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Biological Profiling of Two New Vanillyl Schiff Bases

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Abstract

Background of study: Schiff bases (imines) are characterized by a carbon-nitrogen double bond. Their versatility and documented biological activities contribute to their significance in organic chemistry. **Objectives:** The harmful effects of free-radicals to the human body and prevalence of microbial resistance to current anti-infective drugs led to the search for new molecules with better activities for these conditions. This search for novel pharmaceutically active compounds led to Schiff base synthesis using vanillin and two alkyl amines. Methodology: Vanillin was reacted separately with these amines leading to the imines. The antioxidant activity (IC₅₀) of these compounds was evaluated using the DPPH assay test. A comparison of the obtained antioxidant activities was done.. The agar-diffusion method was adopted for screening the compounds against Staphylococcus aureus, Escherichia coli and Candida albicans for antibacterial and antifungal potentials respectively. Results: The IR spectral technique has revealed that both synthesized imines are new. Consequently, their nomenclatures are vanillyl hexyl imine (Iyadimine A) and vanillyl heptyl imine (Iyadimine B) respectively. Vanillin demonstrated a moderate antioxidant activity of 0.52 µg/mL while vitamin C was significant at 0.48 µg/mL. However, both Iyadimine A and Iyadimine B were marginally active at 1.93 and 1.03 µg/mL respectively with Iyadimine **B** being slightly more antioxidant. The antibacterial and antifungal activities elicited by both imines showed concentration-dependency. Furthermore, Jyadimine A was more antibacterial against S. aureus than E. coli while the converse was observed with Iyadimine B. However, both suppressed C. albicans. Conclusion: The two new imines demonstrated marginal antioxidant activities and good antimicrobial activities. Hence, the two compounds are promising lead templates in the search for newer and more efficacious biological agents especially in synergistic antimicrobial co-administration and formulation studies in drug development.

Keywords: Schiff base, Vanillyl hexyl imine, Vanillyl heptyl imine, Antioxidant, Antimicrobial

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1. Introduction

Vanillin is an aromatic aldehyde. It is the chief component of vanilla bean extract and widely used as a flavoring agent in foods, cosmetics and fragrances (Patel *et al.*, 2007). Its molecule features a benzene ring substituted at the 3-position with a methoxy group (-OCH₃) and at the 4-position with a hydroxyl group (-OH) alongside an aldehyde group (-HC=O) at the 1-position of the ring. The combination of these functional groups is responsible for its distinct flavor. The phenolic capacity of vanillin is due to the presence of the hydroxyl group which contributes to its chemical reactivity hence the antioxidant activity.

The IUPAC nomenclature of vanillin is 4-hydroxy-3-methoxy benzaldehyde (NCBI, 2024). Vanillin has been reported to be a potent free-radical scavenger of ROS as observed in multiple antioxidant assays such as ORAC (Oxygen Radical Absorbance Capacity), ABTS+ (2, 22-azinobis (3-ethyl benzothiazoline-6-sulfonic acid), oxidative haemolysis inhibition where it self-dimerizes contributing to high reaction stoichiometry (Tai *et al.*, 2011). This also has been reflected in the synthesis of a novel Schiff base, vanillyl butyl imine (Walatimine) using the rapid DPPH bench-top assay (Oladimeji *et al.*, 2023). Vanillin exhibits anti-inflammatory potential by inhibiting nitric oxide in the lipo-polysaccharide activated (LPA) RAW264.7 macrophages where the suppression of inducible nitric oxide synthase (iNOS), mRNA in LPA macrophages is clearly indicative of anti-inflammatory activity (Lim and Murtijadi, 2008). Furthermore, vanillin acts as a neuro-protective agent in Huntington's Disease (HD) and ischemia (Gupta and Sharma, 2014) significantly attenuating motor coordination, learning-memory, biochemical impairments (Gupta and Sharma, 2014), 3-nitropropionic acid (3-NPA) induced HD in rats and alleviation of metabolic complications linked to Fe²⁺-induced brain tissue damage (Salau *et al.*, 2020).

