0.771	

SPF values calculated using Mansur's method revealed that serums from maceration exhibited slightly higher UV protection levels than those from ultrasonic extraction. Liberica serums consistently showed the highest SPF values, suggesting their suitability as natural sunscreen agents. The maceration process may better preserve UV-absorbing phenolic compounds, although ultrasonic extraction remains a competitive alternative.

In conclusion, the data demonstrate that green coffee bean serums, particularly those derived from Liberica and Robusta, exhibit promising UV absorption and SPF properties. The maceration method is particularly advantageous for maximizing these properties, making it a favorable extraction technique for developing sunscreen formulations. These findings provide valuable insights into the potential application of green coffee beans serums as natural UV-protective agents.

Table 6. Value of sun protect	tion factor
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Extraction method	Coffee bean	SPF value
	Arabica	8.687
Maceration	Robusta	9.490
	Liberica	9.291
	Arabica	7.759
Ultrasonic	Robusta	7.913
	Liberica	6.634

#### 4. Conclusion

This study emphasizes the relative efficacy of maceration and ultrasonic extraction techniques in extracting antioxidants from green coffee beans (Arabica, Robusta, and Liberica). Ultrasonic extraction exhibited notable benefits, such as increased yields and reduced  $IC_{50}$  values, validating its efficacy in conserving and concentrating bioactive chemicals via cavitation-induced cell wall disintegration. This method also decreases processing time and solvent usage, conforming to sustainable practices and rendering it appropriate for extensive industrial applications. In contrast, the maceration method, although more straightforward and economical for small-scale operations, demonstrated constraints in yield and efficiency. Serums obtained through maceration extraction typically exhibit increased viscosity, rendering them more appropriate for cosmetic formulations that necessitate a denser consistency, such as creams or emulsions. Moreover, the elevated SPF values achieved during maceration extraction indicate its prospective use in natural sunscreen compositions.

Regarding efficacy and commercial acceptability, serums derived from green coffee bean extracts adhered to SNI 16-4399-1996 requirements concerning homogeneity, pH, and active component retention. Microbiological safety assessments indicated that serums obtained using ultrasonic extraction demonstrated a superior safety profile, exhibiting markedly lower total bacteria counts compared to those derived from maceration. This suggests that ultrasonic extraction improves extraction efficiency while also enhancing the stability and safety of serum formulations.

Liberica coffee beans consistently surpassed Arabica and Robusta in antioxidant activity and SPF measurements, highlighting their potential as superior raw materials for cosmetic and medicinal formulations. These findings highlight the potential for utilizing locally obtained green coffee beans to create high-value, natural goods. Additional research is required to refine extraction parameters and formulation methods to enhance economic and environmental advantages.

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# Conflict of interest

The authors declare no conflict of interest in this research.

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