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Identification and antioxidant activity test of β-tocopherol from Dompu corn oil as anti-aging

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ABSTRACT

Corn is a well-cultivated food crop that is grown extensively worldwide. The used portion of corn is its seeds, which are rich in oil. The objective of this study is to separate and characterize tocopherol from corn oil in Dompu, Sumbawa by employing spectroscopic techniques such as ultraviolet (UV) spectrophotometry, ultra-performance liquid chromatography (UPLC), nuclear magnetic resonance (NMR), and liquid chromatography-mass spectrometry (LC-MS). Subsequently, the in-vitro antioxidant activity of the tocopherol was assessed. 1 kilogram of dry maize kernels subjected to 70% ethanol extraction yielded 30 grams (35 ml) of corn oil. The purified isolate obtained from the fractionated extract, using radial chromatography, demonstrated the presence of tocopherol. The isolated sample exhibited the presence of beta-tocopherol. Beta-tocopherol's anti-aging properties were assessed by conducting an in-vitro antioxidant activity test utilizing the tyrosinase enzyme. The IC50 value obtained was 83.954±2.849 ppm. The IC50 value indicates that beta-tocopherol possesses significant antioxidant activity, making it suitable for usage as a primary ingredient in cosmetics and pharmaceutical products.

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1. INTRODUCTION

Corn is widely grown and is one of the world's largest food crops. Corn is a commonly consumed food crop in Indonesia and serves as a prominent item in the agricultural sector of Dompu, Sumbawa, West Nusa Tenggara. According to relevant research, in developed countries, corn is mainly used for animal feed and human consumption. The oil content of the kernel is primarily determined by the percentage of oil present in the germ and the proportion of the germ within the caryopsis. The majority of commercially available maize oil is derived from the germ, which is frequently referred to as "corn germ oil" [1]. Vegetable

oils, which are often consumed globally, are the fundamental sources of lipids in our diet. Enhancing these oils with natural antioxidants could be seen as beneficial. Tocopherols, which possess significant antioxidant properties, are very ideal for enhancing oils [2]. This is due to their lipophilic nature and their presence as minor constituents in vegetable oils [3]. They work as free radical scavengers by donating hydrogen atoms to stabilize free radicals. They are beneficial at low concentrations but lose efficacy as levels rise due to adverse effects with non-peroxyl species. Identifying the ideal antioxidant content of tocopherols in vegetable oils remains difficult [4].

Tocopherols and tocotrienols, known as tocols or vitamin E, are a group of naturally-occurring chemicals that are soluble in fat. This group consists of eight components, namely α-, β-, γ-, and δ-tocopherol (T); α-, β-, γ-, and δ-tocotrienol [5]. Vitamin E is the most powerful lipid-soluble antioxidant found in the plasma of humans. The dietary components that possess antioxidant activity similar to vitamin E are α-, β-, γ-, and δ-tocopherols, as well as α-, β-, γ-, and δ-tocotrienols [6]. These molecules consist of a chromanol ring connected to a saturated phytyl (tocopherols) or unsaturated (tocotrienols) group, and they differ in the number of methyl groups on the chromanol ring [7]. Various techniques have been used so far to extract, separate, and purify vitamin E, especially from vegetable fatty acid distillate (FAD), which is a lucrative by product of the oil refining process [8]. This is due to the recognized health benefits of vitamin E. The vitamin E content in soybean oil FAD is the greatest, ranging from 10-14%. Maize oil follows with a value of 7-10%, while cottonseed oil and sunflower oil have contents of 6-10 and 5-8% respectively [9]. The current methods created to pretreat and extract vitamin E in order to enhance its extraction yield include molecular distillation, esterification, saponification, chromatography, crystallization, solvent extraction, maceration, matrix solid-phase dispersion, pressurized liquid extraction, supercritical fluid extraction (SFE) and ultrasonic-assisted extraction, and combinations thereof [10]. Each approach has advantages and disadvantages, therefore the decision is determined by the physical and chemical features of the sample, as well as the available resources and equipment [11]. Nevertheless, the extraction techniques now used in industry, such as esterification followed by distillation, necessitate the application of elevated temperatures [12]. Unfortunately, these high temperatures can lead to the deterioration of thermosensitive phytonutrients, including vitamin E [12]. Vitamin E acts as an antioxidant specifically targeting cell membrane lipids, circulating low-density lipoprotein (LDL), lungs, liver, and adrenal tissue. Vitamin E serves as an antioxidant, primarily protecting against harmful oxygen, lipid peroxides, and free radicals [13]. It halts the progression of free radicals and safeguards against deoxyribonucleic acid (DNA) damage that leads to mutations. Additionally, it helps manage LDL levels. The consequences of free radical assault include accelerated aging, cancer, atherosclerosis, and other related conditions [14]. Incorporating a higher intake of tocopherol-rich meals can yield advantages such as the treatment and prevention of cardiovascular ailments, including angina, hypertension, and atherosclerosis [15]. Natural forms of tocopherol are better absorbed by the body compared to synthetic versions, highlighting the need to investigate dietary sources, amounts, and stability of tocopherols to better understand their potential health benefits and uses [2].

