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The Synthesis, Infra-Red Analysis and Antimicrobial Studies of the Schiff Base, Oxime, Hydrazone and Azine of Vanillin

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Abstract

Schiff bases, oximes, hydrazones, and azines are organic compounds that contain the imino functional group (C = N). They are obtained by reacting an amine or amino compound and a carbonyl compound in the presence of an acid. This research was focused on synthesizing these four compounds using the primary reactant, Vanillin (4-hydroxy-3-methoxybenzaldehyde), to achieve vanillin derivatives. The Schiff base, Oxime, and hydrazone were synthesized by reacting an equimolar amount of vanillin with aniline, hydroxylamine hydrochloride, and hydrazine hydrate, respectively, in ethanol by stirring under reflux for about 2 hours. However, the azine was obtained by reacting vanillin and hydrazine hydrate in a 2:1 ratio. At the end of the reaction, solid products were obtained and weighed, giving a percentage yield of 52.4%, 88.1%, 85.7%, and 54.9% for the Schiff base, oxime, hydrazone, and azine, respectively. Furthermore, to verify the formation of the imino group, an IR analysis was carried out on the product and the presence of an IR band at 1659 cm^{-1} for Schiff base (C = N) imino, 1689 cm^{-1} for oxime (-C = N-) imino, 1626 cm^{-1} for hydrazone (-C = N--) and 1652 cm^{-1} for azine (-C = N-) alkene, proved that the expected functional formed. In addition, this research showed that these compounds have antimicrobial properties. The Vanillin-Schiff Base was the most effective and broad-spectrum (best against *Pseudomonas sp.*, *Staphylococcus sp.*, and *Bacillus sp.*), Vanillin-Oxime was moderately effective, showing some inhibition against *E. coli* and *Candida sp.*, Vanillin-azine was only strongly effective against *Staphylococcus sp.*, but weak against others, while the Vanillin-Hydrazone was the weakest, with almost no inhibition at lower concentrations. This research simply recommends that scientists should concentrate on developing more eco-friendly methods and solvents for the synthesis of those compounds which will give very high yields.

Keywords

Schiff Base, Imino Group, Functional Group, *Staphylococcus sp.*

1. Introduction

The synthesis of imines, also known as Schiff bases, is a fundamental reaction in organic chemistry formed by the condensation of an aldehyde or ketone with a primary amine. Similarly, the reaction of a carbonyl compound with an amino compound such as a hydroxylamine or a hydrazine yields an oxime or a hydrazone, respectively. This study focuses on the condensation reactions of vanillin with aniline, hydroxylamine hydrochloride, and hydrazine hydrate to produce a series of vanillin-derived compounds. These reactions, which form C = N bonds, are important for the synthesis of various biologically active molecules. The synthesis of these organic compounds, particularly those with potential biological activities, has traditionally relied on methods that involve toxic reagents, hazardous solvents, and energy-intensive processes. These conventional methods pose significant environmental and health risks, contributing to pollution and the depletion of natural resources. Furthermore, Anastas and Warner (2000), stated that, wherever possible, synthetic methods should be designed to employ and create chemicals or products that have virtually no lethal impact on human well-being and the earth, and that the use of additional substances (e.g., separating agents, solvents) should be made unnecessary and harmless when used. Additionally, it is vital to be acquainted with the environmental and financial consequences of the energy needed during synthetic procedures and henceforth, limit it. If achievable, synthetic methods should take place at ambient temperature and pressure [1]. Moreover, the increasing prevalence of antibiotic-resistant microbial strains has necessitated the discovery and development of new antimicrobial agents with novel mechanisms of action [2] [3]. In addition, vanillin-derived Schiff bases, oximes, hydrazones, and azines have shown potential as antimicrobial agents. However, the synthesis of these compounds using green chemistry principles remains underexplored. There is a need to develop sustainable and eco-friendly synthetic routes for these derivatives to minimize environmental impact and enhance their applicability in various fields. Additionally, the antimicrobial efficacy of these compounds needs to be thoroughly investigated to assess their potential as alternatives to conventional antibiotics. This study addresses the gap in the literature by focusing on the green synthesis of Schiff bases, oximes, hydrazones, and azines of vanillin and evaluating their antimicrobial properties. The research aims to provide a comprehensive understanding of the synthetic pathways, structural characteristics, and biological activities of these compounds, thereby contributing to the advancement of green chemistry and antimicrobial research. Imines are the nitrogen analogs of aldehydes and ketones, containing a C = N bond instead of a C = O bond. They are formed by adding a primary amine or an amino compound to an aldehyde or ketone, kicking out a mol-

ecule of water (H_2O). Their general formula is $R_2C = NR$ [4] as shown in **Figure 1**. There are different types of imines and their major differences are in the structural features and the mode with which they are obtained, viz. the group attached to the nitrogen atom.

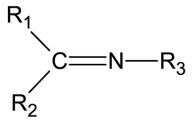


Figure 1. General structure of imines.

The structure above represents the general features of imines. Changes in the group attached to the nitrogen atom (R_3) bring about the different members of the imine family. These members include Schiff base, oxime, hydrazone, azine and semi carbazone [5] as shown in **Figure 2**.

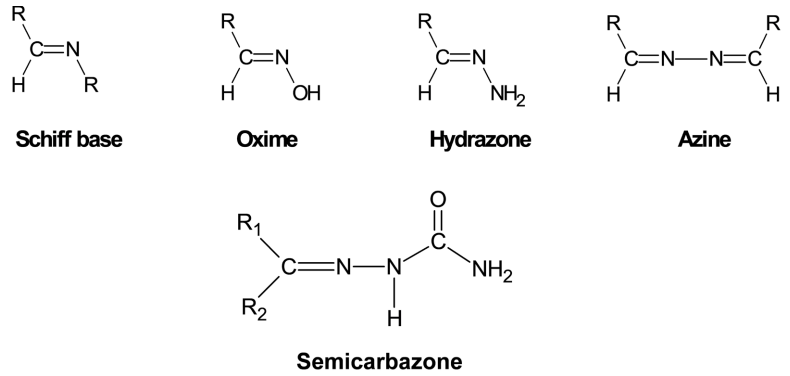


Figure 2. Classes of imines.

These compounds all possess the imino group and their applications in science are known to be a result of this functional group [6]. Imines have been synthesized using a variety of methods and solvents. Most of the solvents that have been used in the past have been toxic to scientists and the environment. Solvents like dichloromethane, benzene and toluene have been used for the synthesis of imines. However, the increasing demand for sustainable and environmentally friendly chemical processes has led to the emergence of green chemistry as a pivotal field in modern scientific research. Green chemistry emphasizes the design of products and processes that minimize the use and generation of hazardous substances. One of the key areas of interest within green chemistry is the synthesis of organic compounds using eco-friendly methods. Among these compounds, Schiff bases, oximes, hydrazones, and azines have earned significant attention due to their diverse biological activities and applications in various fields such as medicine, agriculture, and materials science. They are formed through the condensation reaction between a primary amine ($R-NH_2$) or amino compound and a carbonyl compound (aldehyde or ketone), resulting in the elimination of water (H_2O). The general structure of an imine is $R_2C = NR'$, where R and R' can be alkyl, aryl, or hydrogen groups. This reaction follows

a nucleophilic addition mechanism between the reactants. It can be seen as a condensation reaction because it involves the removal of a water molecule during the dehydration process which then leads to the formation of the imine. Imine formation can occur by acid or base catalysis to affect the removal of water; however, it must be noted that an acid-catalyzed reaction is preferred for an optimal product formation. Careful control of the pH of the reaction mixture is essential for the successful formation of the imine [4] [7].

It is worth mentioning that this reaction is a reversible one and, in most cases, the equilibrium favors the aldehyde or ketone, so the water must be removed as it is formed to ensure a good yield of the imine. Thus, the reaction can be done using a Dean-Stark apparatus to remove the water. Several other dehydrating techniques have been reported including the use of dehydrating agents like sodium sulphate (Na_2SO_4), titanium tetrachloride ($TiCl_4$), molecular sieve and distillation [8]. The mechanism of the reaction is indicated as shown in **Figure 3**.

