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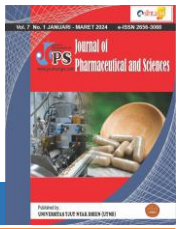

Chlorogenic Acid Quantification and Antioxidant Activity of NADES Extracts from Robusta Green Coffee Beans (*Coffea canephora*) Using MAE and UAE Methods for Cosmetic Raw Material

Penetapan Kadar Asam Klorogenat dan Aktivitas Antioksidan Ekstrak NADES dari Biji Kopi Hijau Robusta (*Coffea canephora*) Menggunakan Metode MAE dan UAE sebagai Bahan Baku Kosmetik

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Abstract

Antioxidants are essential for protecting skin cells from free radicals, causing oxidative damage, and supporting human skin's health. Natural antioxidants are abundant in plants, particularly in Robusta green coffee beans (*Coffea canephora*), which are rich in chlorogenic acid (CA), a key contributor to antioxidant properties. Efficient extraction methods are necessary to obtain these bioactives effectively. Advanced extraction technology, such as Ultrasound-Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE), enhances mass transfer and reduces processing time compared to conventional methods. This study quantified chlorogenic acid using Thin Layer Chromatography-Densitometry (TLC-densitometry) and evaluated the antioxidant activity using the DPPH method of NADES (betaine–triethylene glycol) liquid extracts from Robusta coffee green beans extracted via UAE and MAE. Chlorogenic acid concentration of MAE (3.64 mg CA/g extract ± 0.06) was higher than that of UAE (2.69 mg CA/g extract ± 0.04). The antioxidant activity (IC_{50}) values were 3266.66 µg/mL ± 67.97 (UAE) and 2598.05 µg/mL ± 29.42 (MAE), indicating higher efficiency for MAE. When expressed as chlorogenic acid equivalents in NADES extracts, IC_{50} values were 9.04 µg/mL ± 0.13 (UAE) and 9.44 µg/mL ± 0.07 (MAE). NADES-MAE could be a promising method to acquire raw materials with high antioxidant activity, especially for cosmetic formulation.

***Keywords*: Coffee beans, NADES, MAE, UAE, Antioxidant**

Abstrak

Antioksidan berperan penting dalam melindungi sel-sel kulit dari radikal bebas yang menyebabkan kerusakan oksidatif, sehingga mendukung kesehatan kulit manusia. Antioksidan alami banyak ditemukan dalam tumbuhan, terutama pada biji kopi Robusta hijau (*Coffea canephora*) yang kaya akan asam klorogenat, senyawa utama yang berkontribusi terhadap aktivitas antioksidan. Untuk memperoleh senyawa bioaktif ini secara efektif, diperlukan metode ekstraksi yang efisien. Teknik modern seperti *Ultrasound-Assisted Extraction* (UAE) dan *Microwave-Assisted Extraction* (MAE) dapat meningkatkan perpindahan massa dan mempercepat waktu ekstraksi dibanding metode konvensional. Penelitian ini menentukan kadar asam klorogenat menggunakan *Thin Layer Chromatography-Densitometry* (TLC-densitometri) dan mengukur aktivitas antioksidan dari ekstrak NADES (betaine–triethylene glycol) biji kopi Robusta hijau yang diekstraksi dengan metode UAE dan MAE. Kapasitas antioksidan dianalisis menggunakan uji DPPH. Hasil menunjukkan kadar asam klorogenat pada MAE (3,64 mg AK/g ekstrak ± 0,06) lebih tinggi dibandingkan dengan UAE (2,69 mg

AK/g ekstrak ± 0,04). Nilai IC_{50} aktivitas antioksidan adalah 3266,66 µg/mL ± 67,97 (UAE) dan 2598,05 µg/mL ± 29,42 (MAE). Jika dinyatakan sebagai ekivalen asam klorogenat, nilai IC_{50} masing-masing sebesar 9,04 ± 0,13 µg/mL (UAE) dan 9,44 ± 0,07 µg/mL (MAE). Hasil ini menunjukkan bahwa metode NADES-MAE dapat menjadi metode yang menjanjikan untuk mendapatkan bahan baku kaya antioksidan terutama untuk formulasi kosmetika.

