Preliminary Antibacterial Activity of Eco-Enzymes Derived from Maluku Spice-Plant Residues Against *Staphylococcus aureus* and *Escherichia coli*

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Abstract: Eco-enzymes derived from spice-plant residues may provide an environmentally friendly source of antibacterial compounds. This study evaluated eco-enzymes prepared from nutmeg fruit (BP), nutmeg leaves (DP), eucalyptus leaves (DK), and clove leaves (DC) using a 1:3:10 ratio of coconut sugar, plant residue, and water. Fermentation was conducted anaerobically for 90 days, and the products were screened against *Staphylococcus aureus* and *Escherichia coli* by disk diffusion at 10% concentration. Two commercial 70% alcohol antiseptics served as positive controls. After fermentation, all eco-enzymes were acidic with a final pH of approximately 4.8 and showed distinct sensory profiles. The clove-leaf eco-enzyme produced the largest mean inhibition zones against *E. coli* (14.22 ± 0.31 mm) and *S. aureus* (12.25 ± 0.41 mm). One-way ANOVA indicated that treatment significantly affected inhibition zones against *E. coli* ($p < 0.001$) and *S. aureus* ($p < 0.001$). HPLC screening suggested myristicin as dominant in BP, cineole in DK, and eugenol in DC. Overall, the results indicate preliminary antibacterial potential, especially for clove-leaf eco-enzyme, and support further studies using concentration gradients, MIC/MBC assays, and fully specified chromatographic methods.

Keywords: Eco-Enzyme, Antibacterial Activity, *Staphylococcus aureus*, *Escherichia coli*, Clove Leaves.

INTRODUCTION

Antimicrobial resistance remains a major public-health concern because common bacterial pathogens are increasingly difficult to control, especially in community and healthcare settings (World Health Organization, 2021; Zong *et al*., 2022). *Staphylococcus aureus* is frequently associated with skin and soft-tissue infections, whereas *Escherichia coli* is often involved in gastrointestinal and urinary infections (Zong *et al*., 2022). This problem has encouraged the search for plant-based and environmentally safer antibacterial materials that can complement conventional antiseptics and antibiotics.

Eco-enzyme is a fermentation liquid produced from plant-derived organic residues, water, and a carbohydrate source. Several recent studies have reported that eco-enzymes may show antibacterial properties depending on the substrate composition, fermentation process, and concentration tested (Khadka & Neupane, 2023; Rukmini, 2023; Tallei *et al*., 2023). In addition, eco-enzymes have attracted attention because they combine waste valorization and bioactive-compound recovery in a single low-cost process (Zhang *et al*., 2020; Ismail *et al*., 2024).

Among Maluku spice plants, nutmeg (*Myristica fragrans*), clove (*Syzygium aromaticum*), and eucalyptus (*Eucalyptus globulus*) are relevant because they contain

bioactive compounds that have been linked to antibacterial activity, including myristicin, eugenol, and cineole (Pangestuti & Kim, 2022; Tewari & Malik, 2021; Cortés-Rivera *et al*., 2023; Juergens, 2020). However, comparative information on eco-enzymes prepared specifically from these local residues remains limited. Therefore, this study aimed to evaluate the preliminary antibacterial activity of eco-enzymes derived from nutmeg fruit, nutmeg leaves, eucalyptus leaves, and clove leaves against *S. aureus* and *E. coli*, and to relate the inhibition pattern cautiously to HPLC screening of dominant compounds.

METHODS

This laboratory-based in vitro study evaluated four eco-enzyme preparations: nutmeg fruit (BP), nutmeg leaves (DP), eucalyptus leaves (DK), and clove leaves (DC). The work comprised eco-enzyme preparation, fermentation monitoring, antibacterial testing, HPLC screening, and statistical analysis.

