The Effect of Electric Field on Antioxidant Extraction from Avocado *(Persea americana)* Seed

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***Abstract*−** Avocado seeds have gained attention as a promising natural ingredient for various applications. Utilizing avocado seeds reduces food waste and improves sustainability. Avocado seed contains a high concentration of bioactive chemicals as natural antioxidants that scavenge free radicals. This study investigates the impact of electric field (EF) treatment on avocado seed extraction enhancement. The effect of electric field strength ranging from 2.5 to 7.5 kV/cm with a treatment time of 5 minutes was investigated. Extraction yield, FTIR analysis, antioxidant activity (via the DPPH radical scavenging assay), and IC_{50} values were obtained from the experimental data. The data were analyzed to evaluate extraction performance and to determine free radical scavenging activity. The result indicates that the value of E influences the extraction result. The best extraction conditions was achieved at an electric field strength of 7.5 kV/cm. The highest performance was obtained using an electric field of 7.5 kV/cm with extraction yield 13.29%, antioxidant activity of 95%, and an IC_{50} concentration of 120 μg/mL. The FTIR spectrum shows several peaks at 3206, 2937, 1603, 1402, 1021, and 879 cm^{-1} representing OH, C-H stretch, C=C, -C-H stretch, C-O stretch, and in-plan deformation vibration CH, respectively, to indicate the presence of antioxidants. This study demonstrates that electric field-assisted extraction serves as an eco-friendly alternative to conventional methods for obtaining natural bioactive compounds from avocado seeds.

***Keywords*−Avocado seed, extract, electric field, antioxidant, IC_{50}**

I. INTRODUCTION[1]

Avocado *(Persea americana)* fruits are well-known for their high nutritional and economic value and are widely consumed. Avocado is one of the leading fruit commodities in Indonesia, supported by avocado production amounted to about 668,260 tons in 2021, an increase of 9.89% compared to the previous year [1]. Avocado pulp is widely used for commercial applications, and this fruit processing industry generates many by-products, especially avocado seed. Avocado seeds account for approximately 13–18% of the total fruit weight and currently lack practical application, thus contributing to environmental concerns[2], [3], [4], [5]. On the other hand, the avocado seed contains bioactive compounds, namely antioxidants such as polyphenols, flavonoids, carotenoids, and tocopherols, that contribute to complete nutrition with many health benefits. This bioactive compound can prevent diseases such as cardiovascular insufficiency, hypertension, inflammatory conditions, asthma, diabetes, and Alzheimer's caused by free radicals. It makes avocado seed a cheap and easy source of natural antioxidants [2], [6], [7], [8], [9]. Currently, avocado seed waste is significantly underutilized. Therefore, developing efficient valorization strategies for incorporating this waste into food-related applications is necessary.

Extraction is a crucial step in isolating bioactive compounds from the plant materials. Bioactive compounds extracted from avocado seeds can be used for the needs of the food industry. Up to these days, the current extraction method is conventional methods such as Soxhlet extraction, maceration, and ultrasound-assisted extraction. However, this method has some limitations. Research on extracted essential oil from Neem *(Azadirachta indica A. Juss)* with soxhlet extraction used ethanol-hexane solvent and needed 4 hours at an average temperature of 70 °C. Another research on the extraction of avocado seeds and peels using the Soxhlet extraction method using solvent hexane, ethanol, and Ethyl Acetate takes 6 hours with the average temperature during the extraction process is 75 °C [10], [11]. Several studies show that conventional extraction, such as Soxhlet extraction, requires long-time, high-energy consumption, high temperature, requires high processing costs because it uses organic solvents and is not environmentally friendly because it produces toxic waste [12], [13], [14].

The electric field was recently studied in recent research as a new method with high efficiency, fast process, low energy consumption, and environmentally friendly because it uses a green solvent, leaves no waste, and no pollution [15]. Several studies using electric fields for extraction have been successfully done for lipid extraction from Greenland halibut *(Reinhardtius hippoglossoides)* by-product, squid viscera, and phenolic acids extraction from *Nepeta resemoca* [15], [16], [17]. Extraction of electric field treatment can make the plant material affected by the field, causing cell membrane disruption, and then electric field power leads to faster cell damage. After the cell surface is damaged, it will make the

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bioactive compounds inside able to be extracted quickly and increase the extraction yield [16], [17], [18], [19]. Nevertheless, according to our information, only a few studies report using electric fields in antioxidant extraction from avocado seed waste. This work used an electric field to extract bioactive compounds from avocado seeds and investigate the impact of electric field treatment on avocado seed extraction enhancement. Their extract yield, antioxidant activity, and IC_{50} concentrations were also reported.

