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Enzymatic Synthesis and Antimicrobial Studies of Ethyl Butyrate: A Short Chain Ester for Fragrance Industry

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ABSTRACT

Ethyl butyrate was successfully synthesized via enzymatic esterification of butyric acid and ethanol using immobilized lipase (Novozym 435) as a catalyst. The product was characterized using multiple analytical techniques to confirm its identity and purity. Fourier Transform Infrared (FTIR) spectroscopy revealed a strong absorption band at 1734 cm^{-1}, confirming the presence of the ester carbonyl group (C=O). In the ^{13}C Nuclear Magnetic Resonance (NMR) spectrum, distinct peaks at 174.74, 62.63, 35.89, 18.41, and 13.39 ppm indicated the presence of different carbon environments corresponding to the ethyl butyrate structure. Gas Chromatography-Mass Spectrometry (GC-MS) further confirmed the compound's identity with a molecular ion peak at m/z 116, matching its theoretical molecular mass.The antimicrobial activity of the synthesized ethyl butyrate was evaluated against both Gram-positive and Gramnegative bacteria using the agar diffusion method. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values were determined, revealing strong antimicrobial activity particularly against *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli*, and *Bacillus subtilis*. MBC/MIC ratios were below 4, suggesting effective bactericidal properties. Additionally, the physicochemical properties of ethyl butyrate were assessed. It showed a Sun Protection Factor (SPF) value of 28, a saponification value of 228, and both iodine and peroxide values of 0. These results comply with industrial safety standards, indicating that the compound is stable and safe for consumer use. Overall, the synthesized ethyl butyrate demonstrated desirable chemical characteristics, notable antimicrobial activity, and physicochemical properties suitable for use in cosmetic or fragrance formulations.

1. Introduction

Ethyl butyrate, a volatile ester with a fruity aroma, finds widespread application in diverse industries, including food, fragrance, and pharmaceuticals. Its characteristic scent, reminiscent of fruits like apples, pineapples, and bananas, has long made it a popular choice for flavoring and

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fragrance enhancement. Beyond its olfactory appeal, recent research has shed light on the potential antimicrobial properties of ethyl butyrate, rendering it of particular interest in the food industry for its role in food preservation.

The synthesis of ethyl butyrate can be synthesized via esterification reaction between butyric acid and ethanol, catalyzed by novozyme catalysts. Meticulous control of reaction parameters such as temperature, reaction time, and catalyst concentration is conducted during the chemical synthesis, the synthesis process can be optimized to enhance both the yield and purity of Ethyl butyrate [1]. This synthesis serves as the foundational step, setting the stage for subsequent analyses and applications of Ethyl butyrate. However, in this study, immobilized lipase will be used as biocatalyst to speed up the reaction [2].

Spectroscopy techniques, including Fourier Transform Infrared Spectroscopy (FTIR), Gas Chromatography Mass Spectrometry (GC-MS), and Nuclear Magnetic Resonance spectroscopy (NMR), play pivotal roles in this validation process. FTIR spectroscopy aids in identifying functional groups and confirming the presence of ester bonds in Ethyl butyrate, while GC-MS analysis provides quantitative and qualitative insights into its composition and purity. NMR spectroscopy further elucidates its molecular structure, validating its chemical identity [3].

Other than that, understanding the physicochemical properties of ethyl butyrate is paramount for its diverse applications across industries. Properties such as SPF value, peroxide value, saponification value and iodine value will be rigorously investigated using established methodologies. Additionally, stability studies under varying environmental conditions will provide valuable insights into the robustness and versatility of Ethyl butyrate, guiding its optimization for specific applications [4].

In essence, the proposed research endeavors to holistically explore the synthesis, characterization, and antimicrobial activity of ethyl butyrate, with a view towards unlocking its multifaceted potential across industries. Through systematic experimentation and rigorous analysis, this research aims to advance the understanding and utilization of Ethyl butyrate, paving the way for its enhanced efficacy and application in diverse sectors of food science, fragrance, and pharmaceuticals.

2. Materials and Method

2.1 Synthesis of Ethyl Butyrate

To synthesize Ethyl butyrate via enzymatic esterification, a high purity of 200 mmol butyric acid and 400 mmol of ethanol were used [5]. Next, 3.5 g of immobilized lipase of Novozyme -435 was used as catalyst to speed up the reaction. The reaction mixture were placed in horizontal waterbath shaker at 50 Celcius with continuous shaking speed at 150 rpm for 8 hours, followed by cooling and extraction of the product using a filter paper. Next, the samples were analyzed using a titration method against 0.1 M sodium hydroxide (NaOH) to determine the percentage of Ethyl butyrate (Harris, 2011). Below shows (Equation 1) in determining Ethyl butyrate percentage yield.

$$\text{Ethyl butyrate yield (\%)} = \frac{\text{mol of initial acid} - \text{mol of acid after reaction}}{\text{mol of initial acid}} \times 100 \qquad (1)$$

2.1 Verification of Ethyl Butyrate

Verification of the synthesized Ethyl butyrate was conducted using spectroscopy techniques. Fourier Transform Infrared spectroscopy (FTIR) was employed to identify characteristic absorption bands of functional groups present in Ethyl butyrate. Gas Chromatography Mass Spectrometry (GC-MS) analysis was utilized to determine the purity and composition of the synthesized Ethyl butyrate based on its retention time and mass spectrum. Nuclear Magnetic Resonance spectroscopy (NMR), particularly proton NMR, was employed to confirm the molecular structure and verify the presence of characteristic peaks corresponding to Ethyl butyrate.

