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Formulation of a Bioactive Antiseptic Gel by Incorporating Polyphenol Oxidase Enzyme Extracted from Banana Peel into Aloe Vera Base

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Abstract:
Growing demand for eco-friendly, biocompatible alternatives to synthetic topical antiseptics has accelerated research into plant-derived bioactive molecules. This study presents the formulation of a natural antiseptic gel incorporating polyphenol oxidase (PPO) enzyme extracted from banana peel into an Aloe vera gel base. Banana peel, an abundant agro-waste material, is a rich source of PPO with antioxidant and antimicrobial activity, while Aloe vera possesses established wound-healing and antimicrobial properties. PPO was extracted through cold phosphate buffer extraction, quantified for enzyme activity, and analysed using FTIR spectroscopy. Antibacterial potential was evaluated against *Escherichia coli* using the disc diffusion method. Aloe vera gel formulations containing 1%, 3% and 5% PPO were prepared and evaluated for homogeneity, pH, stability, and applicability. Results demonstrated successful PPO extraction, confirmed enzymatic activity, and moderate antibacterial activity (9 ± 0.2 mm zone of inhibition). Formulated gels exhibited desirable stability, spreading ability, and skin-compatible pH (6.3–6.7). Overall, the study establishes banana peel–derived PPO in Aloe vera gel as a low-cost, sustainable, and bioactive antiseptic topical formulation.

Keywords: Polyphenol oxidase, Banana peel, Aloe vera, Antiseptic gel, Anti-microbial activity, Bioactive formulation, Waste valorisation.

I. Introduction:

Antiseptics are essential in preventing microbial infections, particularly in wound care and skin disinfection. Common chemical antiseptics such as alcohols, iodine compounds, and chlorhexidine exhibit broad-spectrum antimicrobial activity; however, their prolonged or excessive use has been associated with adverse effects including skin irritation, cytotoxicity to healthy tissues, delayed wound healing, and the emergence of microbial resistance. These limitations have prompted growing interest in safer, biocompatible antiseptic alternatives derived from natural sources (D. Deborah Evangeline.,2024).

Natural products, especially plant-derived bioactive compounds and enzymes, have gained increasing attention due to their therapeutic efficacy, sustainability, and reduced toxicity. Enzyme-based formulations offer targeted biological activity and improved safety profiles, making them promising candidates for topical antiseptic and wound-healing applications. Banana peel (*Musa acuminata)*, an abundant agricultural waste, represents a valuable source of bioactive constituents and has been traditionally used to treat minor wounds and skin infections. Phytochemical analyses reveal that banana peel contains phenolic compounds, dopamine, lectins, and enzymes with antioxidant, antimicrobial, and anti-inflammatory properties.

Polyphenol oxidase (PPO), a copper-containing oxidoreductase abundantly present in banana peel, catalyzes the oxidation of phenolics into antimicrobial quinones that disrupt microbial membranes and proteins. Aloe vera, widely used in topical formulations, provides a biocompatible gel base with moisturizing, anti-inflammatory, and wound-healing properties. Incorporating banana peel–derived PPO into an aloe vera gel may yield a synergistic, natural antiseptic formulation with enhanced antimicrobial efficacy and skin compatibility (Ramaza Rizka.,2023).

Although several studies have investigated banana peel extracts and aloe vera-based topical formulations, limited research has focused on the incorporation of enzyme-based bioactives such as polyphenol oxidase into antiseptic gel systems. The development of a bioactive antiseptic gel combining banana peel-derived PPO with an aloe vera base remains underexplored.

Therefore, the present study aims to extract and characterize polyphenol oxidase from banana peel and formulate a bioactive antiseptic gel using aloe vera as a natural base. The formulated gel is evaluated for its physicochemical properties, stability, and antimicrobial activity, highlighting its potential as a natural and sustainable alternative to conventional antiseptic products.

