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Research Article

Antibacterial and Antioxidant Activity of Ecoenzyme Solution Prepared from Papaya, Pineapple, and Kasturi Orange Fruits: Experimental and Molecular Docking Studies

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Organic waste, particularly from fruits, remains an environmental issue if not properly managed. These wastes have the potential to be reprocessed into products, one of which is beneficial for human health. The trend of recycling fruit scraps into ecoenzyme (EE), a fermented product, has increased in recent years. Several health advantages of EE have been highlighted. Therefore, this study is aimed at evaluating the potential antibacterial and antioxidant activity of ecoenzyme solutions derived from papaya, pineapple, and Kasturi orange. In this study, pieces of papaya, pineapple, and Kasturi orange were fermented with brown sugar for 10 days and 3 months. The 10-day- and 3-month-old fermented solutions were used as samples to evaluate their inhibitory abilities against the growth of *Escherichia coli* and *Staphylococcus aureus* using the well diffusion method. In addition, the fermentation solutions were tested for their antioxidant capacity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Furthermore, a molecular docking study was conducted to evaluate the compounds found in these three fruits for their ability to interact with the DNA gyrase of the two indicator bacteria. The enzyme, which is a well-established antimicrobial target, is involved in bacterial DNA replication, repair, and decatenation. The in vitro results revealed that EE, both fermented for 10 days and 3 months, had strong inhibition against *E. coli* and *S. aureus*. The 3-month-old fermentation solution showed stronger inhibitory activity than the 10-day-old fermentation solution. Despite a slight decline in antioxidant activity with increasing fermentation time, both of these samples exhibited extremely potent antioxidant activities. Molecular docking studies revealed that hesperidin and ciprofloxacin interacted more strongly with the DNA gyrase of *S. aureus* than with *E. coli*. Hesperidin can therefore function as a potent antimicrobial. The present study concluded that EE solution fermented from papaya, pineapple, and Kasturi orange exhibits the potential to serve as a source of both antibacterial and antioxidant compounds.

1. Introduction

The Sustainable Development Goals (SDGs) are a global action plan agreed upon by world leaders, including Indonesia, to end poverty, reduce inequality, and protect the environment. The 13th and 14th SDGs are about tackling climate change and protecting terrestrial ecosystems, respectively. Waste, both organic and nonorganic, is a threat to the environment if it is not managed properly. Households are the largest producers of waste. Recycling can be an effective waste management strategy. One method of recycling organic waste is to convert them into ecoenzyme (EE). EE is a liquid that is produced through the fermentation of organic materials, such as fruits, vegetables, and agricultural and household wastes [1, 2].

Fermentation, through the activity of microorganisms, breaks down complex organic compounds into simpler forms and produces bioactive compounds, such as antibacterial and antioxidants [3]. The resulting secondary metabolites include organic acids, phenolic compounds, terpenoids, and alkaloids, which have been found to have antimicrobial properties against pathogenic microorganisms [4, 5]. Polyphenols are recognized as the primary natural antioxidants in food; nevertheless, their efficacy can be hindered by being bound to cell walls, glycosylated, or present in polymeric forms, which can affect their bioavailability. However, during the fermentation process, various metabolic activities are involved in the release or conversion of polyphenols into more active forms [6]. Oxidation is the primary challenge in preserving food products during storage. Hence, the primary function of antioxidant compounds is to extend the shelf life of food products while also conferring health benefits [7].

The EE fermentation is anticipated to provide an alternative method to reduce solid matter, thereby reducing organic waste in the long run [8]. The EE solution has numerous advantages, including for agriculture (as liquid organic fertilizer and natural pesticides) [9, 10], health (as a disinfectant and cleaning fluid) [11, 12], and housekeeping (as an organic soap, floor and bathroom cleaner, and mouthwash/gargle) [13–15]. Products derived from EE can be used as cleaning solutions due to the enzymes they contain, which are capable of breaking down proteins and fats [13, 16, 17].

Papaya, pineapple, and Kasturi oranges are highly prevalent in Indonesia, resulting in suboptimal utilization of these fruits and a significant amount of waste generated from their underutilization. This residual fruit matter possesses the potential to be repurposed through recycling techniques, thereby generating health-enhancing products, such as through the process of fermentation. It has been previously documented that extracts from papaya fruit, including its seeds, peels, and pulps, possess antioxidant and anti-inflammatory properties that may make them suitable raw materials for the production of functional foods [18]. The pineapple fruit contains significant amounts of gallic acid, catechins, epicatechins, and ferulic acids, which are bioactive compounds that can function as active antioxidant ingredients. Furthermore, constituents such as flavonoids, saponins, and tannins have been identified as natural antimicrobials that can aid in reducing food spoilage [19]. Furthermore, it has been reported that the phytochemicals found in citrus fruits exhibit antimicrobial and antioxidant properties [20]. As a disinfectant, EE derived from the fermentation of these fruits exhibits the potential to inhibit the growth of pathogenic bacteria, including *Escherichia coli* and *Staphylococcus aureus*. They are two common pathogenic bacteria that can cause infections in humans. Additionally, they are prevalent foodborne pathogens that are capable of causing food poisoning [21]. Given the described facts, this study is aimed at assessing the growth-inhibiting potential of an EE solution derived from papaya, pineapple, and Kasturi orange fruits against pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus*. Additionally, the study is aimed at measuring the antioxidant activity of the EE solution.