The anti-quorum sensing activity of vanillin has been reported where it inhibited short-chain homoserine lactones and long-chain acyl-homoserine lactones in *Aeromonas hydrophila* (Ponnusamy *et al.*, 2009) and *invitro* silico docking studies involving *Pseudomonas aeruginosa* where vanillin bound to the active site of PQSR (Pqs-binding response regulator) thereby inhibiting pqs expression associated with pyocyanin (quorum sensing molecule) (Mok *et al.*, 2011). Furthermore, its anti-mutagenic properties have also been documented (Tanai *et al.*, 1992; Keshava *et al.*, 1998; Gustafon *et al.*, 2000) where chromosomal aberrations were inhibited and DNA repair and recombination mechanisms enhanced. In addition, this compound has shown anticarcinogenicity where the progression of cancerous cells were down-graded (Kapoor *et al.*, 2013; Bezerra *et al.*, 2016). Schiff bases are compounds containing the azomethine group (-HC=N-) which is a nitrogen analogue of a ketone or aldehyde where the -C=O group has been replaced (Raczuk *et al.*, 2022). They are usually synthesized from the condensation of primary amines with active carbonyl groups by nucleophilic addition forming a hemiaminal (carbinolamine) and followed by dehydration to generate an imine (ketimine or aldimine or hydrazone or azomethine) (Umar *et al.*, 2017; Boulechfar *et al.*, 2023).

These bases are most extensively used organic compounds in pigments, dyes, catalysts, intermediates in organic syntheses and polymer stabilizers (Da Silva *et al.*, 2010). These bases exhibit a wide range of biological activities including but not limited to antifungal, antibacterial, anti-malarial, anti-proliferative, anti-inflammatory, antiviral, antipyretic properties and neurological disorders, such as found in Alzheimer's disease (Rathelot *et al.*, 1995; Pontiki *et al.*, 2008; Kumar *et al.*, 2023). Schiff bases have also found applications in nanotechnology for targeted drug delivery, controlled release of therapeutic agents, improving treatment outcomes while minimizing systemic toxicity (Kumar, 2010). In the light of the foregoing, this present research was conceived with the aim of synthesizing Schiff bases using vanillin and two different aliphatic primary amines (hexyl amine and heptyl amine) separately. The synthesized Schiff bases were evaluated for antioxidant activity (IC₅₀) using the bench-top DPPH reagent assay and the agar-in-hole diffusion method was adopted for determining their anti-bacterial and antifungal sensitivity properties. Comparison of results obtained was done for vanillin, synthesized Schiff bases and positive controls such as Vitamin C (antioxidant drug), chloramphenicol (antibiotic) and fluconazole (antifungal drug) if increase in carbon chain in the synthesized Schiff bases could influence any improvements in their targeted biological activities.

1.1. Reagents/Chemicals

Vanillin and DPPH (2, 2-diphenyl-1-picryl hydrazyl hydrate) both were obtained Sigma Aldrich Chemicals, Germany. Chloramphenicol, fluconazole and Vitamin C tablets were sourced within country from Gemini Pharmaceuticals, Nigeria. The following reagents namely, acetic acid (glacial), acetone, ethanol, ethyl acetate, hydrochloric acid, hexyl amine, heptyl amine, methanol, n-butanol, n-hexane, petroleum-ether and toluene were purchased as AnaLAR Grade Chemicals from British Drug House Chemicals Limited, Poole, England.

1.2. Solubility/Dissolution Tests for Vanillin (with Modifications)

Vanillin (0.04 g) was added to 4 mL each of the following solvents namely, dilute HCl, distilled water, ethyl acetate, ethanol, petroleum ether, n-hexane, n-butanol and methanol separately and observation was made for complete dissolution (solubility) or otherwise.

1.3. Determination of Melting Point

Vanillin (0.04 g) was filled to a quarter of the length of a micro-capillary tube and the melting point determined (B.P., 1993) using an Electro-thermal Melting Point apparatus (Electro-thermal Engineering Limited, England).

1.4. Synthesis of Vanillyl Hexyl Imine

This Schiff base was synthesized as described by (Azam *et al.*, 2007) with slight modifications. Hexyl amine (3 mL) was added to 30 mL of glacial acetic acid to give a mixture. To this mixture was a solution of vanillin (3 g in 10 mL glacial acetic acid) added drop wise whilst stirring and the reaction mixture (green colour) was heated under reflux (glass chamber) for 8 h. At the completion of reaction, the yellow product mixture obtained was refrigerated at 0.4 °C to allow possibly for crystallization to occur. In the absence of the formation of crystals, the mixture was further heated for thirty (30) minutes and a pale yellow oily compound resulted (Figure 1).