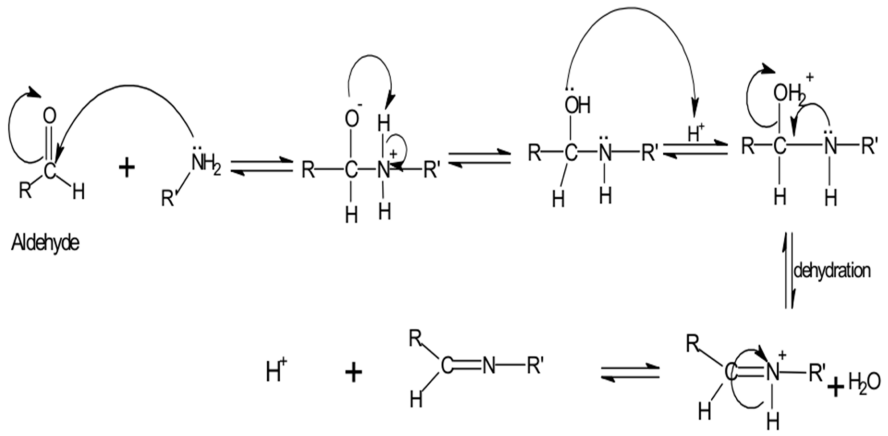


Figure 3. Mechanism for the formation of imines.

Where R' = alkyl or aryl group (for a Schiff base), OH (for an oxime), NH_2 (for hydrazone and N = CRR' (for an azine).

Mechanism for the Formation of Imines [9]

The steps involved in the formation of all imines, following the mechanism in scheme 1 above are as follows.

1. Nucleophilic attack of the electrophilic carbonyl center by the amino compound which leads to movement of the pi electrons to the carbonyl oxygen.
2. The negatively charged oxygen abstracts the hydrogen atom adjacent to it leading to the formation of a carbinolamine intermediate.
3. The oxygen of the carbinolamine picks up the acidic proton (catalyst) to form an unstable water molecule which can be removed simply because of the movement of the lone pair on the nitrogen. This is the dehydration step.
4. The water molecule that was removed then serves as a base in the medium to abstract the imino hydrogen, thus leading to the formation of a stable imine.

2. Materials and Methods

Reagents: All the chemicals/solvents used in this work were commercially bought off the shelve of high reagent grade chemical company Lagos.

2.1. Apparatus

All glassware used in this work were properly washed with detergent, rinsed several times with tap water. They were then soaked in a concentrated solution of hydrochloric acid for about two hours after which they were rinsed 2 - 3 times with distilled water and dried in an oven maintained at 110 oc. All reagents and solvents used were of analytical grade and were used as supplied without further purification. All weighing were carried out on college B154 Meter Toledo electric balance. Melting point and decomposition temperature were carried out on Stuart SMP 10 melting point apparatus. Determination of water of hydration was done on drying oven model DHO-9053A.FT-IR spectra measurements were recorded using Agilent Technology FT-IR spectrophotometer Carry 630, in the region 400 - 4000 cm^{-1}. The thin layer chromatography (TLC) obtained from Christon international/medical science laboratory equipment supplier Lagos was used for rapid screening of various different movement of the four compounds (Schiff base, oxime, hydrazone and azine).

2.2. Synthesis of Vanillin-Derived Schiff Base

Vanillin (0.01 mol, 1.52 g) and aniline (0.01 mol, 0.93 g) dissolved in 50 ml ethanol were poured into a flat-bottom flask (150 ml) and stirred under reflux for an hr (Figure 4). The mixture was a clear solution initially after the combination but on continuous stirring under reflux, the colour changed to light yellow. A few drops of conc. HCl was added after an hour and the mixture stirred for another hour. Thin layer chromatography (TLC) using ethyl acetate was used to monitor the reaction. At completion, the hot mixture was allowed to cool. After about an hour and thirty minutes, yellow crystals formed at room temperature. The product was filtered and purified by recrystallization using ethanol, filtered again, and air-dried.

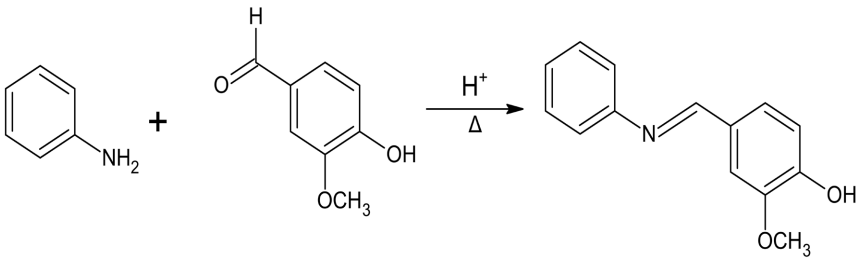


Figure 4. Aniline-vanillin Schiff based.

2.3. Synthesis of Vanillin-Derived Oxime (Figure 5)

Vanillin (0.01 mol, 1.52 g) and hydroxylamine hydrochloride (0.01 mol, 0.695 g)

dissolved in 5N0 ml ethanol were poured into a flat-bottom flask (150 ml) and stirred under reflux for an hour thirty minutes. The mixture was a yellow solution initially after the combination and on stirring under reflux. A few drops of conc. HCl were added after an hour and the mixture stirred for another thirty-minutes. Thin layer chromatography (TLC) using ethyl acetate was used to monitor the reaction. At completion, the solvent was evaporated to give a yellow oily liquid. On standing overnight, the viscous substance solidified. This product was crushed and purified by recrystallization using ethanol.

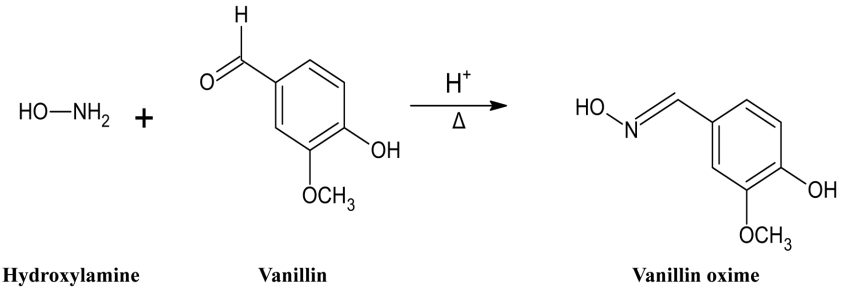


Figure 5. Vanillin oxime.

2.4. Synthesis of Vanillin-Derived Hydrazone

Vanillin (0.01 mol, 1.52 g) and hydrazine hydrate (0.01 mol, 0.50 g) in a ratio of 1:1 was dissolved in 50 ml ethanol were poured into a flat-bottom flask (150 ml). The reaction mixture was stirred under reflux for an hour thirty-minutes, followed by the addition of a few drops of concentrated hydrochloric acid. The mixture was further refluxed for another thirty-minutes and thin layer chromatography (TLC) using ethyl acetate was used to monitor the reaction. At completion, the hot mixture was allowed to cool. On cooling, no precipitate was observed, however, the addition of 50 ml of distilled water led to the formation of an orange-colored precipitate. The precipitate was then filtered, purified by recrystallization using ethanol, the extract from the ethanol using distilled water, filtered again, and air-dried. The chemical reaction as shown in Figure 6 indicates the synthesis of vanillin hydrazine.

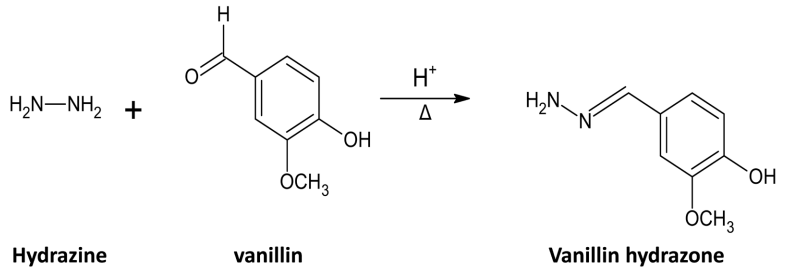


Figure 6. Vanillin hydrazine.

2.5. Synthesis of Vanillin-Derived Azine

Vanillin (0.01 mol, 1.52 g) and hydrazine hydrate (0.01 mol, 0.50 g) in a ratio of

2:1 were dissolved in 50 ml ethanol were poured into a flat-bottom flask (150 ml). At room temperature, precipitation of the azine occurred as an orange-coloured powder in solution as soon as the two reactants came in contact. The reaction mixture was then stirred under reflux for an hour thirty-minutes without the addition of acid. The powder was observed to dissolve in the hot ethanol. Thin layer chromatography (TLC) using ethyl acetate was used to monitor the reaction. At completion, the hot mixture was allowed to cool. The product formed again at room temperature. It was then filtered and purified by recrystallization using ethanol, filtered again, and air-dried.

2.6. Physicochemical Analyses of Compounds

Melting Point Determination: This was done as described by Williamson and Fieser (2004) For Solid samples [10]. A small amount of the dry compound was poured on a watch glass. Some of the dry compound from the watch glass was tightly packed in a cappillary tube and inserted into the melting point apparatus. The temperature range observed as the first and second crystals melted was read and recorded using a thermometer. This constituted the MP of the sample.