Kata Kunci: Biji kopi, NADES, MAE, UAE, Antioksidan.

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Introduction

Cellular metabolism naturally produces reactive species as a result of oxygen utilization. These include reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are generated through redox processes. These radicals, in their outer orbitals, are defined as fragments possessing unpaired electrons, are highly reactive and capable of initiating chain reactions [1]. When excessive radicals accumulate in the skin, they can induce chronic damage, including uneven skin color, premature aging, and cancer. Environmental factors such as radiation, air pollution, vehicle emissions, and smoking exacerbate radical formation and weaken the skin's antioxidant defenses. Consequently, exogenous antioxidants play a vital role in mitigating oxidative stress [2].

Antioxidants counteract free radicals by donating electrons, thereby terminating oxidative chain reactions. These compounds may be endogenous—such as enzymatic antioxidants (e.g., catalase, glutathione peroxidase) and trace elements (e.g., Se, Mn, Cu, Zn)—or exogenous, including vitamins A, C, E, and phytochemical-rich plant extracts [3,4]. Among plant sources, Robusta coffee (*Coffea canephora*) has emerged as a promising candidate due to its bioactive components, including alkaloids, tannins, saponins, and polyphenols. Chlorogenic acid, the major phenolic compound in Robusta coffee, can establish up to 90% of its total phenolic content and has been associated with hepatoprotective, antiviral, and potent antioxidant properties [5,6,7].

Extraction is a critical process to isolate these bioactive constituents, involving the use of appropriate solvents to separate target compounds from solid matrices [8]. Although maceration is widely used, it presents several drawbacks, including prolonged extraction times, large solvent volumes, and the potential use of toxic solvents [9]. More advanced extraction technology, such as UAE and MAE, offers increased efficiency by employing ultrasonic waves or microwave power to enhance the extraction process [10,11].

The choice of solvent is crucial for effective extraction. Natural Deep Eutectic Solvents (NADES), such as a combination of betaine and triethylene glycol, are gaining attention for their non-toxic, thermally stable, recyclable, and environmentally friendly [12]. NADES have demonstrated superior extraction efficiency for chlorogenic acid and caffeine compared to organic solvents [13]. Specifically, NADES, betaine–triethylene glycol, has been defined to yield higher concentrations of chlorogenic acid than methanol and other traditional solvents. NADES extraction results can be directly formulated into a formulation for cosmetic preparation, making it an efficient method because the evaporation process is not needed. Apart from being an extracting solution, betaine and triethylene glycol themselves have a function in cosmetic formulation as a humectant [14].

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This study aims to evaluate the chlorogenic acid content and antioxidant activity of Robusta green coffee bean extracts prepared using NADES betaine–triethylene glycol using UAE and MAE techniques. The antioxidant capacity was assessed using the DPPH assay, selected for its rapidity, simplicity, and stability in polar media [15].

Experimental Section

Materials and Apparatus

The materials used in this study are as follows: Red cherry robusta green bean coffee, obtained from local coffee farmers in Tanggul District, Jember Regency, on May 23, 2024; TLC silica gel 60 F_{254} plates (Merck, Germany); betaine (Thermo Fisher Scientific, Spain); triethylene glycol (Lamurindo, Indonesia); methanol p.a. (Merck, Germany); DPPH (Sigma-Aldrich, USA); chlorogenic acid (Sigma-Aldrich, USA); deionized water (WaterOne, Indonesia); distilled water (aquabidest) (WIDA WITM Unicap, Indonesia); ethyl acetate (Merck, Germany); and formic acid (Merck, Germany). The instrument used in this study includes: pipette, micropipette (Acura manual® 825, Switzerland), stopwatch, Whatmann filter paper No. 42 (11 cm diameter), filter paper, centrifuge (Herml Z206-A, Germany), hot plate (Corning PC-420D, USA), Erlenmeyer flask (Iwaki, Indonesia), ultrasonic bath (Elmasonic Select 100, Germany), microwave oven (Sanyo EM-S105AW, Japan), UV-Vis spectrophotometer (Shimadzu UV1280, Japan), densitometer scanner (Camag TLC Scanner 3, Switzerland), computer with winCATS software, ultraviolet (UV) lamp, analytical balance (Ohaus PX224E Pioneer, USA), and flat bottom chamber (Camag, Switzerland).

