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Synthesis of schiff base from dialdehyde breadfruit (*Artocarpus altilis*) starch and aniline with antibacterial properties

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Abstract. Schiff bases were successfully synthesized via a condensation reaction between dialdehyde starch (DAS), derived from breadfruit (*Artocarpus altilis*) starch, and aniline. DAS was prepared through oxidative cleavage of the vicinal diol groups in native starch using sodium periodate. The formation of DAS was confirmed qualitatively by the appearance of a brick-red precipitate upon addition of Fehling's reagent and a marked increase in its water solubility. Fourier Transform Infrared (FT-IR) spectroscopy revealed a strong absorption band at 1635.64 cm^{-1}, corresponding to the aldehyde C=O stretching vibration, supporting the successful oxidation. The oxidized starch showed a high degree of oxidation (80.9%) and a carbonyl content of 10.36%. Condensation between the aldehyde groups of DAS and the amino group of aniline resulted in the formation of a Schiff base. FT-IR analysis of the resulting compound revealed absorption bands at 1635.64 cm^{-1} and 1604.77 cm^{-1}, indicating the formation of imine (-C=N-) bonds, while a peak at 871.82 cm^{-1} confirmed the presence of aromatic ring structures. The antibacterial activity of the synthesized Schiff base was evaluated using the disk diffusion method, showing strong inhibitory effects against *Escherichia coli* and *Staphylococcus aureus*. These findings showed that starch-based Schiff bases represent a promising class of biodegradable antibacterial agents with potential applications in biomedicine and biomaterials.

Keywords: Antibacterial, Biopolymer, FT-IR spectroscopy, Imine formation, Periodate oxidation

INTRODUCTION

Indonesia is endowed with abundant food crop commodities, many of which hold great potential as raw materials for starch and flour production. One of the underutilized commodities is breadfruit (*Artocarpus altilis*) or *sukun* (Bahasa) [1]. Traditionally, breadfruit has been consumed in various forms of local dishes and sides due to its high carbohydrate content (approximately 28.2%), making it a promising candidate for flour production. Breadfruit flour has been reported to substitute up to 75% of wheat flour in processed food formulations while maintaining relatively stable nutritional content and prolonged shelf life [2].

However, the native starch extracted from breadfruit exhibits several functional limitations that restrict its broader application, particularly in food processing. These include low thermal and mechanical stability, a tendency to swell easily, and reduced viscosity when subjected to heat and mechanical agitation [3]. To enhance its functionality, native starch can be modified through controlled physical or chemical treatments to produce modified starches with desirable properties [4,5]. Chemical modification typically involves introducing new functional groups into the starch polymer backbone, thereby improving its physicochemical and application-specific attributes [6,7].

Schiff bases are a class of compounds formed through the condensation of primary amines with carbonyl compounds, typically aldehydes or ketones, resulting in the formation of imine (–C=N–) linkages. Schiff bases are extensively applied in industrial sectors such as polymer manufacturing, dye production, and pharmaceuticals [8,9]. These compounds and their metal complexes have attracted significant attention due to their wide-ranging applications and biological activities. Additionally, Schiff bases also exhibit numerous therapeutic properties, including antitumor, antibacterial, anti-inflammatory, and antihypertensive effects [10]. In addition to their biomedical potential, their thermal and moisture stability enables their use as effective catalysts in various high-temperature chemical reactions.

Among the various applications of Schiff bases, a notable development involves their synthesis from renewable biopolymers. An example is Schiff base starch, a chemically modified starch produced by the condensation of dialdehyde starch (DAS) with primary amines [11]. DAS is obtained by periodate oxidation of native starch, which selectively cleaves vicinal diol groups in glucose units to generate reactive aldehyde groups. This modification not only enhances the functional properties of starch but also supports the development of bio-based materials with potential biological and industrial applications.