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1.5. Synthesis of Vanillyl Heptyl Imine

Similarly, this Schiff base was synthesized as described by (Azam *et al.*, 2007) with slight modifications. Heptyl amine (3 mL) was added to 30 mL of glacial acetic acid to give a mixture. To this mixture was a solution of vanillin (3 g in 10 mL glacial acetic acid) added drop wise whilst stirring and the reaction mixture (green colour) was heated under reflux (glass chamber) for 8 h. At the completion of reaction, the yellow product mixture obtained was refrigerated at 0.4 °C to allow possibly for crystallization to occur. In the absence of the formation of crystals, the mixture was further heated for thirty (30) minutes and a deep yellow oily compound resulted (Figure 2).



1.6. Determination of Optical Rotation and Refractive Indices of Vanillin and the Synthesized Schiff bases

These experiments were done using a polarimeter (ADP-220, Bellingham Stanley, England) and a refractometer (WAY-15, Abbe, England) respectively with slight modifications. Each sample (0.03 g) was dissolved in methanol (10 mL). The tube of the polarimeter was filled with distilled water and the machine subsequently zeroed. The tube was then refilled with 5 mL of sample and the optical rotation was measured at the wavelength (λ) of sodium D line (589.3 nm) at 20.5 °C. Similarly, the refractive index of sample was obtained on the refractometer at the wavelength (λ) of sodium D line (589.3 nm) at 20.5 °C (Olaniyi and Ogungbamila, 1991; Olaniyi, 2000).

1.7. Thin-Layer Chromatography of Vanillin and Synthesized Schiff Bases

Vanillin (0.03 g) dissolved in methanol (2 mL) and a little portion each of the oily bases were applied on a 20 cm x 10 cm silica gel analytical plate (Merck, Germany) and then developed in a toluene : acetone : water (10:20:1) mixture in a chromatographic tank till optimal separation was observed (Oladimeji *et al.*, 2022).

The retardation factor (R_F) was then computed thus:

 $R_{F} = \frac{Distance moved by spot}{Distance moved by solvent front}$

1.8. Infra-Red Spectroscopy (FTIR) of Vanillin and Synthesized Schiff Bases

Vanillin (0.03 g) and a little portion each of the oily bases were analyzed for IR characteristics using the FTIR 84005 Spectrophotometer (Shimadzu, Japan).

1.9. Ultra-Violet/Visible Spectroscopy of Vanillin and Synthesized Schiff Bases

Vanillin (0.03 g) and a little portion each of the oily bases were analyzed for UV/VS absorption characteristics using the Jenway 6405 UV/VS Spectrophotometer.

2. Evaluation of Antioxidant Activity

2.1. Spectrophotometric Determination of Antioxidant Activity Using DPPH Reagent

Substances which are capable of donating electrons or hydrogen atoms can convert the purple-coloured DPPH radical (2, 2-diphenyl-1-picrylhydrazyl hydrate) to its yellow-coloured non-radical form; 1, 1-diphenyl-2-picryl hydrazine (Guangrong *et al.*, 2008; Nagalapur and Paramjyothi, 2010). This reaction can be monitored by absorption spectrophotometry.

2.2. Preparation of Calibration Curve for DPPH Reagent

This experiment was carried out as described in both (Oladimeji *et al.*, 2016; Oladimeji *et al.*, 2020) with some modifications. DPPH (4 mg) was weighed and dissolved in methanol (100 mL) to produce the stock solution (0.004% w/v). Serial dilutions of the stock solution were then done to obtain the following concentrations *viz*, 0.0004, 0.0008, 0.0012, 0.0016, 0.0020, 0.0024, 0.0028, 0.0032 and 0.0036% w/v. The absorbance of each of the sample was taken at λ_m 517 nm using the Ultra-Violet Spectrophotometer (Jenway 6405, USA). This machine was zeroed after an absorbance had been taken with a solution of methanol without DPPH which served as the blank.