2.2 Preparation of Test Microorganisms

Salmonella typhimurium, Bacillus subtilis, Staphylococcus aureus, and Escherichia coli were among the microorganisms used in this study. Mueller-Hinton broth (MHB) medium is used to cultivate the bacteria for the entire night at 37°C. The ideal density of bacterial growth (OD) was then measured at 600nm by using the Bio Photometer. Next, the bacterial incolumn was then diluted by using Muller-Hinton Broth until the concentration reaches 107 cells/mL before the antimicrobial test.

2.3 Agar Diffusion Method

The agar diffusion test was conducted using the method provided by Smith Palmer [6]. Using sterilised cotton swabs, bacterial cultures are disseminated onto Mueller-Hinton agar (MHA) at a concentration of 107 cells/mL. Every experiment is run twice. Using a cork borer, test wells measuring 6 mm in diameter are made on each agar plate. Next, 10 µL of the test samples at various concentrations were added to each test well. Each test well receives the addition of a positive control, streptomycin sulphate solution at a dosage of 10 mg/mL. Following an overnight incubation at 37°C, the lowest concentration at which an inhibitory zone is seen on the agar was reported. The inhibitory zone's diameter is calculated and reported in millimetres.

2.4 Minimum Inhibitory Concentration (MIC)

After completed with agar diffusion method, the qualitative insight was obtained and proceed to do with the determination of minimum inhibitory concentration (MIC). For this process, a 96-well microtiter plate (Costar, USA) was used. All bacteria that was prepared before (50 µL) was put to each well of the microtiter plate that holds 50 µL of the test sample solutions (Ethyl butyrate). After that, the microtiter plate was covered with a its lid and kept overnight at 37°C for incubation. To determine the presence of bacteria, the MTT solution was used (3-[4,5 dimethyldiazol-2-yl]-2,5-diphenyltetrazolium bromide), the bacteria's survival following exposure to the test compounds was evaluated. A solution of MTT (0.3 mg/mL) was made by dissolving about 0.3 mg of MTT powder in 1 mL of distilled water that has been sterilised. About 40 µL of MTT was added to each test well, and the microtiter plate was then incubated for a further five minutes at 37°C. Minimum inhibitory concentrations (MICs) are those at which formazon blue does not appear to form. Every experiment is run twice [6].

2.5 Minimum Bactericidal Concentration (MBC)

After adding the 2,5-diphenyltetrazolium bromide (MTT) solution and dimethylthiazol-2-yl. In order to prepare the Smith Palmer approach, the value of the Minimum Bactericidal Concentration (MBC) were ascertained. This method involves filling individual wells of a 96-well microtiter plate

with 50 µL of each type of test material and 50 µL of a prepared bacterial inoculum. Next, the microtiter plates are incubated at 37°C for the entire night. Following that, each sample from the test wells was swabbed onto the Mueller-Hinton agar (MHA) medium. After an overnight incubation at 37°C, the growth of the bacteria was measured. The Minimum Bactericidal Concentration, or MBC, is the lowest concentration of any test sample that did not exhibit any bacterial growth [6].

2.6 SPF Value

The samples, each weighing 1 g, were transferred to 100 mL volumetric flasks for SPF value analysis. Subsequently, the solution was diluted with ethanol, then a 5-minute ultrasonification process was conducted, and then filtered through cotton, discarding the first 10 mL for UV- Visible testing. The determination of absorption spectra of the samples in solution was obtained within the 290-450 nm range. Ethanol in a 1cm quartz cell served as a blank, and absorptions were recorded every 5 nm, collecting data in the 290 to 320 nm band. The Mansur equation (Equation 2) is employed to calculate the SPF value.

$$Mansur\ Eq = CF \times \sum 320290 \times EE \times I \times Abs \qquad (2)$$

Where, CF = correction factor
EE = an erythemal effect spectrum I = the solarintensity spectrum Abs = absorbance of the sample

2.7 Peroxide Value

5.0 g of the material was dissolved in 10 mL of chloroform in a 250 mL conical flask and stirred. The mixture was then mixed with 1 mL of potassium iodide solution and 15 mL of acetic acid. The procedure was restarted by adding 30 mL of distilled water and 1 mL of starch indicator after being left in a dark place for five minutes. To find the peroxide value, the solution was titrated with 0.05 molarity sodium thiosulphate until the indicator's blue hue vanished (Equation 3) [7].

$$\text{Peroxide Value} = \frac{[(B - S) \times N \times 1000]}{W} \qquad (3)$$

Where, S = volume of titrant (mL) for the sample B = volume of titrant (mL) for blank
N = normality of sodium thiosulphate solution (mmol/mL)1000 = conversion of the unit (g/kg) W = mass (g) of the sample (Ethyl butyrate)

