Influence of Biotransformation of *Moringa oleifera* Lam. Oil for Oleochemicals, Food, Pharmaceuthical Applications, and Antimicrobial Activity

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This study explores the biotransformation of *Moringa oleifera* Lam. oil using Eversa® Transform 2.0 (E.T. 2.0) lipase immobilized on an organic support derived from moringa residue. In the first reaction step—hydrolysis—the formation of free fatty acids (FFAs) reached approximately 50%, followed by esterification, which achieved a conversion rate of nearly 90%. The resulting bioproducts (oil, FFAs, and esters) were evaluated for antimicrobial activity, demonstrating efficacy against Gram-positive and Gram-negative bacteria and yeasts. These findings underscore the significance of biocatalysis in enhancing the bioactivity and application potential of oleochemical derivatives obtained from *Moringa oleifera* Lam. oil.

Keywords: *Moringa oleifera* Lam. Lipase. Hydroesterification. Antimicrobial Activity.

Moringa oleifera Lam., a plant native to northwestern India, is well known for its rapid growth, adaptability, and resilience. It thrives in arid soils and adjusts easily to diverse climates, making it suitable for cultivation in several regions, including Brazil [1]. Moringa has a cosmopolitan distribution, primarily found in tropical and subtropical regions. Brazil's cultivation is widespread in the Northeast due to its adaptation to arid and semi-arid conditions [2].

Additionally, *Moringa oleifera* Lam. has gained attention for its high nutritional value, as it contains various secondary metabolites such as vitamins (A, B, E), minerals including calcium and iron, polysaccharides, proteins, polyphenols, and flavonoids (e.g., rutin, quercetin, and kaempferol), along with sterols and fatty acids [3]. These metabolites in multiple plant structures—leaves, bark, stems, and seeds—may exhibit various biological activities, including antimicrobial properties [4].

Oil extracted from the seeds is particularly rich in fatty acids such as oleic acid (C18:1, 70%–76%), stearic acid (C18:0, 5%–8%), and palmitic acid (C16:0, 2%–8%) [5]. Oleic acid, in particular, is noted for its ability to reduce superoxide anions, neutralize hydroxyl radicals, and enhance iron ion reduction, all of which may contribute to its antimicrobial potential [6].

Given these properties, exploring methods capable of biotransforming moringa oil into novel bioproducts with potential biological activity is essential. Biocatalysis is a key technology for the sustainable production of biologically derived chemical and pharmaceutical products [7].

Biocatalysis aligns with 10 of the 12 principles of green chemistry, with the remaining two related to product design rather than processing.

Enzymes used in this process are biodegradable, biocompatible, and derived from renewable resources, making them highly suitable for use in a bioeconomy context [8]. Biocatalysis also avoids using precious metals, eliminates the need for costly post-reaction purification steps, generates less waste, and reduces overall environmental and financial costs [9]. However, using lipases in their free form may pose challenges, especially regarding separation from the reaction medium, which increases operational costs and risks enzyme inactivation [10].

These issues can be mitigated through enzyme immobilization, such as physical adsorption, a reversible technique that requires fewer steps, allows reuse of the support material, and facilitates

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the recovery of the enzyme after use. This approach significantly reduces costs and enables multiple catalytic cycles with the same biocatalyst [11].Effective immobilization requires support materials with specific properties—particularly hydrophobic supports, which promote monomeric enzyme conformation, interfacial activation, purification, and stabilization [8]. Organic supports are especially promising, as they exhibit a high affinity for biomolecules, are biodegradable, and yield non-toxic by-products [10].

Thus, this study aims to evaluate the antimicrobial potential of the by-products obtained from the biotransformation of *Moringa oleifera* Lam. oil through the application of Eversa® Transform 2.0 lipase immobilized by physical adsorption on an organic support, aiming to fill existing gaps in the literature regarding the biological activities of the oil and its derivatives.

Materials and Methods

Reflux Extraction

Crude oil was extracted using 25 mg of M*oringa oleifera* Lam. (MO) seeds and 200 mL of n-hexane in a Soxhlet apparatus at 68 °C for 8 hours. The oil was then separated from the solvent by vacuum distillation using a rotary evaporator [9]. The recovered n-hexane was recycled for subsequent extractions of MO seeds.