2.3. Determination of the Antioxidant Activity of Vanillin, Synthesized Schiff Bases and Vitamin C

2 mg of sample was mixed with 50 mL of methanol. Serial dilutions were carried out to obtain the following concentrations; 0.0004 mg mL⁻¹, 0.0008 mg mL⁻¹, 0.0012 mg mL⁻¹, 0.0016 mg mL⁻¹ and 0.0020 mg mL⁻¹ using methanol. 5 mL of each concentration was incubated with 5 mL of 0.004% w/v methanolic DPPH solution for optimal analytical accuracy. After an incubation period of 30 minutes in the dark at room temperature (25 ± 2 °C), observation was made for a change in the colour of the mixture from purple to yellow. The absorbance of each of the samples was then taken at λ_m 517 nm. The Radical Scavenging Activity (RSA%) or Percentage Inhibition (PI%) of free radical DPPH was thus calculated:

 $RSA\%(PI\%) = [(A_{blank} - A_{sample})/A_{blank}] \times 100$

 A_{blank} is the absorbance of the control reaction (DPPH solution without the test sample and A_{sample} is the absorbance of DPPH incubated with the sample. Vanillin/synthesized Schiff base/Vitamin C concentration providing 50% inhibition (IC₅₀) was calculated from a graph of inhibition percentage against the concentration of the vanillin/synthesized Schiff base/Vitamin C (Lebeau *et al.*, 2000; Leitao *et al.*, 2002; Oladimeji *et al.*, 2016) Vitamin C was used as a standard antioxidant drug.

2.4. Antimicrobial Tests

The micro-organisms used in this study, namely; *Staphylococcus aureus* (NCTC 4532), *Escherichia coli* (NCTC 1065) and *Candida albicans* (NCYC 2436) were clinically isolated from specimens of diarrheal stool, abscesses, osteomyelitis, urine, wounds and vaginal swabs obtained from the Medical Laboratory, University of Uyo Health Centre, Uyo. The clinical isolates were collected in sterile bottles, identified and typed by convectional biochemical tests (Gibson and Khoury, 1986; Murray *et al.*, 1995) and then refrigerated at -5 °C at the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy prior to use. The hole-in-agar diffusion method was used with strict adherence to standard procedures for bacteria and fungi respectively. The inoculums of each micro-organism were introduced into separately labeled each petri-dishes (Pyrex, England). Cylindrical plugs were carefully removed from the agar plates by means of sterile cork borers (Pyrex, England) to produce wells with diameter of approximately 6.00 mm. The wells were equidistant from each other and the edge of the plate (Washington, 1995; N.C.C.L.S., 2003). Concentrations of 30 mg mL-¹ of vanillin, 15 mg mL-¹ and 30 mg mL-¹ of synthesized Schiff bases were introduced into the wells. Also, different concentrations of 5 mg mL-¹ chloramphenicol and 1mg mL-¹ of fluconazole both (Gemini Pharmaceuticals, Nigeria) and 50%

methanol were introduced into separate wells as positive and negative controls respectively (Washington, 1995; Oladimeji and Igboasoiyi, 2014). The experiments were carried out in triplicates. The plates were left at room temperature for 2 h to allow for diffusion. The plates were then incubated at 37 ± 2 °C for 24 h. The zones of inhibition were afterwards then measured in millimeters (mm).

3. Results

Blank Absorbance of 0.004 %w/v DPPH reagent: (0.998)

Table 1: Preparation of Calibration Curve for DPPH Reagent at λ_{\max} 517 nm				
Concentration (% w/v)	Average Absorbance (±0.004)			
0.004	0.072			
0.008	0.160			
0.012	0.240			
0.016	0.299			
0.020	0.386			
0.024	0.444			
0.028	0.556			
0.032	0.632			
0.036	0.691			



Vanillin: $C_8 H_8 O_3$; mol. wt. (152.14 g/mol); white crystalline solid; m.pt. (82-84 °C); $[n]_D^{20}$ (1.5774); $[\alpha]_D^{20}$ (0); λ_{max} (248 nm); R_F (0.67); FTIR (cm⁻¹):1078 (-C-O-C), 1590 (-Ar-C=C), 1642 (-C=O), 2943 (-CH stretching in - CH=O), 2968 (-CH bending mode on the aromatic ring) and 3639 (-Ar-OH).