2. Materials and Methods

2.1. Production of EE. The whole fruits of ripe papaya, pineapple, and Kasturi orange were washed and then cut into approximately 2×2 cm pieces. The fruit pieces were placed in a 27 L container afterward. As much as 1.6 kg of brown sugar was cut into pieces, boiled in 16 liters of clean water, and then cooled. Subsequently, the sugar solution was added to a container that contained pieces of fruit, where each fruit had a composition of 1.6 kilograms. The container was sealed tightly, and the fruits were fermented for ten days. After ten days of fermentation, the lid was opened, and the fermented liquid was stirred evenly. The container was then sealed with tape and allowed to ferment for three months. The samples were acquired from the EE which had undergone fermentation for 10 days and 3 months, with the 3-month fermentation product being a continuation of the 10-day fermentation. Measurements taken at 10 days and 3 months during the fermentation process can provide valuable information regarding the short-term and long-term effects of the fermentation on the antibacterial and antioxidant activities. The acidity level of the solution was measured on the first day (prior to fermentation), on the tenth day, and three months after fermentation.

2.2. Evaluation of Inhibition Capacity of EE Solution against Pathogenic Bacteria. Evaluation of the inhibitory ability of EE solution against gram-negative (*Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™)) and gram-positive (*Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 25923TM)) bacteria was carried out using the agar-well diffusion method [22]. This method is commonly used to evaluate whether or not a plant or microbial extract possesses antimicrobial properties [23].

Slanted-agar-grown test bacteria were collected using sterile wire loops and suspended in 2 ml of 0.9% NaCl solution until a turbidity equivalent to the McFarland standard was achieved. All of the test bacteria were given the same treatment. The base layer for the antibacterial test was made by pouring 10 ml of liquid nutrient agar (NA) into a petri dish and allowing it to solidify. After that, several steel cylinders with a diameter of 6 mm were placed on the surface of the base layer, which was arranged so that the observation

distances would not overlap each other. Two hundred μl of the overnight bacterial suspension was then mixed homogeneously with the culture medium. The mixture was poured over the base layer. After the seed layer solidified, the steel cylinders were removed from the medium so that wells could be formed for antibacterial testing.

One μg/μl of ciprofloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid) was used as a positive control. Sterile distilled water served as a negative control. The EE solution was diluted with distilled water until its concentration was 10^{-2} and 10^{-4} times its initial value (10^{0}). Then the solution was heated in the thermoblock at 80°C for one hour and centrifuged for 1 min at 6000 rpm [24]. The supernatant, as well as the positive and negative controls, were added to the well in quantities up to 50 μl. The experiments were conducted in triplicate. The Petri dishes were then incubated in an incubator for 3×24 hours at 37°C. The clear area of inhibition was measured with a caliper and subtracted from the diameter of the well. Based on the Davis and Stout [25] classification, the diameter of the inhibition zone was classified according to its antibacterial potency. An inhibition zone with diameters >20 mm are classified as very strong, 10-20 mm as strong, 5-10 mm as a medium, and 5 mm as having no antibacterial activity.

2.3. Evaluation of the Antioxidant Activity of EE Solution. Both the 10-day- and 3-month-old fermented EE solutions were diluted to 25, 50, 75, 100, and 125 ppm with ethanol. The IC_{50} value of each EE was determined based on these solutions. As a control, a solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with a concentration of 0.1 mM was used. The radical scavenging activity was expressed as a percentage inhibition, which was calculated using the following formula:

$$\text{DPPH scavenging activity}(\%) = \frac{(\text{A0} - \text{A1})}{\text{A0}} \text{x } 100. \quad (1)$$

where A0 represents the absorbance of the control and A1 represents the absorbance of the samples.

In the linear regression equation, the sample concentration and the percentage of inhibition were plotted on the respective x and y axes. The IC_{50} value was calculated in Excel by plotting the inhibition curve and corresponding concentrations using the formula $y = ax + b$, where $y = 50$ and x is the IC_{50} value.