Preparation of Dry Powder Sample

Robusta green coffee beans were manually peeled to remove the epidermal layer, then placed into an oven at 50°C for 2 hours to reduce the moisture content to below 10%. The dried beans were subsequently ground using a mechanical grinder and passed through a No. 60 mesh sieve to obtain a uniform powder. The dried powder (500 mg) was placed into a Moisture Analyzer for moisture content.

Preparation of NADES

The NADES was prepared by combining betaine (as the hydrogen bond acceptor, HBA) and triethylene glycol (as the hydrogen bond donor, HBD) in a 1:2 molar ratio. A total of 70% of this mixture was blended with 30% deionized water in an Erlenmeyer flask. The solution was then stirred continuously at 1000 rpm on a hot plate stirrer at 80°C for 30 minutes until a clear, colorless liquid was formed [16].

Extraction Procedure

The extraction of Robusta green coffee bean dry powder was made using UAE and MAE. Initially, 1 gram of a finely ground Robusta green coffee bean sample was weighed accurately and transferred into an Erlenmeyer flask, followed by the addition of 20 mL of betaine–triethylene glycol-based NADES as the extraction solvent. For the UAE method, the mixture was subjected to ultrasonic extraction at 37 kHz, 50°C for 30 minutes using an ultrasonic bath that was already cleaned before use. In the MAE method, extraction was performed at 50°C for 30 minutes using a microwave oven with 350 W power output [17]. For the separation of solid residues from the liquid phase, the resulting extracts from both methods were then centrifuged at 5000 rpm for 10 minutes. The supernatant was subsequently filtered through Whatman No. 42 filter paper using a Buchner funnel to obtain a clear liquid extract.

Determination of Chlorogenic Acid Content

The determination of chlorogenic acid content was conducted using TLC chromatography, with chlorogenic acid as the standard, and analysis was performed using a densitometric scanner. A total of 3.75 mg of chlorogenic acid standard was dissolved in 25 mL of analytical-grade methanol for a standard solution with a concentration of 150 µg/mL. This solution was diluted to obtain a concentration series of 125, 100, 75, 50, and 25 µg/mL. For the samples, NADES betaine–triethylene glycol liquid extract of Robusta green coffee beans obtained from both UAE and MAE methods was prepared by weighing 0.8 g of extract and dissolving it in 50 mL of methanol p.a to yield a concentration of 16,000 µg/mL. Planar chromatography was carried out by spotting the samples onto silica gel 60 F_{254} TLC plates (20 × 10 cm) using 2.0 µL glass capillaries. The development chamber was saturated with a mobile phase composed of formic acid: ethyl acetate: distilled

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water in a 1:8:1.5 (v/v/v) ratio. The sample spots migrated horizontally to a distance of 8 cm. Densitometric scanning was performed using a TLC Scanner in absorbance mode at a wavelength of 335 nm for all measurements. The Rf of chlorogenic acids from the standard and sample were compared [18].

Determination of Antioxidant Activity

Preparation of DPPH Solution

A total of 4 mg DPPH was dissolved in 100 mL of methanol, resulting in a 40 µg/mL DPPH solution, and then stored in a dark glass bottle wrapped with aluminum foil and placed in a refrigerator [19].

Determination of Maximum Wavelength

A total of 1600 µL of 40 µg/mL DPPH solution was pipetted into a cuvette and mixed with 400 µL of analytical grade methanol. The mixture was homogenized and incubated in the dark for approximately 15 minutes. A UV-Vis spectrophotometer was used to measure the absorbance with a wavelength range of 400–600 nm [20].

