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RESEARCH ARTICLE

Phytochemical Composition, Antioxidant Potential and Antibacterial Efficacy of *Cucumis melo* L., *Citrus sinensis*, and *Musa* spp Peels Ecoenzyme

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Abstract Fruit peel waste offers an eco-friendly alternative to synthetic pesticides for bacterial disease management.This study evaluated Rokmelon (*Cucumis melo* L.), Orange (*Citrus sinensis)*, and Banana (*Musa* spp.) peels for their phytochemical composition, enzymatic activity such as amylase and lipase, antioxidant capacity using Ferric Reducing Antioxidant Power (FRAP) assay, and antibacterial efficacy against *Burkholderia glumae, Bacillus cereus*, and *Bacillus subtilis* using agar well diffusion, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays. Rockmelon ecoenzyme (ER) showed the highest bioactivity with lipase (39.48 U/mL) and amylase (0.202 U/mL) activity. Phytochemical screening showed that phenolic compounds were strongly present (++) only in the rockmelon-based ecoenzyme (ER), but absent (–) in EO, ERO, and EROP samples. Meanwhile for flavonoids and alkaloids, each sample shows (+) result. The mixed formulation of rockmelon, orange and banana (EROP) exhibited peak antioxidant activity (17.992 µmol Fe^{2+}/L). Antibacterial assays revealed rockmelon ecoenzyme (ER) as the most effective. ER demonstrated strongest activity against *B. glumae* (19.67 mm ZOI, p=0.014; MIC=4.17% v/v, MBC=8.33% v/v). All treatments showed significant antibacterial effects versus controls ($p<0.05$). The results demonstrate that single-fruit ecoenzymes, particularly from rockmelon, outperform mixed formulations and exhibit reliable antibacterial activity against key plantation pathogens. These findings support the use of fruit peel-derived ecoenzymes as sustainable, effective alternatives to chemical pesticides in plantation management.

Keywords: Ecoenzyme, FRAP assay, phytochemical screening, *Antibacteria activity,* sustainable agriculture.

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Introduction

Plantation management is of great concern in food security around the globe but is subjected to major threats of bacterial and fungal infections. Due to heavy reliance on chemical pesticides, some significant ecological and economic issues have emerged, including soil deterioration and biodiversity loss, and the global economic loss because of these chemicals is estimated to be around 8 billion dollars every year [1]. Recent studies point to the use of ecoenzymes obtained in fruit peel as a satisfactory alternative to these practices, as they could act as antimicrobials as well as soil enhancements. Research indicates that orange peel eco- enzymes can inhibit *Xanthomonas oryzae* by 80% [2] and banana peel extracts have a broad- spectrum effect against *Staphylococcus aureus* and *E. coli* [3]. Combinations of fruits have been found to enrich soil nutrition by 30% [4]. Nevertheless, there are still considerable knowledge gaps

concerning the comparative efficacy of single and mixed fruit peel formulations as well as their efficacies against Gram-negative pathogens such as *Burkholderia glumae* [5]. Rockmelon (*Cucumis melo* L.), a climbing Cucurbitaceae vine, produces nutrient-rich fruits with thick peels containing valuable bioactive compounds. The peel contains significant phenolics (167.49 mg GAE/100g), flavonoids (79.16 mg QE/100g), and antioxidants (56.92 mg TE/100g) [6], offering potent antimicrobial and antioxidant properties. These phytochemicals make rockmelon peel ideal for developing ecoenzymes and functional food ingredients, supporting sustainable agriculture through waste valorization [7]. Its genetic diversity across cultivars enhances its commercial value in tropical regions while providing ecological benefits through circular agricultural applications. Sweet Orange (*Citrus sinensis*), a Rutaceae family evergreen, is a globally cultivated fruit tree prized for its nutrient-rich, leathery-rind hesperidium fruits. The peel contains over 40 bioactive polyphenols, including potent flavonoids like hesperidin and naringenin [8], delivering exceptional antioxidant and antimicrobial properties. These phytochemicals - phenolics, carotenoids, and essential oils [9] make orange peel ideal for ecoenzyme production, offering dual agricultural benefits as both plant protectants and soil enhancers [10].

Valorizing this abundant food industry byproduct supports sustainable waste management while providing effective, natural alternatives for integrated pest management systems [11]. Banana (*Musa* spp.), a fast-growing herbaceous perennial, produces nutrient-rich berries protected by thick peels with remarkable phytochemical potential. The pseudostemmed plant reaches 3-7m height, bearing easily torn leaves adapted to tropical storms [12]. While the starchy pulp provides vital carbohydrates and vitamins, the peel - often discarded - contains exceptional phenolic (18 mg GAE/g), flavonoid (20.5 mg QE/g), and carotenoid (1 mg/g) concentrations [13], [14], granting potent antioxidant and antimicrobial properties. These bioactive compounds, including saponins and tannins [15], make banana waste ideal for ecoenzyme production, serving as effective biopesticides and biofertilizers [16]. Valorizing banana byproducts supports circular agriculture by transforming waste into plant protection agents and soil amendments while enhancing food security in tropical regions.

Ferric Reducing Antioxidant Power (FRAP) quantifies a sample's electron-donating capacity by measuring its ability to reduce Fe^{3+} to Fe^{2+} [17]. Fruit peels demonstrate exceptional antioxidant potential, with grapefruit peel showing the highest FRAP value (9.22 mg AA/g) among 20 studied fruits, followed by pomegranate and citrus peels [18]. Notably, apple peels exhibit twice the FRAP activity of their flesh [19], underscoring peels as concentrated antioxidant sources. These findings support the valorization of fruit peel waste for sustainable applications as natural food preservatives, crop protectants, and nutritional supplements [16] creating a circular economy that reduces environmental impact while enhancing agricultural productivity [18].

Fruit peel extracts have excellent antibacterial properties because they contain a lot of bioactive compounds. Such compounds as phenolic acids, flavonoids, and alkaloids affect membranes, enzyme inhibition, and prevent bacterial DNA synthesis [20], [21]. The strong multispecies-specific activity pomegranate peel is owed to the presence of ellagitannins [22], [23] and banana peel flavonoids also kill both Gram-positive and Gram- negative bacteria in a dose-dependent fashion [24], Antimicrobial properties are also improved by enzymes such as bromelain (pineapple), papain (papaya), and others in combination with other phytochemicals [25].