II. Experimental Method

A. *Avocado Seed Preparation and Other Materials*

Avocado (*P. americana*) seed was obtained from a local collector in a traditional market in Surabaya, Indonesia. After drying at 80 °C, the size was reduced until passing through the screener at 35 mesh. Methanol (CH_3O; 99.9%, p.a) was purchased from Merck. DPPH (2,2-diphenyl-1-picrylhydrazyl; $C_{18}H_{12}N_5O_6$, reagent grade) was purchased from SMART LAB (Tangerang, Indonesia). Demineralized water used was obtained from UD Sumber Ilmiah Persada Surabaya. All chemicals were used as received without any further purification.

B. *Production of Avocado Seeds Extract by Electric Field*

The production of avocado seed extract using direct current electric field (EF) extraction method. The electric field apparatus comprises a high DC voltage power generator and a batch extraction chamber. 20 g of avocado seed powder and water were put in a batch treatment chamber with dimensions of 12×10×2 cm using a 1 mm thick 316L stainless plate used as the electrodes, placed in parallel with the inter-electrode gap was 2 cm. Electric field strength (E) from 2.5-7.5 kV/cm was applied to the EF extraction method. The extraction was held at room temperature for 5 minutes. After extraction, the sample was filtered, and the liquid obtained was concentrated using a rotary vacuum evaporator (Büchi 461, Switzerland) at 50 °C, 150 mbar to obtain a thick brown liquid called the extract avocado seed. Then, the extract was stored in 4 °C storage for further analyses.

Direct measurements of electrical energy consumption was not performed during the electric field extaction process, but a theoretical estimation was calculated using standard power formula :

$$E = V \times I \times t \tag{1}$$

where E is the energy in joules, V is the applied voltage (7.5 kV), I is the estimated current (10 mA), and t is the treatment time (300 s). Based on this, the estimated energy used during the EF extraction process was:

$$E = 7500\ V \times 0.01\ A \times 300s = 22{,}500\ J\ (\text{or } 6.25\ Wh)$$

C. *Determination of Extraction Yield*

The extraction yield (%) was calculated using the following equation:

$$\text{Yield} = \frac{m_E}{m_S} \times 100\% \tag{2}$$

Where yield, m_E, and m_S are extraction yield (%), the weight of the extract after evaporating (g), and the dry weight of the sample (g).

D. *Fourier Transform Infrared (FTIR) Spectroscopy Analysis*

FTIR (Fourier Transform Infrared Spectroscopy) analysis was used to obtain infrared spectrum absorption, emission, and photoconductivity. FTIR was used for detecting different functional groups in the sample. Fourier transform infrared spectroscopy (FTIR; Shimadzu IRTracer-100) investigated the chemical bonds of the avocado seed extract at wavenumber ranging from 650 to 4,000 cm^{-1}.

E. *Antioxidant Activity*

The scavenging performance of Avocado seed extract was evaluated using the DPPH assay. Methanolic DPPH solution was prepared with a concentration of 0.1 mM as the mother liquor. Next, the avocado seed extract was made at various concentrations. Then, 0.5 mL extract solution was added to 3 mL of methanolic DPPH. The mixture is shaken at room temperature for 30 minutes under light and air insulation. The absorbance was measured using a UV-Vis spectrophotometer at a wavenumber of 517 nm as absorbance of the sample. The antioxidant activity was expressed as DPPH* inhibition percentage using the equation:

$$\text{Antioxidant activity}(\%) = \frac{Abs_{DPPH^*} - Abs_{sample}}{Abs_{DPPH^*}} \times 100\% \tag{3}$$

The sample absorbance *(Abs_{sample})* represents the absorbance value measured after the avocado seed extract has been incubated with the DPPH solution for a certain time period. This value indicates how effectively the sample scavenges DPPH free radicals. Meanwhile, the control absorbance *(Abs_{DPPH})* refers to the absorbance of the DPPH solution without any sample, serving as a baseline to assess the radical scavenging activity of the tested extract.

F. *Determination of IC_{50} Concentration*

In addition, the DPPH assay method was also used to calculate the effective concentration (IC_{50}), which is the concentration of avocado seed extract which can reduce 50% of DPPH free radicals. The extract concentration that corresponds to 50 percent inhibition (IC_{50}) was calculated from the radical scavenging activity curve percentage against the avocado seed extract concentration.