Sitepu (2019) has successfully synthesized Schiff base derivatives from DAS (prepared from cassava starch) and diethylenetriamine via a reflux process in ethanol. FT-IR spectral analysis confirmed aldehyde formation by the presence of a characteristic C=O stretching band at 1635 cm^{-1} [12]. A series of oxazepine-derived Schiff bases synthesized from aromatic aldehydes exhibited notable antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Klebsiella pneumoniae*, with particularly strong effects against *S. typhi* and *K. pneumoniae* [13]. Furthermore, the corrosion inhibition efficiency of these Schiff bases on mild steel in acidic media reached as high as 89.58%. Similarly, Schiff bases derived from 5-bromo-2-hydroxybenzaldehyde and aniline have been studied for their potential in biological, analytical, and coordination chemistry, further supporting their multifunctional applicability [14].

This study aimed to synthesize a Schiff base from dialdehyde breadfruit starch and aniline, characterize the product using FT-IR spectroscopy, and evaluate its antibacterial activity. The use of renewable starch-based materials for Schiff base synthesis not only contributes to value-added utilization of local resources but also offers environmentally friendly alternatives for developing bioactive compounds.

METHODOLOGY

Materials

The materials used in this study included: sodium hydroxide (NaOH, p.a., Merck), hydrochloric acid (HCl, p.a., Merck), mature breadfruit (*Artocarpus altilis*), sodium periodate ($NaIO_4$, p.a., Merck), phenolphthalein indicator, aniline, ethanol 96% (p.a., E'Merck), nutrient agar (NA, p.a., Merck), and bacterial isolates of *Staphylococcus aureus* and *Escherichia coli*.

Isolation of breadfruit starch

Mature breadfruit as indicated from its yellowing skin, was peeled and de-stemmed. The fruits were then washed thoroughly to remove dirt and latex, chopped into small pieces, and blended into a slurry. The resulting pulp was filtered using muslin cloth, and the filtrate was allowed to settle to obtain a starch precipitate. The precipitate was washed repeatedly with distilled water until the supernatant was clear. The starch was subsequently dried in an oven at 45°C for 24 hours, ground, sieved, weighed, and stored in a desiccator. Fourier-transform infrared (FT-IR) spectroscopy was used to characterize the functional group of chemicals in the starch [15].

Synthesis of dialdehyde starch (DAS)

Dialdehyde starch (DAS) was synthesized via oxidation using sodium periodate. Initially, 7 g of $NaIO_4$ was dissolved in 50 mL of distilled water in a 100 mL beaker at room temperature, and the pH was adjusted to 4 using sulfuric acid. Then, 5 g of breadfruit starch was added, and the reaction mixture was stirred and heated at 37°C for 4 hours on a hotplate. The starch slurry was filtered, and the precipitate was washed with distilled water and absolute ethanol. The final product was dried in an oven at 50°C for 8 hours and weighed. Additional syntheses were performed with $NaIO_4$ concentrations of 9, 11, and 13 g to determine the optimal oxidation condition. The products were characterized using FT-IR spectroscopy, and the carbonyl content and degree of oxidation were determined as described in the previous study [16].

Synthesis of Schiff base from DAS and aniline

Schiff base synthesis was carried out by reacting DAS with aniline [17]. A total of 0.352 g (0.002 mol) of the most highly oxidized DAS was dissolved in 20 mL of ethanol in a 250 mL three-neck flask equipped with a magnetic stirrer, thermometer, $CaCl_2$ drying tube, dropping funnel, and a reflux condenser. Then, 0.41 g (0.004 mol) of diethylenetriamine was added dropwise through the funnel under continuous stirring. The mixture was refluxed for 5 hours, after which it was filtered to collect the precipitate. The product was washed with distilled water and hot ethanol, dried in a desiccator, weighed, and analyzed using FT-IR spectroscopy.

Antibacterial test of Schiff base

The antibacterial activity of the synthesized Schiff base was evaluated using the disc diffusion method [18]. Paper disks (6 mm in diameter) were impregnated with the Schiff base compound and placed onto nutrient agar plates previously inoculated with *E. coli* and *S. aureus*. After 24 hours of incubation, inhibition zones were observed. The clear zones around the disks indicated antibacterial activity. The diameter of the inhibition zones was measured using a caliper with millimeter precision.