2.4. Molecular Docking. The CB-Dock2 server (https://cadd.labshare.cn/cb-dock2/php/blinddock.php) [26, 27] based on AutoDock Vina v.1.1.2 [28] was utilized for docking analysis. The sequence of the target proteins, DNA gyrase from *E. coli* and *S. aureus*, was retrieved from GenBank with accession numbers QGJ09419.1 and AID38448.1, respectively. The protein was modeled in the SWISS-MODEL [29] web server (https://swissmodel.expasy.org/interactive) according to the previous study [30]. Rosmarinic acid, caffeic acid, and hesperidin were used as ligands. These compounds were reported to be found in pineapple, papaya, and citrus, respectively [31–33]. The sdf files of the compounds were retrieved from PubChem using their respective CIDs: 5281792 for rosmarinic acid, 689043 for caffeic acid, and 10621 for hesperidin.

Table 1: Inhibition zone (mm) of various concentrations of EE fermented for 10 days against *E. coli* and their efficacy in comparison to 1 μg/μl of ciprofloxacin, which serves as a positive control.

Sample concentration	*Mean* ± *SD*	Inhibition effectiveness (%)
10^{0}	14.00 ± 0.50	49
10^{-2}	11.67 ± 0.76	41.43
10^{-4}	8.83 ± 0.58	28,40
Positive control	28.17 ± 0.29	100
Negative control	0	0

Table 2: Inhibition zone (mm) of various concentrations of EE fermented for 3 months against *E. coli* and their efficacy in comparison to 1 μg/μl of ciprofloxacin, which serves as a positive control.

Sample concentration	Mean ± SD	Inhibition effectiveness (%)
10^{0}	18.17 ± 0.29	62.69
10^{-2}	14.67 ± 0.29	49.18
10^{-4}	10.17 ± 0.29	30.09
Positive control	29.83 ± 0.29	100
Negative control	0	0

Table 3: Inhibition zone (mm) of various concentrations of EE fermented for 10 days against *S. aureus* and their efficacy in comparison to 1 μg/μl of ciprofloxacin, which serves as a positive control.

Sample concentration	*Mean* ± *SD*	Inhibition effectiveness (%)
10^{0}	17.67 ± 0.29	62.73
10^{-2}	14.17 ± 0.58	50.30
10^{-4}	11.67 ± 0.29	41.42
Positive control	28.17 ± 0.29	100
Negative control	0	0

3. Results

3.1. Antibacterial Activity of Ecoenzyme. The results of EE inhibition against E. coli are displayed in Tables 1 and 2, while those against S. aureus bacteria are depicted in Tables 3 and 4. The results indicated that EEs fermented for 10 days or 3 months and were effective against both types of pathogenic bacteria. The ability of EE to inhibit S. aureus was superior to that of E. coli. In addition, the antibacterial activity of EE fermented for three months was greater than that of EE fermented for ten days. Pathogen inhibition was more effective with undiluted EE than with 10^{-2} and 10^{-4} dilutions. The pH level of the EE liquid prior to fermentation

Table 4: Inhibition zone (mm) of various concentrations of EE fermented for 3 months against *S. aureus* and their efficacy in comparison to 1 μg/μl of ciprofloxacin, which serves as a positive control.

Sample concentration	*Mean* ± *SD*	Inhibition effectiveness (%)
10^0	22.3 ± 0.76	74.76
10^{-2}	17.67 ± 0.29	59.24
10^{-4}	12.67 ± 0.29	42.47
Positive control	29.83 ± 0.29	100
Negative control	0	0

Table 5: The IC_{50} of antioxidant activity EE fermented for 10 days.

Sample concentration (ppm)	Absorbance	Inhibition (%)	IC_{50} (ppm)
25	0.409	52.55	
50	0.366	57.34	
75	0.305	64.62	22.54
100	0.243	72.81	
125	0.145	83.18	
DPPH	0.862	—	

Table 6: The IC_{50} of antioxidant activity EE fermented for 3 months.

Sample concentration (ppm)	Absorbance	Inhibition (%)	IC_{50} (ppm)
25	0.436	49.42	
50	0.377	56.26	
75	0.308	64.27	29.56
100	0.244	71.69	
125	0.151	82.48	
DPPH	0.862	—	

was measured at 4.37. During a 10-day fermentation period, the pH of the solution decreased to 3 and remained consistent at this level for the duration of 3 months of fermentation.

3.2. Antioxidant Activity of EE. The antioxidant activity of EE was screened using the scavenging method against stable DPPH. A compound is classified as having very strong antioxidant activity if its IC_{50} value is <50 ppm, strong if it falls within the range of 50-100 ppm, moderate if it falls within the range of 100-150 ppm, and weak if it exceeds 150 ppm [34]. According to the calculation, which is presented in Tables 5 and 6, the antioxidant activity of fermented EE for 10 days was greater than that of fermented EE for 3 months. Nonetheless, these two samples exhibited extremely potent antioxidant activity.