To provide a concise overview of the current literature, Table 1 summarizes the antioxidant compounds and antioxidant activities reported for selected fruit peels, while Table 2 presents the antibacterial activities of ecoenzymes derived from different fruit peels. Collectively, these studies demonstrate that fruit peel-derived ecoenzymes are rich sources of bioactive compounds with considerable potential for sustainable agricultural applications. However, despite the increasing number of studies, direct comparisons between single-fruit and mixed-fruit ecoenzyme formulations remain limited, particularly with respect to their phytochemical composition, enzymatic activities, antioxidant capacity, and antibacterial efficacy against agriculturally important bacterial pathogens. These knowledge gaps warrant further investigation to identify the most effective fruit peel formulations for sustainable crop protection.

This study investigated the phytochemical composition, enzymatic activities, antioxidant capacity, and antibacterial activity of ecoenzymes derived from rockmelon, orange, and banana peels against *B. glumae*, *B. subtilis*, and *B. cereus*. The selected bacterial strains represent agriculturally relevant Gram-negative and Gram-positive bacteria, enabling a broader evaluation of the antibacterial potential of fruit peel-derived ecoenzymes for potential applications in sustainable plantation management. The study further compared the performance of single-fruit and mixed-fruit fermentation formulations, considering differences in phytochemical composition and enzymatic activities. In particular, the enzymatic characteristics of rockmelon peel-derived ecoenzyme were evaluated alongside its antibacterial activity. Ultimately, this study aims to explore the potential of fruit peel-derived ecoenzymes as sustainable

alternatives for bacterial management in plantation systems, thereby supporting the development of environmentally friendly crop protection strategies.

Table 1. Overview of antioxidant compounds and antioxidant activities reported for selected fruit peels [26], [27], [28], [29], [30].

Fruit Peel	Bioactive Compounds	Antioxidant Activity (IC50,DPPH)	Key Findings
Siamese orange	Phenolics, flavonoids	6,727 ppm (3 months)	Weak activity, increases with fermentation time.
Promegranate	Phenolics, flavonoids, tannins	42.52 µg/mL	Strong activity, high phenolic/flavonoid content.
Mango	Phenolic acids, flavonoids	High scavenging capacity	Highest TPC and TFC among tested peels
Grapefruit	Polyphenols, carotenoids	9.17 mg AAE/g	Highest radical scavenging among 20 peels
Citrus (various)	, ascorbic acid, flavonoids	High (varies by variety)	Rich in bioactives, excellent antioxidant activity.

Table 2. Comparative antibacterial activities of ecoenzymes derived from selected fruit peels [31], [32], [33], [34], [35], [36].

Fruit Peel	Family	Antibacterial Properties
Rockmelon (*C. melo*)	Cantaloupe	Inhibits *Escherichia coli* and *Staphylococcus aureus*.
Pomegranate (*Punica granatum*)	Lythraceae	Inhibits *Staphylococcus aureus, Salmonella enterica, Enterococcus faecalis* and *Escherichia coli.*
Orange (*Citrus sinensis*)	Rutaceae	Inhibits *Escherichia coli* and *Staphylococcus aureus*.
Banana (*Musa spp.*)	Musaceae	Inhibits *Pseudomonas aeruginosa* and *Staphylococcus aureus*.
Pineapple (*Ananas comosus*)	Bromeliaceae	Effective against *Escherichia coli* and *Salmonella typhi*
Papaya (*Carica papaya*)	Caricaceae	Inhibits *Staphylococcus aureus* and *B. subtilis*

Materials and Methods

Apparatus

UV-Vis Spectrophotometer (PerkinElmer, USA), cuvettes, water bath, Eppendorf micropipettes, micropipette tips, measuring cylinders, beakers, falcon tube, Glass rode, pH meter (Jenway), Erlenmeyer flask, magnetic stirrer, burette, retort stand, digital analytical balance, Schott bottles (Duran), microcentrifuge tubes, cork borer, volumetric flask, aluminium foil, sterile petri dishes, incubator, weighing boat, spatula, conical flask, dropper, Bunsen burner, inoculating loop, hockey stick, autoclave (ALP), Biological Safety Cabinet (Thermo Fisher), fume hood, universal bottle, syringe, 96 well plates, Parafilm.

Chemical and Sample

Starch solution, Ecoenzyme Rockmelon, Orange, Rockmelon + Orange, and Rockmelon + Orange + Banana sample, Phosphate buffer, 2,5- Dinitrosalicylic acid (DNS) reagent (Merck), Distilled water, olive oil, Ethanol (Merck), acetone (R&M Chemicals), Phenolphthalein indicator solution, Sodium hydroxide (NaOH) (Bendosen), Glacial Acetic Acid (R&M Chemicals), Sodium Acetate Trihydrate (Bendosen), 2,4,6-Tripyridyl-s- triazine (TPTZ), Hydrochloric acid (HCl) (Merck), Ferric chloride ($FeCl_3$) (ABMStore), nutrient agar (Oxoid), nutrient broth (Oxoid), Gram Positive Bacteria (*B. subtilis*, *B. cereus*), Gram Negative (B. glumae), Streptomycin, Dragendorff's Reagent (Merck), Iron (III) chloride (ABMStore).

Ecoenzyme Preparation

Four ecoenzyme formulations were prepared using rockmelon (*C. melo* L.) peels (ER), orange (*Citrus sinensis*) peels (EO), a combination of rockmelon and orange peels (ERO), and a combination of rockmelon, orange, and banana (*Musa* spp.) peels (EROP). The preparation procedure is illustrated in Figure 1. Fresh fruit peels were collected from Universiti Teknologi MARA (UiTM), Jasin Campus,

Melaka, Malaysia. All ecoenzymes were prepared following a peel:molasses:water ratio of 3:1:10 (w/v/v). For the single-fruit formulations (ER and EO), 600 g of either rockmelon or orange peels were mixed with 200 mL molasses and 2 L distilled water. The mixed-fruit formulation (ERO) consisted of 300 g rockmelon peels and 300 g orange peels, whereas the three-fruit formulation (EROP) contained 200 g each of rockmelon, orange, and banana peels. Both mixed formulations were prepared using 200 mL molasses and 2 L distilled water. The mixtures were transferred into airtight plastic containers and allowed to ferment for three months at room temperature. The containers were opened briefly on the tenth day to release gases generated during fermentation before being resealed. Upon completion of the fermentation period, the mixtures were filtered through cheesecloth to obtain the ecoenzyme filtrates for subsequent analyses, following the method described by [37].