The primary biochemical role of tocopherols is to safeguard polyunsaturated fatty acids from peroxidation [16]. The effectiveness of tocopherols may be influenced by the ratio of total tocopherols to polyunsaturated fatty acids. Tocopherols are crucial in inhibiting the oxidation of lipids in almonds, which can extend the storage time of the kernels. In recent times, the beauty industry and clinical dermatology have shown increased interest in tocols because of their photoprotection and antioxidant characteristics [17]. An antioxidant is classified as having very strong activity when its IC50 value is less than 50 µg/mL (IC50 <50 µg/mL), strong activity when the IC50 value is between 50 and 100 µg/mL (50 µg/mL <IC50 <100 µg/mL), moderate activity when the IC50 value is between 100 and 150 µg/mL (100 µg/mL <IC50 <150 µg/mL), weak activity when the IC50 value is between 150 and 200 µg/mL (150 µg/mL <IC50 <200 µg/mL), and very weak activity when the IC50 value is greater than 200 µg/mL (IC50 >200 µg/mL) [18]. Healthy skin is characterized by its ability to shield itself from the harmful effects of sun exposure and prevent premature aging [19]. Melanin has a crucial function in safeguarding against ultraviolet (UV) radiation [20]. Nevertheless, an overabundance of melanin synthesis can result in either hyperpigmentation or hypopigmentation of the skin. The suppression of tyrosinase is the most commonly employed technique for skin depigmentation [21]. Tyrosinase is the pivotal enzyme in the melanin production process [22]. Tocopherol from corn oil and other plant oils has been widely studied [23]–[25], but no one has yet conducted research on tocopherol corn oil from Dompu. The purpose of this study was the identification of tocopherols from Dompu corn oil by UV spectrophotometry, nuclear magnetic resonance (H-NMR), high-performance liquid chromatography (HPLC), and liquid chromatography-tandem mass spectrometry (LC-MS) analysis. Anti-aging testing of the tocopherol content of corn oil using the enzyme tyrosinase was a secondary objective of this study.

2. RESEARCH METHOD

2.1. Time and place of research

This research was conducted during the period of August to December 2023. Research activities were carried out in two main locations, namely the Department of Chemical Engineering, Faculty of Industrial Technology, Universitas Ahmad Dahlan, Yogyakarta, and EBM SciTech, Institut Teknologi Bandung (ITB). The selection of these two locations was based on the availability of adequate facilities and the support of appropriate experts to support the success of the research.

2.2. Research tools and materials

This study utilized corn grains from Dompu, Sumbawa, West Nusa Tenggara, Indonesia. Tyrosinase enzyme, kojic acid, chemicals, and analytical materials for testing were provided by EBM SciTech, Research Center for Bioscience and Biotechnology ITB, Bandung. The instruments utilized in this study consisted of maceration extraction equipment, UV-spectrophotometer, radial chromatography (ERSCH-ITB), H-NMR (NMR Bruker Neo 500 MHz made in Germany), ACQUITY UPLC H-class system with PDA detector and Xevo-TQD MS (Waters, USA), and LC-MS. All of the analysis instruments were facilitated by EBM SciTech ITB.