Vanillyl Hexyl Imine (1-iminohexyl-3-methoxy-4-hydroxy benzene) (lyadimine A): $C_{14} H_{21} NO_2$; mol. wt. (235.07 g/mol); pale yellow oil; $[n]_D^{20}$ (1.5783); $[\alpha]_D^{20}$ (0), λ_{max} (344 nm); R_F (0.83); FTIR (cm⁻¹): 764 and 879 (finger

print region, alkyl bending mode), 1103 (-C-O-C), 1596 (Ar-C=C), 1695 (-HC=N, azomethine), 2977 (-CH bending mode on the aromatic ring) and 3481 (-Ar-OH).

Vanillyl heptyl imine (1-iminoheptyl-3-methoxy-4-hydroxy benzene) (lyadimine B): $C_{15} H_{23} N O_2$; mol. wt. (249.05 g/mol); deep yellow oil ; $[n]_D^{20}$ (1.5814); $[\alpha]_D^{20}$ (0); λ_{max} (401 nm); R_F (0.87); FTIR (cm⁻¹): 801 and 897 (finger print region, alkyl bending mode), 1125 (-C-O-C), 1600 (-Ar-C=C), 1695 (-HC=N, azomethine), 2982 (-CH bending mode on the aromatic ring) and 3482 (-Ar-OH).

4. Discussion

4.1. Spectroscopic/Spectral Analyses

Substances or reagents employed in chemical reactions (synthesis or derivatizations or assays) such as were used in this research must necessarily be put though some monographic evaluations for the purposes of establishing their identity, purity, chemical stability, molecular integrity and suitability for use prior to the commencement of the series of different experiments. Consequent upon this premise vanillin (a pharmaceutical or nutriceutical) which is a white crystalline compound with a sweet vanilla (ice cream) smell and balsamic fragrance was subjected to these determinations. This substance was visibly observed to be soluble in ethanol, ethyl acetate, n-butanol, n-heaxane, methanol and heated water. Conversely, it was not soluble in petroleum ether, dilute hydrochloric acid and dilute sulphuric acid. Furthermore, the determined melting point and refractive index values are consistent with those in literature as previously reported and documented in earlier studies (Oladimeji *et al.*, 2022; Oladimeji *et al.*, 2023). The observed UV absorption characteristic of vanillin at λ_{max} (248 nm) in this study connotes the presence of electron clouds over -Ar-C=C, -OH, -OCH₃ and -HC=O chromophores respectively.

The retardation factor R_F (0.67) indicates that it is somewhat moderately polar and expectedly likewise retarded on the silica plate. In addition, the different peaks in IR spectrum of vanillin as highlighted above are consistent with those obtained in (Oladimeji *et al.*, 2022; Oladimeji *et al.*, 2023) and in literature. The Schiff base condensation reaction between vanillin and hexyl amine resulted in a pale yellow oil which has been identified to be vanillyl hexyl imine by a coupling of physico-chemical values with the FTIR spectral technique. Its IUPAC nomenclature is 1-iminohexyl-3-methoxy-4-hydroxy benzene. It has a faint savouring fragrance. Data-based library searches of organic compounds were carried out. Hence, it can be infer that this imine is novel and consequently denoted by the trivial name of Iyadimine A. It is a Schiff base which belongs to the class of compounds known as aldimines or ketimines. Simply these compounds are obtained by the *in-situ* removal of water through condensation reactions between amines and carbonyl group containing moieties such as ketones or aldehydes or carboxylic acids in the presence of an acid or base and under heat.

The observed UV absorption at λ_{max} (344 nm) which is relatively higher than that shown by vanillin conveys information about the presence of electrons de-localized over -Ar-C=C, -OCH₃, -HC=N (imine, azomethine) and -Ar-OH chemical species. The retardation factor R_F (0.83) is indicative of this base being comparably non-polar because of the attached hexyl group (-CH₂)₅CH₃ thus making it more lipophillic, hence weakly and poorly retarded on an chemically expressive hydrophobic silica gel plate. The IR spectral matrix of lyadimine A reflects absorption peaks at 764, 879, 1103, 1596, 1695, 2977 and 3481cm⁻¹ which are characteristically diagnostic of alkyl bending modes in the finger print region, -C-O-C (ether linkage), -Ar-C=C, -HC=N (imine, azomethine),-CH (bending mode on -Ar ring) and -Ar-OH respectively. In a somewhat similar design, the condensation reaction between vanillin and heptyl amine afforded a deep yellow oily product. Likewise a combination of similar techniques and procedures as mentioned above was employed in its identification. Consequently, this second imine is equally new and its nomenclature is vanillyl heptyl imine.