Determination of R_F value: The compounds were dissolved in dichloromethane and a capillary tube was used to collect the solution. The sample was spotted on the origin drawn on a sheet of precoated silica gel TLC plate and allowed to air-dry for a minute. The plate was placed in a solvent tank containing 100 ml of the solvent (1:3 chloroform-methanol) and allowed to develop. The developed TLC plate was removed from the tank and air-dried. Sulphuric acid (10%) in methanol spray reagent was also used on developed TLC plates to visualize the migration of the spots. The Rf values of detected spots were calculated using equation (1). The spots were marked/circled and the retardation factor calculated thus;

$$\text{Retardation factor } \left(R_f\right) = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}} \quad (1)$$

2.7. Percentage Yield of the Compounds

All products were weighed and their actual yield determined. Using stoichiometric equations, the theoretical yield of all compounds was calculated. Furthermore, the percentage yield was determined through calculations involving these variables, thus;

$$\text{Percentage yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100$$

1)

Actual yield of vanillin-aniline Schiff base = 1.189 g

Theoretical yield of vanillin-aniline Schiff base = 2.27 g

$$\text{Percentage yield of vanillin-aniline Schiff base} = \frac{1.189\ \text{g}}{2.27\ \text{g}} \times 100 = \mathbf{52.4\%}$$

2)

Actual yield of vanillin Oxime = 1.471 g

Theoretical yield of vanillin Oxime = 1.67 g

$$\text{Percentage yield of vanillin Oxime} = \frac{1.471\ \text{g}}{1.67\ \text{g}} \times 100 = \mathbf{88.1\%}$$

3)

Actual yield of vanillin hydrazone = 1.423 g

Theoretical yield of vanillin hydrazone = 1.66 g

$$\text{Percentage yield of vanillin hydrazone} = \frac{1.423\ \text{g}}{1.66\ \text{g}} \times 100 = \mathbf{85.7\%}$$

4)

Actual yield of vanillin azine = 1.647 g

Theoretical yield of vanillin azine = 3.00 g

$$\text{Percentage yield of vanillin azine} = \frac{1.647\ \text{g}}{3.00\ \text{g}} \times 100 = \mathbf{54.9\%}$$

2.8. Spectroscopic Analysis of the Compounds

The Infra-red (IR) spectra of the compounds were determined on a Carry 630 Agilent Technologies FT-IR spectrometer at 650 - 4000 cm^{-1} at the Center for Dryland Agriculture, Bayero University, Gwarzo Road, Kano, Nigeria.

2.9. Antimicrobial Analyses of the Compounds

Source of Organisms: The gram-positive bacteria (*S. aureus and B. subtilis*), gram-negative bacteria (*E. coli and P. aeruginosa*) isolates, and fungi (*A. niger* and *C. albicans*) were characterized, purified, and their viability confirmed at the Department of Microbiology, Rivers State University.

Preparation of Standard Microbial Suspension: A 24-hour-old pure culture of the microbial isolates used for the antimicrobial testing were emulsified in a sterile distilled water in test tube and adjusted to 0.5 McFarland turbidity standard [11].

A 0.5 McFarland standard was prepared according to Cinerman *et al.*, (2000), by mixing 0.05 mL of 1% barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$), with 9.95 mL of 1% sulfuric acid (H_2SO_4) [12].

A 0.5 McFarland standard is a reference solution used in microbiology to standardize the turbidity, or cloudiness, of a bacterial suspension. It's a key tool in clinical and research labs to ensure a consistent and known number of bacteria are used in experiments, most notably for antimicrobial susceptibility testing (AST).

Preparation of the Antimicrobial Disc: The antimicrobial disc used for this study was prepared according to method adopted by Robinson *et al.*, (2023) using sterile perforated filter paper [13]. The perforated filter paper was soaked (impregnated) with the respective vanillin-derived compounds (Oxime, Hydazone, Oxime and Schiff base) and allowed to be absorbed before use.

Antimicrobial Sensitivity Testing: Antimicrobial tests were carried out on two gram-positive bacteria (*S. aureus and B. subtilis*), two gram-negative bacteria (*E. coli and P. aeruginosa*) isolates, and two fungi (*A. niger and C. albicans*) using the disc diffusion method. A sterile swab stick was dipped into the tube containing the microbial suspension adjusted to 0.5 McFarland turbidity standard and streaked on the surface of a prepared Mueller Hinton agar evenly to ensure even distribution of the organism. The agar was allowed to dry for about 3 - 5 minutes before the sterile perforated filter paper impregnated with the test compound in different concentrations was placed on the surface of the inoculated plate with the use of sterile forceps in duplicate. The head of the forceps was used to press down each disc slightly to make contact with the agar. After placing the discs, the plates were incubated in an inverted position aerobically at 35˚C for 16 - 18 hrs except for the fungal plates that were incubated for 72 h. After incubation, the test plates were examined and the diameter of each zone of inhibition was measured in millimeters using a ruler. The results were interpreted as resistant, intermediate, and sensitive according to CLSI, (2017).

3. Results and Discussion

3.1. Synthesis of Schiff Bases

The synthesis of all the compounds was very straightforward and followed what was written in literature. The conventional method was used for the synthesis of all the compounds using ethanol which is an ecofriendly solvent. While the synthetic procedure went as expected, it is worth mentioning that the workup process for the oxime, hydrazone, and azine was quite tedious. The oxime required the solvent to be completely removed before solidification occurred overnight while the hydrazone and azine required that a reasonable amount of distilled water be added to the reaction mixture to cause precipitation of the product before filtration and recrystallization. The Schiff base was the only product that precipitated on its own after the mixture cooled.

3.2. Retardation Factor of the Compounds

The calculated R_f values for each compound are illustrated in Table 1 below.

Table 1. Retardation factor of the vanillin-derived compounds.

COMPOUND	RETARDATION FACTOR (R_f)
Vanillin-aniline Schiff base	0.50
Vanillin oxime	0.80
Vanillin hydrazine	0.40
Vanillin azine	0.42

3.3. Physical State of the Compounds

The physical state of the compounds is illustrated in Table 2 below.

Table 2. Physical state of vanillin-derived compounds.

COMPOUND	COLOUR	PHYSICAL STATE
Vanillin-aniline Schiff base	Yellow	Crystals
Vanillin oxime	Pale-yellow	Powder
Vanillin hydrazone	Orange	Powder
Vanillin azine	Orange	Powder

Based on the Table 2 provided, the physical state and colour of four vanillin-derived compounds are summarized. Vanillin-aniline Schiff base is a yellow solid in the form of crystals. Vanillin oxime, vanillin hydrazone, and vanillin azine are all in the form of a powder. Their colours vary from pale-yellow to orange. The data indicates that while all four compounds are solids at room temperature, their physical appearance differs. The vanillin-aniline Schiff base forms an ordered, crystalline structure, which suggests a high degree of molecular packing and uniformity. The other three compounds—the oxime, hydrazone, and azine—exist as powders. This physical state indicates a less-ordered, amorphous solid structure, or that the individual crystals are too small to be observed with the naked eye. The differences in physical state and colour are a direct result of the specific molecular structure and intermolecular forces of each compound. The presence of different functional groups and the overall molecular geometry of each compound influence how the molecules pack together in the solid state. These structural variations also affect how the compounds absorb and reflect light, leading to their distinct colours. This is similar to Aazam and Thomas 2024 [14].

3.4. Melting Points of Compounds

The uncorrected melting points of the compounds were determined after recrystallization. This is illustrated in Table 3.

Table 3. Melting points of vanillin-derived compounds.

COMPOUND	MELTING POINT (˚C)
Vanillin-aniline Schiff base	251
Vanillin oxime	88 - 89
Vanillin hydrazine	122 - 123
Vanillin azine	125 - 126

Table 3 the melting points of the vanillin-derived compounds vary significantly, from the lowest at 88 - 89˚C for vanillin oxime to the highest at 251˚C for the vanillin-aniline Schiff base. The melting point is a physical property that reflects the amount of energy required to overcome the intermolecular forces holding the molecules in their solid crystalline lattice. The vanillin-aniline Schiff base has a remarkably high melting point of 251˚C. This suggests that the intermolecular forces (such as hydrogen bonding, dipole-dipole interactions, and Van der Waals forces)

in its crystal lattice are exceptionally strong and require a large amount of thermal energy to break. This strong intermolecular attraction is likely due to its specific molecular geometry and the potential for extensive hydrogen bonding or efficient molecular packing. Vanillin oxime has the lowest melting point at 88 - 89˚C. This indicates that the forces holding its molecules together in the solid state are the weakest among the four compounds. Vanillin hydrazine (122 - 123˚C) and vanillin azine (125 - 126˚C) have very similar melting points. This proximity suggests that their crystal structures and the strength of their intermolecular forces are comparable. The minor difference could be due to subtle variations in molecular size, shape, or packing efficiency. The data in the table highlights that even minor changes in a molecule's structure, such as the introduction of a new functional group, can drastically alter its physical properties like melting point. The strength of the intermolecular forces and the efficiency of molecular packing in the solid state are the primary factors that determine a compound's melting point according to Abool 2014 [15].