RESULTS AND DISCUSSION

FT-IR spectrum of breadfruit starch

The starch used in this study was isolated from mature *Artocarpus altilis* fruit obtained from the Universitas Sumatera Utara (USU) plantation. The isolated starch was in the form of a white powder with a yield of 10%. The functional groups of the isolated starch were characterized by FT-IR spectroscopy (Figure 1). The spectrum exhibited vibrational peaks at specific wavenumbers indicating the following functional groups: 3410.15 cm^{-1} (–OH stretching), 2931.80 cm^{-1} (C–H sp^3 stretching), 1635.64 cm^{-1} (C=O stretching), 1411.89 cm^{-1} (C–O stretching), 1157.29 cm^{-1} (symmetric C–O), and 856.39 cm^{-1} (C–H sp^3 bending) [19].

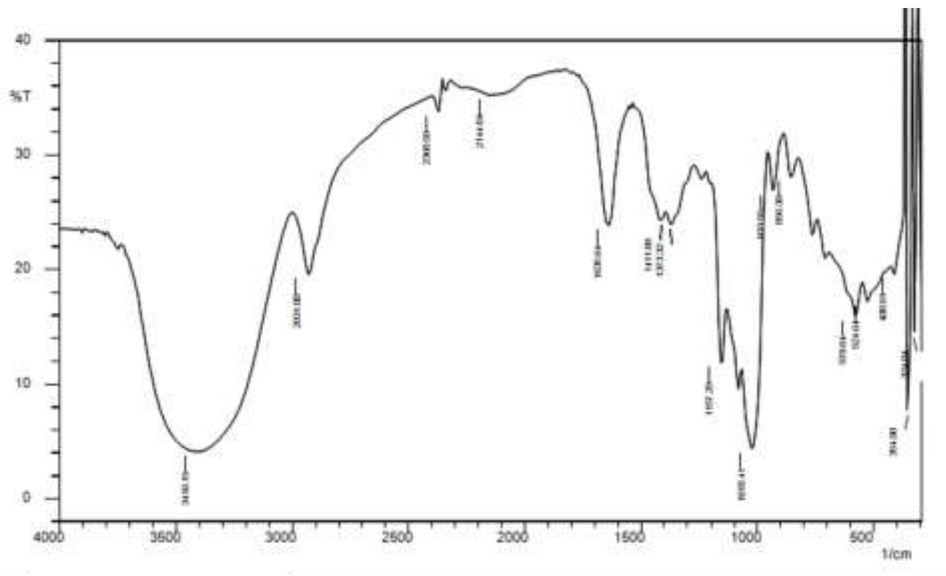

Figure 1. IR spectrum of native (isolated) breadfruit starch.

Synthesis of dialdehyde starch (DAS) from breadfruit

Starch was selectively oxidized using sodium periodate, which cleaves the C2–C3 bond of glucose units in the starch backbone, where secondary hydroxyl groups are present, resulting in the formation of dialdehyde starch (DAS). The reaction mechanism involves nucleophilic attack by an oxygen atom from the periodate ion on the C2 carbon of the starch, followed by proton abstraction and formation of an unstable cyclic intermediate. Ring cleavage then occurs between C2 and C3, producing dialdehyde starch and sodium iodate as a by-product. A proposed reaction mechanism is illustrated in Figure 2. The reaction yielded 4.12 g of a white solid product from an initial 4 g of native starch. The oxidation degree, determined by acid-base titration, was calculated at 80.9%, and the carbonyl content was found to be 10.36%. The formation of DAS was qualitatively supported by the appearance of a brick-red precipitate upon treatment with Fehling's reagent, as well as an increase in water solubility compared to native starch. FT-IR analysis further confirmed the successful oxidation, with the appearance of a sharper aldehyde C=O peak at 1635.64 cm^{-1} compared to the native starch (Figure 3). Other characteristic peaks included 3410.15 cm^{-1} (–OH), 2924.09 cm^{-1} (C–H sp^3), 1018.41 cm^{-1} (symmetric C–O), and 833.25 cm^{-1} (C–H sp^3 bending), consistent with previous findings [20].