Its IUPAC identity is 1-iminoheptyl-3-methoxy-4-hydroxy benzene and hereby accorded the trivial name of Iyadimine B. Its UV absorption at λ_{max} (401 nm) is significantly higher than that of vanillin at 248 nm while it is marginally higher than Iyadimine A at 344 nm. Consequently, the electron-cloud densities are found over -Ar-C=C, -OCH₃₁-HC=N (imine, azomethine) and -Ar-OH chemical species such as are seen in the former

Schiff base. The retardation factor R_c (0.87) of this compound is equally indicative of the non-polar nature of heptyl group (-CH₂),CH₂ evidently making it even more lipophillic than both vanillin and lyadimine A. Hence, it was observed to be weakly and poorly retarded on the silica gel plate. The IR spectral matrix of lyadimine B shows absorption strectchings at 801, 897, 1125, 1600, 1695, 2982 and 3482 cm⁻¹ which are characteristically diagnostic of alkyl bending modes, -C-O-C (ether linkage), -Ar-C=C,-HC=N (imine, azomethine),-CH (bending mode on -Ar ring) and -Ar-OH respectively.

A noticeable observation in both the IR spectra of lyadimine A and lyadimine B is the disappearance of the -C=O peak at 1642 cm⁻¹ which is an indication that both condensation reactions were successful. Physical parameters are important in identifying compounds. In the light of this refractive index and optical rotation are used in the qualitative and quantitative evaluations of substances. Furthermore these parameters are used to confirm the purity, identity and integrity of chemical substances. These physical parameters were both measured at the wavelength (λ) of Na-D light (589.3 nm) and a temperature of 20.5 °C. It is note-worthy that the refractive index of a substance is an indication of the number, type of atoms and chemical groups (species) in the substance. Each atom or group in the substance contributes its individual and distinct refractivity which adds eventually to the refractivities of other groups in a substance.

Hence, the refractive indices of the compounds were then determined. The pro-drug, vanillin and both synthesized bases; lyadimine A and lyadimine B gave refractive indices of 1.5774, 1.5783 and 1.5814 respectively. In addition, vanillin and both synthesized bases demonstrated optical rotation $[\alpha]_{D}^{20}$ of 0 implying that none had inherent chiral centres and therefore optically inactive. In addition, none of these compounds will demonstrate laevorotation (-) (ability of a compound to rotate plane of light in anticlockwise direction) or dextro-rotation (+) (ability of a compound to rotate plane of light in clockwise direction) (Olaniyi and Ogungbamila, 1991; Olaniyi, 2000).

5. Free-Radical Scavenging Activity

5.1. Antioxidant Assays

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Absorption spectrophotometry is routinely employed in evaluating antioxidant potential of compounds such as drugs, plant isolates or synthesized products (Oladimeji and Ahmadu, 2019). The preparation of a calibration curve is of immense necessity before the reagent can be used in such a bench-top assay. Consequently, DPPH (2, 2-diphenyl-1-picryl hydrazyl hydrate) reagent was subjected to this experiment with the aim of ascertaining its purity and suitability prior to the commencement of antioxidant determinations. The Beer-Lambert's Law remains the grundnorm for such determinations (Lebeau et al., 2000; Nagalapur and Paramjyothi, 2010). A calibration curve was obtained which fulfilled all the parameters spelt out in the Law as Figure 3 shows a straight line which passes through the origin. The reduction of the DPPH radical was determined by taking its absorption at a wavelength of λ_m 517 nm. The absorbance of DPPH was observed to decrease as the concentration of added free-radical scavenger (vanillin/Schiff base/Vitamin C) increased which indicated that the DPPH reagent was being reduced as shown in Table 2.