3.5. Percentage Yield of Compounds

Table 4. Percentage yield of the vanillin-derived compounds.

COMPOUND	THEORETICAL YIELD (g)	ACTUAL YIELD (g)	PERCENTAGE YIELD (%)
Vanillin-aniline Schiff base	2.270	1.189	52.4
Vanillin oxime	1.670	1.471	88.1
Vanillin hydrazine	1.660	1.423	85.7
Vanillin azine	3.000	1.647	54.9

The data in Table 4 shows a significant difference in the percentage yields of the synthesized vanillin-derived compounds. The yields for vanillin oxime (88.1%) and vanillin hydrazine (85.7%) are remarkably high, while the yields for vanillin-aniline Schiff base (52.4%) and vanillin azine (54.9%) are considerably lower. This disparity can be attributed to the chemical nature of the reactants and the specific reaction conditions. The formation of Schiff bases, oximes, and hydrazones are all condensation reactions between a carbonyl compound (vanillin) and a nitrogen nucleophile. The nucleophilicity of the nitrogen-containing compound is a key factor in determining the reaction rate and, consequently, the yield. Hydroxylamine (for vanillin oxime) and hydrazine (for vanillin hydrazine and azine) are highly nucleophilic. The lone pair on the nitrogen atom in both hydroxylamine is more readily available for attack on the electrophilic carbonyl carbon of vanillin. This enhanced reactivity leads to a more efficient reaction and higher yields, as observed with the 88.1% and 85.7% yields for vanillin oxime and vanillin hydrazine, respectively. Aniline (for vanillin-aniline Schiff base), in contrast, is an aromatic amine. The lone pair on the nitrogen atom of aniline is delocalized into the benzene ring via resonance. This resonance effect reduces the nucleophilicity of the nitrogen

atom, making it a poorer nucleophile than hydroxylamine or hydrazine. The slower reaction rate and less favorable equilibrium result in a lower yield (52.4%). The synthesis of the vanillin azine compound involves a unique stoichiometric consideration that contributes to its lower yield. Both vanillin hydrazine and vanillin azine are synthesized using vanillin and hydrazine. However, vanillin hydrazine is formed from a 1:1 molar ratio, while vanillin azine is formed from a 2:1 molar ratio (two molecules of vanillin to one molecule of hydrazine). The formation of vanillin azine is a two-step process: the formation of vanillin hydrazine followed by its further condensation with another molecule of vanillin. This second condensation step, while possible, may not go to completion, especially if the reaction conditions (e.g., acid concentration, water removal) are not perfectly optimized. This incomplete conversion to the final azine product likely contributes to the lower yield (54.9%) compared to the vanillin hydrazine intermediate (85.7%). All these reactions are reversible. The lower yields for the Schiff base and azine are likely due to the equilibrium position of their respective reactions. To achieve high yields, water, a by-product of the condensation, must be continuously removed to push the equilibrium toward the products according to Le Châtelier's principle. The higher yields for the oxime and hydrazine suggest that their reactions proceed more favorably and/or are less sensitive to the presence of water compared to the Schiff base and azine syntheses. This is often the case with more reactive nucleophiles, which form the products more quickly, making the reaction less susceptible to reversal.

Table 5. Solubility test of the vanillin-derived compounds.

COMPOUNDS	Ace	EtOH	MeOH	EtOAc	CHL	DCM	DMSO	Hx
Vanillin-aniline Schiff base	IS	IS	IS	S	S	S	S	IS
Vanillin oxime	S	S	S	S	S	S	S	IS
Vanillin hydrazone	S	S	S	S	S	S	S	IS
Vanillin azine	S	S	S	S	S	S	S	IS

S—Soluble, SS—Sparingly soluble, IS—Insoluble; Ace—acetone, EtOH—ethanol, MeOH—methanol, EtOAc—ethyl acetate, CHL—chloroform, DCM—dichloromethane, DMSO—dimethylsulfoxide, Hx—Hexane.

The vanillin-derived oxime, hydrazone, and azine were soluble in all solvents tested at room temperature except hexane. The Schiff base on the other hand was soluble only in ethyl acetate, chloroform, dichloromethane, and dimethylsulphoxide (Table 5).

3.6. IR Spectral Data of the Compounds

All bands on the IR spectrum were recorded in cm^{-1}.

3.7. IR Data for Vanillin-Aniline Schiff Base (Figure 7)

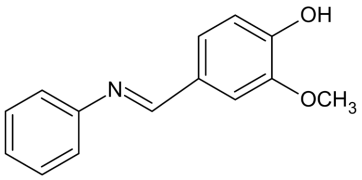


Figure 7. Vanillin-aniline Schiff base structure.

1659 (C = N), 1585 (aromatic C = C stretch), 3000 (aromatic C-H stretch), 1156 (aromatic C-O stretch), 2936 (aliphatic C-H stretch) and 3569 (H-bonded OH stretch).

3.8. IR Data for Vanillin-Oxime (Figure 8)

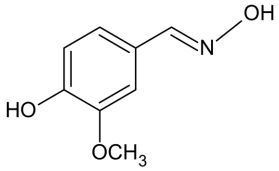


Figure 8. Vanillin oxime structure.

1659 (C = N), 1689 (oxime group, C = N-OH), 1592 (aromatic C = C stretch), 3029 (aromatic C-H stretch), 1153 (aromatic C-O stretch), 2985 (aliphatic C-H stretch) and 3368 (OH stretch).

3.9. IR Data for Vanillin-Hydrazone (Figure 9)

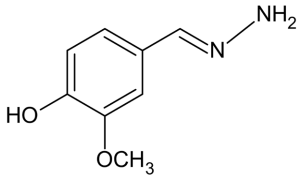


Figure 9. Vanillin hydrazine structure.

1626 (C = N), 1603 (aromatic C = C stretch), 3003 (aromatic C-H stretch), 1156 (aromatic C-O stretch), 2959 (aliphatic C-H stretch) and 3480 (OH stretch).

3.10. IR Data for Vanillin-Azine (Figure 10)

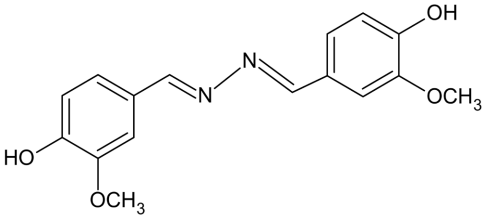


Figure 10. Vanillin azine.

1652 (C = N), 1596 (aromatic C = C stretch), 3056 (aromatic C-H stretch), 1149 (aromatic C-O stretch), 2985 (aliphatic C-H stretch) and 3361 (OH stretch).

For all the compounds the presence of the IR band at 1659 (for Schiff base and oxime), 1626 and 1652 cm^{-1} (for hydrazone and azine respectively) indicated the formation of the imino functional group, the group of interest, hence, proving that the compound formed.

Additionally, absence of the amine nitrogen (NH) IR bands at 3500 - 3400 cm^{-1} and carbonyl (C = O) IR bands at 1730 - 1540 cm^{-1} suggested the total synthesis of the four compounds. Furthermore, the occurrence of the imino (C = N) IR band at 1689 - 1471 cm^{-1} further confirmed their formation [16].

3.11. Antimicrobial Screening Results

The result presented in Table 6 indicates the effect of different concentrations of Vanillin-aniline Schiff base on bacterial and fungal species.

Table 6. Effect of different concentrations of Vanillin-aniline Schiff base on bacterial and fungal species.

Vanillin-aniline Schiff base	Unit	Control (200 mg) 0.5 McFarland std	200 mg	100 mg	50 mg
Escherichia coli	Mm	17.00 ± 2.8^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}
Pseudomonas sp.	Mm	23.00 ± 1.4^{c}	19.00 ± 1.4^{d}	11.00 ± 1.4^{b}	0.00 ± 0.0^{a}
Bacillus sp.	Mm	18.50 ± 2.1^{b}	14.50 ± 3.5^{bc}	10.50 ± 0.7^{b}	8.00 ± 0.0^{b}
Staphylococcus sp.	Mm	27.00 ± 1.4^{d}	17.50 ± 0.7^{cd}	15.00 ± 1.4^{c}	11.50 ± 2.1^{c}
Aspergillus sp.	Mm	17.00 ± 1.4^{b}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}
Candida sp.	Mm	19.00 ± 1.4^{b}	11.00 ± 1.4^{b}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}

Means with similar superscript down the group showed no significant difference ($P > 0.05$). Key: 0 - 13 mm = Resistant, 14 - 16 = Intermediate, 17 and above = Susceptibility.