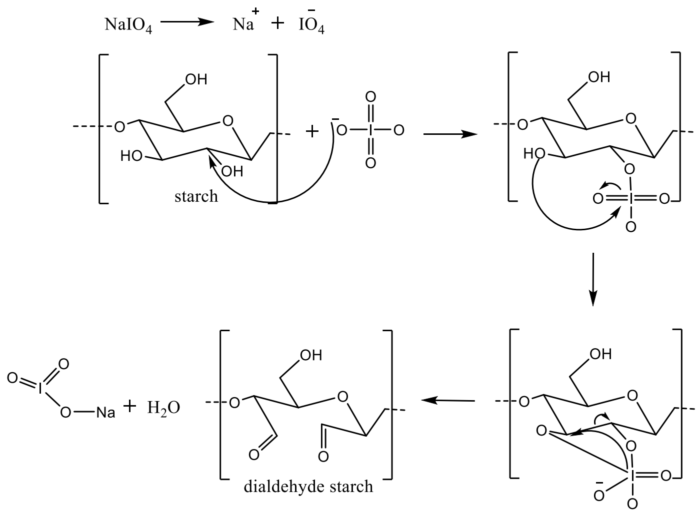

Figure 2. Reaction mechanism of dialdehyde starch synthesis

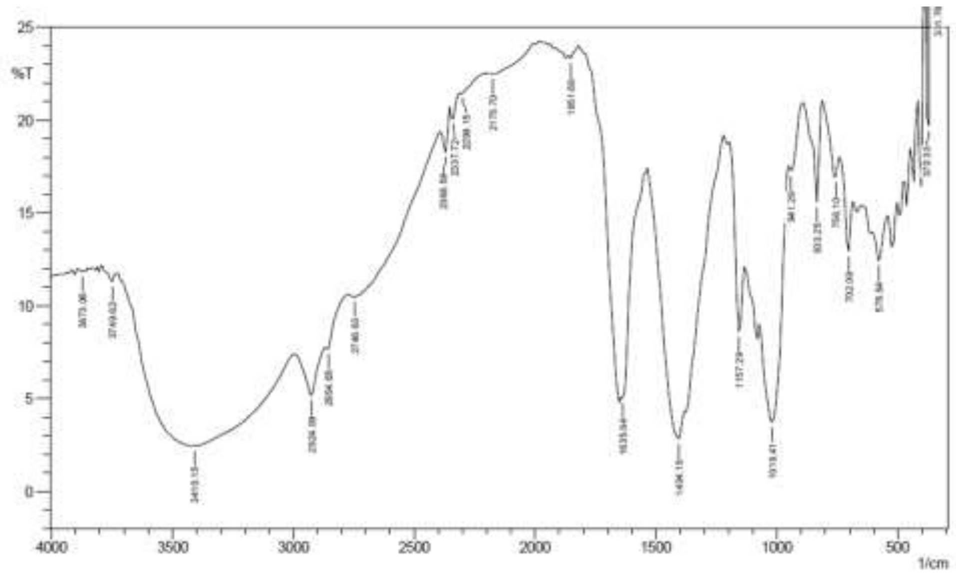

Figure 3. IR spectrum of dialdehyde starch from breadfruit starch.

Synthesis of Schiff base from dialdehyde starch and aniline

The Schiff base was synthesized via condensation between dialdehyde starch and aniline, forming an imine (C=N) linkage as a result of a nucleophilic attack by the amine nitrogen on the aldehyde carbon of DAS. Initially, the nitrogen from the aniline amine group attacks the aldehydic carbon (C2) of DAS, followed by proton transfer and the release of water to form the imine bond. A proposed reaction mechanism is shown in Figure 4. The reaction of 0.35 g DAS with 0.372 g aniline yielded 0.253 g of a yellow-brown powder. Kjeldahl nitrogen analysis indicated a nitrogen content of 1.42%, confirming successful condensation. FT-IR spectral data of the Schiff base revealed peaks corresponding to functional groups including a broad band at 3448.72 cm^{-1} attributed to overlapping –OH and $–NH_2$ stretches, consistent with the expected range for $–NH_2$ (3500–3300 cm^{-1}) and –OH (3400–3200 cm^{-1}). Other characteristic peaks were observed at 2931.80 cm^{-1} ($–CH_2$ stretching), 1635.74 cm^{-1} (C=N stretching), and 1033.85 cm^{-1} (symmetric C–O) (Figure 5). A comparative analysis of FT-IR spectra from native starch, DAS, and Schiff base formation revealed significant changes, especially at 1635 cm^{-1}. The aldehyde C=O peak in DAS became broader and overlapped with the newly formed C=N peak of the Schiff base (Figure 6), indicating successful imine bond formation.