2.8 Saponification Value

Each sample, precisely weighed at 2.0 grams, is introduced into a 250 mL conical flask. Following this, 25 mL of ethanolic potassium hydroxide were added, and the mixture underwent reflux for 60 minutes. Subsequently, 1 mL of phenolphthalein solution is introduced, and the mixture were titrated with 0.5 N of hydrochloric acid, until the indicator's pink color disappeared. (Equation 4) was then applied to calculate the saponification value [8].

$$\text{Saponification value} = \frac{[(\text{B} - \text{S}) \times \text{N} \times 56.1]}{\text{W}} \quad (4)$$

Where, B = volume of titrant (mL) for the blank S = volume of titrant (mL) for the sample
N = normality of hydrochloric acid HCl (mmol/mL)
56.1 = the molecular weight of potassium hydroxide KOH (g/mol)W = mass (g) of sample (Ethyl butyrate)

2.9 Iodine Value

Each sample is weighed precisely at 2.0 grammes and put in a 500 mL stoppered flask with 10 mL of chloroform. After carefully pipetting 25 mL of Wij's solution into the flask, it is capped and stirred to ensure complete mixing. For half an hour, the flask was kept at room temperature in a dark place. Following the addition of 20 millilitres of potassium iodide, 100 millilitres of freshly boiled and cooled distilled water were added. 0.05 M sodium thiosulphate solutions were used to titrate the mixture until the yellow hue was hardly undetectable. The titration was repeated after adding 1 mL of a starch indicator until the indicator's blue hue disappeared. The same criteria were used for a blank determination [9].

$$Iodine\ Value = \frac{[(B - S) \times N \times 126.9]}{W} \quad (5)$$

Where, B = volume of titrant (mL) for the blank S = volume of titrant (mL) for the sample
N = normality of sodium thiosulphate (mmol/mL)
126.9 = the molecular weight of iodine (g/mol)

3. Results

3.1 Enzymatic Synthesis of Ethyl Butyrate

The result from this reaction is the successful synthesis of Ethyl butyrate through the enzymatic esterification reaction between butyric acid and ethanol. Esterification is a chemical process that involves the combination of an alcohol and an acid to produce esters and water as the final products. The most common method for creating esters is by heating a carboxylic acid with an alcohol while removing the water that is formed. In essence, esterification is the reverse of hydrolysis and is influenced by the water content in the reaction mixture. In this study, immobilized lipase of Novozyme -435 was used as catalyst. The catalyst helps to speed up the reaction by react with carboxyl group of butyric acid to form acyl-enzyme as intermediate. Next, ethanol act as nucleophile and attack the acyl enzyme to form ethyl butyrate. Upon completion of the reaction, the formation of Ethyl butyrate should be confirmed by observing the characteristic fruity aroma associated with the compound [5].

3.2 Verification of Synthesized ethyl Butyrate

Verifying synthesized Ethyl butyrate using instruments ensures the product's identity, purity, and structure while confirming reaction success. This process ensures quality, efficiency, and reproducibility especially in industrial. In this experiment, synthesized Ethyl butyrate was successfully achieved as the expected results. The FTIR absorption spectra of Ethyl butyrate were examined, and the summary of these spectra is provided in figure 1. The spectrum indicates the presence of an ester group in the sample, as evidenced by the medium stretching in the 1735.07 cm-1 region for Ethyl butyrate.

For GC-MS, the chromatographic separation demonstrated a well-resolved peak with a retention time window of 4.071-4.713 minutes, encompassing 131 scans, indicating optimal chromatographic conditions and efficient separation. The mass spectral analysis provided definitive structural information through characteristic fragmentation patterns. The molecular ion peak was observed at m/z 116.0, corresponding to the molecular formula $C_6H_{12}O_2$. The base peaks at m/z 43.1 and 71.1 represent the dominant fragments $[CH_3CO]^+$ and $[C_4H_7O]^+$, respectively, which are characteristic fragmentations for ethyl esters. The results is shown in the Figure 3.2.

The NMR analysis of the Ethyl butyrate sample provides detailed insights into its structural composition. In the 1HNMR spectrum, distinct signals corresponding to the protons in the ethyl and butyrate groups are observed. The ethyl group exhibits a triplet around 1.15 ppm, representing the methyl protons CH3, coupled to the adjacent methylene group. This is complemented by a quartet at 4.02 ppm, corresponding to the methylene protons CH3 adjacent to the oxygen atom. The butyrate group shows a triplet at 0.86 ppm for the terminal methyl protons CH3, a multiplet at 1.52 ppm for the middle methylene protons CH2, and a deshielded multiplet at 2.20 ppm for the alpha-methylene protons near the ester group.

In the 13CNMR spectrum, the carbonyl carbon C=O appears at a characteristic downfield region around 174.74 and 173.02 ppm due to its deshielding by oxygenatoms. The methylene carbon in the ethyl group exhibits a resonance at 62.63 ppm, indicative of its attachment to the oxygen atom. For the butyrate chain, the alpha methylene carbon resonates around ~34 ppm, while the middle methylene carbons show signals in the range of ~18-24 ppm. Lastly, the methyl carbons in both the ethyl and butyrate groups resonate at ~14 ppm, consistent with their shielded environment. These observations confirm the structural identity of ethyl butyrate and validate the expected chemical shifts and splitting patterns for this compound.