The results in Table 6 show that vanillin-aniline Schiff base has a varied antimicrobial effect. It is most effective against Gram-positive bacteria (*Bacillus sp.* and *Staphylococcus sp.*), with a clear dose-dependent inhibition. It also shows some activity against *Pseudomonas sp.* and *Candida sp.* but is ineffective against *E. coli* and *Aspergillus sp.* This selectivity can be linked to its molecular structure. The vanillin-aniline Schiff base has a single C = N bond linking a vanillin moiety to an aniline ring. The presence of two aromatic rings and the imine group are key structural features contributing to its biological activity. The compound's effectiveness against Gram-positive bacteria is likely due to their simpler cell wall structure, which allows the compound to penetrate and disrupt cellular functions. The lack of activity against the Gram-negative *E. coli*, which has a more complex outer membrane, highlights the barrier effect of this membrane. When compared to other derivatives, the vanillin-aniline Schiff base's activity against *Candida sp.* is noteworthy. While its activity is limited, it demonstrates that the specific arrangement of its functional groups can have an effect on this fungal species, unlike some other derivatives. Its ineffectiveness against *Aspergillus sp.* further suggests that its mech-

anism of action is highly specific and not broadly effective against all fungi. The overall antimicrobial profile indicates that the vanillin-aniline Schiff base's structure provides some antimicrobial properties, but its activity is less broad-spectrum compared to a positive control (anti-microbial standard) and is highly dependent on the target microbe's cellular structure. Based on the provided data, the "Control (0.5 McFarland std) (200 mg)" is a positive control, which is an anti microbial solution is most likely a standard antibiotic or antifungal agent, rather than a solvent control. This conclusion is drawn because it consistently produces a significant zone of inhibition against all tested microbial species. Generally, the data also shows substantial antimicrobial activity (e.g., 27.00 mm for *Staphylococcus sp.* and 23.00 mm for *Pseudomonas sp.*), its purpose is clearly to serve as a benchmark for comparison, a role filled by a positive control. The table also presents the Vanillin-aniline Schiff base's antimicrobial activity (measured in mm) against different microbial species at varying concentrations (200 mg, 100 mg, and 50 mg). It was observed that the antimicrobial activity generally decreases as the concentration of the Schiff base decreases. However, the 0.5 McFarland standard was prepared and used as control. The (200 mg) of the standard consistently shows the highest zone of inhibition, suggesting that the compound is effective at higher concentrations. The bacteria strains, *Pseudomonas sp.*, *Bacillus sp.*, and *Staphylococcus sp.*, show significant inhibition at higher concentrations, but the effect weakens as the concentration is reduced. For the Fungal species (*Aspergillus sp.* showed no inhibition while *Candida sp.* displayed inhibition only at 200 mg hence no inhibition at lower concentrations, indicating lower sensitivity to the Schiff base. In addition, *Escherichia coli* and *Aspergillus sp.* show no inhibition at any tested concentration, suggesting resistance to the Schiff base. Furthermore, *Staphylococcus sp.* showed the highest inhibition in the control (27.00 ± 1.4 mm) and maintained relatively strong inhibition at 200 mg (17.50 ± 0.7 mm) and 100 mg (15.00 ± 1.4 mm) of the test compound, indicating it is the most sensitive among the tested microorganisms. On the other hand, *Escherichia coli* and *Aspergillus sp.* show complete resistance across all concentrations of the Schiff base. The results of the antimicrobial activity of the Vanillin-derived Schiff base suggest that the compound may have selective antimicrobial properties and is more effective against specific bacterial strains rather than fungi.

Table 7. Effect of different concentrations of vanillin-oxime on bacterial and fungal species.

Vanillin-Oxime	Unit	Control (200 mg)	200 mg	100 mg	50 mg
Escherichia coli	Mm	17.50 ± 0.7^{a}	17.50 ± 2.1^{b}	9.00 ± 1.4^{b}	0.00 ± 0.0^{a}
Pseudomonas sp.	Mm	20.00 ± 0.0^{b}	19.00 ± 1.4^{b}	10.00 ± 0.0^{b}	0.00 ± 0.0^{a}
Bacillus sp.	Mm	18.00 ± 1.4^{b}	18.00 ± 0.0^{b}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}
Staphylococcus sp.	Mm	29.00 ± 1.4^{b}	19.50 ± 2.1^{b}	11.50 ± 1.5^{b}	9.00 ± 1.4^{b}
Aspergillus sp.	Mm	17.00 ± 1.4^{b}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}
Candida sp.	Mm	19.00 ± 1.4^{b}	21.00 ± 1.4^{b}	11.00 ± 1.0^{b}	0.00 ± 0.0^{a}

Means with similar superscript down the group showed no significant difference ($P > 0.05$).
Key: 0 - 13 mm = Resistant, 14 - 16 = Intermediate, 17 and above = Susceptibility.

The results in Table 7 indicate that vanillin-oxime exhibits significant antimicrobial activity against several of the tested bacterial species, particularly Gram-positive bacteria like *Bacillus sp.* and *Staphylococcus sp.*, but also against the Gram-negative species, *E. coli* and *Pseudomonas sp.*. The compound also shows some activity against the fungus *Candida sp.*, while it is ineffective against *Aspergillus sp.*. This diverse range of activity can be attributed to the specific structural features of the vanillin-oxime molecule. The vanillin-oxime molecule contains a complex functional group, the oxime moiety (C = N-OH) which is a key player in its bioactivity. The terminal hydroxyl group (−OH) on the imine nitrogen is a crucial feature. The hydroxyl group can form hydrogen bonds with cellular components, potentially disrupting the cell membranes or inhibiting critical enzymes in susceptible microorganisms. This may explain the observed activity against a broader range of species, including some Gram-negative bacteria and yeast, unlike other derivatives that might lack this specific functional group. The aromatic ring in vanillin provides a stable, hydrophobic core. This can facilitate the compound's passage through the lipid bilayers of cell membranes, allowing it to reach and interact with intracellular targets. The efficacy of vanillin-oxime against Candida sp. is noteworthy when compared to the vanillin-azine and vanillin-hydrazone, which showed no activity against this fungus in the provided tables. This suggests that the oxime functional group is specifically effective against this particular fungal species. Conversely, vanillin-oxime's lack of activity against *Aspergillus sp.* highlights the specificity of its action, indicating that its molecular structure is not suited to disrupting the cellular architecture or metabolism of this mold. Generally, the table also presents the antimicrobial activity (in mm) of Vanillin-Oxime against various microorganisms at different concentrations (200 mg, 100 mg, and 50 mg). The general trend depicts that the antimicrobial activity is highest at 200 mg for most organisms and declines as the concentration decreases. Also, at 50 mg, most microorganisms showed no inhibition, indicating that this concentration is insufficient for antimicrobial action. Except for *Staphylococcus sp.,* the bacteria strains, *Escherichia coli, Pseudomonas sp.,* and *Bacillus sp.,* summarily showed no inhibition with lower concentrations. *Staphylococcus sp.* exhibited the strongest inhibition in the control (29.00 ± 1.4 mm), but its activity at 200 mg of the test compound was notably high (19.50 ± 2.1) and within the susceptible range. Furthermore, it must be stated that *Staphylococcus sp.* was the only organism that showed inhibition at all the concentrations with the highest concentration being in the susceptible range. As for the Fungal strains, *Aspergillus sp.* was completely resistant at all concentrations while *Candida sp.* showed notable inhibition at 200 mg (21.00 ± 1.4) and 100 mg but none at 50 mg. The Vanillin-Oxime exhibits antimicrobial activity, but its effectiveness depends on concentration, with significant inhibition at 200 mg and reduced or no inhibition at lower doses. *Staphylococcus sp.* is the most sensitive to the compound, while *Aspergillus sp.* is completely resistant. The trend suggests that Vanillin-Oxime may be more effective against bacteria than fungi, except for *Candida sp.*, which shows some sensitivity. This data highlights the potential of Vanillin-Oxime as an antimicrobial agent, particularly at higher

concentrations, but suggests that its efficacy varies depending on the microorganisms.

Table 8. Effect of different concentrations of Vanillin-hydrazone on bacterial and fungal species.