3.3. Molecular Docking. DNA gyrase modeling of *E. coli* revealed 100% sequence identity with DNA gyrase subunit A, with QMEAN Z-scores of 0.82 ± 0.05 and a Ramachandran-favored value of 95.8%. Meanwhile, DNA gyrase modeling of *S. aureus* revealed a sequence identity of 99.19% with DNA gyrase subunit A. This was accompanied by QMEAN Z-scores of 0.80 ± 0.05 and a Ramachandran-favored value of 96.88%.

The results of cavity-detection guided blind docking between the DNA gyrase subunit A of *E. coli* and *S. aureus* against the ligands rosmarinic acid, caffeic acid, and hesperidin are presented in Tables 7 and 8. The three-dimensional (3D) conformation of the docking is presented in Figures 1 and 2. The binding-free energy (BFE) of *E. coli* DNA gyrase against rosmarinic acid, caffeic acid, hesperidin, and ciprofloxacin were -7.5, -6.0, -8.3, and -6.9 kcal/mol, respectively. This suggests that rosmarinic acid and hesperidin interact with the DNA gyrase of *E. coli* more strongly than caffeic acid and ciprofloxacin. Meanwhile, the BFE of *S. aureus* DNA gyrase was -7.7, -5.9, -10.1, and 8.0 kcal/mol against rosmarinic acid, caffeic acid, hesperidin, and ciprofloxacin, respectively. These findings suggest that the interaction of *S. aureus* DNA gyrase and hesperidin was stronger than that of rosmarinic acid, caffeic acid, and ciprofloxacin.

Rosmarinic acid, caffeic acid, and hesperidin interact with some of the same residues on *E. coli* DNA gyrase. Only two of the *E. coli* DNA gyrase common residues, ARG91 and TYR266, however, interacted with these four substances. On the other hand, the four compounds appeared to occupy the same cavity in *S. aureus* DNA gyrase, which was indicated by interactions with the same residues in ARG92, GLN95, PHE97, SER98, PHE266, GLN267, VAL268, ASN269, and LYS170. The cavity occupied by these four compounds was also larger in *S. aureus* than in *E. coli*, with volumes of 967 and 351Å^3, respectively.

4. Discussion

In the last decade, there has been an increase in research on natural antibacterial substances. EE derived from pineapple, orange, and papaya has been widely used in health therapy, such as in the treatment of dental disease and caries [13]. Previous studies have shown that papaya, orange, and pineapple have antibacterial properties [35–37]. The ability of EE to inhibit the growth of gram-negative (*E. coli*) and gram-positive (*S. aureus*) bacteria demonstrates its antibacterial activity. However, its effectiveness was still lower than that of ciprofloxacin, a broad-spectrum antibiotic used as a positive control. This antibiotic belongs to the second generation of fluoroquinolones [38]. This study demonstrates that the antibacterial efficacy of EE is proportional to its concentration. The higher the concentration, the higher the efficacy. This is consistent with the findings of the research carried out previously [13]. EE had a bactericidal effect against both pathogens tested.

During fermentation, microorganisms break down complex organic compounds into simpler molecules, resulting in the production of a variety of metabolites and organic acids [39]. As these compounds accumulate, they can alter the pH of the solution. The decrease in pH observed on the 10th day and 3rd month following fermentation is most likely due to the accumulation of these compounds. The precise

Table 7: The docking results of the complex of *E. coli* DNA gyrase subunit A and the ligands.

Compounds	BFE (kcal/Mol)	Cavity volume (Å^3)	Contact residues
Rosmarinic acid	-7.5	351	ARG32, ALA33, ASP39, **GLY40**, **LEU41**, **LYS42**, VAL44, **HIS45**, THR88, **ARG91**, **MET92**, SER97, **LEU98**, **LEU102**, LEU134, **ASN165**, LEU166, **ASN169**, **GLY170**, **SER171**, **SER172**, **TYR266**
Caffeic acid	-6.0	351	ASP39, **GLY40, LEU41, LYS42, HIS45, ARG91, MET92, LEU98, LEU102**, LEU134, **ASN165,** LEU166, **ASN169, GLY170, SER171, SER172,** GLY173, **TYR266,** GLN267,
Hesperidin	-8.3	351	ARG32, ALA33, PRO35, **GLY40, LEU41, LYS42,** VAL44, **HIS45,** SER83, ALA84, VAL85, ASP87, THR88, **ARG91, MET92, LEU98, LEU102**, **ASN165, ASN169, GLY170, SER171, SER172,** GLY173, ILE174, **TYR266,** GLN267
Ciprofloxacin	-6.9	351	TYR86, ASP87, THR88, VAL90, **ARG91,** GLN94, PHE96, SER97, GLY110, SER111, ILE112, GLY114, ASP115, SER116, ALA117, **TYR266,** GLN267, VAL268

Table 8: The docking results of the complex of *S. aureus* DNA gyrase subunit A and the ligands.