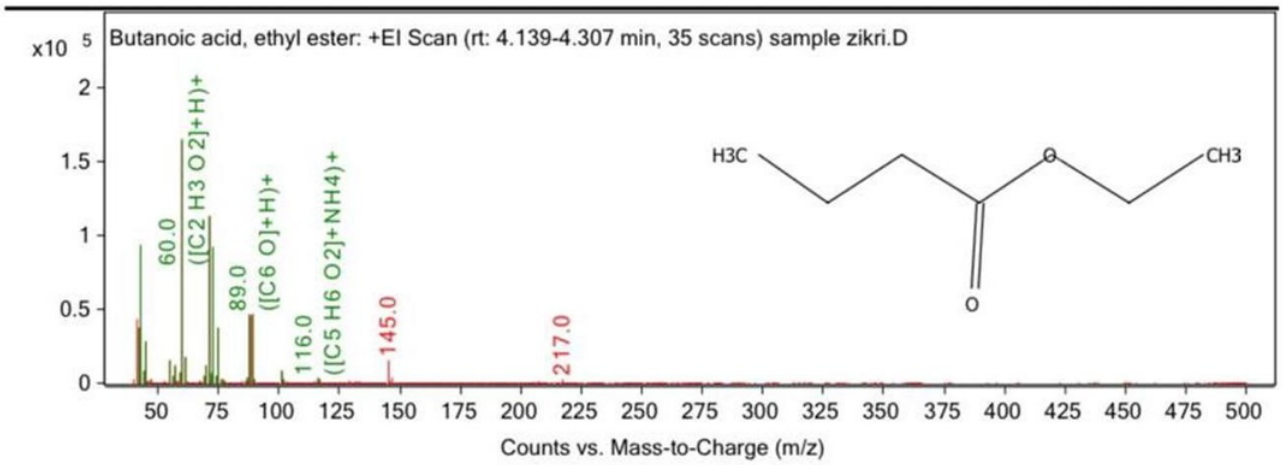

Fig. 1. FTIR absorption spectra of ethyl butyrate

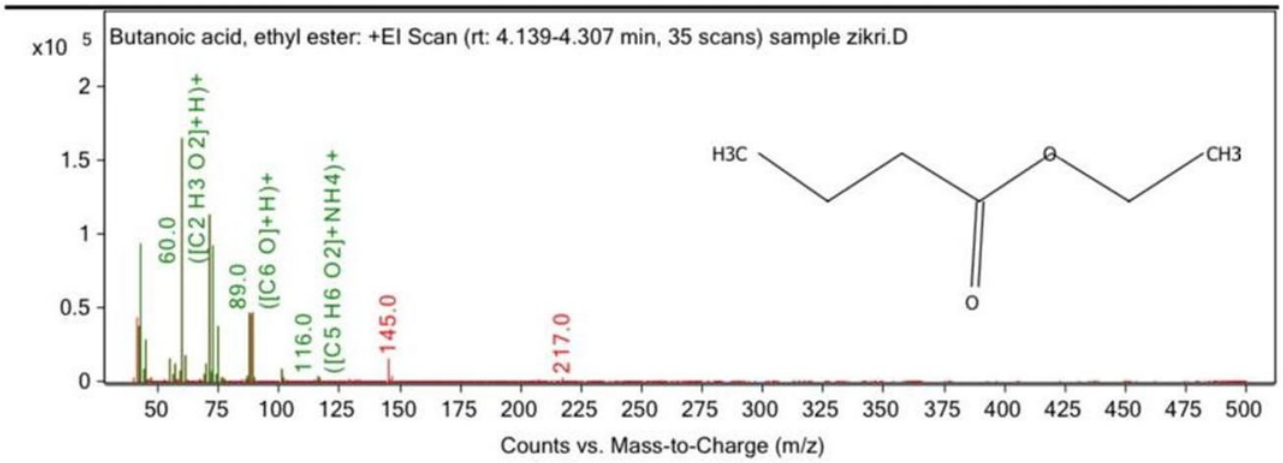

Fig. 2. GC-MS Result of Ethyl Butyrate

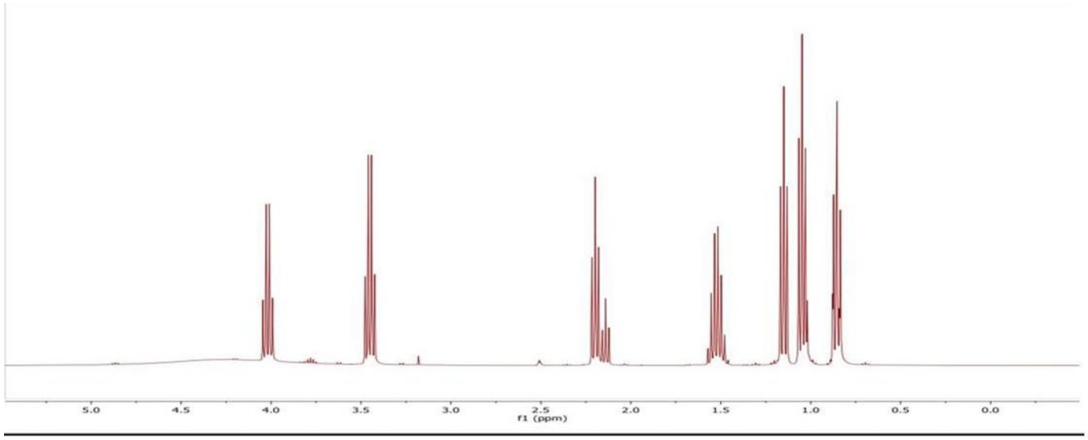

Fig. 3. Result of 13CNMR of Ethyl Butyrate

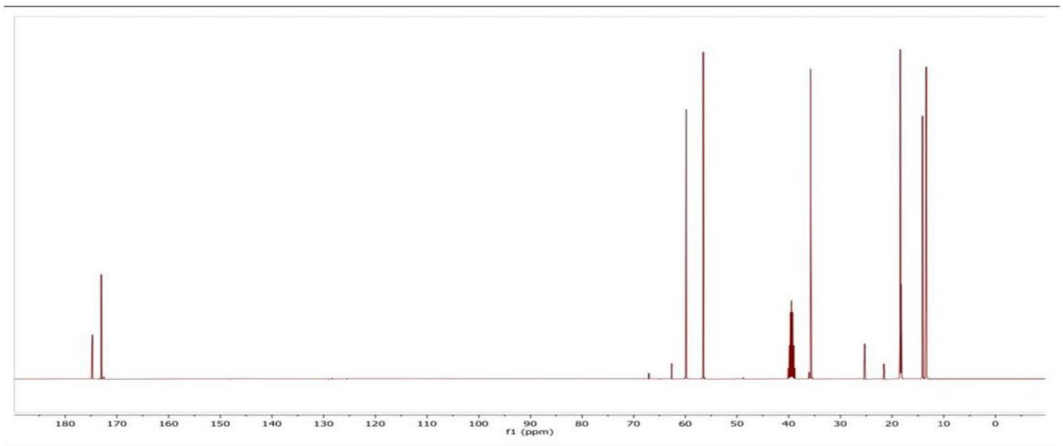

Fig. 4. Result of 1HMR of Ethyl Butyrate

3.3 Antimicrobial Properties of Ethyl Butyrate

Ethyl butyrate demonstrated antimicrobial effects against all the bacteria at pure and 1:1 v/v concentrations (Ester:Broth). However, Ethyl butyrate exhibited strong antibacterial activity with inhibition zones observed even at the fifth concentration (1:5 v/v) for average bacteria tested. The concentration of the flavor ester also influenced their antimicrobial effects, with higher

concentrations showing more significant activity. This is likely because the active compounds responsible for the antimicrobial action are more concentrated in higher doses, whereas lower concentrations may not be sufficient to display noticeable effects [11].

Among the bacteria tested, S. aureus (Gram-positive), and B. subtilis (Gram positive) were the most susceptible to the flavor ester, while S. typhimurium (Gram negative) and E. coli (Gram-negative) were more resistant. Some compounds may lose their effectiveness after the active ingredients are separated or synthesized, possibly due to the synergistic effects of components in the original compound that act together or inhibit each other. In other hands, the result observed is because Gram-negative contain outer membrane of the cell walls that make it more resistant to the ester [11].

Table 1
Diameter of Inhibition Zone (mm) for Antimicrobial Test of Ethyl butyrate via Agar-Well Diffusion Assay