III. Results and Discussion

Figure 1 illustrates the effect of increasing electric field strength on the extraction yield. As the electric field strength increased, the extract yield also increased. The yields were 10.82, 12.28, and 13.13% for the 2.5, 5.0, and 7.5 kV/cm electric field strength, respectively. These findings indicate that the electric field strength impacts the amount and yield of avocado seed extract. Increasing the voltage from 2.5 kV/cm to 5 kV/cm results in a more considerable extract amount and yield than the difference observed when increasing the voltage from 5 kV/cm to 7.5 kV/cm. The increase in yield is not linear because the increase in the intensity of a larger electric field can cause more cell membrane disruption. Electroporation occurs when electric fields produce transient pores in cell membranes. These pores let the extraction solvent penetrate the cells more efficiently, releasing intracellular bioactive compounds into the extracted solvent. A larger electric field can affect more cell membranes, causing a higher yield of extracted bioactive compounds [17], [20]

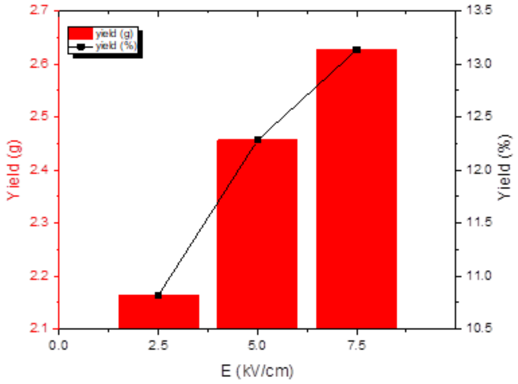

Figure 1. Extraction yield of avocado seed extract

Fourier-transform infrared spectroscopy (FTIR) analysis presents information on the functional groups and chemical bonds found in the samples. FTIR spectroscopy may detect and identify some functional groups found in antioxidants. Figure 2 presents the FTIR spectrum of avocado seed extract. The figure shows that avocado seed extract with electric fields varying from 2.5 to 7.5 kV/cm has the same peak tendency. Peaks at 3,206 cm^{-1} and 2,937 cm^{-1} are specific for the OH group and CH_2. The peak at 1,602.8 cm^{-1}, 1,402 cm^{-1}, and 1,312 cm^{-1} represents C=C, -C-H alkane, and OH phenolic group, respectively. Flavonoid structures were identified through C–O peaks at 1,021 cm^{-1} and in-plane deformation vibrations of C–H at 879 cm^{-1} [21]. The results indicate that electric field extraction produces bioactive components with antioxidant properties. However, increasing the electric field intensity has little impact and does not affect the chemical bonds of the produced bioactive substance. Based on the IR spectra, it is possible to conclude that avocado seed extract contains bioactive antioxidant molecules [19], [21], [22], [23], [24].

In this study, FTIR analysis was used to confirm the presence of functional groups commonly found in antioxidant compounds, such as hydroxyl (–OH), carbonyl (C=O), and aromatic rings. However, this technique does not allow for the identification of specific bioactive compounds. Based on findings from previous studies, the most dominant antioxidant constituents in avocado seed are theoretically phenolic compounds and flavonoids. The extract analyzed in this research was a crude extract, and the focus was limited to overall yield and antioxidant activity.

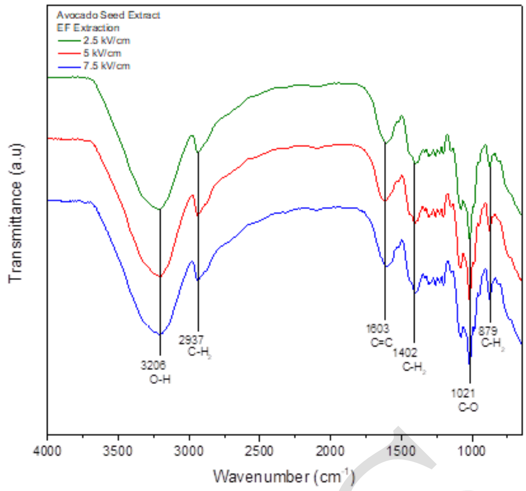

Figure 2. FTIR Spectra of avocado seed extracts at varied electric field strength