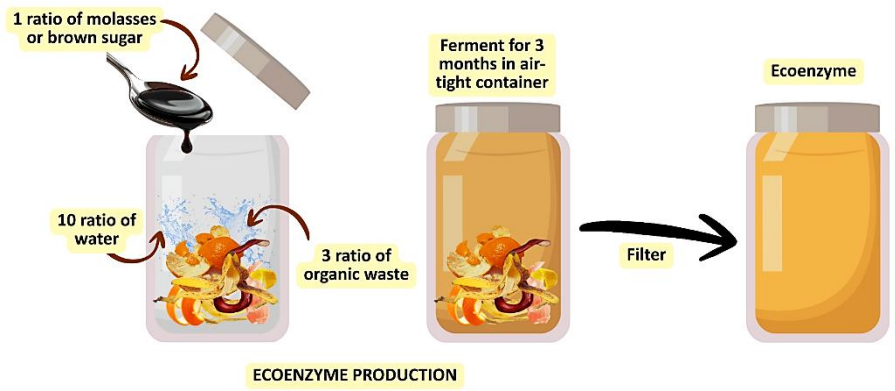


Figure 1. Preparation process of fruit peel-derived ecoenzymes.

Lipase Test

Lipase activity was determined using a titrimetric method as described by [38], and the experimental workflow is illustrated in Figure 2. Briefly, 1 g of olive oil was added to an Erlenmeyer flask containing 4 mL of phosphate buffer (pH 6.5) and 1 mL of ecoenzyme sample. The reaction mixture was homogenized in a water bath at 45 °C for 10 min. Subsequently, an ethanol–acetone solution (1:1, v/v) and three drops of phenolphthalein indicator were added before titration with 0.1 N NaOH to the endpoint. The volume of NaOH consumed was recorded and used to calculate lipase activity according to Equation (1).

$$\text{Enzyme Activity (EA)} = \frac{(\text{Vs} - \text{Vb}) \times \text{N} \times 1000}{\text{V}_\text{e} \times \text{t}}$$

where EA is the lipase activity (U mL^{-1}), V_s is the volume of NaOH used for the sample titration (mL), V_β is the volume of NaOH used for the blank titration (mL), N is the normality of NaOH, V_e is the volume of enzyme extract (mL), and t is the reaction time (min).

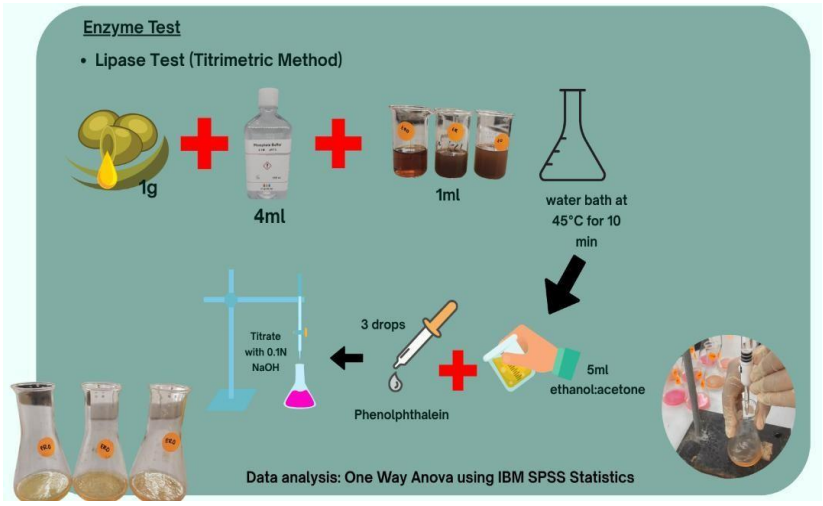


Figure 2. Experimental procedure for lipase activity determination using the titrimetric method.

Amylase Test

Amylase activity was determined using the 3,5-dinitrosalicylic acid (DNS) method as described by [38], and the experimental procedure is illustrated in Figure 3. Briefly, 0.5 mL of 1% (w/v) starch, 0.5 mL of ecoenzyme sample, and 1.0 mL of phosphate buffer were mixed and incubated for 10 min. Subsequently, 0.5 mL of DNS reagent was added to terminate the reaction, followed by incubation in a boiling water bath for 30 min. After cooling to room temperature, 2.5 mL of distilled water was added, and the absorbance was measured spectrophotometrically using maltose as the standard. Amylase activity (U mL^{-1}) was calculated using Equation (2), where a and b represent the slope and y-intercept of the maltose standard curve, respectively, V_t is the total reaction volume (mL), t is the incubation time (min), and V_e is the volume of enzyme extract (mL).

$$\text{Enzyme Activity (U mL}^{-1}) = \frac{(\frac{\text{Absorbance} - \text{b}}{\text{a}}) \times \text{Vt}}{\text{t} \times \text{V}_\text{e}}$$

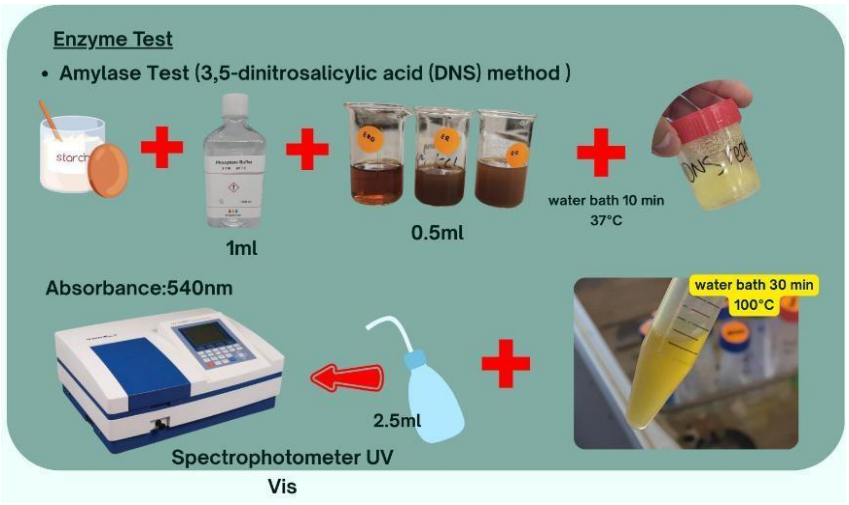


Figure 3. Experimental procedure for amylase activity determination using the DNS method.

Phytochemical Screening

Alkaloid Test

1 ml of each of the soap samples will be reacted with 5 drops of Dragendorff's reagent in a test tube. A positive result will be indicated if the formation of an orange color in the sample appears [39], [40].