Biomass Treatment for Biocatalyst Preparation

After reflux extraction, the biomass was dried at 40 °C for 8 hours to remove residual solvent. Subsequently, it was sieved to obtain particles within the 100–120 mesh range. For biocatalyst synthesis, 1 g of this support material was immersed in 25 mL of ethanol for 24 hours. Afterward, the biomass was vacuum-filtered using a Büchner funnel and Whatman No. 41 filter paper. The filtered support was then prepared for the immobilization process.

Lipase Immobilization by Physical Adsorption

The immobilization of Eversa® Transform 2.0 lipase on the organic support followed the methodology described by Kumar and colleagues [4]. A suspension was prepared using a lipase solution at pH 5.0 (5 mM sodium phosphate buffer) and the support at a 1:19 (w/v) ratio (support: lipase solution), with an initial protein loading of 5 mg/g of support [10].The suspension was stirred at 200 rpm in an orbital shaker for 24 hours. The resulting heterogeneous biocatalyst was vacuum-filtered using a Büchner funnel with Whatman No. 41 filter paper, washed with distilled water, and stored at 4 °C for 24 hours.

Enzymatic Hydrolysis of *Moringa oleifera* Lam. Seed Oil

The enzymatic hydrolysis reaction was conducted to produce free fatty acids (FFAs) from *Moringa oleifera* Lam. seed oil using the immobilized Eversa® Transform 2.0 lipase, with modifications based on the method described by Barbosa (2019). The reaction was carried out at 37 °C, 1000 rpm, for 120 minutes, with the oil comprising 25% of the total reaction volume. For complete FFA conversion in preparation for esterification, Candida rugosa lipase was used under the same temperature and agitation conditions, with 25% oil, 75% water, and an enzyme activity of 550 U/g oil for 60 minutes.

Purification of Free Fatty Acids

Following hydrolysis, the reaction mixture was transferred to a separatory funnel. The upper organic phase was washed five times with boiling distilled water (1:2 v/v). The organic phase (fatty acids) was then separated from the aqueous layer (containing water, glycerol, and biocatalyst) and passed through a column packed with glass wool and anhydrous sodium sulfate to remove residual moisture.

Enzymatic Esterification of Fatty Acids

The purified FFAs were carried out in a reactor containing immobilized enzymes. The FFAs were reacted with isoamyl alcohol at 40 °C and 300 rpm, using a 1:1 molar ratio (oil: alcohol) and a protein concentration of 5% (w/v) relative to the system's total mass. Samples were collected over time and analyzed to determine ester content.

Ester Purification

The purification of esters followed the procedure described by Lage and colleagues (2016) [12]. To eliminate residual fatty acids, the organic phase was neutralized using a 15% (m/v) sodium carbonate (Na_2CO_3) solution at a 1:1 volume ratio. The mixture was then washed five times with distilled water at 40 °C (1:5 v/v). Water was removed using a rotary evaporator under vacuum at 50 °C, followed by incubation at room temperature with 30% (m/v) activated molecular sieves (pre-activated for 24 hours at 250 °C) to eliminate remaining moisture.

Antimicrobial Activity

The antimicrobial and antifungal activities of *Moringa oleifera* Lam. oil, hydrolysis products, and esterification products were assessed using the disk diffusion method according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS)[13].

Microbial suspensions (0.5 McFarland standard) containing 1.5×10^8 CFU/mL for bacteria and 1.5×10^6 CFU/mL for fungal strains were prepared. Mueller-Hinton agar (Merck KGaA, Darmstadt, Germany) and Sabouraud dextrose agar were used for bacterial and fungal inoculations, respectively. The test solutions (1:1 dilution in acetone) were applied in 10 µL aliquots onto sterile paper disks placed on the inoculated agar surfaces. The test organisms included *Escherichia coli*, *Candida albicans*, and *Staphylococcus aureus*. Fluconazole (20 and 40 µg/mL) and commercial gentamicin discs were positive controls for antifungal and antibacterial activities, respectively. Discs containing only acetone served as negative controls.