Compounds	BFE (kcal/Mol)	Cavity volume (Å^3)	Contact residues
Rosmarinic acid	-7.7	967	**ARG92**, **GLN95, PHE97, SER98,** TYR99, GLY111, **SER112, MET113, GLY115,** ASP116, ASN170, PRO219, **THR220, ILE264, PRO265, PHE266, GLN267, VAL268, ASN269, LYS270**
Caffeic acid	-5.9	967	**ARG92, GLN95, PHE97,** SER98, **SER112, MET113,** ASP114, **GLY115, THR220, ILE264, PRO265, PHE266, GLN267, VAL268, ASN269, LYS270**
Hesperidin	-10.1	967	GLY41, LEU42, LYS43, HIS46, GLU88, VAL91, **ARG92, GLN95, PHE97, SER98,** TYR99, PHE110, GLY111, **SER112, MET113,** ASP114, **GLY115,** ASP116, GLY117, ASN170, GLY171, ALA172, SER173, **THR220, ILE264, PRO265, PHE266, GLN267, VAL268, ASN269, LYS270**
Ciprofloxacin	-8.0	967	LYS43, HIS46, **ARG92, GLN95, PHE97, SER98,** TYR9,9 **SER112, MET113,** ASP114, **GLY115,** SER173, THR220, **PHE266, GLN267, VAL268, ASN269, LYS270**

composition and concentration of metabolites and organic acids can vary based on the microorganisms involved, fermentation conditions, and other variables [40]. A previous study demonstrated a significant increase in the levels of organic acids, total free amino acids, total phenolic compounds, and flavonoids during the fermentation process of pineapple by-products. Additionally, the final product was found to contain 152 distinct peptides [41].

For the production of EE from fruits, three months of fermentation are the bare minimum necessary to achieve the highest concentrations of hydrolytic enzymes and fermented organic acids [42]. It has been hypothesized that the concentrations of these compounds after prolonged fermentation will increase the antimicrobial activity of fruit-based EE [1]. The antimicrobial activity of papain contained in papaya is generally related to its enzymatic actions, such as amidase and esterase, which increase in a more acidic environment with a fermentation period of more than 3 months [43, 44]. However, EE maturity with a peak of hydrolytic enzymes occurred in the third month of fermentation as a result of synergistic interactions between EE in pineapple and orange [45].

Ethanol, as a by-product of the fermentation process, theoretically has antibacterial activity against various pathogenic microorganisms. However, fruit fermentation produces low levels of ethanol. The pH of a fermented liquid can affect its antibacterial properties. Typically, the EE of fermented fruit falls between 2.8 and 3.6 [2]. The antibacterial activity of fermented fruits can also be contributed by the presence of beneficial microorganisms, including lactic acid bacteria (LAB). Previous research demonstrated that LAB was successfully isolated from naturally fermented pineapple juice samples [46]. Fermented pineapple juice contained approximately 150 mM lactic acid (LA) and 30 mM acetic acid (AA), both of which were produced by LAB [47]. In terms of bactericidal efficacy, weak organic acids like LA are superior to inorganic acids [48].

The DPPH free radical scavenging method is a simple, fast, and inexpensive method that is widely used to evaluate the antioxidant potential of a compound, an extract, or other biological sources [49]. Moreover, *Caenorhabditis elegans* was used in studies on in vivo antioxidant activity, and the results showed comparable residual values against DPPH [50]. The antioxidant IC_{50} values for EE fermented for 10 days and 3 months as measured by the DPPH method, were 22.54 and 29.56 ppm, respectively. Using the same method, Trolox had an IC_{50} of 64.69 ppm, whereas ascorbic acid (vitamin C) had an IC_{50} of 41.25 ppm. The antioxidant activity decreases with increasing IC_{50} values [51]. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) is a water-soluble analog of vitamin E. This indicates that EE fermented for 10 days and 3 months has high antioxidant activity, exceeding that of vitamin E and vitamin C. The phenolic compounds in the fermented fruit likely play a role in this antioxidant activity [52, 53]. Using ultrahigh-performance liquid chromatography, the bioactive compound present in the extracts of fermented golden pineapple peel was able to be identified and quantified (UHPLC). This