2.3. Research procedure

2.3.1. Oil extraction from corn grains

1 kilogram of dehydrated maize kernels sourced from Dompu, Sumbawa, West Nusa Tenggara, Indonesia was mixed together and thereafter subjected to maceration using 70% ethanol for a duration of 24 hours. Subsequently, the process of filtration was carried out using filter paper to acquire a filtrate. The solvent in the filter was evaporated using an evaporator in order to get a concentrated extract. Subsequently, the process of evaporation was prolonged by employing an oven, resulting in the acquisition of a 30-gram quantity of extract with a yellow oil-like appearance.

2.3.2. Conducting a spectroscopic analysis

The analysis techniques utilized in the study encompass UV spectrophotometry, radial chromatography, H-NMR, and advanced systems such as UPLC H-Class with detector, Xevo-TQD MS, and LC-MS. These methods provide comprehensive data on molecular composition and structural elucidation, enhancing the accuracy of analytical outcomes. The approach aligns with the techniques described by Saini and Keum [11], demonstrating their reliability and relevance in modern research. Each technique contributes uniquely to identifying and quantifying compounds, ensuring robust analytical validation. This combination of techniques represents a gold standard for studies requiring precise chemical and molecular analysis.

2.3.3. Tyrosinase enzyme inhibition test procedure

Reagents used include i) Phosphate Buffer 0.1 M pH 6.8 consisting of 0.05104 M NaH2PO4. H2O (352.2 mg) and 0.04896 Na2HPO4 (347.5 mg) in 50 ml of distilled water; ii) 12.5 mM L-DOPA solution (substrate), a total of 2.464 mg L-DOPA powder was dissolved in 1 ml phosphate buffer; and iii) 500 U/ml tyrosinase enzyme, 20 µL of 25,000 U/ml tyrosinase enzyme stock was taken and 980 µL phosphate buffer was added.

The working method used was a stock solution of the sample or comparator (kojic acid) prepared at a concentration of 10,000 ppm in 5% DMSO. The test solution for the comparator was made from dilution of the stock solution with a concentration of 5-50 ppm (µg/mL) while the test solution for beta tocopherol was made in the range of 10-160 ppm and 200-700 ppm for royal jelly acid. A total of 40 µL phosphate buffer, 40 µL sample solution or comparator, and 40 µL tyrosinase enzyme were put into 96 wells and then incubated for 15 minutes at 37 °C. A total of 40 µL of L-DOPA substrate was added to the well and the mixture was incubated for 15 minutes at room temperature absorbance was read using a spectrophotometer at a wavelength of 475 nm. Tyrosinase inhibition calculated by (1).

$$\text{Tyrosinase inhibition } (\%) = \left(\frac{(Abs_A - Abs_B) - (Abs_C - ABs_dD)}{Abs_A - Abs_B}\right) \times 100 \quad (1)$$

Where Abs_A is absorbance blank (phosphate buffer, enzyme, substrate, and no sample), Abs_B is the absorbance of blank (phosphate buffer, substrate, without sample and enzyme), Abs_C is the absorbance of sample, enzyme, and substrate, Abs_D is the absorbance of sample and substrate without enzyme. IC50 was calculated using the linear regression equation $y = a + bx$, where the sample concentration is the x-axis and % inhibition is the y-axis.

3. RESULTS AND DISCUSSION

The process of extracting dehydrated corn kernels results in the production of 30 grams (35 ml) of corn oil, which corresponds to a yield of 3%. Enhancing corn oil output in the future can be achieved through the utilization of non-dry, aged maize kernels and employing advanced extraction techniques like SFE. Oil extraction from corn grains can be seen in Figure 1, which shows the stages of the extraction process from dried corn kernels, including the raw materials sourced from dried corn from Dompu, Sumbawa, West Nusa Tenggara, Indonesia (Figure 1(a)), the crushed corn kernels reduced in size using a blender to facilitate extraction (Figure 1(b)), the maceration of the crushed kernels with 70% ethanol to extract oil components (Figure 1(c)), and the resulting corn oil obtained after filtration and evaporation (Figure 1(d)).