Flavonoid Identification

In this analysis, alkaline reagent test was used. Two to three drops of sodium hydroxide NaOH were added into 2 ml of samples. Initially, a mixture solution has a deep yellow colour appeared however it was gradually becoming colourless after adding few drops of diluted HCl that indicating flavonoids were present [41]

Phenolic Identification

A total of 2 mL of the samples will be added to 1-2 drops of 1% iron (III) chloride solution. If a blue or purple colour is formed, it indicates a phenolic compound [42]

Ferric Reducing Power Antioxidant (FRAP) Assay

The ferric reducing antioxidant power (FRAP) assay was performed according to the method described by [43], and the experimental workflow is illustrated in Figure 4. The FRAP reagent was freshly prepared by mixing acetate buffer, 2,4,6-tripyridyl-*s*-triazine (TPTZ) solution in HCl, and ferric chloride solution, followed by incubation at 37 °C for 10 min. Briefly, 80 µL of ecoenzyme sample, 1.0 mL of distilled water, and 600 µL of FRAP reagent were mixed in a cuvette. A reagent blank was prepared by replacing the sample with distilled water. After incubation for 4 min, the absorbance was measured at 593 nm using a UV–Vis spectrophotometer. All samples were analysed in triplicate, and FRAP values (µmol Fe^{2+} L^{-1}) were calculated using Equation (3).

$$\mathrm{FRAP}(\mu\mathrm{mol\ Fe^{2+}/L}) = \frac{\left(\frac{\mathrm{SA}-\mathrm{BA}}{\mathrm{Slope}}\right)\times 10}{2}\times 1000$$

where SA is the sample absorbance, BA is the blank absorbance, and slope is the slope of the Fe^{2+} standard calibration curve.

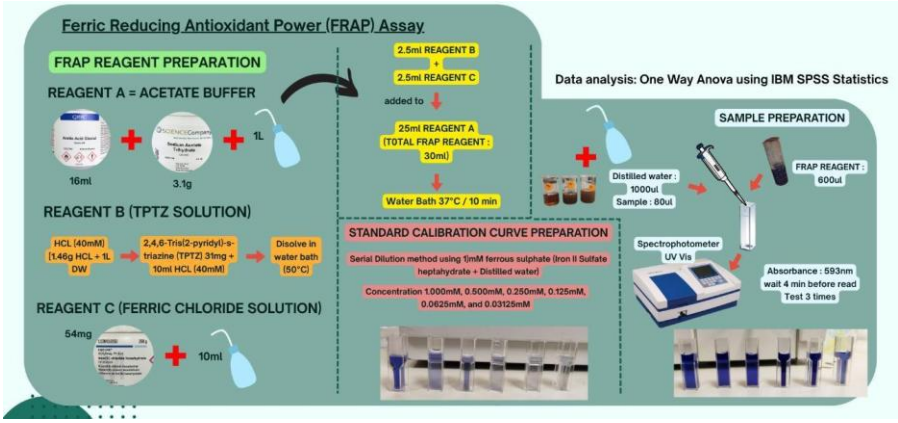


Figure 4. Schematic workflow of the ferric reducing antioxidant power (FRAP) assay used for antioxidant activity determination.

Antibacterial Screening

The antibacterial activity of the ecoenzyme formulations was evaluated using the agar well diffusion method as described by [40], [44], and the experimental workflow is illustrated in Figure 5. Four ecoenzyme formulations were tested at 100% (v/v) against *B. glumae*, *B. subtilis*, and *B. cereus*. *B. glumae* was selected as an economically important Gram-negative phytopathogen, whereas *B. subtilis* and *B. cereus* were included as representative Gram-positive bacteria commonly associated with agricultural environments. Nutrient broth (13 g L^{-1}) and nutrient agar (28 g L^{-1}) (Oxoid, UK) were prepared according to the manufacturer's instructions and sterilized by autoclaving at 121 °C for 15 min. Each bacterial strain was subcultured on nutrient agar and incubated at 37 °C for 24 h. A single colony was then inoculated into 10 mL nutrient broth and incubated overnight at 37 °C to obtain fresh bacterial cultures. For the agar well diffusion assay, 100 µL of each bacterial suspension was evenly spread onto

nutrient agar plates. Wells (6 mm diameter) were aseptically prepared using a sterile cork borer and filled with 30 µL of ecoenzyme sample. Streptomycin and sterile distilled water served as the positive and negative controls, respectively. Following incubation at 37 °C for 24 h, the diameter of the inhibition zone was measured. All assays were performed in triplicate.

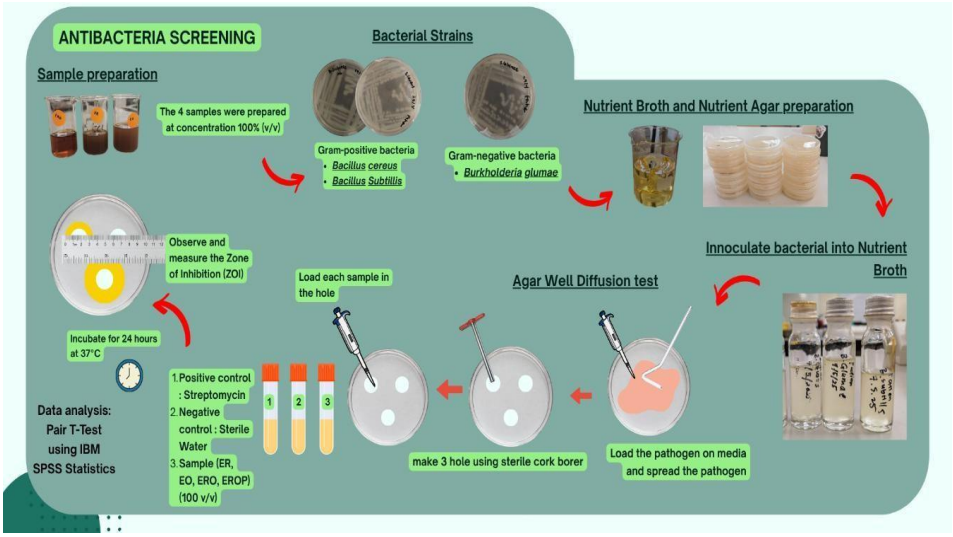


Figure 5. Schematic overview of the antibacterial screening procedure using the agar well diffusion method.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the ecoenzyme formulations were determined using the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines and the method described by [45]. The experimental workflow is illustrated in Figure 6. For MIC determination, two-fold serial dilutions of each ecoenzyme sample were prepared in sterile nutrient broth using 96-well microplates. A standardized bacterial suspension (approximately 1×10^6 CFU mL^{-1}) was added to each well, while broth containing bacteria only and sterile broth served as the negative and blank controls, respectively. Plates were incubated at 37 °C for 18–24 h, and the MIC was recorded as the lowest concentration showing no visible bacterial growth. For MBC determination, 10 µL aliquots from wells showing no visible growth in the MIC assay were spotted onto nutrient agar plates and incubated at 37 °C for 18–24 h. The MBC was defined as the lowest concentration at which no bacterial colonies were observed after incubation [45].