Preparation of Chlorogenic Acid Positive Control Solution

Chlorogenic acid (3 mg) was dissolved in 50 mL of analytical grade methanol to obtain a 60 µg/mL stock solution. This solution was diluted to become a series of concentrations: 50, 40, 30, 20, and 10 µg/mL.

Determination of Incubation Time

A total of 250 µL of chlorogenic acid positive control solution was mixed with 0.1 mM DPPH solution (1000 µL). The absorbance was measured every 5 minutes for 1 hour, starting from minute 0 to minute 60 at the previously determined maximum wavelength. The operational incubation time was defined as the point when the DPPH radical absorbance reached maximum stability [21].

Effect of NADES Betaine–Triethylene Glycol on 0.1 mM DPPH Solution

A total of 250 µL of NADES betaine–triethylene glycol (negative control) was mixed with 0.1 mM DPPH solution (1000 µL) and homogenized using a vortex for 1 minute, then incubated according to the determined incubation time, followed by absorbance measurement.

Preparation of NADES Betaine–Triethylene Glycol Green Robusta Coffee Bean Extract Solution

A total of 0.8 g of NADES betaine–triethylene glycol green Robusta coffee bean liquid extract was weighed and dissolved in 50 mL of analytical grade methanol to obtain a standard concentration of 16,000 µg/mL. This solution was then diluted to prepare a series of concentrations: 10,000, 8,000, 4,000, 2,000, and 1,000 µg/mL. Each concentration was tested in triplicate [22].

Determination of Antioxidant Activity

Analytical grade methanol was used to dissolve the test samples for the DPPH assay. Each series of test concentrations (10 mL) was prepared. From each solution, 250 µL was pipetted and mixed with 1000 µL of DPPH solution, then incubated for the previously determined optimal incubation time. After incubation, a UV-Vis spectrophotometer was used to measure the absorbance at the maximum wavelength.

Calculation of IC_{50} value

The absorbance values from each sample concentration were used to calculate the DPPH radical scavenging activity using the following equation:

$$DPPH\ Scavenging\ (\%) = \frac{Abs\ DPPH - Abs\ Sample}{Abs\ DPPH}\ x\ 100\% \qquad (1)$$

The percentage of DPPH scavenging was then used to construct a regression equation (Y-axis: % scavenging; X-axis: sample concentration). The regression equation y = bx + a was applied to determine the IC_{50} value using the formula:

$$IC50 = \frac{50-a}{b} \qquad (2)$$

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Conversion of IC_{50} Value of Chlorogenic Acid in Betaine–Triethylene Glycol NADES Extract of Green Robusta Coffee Beans

The chlorogenic acid content in the extract of NADES betaine–triethylene glycol green Robusta coffee beans obtained from MAE and UAE was converted into IC_{50} values of chlorogenic acid in the NADES extract by transforming the extract into a series of concentrations, using the NADES extract concentration series as a reference (10,000; 8,000; 6,000; 4,000; 2,000 µg/mL), with the following formula:

$$CA\ conc. = \frac{Conc.of\ NADES\ extract}{1\ g\ of\ NADES\ extract}\ x\ CA\ content \qquad (1)$$

After obtaining the converted values from the various concentrations extracted from NADES betaine–triethylene glycol of green Robusta coffee beans, a regression equation was generated from the Ln of the converted concentration series versus % inhibition, which was then used to determine the IC_{50} value using the formula:

$$IC50 = \frac{50-a}{b} \qquad (2)$$

Data Analysis

This study investigates the antioxidant potential of various concentrations of NADES betaine–triethylene glycol liquid extracts from green Robusta coffee beans, extracted using Microwave-Assisted Extraction (MAE) and Ultrasonic-Assisted Extraction (UAE) methods, through IC_{50} analysis. The IC_{50} test results will first be assessed for normality using the Shapiro–Wilk test, followed by an evaluation of variance homogeneity. If the data meet the assumptions of normal distribution and homogenous variance, further analysis will be conducted using a parametric one-way ANOVA. In cases where $p < 0.05$ is obtained, an LSD Post-Hoc test will be performed. However, if the data do not meet the requirements for normality and homogeneity, a data transformation will be applied. If, after transformation, the data return to a normal distribution and exhibit homogeneous variance, analysis may proceed with a one-way ANOVA, followed by an LSD Post-Hoc test if $p < 0.05$. Furthermore, an Independent Samples T-Test will be applied to assess the significance (sig. (2-tailed)) of the difference in chlorogenic acid content between MAE and UAE extracts of the NADES betaine–triethylene glycol liquid extract of Robusta green coffee beans, as well as the difference in IC_{50} values of chlorogenic acid between the two extraction methods.

Results and Discussion

Dry Powder Appearance and Its Moisture Content

In this study, the sample powder was prepared through a drying process aimed at extending the shelf life of Robusta green coffee beans by reducing the moisture content to below 10% [23]. This reduction in moisture content helps inhibit enzymatic reactions, thereby improving the quality of the resulting dry powder sample [24]. The powdered form of the sample and the results of the moisture content test of the sample powder can be seen in Figure 1 and Table 1.

Figure 1. Dry powder sample of green robusta coffee beans

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Table 1. Moisture Content Test Results

Sample	$\bar{x}$ Moisture Content (%)	SD
Green Robusta Coffee beans	8,291	0,428

Liquid Extract of NADES Betaine-Triethlyene Glycol Green Robusta Coffee Beans

A liquid extract was used in this study. The use of a liquid extract refers to the method by Alishlah et al. (2024), who extracted oxyresveratrol from mulberry root in a liquid extract using NADES urea-glycerol as the solvent [22]. The purpose of using a liquid extract in this study was to improve the quality of Robusta green coffee bean extract as a raw material for cosmetics, as the NADES components, betaine and triethylene glycol, function as humectants that help maintain skin moisture [25,26,27]. Figure 2 shows the NADES betaine–triethylene glycol liquid extracts of Robusta green coffee beans obtained using the MAE and UAE methods. Respectively, the resulting liquid extracts appeared clear with a dark brown color for both extraction methods.

(a) (b)

Figure 2. (a) NADES liquid extract – MAE; (b) NADES liquid extract – UAE

Total Chlorogenic Acid Content

The result of TLC chromatography showed that the sample and standard have the same Rf value of 0,36. In the quantification of total chlorogenic acid content in NADES betaine-triethylene glycol extract of Robusta green coffee beans, a significant difference was observed between UAE and MAE. MAE yielded a higher chlorogenic acid content than UAE, with successive content values of 3.64 ± 0.06 mgCA/g of NADES extract and 2.69 ± 0.04 mgCA/g of NADES extract. This is attributed to the electric field generated by microwaves, which produces heat and causes changes in plant tissue, significantly increasing the yield of target compounds through two working mechanisms, which are rotation of the dipole and conduction of ionic [27]. MAE shows high efficiency, low solvent consumption, and a rapid procedure [40]. The chlorogenic acid content in the NADES betaine–triethylene glycol liquid extracts of Robusta green coffee beans obtained using MAE and UAE is presented in Figure 3.

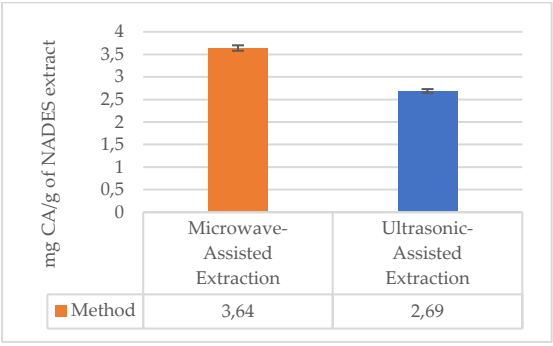

Figure 3. Total Chlorogenic Acid Content from extract NADES betaine-triethylene glycol Robusta Green Coffee Beans. Bars are expressed as mean ± SD (each sample was replicated 3 times).