Table 2: Absorbance of Samples Incubated with DPPH at Different Concentrations (mg mL ⁻¹) at λ_{max} 517 nm (Blank Absorbance of 0.004 % w/v DPPH Reagent: 0.998) (± 0.004)							
Sample	0.0008	0.0012	0.0016	0.0020	0.0024		
Vitamin C	0.086	0.068	0.058	0.054	0.047		
Vanillin	0.363	0.272	0.270	0.269	0.268		
lyadimine A	0.618	0.578	0.538	0.500	0.486		
lyadimine B	0.555	0.497	0.484	0.475	0.463		
Key: Iyadimine A = Vanillyl hexyl imine (1-iminohexyl-3-methoxy-4-hydroxy benzene); Iyadimine B = Vanillyl heptyl imine (1-iminoheptyl-3-methoxy-4-hydroxy benzene)							

Furthermore, Table 3 displays the Radical Scavenging Activity (RSA%) or Percentage Inhibition (PI%) and the computed IC_{50} values of vanillin/Schiff base/Vitamin C. The RSA% is an indicator or a measure of the antioxidant activity of vanillin/Schiff base/Vitamin C. Interestingly, vanillin demonstrated a moderate antioxidant activity (IC_{50}) of 0.52 ig mL⁻¹ just as was obtained in (Oladimeji *et al.*, 2023). In addition, vanillin like most low molecular weight phenolic compounds possesses weak or moderate antioxidant properties (Oladimeji *et al.*, 2022). A combination of both observations is somewhat indicative of consistency and reproducibility of results. In this study the antioxidant activity given by vanillin is comparable to that elicited by Vitamin C at 0.48 ig mL⁻¹. It should also be mentioned that the value of obtained activity of Vitamin C in this study shows significant compatibility with 0.44 ig mL⁻¹ obtained in (Oladimeji *et al.*, 2022). However, both Iyadimine A and Iyadimine B were less active than both vanillin and Vitamin C at 1.95 and 1.03 μ g mL⁻¹ respectively.

Table 3: Radical Scavenging Activity (Percentage Inhibition %) of Samples at Different Oncentration	۱S
(mg mL ⁻¹) and IC ₅₀ of Samples (\pm 0.02)	

Sample	0.0008	0.0012	0.0016	0.0020	0.0024	IC ₅₀ (µgmL-1)
Vitamin C	91.40	93.19	94.15	94.28	95.26	0.48
Vanillin	63.60	72.75	72.96	73.05	73.15	0.52
Iyadimine A	38.42	42.41	46.10	49.10	51.30	1.95
lyadimine B	44.38	50.20	51.50	52.50	53.60	1.03

Key: Refer to Table 2, RSA% (PI%) = Radical scavenging activity (percentage inhibition %); IC₅₀ = Concentration at which 50% of DPPH is scavenged or inhibited.

Vanillyl heptyl imine (1-iminoheptyl-3-methoxy-4-hydroxy benzene) (Iyadimine B) is more lipophillic than vanillyl hexyl imine (1-iminohexyl-3-methoxy-4-hydroxy benzene) (Iyadimine A) principally on account of the heptyl group $(-C_7 H_{15})$ compared with a hexyl unit $(-C_6 H_{13})$. This molecular/chemical feature will engender lyadimine B to be more lipophillic and consequently will be able to traverse the lipodial membrane readily much quickly to the active or allosteric sites than lyadimine A where the pharmacological action of anti-oxidation is effectuated. Accordingly, Iyadimine B has a relatively better antioxidant activity than lyadimine A. The results of antioxidant tests are not surprising because vanillin and Schiff bases with metallic ligands have been reported to be strongly antioxidant (Wu *et al.*, 2014). Other equally efficient antioxidant assays outside of the DPPH test for determining the antioxidant activity of compounds include the hydrogen peroxide, nitric oxide, conjugated diene, superoxide, phosphomolybdenum, peroxynitrile and xanthine oxidase assays methods amongst many others (Bunta and Ingold, 1984; Dapkevicius *et al.*, 1998).