Concentration of test samples (v/v) (Ester:Broth)	Diameter of Inhibition Zone (mm)			
	Staphylococcus aureus	*Salmonella thyphirium*	*Bacillus subtilis*	*Eshericia coli*
aPure	b9.0±0.0	20.0±0.0	24.0±0.5	24.0±0.0
1:1	18.0±0.5	18.0±0.0	19.0±0.5	18.0±0.0
1:2	-	16.0±0.0	18.0±0.5	16.0±0.5
1:3	[c]-	15.0±0.0	17.0±0.0	13.0±0.5
1:4	-	14.0±0.0	16.0±0.0	12.0±0.5
1:5	-	13.0±0.0	16.0±0.0	11.0±0.5
1:6	-	-	15.0±0.0	-
1:7	-	-	15.0±0.0	-
1:8	-	-	15.0±0.0	-
1:9	-	-	15.0±0.0	-
[d]S10	28.0±0.0	26.0±0.0	28.0±0.0	38.0±0.0

[a]Concentration of test sample (v/v); [b]Diameter of inhibition zone (mm); [c]No zone of inhibition; [d]Streptomycin (10mg/mL)

Fig. 5. Formation of inhibition zone at positive control and no clear zone at negative control

However, the mere formation of an inhibition zone in the agar diffusion test does not necessarily indicate the full effectiveness of an antimicrobial agent. Other factors, such as bacterial inoculum volume, choice of culture media, bacterial growth phase, medium pH, incubation time, temperature, and the diffusion properties of the sample through the agar, can all affect the outcome of the test [12].

3.4 Minimum Inhibitory Concentration (MIC)

The microdilution method was used as an alternative approach to determine the minimum inhibitory concentration (MIC) of the flavor esters. The results from the two methods differed, with the microdilution technique proving to be more effective for MIC determination. This test evaluated bacterial viability using 3-[4,5-dimethylthiazol-2-yl] 2,5- diphenyltetrazolium bromide (MTT, thiazolyl blue), which was dissolved in each well and incubated at 37°C for 30 minutes. The formation of a blue color indicated bacterial growth in the wells (Tables 2).