Dipole rotation occurs in polar molecules induced by electromagnetic waves in MAE, causing continuous rotation as the molecules attempt to align their dipoles with the oscillating electric field. This generates heat and disrupts hydrogen bonds, enhancing the migration of dissolved ions and facilitating solvent penetration into the matrix, thereby easing the release of chemical compounds [29,30]. In ionic conduction, ions move translationally through space, trying to adjust to the changing electric field. Like dipole

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rotation, the friction from this movement also generates heat. These mechanisms allow MAE to produce a heating rate of 10–15°C/sec [28,31].

A similar result was reported by Wang et al. (2017); the extraction of blueberry leaves with NADES using MAE provided better extraction efficiency of chlorogenic acid compared to heat reflux extraction (HRE) and UAE. Peng et al. (2016) found better extraction to get phenolic compounds (chlorogenic acid, caffeic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid) from *Lonicera japonica* Thunb. Flos in NADES using MAE compared to HRE and UAE [32,33]. The MAE extraction also required less time than UAE [34]. The recovery of target compounds using MAE depends on the physicochemical properties of the NADES used and the chemical molecules of the target compounds. A suitable combination of the NADES and extraction method could enlarge the solubility of the target compound in NADES [35].

Antioxidant Activity

The optimization of incubation time was carried out using the maximum wavelength of 516 nm obtained from the 0.1 mM DPPH solution, as shown in Figure 4. The results revealed that the optimal incubation times for achieving stable absorbance were 45 minutes for the chlorogenic acid standard as positive control, 20 minutes for the UAE extract, and 40 minutes for the MAE extract. The NADES negative control showed a decrease in absorbance values corresponding to the incubation time of each sample. These optimization results are presented in Figure 5.