Vanillin-Hydazone	Unit	Control (200 mg) 0.5 McFarland standard	200 mg	100 mg	50 mg
Escherichia coli	Mm	16.50 ± 07[a]	0.00 ± 0.0[a]	0.00 ± 0.0[a]	0.00 ± 0.0[a]
Pseudomonas sp.	Mm	19.00 ± 1.4[b]	8.00 ± 0.0[b]	0.00 ± 0.0[a]	0.00 ± 0.0[a]
Bacillus sp.	Mm	17.50 ± 0.7[a]	14.50 ± 3.5[c]	10.50 ± 0.5[b]	8.00 ± 0.0[b]
Staphylococcus sp.	Mm	26.00 ± 1.4[c]	16.50 ± 0.0[c]	0.00 ± 0.0[a]	0.00 ± 0.0[a]
Aspergillus sp.	Mm	17.00 ± 1.4[a]	0.00 ± 0.0[a]	0.00 ± 0.0[a]	0.00 ± 0.0[a]
Candida sp.	Mm	21.00 ± 1.4[b]	0.00 ± 0.0[a]	0.00 ± 0.0[a]	0.00 ± 0.0[a]

Means with similar superscript down the group showed no significant difference ($P > 0.05$).
Key: 0 - 13 mm = Resistant, 14 - 16 = Intermediate, 17 and above = Susceptibility.

The results in Table 8 reveal that vanillin-hydrazone has a narrow antimicrobial spectrum. It exhibits activity primarily against Gram-positive bacteria like *Bacillus sp.* and, to a lesser extent, *Pseudomonas sp.*, but shows no effect on Gram-negative bacteria (*E. coli*) or the tested fungal species (*Aspergillus sp.* and *Candida sp.*). This selectivity is directly related to the compound's molecular structure. The vanillin-hydrazone molecule contains an aromatic ring, a methoxy group, a hydroxyl group, and a hydrazone moiety ($C = N\text{-}NH_2$). The presence of the hydrazone group, with its free primary amine, likely contributes to its antimicrobial properties, possibly by interacting with specific bacterial enzymes or disrupting cell membranes. The observed activity against Gram-positive bacteria, particularly *Bacillus sp.*, can be attributed to their less complex cell wall structure compared to Gram-negative bacteria. The outer membrane of Gram-negative bacteria like *E. coli* and *Pseudomonas sp.* acts as a barrier, preventing the compound from reaching its intracellular targets. The lack of activity against the fungal species suggests that the vanillin-hydrazone structure is not effective at disrupting fungal cell walls or inhibiting key fungal metabolic pathways. Comparing these results to those of other vanillin derivatives (like the oxime or Schiff base) would provide further insight into how minor structural changes can drastically alter antimicrobial efficacy. For instance, the vanillin-azine, with its dimeric structure and two imine groups, often shows greater activity against some of the same bacterial species, demonstrating that the presence and arrangement of functional groups are critical for biological activity. The data also shows that the vanillin-hydrazone compound, even at the highest concentration of 200 mg, is significantly less effective than the positive control which an antimicrobial substance. For most species, including *E. coli*, *Aspergillus sp.*, and *Candida sp.*, the compound shows no antimicrobial activity at all (0.00 mm zone of inhibition). It only demonstrates some effectiveness against

Pseudomonas sp. and *Bacillus sp.*, and even then, its activity is considerably weaker than the control. For example, the control inhibits *Bacillus sp.* with a 17.50 mm zone, while the vanillin-hydrazone at 200 mg only achieves a 14.50 mm zone, and its activity drops off at lower concentrations. Furthermore, the antimicrobial activity (in mm) of Vanillin-Hydrazone against various microorganisms at different concentrations (200 mg, 100 mg, and 50 mg) Vanillin-Hydrazone. The general observation for the test of vanillin-derived hydrazone on the test organisms was the inactivity of *E. Coli* and the two fungi, *Aspergillus sp.* and *candida sp.*, even at the highest tested concentration of 200 mg indicating weak antimicrobial potential for this compound. Bacteria (*Pseudomonas sp., Bacillus sp., Staphylococcus sp.*) exhibit moderate inhibition at 200 mg Vanillin-Hydrazone but show little to no inhibition at lower concentrations. *Pseudomonas sp.* shows weak inhibition at 200 mg (8.00 ± 0 mm) and no activity at lower concentrations while *Bacillus sp.* shows inhibition at 200 mg and 100 mg but loses effectiveness at 50 mg. In addition, *Staphylococcus sp.* exhibits the highest inhibition at 200 mg but loses effectiveness at lower concentrations. The results also show that vanillin-hydrazone exhibits weak overall antimicrobial activity, with no inhibition at lower concentrations (100 mg and 50 mg) for most microorganisms. The compound is ineffective against fungi (*Aspergillus sp.* and *Candida sp.*). *Bacillus sp.* and *Staphylococcus sp.* show intermediate inhibition at higher concentrations. The drastic drop-in activity at lower concentrations suggests that Vanillin-Hydrazone is not an effective antimicrobial agent unless used at very high doses. This data suggests that Vanillin-Hydrazone has limited antimicrobial potential, especially compared to Vanillin-Schiff base and Vanillin-Oxime, which showed more consistent activity.

Table 9. Effect of different concentrations of vanillin-azine on bacterial and fungal species.

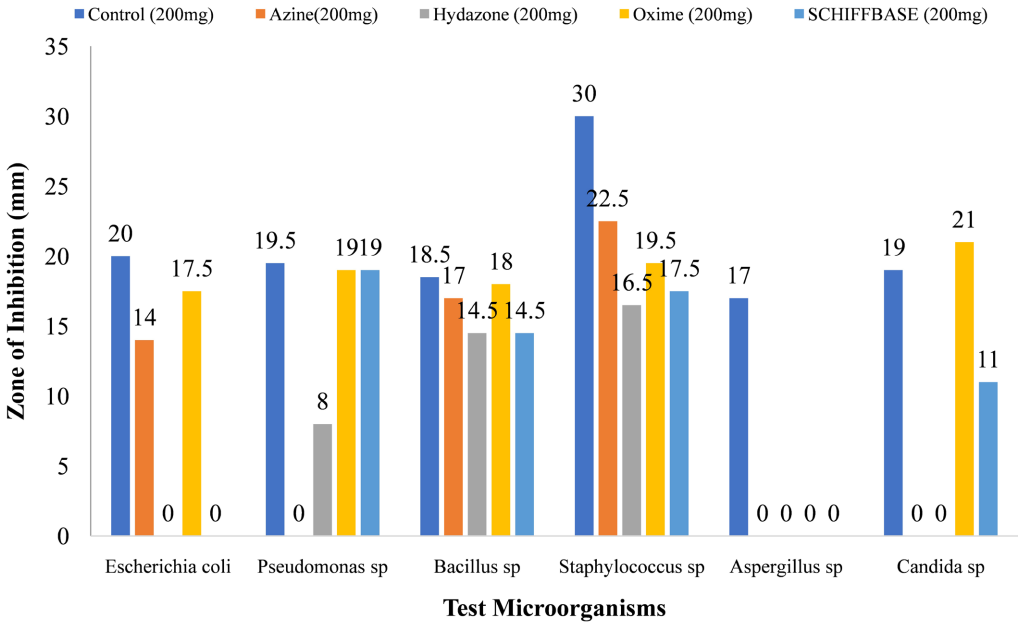
Vanillin-Azine	Unit	Control (200 mg) 0.5 McFarland standard	200 mg	100 mg	50 mg
Escherichia coli	Mm	20.00 ± 2.8^{a}	14.00 ± 1.4^{b}	0.00 ± 0.0a	0.00 ± 0.0^{a}
Pseudomonas sp.	Mm	19.50 ± 0.7^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0a	0.00 ± 0.0^{a}
Bacillus sp.	Mm	18.50 ± 0.7^{a}	17.00 ± 0.0^{b}	8.00 ± 0.0^{b}	0.00 ± 0.0^{a}
Staphylococcus sp.	Mm	30.00 ± 0.0^{b}	22.50 ± 3.5^{c}	18.50 ± 2.1^{c}	11.0 ± 1.4^{b}
Aspergillus sp.	Mm	17.00 ± 1.4^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0a	0.00 ± 0.0^{a}
Candida sp.	Mm	19.00 ± 1.4^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0a	0.00 ± 0.0^{a}

Means with similar superscript down the group showed no significant difference ($P > 0.05$).
Key: 0 - 13 mm = Resistant, 14 - 16= Intermediate, 17 and above = Susceptibility.