II. Methodology:

2.1 . Materials:

Banana peels (*Musa spp.*) were obtained from ripe bananas sourced from a local market, while fresh Aloe vera leaves were collected from healthy plants, thoroughly washed, and processed for gel extraction. The chemicals and reagents used in the study included phosphate buffer solution (0.1 M, pH 7.0), catechol as the substrate for enzyme activity assays, Tris-HCl buffer, ethanol (95%), glycerin, Carbopol 940 as the gelling agent, triethanolamine as the neutralizing agent, and distilled water.

2.2 . Extraction of *Polyphenol oxidase* from Banana Peel:

Fresh banana peels were washed thoroughly with distilled water to remove adhering dirt and contaminants and then cut into small pieces. The chopped peels were homogenized in cold phosphate buffer solution (0.1 M, pH 7.0) at a ratio of 1:2 (w/v) using a blender under chilled conditions to minimize enzyme denaturation. The homogenate was filtered through muslin cloth to remove particulate matter and subsequently centrifuged at 10,000 rpm for 15 min at 4 °C. The clear supernatant obtained after centrifugation was collected as the crude polyphenol oxidase enzyme extract and stored at 4 °C for further analysis (Ghazi M. Aziz.,2015).

2.3 . Determination of *Polyphenol oxidase* Activity:

Polyphenol oxidase activity was determined spectrophotometrically using catechol as the substrate. The reaction mixture consisted of 2.5 mL of phosphate buffer (0.1 M, pH 7.0), 0.3 mL of catechol solution, and 0.2 mL of the enzyme extract. The reaction was carried out at 25 °C, and the increase in absorbance due to the formation of o-quinones was recorded at 420 nm at 30-second intervals for a duration of 3 min using a UV–visible spectrophotometer. One unit of PPO activity was defined as the amount of enzyme required to produce an increase of 0.001 in absorbance per minute under the assay conditions (Daria Wohlt.,2021).

2.4. Antimicrobial Activity Assay:

The disc diffusion method was used to assess the antimicrobial properties of the polyphenol oxidase (PPO) enzyme extract. Escherichia coli was chosen as the test microorganism because of its significance as a prevalent Gram-negative bacterial pathogen. The bacterial culture was cultivated in nutrient broth and incubated at 37 °C for 18 to 24 hours. The culture's turbidity was calibrated to align with the 0.5 McFarland standard to guarantee consistent bacterial concentration. Sterile Mueller–Hinton agar plates were inoculated with a standardized E. coli suspension using a sterile cotton swab to achieve a uniform bacterial lawn. Sterile Whatman No. 1 filter paper discs (6 mm diameter) were treated with 20 µL of the PPO enzyme extract and aseptically positioned on the agar plates that had been inoculated. Tetracycline (10 µg/mL) acted as the positive control, whereas blank discs were utilized as the negative control. The dishes were kept at 37 °C for 24 hours. Following incubation, the measurement of the inhibition zone diameter surrounding each disc was recorded in millimeters to assess the antibacterial efficacy of the PPO enzyme extract (Ali Ghasemzadeh.,2016).

2.5. Characterization studies:

2.5.1. FTIR Analysis:

Fourier Transform Infrared (FTIR) spectroscopy was utilized to determine the functional groups in the polyphenol oxidase enzyme extract. The enzyme extract was dried using freeze-drying to produce a powdered sample. About 2 mg of the dried sample was combined with spectroscopic-grade potassium bromide (KBr) and formed into a clear pellet with a hydraulic press. FTIR spectra were obtained with an FTIR spectrophotometer over a wavelength range of 4000–600 cm^{-1}, at a resolution of 4 cm^{-1}, utilizing 32 scans per sample. A KBr pellet of high purity was employed as the reference for the background.

2.6. Aloe vera Gel Extraction :

Fresh Aloe vera leaves were washed thoroughly with distilled water, and the outer green rind was carefully removed. The transparent inner gel was collected, homogenized using a blender to obtain a uniform consistency, and filtered by centrifugation to remove fibrous materials. The resulting Aloe vera gel was stored at 4 °C until further use in gel formulation (T Reynolds.,1999).