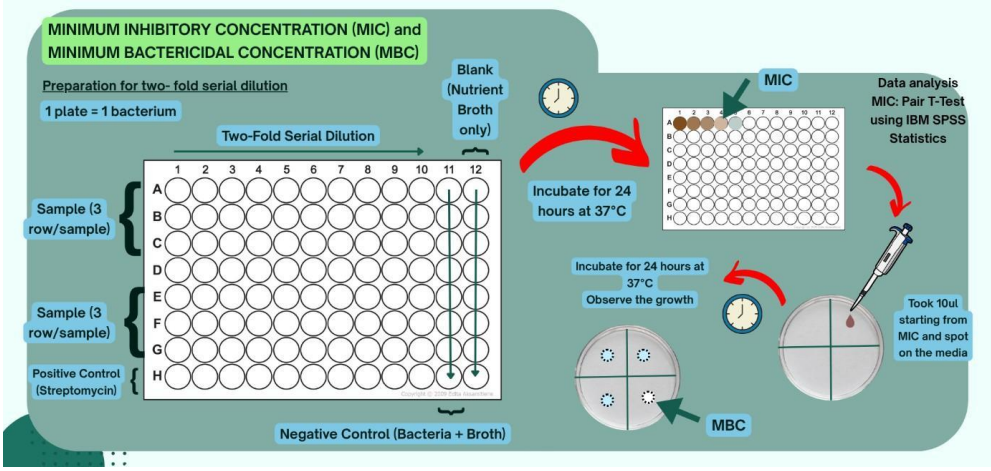


Figure 6. Schematic workflow of the broth microdilution assay for determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of fruit peel-derived ecoenzymes.

Results and Discussion

Lipase Test

The lipase activities of the four ecoenzyme formulations are presented in Table 3. Among the tested formulations, the rockmelon peel ecoenzyme (ER) exhibited the highest lipase activity (39.48 U mL^{-1}), followed by the orange peel ecoenzyme (EO) (30.21 U mL^{-1}). In contrast, the mixed-fruit formulations, ERO and EROP, showed lower lipase activities of 23.35 and 16.14 U mL^{-1}, respectively, indicating a progressive decline in enzyme activity with increasing substrate complexity. This indicates that the ecoenzyme lipase generated by rockmelon peel (ER) indicates good biochemical and microbial environment conducive to the production of lipase during fermentation. The composition of lipids, proteins, and natural sugars that are conducive producers and provide nutrients to lipase- producing microbes is probably well balanced in Rockmelon peel leading to better lipase activity [46], [47]. Though orange peel (EO) exhibited lipase activity as well, its acidic nature of pH and varied phytochemical composition might have reduced the microbial efficiency or lack the stability of enzyme activity [48]. The lower enzymatic activity in the blending preparation like ERO and EROP could be linked either to dilution of the substances or phytochemical interferences or antagonistic activities of micro-organisms and hence interferes with an ideal enzyme fermentation condition [49]. Parallel results were attained in research concerning the fermentation of mixed fruit waste, where no synergistic effect was always achieved by mixes and enzyme expression was even impaired [50]. The results are congruent with literature that single- substrate systems particularly those that are lipid precursor-laden ones such as rockmelon are more adequate in maximizing the lipase yield in the process of ecoenzyme production [51], [52], [53], [54].

Table 3. Lipase activity of ecoenzymes produced from different fruit peel formulations.

Samples	Mean Final Volume (mL)	Enzyme Activity (EA) (U/mL)
Ecoenzyme Rockmelon (ER)	3.8	39.48
Ecoenzyme Orange (EO)	2.9	30.21
Ecoenzyme Rockmelon + Orange (ERO)	2.3	23.35
Ecoenzyme Rockmelon + Orange + Banana (EROP)	1.6	16.14

Amylase Test

The amylase activities of the different ecoenzyme formulations are presented in **Table 4**. The rockmelon peel ecoenzyme (ER) exhibited the highest amylase activity (0.202 U mL^{-1}), followed by the rockmelon–orange formulation (ERO) (0.179 U mL^{-1}). Lower activities were observed for the rockmelon–orange–banana formulation (EROP) (0.127 U mL^{-1}) and the orange peel ecoenzyme (EO), which recorded the lowest activity (0.095 U mL^{-1}), respectively.

The highest activity of amylase was observed in the rockmelon based ecoenzyme (ER) which shows that this substrate contains the best carbon sources and fermentation conditions of starch degrading microbial populations. The availability of easily breakdown polysaccharides and moderate acidity found in the peel of rockmelon favor efficient growth of microbes and biosynthesis of enzymes [55]. Conversely, there was less activity when using orange peel (EO) which could be attributed to the fact that EO is high in citric acid that is known to inhibit the activity of microorganisms that produce enzymes or even denature the amylase under low pH conditions [56], [57]. Combined mixtures of ERO and EROP did not increase the activity of amylase production because of variable nutrient conditions or fermentation imbalances [58], [59]. The results confirmed the results of the earlier reports that single-fruit substrates usually perform better than mixtures in enzyme production because of the consistent biochemical composition and stable behavior of fermentation [60], [61]. Thus, in the case of targeted food applications where the target is starch degradation, the most effective strategy can be to use a single substrate (such as the rockmelon peel) instead of a mixed peels.

Table 4. Amylase activity of ecoenzymes produced from different fruit peel formulations.