In the serial dilutions (ranging from 1:1 to 1:10 v/v), MIC values could not be determined by using bare eyes for Ethyl butyrate across all tested bacteria in the microtiter plates, indicating its strong antimicrobial activity. The information of MIC obtained by using Elisa Reader. The information shown in the (Table 2). MIC value of Ethyl butyrate is 1:7 (v/v) for E. coli, 1:5 (v/v) for B. Subtilis, 1:6 (v/v) for S. thyphimurium and 1:5 (v/v) for Salmonella. So in this experiment, the blue color in the microtiter plate may be observed by using lower concentration of Ethyl butyrate (ranging from 1:10 to 1:100 v/v) for all bacteria tested.

3.5 Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) values were determined for various bacterial strains to assess the bactericidal properties of ethyl butyrate. MBC is defined as the lowest concentration of an antimicrobial agent that eliminates more than 99.99% of the initial bacterial population, with no visible bacterial growth on the agar plates. In this study, Ethyl butyrate was tested at different concentrations, revealing either bactericidal (bacteria- killing) or bacteriostatic (bacteria-inhibiting) effects. A bactericidal concentration refers to the lowest concentration at which bacterial growth is completely inhibited in broth, whereas a bacteriostatic concentration refers to the lowest concentration at which bacterial growth is prevented in broth, but growth resumes when the broth is plated onto agar [5]. The re-growth of bacterial colonies on Mueller-Hinton Agar (MHA) plates following treatment with specific concentrations of Ethyl butyrate indicated bacteriostatic effects, while no colony growth observed at higher concentrations signified bactericidal activity.

In this investigation, the antimicrobial efficacy of Ethyl butyrate was evaluated using both the microdilution method and the MTT assay to determine the Minimum Inhibitory Concentration (MIC) and MBC values. The results demonstrated strong antimicrobial activity of ethyl butyrate. The MTT assay showed no bacterial growth, even at a dilution of 1:10 (v/v), suggesting that Ethyl butyrate effectively inhibited bacterial growth at these concentrations. But after using Elisa Reader, there were significant difference between the value of serial dilution with positive control indicate that there was bacterial growth but at low concentration. The MIC values obtained further confirmed the potent antimicrobial activity of Ethyl butyrate, as it successfully inhibited bacterial growth at very low concentrations.

Table 2
The Minimum Inhibition Concentration (MIC) values of Ethyl butyrate

Concentration of test samples (v/v) (Ester-Broth)	Inhibitors			
	Staphylococcus aureus	*Salmonella thyphirium*	*Bacillus subtilis*	*Eshericia coli*
[a]Pure	-[a]	-	-	-
1:1	-	-	-	-
1:2	-	-	-	-

1:3	-	-	-	-
1:4	-	-	-	-
1:5	-	-	-	-
1:6	+	-	+	-
1:7	+	+	+	-
1:8	+	+	+	+
1:9	+	+	+	+
S10[d]	-	-	-	-
C11[e]	++	++	++	++

-[a] No bacterial growth; +[b] Bacterial growth with slightly turbidity; ++[c] Bacterial growth with highly turbidity; S10[d] Streptomycin (10 mg/ml). C11e Negative Control (MHB)

Table 3
MIC and MBC Values from Microdilution Test and MBC/MIC Ratio for Ethyl butyrate

Test bacteria	[a]MIC (v/v)	[b]MBC (v/v)	[c]MBC/MIC
Staphylococcus aureus	0.2	0.25	1
Salmonella typhimurium	0.17	0.2	1
Bacillus subtilis	0.2	0.25	1
Eschericia coli	0.14	0.17	1

[a]Minimum inhibition concentration (MIC); [b]Minimum bacteriacidal concentration (MBC); [c]Ration values of Minimum bacteriacidal concentration per minimum inhibition concentration

Ethyl butyrate exhibited the highest bactericidal activity against all bacteria, with an MBC value of 1:6 (v/v) for E. coli, 1:4 (v/v) for B. Subtilis, 1:5 (v/v) for S. thyphimurium and 1:4 (v/v) for S. Aureus, indicating that this concentration was sufficient to eliminate the bacteria. The MBC/MIC ratio for all tested bacteria was ≤ 4, supporting the conclusion that ethyl butyrate demonstrates bactericidal properties effectively killing the bacteria rather than merely inhibiting their growth. Additionally, the data revealed that Gram-positive bacteria exhibited greater sensitivity to ethyl butyrate compared to Gram-negative bacteria, suggesting a higher effectiveness of ethyl butyrate against Gram-positive strains. These findings underscore the significant antimicrobial potential of ethyl butyrate, particularly against Gram-positive bacteria.

3.6 SPF Value

The Sun Protection Factor (SPF) is a widely accepted parameter for estimating the effectiveness of sunscreen products in protecting the skin from ultraviolet (UV) radiation. Higher SPF values typically indicate greater levels of protection [6]. Products with SPF values between 2 and 12 are classified as offering minimal protection, 12 to below 30 as moderate protection, and values above 30 as providing high protection.