The DPPH assay is used as an experiment to investigate antioxidant activity and radical scavenging performance of bioactive compounds from avocado seed extract because it is quite stable in a standard laboratory environment. Figure 3 displays an optical image of the DPPH treated by avocado seed extract. The change in color in the DPPH radical solution with the addition of avocado seed extract can be seen in this picture. DPPH solution is intensely purple-colored, shows a strong absorption band at 517 nm, and will change color to yellow due to a reduced absorption intensity of DPPH itself [25].The DPPH radical assay of avocado seed extract treated at 2.5 and 5 kV/cm showed a color change from purple at 25 – 150 µg/ml extract to light purple as the transition color at 200 – 400 µg/ml extract, then changed to light yellow at the addition of the extract concentration at 600 µg/ml until the last addition at 1000 µg/ml. The addition of 7.5 kV/cm extract has the transition color at 200 and 300 µg/ml and changed color to yellow at a concentration of 400 - 1000 µg/ml. It indicates that the color phase transition occurs faster than other extracts. The purple solution of the DPPH radical indicates high DPPH radical concentration and low or no antioxidant activity. Then the solution with yellow color is a sign of high antioxidant activity and low or no DPPH radical scavenging. The color transformation from purple to yellow and the increase in extract concentration indicates the existence of antioxidants in avocado seed extract. The phenomena correspond to the results of other antioxidant analyses reported by [26], [27]; this experiment showed more apparent results by observing the color change due to more variations in the addition of extract concentration.

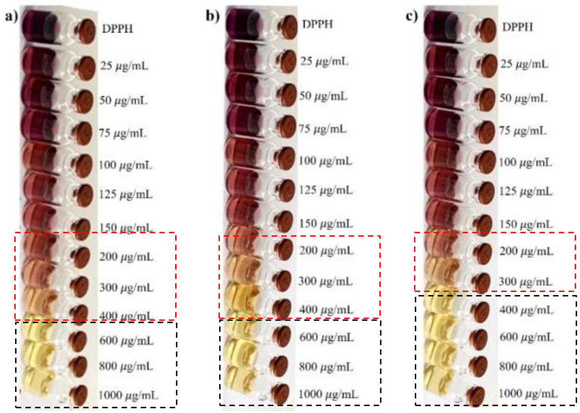

Figure 3. Optical picture of the DPPH radical versus avocado seed extract a) 2.5 kV/cm, b)5 kV/cm, and c)7.5 kV/cm.

By increasing the concentration of the avocado seed extract, there is a change of color and transition from dark purple to bright yellow and a decrease in DPPH D Figure 4 radical absorbances. Figure 4 shows the Absorption spectra of DPPH and avocado seed extract with concentrations from 25 – 1000 µg/ml using extraction at 2.5, 5, and 7.5 kV/cm. According to the picture, the DPPH peak reduced as the avocado seed extract concentration increased. Avocado seed extract contains antioxidant compounds that can transfer electrons or hydrogen atoms. They can transfer an electron to the DPPH radical and act as reducing agents [28]. The decrease in DPPH absorbance and color changes, along with the increase in the concentration of avocado seed extract in this study, follows the result of several studies on the identification of antioxidants with a decrease in absorbance and color intensity [25], [29], [30], [31].

Figure 5 shows the inhibition percentage of the DPPH radical treated by avocado seed extract using EF a) 2.5 kV/cm, b) 5 kV/cm, and c) 7.5 kV/cm. The inhibition can be up to 90% by plotting the inhibition ability of the