Samples	Mean Absorbance (NM)	Enzyme Activity (EA) (U/mL)
Ecoenzyme Rockmelon (ER)	0.545	0.202
Ecoenzyme Orange (EO)	0.257	0.095
Ecoenzyme Rockmelon + Orange (ERO)	0.484	0.179
Ecoenzyme Rockmelon + Orange + Banana (EROP)	0.343	0.127

Phytochemical Screening Test

The qualitative phytochemical screening results are summarized in Table 5. Among the four ecoenzyme formulations, only the rockmelon peel ecoenzyme (ER) showed a positive reaction (++) for phenolic compounds, whereas phenolic compounds were not detected in the orange (EO), rockmelon + orange (ERO), and rockmelon + orange + banana (EROP) formulations. Flavonoids and alkaloids showed weak positive reactions (+) across all formulations. As the assay was qualitative, these findings indicate the presence or absence of specific phytochemical groups rather than their concentrations. The exclusive detection of phenolic compounds in ER suggests compositional differences among the fermented fruit peel formulations that may contribute to their observed biological activities.

Previous studies have reported that rockmelon peel contains phenolic compounds, including gallic acid, which are associated with antioxidant and antimicrobial activities [62], [63]. Consistent with these reports, phenolic compounds were qualitatively detected only in the ER formulation. However, as the present phytochemical screening was qualitative, no inference regarding phenolic concentration can be made. Flavonoids and alkaloids were detected in all formulations, indicating that these phytochemical groups remained detectable following fermentation. These compounds have been widely reported to exhibit antioxidant, antimicrobial, and other biological activities, which may contribute, together with fermentation-derived metabolites, to the overall bioactivity of the ecoenzymes. Nevertheless, quantitative phytochemical analyses are required to establish the abundance of these compounds and their relationship with the observed biological activities.

Table 5. Qualitative phytochemical screening of ecoenzymes produced from different fruit peel formulations.

Test/Samples	ER	EO	ERO	EROP
Flavonoids	+	+	+	+
Phenols	++	-	-	-
Alkaloids	+	+	+	+

Indicator: (-) absent, (+) weakly positive reaction, (++) positive reaction [64]

Ferric Reducing Antioxidant Power (FRAP)

The FRAP values of the different ecoenzyme formulations are presented in Figure 7. Among the tested formulations, EROP exhibited the highest antioxidant capacity (17.992 µmol Fe^{2+}/L), followed by ER (13.9512 µmol Fe^{2+}/L), whereas EO (5.9122 µmol Fe^{2+}/L) and ERO (5.8742 µmol Fe^{2+}/L) showed considerably lower FRAP values. These findings demonstrate that antioxidant capacity varied markedly among the ecoenzyme formulations, indicating that both substrate composition and formulation influence the reducing capacity of the fermented products.

The superior antioxidant capacity of EROP suggests that the combination of rockmelon, orange, and banana peels produced a fermented formulation with greater reducing capacity than either the individual substrate (ER and EO) or the binary formulation (ERO). In contrast, the lower FRAP value observed for ERO indicates that combining fruit peel substrates does not necessarily enhance antioxidant activity. This finding suggests that the antioxidant potential of ecoenzymes is formulation-dependent and may be influenced by interactions occurring during the fermentation process rather than simply by increasing substrate diversity.

These findings are consistent with previous reports demonstrating that the antioxidant capacity of fermented fruit-derived products is influenced by substrate composition and fermentation conditions, which collectively determine the phytochemical profile of the final product [64], [65], [66]. However, unlike the general expectation that mixed substrates enhance antioxidant activity, the present study demonstrated contrasting responses between the two mixed formulations, with EROP exhibiting the highest FRAP value whereas ERO showed one of the lowest. This highlights that the effect of substrate combination is not necessarily additive and may vary according to the specific formulation used.

It should be noted that the present study evaluated the overall antioxidant capacity using the FRAP assay and did not identify or quantify individual antioxidant constituents. Therefore, the higher antioxidant activity observed in EROP should be interpreted as the overall reducing capacity of the fermented ecoenzyme rather than being attributed to specific phytochemical compounds. Further phytochemical characterization is warranted to identify the bioactive constituents contributing to the observed antioxidant activity and to better understand their contribution to the biological activities of fruit peel-derived ecoenzymes.

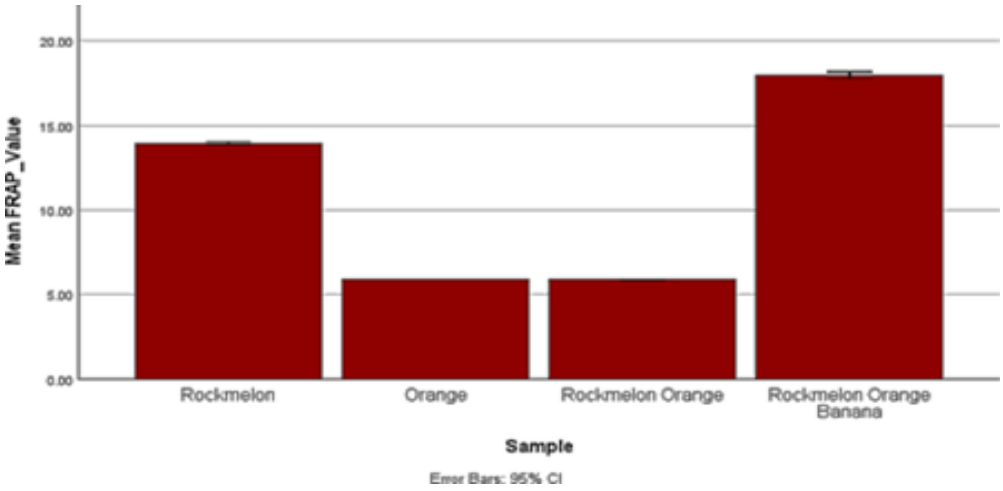


Figure 7. Ferric reducing antioxidant power (FRAP) values of ecoenzymes produced from different fruit peel formulations.

Antibacterial screening

Antibacterial activity for *B. glumae*

The antibacterial activities of the ecoenzyme formulations against *B. glumae* are presented in Figure 8. The zone of inhibition (ZOI) analysis revealed that all ecoenzyme formulations exhibited significantly lower antibacterial activity against *B. glumae* than the streptomycin control. Among the tested formulations, the orange peel-derived ecoenzyme (EO) produced the largest mean inhibition zone, although its activity remained significantly lower than that of the positive control. Specifically, the rockmelon-based ecoenzyme (ER) exhibited a mean ZOI difference of 19.67 mm (p = 0.008), while the rockmelon–orange–banana formulation (EROP) showed a mean difference of 19.67 mm (p = 0.014) relative to the control. Overall, all ecoenzyme formulations differed significantly from streptomycin (p < 0.05), indicating limited antibacterial activity against *B. glumae* under the conditions evaluated.