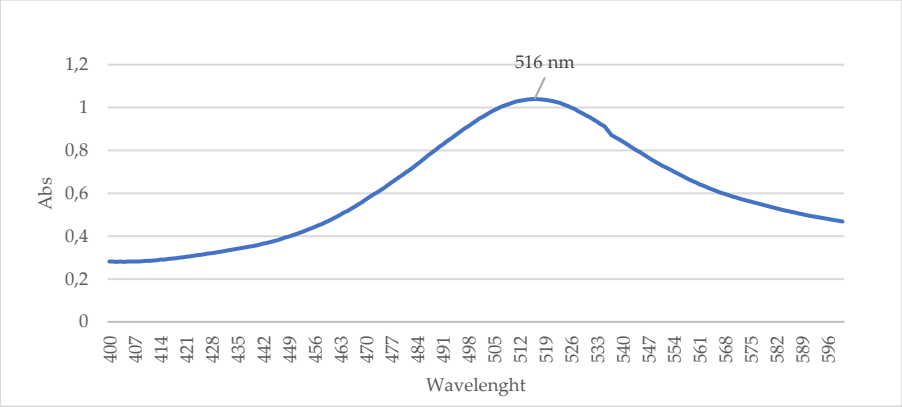

Figure 4. Maximum Wavelength of DPPH

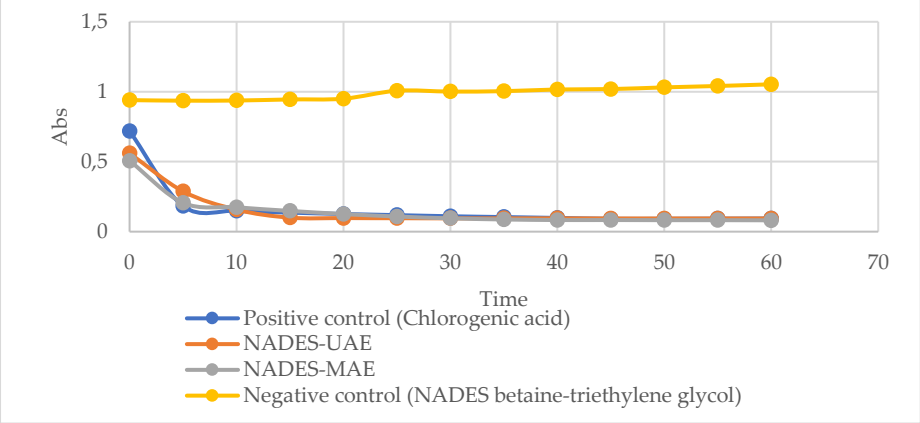

Figure 5. Optimization of Incubation

Antioxidant Activity

The IC_{50} value of the NADES betaine–triethylene glycol liquid extracts of Robusta green coffee beans obtained using MAE and UAE, as well as the IC_{50} values of the chlorogenic acid content within those extracts. The best IC_{50} value of the liquid NADES extract (betaine–triethylene glycol) of Robusta green coffee beans was achieved by the MAE extract, with an IC_{50} of 2598.05 µg/mL ± 29.42. Meanwhile, the UAE extract showed a

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higher IC_{50} value of 3266.63 µg/mL ± 67.97. Although the direct NADES extracts showed relatively weak antioxidant activity (high IC_{50} values), the MAE method was more efficient in extracting chlorogenic acid, resulting in an extract with a higher concentration of the active compound and consequently a better antioxidant activity yield per gram of coffee bean. These data are presented in Table 2.

Table 2. IC_{50} Value of extract NADES betaine-triethylene glycol Green Robusta Coffee Beans

Sample	IC_{50} (µg/mL) ± SD	
	NADES extract	Based on the chlorogenic acid content in the NADES extracts
Microwave-Assisted Extraction	2598,05 ± 29,42	9,44 ± 0,07
Ultrasonic-Assisted Extraction	3266,63 ± 67,97	9,04 ± 0,13
Chlorogenic Acid standard	16,31 ± 0,355	-

The IC_{50} values of the NADES liquid extracts were analyzed using a one-way ANOVA followed by an LSD post-hoc test. Before conducting the one-way ANOVA, the data were subjected to normality and homogeneity tests. The Shapiro-Wilk test was employed to assess data normality, and the results indicated that the data were normally distributed (Sig. (p) > 0,05, pMAE = 0,608; pUAE = 0,300; pCA = 0,922). Levene's statistic was used to evaluate homogeneity, revealing that the data were not homogeneously distributed. Consequently, data transformation was performed to homogenize the variance. Following transformation, the significance value increased to >0.05, confirming that the variance had become homogeneous (Sig. (p) > 0,05, p = 0,562). One-way ANOVA was then used to determine whether statistically significant differences existed among the means of three or more independent groups. The results demonstrated a significant difference in IC_{50} values across the three sample groups, MAE, UAE, and chlorogenic acid (Sig. (p) < 0,05, pANOVA = 0,000).

Additionally, an Independent Samples T-Test was performed to evaluate whether significant differences existed between the two extraction methods based on the significance value (sig. (2-tailed)). The results indicated a statistically significant difference between the two groups (MAE and UAE), leading to the conclusion that the two extraction methods yielded significantly different levels of chlorogenic acid and IC_{50} values of chlorogenic acid in the NADES betaine–triethylene glycol liquid extract of green Robusta coffee beans (Sig. (2-tailed) < 0,05, sig. (2-tailed) = 0,000).

Research by Anita (2024) showed that MAE resulted in a correspondence with the IC_{50} values of the NADES MAE and UAE extracts, where the MAE extract exhibited better antioxidant activity than the UAE extract [36]. In Robusta green bean extract, this could be related to the higher chlorogenic acid content in the MAE extract than in the UAE extract. The superiority of MAE in yielding higher chlorogenic acid concentrations than UAE highlights the potential for optimizing extraction processes to maximize the recovery of bioactive compounds. The chlorogenic acid is the most abundant compound compared to other compounds in Robusta green bean extract, accounting for approximately 6–12% of Robusta green coffee beans [37]. This is significantly higher than the levels of other phenolic compounds such as caffeic acid (0.033–0.141%), ferulic acid (0.006–0.035%), and p-coumaric acid (0.0056%). The antioxidant potency of Robusta green coffee beans is thought to be due to the presence of chlorogenic acid as a polyphenolic compound.