Eco-enzymes were prepared using a 1:3:10 ratio of coconut sugar, spice-plant residues, and water. In practical terms, 1 part coconut sugar was mixed with 3 parts organic material and 10 parts water. The organic materials consisted of nutmeg fruit, nutmeg leaves, eucalyptus leaves, and clove leaves. Fermentation was carried out anaerobically in closed containers for 90 days. During fermentation, pH and sensory characteristics were monitored periodically, and the final fermented liquid was filtered to separate the liquid fraction from the remaining solids.

Bacterial cultures of *S. aureus* and *E. coli* were obtained from a microbiology laboratory collection. The bacteria were cultivated on nutrient agar and adjusted to 0.5 McFarland in sterile saline. Disk diffusion was then performed by placing sterile paper disks impregnated with 10% eco-enzyme on inoculated agar surfaces, followed by incubation at 37 °C for 24 h. Two commercial antiseptics containing 70% alcohol were used as positive controls and are reported as brand A (K+1) and brand B (K+2). Sterile water served as the negative control in the original protocol; however, because its inhibition-zone values were not documented in the archived dataset, the statistical analysis focused on BP, DP, DK, DC, K+1, and K+2. Each treatment-bacterium combination was measured in five replicates.

HPLC screening was used to identify the dominant compounds associated with each eco-enzyme material. The source manuscript reported the dominant compounds as myristicin, cineole, and eugenol, but did not provide full chromatographic details such as column type, mobile phase, detection wavelength, retention time, or calibration information. Therefore, the HPLC data in this article are interpreted as compound-screening results rather than complete quantitative validation.

Inhibition-zone data were summarized as mean ± standard deviation (SD). One-way ANOVA was conducted separately for *E. coli* and *S. aureus* to test treatment differences among BP, DP, DK, DC, K+1, and K+2. The findings were interpreted descriptively and comparatively because only one eco-enzyme concentration was tested.

RESULTS AND DISCUSSION

After 90 days of fermentation, all eco-enzyme preparations showed acidic sensory characteristics and a final pH of approximately 4.8. Their dominant color and aroma profiles are presented in Table 1.

Table 1. Dominant Sensory Characteristics Of Eco-Enzymes After 90 Days of Fermentation

Treatment	Raw Material	Dominant Color	Dominant Aroma After 90 Days
BP	Nutmeg fruit	Clear reddish-brown	Acidic, strong nutmeg aroma
DP	Nutmeg leaves	Clear brown	Acidic, nutmeg aroma
DK	Eucalyptus leaves	Clear brown	Acidic, eucalyptus aroma
DC	Clove leaves	Clear dark brown	Acidic, clove aroma

The sensory profiles indicate that the four eco-enzymes were all acidic after fermentation, but they differed in dominant color and aroma. These observations are useful as preliminary descriptors of fermentation outcomes. However, color and aroma should not be interpreted as direct proof of chemical composition, because the source manuscript did not include quantitative sensory analysis or a statistical correlation between sensory traits and compound levels.

The inhibition-zone diameters measured at the tested concentration of 10% are summarized in Table 2 as mean ± SD. The clove-leaf eco-enzyme (DC) produced the largest mean inhibition zones against both *E. coli* and *S. aureus*.

Table 2. Mean Inhibition-Zone Diameter (Mean ± SD, N = 5, Mm) of Eco-Enzymes Against Test Bacteria at 10% Concentration

Treatment	*Escherichia coli* (mm)	*Staphylococcus aureus* (mm)
BP	9.70 ± 0.71	10.22 ± 0.42
DP	9.81 ± 0.32	9.64 ± 0.39
DK	10.14 ± 0.15	10.68 ± 0.29
DC	14.22 ± 0.31	12.25 ± 0.41
K+1	11.10 ± 0.25	6.21 ± 0.16
K+2	11.89 ± 0.30	7.18 ± 0.27

At the tested concentration, all four eco-enzyme preparations produced measurable inhibition zones against both bacteria. Descriptively, DC showed the strongest antibacterial performance among the eco-enzyme treatments, whereas DP showed the lowest mean inhibition against *Staphylococcus aureus* and BP showed slightly lower inhibition against *Escherichia coli* than the other eco-enzymes. The two 70% alcohol antiseptics behaved differently across bacteria, with larger mean inhibition against *Escherichia coli* than against *Staphylococcus aureus* in the archived dataset.