(a) (b) (c) (d)

Figure 1. Oil extraction from corn grains of (a) corn grains, (b) blended corn, (c) maceration of blended corn, and (d) corn oil extract

Figures 2-5 illustrate the identification of pure isolates through various spectroscopic techniques. These include a UV spectrophotometry profile that shows two peaks at maximum wavelengths, indicative of conjugated chromophore bonds (Figure 2). Additionally, the UPLC profile reveals a chromatogram demonstrating compound purity of less than 97% (Figure 3). Furthermore, the H-NMR profile displays signals corresponding to the structure of β-tocopherol, confirming the compound's distinct chemical shifts (Figure 4). Lastly, the mass spectrogram analysis validates the molecular weight of the β-tocopherol compound (Figure 5). These combined results provide a comprehensive understanding of the structural and purity characteristics of the isolates.

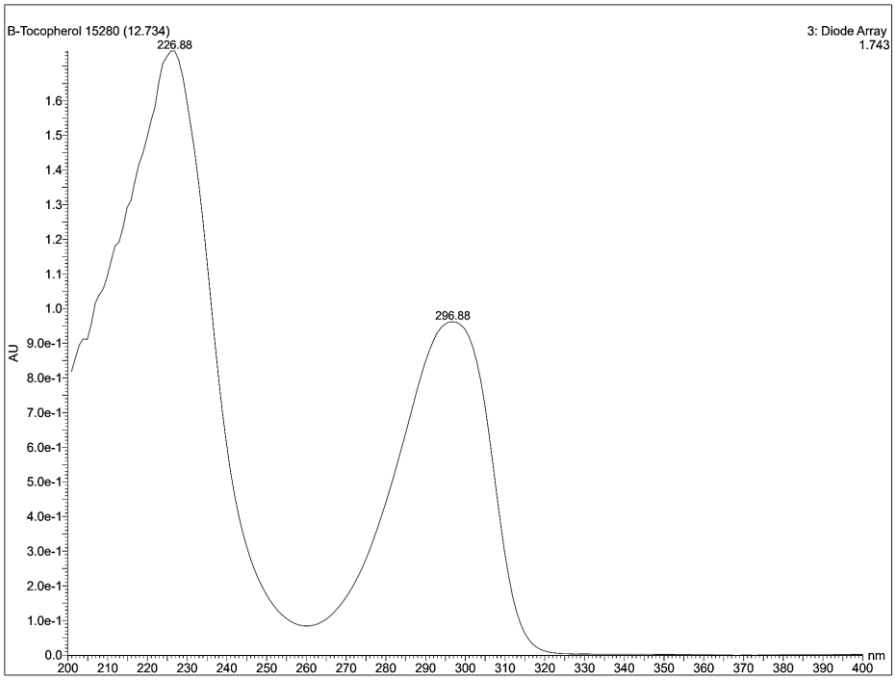


Figure 2. Spectrophotometry profile

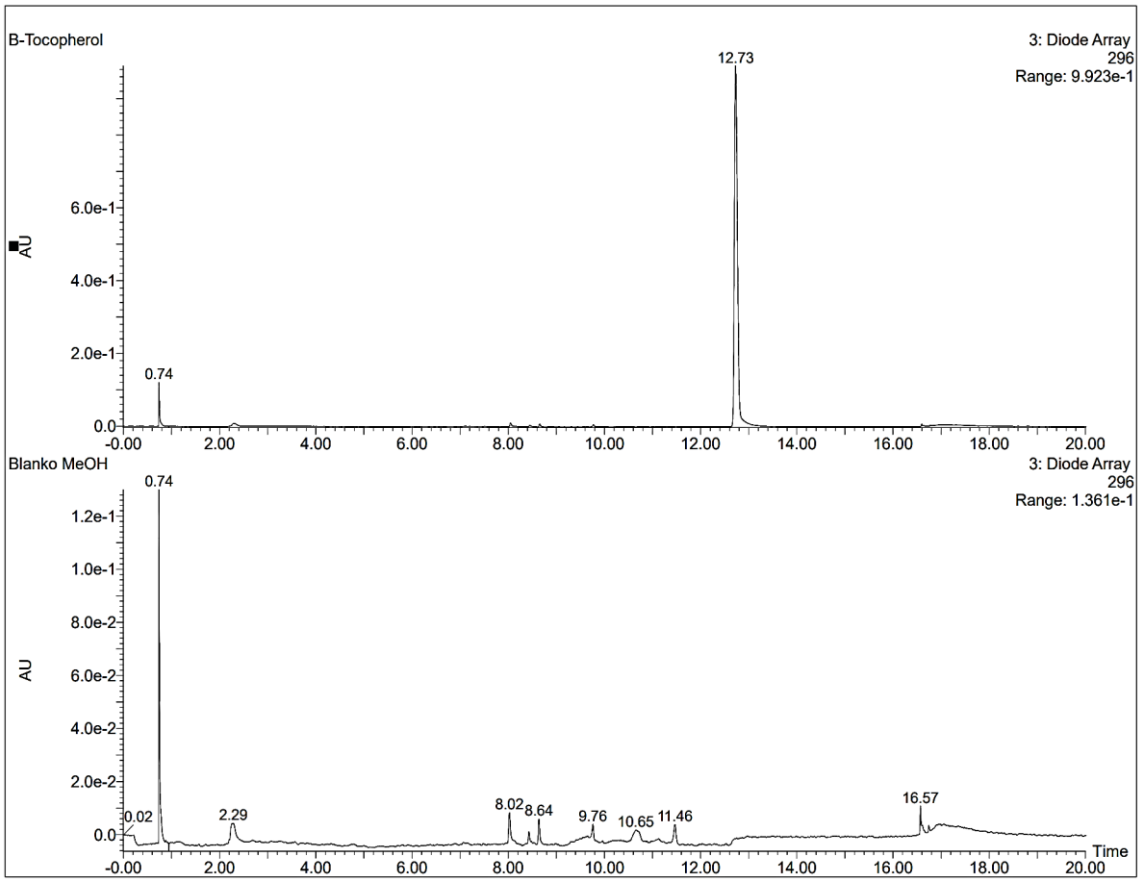


Figure 3. UPLC profile of the isolate

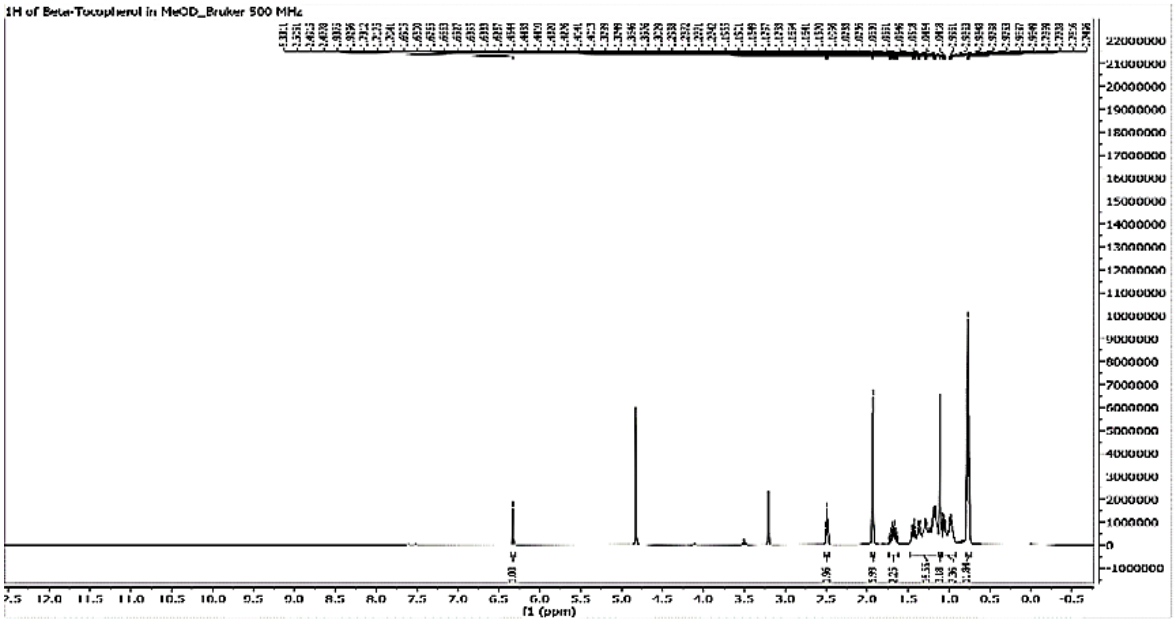


Figure 4. H-NMR profile of the isolate

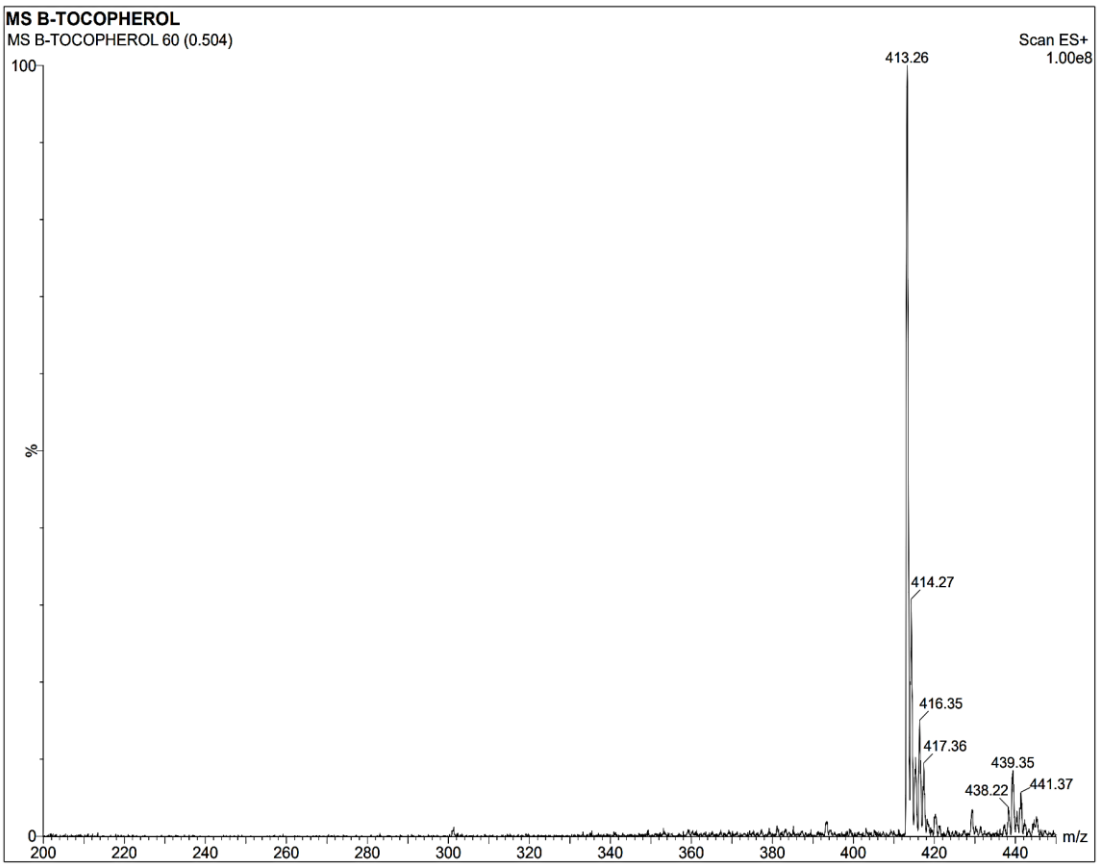


Figure 5. Mass spectrogram profile of the isolate

Fractionation and purification of tocopherol compounds from corn oil with eluted using n-Hexane solvent, ethyl acetate volume ratio (8:2) using radial chromatography with a plate thickness of 4 mm (stationary phase). Several spectroscopy techniques, including spectrophotometry UV, UPLC, NMR, and LC-MS, were used to determine the chemical structure. The specific data results can be seen in Figures 2-5. In order to determine the retention duration of tocopherols in chromatography, pure oils were employed. The kind and proportion of tocopherols were determined by analyzing the retention