All Petri dishes were incubated at 37 °C for 24 hours (bacteria) or 48 hours (fungi). Antimicrobial susceptibility was evaluated by measuring the diameters of the inhibition zones (in mm) around the disks.

Results and Discussion

After *Moringa oleifera* seed oil was extracted, a two-step hydroesterification process was carried out. The first step involved the enzymatic hydrolysis of the oil to produce free fatty acids (FFAs), followed by enzymatic esterification to form esters. Both reactions were catalyzed using Eversa® Transform 2.0 lipase immobilized on an organic support. Table 1 presents the results of the hydroesterification reactions—comparing the use of free and immobilized enzymes.

The immobilization of Eversa® Transform 2.0 demonstrated significant advantages in both hydrolysis and esterification reactions. In hydrolysis, the immobilized enzyme increased the conversion rate from approximately 39% (free enzyme) to 46%. During esterification, conversion improved by about 10%, reaching 83%, and the reaction time was notably reduced from 24 hours to just 3 hours. These improvements are consistent with findings in the literature, which highlight the benefits of enzyme immobilization in biocatalytic processes, particularly in esterification reactions involving lipases [4,7]. The enhanced performance observed can be attributed to the physicochemical characteristics of the organic support used for immobilization. The support interacts with the enzyme's polymer matrix, modulating its surface conformation and increasing the accessibility of catalytic sites. This modulation facilitates interfacial activation of the lipase, exposing the active sites and improving the interaction with substrates, thereby enhancing catalytic efficiency [14]. Moreover, the organic support used in this study

Table 1. Performance of Eversa® Transform 2.0 (free and immobilized) in the hydroesterification of *Moringa oleifera* oil.

Lipase form	Hydrolysis (%)	Time of Hydrolysis (min)	Esterification (%)	Time (h)
Free Eversa Transform 2.0	39	45	75	24
Organic support	46,4	180	83	3

offers practical advantages. It is cost-effective, derived from renewable and readily available secondary products, and requires no pre-treatment before immobilization. These features make it a sustainable and scalable option for industrial applications in biocatalysis [14].

Antimicrobial Activity

The oil extracted from *Moringa oleifera* Lam. seeds did not exhibit antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, or *Candida albicans* (Figure 1). This finding agrees with previous reports by Özcan (2020) [2], which indicated that extracts from *Moringa oleifera* leaves demonstrated antimicrobial activity against a broad spectrum of microorganisms, while seed extracts generally lacked such effects. The absence of activity may be attributed to seasonal variation in seed composition, which can influence key chemical parameters such as acidity index, iodine content, and the proportions of mono-, di-, and triacylglycerols [15].

In contrast, Figure 2 shows the formation of inhibition zones in areas treated with the fatty acid and ester products obtained from the biotransformation of *Moringa oleifera* seed oil. The fatty acid exhibited an average inhibition zone of 14.04 mm against *E. coli* (A), no inhibition against *S. aureus* (B), and 13.09 mm against *C. albicans* (C). The ester product demonstrated broader antimicrobial effects, with average inhibition zones of 14.44 mm for *E. coli* (D), 9.35 mm for *S. aureus* (E), and 14.56 mm for *C. albicans* (F).

These antimicrobial effects can be attributed to the amphipathic nature of fatty acids and monoglycerides, which are known to disrupt bacterial membranes and increase cell permeability. Such biophysical interactions can lead to loss of cellular integrity and, ultimately, inhibition of microbial growth and proliferation [16].

Notably, the fatty acid and esterified products demonstrated antimicrobial activity, starkly contrasting with the original seed oil. This highlights the potential of enzymatic biotransformation as a strategy to enhance the biological activity of vegetable oils. Although promising, the literature still lacks comprehensive studies evaluating the antimicrobial potential of such biocatalytic derived products, underscoring the relevance and originality of the present work.

Conclusion

Biocatalysis using immobilized Eversa® Transform 2.0 lipase proved effective for operational optimization and enhancing antimicrobial activity. The immobilization of renewable and untreated organic support increased conversion efficiency in hydrolysis and esterification reactions and eliminated the need for chemical pre-treatment during enzyme immobilization.