Table 3. One-Way ANOVA Summary for Inhibition-Zone Diameter by Treatment

Bacterium	df Between	df Error	F	p Value
Escherichia coli	5	24	102.15	< 0.001
Staphylococcus aureus	5	24	226.82	< 0.001

One-way ANOVA showed that treatment significantly affected the inhibition zones against *E. coli* ($F(5,24) = 102.15$, $p < 0.001$) and *S. aureus* ($F(5,24) = 226.82$, $p < 0.001$). This finding supports the conclusion that the treatments were not equivalent under the tested conditions. Nevertheless, because only one eco-enzyme concentration was

examined and negative-control values were not retained in the archived dataset, the present results should still be interpreted as preliminary antibacterial evidence rather than as a full efficacy assessment.

Table 4. Dominant Compounds Detected by HPLC Screening in Each Eco-Enzyme Material

Raw Material	Dominant Compound	Concentration (µg/mL)
BP	Myristicin	6605.27197
DP	Myristicin	46.26635
DK	Cineole	1877.87784
DC	Eugenol	5113.45283

HPLC screening suggested that myristicin was dominant in nutmeg-based materials, cineole in eucalyptus leaves, and eugenol in clove leaves. This pattern is biologically plausible because clove leaves are widely associated with eugenol and strong antimicrobial activity, whereas nutmeg and eucalyptus are associated with myristicin and cineole, respectively (Pangestuti & Kim, 2022; Cortés-Rivera *et al*., 2023; Juergens, 2020). Even so, the HPLC results should be interpreted cautiously. The source record did not provide chromatograms, retention times, wavelength settings, or calibration data, so the present article treats the HPLC findings as compound-screening results rather than fully validated quantitative chemical analysis.

Taken together, the descriptive inhibition pattern and the ANOVA results indicate that the clove-leaf eco-enzyme had the strongest antibacterial performance among the eco-enzyme treatments at 10% concentration. This pattern is consistent with the reported eugenol signal in clove leaves and with the broader literature on clove-derived antibacterial compounds (Cortés-Rivera *et al.*, 2023; Hyldgaard *et al*., 2015). However, the relationship between HPLC-screened compounds and inhibition zones was not tested statistically in the source dataset. Therefore, the discussion should remain at the level of plausible association rather than proven cause-and-effect. In addition, because the assay used only one concentration and a nutrient agar base from the original protocol, future studies should adopt a concentration series, standardized susceptibility medium, and MIC/MBC testing to strengthen comparability with antimicrobial testing standards (Mavani *et al*., 2023; Ningrum *et al*., 2024).

CONCLUSION

At the tested concentration of 10%, all eco-enzyme preparations showed measurable inhibition against *Staphylococcus aureus* and *Escherichia coli*. Based on mean inhibition zones and one-way ANOVA, the clove-leaf eco-enzyme showed the strongest antibacterial performance among the eco-enzyme treatments, while the nutmeg-leaf eco-enzyme tended to show the weakest response. The HPLC screening results were consistent with the presence of dominant compounds such as eugenol, myristicin, and cineole, but these chemical findings should be interpreted as preliminary screening rather than complete compound validation. Overall, the eco-enzymes studied here show preliminary antibacterial potential and merit further investigation under more standardized and fully documented test conditions.

SUGGESTIONS

Future studies should evaluate multiple eco-enzyme concentrations, include MIC/MBC testing, report complete negative-control data, and apply a fully specified HPLC method with chromatograms and calibration standards. For practical development, clove-leaf and nutmeg-fruit eco-enzymes may be prioritized for follow-up formulation studies because they showed the strongest preliminary antibacterial responses in the present dataset.

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