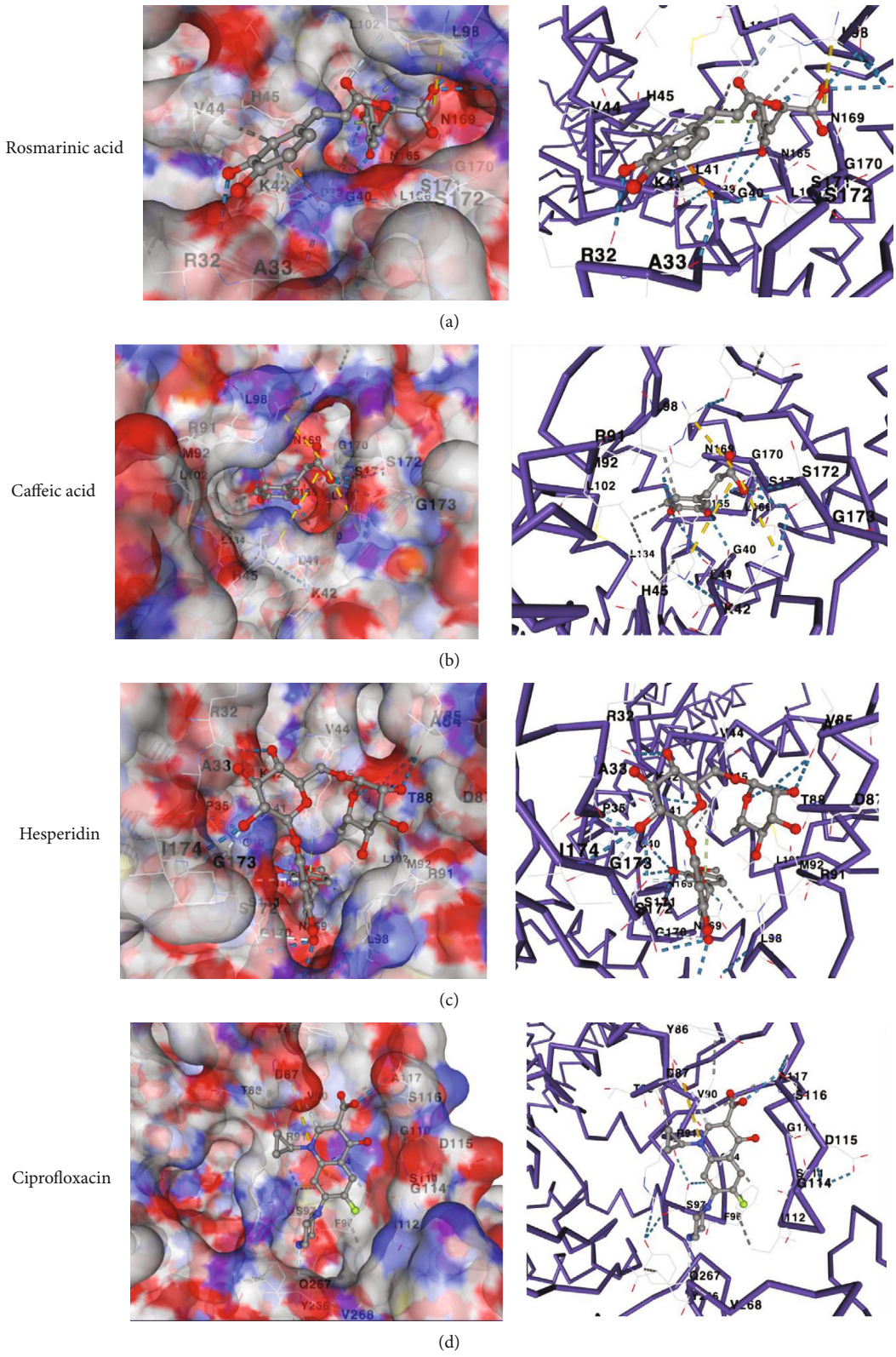

Figure 1: The three- and two-dimensions of the docking results of DNA gyrase subunit A of *E. coli* and rosmarinic acid (a), caffeic acid (b), hesperidin (c), and ciprofloxacin (d).

method demonstrated the presence of rosmarinic acid, caffeic acid, vanillic acid, p-coumaric acid, ferulic acid, quercetin-3 glucoside, rutin, quercetin, kaempferol-3 glucoside, and gallic acid, which demonstrates the great potential of these by-products to download components that are beneficial to the consumer's health [31].