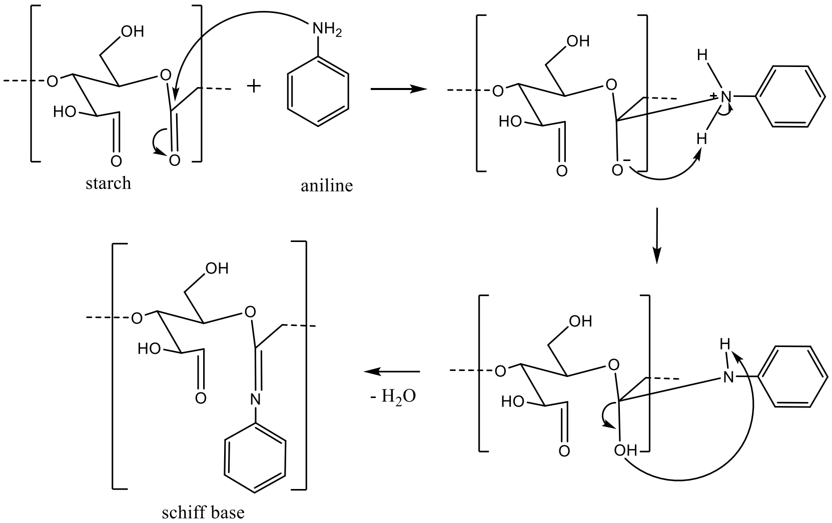

Figure 4. Reaction mechanism of Schiff base formation through condensation between dialdehyde starch and aniline.

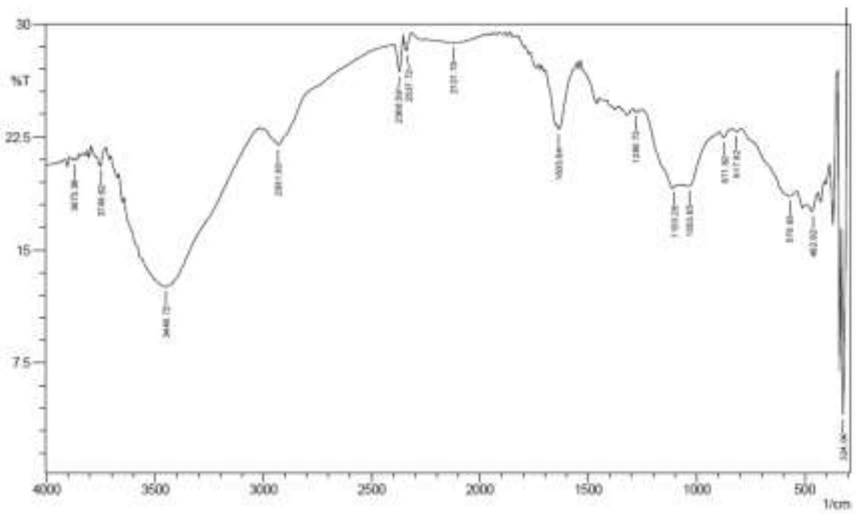

Figure 5. IR spectrum of Schiff base synthesized from dialdehyde starch and aniline.

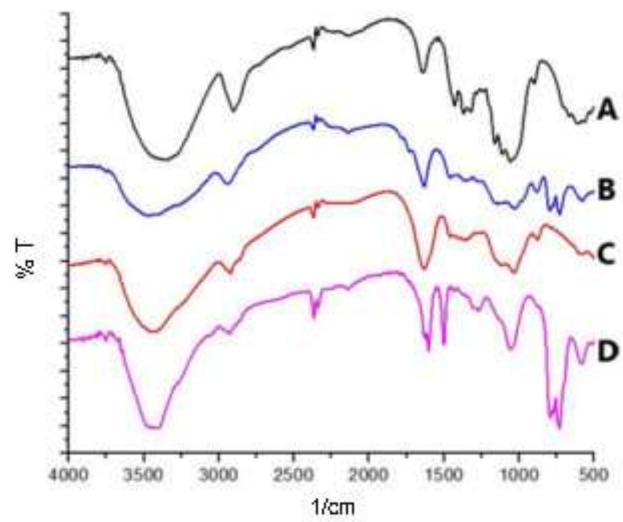

Figure 6. IR spectral profile of native breadfruit starch (A), dialdehyde starch (B), and Schiff bases (C, D).