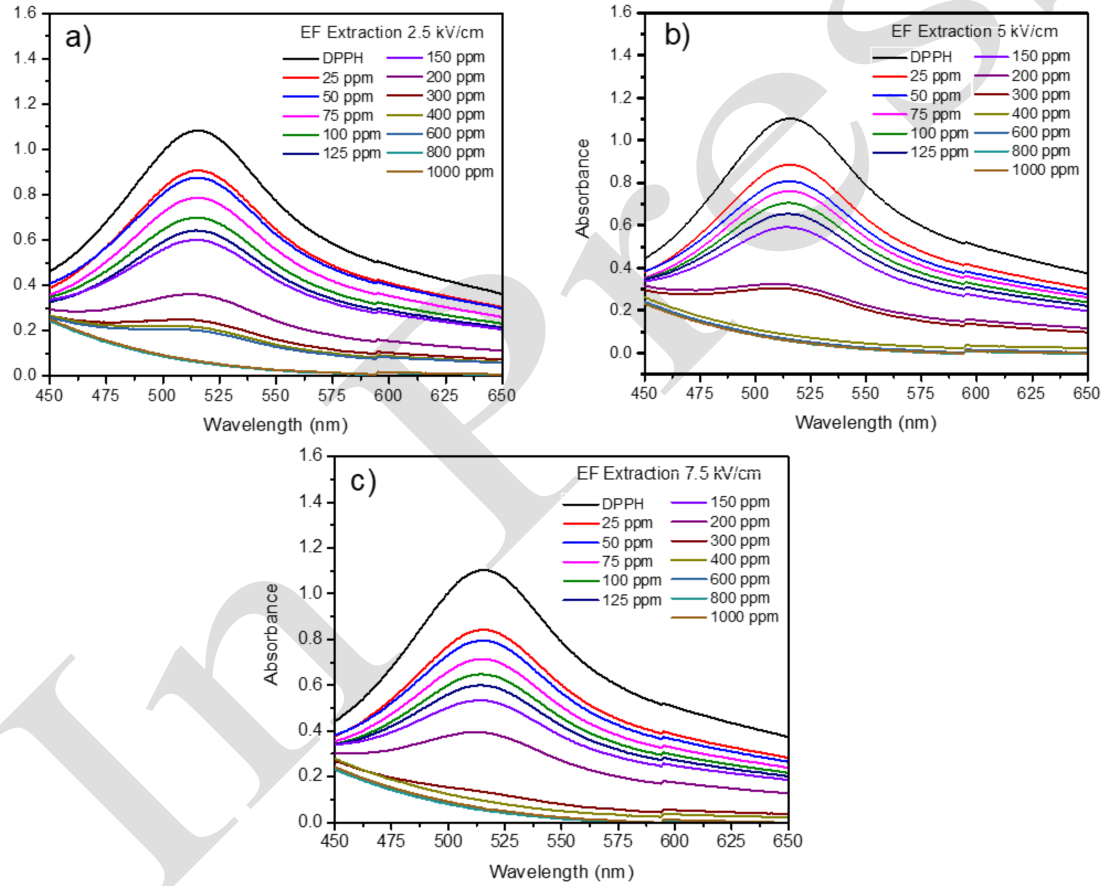

Figure 4. Absorption spectra of DPPH and avocado seed extract with concentration from.

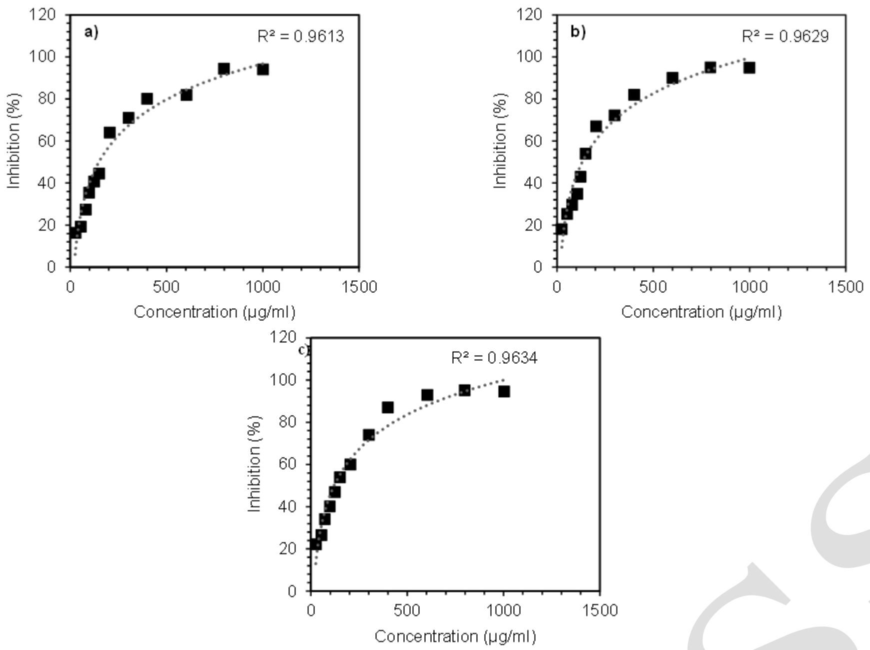

Figure 5. Inhibition percentage of the DPPH radical under various avocado seed extract concentrations using EF a) 2.5 kV/cm, b) 5 kV/cm, and c) 7.5 kV/cm.