5.2. Antibacterial Activity

The microbes employed in this screening reflected the antibacterial spectrum encompassing one (1) gram (+) bacterium namely, *S. aureus* and one (1) gram (-) bacterial species *E. coli*. Vanillin was observed to be bacteriostatic

Table 4: Antibacterial Screening of Vanillin and Synthesized Schiff Bases at Different Concentrations on Test Microbes in 50% Methanol (± 0.01 mm)

Microbe	Vanillin 30 mg L-1	Iyadimine A 15 mg mL-1	Iyadimine A 30 mg L-1	Iyadimine B 15 mg mL- ¹	Iyadimine B 30 mg mL-1	Chloramphenicol 5 µg mL-1	MeOH/ H ₂ O (1:1)
S. aureus (NCTC 4532)	41.27	37.32	38.65	34.32	37.02	49.23	6.00
E. coli (NCTC 1065)	43.35	34.67	37.39	34.42	37.21	50.59	6.00
Key: Refer to Table 2. *The zone diameter recorded is zone of inhibition + size of cup (zone of inhibition +6.00) mm NCTC - National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London NW9, UK.							

against the two bacteria tested. However, it was more suppressive of *E. coli* than *S. aureus* which surprisingly corroborated earlier findings in (Oladimeji *et al.*, 2023). The two synthesized vanillyl imines demonstrated concentration-dependent antibacterial activities at 15 and 30 mg mL-¹ respectively as can be viewed in Table 4. In addition lyadimine A was more antibacterial against *S. aureus* than *E. coli* at both concentrations. Conversely, lyadimine B was slightly more active against *E. coli* than *S. aureus* Schiff bases have been reported and documented to be antibacterial (Mishra *et al.*, 2020). It is probably possible to infer from these observations that the two imines could be promising lead compounds in the search for newer and more efficacious antibacterial agents especially for the purpose of synergistic activity when co-administered in situations of chronically prevalent microbial resistance as witnessed currently in bactericidal chemotherapy.

5.3. Antifungal Potential

The evaluation of antifungal test was done with *C. albicans.* Similarly, the activities elicited by the two synthesized compounds were concentration-dependent at 15 and 30 mg L⁻¹ respectively just as observed in the antibacterial screening. Iyadimine A was more anti-candidal than Iyadimine B at both concentrations. In addition Iyadimine A demonstrated slightly better activity than vanillin at 30 mg L⁻¹ as Table 5 attests. Previously reported and documented studies on some cinnamyl Schiff bases have shown remarkable antifungal activity against both *C. albicans* and *Aspergillus fonsecaea* (Magalhaes *et al.*, 2020). Hence, the results obtained from this present research were not surprising. However, further studies especially on those involving synergistic co-administration of these new compounds are recommended.

Table 5: Antifungal Evaluations of Vanillin and Synthesized Schiff Bases at Different Concentration	۱S
on Test Microbes in 50% Methanol (± 0.01 mm)	

Microbe	Vanillin 30 mg L- ¹	Iyadimine A (15 mg mL-1)	Iyadimine A (30 mg L-1)	Iyadimine B 15 mg mL- ¹	Iyadimine B 30 mg mL- ¹	Fluconazole 1 mg mL- ¹	MeOH/ H ₂ O (1:1)
C. albicans (NCYC 2436)	24.71	18.62	25.83	15.12	22.34	42.89	6.00

Key: Refer to Table 2. *The zone diameter recorded is zone of inhibition + size of cup (zone of inhibition + 6.00) mm. NCYC - National Collection of Yeast Cultures, UK.

6. Conclusion

This present study reports for the first time the syntheses of two new vanillyl imines namely; 1-iminohexyl-3-methoxy-4-hydroxy benzene or vanillyl hexyl imine (Iyadimine A) and 1-iminoheptyl-3-methoxy-4-hydroxy benzene) or vanillyl heptyl imine (Iyadimine B) respectively. Both Schiff bases gave comparably marginal anti-oxidant activity of 1.95 and 1.03 μ g mL⁻¹ while vanillin and vitamin C elicited moderate activities at 0.52 and 0.48 μ g mL⁻¹ respectively. The two imines were both significantly suppressive of both bacteria tested.

Furthermore, Iyadimine A was more active against *S. aureus* than *E. coli* while the converse was true for Iyadimine B being relatively more bacteriostatic against *E. coli* than *S. aureus*. In addition both compounds also demonstrated some anti-candidal potential. Hence both imines could be lead drug templates for further considerations especially in more expanded synergistic antibacterial and antifungal co-administration studies to tackle the rising prevalence of microbial resistance to antibiotics and antifungal molecules already in clinical drug therapy.

Conflict of Interest

The authors declare no conflict of interest financial or otherwise.

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