The results in Table 9 indicate that vanillin-azine possesses varied antimicrobial activity against the tested microorganisms. Its effectiveness is most pronounced against *Staphylococcus sp.* and *Bacillus sp.*, showing a clear dose-dependent inhibition. However, it shows no activity against *Pseudomonas sp.*, *Aspergillus sp.*, and

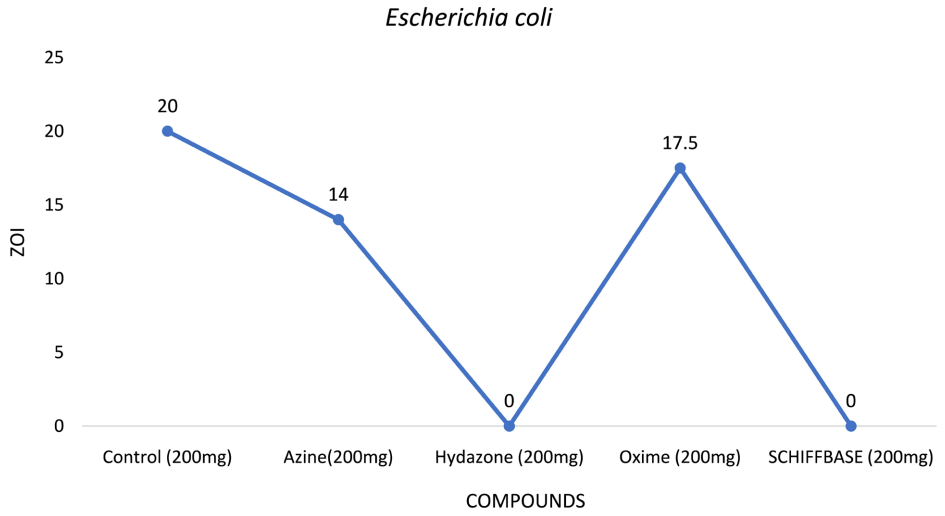
Candida sp., and limited activity against *E. coli*. These findings, when combined with the data for the other derivatives, reveal a clear structure-activity relationship. The azine derivative, with its unique (C = N-N = C) bridge, appears to be the most potent antimicrobial among the tested vanillin compounds, particularly against Gram-positive bacteria like *Staphylococcus sp.* and *Bacillus sp.*. The extended conjugated system and the presence of the two imine groups and a central N-N bond likely enhance its ability to interact with and disrupt bacterial cellular components. This is a significant improvement over the other derivatives. For instance, the vanillin-hydrazine (a precursor to vanillin-azine), which contains a single vanillin unit attached to a hydrazine, showed lower or no activity against most species in a previous table (e.g., 0.00 mm inhibition for *E. coli*). This suggests that the formation of the full azine structure, with two vanillin moieties, is critical for achieving this enhanced antimicrobial effect. Comparatively, Vanillin-aniline Schiff base (a simple imine with a single C = N bond) typically shows limited antimicrobial activity because its aromatic structure does not provide sufficient functional groups to interact with a broad range of microbial targets. Vanillin oxime contains a single imine group with a terminal–OH group. While this hydroxyl group can contribute to some activity through hydrogen bonding, its overall efficacy is often limited compared to the azine's more complex and extended structure. The higher efficacy of the vanillin-azine can be attributed to the combined effect of two imine groups and the presence of two electron-rich vanillin moieties. the specific molecular architecture of the vanillin-azine, characterized by its dimeric nature and two imine functionalities, is directly responsible for its enhanced antimicrobial properties, particularly against Gram-positive bacteria. This indicates that modifying the vanillin backbone through complex condensation reactions can effectively create compounds with potent and targeted antimicrobial activities. Generally, the data presents the antimicrobial activity (in mm) of Vanillin-Azine against various microorganisms at different concentrations (200 mg, 100 mg, and 50 mg). The trend shows some microorganisms inhibition at 200 mg, but activity is lost completely at 100 mg and 50 mg for most microorganisms. Bacteria (*E. coli, Bacillus sp., Staphylococcus sp.*) show varying degrees of inhibition, with *Staphylococcus sp.* being the most sensitive. However, Fungi (*Aspergillus sp., Candida sp.*) show no inhibition at any tested concentration, indicating complete resistance. *Escherichia coli* Shows intermediate inhibition at 200 mg (14.00 ± 1.4 mm) but is inactive at lower concentrations. *Pseudomonas sp.* is resistant at all concentrations of the test compound, hence, no inhibition at 200 mg and lower. In addition, *Bacillus sp.* shows inhibition at 200 mg and 100 mg, but loses activity at 50 mg. Furthermore, *Staphylococcus sp.* being the most sensitive organism, inhibited at 200 mg (22.50 ± 3.5 mm), 100 mg (18.50 ± 2.1 mm), and 50 mg (11.00 ± 1.4 mm), thus showing that the compound is susceptible at 200 mg and 100 mg against it (**Figure 11**). The result suggests that Vanillin-azine could have potential antibacterial applications, but only at higher concentrations. It is noteworthy to mention that on comparison of all the test compounds, the following trend was observed showing

from strongest to weakest inhibition: Vanillin-Schiff Base → Vanillin-Oxime → Vanillin-Azine → Vanillin-Hydrazone. The Vanillin-Schiff Base is the most effective and broad-spectrum (best against *Pseudomonas sp.*, *Staphylococcus sp.*, and *Bacillus sp.*). Vanillin-Oxime is moderately effective, showing some inhibition against *E. coli* and *Candida sp.* Vanillin-azine is only strongly effective against *Staphylococcus sp.* but weak against others. Vanillin-Hydrazone is the weakest, with almost no inhibition at lower concentrations.



Key: 0 - 13 mm = Resistant, 14 - 16 = Intermediate, 17 and above = Susceptibility.

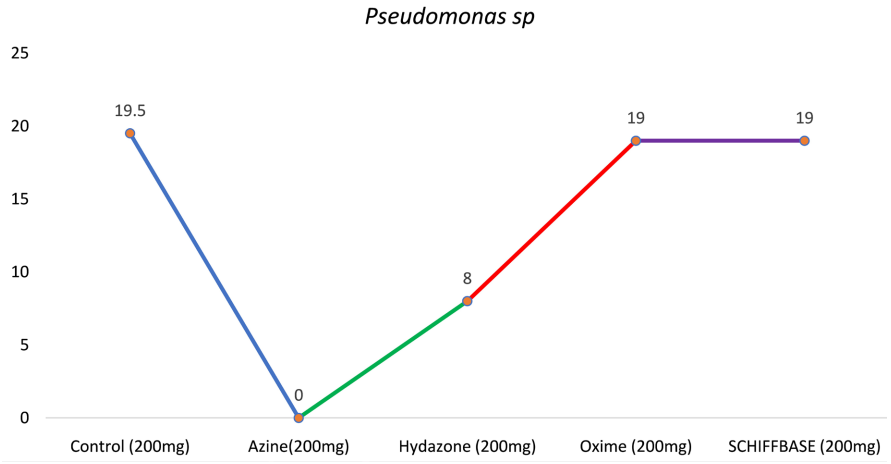
Figure 11. Effect of the different compounds at 200 mg concentration on the Test Microorganisms.



Key: 0 - 13 mm = Resistant, 14 - 16 = Intermediate, 17 and above = Susceptibility.

Figure 12. Effect of the difference compounds at 200 mg concentration on *Escherichia coli*.

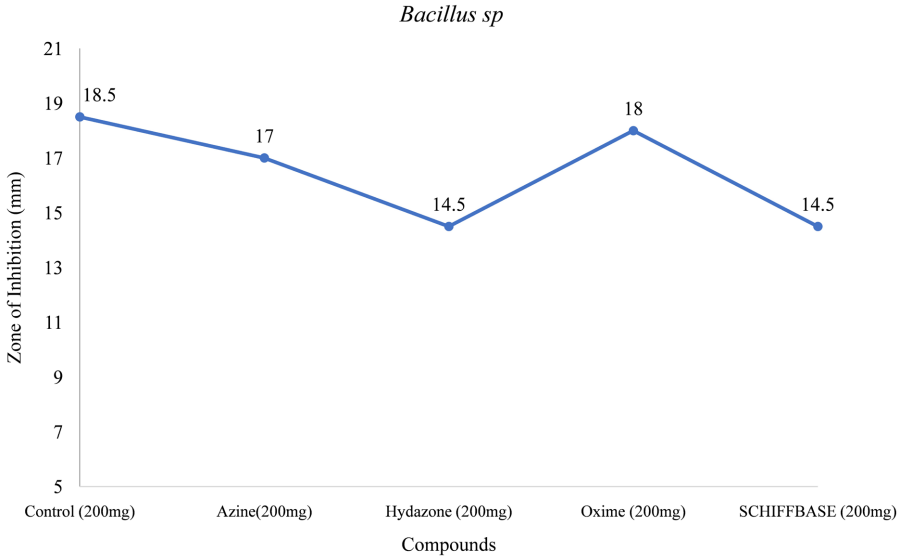
For an organism-specific trend concerning which compound has the most and least effect at the highest test concentration of 200 mg (Figure 12). The control drug was observed to be the most effective against all organisms. For the vanillin-derived compounds, Oxime (17.5 mm) had the most effect against *E. coli* while hydrazone and Schiff base had the least effect (0 mm).