2.7. Formulation of Bioactive Antiseptic Gel:
The Aloe vera gel was used as the base for the formulation of the bioactive antiseptic gel. Carbopol 940 was dispersed in distilled water and allowed to hydrate completely, after which glycerin was added as a humectant. The pH of the gel base was adjusted using triethanolamine to obtain a clear and stable gel. The PPO enzyme extract was then incorporated into the gel base at different concentrations (1%, 3%, and 5% v/v) with gentle stirring to ensure uniform distribution. The formulations were coded as F1 (1% PPO), F2 (3% PPO), and F3 (5% PPO) and stored under refrigerated conditions for further evaluation (Barbara R. Conway.,2021).

III. Results and Discussion:

3.1. Extraction and Activity of *Polyphenol oxidase* from Banana Peel:
Polyphenol oxidase (PPO) was successfully extracted from fresh banana peel using a cold phosphate buffer extraction method. The extraction yielded a clear, light brown supernatant after centrifugation, indicating the presence of soluble enzyme components. The enzyme extract was stored at 4 °C to preserve its activity prior to analysis. The enzymatic activity of PPO was evaluated spectrophotometrically using catechol as the substrate, and absorbance was measured at 420 nm. A gradual increase in absorbance was observed over time, confirming active enzymatic oxidation of catechol to o-quinones. The absorbance values increased from 3.414 at T1 to 3.442 at T2, and further to 3.446 at T3, indicating sustained PPO activity throughout the assay period.

Table 1: Polyphenol oxidase (PPO) activity assay showing absorbance change over time

Time Interval	Absorbance at 420 nm (A_{420})	Mean ± SD	Interpretation
T1	3.414	3.414 ± 0.002	Initial enzyme activity observed; substrate oxidation begins
T2	3.442	3.442 ± 0.003	Moderate increase in absorbance indicating steady enzyme catalysis
T3	3.446	3.446 ± 0.001	Slight rise in absorbance; enzyme activity maintained over time