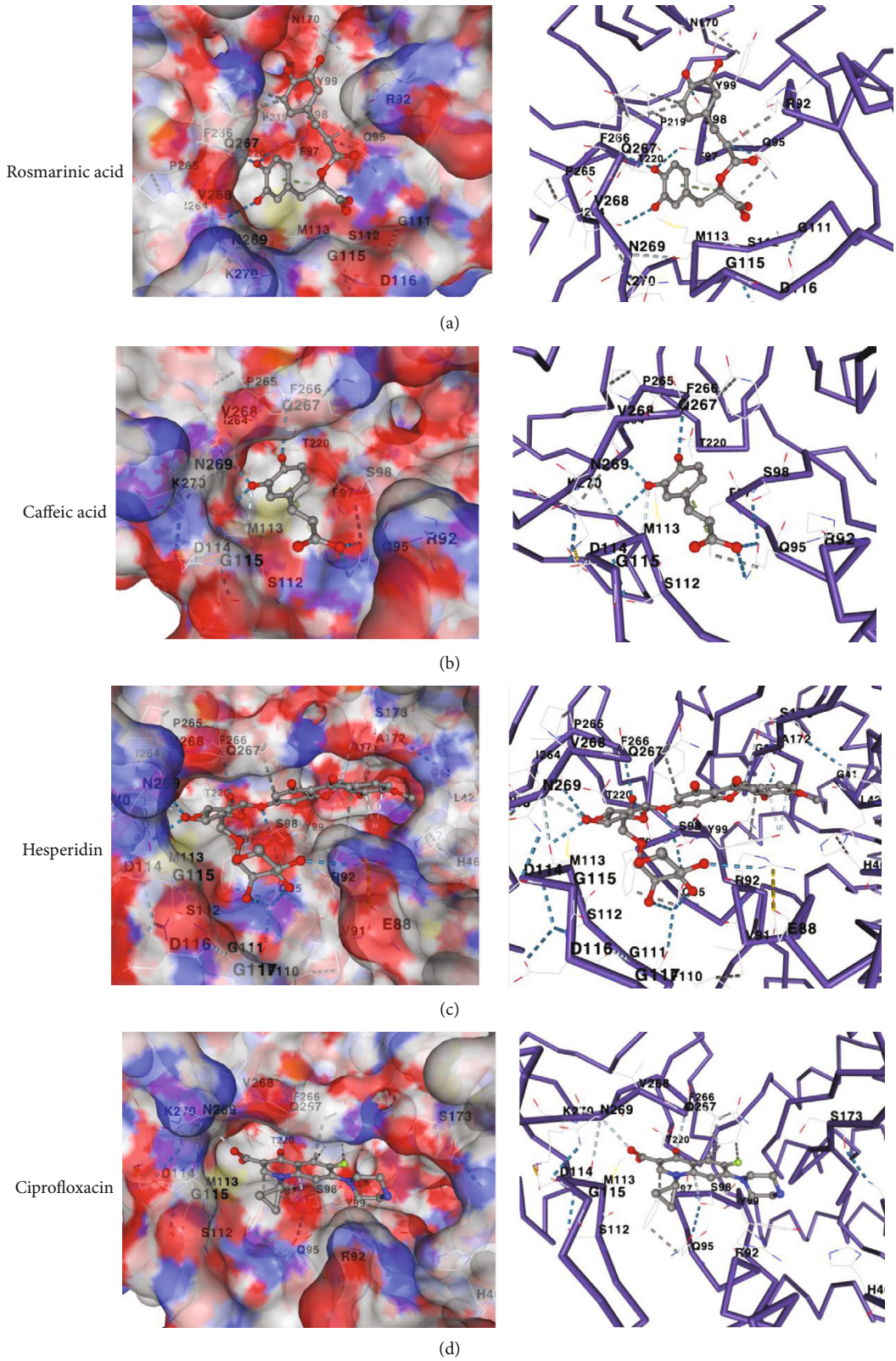

FIGURE 2: The three- and two-dimensions of the docking results of DNA gyrase subunit A of *S. aureus* and rosmarinic acid (a), caffeic acid (b), hesperidin (c), and ciprofloxacin (d).

Gallic acid, caffeic acid, p-coumaric acid, ferulic acid, and quercetin are the major polyphenols quantified in papaya peel extracts [32]. The extract showed a high antioxidant activity [54]. Moreover, citrus contains important phenolic compounds, such as hydroxycinnamic acids (HCA) and hesperidin. Generally, citrus peels contain more

antioxidants than fruit flesh [33]. It has been suggested that fermentation can result in an increase in antioxidant properties, with the potential for this increase depending on the metabolic activities of the starter that was used [6]. It has also been reported that fermented blueberry fruit juice has a greater antioxidant capacity than unfermented juice. Moreover, this fermented product exhibited potent antibacterial activity against *E. coli*, *S. aureus*, and *Salmonella typhimurium* [55]. The antioxidant activity of fermented citrus fruits is a result of the increased activity of small-molecule phenolic compounds [56]. This fact suggests that compounds with low molecular weight and potent antioxidant properties are produced during the fermentation process. Their antioxidant capacity is related to their ability to reduce reactive oxygen species, which are widely responsible for age-related diseases [50].