The comparatively lower antibacterial activity observed against *B. glumae* is consistent with the characteristics of Gram-negative bacteria, whose outer membrane rich in lipopolysaccharides acts as an effective permeability barrier that restricts the penetration of many antimicrobial agents [67]. Similar, observations have been reported for plant-derived antimicrobial products, which generally exhibit lower antibacterial activity against Gram-negative bacteria than Gram-positive bacteria because of this

structural barrier [67].

Among the ecoenzyme formulations, EO exhibited the greatest antibacterial activity against *B. glumae*, whereas the remaining formulations produced relatively smaller inhibition zones. These differences may be associated with variations in fruit peel composition and fermentation characteristics, which influence the overall antibacterial properties of fermented ecoenzymes. Similar variability among fruit peel-derived fermented products has been reported in previous studies, where substrate selection and fermentation conditions influenced antibacterial efficacy [68], [69]. Although the antibacterial activity remained lower than that of the antibiotic control, the measurable inhibition observed suggests that optimization of ecoenzyme formulation and fermentation conditions may further improve antibacterial performance against phytopathogenic bacteria.

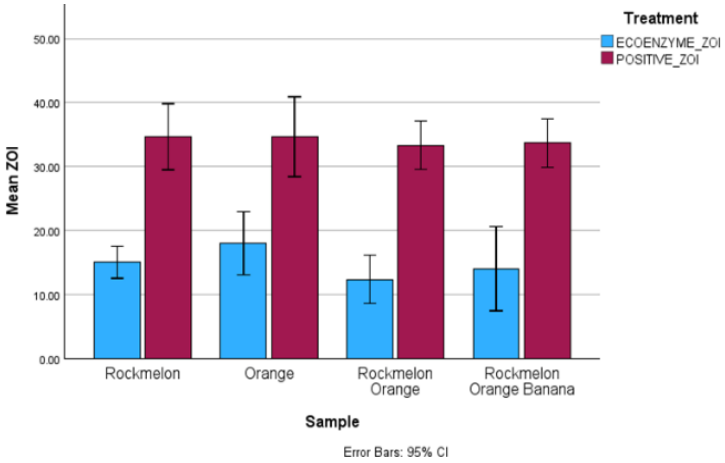


Figure 8 : Antibacterial activity of ecoenzyme formulations against *B. glumae* determined using the agar well diffusion assay.

Antibacterial activity for *B. subtilis*

The ecoenzymes demonstrated antibacterial activity against *B. subtilis* in Figure 9, although the inhibitory effects were slightly lower compared to their activity against *B. glumae* and remained less effective than the positive control. According to the agar well diffusion test, all ecoenzyme treatments produced measurable zones of inhibition (ZOI) against B. *subtilis*, with rockmelon (ER) ecoenzymes exhibiting relatively higher inhibitory effects among the tested treatments. Paired samples t-test confirmed significant differences between each ecoenzyme treatment and streptomycin (positive control) ($p<0.05$). Notably, the rockmelon peel extract showed the highest mean ZOI difference of 15.50 mm with a low standard deviation of 1.00 ($p<0.001$), indicating consistent antibacterial performance across replicates.

The effect of ecoenzymes towards the *B. subtilis* can be explained by the fact that this Gram-positive organism has no outer membrane as the Gram negative ones do, which serves as a protective layer. This simplicity of structure enables the bioactive compounds, e.g., phenolics, flavonoids, and organic acids to be developed in the fermentation process to penetrate more easily [70]. Fermentation may alter the chemical composition of fruit peel substrates, resulting in the formation of organic acids and other fermentation-derived metabolites that have been reported to contribute to the antimicrobial activity of fermented products [71]. The robust and stable ZOIs indicate that ecoenzyme, particularly that of rockmelon peels or a particular combination, may emerge as encouraging Gram positive antimicrobial agents. Even though they are still not as effective as positive control group, they have statistically significant results with sustainability, which is enough motive that they may be good alternatives to the chemical disinfectants or antibiotics where moderate antibacterial efficacy is required.

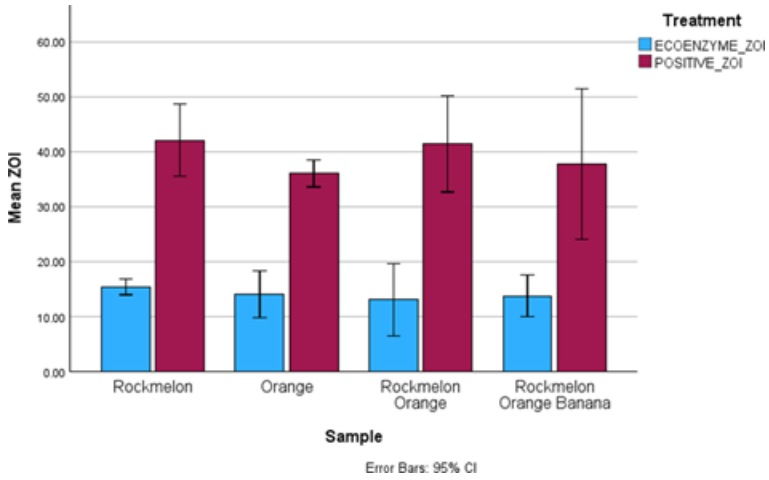


Figure 9 Antibacterial Activity for *B. subtilis*

Antibacterial activity for *B. cereus*

Ecoenzyme treatments exhibited the strongest antibacterial effects against *B. cereus* among the three tested bacteria, although still less effective than the streptomycin. The zone of inhibition (ZOI) results in Figure 10 revealed that all treatments demonstrated significant and reproducible antibacterial activity. The orange (EO) and rockmelon (ER) ecoenzymes produced the highest average ZOI, closely followed by the rockmelon + orange (ERO) combination. Paired t-test analysis confirmed that each ecoenzyme sample differed significantly from the streptomycin (positive control) with mean ZOI differences ranging from 13.67 mm to 17.00 mm. The orange peel extract again showed strong consistency, recording a standard deviation of 1.53 and a p-value of 0.002, highlighting its reliability in antibacterial performance. Similarly, the rockmelon peel extract exhibited a comparable high inhibition zone, with a standard deviation of 2.74 and a p-value of 0.004, indicating significant antibacterial activity despite slightly greater variability than the orange peel extract.