duration and quantities of these compounds in the pure oil samples. Based on the UV spectrum showed two peaks at maximum wavelength λ=226.88 and 296.88 nm which means this compound has a conjugated chromophore bond with purity <97% when viewed on UPLC chromatogram. To confirm the structural form of this compound, 1H NMR_Bruker 500 MHz (MeOD) analysis was carried out which showed 12 signal groups representing 47 carbon numbers. There is one signal (H-7) at the aromatic sp2 chemical shift δH 6.33 (s, 1H), two neighboring signals forming a pyran ring at δH 2.49 (t, 2H) (H-3) and 1.68 (m, 2H) (H-4). This compound also has 7 methyl (CH3) at δH 1.93 (d, 6H) (H-28 and H-29), 1.12 (s, 3H) (H-25), 0.78 (s, 3H) (H-23), 0.77 (s, 3H) (H-24), 0.76 (d, 6H) (H-26 and H-27). The latter is the sp3 signal from 1.45-0.96 ppm which stretches to form CH2 and CH aliphatic. Based on NMR data showed that this compound is beta-tocopherol which was confirmed by molecular weight analysis which is [M+H]+m/z 417. The chemical structure of β-tocopherol can be seen in Figure 6.

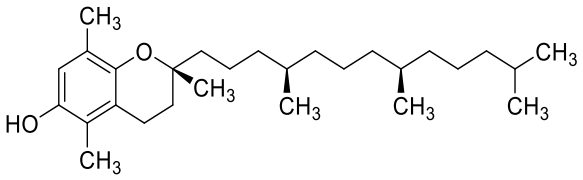
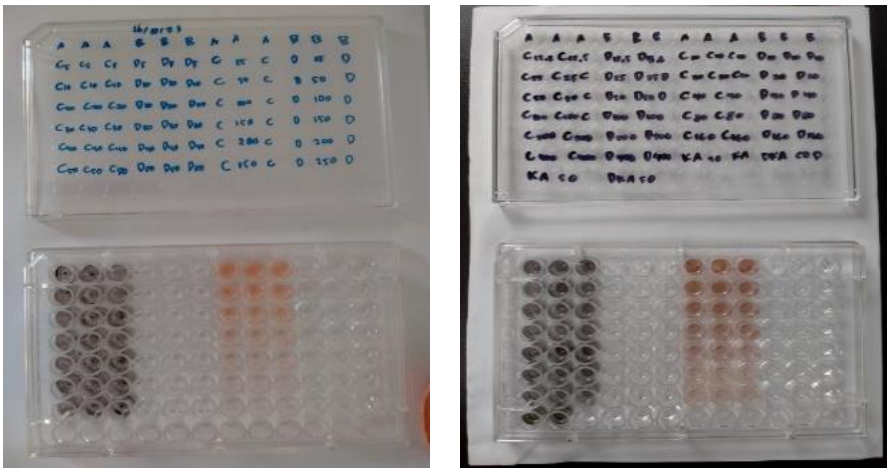


Figure 6. β-tocopherol chemical structure

β-tocopherol has received less attention independently and is frequently compared to α-tocopherol. It has similar antioxidant characteristics as α-tocopherol but does not impede protein kinase C activity, cell proliferation, or gene expression. β-tocopherol has lower inhibition of thrombin-induced PKC activity and does not affect IL-1β production in monocytes or caspase-3 activity in endothelial cells. β-tocopherol, unlike α-tocopherol, has no effect on cell adhesion or integrin expression in human erythroleukemia cells, but it does, along with γ-tocopherol, effectively reduce intracellular tyrosinase activity in mouse melanoma cells [26].