In this study, the SPF of ethyl butyrate was determined using UV-Vis spectroscopy, an established in vitro method known for its cost-effectiveness and suitability for preliminary screening in cosmetic formulations prior to in vivo testing. Using a correction factor (CF) of 10, the maximum absorbance of ethyl butyrate was observed at 228 nm with a value of 2.826, resulting in a calculated SPF of 28.26, indicating a moderate level of UV absorption.

3.7 Peroxide Value

The peroxide value (PV) is a key indicator used to assess the oxidative stability of ester compounds, particularly in evaluating the formation of primary oxidation). It is typically expressed in milliequivalents (mEq) of peroxide per kilogram of sample. According to Nielsen (2023), peroxide

values above 20 mEq/kg are indicative of poor quality and are often associated with off-flavors and product degradation. In contrast, high-quality materials generally exhibit low or zero peroxide values.

In this study, the synthesized ethyl butyrate ester demonstrated a peroxide value of 0, indicating excellent oxidative stability. This is particularly favorable in the fragrance industry, where a peroxide value of zero is considered ideal, as it suggests the product is free from peroxides unwanted byproducts that can lead to rancidity and reduce shelf life. The absence of peroxides in ethyl butyrate can be attributed to its fully saturated structure, containing no double bonds, which significantly limits its susceptibility to oxidation. Reactive oxygen species, such as peroxides, commonly form in unsaturated oils or esters upon exposure to air, heat, or light, leading to undesirable changes in odor and stability. Therefore, a peroxide value of zero not only confirms the chemical stability of ethyl butyrate but also supports its suitability for use in oxidation-sensitive fragrance formulations [7].

3.8 Saponification Value

The saponification value is defined as the amount of potassium hydroxide (KOH), in grams, required to saponify 1 gram of ester. A higher amount of KOH indicates the saponification of ester bonds in the molecule. Wax esters with high molecular weights of triacylglycerols are associated with low saponification values. It is stated that high 44 saponification values correspond to low molecular weight samples, as short-chain fatty acids are predominant. The same study found that high saponification values reflect the presence of esters containing short-chain fatty acids [12].

In this experiment, the saponification value of ethyl butyrate was determined to be 226, indicating a relatively high saponification value due to its shorter molecular chain. Long-chain fatty acids, in contrast, tend to have lower saponification values due to a reduced number of carboxylic functional groups per unit mass compared to short-chain fatty acids.

3.9 Iodine Value

The iodine value measured in this experiment was successfully determined using the method previously described. The iodine value is crucial for assessing the degree of unsaturation in fatty acids within the samples. In this experiment, the iodine value of Ethyl butyrate was determined to be 0. A higher iodine value indicates a higher degree of unsaturation in fatty acids [13]. Additionally, it is suggested that esters with higher iodine values typically demonstrate better liquidity properties. It is also well-established that an increase in iodine value correlates with a higher number of double bonds in the compound [13]. Based on the value determined for ethyl butyrate, it can be concluded that the compound is saturated compound, as indicated by its iodine value of 0.

Since there are no double bonds or unsaturated sites for iodine to react with, a value of 0 indicates that the oil or fat is fully saturated. Most natural oils, including those used in perfumes (such carrier oils or essential oils), usually have iodine values that are not zero, which suggests some degree of unsaturation. A zero-iodine result does not always indicate a problem, even though it is uncommon in many oils used in perfumes. In addition to being less reactive and less likely to mix well with other unsaturated scent compounds, it may signify a highly saturated material, which may be more stable [13]. Its acceptability would depend on the situation in which it is used. Even though it contains very low iodine value, it could be used in many others formulation and application. Table 4 shows the summary of physicochemical results of Ethyl butyrate.

Table 4
Summary of physicochemical results of ethyl butyrate

Test	Value	Explaination
Peroxide value	0 (mEq/kg)	No presence of double bond
Iodine value	0 (g og I2/100g)	No presence of double bond
Saponification value	226 (KOH/g)	Short chain ester
SPF Value	28	Presence of ester group

4. Conclusion

The synthesis of Ethyl butyrate using conventional method were accomplished using butyric acid and ethanol. Immobilized lipase (Novozyme -435) was used in the experiment as catalyst to speed up the reaction. In the concept of green chemistry, the method used was suitable for the current trend of sustainable of fragrance industry.

The experiment was well conducted as the sample was verified using various instrument including NMR, GC-MS and FTIR. The sample's result of the every instrument matched with the standard indicate the successful in synthesis of Ethyl butyrate. For the safety aspects, bioassay study was conducted. The bioassay including agar diffusion and serial dilution techniques. From the antimicrobial study, the results shows that the Ethyl butyrate have antimicrobial activity against four selected bacteria which are Escherichia coli, Salmonella typhimurium, Bacillus subtilis and Staphylococcus aureus.