Antibacterial activity of Schiff base

The antibacterial activity of the synthesized Schiff base, produced via condensation of dialdehyde starch (DAS) with aniline, was evaluated against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). Inhibition zones were interpreted as follows: diameters >20 mm indicate very strong activity, 11–20 mm strong, 6–10 mm moderate, and <5 mm weak [21]. The results demonstrated that the Schiff base exhibited strong antibacterial activities to both tested bacteria, which are common human pathogens (Figure 7).

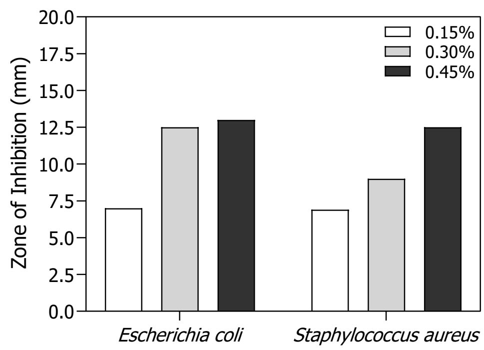

Figure 7. Antibacterial activities of Schiff base against human pathogenic bacteria.

The research results show that at a concentration of 0.45%, the Schiff base compound formed an inhibition zone of 13% for both *Escherichia coli* and *Staphylococcus aureus* bacteria. However, there was a difference in the response between the two bacteria. The inhibition zone for *E. coli* appeared to be stagnant or did not increase significantly at concentrations of 0.30% and 0.45%. Meanwhile, the inhibition zone for *S. aureus* still showed a significant increase with a rise in concentration. Overall, these findings conclude that the Schiff base compound has good antibacterial potential, especially against *E. coli*.

Previous investigations have reported similar findings regarding starch-based Schiff bases and their metal complexes. For instance, a carboxymethyl chitosan dialdehyde starch Schiff base (CMCDAS) and its associated metal complexes displayed significant antibacterial properties against both *E. coli* and *S. aureus*, with the zinc complex showing the lowest minimum inhibitory concentration (MIC) [22]. Likewise, dodecylamine dialdehyde starch (DASDA) derivatives showed strong antibacterial effects, especially against *E. coli* [23]. Schiff bases synthesized from phenylhydrazine and aromatic aldehydes have also demonstrated potent inhibition zones against *S. aureus* and *Pseudomonas* *aeruginosa* [24]. Biologically, Schiff bases (imines) are known to possess various pharmacological activities, including antioxidant, anti-inflammatory, analgesic, antifungal, and antibacterial properties. These bioactivities are attributed to the azomethine group (C=N), which can engage in hydrogen bonding, thereby increasing the molecule's polarity. As a result, Schiff base compounds are capable of penetrating lipid membranes and interfering with the active sites of enzymes, potentially disrupting microbial metabolism and ultimately leading to cell death [25]. Therefore, the presence of the azomethine group in the synthesized Schiff base is hypothesized to contribute to its observed antibacterial activity, particularly through mechanisms involving increased membrane permeability and enzyme inhibition.

CONCLUSION

The Schiff base compound synthesized from breadfruit starch and sodium periodate exhibits antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. This activity is attributed to the presence of the antibacterial azomethine (C=N) functional group, which was confirmed by Fourier-transform infrared (FTIR) analysis. The use of Schiff bases as antibacterial agents is appealing due to their low-cost and simple synthesis. Therefore, further research on the Schiff base derived from breadfruit starch is warranted. Future studies could focus on its antifungal activity or its development into nanoparticles. This would provide more in-depth information about the compound's effectiveness, especially for medical applications.

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