The tyrosinase enzyme inhibitor activity test is an in-vitro test using a microplate reader to read the absorbance of melanin formed after inhibition by the sample [27]. Figure 7 shows an in-vitro test using a well microplate between kojic acid and β-tocopherol. A study was conducted to test the inhibitory action of the tyrosinase enzyme by beta-tocopherol from Dompu maize oil. Kojic acid was used as a comparator in this study. The IC50 test findings for β-tocopherol using the tyrosinase enzyme yielded a value of 83.954±2.849 ppm. When compared to the comparator, specifically, kojic acid exhibits an IC50 value of 21.531±0.089 ppm with the tyrosinase enzyme. The IC50 of β-tocopherol is significantly lower than that of the normal kojic acid. The IC50 value obtained from β-tocopherol was 83.954±2.849 ppm. This means that the antioxidant activity possessed by β-tocopherol from corn oil from Dompu has a strong antioxidant category [18], [28].

Figure 7 depicts an in-vitro test for tyrosinase enzyme inhibition activity using a well microplate. This test compares the inhibitory activity of β-tocopherol produced from Dompu maize oil to kojic acid, a well-known tyrosinase inhibitor commonly used in cosmetic and medicinal formulations. The absorbance of the melanin generated after being inhibited by the sample was measured using a microplate reader, which aids in determining the inhibitory activity of each compound. Figure 7(a) displays microplates labeled with sample codes and concentrations, indicating a controlled experimental design. The darker wells imply higher amounts of melanin synthesis, but the lighter wells indicate stronger inhibitory effects, maybe from kojic acid. In the second image, the wells appear cleaner in some regions, indicating that the tested chemicals prevent melanin synthesis. The difference in color intensity aids in determining the efficacy of the tested inhibitors. Figure 7(b) shows the wells appear cleaner in some regions, indicating that the tested chemicals prevent melanin synthesis. The difference in color intensity aids in determining the efficacy of the tested inhibitors.



(a) (b)

Figure 7. In-vitro test using a well microplate of (a) kojic acid well microplate and (b) β-tocopherol well microplate

Kojic acid, a well-known tyrosinase inhibitor, is frequently utilized in cosmetics and pharmaceuticals because of its great potency [29]–[31]. β-tocopherol's moderate tyrosinase inhibition and strong antioxidant activity indicate that it could be used with more potent inhibitors, such as kojic acid, to improve overall performance in cosmetic and medicinal formulations. The significant difference in IC50 values between β-tocopherol and kojic acid highlights the need to investigate synergistic effects or combination techniques, as β-tocopherol may increase total antioxidant activity when combined with other substances. Although β-tocopherol has a weaker inhibitory activity than kojic acid, its antioxidant qualities could be beneficial in formulations for lowering oxidative stress. Such combinations could be especially useful in skincare and anti-aging treatments, where the combination of antioxidants and modest tyrosinase

inhibition produces synergistic benefits, resulting in brighter, healthier skin. Optimizing the usage of β-tocopherol with other powerful inhibitors or antioxidants, as well as researching their stability and combined effects, could improve their practical applications in cosmetic formulations.

4. CONCLUSION

This research achieved the extraction of 30 grams (35 ml) of corn oil from 1 kilogram of dried corn kernels sourced from Dompu, utilizing maceration followed by purification through radial chromatography. Tocopherol compounds, particularly β-tocopherol, were identified using UV spectroscopy, UPLC, NMR, and LC-MS. The in-vitro antioxidant activity assessment of β-tocopherol resulted in an IC50 value of 83.954±2849 ppm, demonstrating its strong antioxidant capabilities. These results suggest that β-tocopherol extracted from corn oil holds significant potential as a key component in cosmetic formulations and pharmaceutical materials due to its potent antioxidant effects. However, the study's limitations include the relatively low extraction efficiency and the limited scope of antioxidant testing. Future studies should investigate more efficient extraction methods, such as SFE, and include in-vivo testing to further validate the benefits of β-tocopherol for health and cosmetic uses.

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