A greater sensitivity of *B. cereus* to eco- enzymes is described by its Gram-positive property, since it does not have the outer layer that Gram-negative bacteria must generally protect them against bioactive substances, including tannins, flavonoids, and acids that were produced during fermentation, thus being more vulnerable to the attacks of the bioactive products [72], [73]. These agents interfere with the peptidoglycan layer of the bacteria, damage the integrity of the cell wall and make the interior contents leak. Based on the increased activity of combination treatment, there appears to be synergism between phytochemicals found in various fruit peels. Although there was a narrow difference in the standard deviation of some formulations, especially the triple blend, all the formulations were relatively reproducible to show that they can be usable in practice. Since *B. cereus* is one of the most used foodborne pathogens, the capacity of ecoenzymes to arrest its development presents a prospect in sustainable food hygiene, waste management procedures, and non-virtual disinfection manifestations.

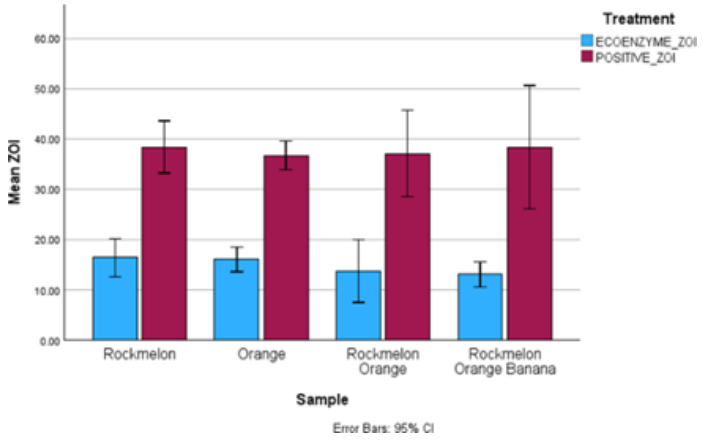


Figure 10 Antibacterial activity of ecoenzyme formulations against *B. cereus*.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Table 6 presents the antibacterial effects of different ecoenzyme treatments ER (rockmelon peel), EO (orange peel), ERO (rockmelon + orange), and EROP (rockmelon + orange + banana) against *B. glumae*, *B. subtilis*, and *B. cereus*. Both single-peel ecoenzymes, ER and EO, showed notable antibacterial activity. ER had the lowest MIC (4.17% v/v) and MBC (8.33% v/v) against *B. glumae*, with moderate effects against *B. subtilis* and *B. cereus*. Similarly, EO showed effective MIC and MBC values against *B. glumae* and *B. cereus*. In contrast, the combined peel formulations (ERO and EROP) displayed no antibacterial activity, with even 100% concentration showing no inhibition.

The apparent differences between the agar well diffusion and broth microdilution assays should be interpreted with caution because these methods assess antibacterial activity using different principles. Agar well diffusion is influenced by the diffusion characteristics of antimicrobial compounds through solid media, whereas broth microdilution measures bacterial growth inhibition under direct liquid exposure. Consequently, inhibition zones observed in agar diffusion do not necessarily correspond to low MIC or MBC values, particularly for complex natural products such as ecoenzymes that contain multiple constituents with different physicochemical properties.

The greater antibacterial activity of ER and EO may be associated with the combined effects of fermentation-derived organic acids, the naturally acidic fermentation matrix, and bioactive phytochemicals, including phenolic compounds, acting synergistically to inhibit bacterial growth [74], [75], [76]. In contrast, the absence of detectable antibacterial activity in ERO and EROP under broth microdilution conditions indicates that combining multiple fruit substrates did not improve antibacterial performance. The mechanisms underlying this observation remain unclear but may involve differences in fermentation-derived metabolites, substrate composition, or microbial interactions during mixed-substrate fermentation [78], [79]. However, these possibilities were not investigated in the present study and therefore require further verification.

Because pH-neutralized and acidity-matched controls were not included, the relative contributions of acidity, organic acids, and phytochemical constituents could not be distinguished. Therefore, the antibacterial activity observed in this study should be interpreted as the overall biological effect of the native fermented ecoenzyme rather than being attributed to any single component. Future studies incorporating pH-neutralized and acidity-matched controls, together with quantitative organic acid profiling and metabolomic analyses, are warranted to elucidate the mechanisms underlying the observed antibacterial activity.

Table 6 : Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ecoenzyme formulations against *B. glumae*, *B. subtilis*, and *B. cereus*.

Samples	Bacteria	MIC (% v/v)	MBC (% v/v)
Ecoenzyme Rockmelon (ER)	*B. glumae*	4.17	8.33
	B. subtilis	16.67	16.67
	B. cereus	8.33	22.22
Ecoenzyme Orange (EO)	*B. glumae*	4.17	27.78
	B. subtilis	16.67	16.67
	B. cereus	7.29	16.67
Ecoenzyme Rockmelon + Orange (ERO)	*B. glumae*	>100.00	>100.00
	B. subtilis	>100.00	>100.00
	B. cereus	>100.00	>100.00
Ecoenzyme Rockmelon + Orange + Banana (EROP)	*B. glumae*	>100.00	>100.00
	B. subtilis	>100.00	>100.00
	B. cereus	>100.00	>100.00

Conclusions

This study demonstrated that ecoenzymes derived from rockmelon, orange, and banana peels exhibited distinct phytochemical profiles, enzymatic activities, antioxidant capacities, and in vitro antibacterial activities. Rockmelon peel ecoenzyme (ER) exhibited the highest enzymatic activities (amylase: 0.202 U/mL; lipase: 39.48 U/mL) and was the only formulation showing a strong positive reaction for phenolic compounds in the qualitative phytochemical screening. ER also exhibited the greatest antibacterial activity against the tested Gram-positive bacteria (*B. subtilis*: 15.50 mm ZOI; *B. cereus*: 17.00 mm ZOI), whereas the mixed formulation containing rockmelon, orange, and banana peels (EROP) demonstrated the highest antioxidant capacity (17.992 µmol Fe^{2+}/L). However, the mixed formulations generally exhibited lower antibacterial and enzymatic activities than the single-fruit formulations under the conditions evaluated. The comparatively lower antibacterial activity observed against *B. glumae* suggests that further optimization of ecoenzyme formulation may be required to improve activity against Gram-negative bacteria. Overall, these findings provide a basis for further investigation of fruit peel-derived ecoenzymes as biologically active fermented products. Future studies should include comprehensive phytochemical characterization, optimization of fermentation conditions, and greenhouse and field evaluations to validate their potential agricultural applications.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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