Notably, the biotransformed products (fatty acids and esters) exhibited antimicrobial activity against *E. coli*, *S. aureus*, and *C. albicans*, suggesting potential applications in developing bioactive formulations. These may include natural deodorants, antiseptic soaps, and vaginal

Figure 1. Average inhibition diameter of *Moringa oleifera* Lam. oil and its products (fatty acid and ester) obtained by hydro esterification reaction with ET 2.0 immobilized on organic support by disk diffusion method, using *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*.

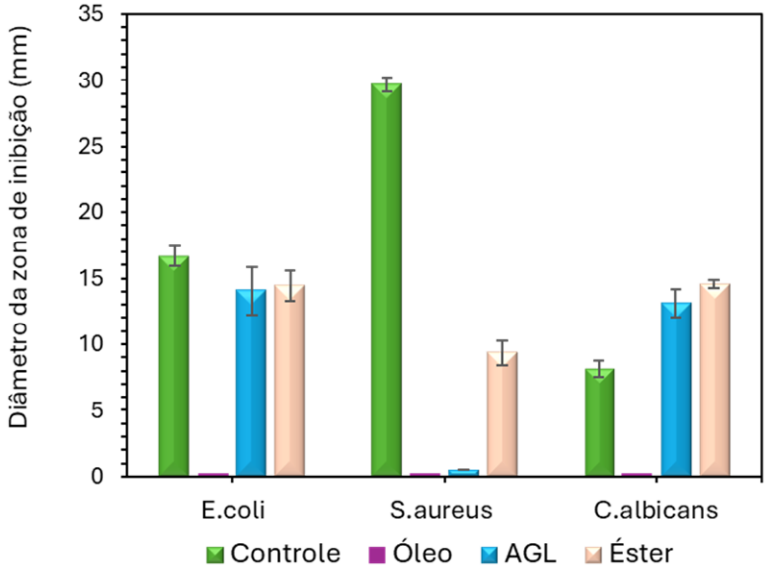
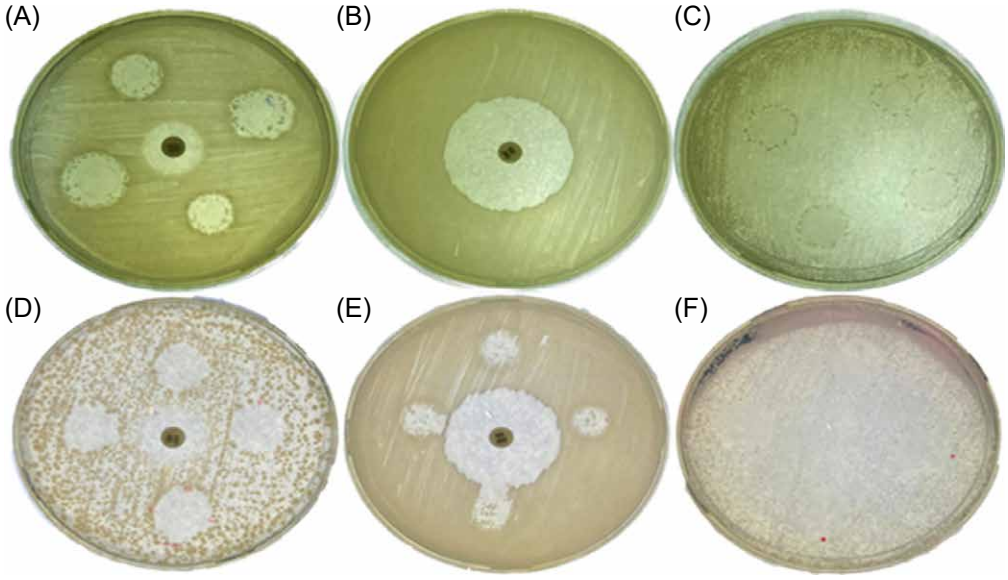


Figure 2. Result of the disk diffusion test with the products of *Moringa oleifera* Lam. oil (fatty acid and ester) obtained by hydroesterification reaction with ET 2.0 immobilized on organic support by disk diffusion method using *Escherichia coli* in A, *Staphylococcus aureus* in B and *Candida albicans* in C, respectively.



creams, positioning enzymatically modified oils as valuable alternatives in pharmaceutical and cosmetic industries.