When the extract concentration was converted to chlorogenic acid equivalents in NADES extracts, IC_{50} values from UAE showed 9.04 µg CA/mL ± 0.13, higher than MAE (9.44 µg CA/mL ± 0.07). These results were comparable to the IC_{50} of the chlorogenic acid standard (Table 2). It showed that antioxidant activity from Robusta green coffee beans not only depends on chlorogenic acid content. Other polyphenols also found in coffee, including caffeic acid, p-coumaric acid, and ferulic acid, make an important contribution to antioxidant activity. Caffeine was reported as an antioxidant compound that manages neurodegenerative diseases in a beneficial way [38]. Chlorogenic and caffeic acids, two major phenolics in green coffee beans, exhibit antioxidant activities, anticarcinogenic, and antimutagenic, which are related to the ROS scavenging ability [39]. These polyphenols act together as antioxidants that play a crucial role in protecting organisms from oxidative damage and slowing the formation of free radicals. It can be concluded that the antioxidant strength in coffee is not solely influenced by chlorogenic acid, but chlorogenic acid is the major contributor to the resulting antioxidant activity.

Another study also showed that the NADES extracts have antioxidant activity. Alishlah et al. (2024) reported the antioxidant activity of NADES (glycerin-urea) mulberry root extract, which contains oxyresveratrol, obtaining an IC_{50} value of 1392.14 µg/mL [22]. The significant difference in IC_{50} values observed

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between these two studies can be attributed to the difference in potency between the two primary antioxidant compounds examined, namely oxyresveratrol (in root mulberry extract) and chlorogenic acid (in robusta green coffee extract). Nayak et al. (2017) evaluated the antioxidant activity of pure oxyresveratrol and reported an IC_{50} value of 4.3 µg/mL [44]. In contrast, the present study revealed that pure chlorogenic acid exhibited a markedly lower antioxidant potency, with an IC_{50} value of 16.31 µg/mL. Furthermore, when compared to the IC_{50} values of the pure compounds, the IC_{50} values of the liquid extracts in both studies were consistently higher. The high IC_{50} values of the NADES extract may be due to the target compounds in the sample not being fully concentrated, since a liquid extract was used. Therefore, the measured activity reflects a combination of the solvent and sample compound [43]. NADES used in this study was betaine–triethylene glycol mixture, whereas Alishlah et al. used glycerin-urea mixture. The data comparison between two NADES has not yet been done. Further research will be needed to compare some NADES to optimize the extraction of green bean coffee. Beyond that, NADES has benefits in extracting the phenolic compounds from cornelian cherry fruits (*Cornus mas* L.), Wild thyme (*Thymus serpyllum* L.), and Scutellariae Radix, which showed antioxidant activity [40-42]. Extraction using NADES could be a promising method to acquire raw material with high antioxidant activity, especially for cosmetic formulation.

Conclusion

This study highlights the superior performance of the MAE method over UAE in extracting chlorogenic acid and enhancing the antioxidant potential of Robusta green coffee beans when utilizing NADES (betaine–triethylene glycol) as the extraction medium. Despite the relatively weak antioxidant activity exhibited by the direct NADES extracts (as indicated by high IC_{50} values), MAE demonstrated greater extraction efficiency by yielding higher concentrations of chlorogenic acid, thereby achieving a higher antioxidant activity output per gram of coffee bean. These results reinforce the pivotal contribution of chlorogenic acid to the extract's antioxidant properties, while also suggesting a potential synergistic influence of other polyphenolic constituents. The application of NADES as an environmentally benign and non-toxic solvent underscores its promise in advancing sustainable extraction technologies for use in the cosmetic and pharmaceutical sectors. Building on these insights, future research should aim to optimize MAE operational parameters to further enhance the extraction efficiency, integrate the NADES-derived extracts into product formulations with validated stability and bioactivity, and perform comprehensive phytochemical profiling to fully elucidate the spectrum of bioactive components underlying the observed effects.

Conflict of Interest

The authors have no conflicts of interest regarding this investigation.

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Supplementary Materials

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