The EE solution derived from fermented papaya, pineapple, and Kasturi orange is purported to contain antioxidants that have the potential to provide a range of health benefits. These antioxidants may function by neutralizing free radicals, enhancing immune function, reducing inflammation, shielding cells from damage, and lowering the risk of chronic diseases, such as cancer, heart disease, and Alzheimer's disease. Furthermore, these EE may hold promise for promoting skin health, as they comprise vitamin C, carotenoids, and natural enzymes that safeguard skin cells from harm, decrease inflammation, and minimize wrinkles. Additionally, they are abundant in nutrients that may have the potential to protect eye health by lessening oxidative stress and safeguarding against age-related vision problems. Nonetheless, the particular antioxidant compounds found in EE fermented for 10 days and 3 months necessitate further investigation.

Molecular docking is an established structure-based in silico technique widely employed in drug discovery [57], owing to its capacity to predict the binding-conformation of small molecule ligands to the correct target binding site [58]. According to the results of the molecular docking study, rosmarinic acid, caffeic acid, hesperidin, and ciprofloxacin interacted more strongly with the DNA gyrase of *S. aureus* than *E. coli*. ciprofloxacin and other quinolone-derived antibiotics have been shown to bind bacterial DNA gyrase, causing it to introduce double-stranded breaks into DNA [59, 60]. Based on the docking study and inhibition test results, it is evident that ciprofloxacin is more likely to inhibit *S. aureus* than *E. coli*. Similarly, EE solutions fermented for 10 days and 3 months were more effective at inhibiting *S. aureus* than *E. coli*. This finding, however, contradicts earlier studies that found ciprofloxacin to be the most effective medication for treating gram-negative bacteria [61].

The results of this research demonstrate that EE has the potential to serve as a source of antibacterial and antioxidant properties. However, the methanol content of this EE product needs to be investigated further. This is due to a disagreement among EE practitioners; some allow raw EE consumption, while others prohibit it. Methanol contamination must be taken into account during the EE fermentation process because this compound, which can be fatal if levels exceed the threshold level, can be produced during the fermentation of fruit-based beverages [62]. The production of methanol itself can be related to the activities of pectinase-producing yeast, fungi, and bacteria [63]. Methanol concentrations in typical ranges of 6-27 mg/l for beer and 10-220 mg/l for spirits are not harmful, according to the WHO [62].

Natural antibacterial agents and antioxidants have multiple advantages over synthetic antibiotics. They are derived from natural sources, have a broader spectrum of activity, are safer, better tolerated, have fewer side effects, and are more sustainable. These natural compounds have been extensively studied and tested for safety and efficacy, and their use can help protect cells from damage caused by free radicals, prevent chronic diseases, and fight against bacterial infections. Therefore, the use of natural antibacterial agents and antioxidants represents an important area of research and development in the healthcare industry.

5. Conclusions

The present study investigated the potential of ecoenzyme (EE) produced by fermenting fruit waste as a source of antibacterial and antioxidant compounds. Papaya, pineapple, and Kasturi orange were fermented for 10 days and 3 months to produce EE solutions. The EE solutions had broad-spectrum antibacterial activity and were more effective against S. aureus than E. coli. The 3-month EE solution had a higher antibacterial activity than the 10-day EE solution. Undiluted EE was more effective than diluted EE. Molecular docking revealed that rosmarinic acid and hesperidin had a stronger binding affinity to the DNA gyrase of E. coli and S. aureus than caffeic acid and ciprofloxacin. The EE solution also displayed exceptional antioxidant activity. The study suggests that the EE solution produced from papaya, pineapple, and Kasturi orange has significant potential for further investigation due to its antibacterial and antioxidant properties.

Abbreviations

AA: Acetic acid
ATCC: American-type culture collection
BFE: Binding-free energy
CID: Compound identifier
DNA: Deoxyribonucleic acid
DPPH: 2,2-diphenyl-1-picrylhydrazyl
EE: Ecoenzyme
HCA: Hydroxycinnamic acids
IC: Inhibition concentration
LAB: Lactic acid bacteria
NA: Nutrient agar
NaCl: Natrium chloride
QMEAN: Qualitative model energy analysis
SD: Standard deviation
SDGs: Sustainable development goals
UHPLC: Ultrahigh performance liquid chromatography.

Data Availability

The data that support the findings of this study are available upon reasonable request from the corresponding author.

Conflicts of Interest

No conflict of interest to declare.

Authors' Contributions

Trina Ekawati Tallei worked on conceptualization, investigation, formal analysis, writing the original draft, and reviewing and editing. Fatimawali performed the investigation, formal analysis, writing—review, and editing. Nurdjannah Jane Niode conducted the investigation, validation, writing—review, and editing. Wadiah M. Alsaihati was assigned to the formal analysis, and writing—review and editing. Mohammed Alissa did the formal analysis and writing—review and editing. Christina Leta Salaki executed the investigation and formal analysis. Marlon Kamagi was focused on conceptualization, supervision, and project administration. Ali A. Rabaan was assigned to formal analysis and writing—review and editing.

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