Key: 0 - 13 mm = Resistant, 14 - 16 = intermediate, 17 and above = Susceptibility.

Figure 13. Effect of the different compounds at 200 mg concentration on *Pseudomonas species*.

For *Pseudomonas sp.,* the oxime and Schiff base had the highest inhibition (19 mm) while the azine showed no inhibition (Figure 13).

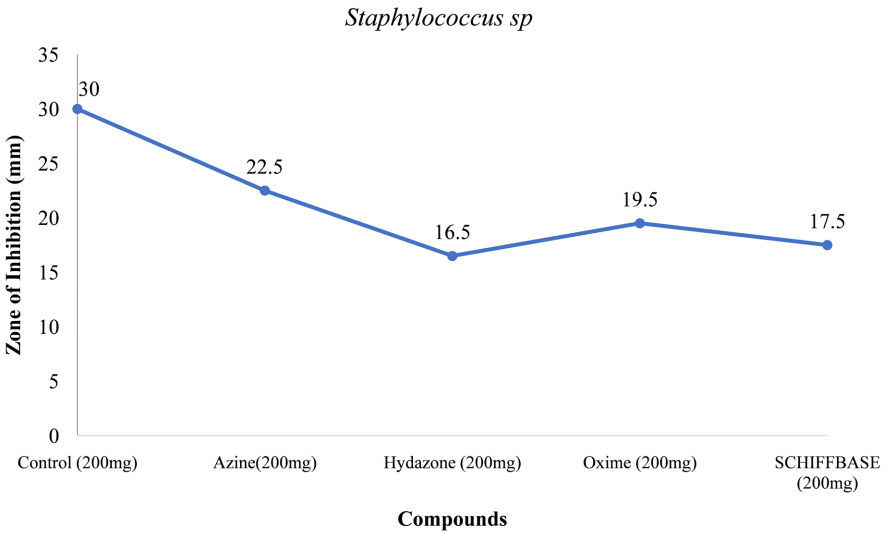


Key: 0 - 13 mm = Resistant, 14 - 16 = intermediate, 17 and above = Susceptibility.

Figure 14. Effect of the different compounds at 200 mg concentration on *Bacillus species*.

Oxime had the highest effect (18 mm) against *Bacillus sp.* while the hydrazone

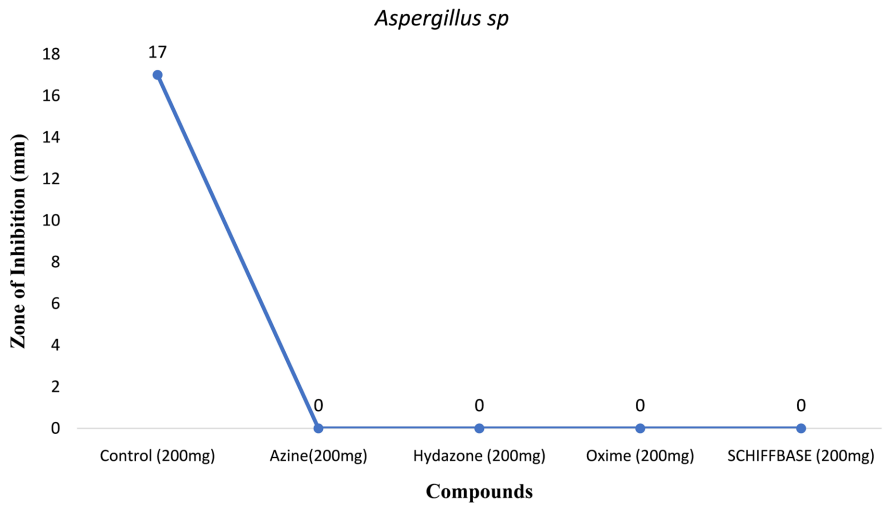
and Schiff base had the least effect (18.5 mm). All the compounds were active against this bacterium (Figure 14).



Key: 0 - 13 mm = Resistant, 14 - 16 = intermediate, 17 and above = Susceptibility.

Figure 15. Effect of the different compounds at 200 mg concentration on *Staphylococcus species*.

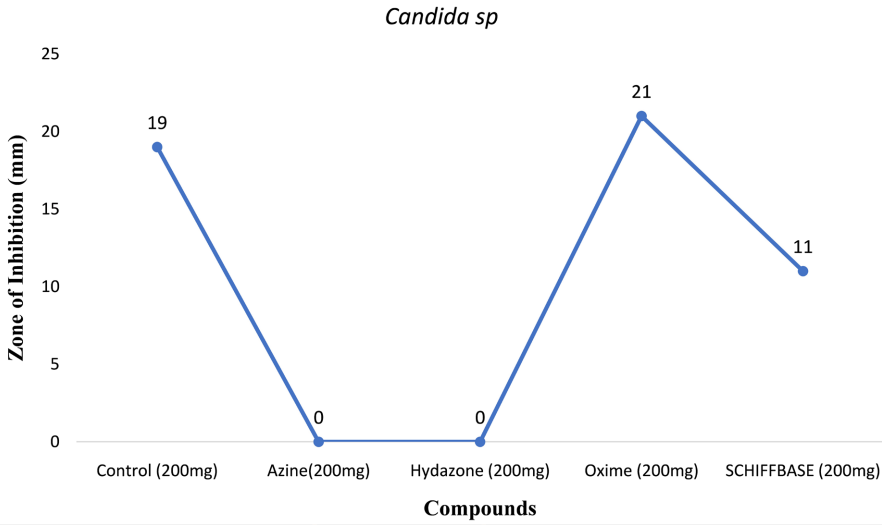
The azine had the greatest inhibition (22.5 mm) for *Staphylococcus sp.* while the hydrazone had the least effect (16.5 mm). All the compounds showed inhibition against this organism (Figure 15).



Key: 0 - 13 mm = Resistant, 14 - 16 = intermediate, 17 and above = Susceptibility.

Figure 16. Effect of the Different compounds at 200 mg Concentration on *Aspergillus species*.

All vanillin-derived compounds were observed to be inactive against the fungus, *Aspergillus sp.* (Figure 16).



Key: 0 - 13 mm = Resistant, 14 - 16 = intermediate, 17 and above = Susceptibility.

Figure 17. Effect of the different compounds at 200 mg concentration on *Candida species*.

Candida sp. was most susceptible to the vanillin-oxime (21 mm) and showed no inhibition for the azine and hydrazine (**Figure 17**).

4. Conclusion and Recommendation

4.1. Conclusion

The results achieved from this research show that Vanillin-derived Schiff base, Oxime, Hydrazone, and Azine can be synthesized at relatively high yields using an eco-friendly method (*i.e.* by condensation of the use of ethanol which is a non-toxic substance) and solvent rather than using methods that can be harmful to humans and the environment. The Vanillin-Schiff Base was the most effective and broad-spectrum (best against *Pseudomonas sp.*, *Staphylococcus sp.*, and *Bacillus sp.*), Vanillin-Oxime was moderately effective, showing some inhibition against *E. coli* and *Candida sp.*, Vanillin-azine was only strongly effective against *Staphylococcus sp.,* but weak against others, while the Vanillin-Hydrazone was the weakest, with almost no inhibition at lower concentrations. Furthermore, the findings of this work have proven that these compounds have antimicrobial properties against the tested strains of bacteria and fungi to varying extents, hence expressing their possible use for drug discovery and development. Vanillin oxime and vanillin hydrazine show high percentage yields of 88.1% and 85.7%, respectively. This indicates that their synthesis reactions were highly efficient, with a large portion of the reactants successfully converted into the desired product. High yields are generally desirable in chemical synthesis as they minimize waste and maximize productivity.

4.2. Recommendations

From the observations and results achieved from this research, the following recommendations must be mentioned:

1. Scientists should concentrate on developing more eco-friendly methods and

solvents for the synthesis of these compounds which will give very high yields.
2. An investigation of the effect of metal ion complexation with these compounds should be done to check if the antimicrobial activity will be enhanced.
3. The susceptibility tests should be carried out on more bacterial and fungal strains to check their efficacy against order disease-causing organisms.
4. The pharmacological importance of vanillin-derived imines is not limited to their antibacterial and antifungal properties. These compounds may also have other pharmacological properties like antitubercular, antitumor, antiviral activities, etc. Hence, the four compounds should be explored.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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