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References

1. Shi H, Yang E, Yang H et al. Dynamic changes in the chemical composition and metabolite profiles of Drumstick (*Moringa oleifera* Lam.) leaf flour during fermentation. LWT 2022:155.
2. Özcan MM, Ghafoor K, Al Juhaimi F, Ahmed IAM, Babiker EE. Effect of cold-press and soxhlet extraction on fatty acids, tocopherols and sterol contents of the Moringa seed oils. South African J Bot 2019;124:333–337.
3. Sharma P, Wichaphon J, Klangpetch W. Antimicrobial and antioxidant activities of defatted *Moringa oleifera* seed meal extract obtained by ultrasound-assisted extraction and application as a natural antimicrobial coating for raw chicken sausages. International Journal of Food Microbiology 2020;332:108770.
4. Kumar M, Selvasekaran P, Kapoor S et al. *Moringa oleifera* Lam. seed proteins: Extraction, preparation of protein hydrolysates, bioactivities, functional food properties, and industrial application. Food Hydrocolloids 2022;131:107791.
5. Fernandes DM, Squissato AL, Lima AF, Richter EM, Munoz RAA. Corrosive character of *Moringa oleifera* Lam biodiesel exposed to carbon steel under simulated storage conditions. Renewable Energy 2019;139:1263–1271.
6 Abdulmalek SA, Yan Y. Recent developments of lipase immobilization technology and application of immobilized lipase mixtures for biodiesel production. Biofuels, Bioproducts and Biorefining 2022;16(4):1062–1094.
7. Mendes AA, Soares CMF, Tardioli PW. Recent advances and future prospects for biolubricant base stocks production using lipases as environmentally friendly catalysts: A mini-review. World J Microbiol Biotechnol 2023;39:25.
8. Rodrigues RC, Berenguer-Murcia Á, Carballares D et al. Stabilization of Enzymes via Immobilization: multipoint covalent attachment and other stabilization strategies. Biotechnol Adv 2021;52:107821.
9. Carvalho WCA, Luiz JHH, Fernandez-Lafuente R et al. Eco-friendly production of trimethylolpropane triesters from refined and used soybean cooking oils using an immobilized low-cost lipase (Eversa>® Transform 2.0) as heterogeneous catalyst. Biomass and Bioenergy 2021;155:106302.
10. Zdarta J, Meyer A, Jesionowski T, Pinheiro M. A general overview of support materials for enzyme immobilization: characteristics, properties, practical utility. Catalysts 2018;8(2):92.
11. Barbosa MS, Freire CCC, Brandão LMS et al. Biolubricant production under zero-waste *Moringa oleifera* Lam biorefinery approach for boosting circular economy. Ind Crops Prod 2021;167:113542.
12. Lage FAP, Bassi JJ, Corradini MCC, Todero LM, Luiz JHH, Mendes AA. Preparation of a biocatalyst via physical adsorption of lipase from Thermomyces lanuginosus on hydrophobic support to catalyze biolubricant synthesis by esterification reaction in a solvent-free system. Enzyme and Microbial Technology. 2016;84:56–67. doi: 10.1016/j.enzmictec.2015.12.007.
13. Sharifi-Rad M; Pawel P. Synthesis of Biogenic Silver Nanoparticles (AgCl-NPs) Using a Pulicaria vulgaris Gaertn. Aerial Part Extract and Their Application as Antibacterial, Antifungal and Antioxidant Agents. Nanomaterials 2020;10(4):638.
14. Ismail Baek AR; Baek K. Lipase immobilization with support materials, preparation techniques, and applications: Present and future aspects. International Journal of Biological Macromolecules 2020;163: 1624-1639.
15. Wilshire FMS; Santos AF; Silva LKB et al. Influence of seasonality on the physicochemical properties of Moringa oleifera Lam. Seed oil and their oleochemical potential. Food Chemistry: Molecular Sciences 2022; 4(30):100068.
16. Yoon BK; Jackman JA; Valle-González ER et al. Antibacterial free fatty acids and monoglycerides: biological activities, experimental testing, and therapeutic applications. International Journal of Molecular Sciences;19(4):1114.