Different concentration of Ethyl butyrate inhibited all examined microorganisms, even the agar diffusion essay showed a limited inhibition zone. To improve the outcome of minimal inhibition concentration (MIC), serial dilution methods were used as a backup approach. When compared to the agar diffusion approach, the serial dilution technique proved to be more successful. By altering sample concentrations from 1:1 until 1:10, the minimum bactericidal concentration (MBC) of Ethyl butyrate was also investigated. The result shows that the average MIC and MBC value for inhibite and killing bacteria is at 1:6 meaning that only small amount of Ethyl butyrate needed to prevent bacteria from growing. The MBC/MIC value for all bacteria tested was determined to be <4 indicate that ethyl butyrate is a promising compound to inhibit bacteria.

The physicochemical properties such as SPF value, peroxide value, iodine value and saponification value of Ethyl butyrate were also determined in this study. The physicochemical assay done suggest that the synthesized Ethyl butyrate can be used as main ingredients in fragrance formulation. The SPF value obtained was 28, the iodine and peroxide value was 0 and the saponification value obtained was 226.

4.1 Suggestions and Recommendations

The findings have discovered new area of research, which can be developed further. The following areas were identified to be worthy for further investigation. In the enzymatic esterification of butyric acid and ethanol, one molecule of water is produced as a by-product, which can shift the reaction equilibrium unfavorably. Therefore, an effective method for removing water during the reaction is essential to drive the reaction toward ester synthesis and enhance the overall conversion rate. However, the use of a rotary evaporator is unsuitable, as the boiling point of ethyl butyrate is close to that of water, making selective removal difficult. In addition, key reaction parameters such as reaction time, temperature, substrate molar ratio, and the use of organic solvents should be systematically optimized using Response Surface Methodology (RSM) to achieve the highest possible product yield. The reaction has also demonstrated effective conversion under solvent-free conditions, making this approach valuable for scale-up processes, especially in food and

pharmaceutical applications where solvent toxicity, safety regulations, and disposal concerns are critical. Lastly, the potential applications of ethyl butyrate in flavor, fragrance, and pharmaceutical formulations should be further explored, as its favorable characteristics may contribute to the development of safe, high-quality consumer products.

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References

[1] Foukis, Athanasios, Olga A. Gkini, Panagiota-Yiolanda Stergiou, and Emmanuel M. Papamichael. "New insights and tools for the elucidation of lipase catalyzed esterification reaction mechanism in n-hexane: The synthesis of ethyl butyrate." *Molecular Catalysis* 455 (2018): 159-163. doi: 10.1016/j.mcat.2018.06.004.

[2] Api, A. M., D. Belsito, D. Botelho, M. Bruze, G. A. Burton Jr, M. A. Cancellieri, H. Chon et al. "RIFM fragrance ingredient safety assessment, ethyl butyrate, CAS registry number 105-54-4." *Food and Chemical Toxicology* 167 (2022): 113344.doi: 10.1016/j.fct.2022.113344.

[3] Cai, Jingwei, Jingtao Zhang, Yuan Tian, Limin Zhang, Emmanuel Hatzakis, Kristopher W. Krausz, Philip B. Smith, Frank J. Gonzalez, and Andrew D. Patterson. "Orthogonal comparison of GC–MS and 1H NMR spectroscopy for short chain fatty acid quantitation." *Analytical chemistry* 89, no. 15 (2017): 7900-7906. doi: 10.1021/acs.analchem.7b00848.

[4] Esipovich, Anton L., Evgeny A. Kanakov, Tatyana A. Charykova, Ksenia V. Otopkova, Mikhail A. Smirnov, Yulia A. Mityukova, and Artem S. Belousov. "A comprehensive study on physicochemical properties of fatty acid esters derived from different vegetable oils and alcohols and their potential application." *Energies* 17, no. 24 (2024): 6407. doi: 10.3390/en17246407.

[5] Salina Mat Radzi et al., World Journal of Pharmacy and Pharmaceutical Sciences, Vol. 4, pp. 1143-1151, 2015

[6] Zarkogianni, Maria, and Nikolaos Nikolaidis. "Determination of sun protection factor (SPF) and stability of oil-in-water emulsions containing Greek red saffron (Crocus Sativus L.) as a main antisolar agent." *International Journal of Advanced Research in Chemical Science* 3, no. 7 (2016): 1-7. doi: 10.20431/2349-0403.0307001.

[7] Barthel, G., and W. Grosch. "Peroxide value determination—Comparison of some methods." *Journal of the American Oil Chemists Society* 51, no. 12 (1974): 540-544. doi: 10.1007/bf02636025.

[8] A. Choudhary, "Determination of saponification value," Pharmaguideline, May 23, 2018. https://www.pharmaguideline.com/2011/07/determination-of-saponification- value.html

[9] S. K. C, "Determination of iodine value of fats and oils," The Science Notes, Jul. 16, 2023. https://thesciencenotes.com/determination-iodine-value-fats-oils/

[10] Fadli, M., J. Chevalier, L. Hassani, N-E. Mezrioui, and J-M. Pagès. "Natural extracts stimulate membrane-associated mechanisms of resistance in Gram-negative bacteria." *Letters in applied microbiology* 58, no. 5 (2014): 472-477. doi: 10.1111/lam.12216.

[11] Friedman, Mendel, Philip R. Henika, and Robert E. Mandrell. "Bactericidal activities of plant essential oils and some of their isolated constituents against Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, and Salmonella enterica." *Journal of food protection* 65, no. 10 (2002): 1545-1560.