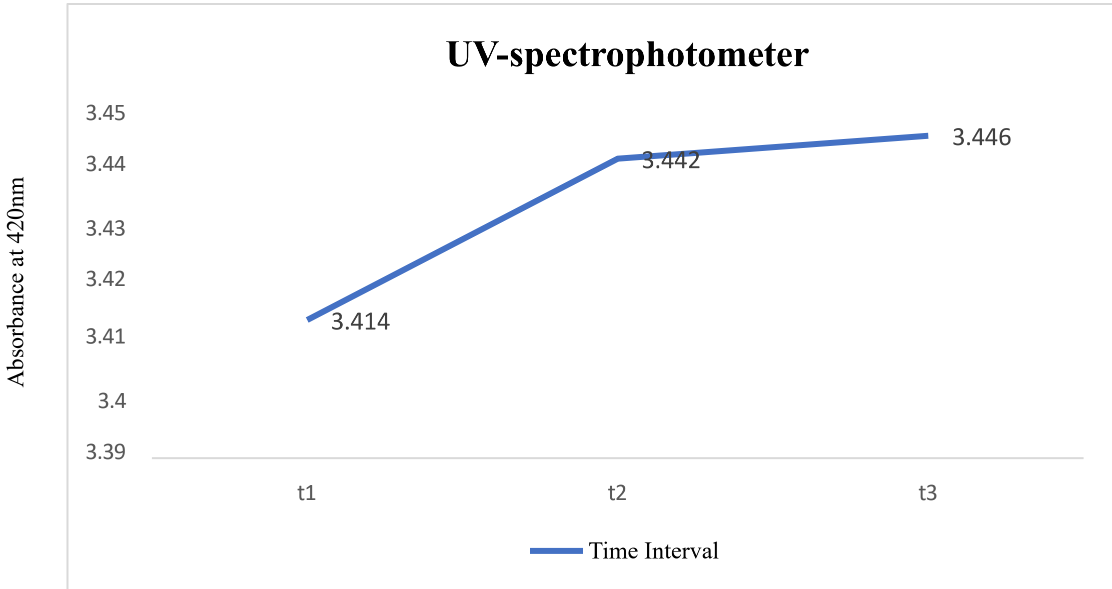

Fig 1. UV-Vis Spectrometer Graph

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The observed PPO activity is consistent with earlier studies reporting banana peel as a rich source of polyphenol oxidase and supports its suitability for incorporation into bioactive topical formulations.

3.2. Antimicrobial Activity of PPO Enzyme Extract:

The antibacterial activity of the PPO enzyme extract was evaluated against *Escherichia coli* using the disc diffusion method. The PPO-impregnated discs produced a measurable zone of inhibition with a mean diameter of 9 ± 0.2 mm, indicating moderate antibacterial activity. Tetracycline, used as the positive control, exhibited a larger zone of inhibition measuring 11 ± 0.1 mm, confirming its strong antibacterial efficacy.

Table 2. Antimicrobial susceptibility of PPO enzyme extract and tetracycline

SAMPLE	MEAN ZONE OF INHIBITION	INTERPRETATION
PPO enzyme extract	9 ± 0.2	Moderate activity
Tetracycline (standard)	11 ± 0.1	Strong activity

Although the antibacterial activity of the PPO enzyme extract was lower than that of the synthetic antibiotic, the observed inhibition is significant considering the natural and enzymatic origin of the compound. The antimicrobial mechanism of PPO is primarily attributed to the enzymatic oxidation of phenolic compounds into reactive quinones. These quinones are known to interact with bacterial cell wall proteins, enzymes, and nucleic acids, leading to membrane disruption, protein inactivation, and inhibition of metabolic processes. The results suggest that banana peel-derived PPO exhibits effective antibacterial activity against E. coli and holds promise as a natural antimicrobial agent for topical formulations.

3.3. Characterization of Polyphenol oxidase Enzyme by FTIR Analysis:

Fourier Transform Infrared (FTIR) spectroscopy was performed to identify the functional groups present in the PPO enzyme extract and to confirm its proteinaceous nature. The FTIR spectrum exhibited a broad absorption band around 3309 cm^{-1}, corresponding to O–H and N–H stretching vibrations, which are characteristic of hydroxyl and amide groups present in enzyme proteins.

The prominent peak observed at 1634 cm^{-1} was assigned to the amide I band, arising from C=O stretching vibrations of peptide bonds, while the band at 1505 cm^{-1} corresponded to the amide II band associated with N–H bending and C–N stretching vibrations. Additional peaks in the range of 2800–3000 cm^{-1} indicated aliphatic C–H stretching vibrations from amino acid side chains. The presence of these characteristic peaks confirms the protein structure of the extracted PPO enzyme and indicates that its structural integrity was preserved during the extraction process.

Table 3: FTIR Peak Assignments for PPO Enzyme Extract

Wavenumber (cm^{-1})	Functional Group / Vibration	Interpretation
3309.25	O–H / N–H stretching	Indicates hydroxyl groups and amide A bands in enzyme proteins
2899.45	C–H stretching	Aliphatic ($–CH_2–$, $–CH_3$) groups from amino acid side chains
2120.35	C≡C stretching	Possibly unsaturated or alkyne bonds from aromatic amino acids
1634.38	C=O stretching (amide I band)	Characteristic of peptide linkages in enzyme backbone
1505.17	N–H bending (amide II band)	Confirms presence of protein amide linkages
1073.19	C–O–C stretching	Indicates carbohydrate residues associated with glycoproteins

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929.54	C–H bending / skeletal vibration	Associated with aliphatic or carbohydrate structures