DPPH radical under the gradient concentration of the avocado seed extract. According to the figure, the highest antioxidant activity was observed in the avocado seed extract treated at 7.5 kV/cm, with scavenging values of 22%, 27%, 34%, 40%, 47%, 54%, 60%, 74%, 87%, 93%, 95%, and 95% of scavenging activity at concentrations 25 µg/ml until 1000 µg/ml, respectively. All the results established that Avocado seed extract showed intense radical scavenging activity. However, at higher concentrations, such as 600, 800, and 1000 µg/ml. The inhibition percentage can reach 90% at extraction with electric field from 5 and 7.5 kV/cm, unlike avocado seed extract from extraction with 2.5 kV/cm, which has only reached up to 90% inhibition percentage at a concentration of 800 µg/ml. The absorption spectra of DPPH and avocado seed extract result is better than other research of antioxidant extraction from Soxhlet extraction that reaches 92% of scavenging activity at a concentration of 600 µg/ml. This result is in accordance with Figures 3 and 4 that the dark purple color indicates low or no antioxidant activity. The color will change to light purple, along with antioxidant activity. The solution with yellow color is a sign of high antioxidant activity followed by a decrease in DPPH radical absorbances [32].

In addition to evaluating extraction yield and antioxidant activity, an assessment of the energy efficiency of the electric field extraction method was also considered. A theoretical estimation based on the applied voltage (7.5 kV), current (10 mA), and treatment time (5 minutes) yielded a total energy consumption of approximately 6.25 Wh. This value is significantly lower than that of conventional Soxhlet extraction, which can require 4 to 16 hours of operation and consume up to 3.0 kWh of energy [33]. This comparison reinforces the claim that electric field-assisted extraction provides a more energy-efficient and sustainable approach for recovering bioactive compounds from natural sources.

TABLE 1.
ANTIOXIDANT ACTIVITY AND IC_{50} VALUES OF VARIOUS EXTRACTS OF AVOCADO SEED BY DPPH RADICAL SCAVENGING ASSAY

E (kV/cm)	Antioxidant Activity (%)	IC_{50} (µg/ml)
2.5	94	149
5	95	131
7.5	95	120

The IC_{50} concentration was analysed to determine the amount of antioxidant extract required to reduce DPPH radical absorbance by 50%. [34]. Table 1 summarizes avocado seed extract's antioxidant activity and IC_{50} values at different electric field strengths applied. Avocado seed extract from 2.5, 5, and 7.5 kV/cm showed 149, 131, and 120 µg/ml of IC_{50} concentration, respectively. Followed by antioxidant activity values of 94, 95, and 95% for avocado seed extract from 2.5, 5, and 7.5 kV/cm. The IC_{50} concentration correlated with the potency of a bioactive compound, the amount of extract needed to give an antioxidant effect. The lower the IC_{50} value, the more potential for the bioactive compound. A lower amount of extract is required to achieve high antioxidant activity. The result of this experiment was better than an avocado seed extract from another research. Antioxidant extract from avocado seed with Soxhlet extraction requires an IC_{50} value of 187.66 µg/ml [35]. According to a recent study on the effect of an electric field on the extraction, it was found that electric treatment has a pronounced cell-breaking effect in the sample and can collect more antioxidant substances, which is also in line with previous research on lipid extraction by electric field extraction. The increase of the electric field will increase the electric field intensity and cell damage and resulting in lower IC_{50} concentration [15], [16], [36]. Therefore, from the results of this study, it can be concluded that electric field extraction can be used as an alternative to green technology for antioxidant extraction. The concentration of antioxidant compounds in this study was indirectly inferred from the sample's ability to inhibit DPPH radicals at varying extract concentrations.

Antioxidant activity was quantified through the percentage of inhibition and IC_{50} values, which reflect the amount of extract required to reduce 50% of DPPH absorbance. Since this study did not isolate specific compounds, the extract concentration (in µg/mL) used in DPPH assays was considered representative of the bioactive content. Although this is an indirect approach, it is commonly used in preliminary antioxidant screening.

IV. CONCLUSION

This study investigated the extraction of avocado seeds using the electric field-assisted extraction method. The effect of electric field strength ranging from 2.5 to 7.5 kV/cm was investigated. Extraction yield, FTIR spectra, antioxidant activity (via DPPH radical scavenging assay), and IC_{50} values were effectively assessed. Increasing the electric field during extraction leads to a higher yield of extracted compounds but may not always result in a proportionally significant change in yield. The optimal extraction conditions was achieved at 7.5 kV/cm for 5 minutes, resulting in a 13.29% yield, 95% antioxidant activity, and an IC_{50} value of 120 µg/mL. The FTIR spectrum also shows several peaks representing the presence of antioxidants. These results demonstrate that electric field-assisted extraction is a promising green technology for the recovery of natural bioactive compounds from avocado seeds.

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