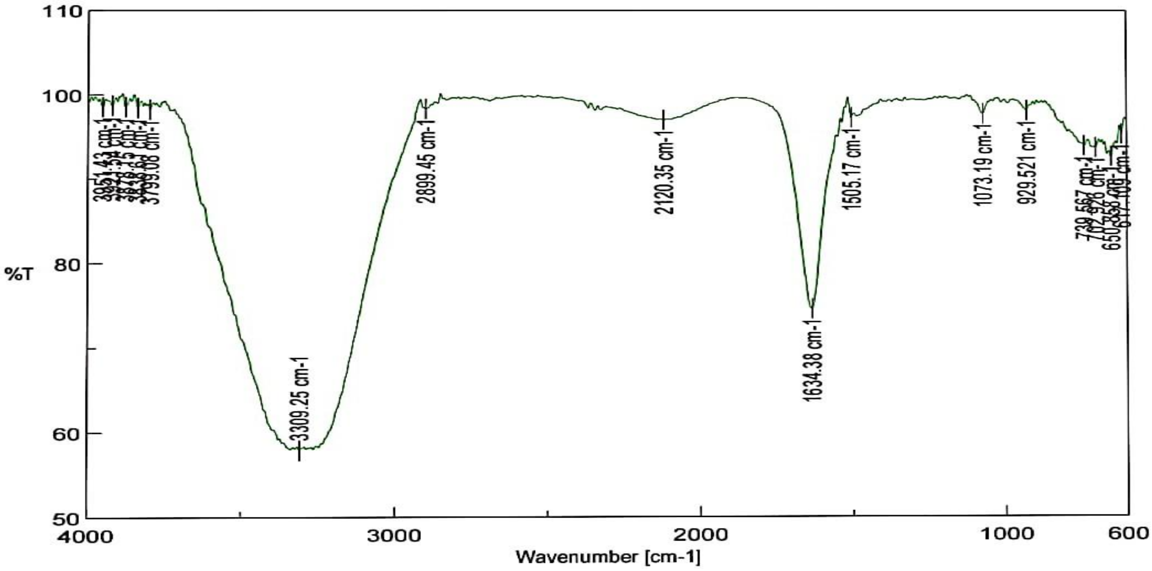

Fig 4 FTIR Graph

3.4. Formulation and Evaluation of Bioactive Antiseptic Gel:

The PPO enzyme extract was successfully incorporated into the Aloe vera gel base to formulate a bioactive antiseptic gel. All formulations were visually smooth, homogeneous, and light brown in color, with no evidence of phase separation or precipitation. The pH of the formulated gels ranged between 6.3 and 6.7, which falls within the normal skin pH range and indicates suitability for topical application.

An increase in PPO concentration resulted in a slight decrease in viscosity, which enhanced the spreading ability of the gel and facilitated easy application on the skin. The Aloe vera gel base contributed to the moisturizing and soothing properties of the formulation while also acting as a stabilizing matrix for the enzyme. The results demonstrate that Aloe vera is an effective natural carrier for PPO incorporation and supports the development of a stable, skin-compatible, and bioactive antiseptic gel.

IV. CONCLUSION:

This study successfully formulated a bioactive antiseptic gel by incorporating *polyphenol oxidase* (PPO) enzyme extracted from banana peel into an Aloe vera gel base. The extracted PPO exhibited enzymatic activity as confirmed by spectrophotometric analysis and showed moderate antibacterial activity against Escherichia coli when compared with tetracycline as the positive control. FTIR analysis confirmed the proteinaceous nature and structural integrity of the enzyme. The formulated gel was homogeneous, stable, and exhibited a skin-compatible pH, indicating its suitability for topical application. Overall, the findings demonstrate the potential of banana peel-derived PPO and Aloe vera for the development of an eco-friendly and natural antiseptic gel, supporting waste valorization and sustainable product development.

DECLARATIONS:

Conflict of interest: The authors report no conflicts of interest.

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